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U.S. FOOD & DRUG ADMINISTRATION

Memorandum

To: File

From: Salome Bhagan, Ph.D.
Chemist
Chemistry Branch I, Division of Product Science
Office of Science, Center for Tobacco Products

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Date: 2023.11.06 12:14:21 -05'00'

Through: Matthew Hassink, Ph.D.
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Matthew J. Walters, Ph.D., M.P.H.
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Office of Science, Center for Tobacco Products

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Date: 2023.11.06 16:02:44 -05'00'

Subject: Considerations for Tobacco Specific Nitrosamines (TSNAs) in cigar filler, wrapper and binder, and mainstream smoke during premarket application product review

Purpose

The purpose of this memo is to provide information on the measured levels of tobacco specific nitrosamines (TSNAs): N'-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT) in the filler, wrapper and binder, and mainstream smoke (referred to as smoke) for filtered little cigars (FLCs), small cigars (such as cigarillos), and large cigars. The information provided in this memo may be helpful to the chemistry reviewer to (1) understand the relationship between TSNAs in ground cigars and cigar smoke, (2) inform a reviewer's determination of when TSNA data is needed to fully evaluate the impact of reported changes (such as wrapper and binder changes), and (3) to provide data for comparison products including cigarettes, smokeless tobacco products, electronic nicotine delivery system liquids (e-liquids), and different types of cigars that may be relevant for premarket application review (i.e., SE/PMTA).

Introduction

Combusted tobacco products, like cigars and cigarettes, release hundreds of volatile organic compounds including at least 60 compounds confirmed to have carcinogenic activity, including benzene, aromatic amines, formaldehyde, acetaldehyde, 1,3-butadiene, polyaromatic hydrocarbons, and tobacco-specific nitrosamines (TSNAs).[1] The levels of TSNAs in smokeless tobacco products have been thoroughly investigated [2] but levels in cigars have been less studied.[3] Of the TSNA cigar studies found in literature, one TSNA cigar study focused on

[1] Centers for Disease Control and Prevention (US); National Center for Chronic Disease Prevention and Health Promotion (US); Office on Smoking and Health (US). How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US); 2010. 5, Cancer. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53010/

[2] E. Konstantinou, F. Fotopoulou, A. Drosos, N. Dimakopoulou, Z. Zagoriti, A. Niarchos, D. Makrynioti, D. Kouretas, K. Farsalinos, G. Lagoumintzis, K. Poulas, Tobacco-specific nitrosamines: A literature review, Food Chem. Toxicol. 118 (2018) 198–203.

[3] The National Academies of Sciences, Engineering, and Medicine Report on Premium Cigars: Patterns of Use, Marketing, and Health Effects. 2022

FLCs using cigarette smoking methods[4] and another reported the total alkaloids in large cigars and not individual TSNA levels[5]. A previous memo has shown NNN and NNK smoke yields can be influenced by saccharides present in cigars.[6] To aid a reviewers understanding of TSNA levels in cigars, preliminary data from FDA's Co-operative Cigar Reference Program (CRP) with the University of Kentucky (RFA-FD-20-002) on the TSNA levels in ground cigars (i.e., all tobacco derived components such as wrapper/binder, and filler) and in smoke using cigar smoking methods and machines are presented and evaluated in this memo.

TSNA Formation

The mechanistic pathways for the formation of TSNAs are well studied.[7] TSNAs are predominantly formed from the reaction of tobacco alkaloid precursors with nitrite or nitrate during tobacco curing, fermentation, and ageing. The majority of NNK and some NNN are formed from the tertiary amine of nicotine by oxidation in the 1', 2' bond of the pyrrole ring to form pseudo-oxo nicotine (PON) followed by nitrosation to produce NNK at the later stages of tobacco curing and fermentation. NNN, NAB and NAT are formed primarily from the corresponding secondary amines (i.e., nornicotine, anatabine, and anabasine, respectively) during the early stages of tobacco curing and processing. Figure 1 shows the structure of TNSAs formed through nitrosation.[8]

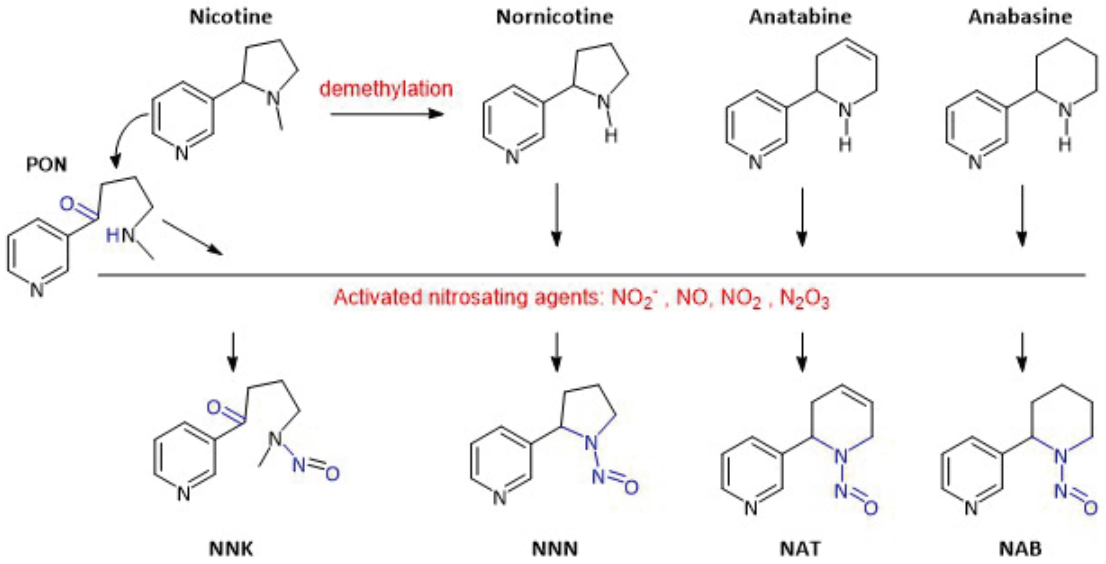

Figure 1. Structure of TSNAs formed by nitrosation of tobacco alkaloids; nitrosamine functional group shown in blue.

Four main factors that influence TSNA formation[9] in tobacco products are discussed here to provide relevant background for chemistry reviewers when evaluating NNN and NNK quantities and formation in cigars. The four main factors are:

[4] Edwards, S. H., M. D. Hassink, K. M. Taylor, C. H. Watson, P. Kuklenyik, B. Kimbrell, L. Wang, P. Chen, and L. Valentín-Blasini. Tobaccospecific nitrosamines in the tobacco and mainstream smoke of commercial little cigars. Chemical Research in Toxicology 34(4), 2021, 1034–1045.

[5] Dethloff, O., C. Mueller, X. Cahours, and S. Colard. Cigar burning under different smoking intensities and effects on emissions. Regulatory Toxicology and Pharmacology 91, 2017, 190–196.

[6] Addendum to July 17, 2017 Memo "Review of Saccharides as Tobacco Ingredients: Effects on Smoke Chemistry" - Recommendations for Chemistry SE Review of Cigar Tobacco Products (2020)

[7] (a) Rostkowska, K.A., Zwierz, K., Rózański, A., Moniuszko-Jakoniuk, J., & Roszczenko, A.Formation and metabolism of N-nitrosamines. Polish Journal of Environmental Studies, 07, 1998. 321-325. (b) Veena, S., Rashmi, S., A Review on Mechanism of Nitrosamine Formation, Metabolism and Toxicity in In Vivo. International Journal of Toxicological and Pharmacological Research 2014; 6(4): 86-96.

[8] T.A. Perfetti, A. Rodgman. The complexity of tobacco and tobacco smoke. Beitrage zur Tabakforschung Int. Contributions to Tobacco Res., 24 (2011), pp. 215-232

[9] Gupta, A., Tulsyan, S., Bharadwaj, M., Mehrotra, R., Grass roots approach to control levels of carcinogenic nitrosamines, NNN and NNK in smokeless tobacco products, Food and Chemical Toxicology, 124, 2019, 359-36.

(1) Agronomic practices: During tobacco growth, nitrogen is incorporated in tobacco leaves in the form of nitrate. However, TSNAs are undetectable or at a very low level in fresh leaves before harvest.[10] Higher NNN and NNK levels are found in cured tobacco from tobacco crops fertilized with nitrogen rich fertilizers (i.e., nitrate and nitrite accumulates in tobacco leaves). Burley and other dark tobacco require more nitrogenous fertilizer to achieve an economically viable yield, thus high levels of nitrate accumulate in the leaves resulting in increased levels of TSNAs postharvest (Burley has approx. 10x more nitrate content than flue cured).[11] Nitrate is chemically unstable and may produce gaseous nitrogen oxides which could easily react with alkaloids to form TSNAs by nitrosation. Relatively high levels of NNN and NNK also occur in the midribs of air-cured tobacco or lamina from flue-cured tobacco[12].

(2) Leaf Genetics and Curing Method: The method for curing tobacco (e.g. air-, fire-, flue- or sun-curing) and the various steps of processing (such as heating with propane during curing) are important factors influencing quantities of TSNA in tobacco.[13] During traditional air curing, nitrite is formed after approximately two to three weeks of curing, i.e. at the end of the yellowing stage when the leaf turns brown and the cell membranes break down due to moisture loss making the cell contents available to microorganisms that naturally exist on or in the tobacco leaf. TSNAs are formed during curing from the reaction of tobacco alkaloids with microbially generated nitrite. [14] Studies have shown that curing of tobacco leaves at higher temperatures and humidity increases the accumulation of nitrate/nitrite which impact TNSA formation.[15]

(3) Manufacturing Practices: During manufacturing, use of alkaline agents can adjust the pH which impacts the equilibrium distribution of nicotine free base species which are the precursors to NNN, NAT and NAB. During tobacco fermentation, microbial-mediated reduction of nitrate generates nitrite, which further reacts with alkaloids present in tobacco to produce TSNAs.[16]. Non-sterilized manufacturing equipment can retain and transfer nitrate reducing bacteria commonly on tobacco plants, such as *Staphylococcus*, and *Corne bacterium*, which may also impact NNN and NNK formation.[17]

(4) Storage Conditions (prior to manufacturing): Before harvest, TNSAs are undetectable or at very low levels in fresh leaves.[18] Post harvest, tobacco leaves are stored on the farm (usually for approximately 3 months) and then subsequently stored at the manufacturer for an extended period, usually an additional 18 months, before being used in manufacturing. Post manufacturing humid storage conditions and insufficient air flow support micro-organism growth often resulting in increased microbial activity with increased nitrate to nitrite conversion and TSNA formation.[19]

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[10] Jiang Y, Gong J, Chen Y, Hu B, Sun J, Zhu Y, Xia Z, Zou C. Biodegradation of Nicotine and TSNAs by Bacterium sp. Strain J54. Iran J Biotechnol. 2021 Jul 1;19(3):2812

[11] Li, Y., Chang, D., Zhang, X. et al. RNA-Seq, physiological, and biochemical analysis of burley tobacco response to nitrogen deficiency. Sci Rep 11, 16802 (2021).

[12] Burton, H. R., Dye, N.K., and Bush, L.P., Relationship between Tobacco-Specific Nitrosamines and Nitrite from Different Air-Cured Tobacco Varieties. J. Agric. Food Chem, 1994 42 (9), 2007-2011

[13] Peele, D.M., Riddick, M.G. & Edwards, M.E. Formation of tobacco-specific nitrosamines in flue-cured tobacco. Recent Adv. Tob. Sci., 2001, 27, 3–12. Bush, L.P., Cui, M., Shi, H., Burton, H.R., Fannin, F.F., Lei, L. & Dye, N. Formation of tobacco-specific nitrosamines in air-cured tobacco. Recent Adv. Tob. Sci., 2001, 27, 23–46

[14] Staaf, M., Back, S., Wiernik, A., et al., 2005. Formation of Tobacco-specific Nitrosamines (TSNA) during Air-curing: Conditions and Control. Contributions to Tobacco & Nicotine Research, vol.21, no.6, 2005, pp.321-330

[15] (a) Burton HR, Childs Jr.GH, Andersen RA, Fleming PD. 1989. Changes in chemical composition of burley tobacco during senescence and curing. 3. Tobacco-specific nitrosamines. J Agric Food Chem 37:426–430. (b) Weirnik A, Christakopoulos A, Johansson L, Wahlberg I. 1995. Effect of air-curing on chemical composition of tobacco. Rec Adv Tob Sci, 1995, 21, 39–80..

[16] (a) Fisher MT, Bennett CB, Hayes A, et al. Sources of and technical approaches for the abatement of tobacco specific nitrosamine formation in moist smokeless tobacco products. Food Chem Toxicol. Mar 2012;50(3-4):942-8. (b) Di Giacomo M, Paolino M, Silvestro D, et al. Microbial community structure and dynamics of dark fire-cured tobacco fermentation. Appl Environ Microbiol. Feb 2007;73(3):825-37.

[17] Tyx, R.E., Stanfill, S.B., Keong, L.M., et al., Characterization of bacterial communities in selected smokeless tobacco products using 16S rDNA analysis. PLoS ONE 11(1), 2016, e0146939.

[18] Shi, H. et al. Changes in TSNA contents during tobacco storage and the effect of temperature and nitrate level on TSNA formation. J. Agric. Food Chem. 2013, 61, 11588−11594.

[19] Smokeless tobacco and some tobacco-specific N-nitrosamines. IARC Monogr Eval Carcinog Risks Hum. 2007; 89: 1–592. – Various excerpts used throughout.

In this memo, we evaluate TSNA levels in the wrapper and binder, tobacco filler, and smoke in FLCs, small cigars, and large cigars. Although agricultural and manufacturing practices, curing methods, and storage conditions impact TSNA levels in tobacco and tobacco products, these factors are largely manufacturer trade secrets or propriety information. Therefore, this memo will focus on TSNA levels and other relevant product data measured from 52 cigars including FLCs, small cigars, and large cigars.

Public Health Perspective on TSNAs

Metabolic activation of TSNAs in the human cell results in several intermediates that can directly bind with or alkylate DNA (via CYP450 catalyzed α-hydroxylation to form reactive oxygen species) or react with another compound to form alkylating agents that may cause mutation to DNA or formation of cancerous tumor cells. [20] Of the TNSAs present in tobacco, NNN and NNK are identified and classified as human carcinogens (IARC Group 1) by the International Agency for Research on Cancer.[21]

On January 23, 2017, FDA issued a proposed product standard rule on the NNN level in smokeless tobacco products[22] which stated NNN is a potent carcinogenic agent found in smokeless tobacco products and is a major contributor to the elevated cancer risks associated with smokeless tobacco use. NNK and its primary metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), are thought to be particularly important in the induction of adenocarcinoma, now the leading lung cancer in the United States.[23] The proposed mechanism for the formation of NNN and NNK from nicotine is shown in Figure 2 (contribution from Saibal Chakraborty). NAB has been reported to possibly cause tumors in the esophagus.[24] NAT is reported to have little or no carcinogenic activity.[25] As a result, in this memo, a majority of the evaluation is focused on NNN and NNK, with information on NAT and NAB as relevant.

Because of the cancer health risks identified by IARC, *NNN and NNK levels in cigars are the regulatory focus for reviewers*. As NNN and NNK quantities and quantity differences are also attributed to microbial mediated nitrosation reactions and chemical pathways, data is presented in both mass per gram of product and mass per unit cigar to be most useful to the microbiology and chemistry reviewer, respectively.

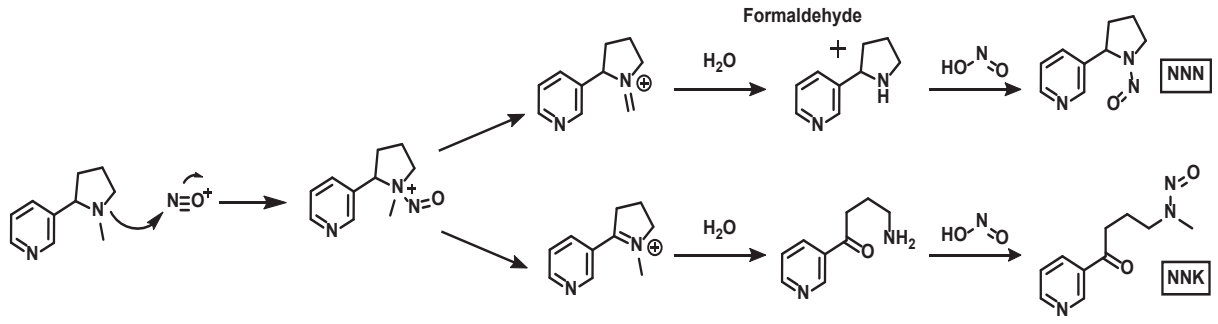

Figure 2. Proposed pathway for the conversion of nicotine to NNN and NNK

[20] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Personal Habits and Indoor Combustions. Lyon (FR): International Agency for Research on Cancer; 2012. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 100E.) N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

[21] International Agency for Research on Cancer (IRAC), 2012. Review of Human Carcinogens. World Health Organization. http://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Evaluation-Of-Carcinogenic-Risks-To-Humans/Review-Of-Human-Carcinogens-Package-Of-6-Volumes-A-B-C-D-E-F-2012.

[22] 82 FR 8004-8053: Tobacco Product Standard for N-Nitrosonornicotine Level in Finished Smokeless Tobacco Products (Proposed Rule)

[23] Thun, M. J., Lally, C. A., Flannery, J. T., Calle, E. E., Flanders, W. D., & Heath, C. W. Jr. (1997). Cigarette smoking and changes in the histopathology of lung cancer. Journal of the National Cancer Institute, 89, 1580–1586.

[24] Boyland E, Roe FJC, Gorrod JW, Mitchley BCV. The carcinogenicity of nitrosoanabasine, a possible constituent of tobacco smoke. British Journal of Cancer 1964; 18(2): 2265-270

[25] Hoffman D, Rivenson A, Amin S, Hecht SS. Dose response study of the carcinogenicity of tobacco specific N- nitrosamine in F344 rats. Journal of cancer research and clinical oncology 1984; 108: 81-86. Padma PR, Amonkar AJ, Bhide SV. Mutagenic and cytogenetic studies of N-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl) -1-butanone. Cancer letters 1989; 46: 173-180.

Experimental & Data Analysis

Fifty-two marketed cigars (25 FLCs, 16 small cigars, and 11 large cigars)[26] were purchased and stored in a freezer at –20°C in the original packaging until the time of testing; see Table A (Appendix) for a list of the cigars used. Marketed cigars in this study were limited to machine–made cigars and categorized as described in the November 16, 2021, memo on FLCs, small cigars, and large cigars.[27] Three days prior to analysis, cigars were conditioned as prescribed in CORESTA Recommended Method (CRM) N°46[28] at 22°C and 60% relative humidity (RH). The temperature and RH during smoking are maintained in accordance with CRM N°65[29]. All cigars were smoked (10 replicates for FLCs, and 8 replicates for small and large cigars) using CRM N°64 (20 mL puff volume for cigars ≤ 12mm diameter or puff volume =0.139*diameter^2 for cigars > 12mm diameter), 1.5 s puff duration, 40 s inter-puff interval).[30] For FLCs, smoke generation was performed mechanically by a linear Cerulean SM-450 smoke machine. Smoke generation for small cigars and large cigars was performed using a LM5C cigar smoking machine. Tobacco alkaloids in ground cigars were determined individually for the cigar filler and for the wrapper and binder using an in-house method adapted from CRM N°62 using methyl-tert-butyl ether (MTBE) as the extract solution and then subsequently analyzed using gas chromatography/flame ionization detection.[31] Average TSNA transfer (ATT) was calculated by dividing the total TSNAs in smoke by the total TSNAs in the ground cigar (i.e., the sum of NNN, NNK, NAT, and NAB measured in the ground filler and the wrapper and binder). Note: Non-tobacco components such as the filter and tipping paper in FLCs are not included in the ground cigar. Ground cigar refers to tobacco derived filler, wrapper, and binder of cigars.

Results and Discussion

Evaluation of the TSNA levels in ground cigars (wrapper+binder and filler) and in cigar smoke of FLCs, small cigars, and large cigars is discussed in this section. The amount of TSNAs in the ground cigar transferred to the cigar smoke is calculated and reported as % ATT. Additionally, TSNA levels in the three types of cigars is compared to other tobacco products.

NNN and NNK levels in Whole Ground Cigars

TNSA levels in ground cigars are evaluated by the microbiology reviewer to determine if the cigar, due to microbial activity, raise public health concerns. In reviews, microbiology typically compares TNSA levels on a per gram basis in order to compare different products on a uniform basis. The results, on a per gram basis, are illustrated in Figure 3. The TSNA levels, based on contributions from the tobacco filler compared to the wrapper/binder, are shown in Figure 4. In general, TSNA levels follow the following trend NAB < NNK <NAT<NNN in ground cigars and tend to increase on a per gram basis, such that FLCs < small cigars< large cigars, see Table 1. Total TSNAs in ground cigars was found to range from 4–30.2 μg/g for FLCs, 7.1–104.4 μg/g for small cigars, and 21.8–79.4 μg/g for large cigars. NNN accounted for 41–68% of the total TSNAs while NNK accounted for 7–39% for all cigar types. The ranges of NNN and NNK were found to be 2.7 – 12.9 μg/g and 0.4–11.7 μg/g, respectively, for FLCs, 3.8 –56.9 μg/g and 1.1–10.7 μg/g, respectively, for small cigars and 11.1– 44.8 μg/g and 1.9–9.4 μg/g, respectively, for large cigars.

As noted above, NNN levels were higher than NNK levels, as high as 537% more in FLCs, 664% more in small cigars, and 790% more in large cigars, Table 1. The ratios of NNN to NNK are 1.1 – 5.4 to 1 for FLCs[32], 1.9 – 6.6 to 1 for small cigars, and 2.4 – 7.9 to 1 for large cigars and illustrate the tendency of NNN quantities to exceed NNK quantities from 1.1x more up to 7.9x more in large cigars.

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[26] See Appendix for full list of products in study. Note: Large cigars selected for this study were 40–44 ring gauge, machine made cigars with shapes described by the manufacturer as corona, parejo, perfecto, or presidente.

[27] Memo titled "Considerations on the use of the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Recommended Method N°64 to measure Tar, Nicotine, and Carbon Monoxide (TNCO) smoke yields for filtered little cigars (FLCs)".

[28] CRM No 46 - Atmosphere for conditioning and testing cigars of all sizes and shapes

[29] CRM No 65 - Determination of total and Nicotine-free dry particulate Matter using a routine Analytical cigar-smoking Machine – Determination of Total particulate matter and Preparation for water and Nicotine measurements

[30] CRM No. 64 Routine analytical cigar-smoking machine - specifications, definitions, and standard conditions

[31] CRM No. 62 Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis

[32] Except Djarum which is an Indonesian kretek cigar

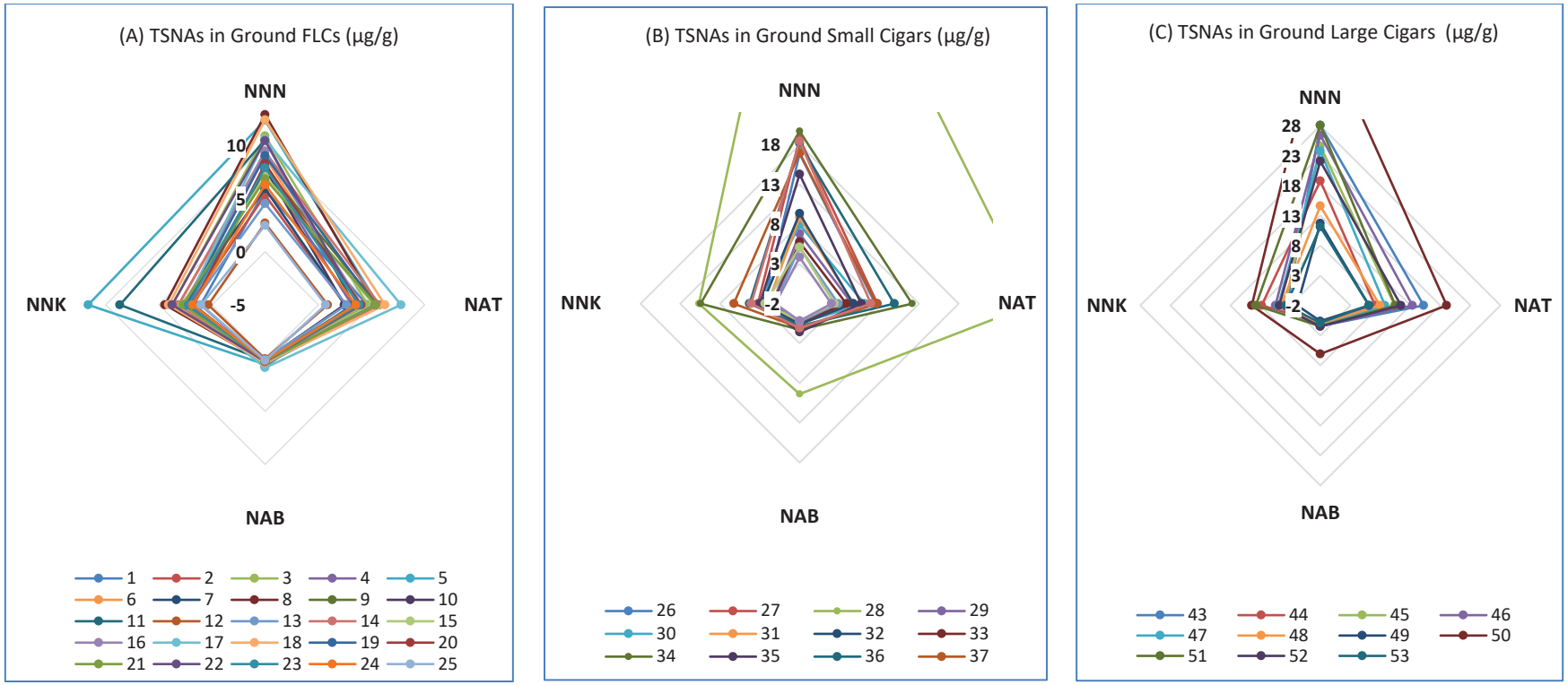

Figure 3. Distribution of NNN, NNK, NAB, and NAT in cigar sample #'s of ground (A) FLCs, (B) Small Cigars, and (C) Large Cigars in µg/g.

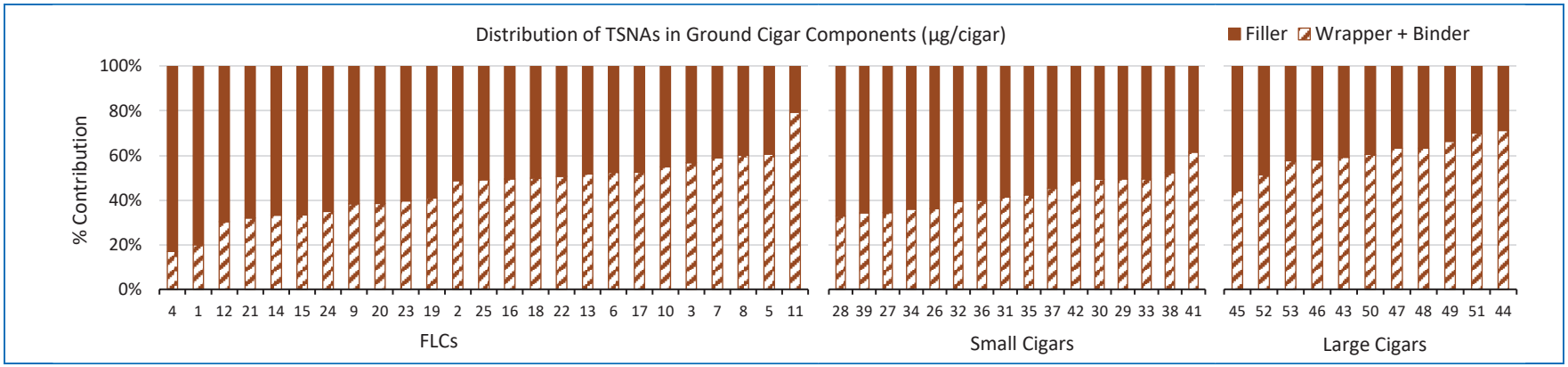

Figure 4. Contribution of total TSNAs in the ground wrapper and binder compared to the filler of FLCs, small cigars, and large cigars sorted by increasing total TSNAs in the cigar wrapper and binder in µg/cigar.

Because NNN and NNK are directly derived from nicotine in the tobacco; NNN and NNK levels as a percent of the overall nicotine, in the ground cigars, were evaluated. The minimum percentage of NNN and NNK relative to nicotine increase in order from FLCs (0.0025% and 0.0006%, respectively) to small cigars (0.0033% and 0.0007%, respectively), finally to large cigars (0.0046% and 0.0008%, respectively). The maximum NNN% relative to nicotine were similar for all cigar types, i.e., 0.0072% for FLCs and 0.0068% for small and large cigars. The maximum NNK% compared to nicotine levels were 0.0025% for both small and large cigars but higher in FLCs, 0.0043%.

Table 1. NNN and NNK as a function of total TSNAs and percent relative to other HPHCs by cigar type.

	Total TSNAs (µg/g)	% NNN relative to total TSNAs	% NNK relative to total TSNAs	% NNN relative to NNK	% NNN relative to nicotine	% NNK relative to nicotine
FLCs	4 – 30.2	41 – 68	9 – 39	106 – 537	0.0025 – 0.0072	0.0006 – 0.0043
Small Cigars	7.1 – 104.4	45 – 61	9 – 24	188 – 664	0.0033 – 0.0068	0.0007 – 0.0024
Large cigars	21.8 – 79.4	51 – 62	7 – 23	241 – 790	0.0046 – 0.0068	0.0008 – 0.0025

Evaluation of the relationship between total nicotine compared to NNN and NNK levels in the ground cigar showed linear relationships with a goodness of fit of 0.72–0.76, as shown in Figure 5. As nicotine in the ground cigar increases, the NNN and NNK levels are observed to also increase, however there is overlap in the nicotine concentrations among different cigar types and reviewers should not rely solely on nicotine concentration to distinguish cigar types or subcategories, Figure 5.[33]

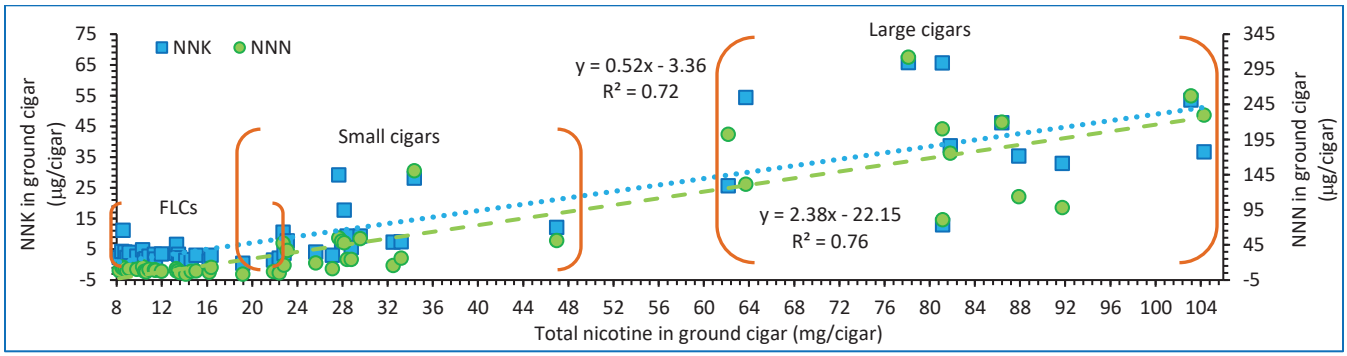

Figure 5. Relationship of nicotine and NNN and NNK levels showing overlap among ground cigar types.

Figure 6 shows NNN and NNK levels per cigar relative to tobacco rod mass (intercept = 0). As shown in Figure 6, the linear relationship is accompanied by clustering of data based on cigar as clear demarcations between cigar types due to similarities in tobacco rod mass are observed. The cigar mass ranges for each cigar type do not overlap and have gaps of approximately 1 -3 g/cigar, and so the mass data for this study lacks a dynamic range, however, this allows us to identify that NNN and NNK are not independent of cigar type. Therefore, reviewers should cautiously estimate NNN and NNK using the equations shown in Table 2 because NNN and NNK levels in the ground cigar , as shown in Figure 6, relationship to cigar is not independent of cigar type (i.e., data clusters).

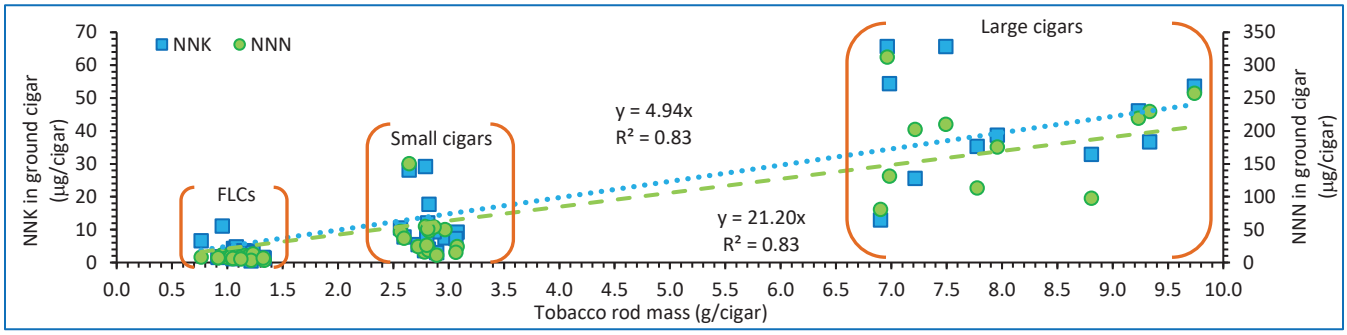

Figure 6. Relationship of cigar mass and NNN and NNK (ground cigar) levels clustering based on cigar type.

[33] CTP Internal Memorandum: Unique Identification of Tobacco Products, April 20, 2021

Table 2. Estimation of NNN and NNK in cigars using tobacco rod mass or nicotine in the ground cigar

	Variable (x)	R^2	NNN (μg/cigar)	R^2	NNK (μg/cigar)
Filler *only*	nicotine (mg/cigar)	0.77	y = 1.10x – 8.61	0.86	y = 0.25x – 1.84
	mass (g/cigar)	0.81	y = 11.06x – 6.55	0.89	y = 2.50x – 1.36
Wrapper+Binder *only*	nicotine (mg/cigar)	0.78	y = 7.57x – 1.63	0.58	y = 1.50x + 0.59
	mass (g/cigar)	0.67	y = 108.49x – 12.44	0.48	y = 21.0x – 1.36
Tobacco Rod (filler + wrapper/binder)	nicotine (mg/cigar)	0.76	y = 2.38x – 22.15	0.72	y = 0.52x – 3.36
	mass (g/cigar)	0.83	y = 21.20x	0.83	y = 4.94x

The TSNAs present in the wrapper+binder and filler were analyzed to evaluate the contribution of different components to the total TSNA quantities. NNN in the wrapper+binder accounts for 17– 59 % (FLCs), 31–62% (small cigars), and 43–68% (large cigars) of the total NNN in the ground cigar. The contribution of NNN from the wrapper+binder is a significant source of NNN in the ground cigars. NNK in the wrapper+binder accounts for 1–25 % (FLCs), 2–15% (small cigars), and 3–16% (large cigars) of the total NNK. The wrapper+binder contribution to total NNK in the ground cigar is lower than that for NNN in the ground cigar. The wrapper+binder TSNA contribution to the overall cigar TSNAs increase per cigar type from 17–61% for FLCs , to 33–51% for small cigars, and to 44–71% for large cigars. Despite the physcial differences among different cigars types (e.g., rod mass), cigar types are not distinguishable using HPHC (nicotine, NNN, and NNK) levels. Reviewers may estimate the NNN and NNK levels in wrapper+binder in a ground cigar using the equations in Table 2.

Product moisture and pH are typically measured so they can be evaluated with TNSA levels. Therefore, pH and oven volatiles (in lieu of moisture) were also measured in this study.[34] Figure A (Appendix) shows oven volatiles as function of NNN and NNK in ground cigars and smoke. In this study, oven volatiles in cigars are observed to increase from FLCs, to small cigars to large cigars. Percent oven volatiles range from 9.9–14% for FLCs, 12.2–17.5% for small cigars, and 14.1–16.6% for large cigars, see Figure A (Appendix). Based on this trend of increasing oven volatiles with increasing cigar size, premium cigars which are generally larger in mass than machine made large cigars (tested in this study), are anticipated to have even higher oven volatiles (i.e., greater than 14–16.6 % oven volatiles observed in large cigars) through extrapolation of this data trend.

For the cigars in this study, the data and the poor linear regression fit (R^2 = 0.30), in Figure 7A, do not show a strong relationship between percent oven volatiles and NNN and NNK levels. The filler pH compared to the NNN and NNK levels per cigar also shows a poor linear regression fit where R^2 = 0.31–0.33 (see Figure 7B), and therefore the pH (4.9–7.1) does not have a strong relationship to NNN and NNK levels in cigars; therefore, filler pH NNN and NNK should not be used to extrapolate smoke yields in cigar reviews.

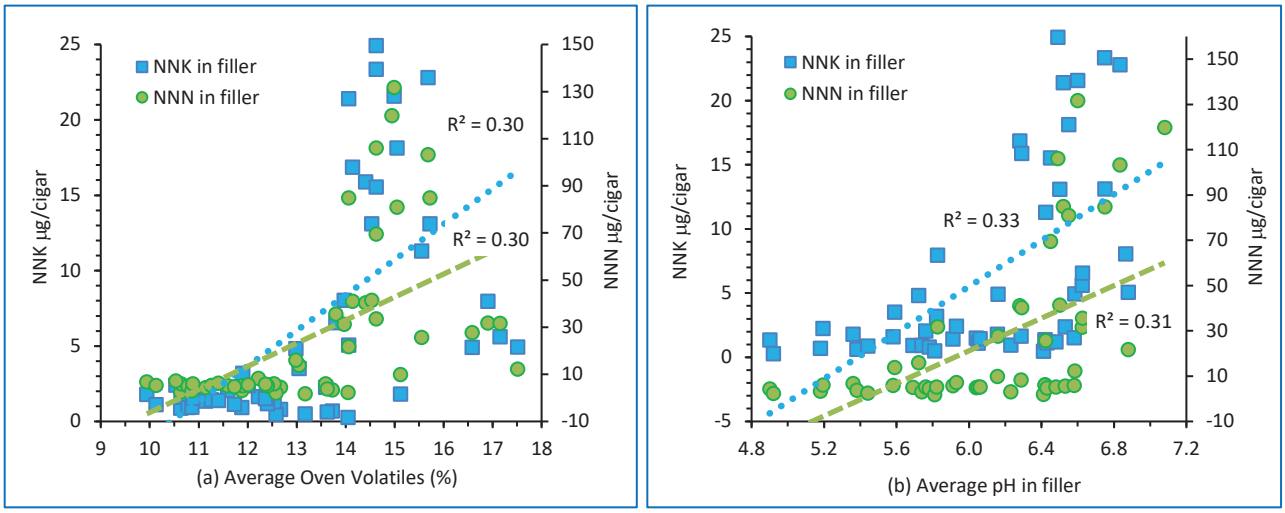

Figure 7. Relationship of NNN and NNK levels to (A) oven volatiles (%) and (B) pH in cigars

[34] Edwards SH, Hassink MD, Taylor KM, Vu AT. Quantitative measurement of harmful and potentially harmful constituents, pH, and moisture content in 16 commercial smokeless tobacco products. Regulatory Toxicology and Pharmacology. 2022; 133, 105199.

Based on the analysis of NNN and NNK levels measured in ground cigars (note: cigars studied were purchased from stores and did not include manufacture date), the following chemistry key findings:

- Reviewers should pay particular attention to differences due to changes in wrapper+binder as wrapper+binder are a significant source of NNN and NNK in cigars.
- Oven volatiles increase from FLCs, to small cigars, to large cigars, however, oven volatiles do not have a strong relationship with NNN and NNK levels.
- NNN and NNK lack an apparent relationship to cigar filler pH based on the data in this study.
- Cigar mass and nicotine are proportional to NNN and NNK in the ground cigar (or ground cigar components). If cigar mass and nicotine in the ground cigar are provided (for the whole ground cigar, filler only, or wrapper+binder only), reviewers can use the equations in Table 2 to cautiously estimate NNN and NNK in various components of the cigar using the tobacco mass or nicotine.

TSNA Smoke Yields of Cigars

All four TSNAs are present in cigar smoke at various concentrations; the percent distribution of individual TSNAs in cigar smoke is shown in Figure 8A. NNN accounts for the majority of TSNAs in cigar smoke (32–55%), similar to its majority in cigar filler, and NNK accounts for 13–36%. NAT contributes 18–41% and NAB is the lowest contributor with 2–12% of the total TSNAs. Figure 8 B–D show the standard deviation associated with each TSNA measured in cigar smoke based on cigar types. In all cigars measured, NAB and NAT have small relative standard deviations (RSD) compared to NNN and NNK. Generally, the measurements of NNK in FLCs showed greater RSDs than NNN, however, for small and large cigars NNN showed larger RSDs than NNK. These differences may be due to the impact of pyrosynthesis on TSNA smoke yields; however, pyrosynthesis role is not completely understood at this time.[35] NNN and NNK cigar smoke yields are discussed further in the following subsection.

NNN and NNK Smoke Yields of Cigars

The following analysis is intended to provide the scientific reviewer with useful information to address questions which may occur in reviews or research proposals regarding the presence of NNN and NNK in cigars.

What are the levels of NNN and NNK in different cigar types? Table 3 shows the amounts of NNN and NNK in each type of cigar as measured in the filler, wrapper and binder, and smoke and can be a helpful reference data for future reviews and research projects at CTP. Generally, the levels of NNN and NNK increase from FLCs, to small cigars, and are greatest in large cigars.

Table 3. Quantites of NNN and NNK measured in cigar components and in the smoke of FLCs, small cigars, and large cigars

	FLCs			Small Cigars			Large Cigars		
	Filler	Wrapper + Binder	Smoke	Filler	Wrapper + Binder	Smoke	Filler	Wrapper + Binder	Smoke
NNK (µg/cigar)	0.27 – 3.2	0.17 – 7.9	0.06 – 0.44	0.7 – 23	0.83 – 6.4	0.11 – 2.4	4.9 – 34	8.0 – 50	0.66 – 4.0
NNN (µg/cigar)	1.8 – 7.2	0.98 – 8.2	0.18 – 0.65	3.4 – 103	3.3 – 47	0.21 – 2.8	28 – 132	53 – 192	1.9 – 6.6
NNK (µg/g)	0.22 – 3.35	0.14 – 8.3	0.05 – 0.42	0.45 – 8.6	0.60 – 2.3	0.04 – 0.86	0.71 – 4.9	1.2 – 6.7	0.10 – 0.56
NNN (µg/g)	1.3 – 6.5	0.81 – 7.9	0.13 – 0.52	2.0 – 39	1.8 – 18	0.11 – 0.98	4.0 – 17	6.5 – 28	0.21 – 0.94

Can we extrapolate cigar filler measurements to smoke yields? The relationship between NNN and NNK in filler, NNN and NNK in ground cigar, nicotine in ground cigar, and the tobacco rod mass of cigars to the NNN and NNK measured in smoke is summarized in Table 4 and example graphs are shown in Figure 10. Evaluation of the relationship between ground cigar measurements and NNN and NNK smoke yields resulted in four outliers[36] which were Garcia y Vega Game Cigarillo Black, Pom Pom Sweet cigarillo, Swisher Sweets Blunts Natural Petite Corona Sweet Large Cigar, and William Penn Perfecto large cigar.

[35] Lipowicz PJ, Seeman JI. A Model to Estimate the Sources of Tobacco-Specific Nitrosamines in Cigarette Smoke. Chem Res Toxicol. 2017;30(8):1556-1561. Wu W, Zhang L, Jain RB, Ashley DL, Watson CH. Determination of carcinogenic tobacco-specific nitrosamines in mainstream smoke from U.S.-brand and non-U.S.-brand cigarettes from 14 countries. Nicotine Tob Res. 2005;7(3):443-451.

[36] Identified by visual inspection of the graphed data

Figure 8. (A)Distrubition of TSNAS in cigar smoke showing standard deviations measured for (B) FLCs, (C) small cigars, (D) large cigars..

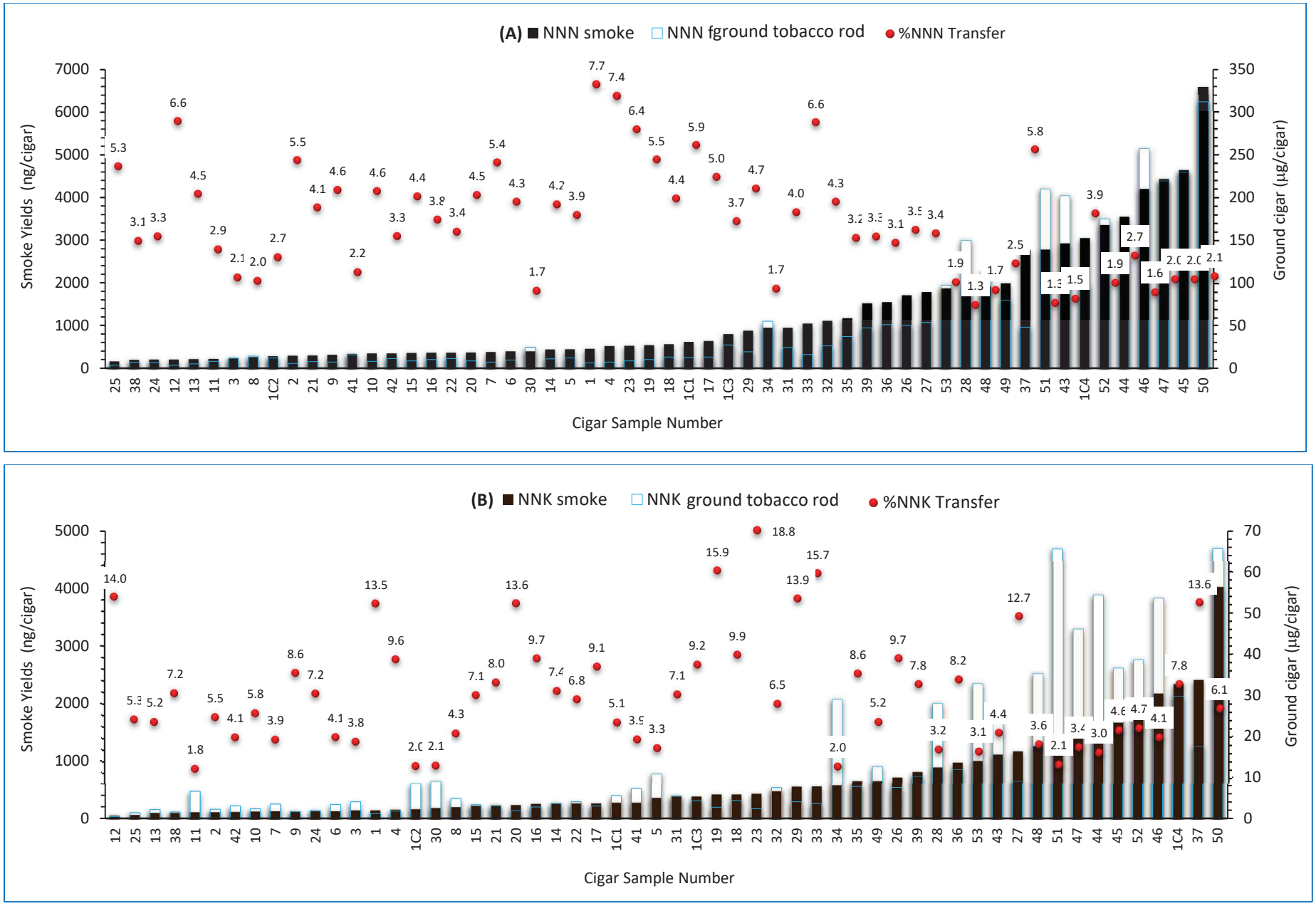

Figure 9. (A) NNN and (B) NNK in the ground cigar (blue stacks), cigar smoke (black stacks), and the percent of NNN and NNK transferred from the ground cigar to cigar smoke (red circles).

Table 4. Estimation of NNN and NNK in cigars using tobacco rod mass or nicotine in the ground cigar

Variable (x)	NNN (y)		NNK (y)	
	R^2	Smoke (ng/cigar)	R^2	Smoke (ng/cigar)
NNN/NNK in filler (mg/cigar)	**0.94**	**y = 37.41x + 264.88**	**0.91**	**y =76.51x + 149.89**
NNN/NNK in ground cigar (mg/cigar)	0.93	y = 37.56x – 46.12	0.78	y = 30.60x + 202.04
Nicotine in ground cigar (mg/cigar):	0.81	y = 37.56x – 46.12	0.76	y = 16.44x + 43.82
Total tobacco rod mass (g/cigar)	0.92	y = 380.48x	0.90	y = 177.63x

Nicotine in the ground cigar (Figure 10A) and total tobacco rod mass shows (intercept = zero) good linearity to NNN and NNK smoke yields, respectively (correlation coefficient square values (R^2) of 0.76–0.92). However, the data scatters widely on either side the trendline at higher concentrations and clusters together at lower concentrations. Only the relationships of NNN/NNK in the filler (Fig 10B) and NNN/NNK in the ground cigar to NNN or NNK in smoke showed good alignment of the data points to the trendline with near linearity of R^2 = 0.91–0.94 and good linearity of R^2 = 0.78–0.93, respectively. These two equations, but especially the relationship of NNN or NNK in the filler can be used to estimate NNN or NNK smoke yields for all cigar types, Table 4.

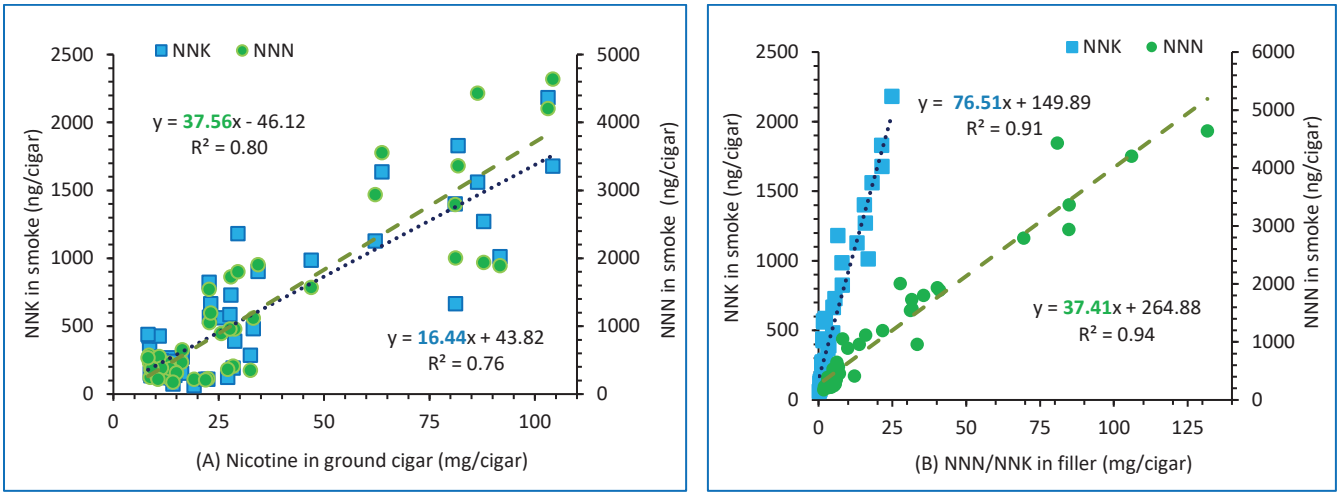

Figure 10. Relationship of the (a) nicotine in the ground cigar and (b) NNN and NNK in the cigar filler to NNN and NNK in smoke.

For all cigar types collectively, approximately 2–18 % of NNN in a cigar is transferred to smoke which ranges from 0.18 –6.6 μg/cigar (0.13 –0.98 μg/g). NNK is present in cigars in much lower quantities and smoke yields range from 0.06 – 4.0 μg/cigar (0.05 –0.86 μg/g) which is approximately 1–8 % from the whole cigar transferred to the smoke of the cigar, as seen in Figure 9. NNN and NNK smoke yields can be reasonably estimated given the NNN and NNK quantity in the cigar filler.

Comparison of TSNAs in Cigars to Other Tobacco Products

A survey of current literature was conducted to develop Table 5 which compares TSNAs levels in cigar smoke, cigarettes smoke, smokeless tobacco products, and eliquids. This information may be useful to scientific chemistry reviewers for comparison data in premarket tobacco application reviews.

Of the comparison products noted in Table 5, cigarettes are the most like cigars; both cigars and cigarettes are rods of tobacco combusted by the user. In a previous study, to account for the differences in ventilation levels in different cigarettes, a regression model analysis was conducted with the mean TSNA level (NNN +NNK) in smoke as the dependent variable and mean TSNA level (NNN +NNK) in tobacco filler and ventilation level as independent variables.[37] A good correlation was found between TSNA levels (NNN + NNK) in the tobacco filler and the corresponding levels in mainstream smoke in cigarettes, Figure 11A.

[37] Ashley, D. L., Beeson, M. D., Johnson, D. R., McCraw, J. M., Richter, P., Pirkle, J. L., Pechacek, T. F., Song, S., & Watson, C. H. Tobacco-specific nitrosamines in tobacco from U.S. brand and non-U.S. brand cigarettes. Nicotine & Tobacco Research, 5, 2003, 323–331.

Table 5. TSNA levels in different tobacco product by product categories

	Cigarette Smoke[38]	Smokeless Tobacco (µg/g)[38,39]			E–liquid[40]	Cigar Smoke (µg/cigar)		
	(µg/cigarette)	Chewing	Moist Snuff	Dry Snuff	(µg/L)	FLCs	Small Cigars	Large Cigars
NNN	0.033 – 0.32	0.67 – 4.1	0.60 – 13	0.81 – 14	0.34 – 60	0.18 – 0.65	0.21 – 2.8	1.9 – 6.6
NNK	0.04 – 0.25	0.03 – 0.38	0.12 – 3.1	0.12 – 7.4	0.22 – 9.84	0.06 – 0.44	0.11 – 2.4	0.66 – 4.0
NAB	0.006 – 0.04	0.03 – 0.14	0.2 – 2.2	0.05 – 3.0	0.11 – 11.1	0.03 – 0.10	0.04 – 0.2	0.14 – 0.56
NAT	0.044– 0.29	0.33 – 2.3	0.49 – 14	0.57 –16	0.09 –62	0.09 – 0.43	0.23 – 1.3	1.2 – 3.1

A similar analysis, to account for the differences in ventilation levels in different cigars in this study is shown in Figure 11B where the cigar TSNAs (NNN +NNK) in the ground cigar (wrapper/binder, and filler) as a function of TSNA (NNN +NNK) in smoke results in a linear fit of $R^2 = 0.78$ (Figure 11B). The linear fit seen for cigars is poorer than the good fit observed for cigarettes ($R^2 = 0.86$). Differences in goodness of fit between TSNAs in ground/smoke in cigarettes compared to cigars maybe due to differences in wrapping paper of cigarettes compared to cigars. Cigarettes are made with cigarette paper, but cigars have wrappers and binders made of whole leaf or homogenized tobacco leaf which may be one factor that accounts for the different rate of TSNA transfer to smoke from cigars compared to cigarettes.[41] Overall, Figure 11 illustrates a difference between cigar and cigarette product chemistry.

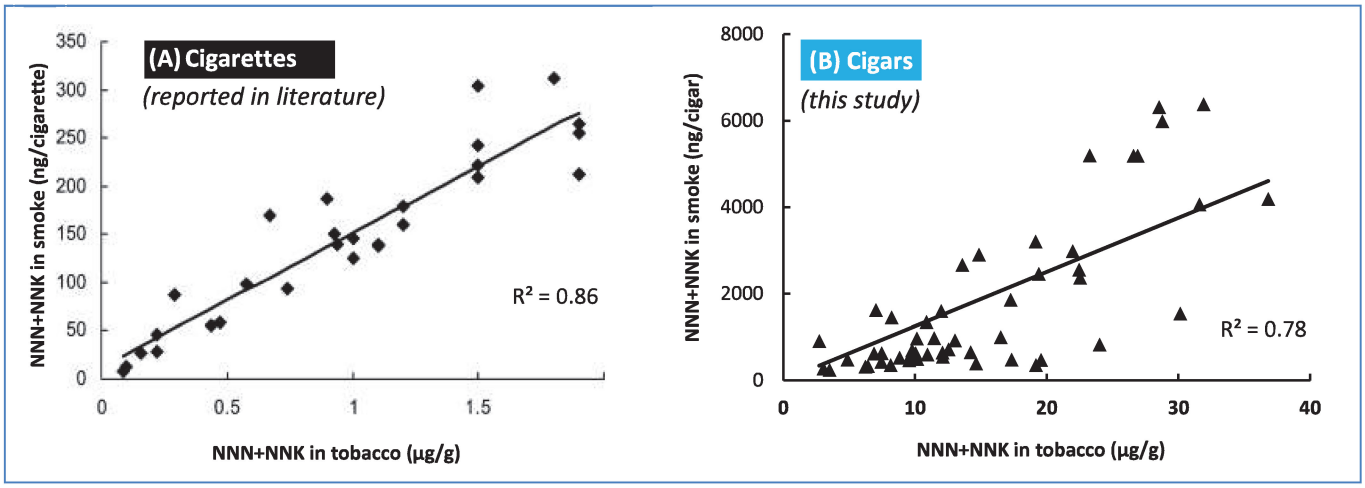

Figure 11. Relation of tobacco-specific nitrosamine (sum of NNN and NNK) levels between tobacco and corresponding smoke yields in (A) cigarettes tobacco filler reported in literature [42] and (B) cigars tobacco filler from the UK Cigar Co-op used in this study.

[38]Data reported in table is based intense smoking regimen study reported by FDA: Edwards SH, Rossiter LM, Taylor KM, Holman MR, Zhang L, Ding YS, Watson CH. Tobacco-Specific Nitrosamines in the Tobacco and Mainstream Smoke of U.S. Commercial Cigarettes. Chem Res Toxicol. 2017 Feb 20;30(2):540-551. References for earlier studies of TSNA levels in cigarettes:(a) Stephen S. Hecht, Dietrich Hoffmann, Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke, Carcinogenesis, *9 (6)*, 1988, pp 875–884. (b) Tricker, A.R., Ditrich, C. & Preussmann, R. N-Nitroso compounds in cigarette tobacco and their occurrence in mainstream tobacco smoke. Carcinogenesis, *12*, 1991, 257–261

[39] M.F. Borgerding, J.A. Bodnar, G.M. Curtin, et al. The chemical composition of smokeless tobacco: a survey of products sold in the United States in 2006 and 200.7 Regulatory Toxicology and Pharmacology *64*, 2012, pp 367–387

[40] H.J. Kim and H.S. Shin. Determination of tobacco-specific nitrosamines in replacement liquids of electronic cigarettes by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A, *1291*, 2013, pp. 48-55

[41] Dethloff, O., Mueller, C., Cahours, X., & Colard, S.. Cigar burning under different smoking intensities and effects on emissions. Regulatory Toxicology and Pharmacology, 91, 2017, 190-196

[42] Wu, W., Zhang, L., Jain, R., Ashley, D. & Watson, C. Determination of carcinogenic tobacco specific nitrosamines in mainstream smoke from U.S.-brand and non-U.S.-brand cigarettes from 14 countries. Nicotine Tob. Res., 7, 2005, 443–451

Recommendations

The three primary result sections (1) NNN and NNK in whole ground cigars, (2) NNN and NNK smoke yields of cigars, and (3) Comparison of TSNAs in cigars compared to other tobacco products include summary statements with key findings which could be referenced as needed. The following are the primary key findings and recommendations to the scientific reviewer:

- Wrappers and binders of cigars contribute notable quantities of NNN and NNK to the total ground cigar. However, this study illustrates NNN and NNK smoke yields are more closely related to the NNN and NNK in cigar fillers than that of the wrappers and binders. In reviews, differences in wrappers and binders may not have a discernable impact on NNN and NNK smoke yields.
- In the ground cigar, cigar mass and nicotine are directly proportional to NNN and NNK in the ground cigar (or ground cigar components). Equations in Table 2 can be used to cautiously estimate NNN and NNK in various components of the ground cigar using the tobacco mass or nicotine.
- Reviewers may use NNN and NNK levels in the ground cigar filler to estimate NNN and NNK cigar smoke yields using the appropriate equation in Table 4. These equations show near linearity with R^2 of 0.91– 0.94 based on this study.
- The compiled comparison data showing differences among cigar types and other tobacco products may be helpful in your reviews or research proposals, see Table 5.

Appendix

Table A: List of cigars included in FDA's Co-operative Cigar Reference Program with the University of Kentucky.

Sample #	Cigar Type	Brand	Manufacturer/Distributor/Shape
1	FLC	Talon Regular Filtered Regular	Scandinavian Tobacco Group
2	FLC	Phillies Filter Tipped 100M Regular	ITG Brands
3	FLC	Cheyenne Filtered Cigar Classic	Cheyenne International
4	FLC	Cherokee Filtered Cigars Full Flavor Red 100s	Cherokee Tobacco Company
5	FLC	Swisher Sweets Little Cigars Regular	Swisher International, Inc.
6	FLC	Santa Fe Filtered Cigars Original	Swisher International, Inc.
7	FLC	Seneca Full Flavor Filtered Cigar Red	Lake Erie Tobacco Company
8	FLC	Derringer Filtered Cigars Classic	Cheyenne International, LLC
9	FLC	Captain Black Little Filtered Cigars	Scandinavian Tobacco Group (Lane Limited)
10	FLC	King Edward Filtered Cigars Regular	Swisher International
11	FLC	Winchester Little Cigars Classic King Box	Scandinavian Tobacco Group, Tucker GA, USA
12	FLC	Djarum Filtered Clove Cigars Special	PT Djarum. Indonesia Distributor Kretek Intl, Moorpark CA
13	FLC	305's Filtered Cigars Full Flavor	Dosal Tobacco Corp., Miami FL, USA
14	FLC	Vaquero Filtered Cigars Original (Natural)	Sunshine Tobacco, Miami FL, USA
15	FLC	Bella Orchid Filtered Cigars Full Flavor	Sunshine Tobacco, Miami FL, USA
16	FLC	Clipper Filtered Cigars Full Flavor	Distributor: Global Tobacco LLC, Dallas TX, USA
17	FLC	Smokers Choice Filtered Cigars Red	Distributor Prime Time International Distributing, Inc.;
18	FLC	Remington Filtered Cigars Full Flavor	Good Times USA, LLC, Tampa FL Dominican Republic
19	FLC	Red Buck Filtered Cigars Full Flavor	Distributor: Xcaliber International, Ltd, Pryor OK
20	FLC	Supreme Blend Filtered Cigars Full Flavor	Distributor: Global Tobacco LLC, Dallas TX USA
21	FLC	Wrangler Filtered Cigars Full Flavor	Sunshine Tobacco, Miami FL USA
22	FLC	Racer Filtered Cigar Full Flavor	License: Global Tobacco LLC, Dallas TX, USA
23	FLC	Westport Filtered Cigars Original	Distributor: Inter-Continental Trading USA, Inc, Mt.
24	FLC	Richmond Filtered Cigars Full Flavor 100's	Ohserase Manufacturing LLC, Akwesasne NY
25	FLC	John Black 100's Box Black Cherry	possibly Golden Tobacco Ltd., India
26	Small	Antonio y Cleopatra Minis	ITG Brands, LLC
27	Small	Dutch Masters Cigarillos De Luxe	ITG Brands, LLC
28	Small	Garcia y Vega Game Cigarillo Black	Swedish Match
29	Small	King Edward Special	Swisher International, Inc.
30	Small	Optimo Cigars Sweet	Swisher International, Inc.
31	Small	Phillies Cigars Cigarillos Black	ITG Brands, LLC
32	Small	Phillies Cigars Cigarillos Sweet	ITG Brands, LLC
33	Small	Swisher Sweets Cigarillos Original	Swisher International, Inc.
34	Small	White Owl Cigarillos Black	Swedish Match
35	Small	White Owl Cigarillos Sweets	Swedish Match
36	Small	Backwoods Original	ITG Brands, LLC
37	Small	Pom Sweet	Swisher International, Inc.
38	Small	Al Capone Sweets (Cognac Dipped)	ICC Inter-Continental Cigar Corporation
39	Small	Jackpot Sweets	SMCI Holdings, Inc.
40	Small	Zig-Zag Sweets	National Tobacco Company, L.P.
41	Small	Black & Mild Original	For: John Middleton Co.
42	Small	Hav-A-Tampa Jewels Original	ITG Brands, LLC
43	Large	Phillies	Shape: Blunt Petite Corona Natural
44	Large	Swisher Sweets	Shape: Blunts Natural Petite Corona Sweet
45	Large	Dutch Masters	Shape: Collection Palma Corona Natural
46	Large	Dutch Masters	Shape: Corona Deluxe Natural
47	Large	Antonio y Cleopatra	Shape: Coronas
48	Large	Garcia y Vega	Shape: English Corona
49	Large	White Owl	Shape: Invincible Natural
50	Large	William Penn	Shape: Perfecto
51	Large	Swisher Sweets	Shape: Perfecto Natural Sweet
52	Large	Dutch Masters	Shape: President
53	Large	Garcia y Vega	Shape: President

Sample 40 has incomplete datasets and could not be included in this memo

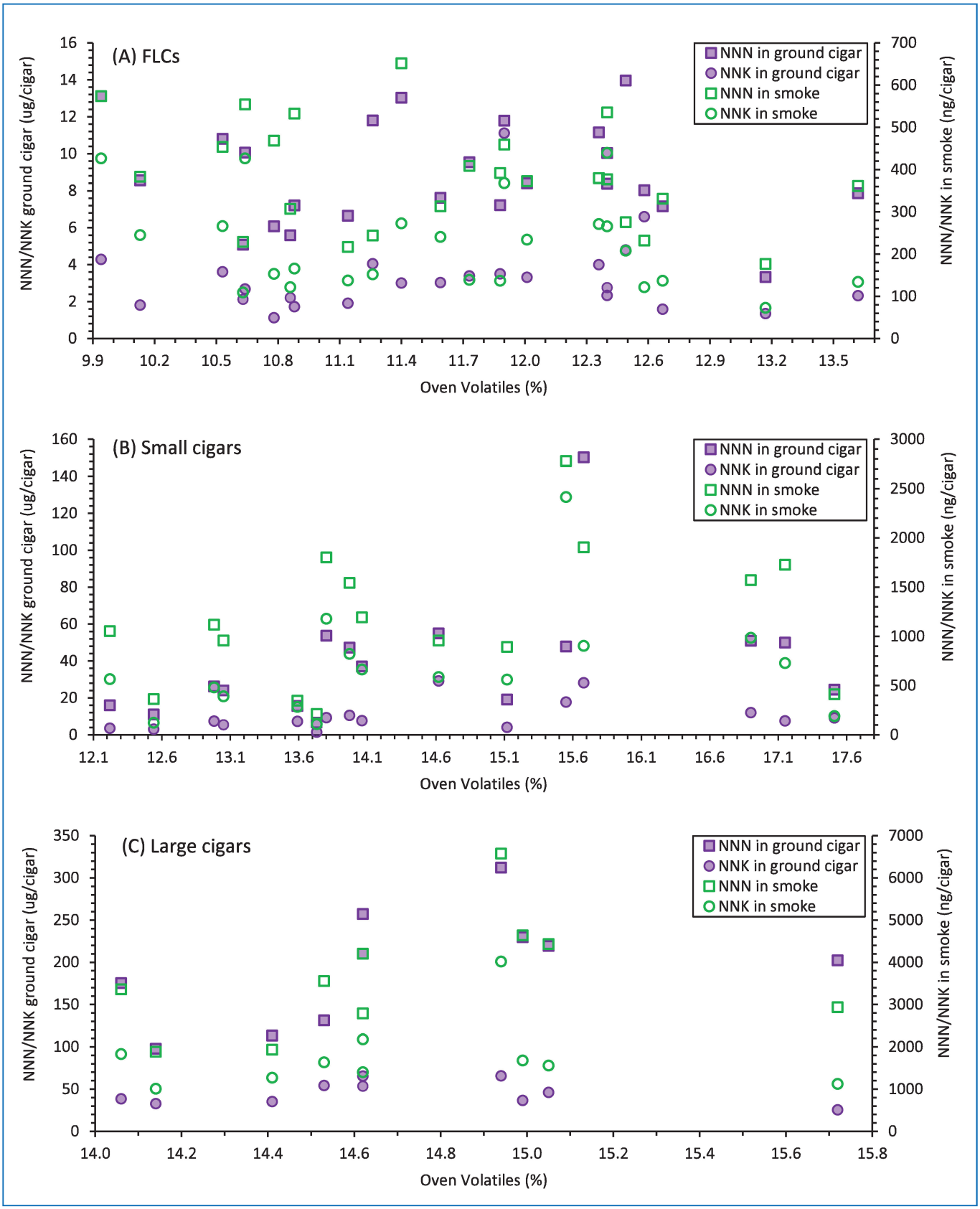

Figure A. Average oven volatiles (%) and levels of NNN and NNK in the ground cigar and smoke by cigar type.