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Comprehensive Chemical Characterization of Natural American Spirit Cigarettes

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Abstract

Objectives: Marketing of the Natural American Spirit (NAS) cigarettes implies reduced risk of toxic exposures. We aimed to provide a comprehensive chemical characterization of these cigarettes.

Methods: We analyzed 13 varieties of NAS for a range of tobacco- and combustion-derived constituents. Cigarettes were smoked by 2 standard regimens and analyzed using our routine analytical procedures. We also analyzed tobacco filler and physical cigarette characteristics.

Results: Under intense smoking conditions, nicotine in smoke of NAS cigarettes averaged 3.3(±0.7) mg/cigarette, compared to 2.4(±0.4) in other brands. The levels of carcinogenic nitrosamines NNN and NNK varied extensively across NAS varieties, their sum ranging from 71 to 443 ng/cigarette. Levels of volatile toxicants were generally similar to, or higher than those found in other commercial US cigarettes.

Conclusions: High nicotine content suggests that NAS cigarettes may be more addictive than many other brands. Similarly low TSNA levels were measured in some NAS varieties, independent of whether or not they were labeled as organic. Levels of other toxicants were similar to other brands. Consumer education and additional regulatory measures are needed to address the misperceptions that NAS cigarettes are safer than other commercial cigarette brands.

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Human Subjects Statement
This study did not involve human subject research.

Conflict of Interest Disclosure
The authors report no conflicts of interest.

Keywords

Natural American Spirit cigarettes; harmful constituents; analysis; tobacco smoke

Natural American Spirit (NAS) cigarettes have been marketed as made from "natural" or "organic" tobacco and "100% additive-free," implying reduced risk of toxic exposures.[1,2] Indeed, studies show that NAS cigarettes are perceived by smokers as posing lower health risks than other brands and those smokers who use NAS, being more concerned about health than other smokers, are more likely to have these beliefs and choose NAS because of them.[3–5] Whereas some of the misleading descriptors are no longer allowed in the NAS advertisements, words such as "natural," "organic," and "tobacco and water" are still used in the brand's name, packaging, or advertising, contributing to sustained misperceptions of relative safety of NAS cigarettes.[6]

Detrimental health outcomes associated with smoking, such as 19 types of cancer, respiratory diseases, and cardiovascular diseases, are caused by the numerous harmful constituents that are either derived from tobacco itself or are formed during the process of combustion.[7–9] A substantial amount of research provides clear evidence that levels of these constituents in smoke depend on factors other than tobacco being "organic," "natural," or "additive-free." For instance, levels of the addictive tobacco alkaloid nicotine and the carcinogenic tobacco-specific *N*-nitrosamines (TSNA) *N*′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in cigarette smoke depend on the type of tobacco plant and on how it has been cured.[10–12] Carcinogenic metals are being absorbed from soil into the tobacco plant, and their levels will depend on the soil rather than tobacco cultivation practices.[13,14] Lastly, a wide range of polycyclic aromatic hydrocarbons (PAH), carbonyls, and other volatile toxicants are formed upon the combustion of any organic matter.[8,15] Therefore, it is plausible to expect that, even if made with organically-grown tobacco and without additives, NAS cigarettes have similar toxicity and carcinogenic potency as the majority of other commercial cigarette brands.

Comprehensive characterization of key harmful chemical constituents in tobacco and smoke of NAS cigarettes, and their comparison with other brands, is essential for developing accurate and effective communication of health risks associated with NAS use, for interpreting biomarker data, and for supporting regulatory measures. However, data on the levels of many important toxicants and carcinogens in various NAS cigarettes is critically lacking. Although there are at least 13 varieties of NAS cigarettes, most publications that report on the chemical constituents in specific brands and sub-brands of cigarettes in the United States (US) include one, sometimes unidentified NAS variety.[16–18] Other publications included a range of NAS varieties, but the analyses were focused on a limited set of constituents, such as particulate matter, nicotine, or ammonia.[19,20] To address this important gap, we analyzed a range of tobacco-derived constituents, such as nicotine, other tobacco alkaloids, beta-carbolines, and TSNA, as well as a panel of combustion-derived constituents in smoke, and some of the same and other important constituents in tobacco filler of 13 NAS cigarette varieties. These results, together with some physical characteristics of cigarettes, are compared to a limited set of other commercial cigarette brands.

METHODS

Cigarettes

A convenience sample of 13 varieties of NAS and 5 other popular cigarette brands were purchased from retail stores in the Minneapolis, MN metropolitan area in 2017 and analyzed in this study. Reference cigarettes (1R5F and 3R4F) were obtained from The Center for Tobacco Reference Products (CTRP), University of Kentucky, Lexington, KY. To generate representative average values for each measurement, 3 packs of each commercial cigarette variety were purchased. For most of the measurements, one cigarette was randomly taken out of each pack of a particular cigarette variety and analyzed separately to generate triplicate data per analysis. Carbonyls in cigarette smoke and anions (nitrate, nitrite, and ammonia) and metals in tobacco filler were analyzed in duplicate by taking cigarettes from 2 out of the 3 packs of each cigarette variety. Prior to analyses, all cigarettes were stored refrigerated in their original packs, in sealed plastic sleeves.

Chemicals

Nicotine, minor tobacco alkaloids, TSNA, beta-carbolines, and their isotopically labeled analogues were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Mixtures of PAH and ^{13}C-labeled PAH were purchased from Cambridge Isotope Laboratories (Andover, MA). A standard mix of carbonyl-DNPH derivatives was purchased from AccuStandard (New Haven, CT). All other chemicals and solvents were purchased from either Sigma-Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Fairlawn, NJ). All aqueous solutions were prepared with water purified on a 0.22 μm Millipore system (Billerica, MA).

Physical Parameter Measurements

Cigarette length, filter length, and tobacco filler weights were measured for all the cigarettes. Tobacco weight per cigarette was determined as the difference between the whole cigarette weight and the paper and filter weight after removal of the tobacco filler.

Cigarette Smoke Analyses

Smoke generation and collection for constituent analyses.—Prior to smoking, cigarettes were conditioned for 48 hours in an environmental chamber at 22 °C and 60% relative humidity. Cigarettes were then smoked on a Borgwaldt LX1 linear single port smoking machine under ISO (35-mL puff volume, 2-s puff duration and 60 s puff interval) and Canadian Intense (55-mL puff volume, 2-s puff duration, 30-s puff interval, and 100% blocked ventilation holes) smoking regimens.[21,22] For the analyses of alkaloids, TSNA, beta-carbolines, and PAH, the mainstream smoke was collected on Cambridge filter pads. For the analysis of carbonyl compounds Cambridge filter pads were not used and cigarette smoke was passed through 2 consecutively connected impingers containing acidified solution of 2,4-dinitrophenylhydrazine (DNPH). Puff numbers were recorded by the smoking machine software. Total particulate matter (TPM) was measured by gravimetric analysis by weighing filter pads before and after smoking.

Nicotine and minor alkaloids.—Filter pads were extracted in 15 mL of 10 mM ammonium acetate buffer by sonication for one hour. Samples were prepared by serial dilution of the extract with 10 mM ammonium acetate, and addition of [D_3]nicotine, [D_4]nornicotine, [D_4]anabasine and [D_4]anatabine as internal standards. The prepared samples were analyzed by liquid chromatography (LC)-tandem mass spectrometry (MS/MS) on a Hypercarb column (Thermo Scientific), using 10 mM ammonium acetate (with 0.01% formic acid) and methanol as mobile phase as previously described.[23,24]

Beta-carbolines.—Harman and norharman were analyzed by using the same 10 mM ammonium acetate filter pad extracts that were prepared for nicotine and minor alkaloid analyses. A 250 μL of the extract was mixed with [$^{13}C_2{}^{15}N$]-harman and [D_7]-norharman internal standards and diluted to 5 mL with water. The mixture was loaded on ChemElut cartridges (Agilent, Santa Clara, CA) and eluted twice with 8 mL methylene chloride. The eluates were dried in SpeedVac, reconstituted in water, and analyzed by LC-MS/MS on a Zorbax SB C18 (Agilent, Santa Clara, CA) column, using water (with 0.1% trifluoroacetic acid) and acetonitrile (with 0.1% trifluoroacetic acid) as mobile phase. The mass spectrometer was set in the positive ion with selective reaction monitoring (SRM) mode at *m/z* 169 ®115 for norharman, *m/z* 183® 115 for harman, and corresponding transitions for respective internal standards.

TSNA.—Four TSNA were analyzed: NNN, NNK, *N′*-nitrosoanatabine (NAT) and *N′*-nitrosoanabasine (NAB). Briefly, [$^{13}C_6$]NNN and [pyridine-D_4]NNK internal standards were applied directly to the filter pads which were then extracted in 15 ml 10 mM ammonium acetate buffer by sonication for one hour. The extracts were then purified on ChemElut (Varian, Harbor City, CA) and Sep-Pak Plus silica cartridges (Waters, Milford, MA). The purified samples were analyzed by LC-MS/MS in selected reaction monitoring mode as previously described.[25]

PAH.—Eleven PAH were analyzed using our previously described gas chromatography (GC)-MS method with slight modifications.[26] Briefly,[13] C-labeled internal standard mix was added to Cambridge filter pads which were then extracted in 12 mL cyclohexane on a benchtop shaker for an hour, followed by sonication for 10 min. The extracts were purified on SepPak 500 mg silica cartridges (Waters), concentrated in SpeedVac to a final volume of 200 μL, and analyzed by GC-MS as described.[26]

Carbonyl compounds.—The content of the 2 DNPH-filled impingers (see smoke collection procedure above) was combined and analyzed for 8 carbonyl compounds by HPLC-UV.[27,28] Briefly, an aliquot of the DNPH solution was mixed with 1% Trizma base solution to quench the DNPH reaction. The samples were then analyzed by HPLC-UV on a Phenomenex C18(2) 250×4.6 mm column, using 30% acetonitrile/10% tetrahydrofuran/1% isopropanol/59% water as mobile phase A and 65% acetonitrile/1% tetrahydrofuran/1% isopropanol/33% water as mobile phase B, with the UV detector set at 365 nm.

Tobacco Filler Analysis

Alkaloids and TSNA.—For each cigarette, tobacco filler was removed from the cigarette rod and 200 mg were weighed and extracted in 10 mL of 10 mM ammonium acetate buffer by sonicating for one hour. Tobacco particles were then precipitated by centrifugation and the extracts were analyzed for nicotine, minor alkaloids, and TSNA as described above for cigarette smoke analyses.

Nitrate, nitrite, and ammonia.—For these analyses, samples were prepared as previously described.[29] Briefly, approximately 100 mg of tobacco filler was extracted by sonication for 30 min with 10 mL of reagent grade water (Milli-Q, Millipore Corp.); tobacco particles were precipitated by centrifugation, and the extracts were purified on C-18 SPE cartridges (Waters Corp., Milford, MA). Prepared samples were analyzed colorimetrically by the Research Analytical Laboratory, University of Minnesota.

Metals and metalloids.—Tobacco samples were subjected to microwave-assisted digestion in 4:1 mixture of hydrogen peroxide and nitric acid, and analyzed by inductively-coupled plasma mass-spectrometry at the Research Analytical Laboratory, University of Minnesota, as previously described.[30]

Measurement of pH.—Approximately 200 mg of tobacco filler was extracted in 2 mL HPLC-grade water by sonication for 10 min and allowed to stand at room temperature for an additional 20 min. The pH of the aqueous extract was measured with a calibrated pH meter in duplicates and the mean of 2 measurements was calculated.

Filter ventilation.—Borgwaldt KC-3 Ventilation Machine was used to record the ventilation of the cigarette filters.

RESULTS

Table 1 summarizes general characteristics of the tested cigarettes. All varieties of NAS cigarettes, including the non-filtered NAS Brown were 84-mm long. Filtered NAS varieties had generally shorter filters than other commercial brands, and filter ventilation ranged widely, from 0.4% in Dark Green to 58.6% in Orange. Filtered NAS cigarettes had higher tobacco filler weight than other filtered cigarette brands, averaging (±SD) 845(±18) mg, compared to 669(±36) mg, respectively. The number of puffs for NAS filtered cigarettes averaged 10.9(±0.9) under ISO and 13.7(±1.1) under CI smoking conditions; these numbers were 7.4(±0.4) and 9.4(±1.1), respectively, for other filtered commercial brands. The average TPM yields for NAS filtered cigarettes averaged 14.2(± 4.8) mg under ISO and 45.4(± 7.7) mg under CI conditions, compared to 15.4(± 3.5) mg and 39.6(± 5.1) mg, respectively, in other filtered commercial brands. The non-filtered NAS Brown contained ~300 mg more tobacco and, under CI regimen, generated 8 more puffs and ~40% more TPM than Camel non-filtered cigarette (Table 1).

Constituents in Cigarette Smoke

Tables 2–5 show the results of constituent analyses in smoke generated under CI conditions. Levels of the same constituents in smoke generated under ISO conditions are available as supplementary tables S1–S4.

Table 2 summarizes the levels of tobacco alkaloids and beta-carbolines under CI conditions. Nicotine levels in NAS varieties ranged from 2.2 to 4.4 mg/cigarette, averaging 3.3 (±0.7) mg/cigarette. In other commercial brands, nicotine yield averaged 2.4 (±0.4) mg/cigarette. Levels of nornicotine were somewhat lower, whereas levels of anabasine were higher, in the smoke of NAS cigarettes compared to other brands. Beta-carbolines harman and norharman in NAS varieties averaged 3.5(±1.0) and 10.0 (±2.0) μg/cigarette, similar to the levels measured in other brands. The non-filtered cigarettes, NAS Brown and Camel Non-Filter, had the highest yields of harman and norharman among all tested varieties.

Table 3 presents the levels of TSNA measured under CI conditions. There was substantial variation of these constituents across NAS varieties: levels of NNN ranged from 32 ng/cigarette in NAS Tan to 323 ng/cigarette in NAS Gray, and levels of NNK ranged from 38 ng/cigarette in NAS Orange to 128 ng/cigarette in NAS Black. Levels of these carcinogens in other commercial brands averaged 288(±55) and 153(±33) ng/cigarette, respectively (Table 3).

Levels of PAH were somewhat higher in NAS cigarettes than in other commercial brands (Table 4). The largest differences between NAS and other cigarettes were observed for phenanthrene, anthracene, and the representative carcinogenic PAH benzo[*a*]pyrene: 432(±112) versus 307(±57) ng/cigarette, 176(±37) versus 136(±36) ng/cigarette, and 25(±5) versus 20(±3) ng/cigarette, respectively. The highest levels of all PAH were found in the smoke of non-filter NAS Brown cigarettes, with the levels of benzo[*a*]pyrene in this variety being almost 2-fold higher than in Camel Non-Filter. Among the commercial brands analyzed for comparison, Camel Non-Filter had the highest total PAH content (sum of all 11 PAH) at 888 ng/cigarette.

Table 5 presents the levels of 8 carbonyls analyzed under CI conditions. Overall, levels of these constituents were comparable in NAS cigarettes and other tested brands, with a few notable differences; levels of formaldehyde varied substantially (more than 4-fold) across NAS varieties, and levels of butyraldehyde were generally higher in NAS cigarettes than in other commercial brands (88(±13) versus 67(±8) μg/cigarette, respectively). Total carbonyl content averaged 2469(±212) μg/cigarette in NAS varieties and 2304(±251) in other commercial brands.

Constituents in Tobacco Filler

Tables 6 and 7 summarize the results of tobacco filler analyses. The pH of NAS tobacco filler was lower compared to other tested brands; it ranged from 4.95 to 5.13 across NAS varieties, whereas the lowest pH of tobacco from other brands was 5.24 (Table 6). Nicotine levels in the NAS filler were higher than in other brands; it ranged from 16.9 to 24.9 mg/g tobacco across NAS varieties and from 13.2 mg/g to 14.8 mg/g tobacco in other brands (Table 6). Similar to smoke minor alkaloid data, levels of anabasine were somewhat higher in tobacco filler of NAS cigarettes than in other brands; the levels averaged 101(±20) and 77(±5) µg/g tobacco, respectively. Levels of TSNA in tobacco filler of NAS cigarettes varied substantially, with NNN ranging from 0.14 µg/g to 1.76 µg/g tobacco, and NNK levels ranging from 0.11 µg/g to 0.35 µg/g tobacco (Table 6). The highest total TSNA content was in Black and Gray NAS varieties, 3.4 µg/g and 2.8 µg/g tobacco, respectively, which is comparable to levels found in other commercial brands.

Levels of nitrites and nitrates were substantially lower in NAS varieties than in other commercial brands; nitrites averaged 4.0(±0.9) µg/g tobacco in NAS cigarettes and 8.6(±6.7) µg/g tobacco in other brands, and nitrates averaged 1.2(±0.6) mg/g and 9.9(±0.8) mg/g tobacco, respectively (Table 7). Similarly, ammonia levels were approximately 4-fold lower in NAS cigarettes than in other brands (Table 7). Levels of chromium and nickel were lower in NAS varieties, whereas other measured elements did not differ between NAS and other brands (Table 7). Highest levels of cadmium were measured in NAS Black and Gray.

DISCUSSION

Cigarette brands that strongly appeal to certain smoker sub-populations can potentially interfere with and slow down the overall rate of decline in smoking prevalence and cigarette sales in the US. Natural American Spirit is one such brand as it is perceived by health-concerned smokers as less hazardous than other commercially available cigarettes. This misperception is primarily driven by the original marketing which implied reduced toxicity by emphasizing the organic and additive-free nature of NAS cigarettes. Data on harmful constituent yields in these cigarettes could help to inform consumers and public health professionals and correct this misperception; however, such data is critically lacking in published literature. Our study aimed to address this important gap by carrying out comprehensive chemical analysis of NAS cigarettes. We report here the results of our study in which smoke and tobacco filler of NAS cigarettes were analyzed for tobacco alkaloids, beta-carbolines, TSNA, PAH, carbonyls, anions, metals, as well as key physical characteristics, and compared to 5 popular commercial cigarette brands.

Analysis of physical characteristics of cigarettes can be helpful in interpreting data on constituent yields in the smoke and use patterns and exposures in smokers. For instance, whereas NAS cigarettes appear to have similar dimensions to other king-size cigarettes, they feature greater tobacco filler mass, and as the result, generally produce a higher number of puffs and more TPM per cigarette than other commercial brands. In addition, filters of 7 out of 13 tested NAS varieties had more than 30% ventilation, suggesting that such varieties may be smoked with relatively high intensity to compensate for smoke dilution. Therefore, it is plausible to expect that, due to the physical characteristics alone, smokers of NAS cigarettes may be exposed to higher levels of some tobacco constituents on a per cigarette basis than smokers of other brands. However, the actual exposures will depend on the actual constituent yields in NAS cigarette smoke and on smokers' topography. For instance, as Carroll et al report in their paper in this supplemental issue, biomarkers of specific chemicals in NAS smokers were either lower, higher, or similar to the levels found in smokers of other brands.[31]

The consistently high level of nicotine in all varieties of NAS cigarettes is in agreement with the biomarker data for NAS smokers,[31] and is an important finding. Nicotine is the major addictive agent in tobacco and cigarette smoke, and its levels are important in defining the abuse liability of tobacco products.[32,33] Acknowledging its central role in driving tobacco use (and as a consequence, the associated morbidity and mortality), substantial reduction of nicotine content in cigarettes is being considered by the US Food and Drug Administration (FDA) as an approach to reduce the addictiveness and eventually eliminate the use of combusted tobacco products.[34,35] Besides its addictive properties, nicotine also stimulates the sympathetic nervous system, decreases coronary blood flow, and induces other pharmacological effects that can contribute to cardiovascular events in tobacco users.[36] The high levels of nicotine in the tobacco filler of NAS brands (Table 6) indicate that tobacco type, in addition to the greater mass of tobacco per cigarette rod, contributes to the high levels of nicotine in the smoke of these cigarettes. Research also suggests that nicotine addiction in tobacco users may be reinforced by some minor tobacco alkaloids and by beta-carbolines harman and norharman which are monoamine oxidase inhibitors.[37,38] Levels of these constituents in NAS cigarettes are generally similar to those found in other cigarette brands (Tables 2 and 6). However, the slightly elevated levels of anatabine and anabasine, in combination with high levels of nicotine, could have a potential impact on abuse liability of NAS cigarettes.

Because of their specificity to tobacco and strong carcinogenic potency, TSNA are believed to be among the most important constituents in tobacco and cigarette smoke.[11] Levels of these constituents are highly variable across NAS varieties, in both the tobacco and the smoke, with most of the varieties containing much lower levels than the majority of commercial US cigarette brands (Tables 3, 6, and S2). Tobacco type, processing methods, and nitrate and nitrite content are among major factors affecting TSNA levels in tobacco,[39–41] and the tobacco type is the most likely determinant of TSNA variation across NAS varieties. Indeed, the average ratio of NNN to NNK, which varies significantly by tobacco plant type, is 1.2 (±0.3) in the tobacco filler of the low-TSNA NAS varieties, which is typical of cigarettes made with Virginia-type bright tobacco.[40] This ratio in other brands analyzed here is 3.2 (±0.8), which is commonly observed in the American-blended cigarettes; while Black and Gray NAS varieties, which are made with Perique tobacco and contain high TSNA levels, have NNN to NNK ratios of 6.3 and 10.6, respectively. The relatively low levels of nitrates and nitrites may also be contributing to the low TSNA levels in most NAS varieties (Table 7). It is important to note that low levels of TSNA measured in most NAS cigarettes are consistent with the urinary biomarker findings in NAS smokers,[31] and that biomarker-assessed level of NNN and NNK intake has been associated with the risk of lung and esophageal cancer in prospective epidemiological studies.[42,43] However, TSNA levels in the smoke of NAS cigarettes are generally higher than in the smoke of cigarettes smoked by the participants of those studies, and urinary biomarker levels in NAS smokers are present at levels that have been associated with increases cancer risk.[31,44]

Many PAH are potent carcinogens or toxicants in laboratory animals and are widely accepted as major contributors to lung cancer in smokers.[45–48] Carbonyls are irritants and respiratory toxicants and tumorigens,[49–53] and damage DNA in a dose-response manner.[54–57] The slightly higher levels of some PAH and carbonyls in the smoke of NAS cigarettes than in other tested brands could potentially be due to the greater mass of tobacco and the resulting larger number of puffs and higher amount of TPM. The relatively low levels of nitrates could be another contributing factor to the higher PAH levels, because nitrogen oxides derived from nitrates during tobacco combustion can prevent PAH formation in the smoke.[58] Given the role of nitrate in TSNA formation, our results are in agreement with the observation that various cigarette brands generally deliver increased amounts of PAH as TSNA levels decrease.[59]

In addition to lower levels of nitrates and nitrites, tobacco filler of NAS cigarettes contained lower levels of ammonia (Table 7). Low levels of these constituents could potentially be the consequence of not using fertilizers and additives during tobacco growing and cigarette manufacturing. For instance, ammonia is used as an additive to increase smoke pH, and thus, the bioavailability of nicotine. The slightly lower pH of NAS tobacco filler suggests that such additives may not be part of NAS blend. It is important to note that filler pH of all brands is slightly acidic, resulting in less than 1% of nicotine being in biologically available form; therefore, it is not informative of the nicotine bioavailability in the smoke. We did not measure smoke pH in this study, and it is not clear whether there are differences in nicotine bioavailability between NAS and other cigarette brands. However, as Carroll et al[31] report in this issue, biomarker data show that smokers of NAS cigarettes have higher levels of nicotine intake per cigarette than smokers of other brands, consistent with the high nicotine yields in the smoke of NAS cigarettes measured in our study. The lower levels of some metals in the tobacco filler of NAS cigarettes as compared to other brands also could be due to agricultural or manufacturing practices; however, elevated levels of cadmium, a lung carcinogen, in some NAS varieties, is of concern.

In summary, we report here on comprehensive chemical analyses of 13 NAS cigarette varieties, addressing an important gap in the published literature. Our results suggest that NAS cigarettes may be more addictive than many other cigarette brands, and show that most of the key harmful constituents are present in the smoke of NAS cigarettes at levels comparable or higher than other brands. The lower levels of TSNA in some NAS varieties, although encouraging, are not due to “natural” or “organic” properties of tobacco. Consumer education and additional regulatory measures are urgently needed to address the misperceptions that NAS cigarettes are safer than other commercial cigarette brands.

IMPLICATIONS FOR TOBACCO PRODUCT REGULATION

This paper provides important information that addresses several issues relevant to tobacco product regulation. The Tobacco Control Act prohibits both unauthorized modified risk claims and false or misleading labeling and advertising of tobacco products. Although the misleading descriptors “additive free” and “natural” are no longer allowed in NAS advertisements, “natural” is still used in the brand name, and other terms such as “organic” and “tobacco and water” may still be used, implying a lack of certain toxic ingredients and/or contamination that may arise from non-organic agricultural practices and less harm. However, our findings show that several toxicants and carcinogens from the FDA harmful and potentially harmful constituent (HPHC) list are present at levels mostly comparable to other commercial cigarette brands. Combined with continued evidence that NAS advertising leads to consumer misperceptions about the relative harms of NAS cigarettes (even despite required disclaimers), regulatory authorities like the FDA should consider further enforcement action prohibiting such unauthorized modified risk claims and/or deeming NAS cigarettes misbranded for false or misleading labeling and advertising.

Another provision in the Tobacco Control Act requires companies to report to the FDA the levels of HPHCs in their products. In turn, the FDA must make this information public in a format that is understandable and not misleading to a lay person. Currently, even if HPHC levels are reported to the FDA, they are not being communicated to the public because of the difficulty of presenting this information in an understandable and not misleading way. However, NAS cigarettes are an example of how the absence of publically available information on constituent levels allows manufacturers to benefit from misperceptions about their products and continue to recruit and retain consumers. Therefore, there is an urgency to develop constituent communication and education strategies so that this information becomes available and understandable to the public.

Lastly, the FDA has the authority to regulate tobacco products by adopting tobacco product standards that are appropriate for the protection of public health, such as setting limits on the levels of HPHCs. Our data on the chemical composition of NAS cigarettes exemplify how the absence of such regulation can result in unnecessarily high or variable levels of important harmful constituents in tobacco products. For instance, the use of high-nicotine tobacco in the manufacture of NAS cigarettes is in direct conflict with the FDA’s plan to require substantial reduction of nicotine content in cigarettes to non-addictive levels.[34,35] The low levels of TSNA in some NAS varieties and the approximately 10-fold higher levels in other NAS varieties are an example of how methods to achieve lower levels of potent carcinogens can be available but not always implemented. It is also important to note that, whereas NAS cigarettes follow the general trend of reverse association between TSNA and PAH content in smoke, lower levels of TSNA can be achieved without increasing PAH yields.[44] These considerations emphasize the importance of issuing tobacco product standards so that companies use available technologies and manufacturing practices that result in the lowest achievable levels of HPHCs in their products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Physical Characteristics of Cigarettes Analyzed in this Study

Cigarette Variety	Length, mm[a]		Filter Ventilation, %[b]	Tobacco Filler Weight, mg[b]	Total Particulate Matter, mg[a]		Puff Count[b]	
	Cigarette	Filter			ISO	Canadian Intense	ISO	Canadian Intense
Natrual American Spirit								
Black	84	23	19.8	857	15.8	45.5	11.0	13.5
Blue	84	22	21.4	851	19.1	51.9	12.0	15.0
Celadon	84	22	34.9	873	14.5	42.5	11.0	14.3
Dark Blue	84	23	22.9	805	17.0	47.9	10.6	12.7
Dark Green	84	23	0.4	865	22.0	64.2	11.0	13.6
Gold	84	23	54.6	835	6.8	39.0	11.0	15.0
Green	84	23	29.0	834	18.7	45.5	10.0	13.0
Gray	84	23	43.1	831	12.3	46.8	10.3	13.1
Orange	84	23	58.6	855	6.1	35.5	12.3	14.7
Tan	84	23	40.3	837	10.1	36.4	11.0	11.4
Turquoise	84	23	31.3	843	14.4	46.9	12.0	14.6
Yellow	84	22	34.9	855	13.7	42.1	9.0	14.0
Brown (non-filter)	84	n/a	n/a	1129	35.8	77.3	13	17.0
Average (SD), filter	*84 (0)*	*22.8 (0.5)*	*32.6 (15.9)*	*845 (18)*	*14.2 (4.8)*	*45.4 (7.7)*	*10.9.(0.9)*	*13.7 (1.1)*
Average (SD), all NAS	*84 (0)*			*867 (80)*	*15.9 (7.6)*	*47.8 (11.5)*	*11.1 (1.0)*	*14 (1.4)*
Other Brands								
Newport Menthol	80	20	0.2	659	19.5	38.8	7.2	9.0
Marlboro Red	83	27	20.7	646	13.8	40.1	7.0	8.8
Marlboro Gold	83	27	23.8	648	11.5	33.5	7.4	8.9
Camel Filter	83	21	21.2	722	16.9	46	8.0	11
Camel Non-Filter	69	n/a	n/a	806	25.6	55.8	7.5	8.9
Average (SD), filter	*82.3 (1.5)*	*23.8 (3.8)*	*16.5 (10.9)*	*669 (36)*	*15.4 (3.5)*	*39.6 (5.1)*	*7.4 (0.4)*	*9.4 (1.1)*
Average (SD), all other	*79.6 (6.1)*			*696 (69)*	*17.5 (5.5)*	*42.8 (8.5)*	*7.4 (0.4)*	*9.3 (0.9)*
Reference Cigarettes								
1R5F	84	26	68.8	577	5.3	24.1	7.0	12.0
3R4F	84	26	40.6	736	9.2	33.2	8.0	9.4

Note.

[a]:Values are from single analysis.

[b]:Values are averages of triplicate analyses.

Table 2

Levels of Nicotine, Minor Alkaloids, and Beta-Carbolines in Cigarette Smoke (Canadian Intense Regimen)[a]

Cigarette Variety	Nicotine, mg/cigarette	Minor Alkaloids, μg/cigarette			Beta-carbolines, μg/cigarette	
		Nornicotine	Anatabine	Anabasine	Harman	Norharman
Natural American Spirit						
Black	3.9	12.8	13.0	3.0	3.3	9.7
Blue	3.4	9.1	12.5	2.7	3.6	9.4
Celadon	3.3	8.1	9.4	2.1	3.2	9.6
Dark Blue	3.0	7.7	7.3	1.7	2.6	8.9
Dark Green	4.4	14.6	17.2	4.3	4.3	12.2
Gold	2.9	13.4	13.8	3.5	3.6	9.0
Green	3.4	12.6	13.7	3.7	4.0	11.5
Gray	3.0	7.6	7.8	2.3	2.8	9.0
Orange	2.2	6.3	6.4	1.6	2.4	7.4
Tan	2.6	6.6	6.2	1.5	2.4	7.9
Turquoise	3.8	14.4	16.1	4.0	3.7	10.9
Yellow	2.8	9.3	8.6	1.6	3.5	9.2
Brown (non-filter)	4.3	12.1	12.1	3.1	6.1	14.9
Average (SD), filter	*3.2 (0.6)*	*10.2 (3.1)*	*11.0 (3.8)*	*2.7 (1.0)*	*3.3 (0.6)*	*9.6 (1.4)*
Average (SD), all NAS	*3.3 (0.7)*	*10.4 (3.0)*	*11.1 (3.7)*	*2.7 (1.0)*	*3.5 (1.0)*	*10.0 (2.0)*
Other Brands						
Newport Menthol	2.2	15.1	8.5	1.3	3.4	11.2
Marlboro Red	2.1	12.8	9.7	1.6	3.1	11.5
Marlboro Gold	1.9	13.3	8.1	1.4	2.9	9.7
Camel Filter	2.6	15.6	9.9	1.3	4.0	13.0
Camel Non-Filter	3.0	17.4	12.7	1.7	5.9	17.6
Average (SD), filter	*2.2 (0.3)*	*14.2 (1.4)*	*9.1 (0.9)*	*1.4 (0.1)*	*3.3 (0.5)*	*11.4 (1.4)*
Average (SD), all other	*2.4 (0.4)*	*14.8 (1.8)*	*9.8 (1.8)*	*1.5 (0.2)*	*3.9 (1.2)*	*12.6 (3.0)*
Reference Cigarettes						
1R5F	0.8	13.2	7.9	1.0	1.8	5.6
3R4F	1.8	12.4	10.0	1.5	2.6	7.0

Note.

[a] All values are averages of triplicate analyses.

Table 3

Levels of Tobacco-Specific *N*-nitrosamines (TSNA) in Cigarette Smoke (Canadian Intense Regimen)[a]

Cigarette Variety	TSNA, ng/cigarette				
	NNN	NNK	NAT	NAB	Total
Natural American Spirit					
Black	315	128	226	58	726
Blue	64	60	133	21	277
Celadon	48	59	65	13	185
Dark Blue	48	49	88	12	195
Dark Green	95	97	173	34	340
Gold	93	62	132	24	311
Green	79	55	121	24	280
Gray	323	112	170	49	654
Orange	33	38	59	8	139
Tan	32	41	57	7	137
Turquoise	118	64	163	34	379
Yellow	48	54	81	11	194
Brown (non-filter)	80	69	134	19	301
Average (SD), filter	*108 (102)*	*68 (28)*	*122 (54)*	*24 (16)*	*323 (191)*
Average (SD), all NAS	*106 (98)*	*68 (27)*	*123 (52)*	*24 (16)*	*321 (183)*
Other Brands					
Newport Menthol	237	129	359	68	793
Marlboro Red	382	198	513	81	1175
Marlboro Gold	276	173	406	69	923
Camel Filter	261	117	403	65	845
Camel Non-filter	286	151	535	87	1058
Average (SD), filter	*289 (64)*	*154 (38)*	*420 (65)*	*71 (7)*	*934 (169)*
Average (SD), all other	*288 (55)*	*153 (33)*	*443 (76)*	*74 (9)*	*959 (157)*
Reference Cigarettes					
1R5F	297	149	415	59	919
3R4F	379	244	497	65	1184

Note.

[a:]All values are averages of triplicate analyses.

TSNA, tobacco-specific *N*-nitrosamines; NNN, *N′*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N′*-nitrosoanatabine; NAB, *N′*-nitrosoanabasine

Table 4

Levels of Polycyclic Aromatic Hydrocarbons (PAH) in Cigarette Smoke (Canadian Intense Regimen)[a]

Cigarette Variety	PAH, ng/cigarette											
	PHE	ANT	PY	B[a]A	CHR	B[b+j]F	B[k]F	B[e]P	B[a]P	I[cd]P	DB[ah]A	Total PAH
Natural American Spirit												
Black	528	186	63	64	93	36	5.7	11	28	13	9.1	1037
Blue	539	199	67	67	97	35	5.6	11	28	15	10	1074
Celadon	429	205	66	65	92	35	6.1	11	26	15	7.1	955
Dark Blue	383	172	59	58	84	30	4.7	9.1	24	12	7.2	842
Dark Green	425	194	77	75	109	46	6.5	12	29	17	7.1	998
Gold	288	130	48	35	52	23	2.9	6.3	15	7.4	6.3	613
Green	432	158	69	54	78	31	4.7	10	24	12	7.3	881
Gray	404	156	58	55	78	29	4.7	9.0	24	12	7.4	839
Orange	364	148	57	47	69	27	4.2	8.5	21	11	6.1	764
Tan	382	164	57	56	82	30	5.0	9.3	24	11	6.8	826
Turquoise	330	147	53	42	63	26	3.5	7.8	19	8.0	7.2	706
Yellow	383	160	61	54	83	34	5.4	10	25	13	7.2	836
Brown (non-filter)	725	274	78	100	145	50	7.1	15	38	23	10	1466
Average (SD), filter	*407 (72)*	*168 (23)*	*61 (8)*	*56 (11)*	*82 (15)*	*32 (6)*	5 *(10)*	*10 (1.6)*	*24 (4)*	*12 (3)*	7 *(1.1)*	*864 (135)*
Average (SD), all NAS	*432 (112)*	*176 (37)*	*63 (9)*	*59 (16)*	*87 (23)*	*33 (8)*	*5 (1.1)*	*10 (2.1)*	*25 (5)*	*13 (4)*	*8 (1.2)*	*911 (211)*
Other Brands												
Newport Menthol	264	103	65	35	50	28	5.4	8.6	18	9.7	6.7	593
Marlboro Red	303	121	55	33	53	27	4.1	7.6	18	8.4	5.8	637
Marlboro Gold	261	114	59	39	51	28	4.8	7.3	18	9.7	5.0	596
Camel Filter	305	147	70	44	63	31	5.9	9.6	22	10	5.7	712
Camel Non-Filter	401	194	74	53	69	37	6.5	10	23	13	6.9	888
Average (SD), filter	283 (24)	121 (19)	62 (7)	38 (5)	54 (6)	29 (2)	5 (0.8)	8 (1)	19 (2)	10 (0.7)	6 (0.7)	635 (55)
Average (SD), all other	307 (57)	136 (36)	65 (8)	41 (8)	57 (8)	30 (4)	5 (0.9)	9 (1)	20 (3)	10 (1.2)	6 (0.8)	685 (123)
Reference Cigarettes												
1R5F	133	65	33	18	23	15	2.0	5.0	10	4.0	4.0	312
3R4F	275	116	51	36	47	26	4.0	7.0	17	8.0	6.0	594

Note.

[a:] All values are averages of triplicate analyses. PHE, phenanthrene; ANT, anthracene; PY, pyrene; B[a]A, benz[*a*]anthracene; CHR, chrysene; B[b+j]F, benzo[*b*]fluoranthene plus benzo[*j*]fluoranthene; B[k]F, benzo[*k*]fluoranthene; B[e]P, benzo[*e*]pyrene; B[a]P, benzo[*a*]pyrene; I[cd]P, indeno[1,2,3-*cd*]pyrene; DB[ah]A, dibenz[*a,h*]anthracene

Table 5

Levels of Carbonyl Compounds in Cigarette Smoke (Canadian Intense Regimen)[a]

Cigarette Variety	Carbonyl Compounds, µg/cigarette								
	Formaldehyde	**Acetaldehyde**	**Acetone**	**Acrolein**	**Propionaldehyde**	**Crotonaldehyde**	**Butanone**	**Butyraldehyde**	**Total**
Natural American Spirit									
Black	86	1245	452	103	88	37	122	78	2212
Blue	82	1387	491	140	98	39	159	103	2499
Celadon	97	1302	389	115	86	20	68	63	2140
Dark Blue	70	1355	434	117	100	45	115	104	2339
Dark Green	84	1456	428	113	100	46	104	84	2416
Gold	128	1428	452	143	103	45	132	93	2525
Green	61	1393	440	115	101	40	117	88	2355
Gray	129	1477	496	151	118	44	110	93	2619
Orange	88	1523	550	139	120	44	143	87	2695
Tan	63	1501	500	130	105	40	127	75	2540
Turquoise	267	1601	494	158	128	52	120	104	2924
Yellow	109	1370	479	126	108	44	129	81	2445
Brown (non-filter)	98	1340	480	122	96	45	114	93	2387
Average (SD), filter	*105 (53)*	*1420 (97)*	*467 (41)*	*129 (17)*	*105 (12)*	*41 (8)*	*121 (21)*	*88 (12)*	*2476 (205)*
Average (SD), all NAS	*105 (56)*	*1414 (99)*	*468 (43)*	*129 (17)*	*104 (12)*	*42 (8)*	*120 (22)*	*88 (13)*	*2469 (212)*
Other Brands									
Newport Menthol	76	1333	376	100	84	41	101	62	2173
Marlboro Red	65	1250	406	117	98	42	100	76	2154
Marlboro Gold	73	1323	439	141	106	43	102	63	2290
Camel Filter	100	1521	506	162	123	51	155	75	2693
Camel Unfiltered	149	1318	362	110	74	63	73	59	2208
Average (SD), filter	*78 (34)*	*1357 (101)*	*432 (58)*	*130 (25)*	*103 (19)*	*44 (10)*	*115 (30)*	*69 (8)*	*2327 (224)*
Average (SD), all other	*93 (15)*	*1349 (115)*	*418 (56)*	*126 (27)*	*97 (16)*	*48 (5)*	*106 (28)*	*67 (8)*	*2304 (251)*
Reference Cigarettes									
1R5F	34	1500	413	129	97	31	89	93	2387
3R4F	67	1522	501	157	114	37	109	72	2578

Note.

[a]: All values are means of duplicate analyses.

Table 6

Levels of Alkaloids, Tobacco-specific *N*-nitrosamines, and pH in Tobacco Filler of Cigarettes Analyzed in this Study

Cigarette Variety	pH[a]	Nicotine, mg/g tobacco[b]	Minor alkaloids, μg/g Tobacco[b]			TSNA, μg/g Tobacco[b,c]				
			Nornicotine	Anatabine	Anabasine	NNN	NNK	NAT	NAB	Total
Natural American Spirit										
Black	5.13	21.2	764	964	112	1.76	0.28	1.13	0.20	3.37
Blue	5.04	18.7	624	848	95	0.23	0.24	0.41	0.03	0.90
Celadon	5.10	16.9	438	800	79	0.14	0.11	0.25	0.02	0.52
Dark Blue	4.98	19.4	559	780	93	0.19	0.15	0.32	0.03	0.69
Dark Green	5.01	22.7	766	907	110	0.28	0.19	0.41	0.03	0.91
Gold	5.06	24.1	702	913	138	0.35	0.21	0.53	0.04	1.14
Green	4.95	23.0	733	888	129	0.35	0.19	0.49	0.05	1.08
Gray	5.02	18.7	456	850	101	1.69	0.16	0.74	0.18	2.77
Orange	5.05	17.2	462	770	81	0.17	0.26	0.31	0.02	0.76
Tan	5.08	17.8	595	754	75	0.16	0.14	0.28	0.02	0.59
Turquoise	5.07	24.9	848	967	123	0.37	0.35	0.56	0.05	1.33
Yellow	5.08	19.2	579	851	96	0.20	0.16	0.32	0.02	0.71
Brown (non-filter)	5.01	17.5	405	792	84	0.14	0.13	0.25	0.01	0.54
Average (SD),filter	*5.05 (0.05)*	*20.3 (2.8)*	*627 (136)*	*858 (72)*	*103 (20)*	*0.49 (0.58)*	*0.20 (0.07)*	*0.48 (0.25)*	*0.06 (0.06)*	*1.23 (0.90)*
Average (SD), all NAS	*5.04 (0.05)*	*20.1 (2.8)*	*610 (144)*	*853 (72)*	*101 (20)*	*0.46 (0.57)*	*0.20 (0.07)*	*0.46 (0.25)*	*0.06 (0.06)*	*1.18 (0.88)*
Other Brands										
Newport Menthol	5.35	14.1	916	708	78	1.93	0.43	1.52	0.07	3.94
Marlboro Red	5.61	13.5	538	614	71	2.06	0.65	1.69	0.07	4.47
Marlboro Gold	5.54	13.2	758	681	74	1.89	0.57	1.45	0.06	3.97
Camel Filter	5.38	14.8	503	643	79	1.08	0.47	1.68	0.06	3.28
Camel Non-Filter	5.24	14.2	544	671	84	0.99	0.34	1.62	0.05	3.00
Average (SD),filter	*5.47 (0.12)*	*13.9 (0.7)*	*679 (194)*	*662 (41)*	*76 (4)*	*1.74 (0.46)*	*0.53 (0.10)*	*1.59 (0.12)*	*0.06 (0.01)*	*3.92 (0.49)*
Average (SD), all other	*5.42 (0.15)*	*13.9 (0.6)*	*652 (179)*	*663 (36)*	*77 (5)*	*1.59 (0.51)*	*0.49 (0.12)*	*1.59 (0.10)*	*0.06 (0.01)*	*3.74 (0.59)*
Reference Cigarettes										
1R5F	5.30	12.7	788	646	71	2.59	0.90	1.94	0.08	5.50
3R4F	5.33	14.6	645	581	71	2.65	0.88	2.22	0.09	5.85

Note.

[a] Values are means of duplicate analyses.

[b] Values are average (SD) of triplicate analyses.

[c] NNN, *N′*-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT, *N′*-nitrosoanatabine; NAB, *N′*-nitrosoanabasine

Table 7

Levels of Nitrites, Nitrates, Ammonia and Metals in Tobacco Filler of Cigarettes Analyzed in this Study[a]

Cigarette Variety	Nitrites, µg/g Tobacco	Nitrates, mg/g Tobacco	Ammonia, mg/g Tobacco	Metals, µg/g Tobacco				
				As	Cd	Cr	Ni	Pb
Natural American Spirit								
Black	3.3	0.5	0.42	0.57	1.74	0.42	0.88	0.63
Blue	3.3	1.4	0.31	0.55	1.09	0.94	1.60	0.78
Celadon	3.2	0.6	0.26	0.64	1.07	0.93	1.65	0.76
Dark Blue	3.3	0.7	0.28	0.57	1.32	0.50	0.56	0.72
Dark Green	3.3	1.8	0.26	0.53	0.90	0.95	1.30	0.59
Gold	4.9	2.0	0.23	0.50	1.04	0.49	0.59	0.69
Green	4.9	1.9	0.27	0.48	0.93	0.86	1.18	0.73
Gray	4.9	0.7	0.42	0.55	1.65	0.57	0.88	0.67
Orange	3.2	0.5	0.27	0.61	1.07	0.76	1.56	0.75
Tan	5.0	0.8	0.26	0.67	1.34	0.57	0.57	0.63
Turquoise	3.2	1.3	0.25	0.42	0.92	0.50	0.56	0.61
Yellow	4.9	2.1	0.29	0.59	1.10	0.68	1.25	0.75
Brown (unfiltered)	4.8	0.8	0.23	0.58	1.14	0.74	1.19	0.68
Average (SD), filter	*3.9 (0.9)*	*1.2 (0.6)*	*0.29 (0.06)*	*0.56 (0.07)*	*1.18 (0.27)*	*0.68 (0.19)*	*1.05 (0.41)*	*0.69 (0.06)*
Average (SD), all NAS	*4.0 (0.9)*	*1.2 (0.6)*	*0.29 (0.06)*	*0.56 (0.07)*	*1.18 (0.28)*	*0.69 (0.20)*	*1.06 (0.43)*	*0.69 (0.06)*
Other Brands								
Newport Menthol	8.1	8.7	0.99	0.42	1.01	1.07	2.11	0.66
Marlboro Red	8.0	9.5	1.28	0.67	1.10	2.06	3.24	1.02
Marlboro Gold	3.2	10.6	1.53	0.50	1.11	1.45	2.50	0.74
Camel Filter	19.0	9.5	0.82	0.64	1.05	1.16	2.32	0.95
Camel Non-Filter	4.8	11.1	0.72	0.55	1.05	2.15	2.68	1.03
Average (SD), filter	*9.6 (6.2)*	*9.6 (0.9)*	*1.16 (0.03)*	*0.56 (0.10)*	*1.06 (0.04)*	*1.44 (0.50)*	*2.54 (0.43)*	*0.84 (0.17)*
Average (SD), all other	*8.6 (6.7)*	*9.9 (0.8)*	*1.07 (0.03)*	*0.56 (0.11)*	*1.06 (0.04)*	*1.58 (0.45)*	*2.57 (0.49)*	*0.88 (0.17)*
Reference Cigarettes								
1R5F	4.8	16.6	1.11	0.70	1.50	1.28	2.28	1.61
3R4F	4.7	10.0	0.83	0.36	1.08	1.33	2.25	0.57

Note.

[a]: All values are means of duplicate analyses.

As, arsenic; Cd, cadmium; Cr, chromium; Ni, nickel; Pb, lead.