Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

HHS Public Access
Author manuscript
Int J Cancer. Author manuscript; available in PMC 2017 September 15.

Published in final edited form as:
Int J Cancer. 2016 September 15; 139(6): 1261–1269. doi:10.1002/ijc.30178.

Tobacco-specific *N*-nitrosamines and polycyclic aromatic hydrocarbons in cigarettes smoked by the participants of the Shanghai Cohort Study

Katrina Yershova[1], **Jian-Min Yuan**[2,3], **Renwei Wang**[2], **Liza Valentin**[4], **Clifford Watson**[4], **Yu-Tang Gao**[5], **Stephen S. Hecht**[1], and **Irina Stepanov**[1,6,*]

[1]Masonic Cancer Center, University of Minnesota, 2231 Sixth Street SE, Minneapolis, Minnesota 55455, USA

[2]University of Pittsburgh Cancer Institute, 5150 Centre Avenue, Pittsburgh, Pennsylvania 15232, USA

[3]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, USA

[4]Centers for Disease Control and Prevention, Atlanta, GA, USA

[5]Department of Epidemiology, Shanghai Cancer Institute, Shanghai Jiaotong University, Shanghai, 200032, China

[6]Division of Environmental Health Sciences, University of Minnesota, 420 Delaware Street SE, Minneapolis, Minnesota 55455, USA

Abstract

Our recent studies on tobacco smoke carcinogen and toxicant biomarkers and cancer risk among male smokers in the Shanghai Cohort Study showed that exposure to tobacco-specific nitrosamines (TSNA) and polycyclic aromatic hydrocarbons (PAH) is prospectively associated with the risk of cancer. These findings support the hypothesis that the smokers' cancer risk is a function of the dose of select tobacco carcinogens and highlight the importance of understanding the factors that affect the intake of these carcinogens by smokers. Given that tobacco constituent exposures are driven, at least in part, by the levels of these constituents in cigarette smoke, we measured mainstream smoke TSNA and PAH levels in 43 Chinese cigarette brands that participants of the Shanghai Cohort Study reported to smoke. In all brands analyzed here, mainstream smoke levels of NNN and NNK, the two carcinogenic TSNA, were generally relatively low, averaging (±SD) 16.8(±25.1) and 14.2(±9.5) ng/cigarette, respectively. The levels of PAH were comparable to those found in U.S. cigarettes, averaging 15(±9) ng/cigarette for benzo[*a*]pyrene, 119(±66) ng/cigarette for phenanthrene, and 37(±19) ng/cigarette for pyrene. Our findings indicate that the generally low levels of NNN and NNK are most likely responsible for the relatively low levels of the corresponding biomarkers in the urine of the Shanghai Cohort Study participants as compared to those found in the U.S. smokers, supporting the role of the

Requests for reprints: Irina Stepanov, Masonic Cancer Center, University of Minnesota, 2231 6th Street SE, Room 2-140 CCRB, Minneapolis, MN 55455, USA. Phone: 612-624-4998; Fax: 612-624-3869. stepa011@umn.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

levels of these constituents in cigarette smoke in smokers' exposures. Our findings also suggest that, in addition to smoking, other sources contribute to Chinese smokers' exposure to PAH.

Keywords

TSNA; PAH; nicotine; nitrate; nitrite; tobacco; smoke

Introduction

Tobacco-specific *N*-nitrosamines (TSNA) and polycyclic aromatic hydrocarbons (PAH) are believed to play important roles in the development of cancers associated with smoking. In laboratory animals, the carcinogenic TSNA 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN) cause cancers of the lung, pancreas, oral cavity, esophagus, and nasal cavity.[1,2] Many PAH are also potent carcinogens or toxicants in laboratory animals and are widely accepted as major contributors to lung cancer in smokers.[3,4] Based on the extensive laboratory animal, mechanistic, and epidemiological evidence, NNN and NNK, as well as the prototypic PAH benzo[*a*]pyrene (BaP) are classified by the International Agency for Research on Cancer (IARC) as Group 1 carcinogens (carcinogenic to humans).[1,2,4–8]

Our recent studies on tobacco smoke carcinogen and toxicant biomarkers and cancer risk among male smokers in the Shanghai Cohort Study showed that the intake of TSNA and PAH is prospectively associated with the risk of cancer, providing further support for the role of these constituents in cancer development in smokers.[9] Specifically, we observed a significant dose-dependent association between prospectively measured urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a biomarker of exposure to NNK, and the risk of lung cancer in that cohort.[10] This association was also found in a prospective cohort of U.S. smokers.[11] Similarly, urinary *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), a metabolite of the non-carcinogenic PAH phenanthrene, was found to be significantly associated with lung cancer risk in the Shanghai Cohort Study.[12] Phenanthrene is structurally related to the carcinogenic BaP and is always part of PAH mixtures present in various environmental sources, including cigarette smoke.[13] In addition, urinary total NNN – a biomarker of exposure to NNN – was shown to be a strong predictor of esophageal cancer in smokers in the same cohort.[14] All three biomarkers were independently associated with cancer risk, even after adjustment for number of cigarettes smoked per day, number of years of smoking, and nicotine intake.

The findings of the Shanghai Cohort Study strongly support the hypothesis that smokers' cancer risk is a function of the dose of select tobacco carcinogens. Therefore, it is important to understand factors affecting the intake of these carcinogens by smokers. Tobacco constituent exposures in smokers are driven, at least in part, by the levels of these constituents in cigarette smoke. Limited studies reported that Chinese cigarettes contain relatively low levels of TSNA,[15] while information on PAH content is lacking. In this study we analyzed TSNA, PAH, and nicotine in the smoke of 43 samples of Chinese cigarettes representing 40 brands smoked by the smokers in the Shanghai Cohort Study. It has been

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

previously shown that various cigarette brands generally deliver increased amounts of PAH as TSNA levels decrease, which is suggested to be in part due to the contrasting effect of nitrate content in tobacco on TSNA formation and PAH pyrosynthesis in smoke.[16,17] Therefore, we also analyzed tobacco filler TSNA, nitrate, and nitrite levels, as factors known to affect TSNA and PAH content in cigarette smoke.

Materials and Methods

Cigarettes

The cigarettes were purchased in May 2011 from four shops across a wide area of the city of Shanghai, China. Most of the common brands were manufactured in Shanghai, Beijing, Tianjin, Qingdao, and Guizhou, China. These brands were chosen based on the in-person interview results of all 1,356 male current smokers in 2010–2011 who were participants of the Shanghai Cohort Study. Among all the brands, the Double Happiness brand manufactured by the Shanghai Tobacco Co. (Shanghai, China) was most frequently smoked brand (63.3% of the Shanghai Cohort Study smokers), followed by the Daqianmen brand (7.5%).

The description of cigarette brands along with the frequencies of each brand use among the Shanghai Cohort Study smokers are summarized in Table 1. Levels of tar, nicotine, and CO printed on each pack are provided in Supplementary Table S1. Unopened sealed packs of the purchased cigarettes were stored at −20 °C until the transport to the University of Minnesota for analyses. The analyses were performed within a year from the time of their purchase.

Analyses

Cigarettes were smoked under US Federal Trade Commission (FTC) standard conditions and the mainstream smoke was collected on Cambridge filter pads as previously described.[18]

TSNA analyses—The four commonly analyzed TSNA – NNN, NNK, *N′*-nitrosoanatabine (NAT) and *N′*-nitrosoanabasine (NAB) – were analyzed by liquid chromatography (LC)-tandem mass spectrometry (MS/MS) in positive ion electrospray mode as previously described.[18] Briefly, internal standards [$^{13}C_6$]NNN and [pyridine-D_4]NNK were added to either cigarette filler samples or smoke filter pads, followed by extraction with citrate-phosphate buffer and purification of the extracts on ChemElut cartridges (Varian, Harbor City, California, USA) and Sep-Pak Plus silica cartridges (Waters, Milford, Massachusetts, USA). The purified samples were analyzed by LC-MS/MS in selected reaction monitoring mode as described.[18]

PAH analyses—BaP, phenanthrene, and pyrene were analyzed using our previously described gas chromatography (GC)-MS method.[19] Briefly, an internal standard mix containing [$^{13}C_4$]BaP, [$^{13}C_6$]phenanthrene, and [$^{13}C_6$]pyrene was added to Cambridge filter pads, and the pads were extracted with hexane on a benchtop shaker for 3 hours. The extracts were purified on BondElut Silica cartridges (Varian), concentrated under a gentle stream of N_2 to a final volume of 20 μL, and analyzed by GC-MS as described.[19]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Nicotine—Tobacco filler was extracted with methanol containing potassium hydroxide, and an aliquot of the extract was diluted with 100 mM ammonium acetate. Smoke pads were extracted with 15 mM ammonium acetate and an aliquot was diluted with 100 mM ammonium acetate. [CD_3]Nicotine internal standard was used for both sample types. The prepared samples were analyzed by LC-MS/MS essentially as previously described, except that samples were eluted isocratically with acetonitrile:water:formic acid (85.6:13:1.4) containing 0.01% trifluoroacetic acid.[20]

Nitrate and nitrite analyses—These were analyzed essentially as previously described.[21] Briefly, tobacco filler (~100mg) was extracted with deionized H_2O and purified on C-18 SPE cartridges (Waters Corp., Milford, MA) prior to analysis by ion chromatography at the University of Minnesota Geochemical Analysis Facility.

Moisture content—The moisture content of cigarette filler was analyzed by a gravimetric method as previously described.[22]

Statistical analyses—Pearson correlations were determined using Sigma Plot 2001, v. 7.101 (SPSS, Inc., Chicago, IL).

Results

The results of cigarette smoke analyses are summarized in Table 2, and the results of tobacco filler analyses are summarized in Table 3.

The results for cigarette smoke in Table 2 are presented on a ‘per cigarette’ basis. The levels of NNN ranged from 1.8 to 135 ng/cigarette, and the levels of NNK ranged from 3.3 to 63.9 ng/cigarette. There was also variation in the measured PAH levels, which ranged 4–44 ng/cigarette, 34–307 ng/cigarette, and 11–91 ng/cigarette, respectively, for BaP, phenanthrene, and pyrene. Nicotine levels in cigarette smoke ranged from 0.31 to 1.94 mg/cigarette.

All results for cigarette filler in Table 3 are presented per gram wet weight. Moisture content in the filler of all brands averaged 14.8±1.7% (SD). NNN levels in the filler of tested cigarettes ranged from 0.02 to 4.67 μg/g, and NNK levels ranged from 0.032 to 1.35 μg/g tobacco. Total TSNA – the sum of all four nitrosamines analyzed here – varied from 0.103 to 6.38 μg/g tobacco. The levels of nitrate and nitrite also varied widely: 0.2–32.8 mg/g tobacco for nitrate and from non-detected to 0.099 mg/g tobacco for nitrite. Nicotine levels in the filler ranged from 7.45 to 23.3 mg/g tobacco.

Relations among the tested constituents are presented in Table 4. The TSNA levels in the tobacco filler correlated with TSNA levels in cigarette smoke and with tobacco filler nitrate, but not nitrite, levels. Cigarette smoke TSNA levels also correlated with nitrate levels in tobacco filler. The negative correlation between nitrate levels in tobacco filler and PAH levels in the smoke was not statistically significant. Levels of NNN and total TSNA in tobacco filler negatively correlated with PAH levels in cigarette smoke, while negative relation between filler NNK and smoke PAH levels was not statistically significant. There was no statistically significant relation between cigarette smoke TSNA and PAH levels. Levels of various measured PAH strongly correlated with tobacco and smoke levels of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

nicotine and among each other. The positive correlation between nicotine levels in the tobacco filler and smoke was not significant.

Discussion

Tobacco constituent intake in smokers can be affected by a variety of factors, including the levels of the constituents in cigarette smoke, individual smoking topography, and other individual characteristics of smokers. To provide insights into the potential contribution of cigarette smoke content to carcinogen intake by smokers in the Shanghai Cohort Study, we analyzed TSNA and PAH – the pertinent carcinogens – in cigarette brands smoked by the cohort participants. This is the first study to characterize multiple constituents in both the tobacco filler and the smoke of a wide range of Chinese cigarette brands.

The levels of TSNA in the smoke of cigarettes analyzed in this study were generally relatively low, in the range that is typically associated with Virginia tobacco.[23] A histogram showing the distribution of smoke TSNA levels in the brands analyzed here is illustrated in Figure 1A. It demonstrates that, while some of the analyzed brands contained higher levels of NNN and NNK, the sum of these carcinogens in the smoke of 88% of the brands is less than 50 ng/cigarette. These results are consistent with the levels reported in a previous study that examined the smoke of 39 unspecified Chinese cigarette brands.[15] For comparison, we recently reported that the sum of NNN and NNK in U.S. cigarettes ranged from 45 to 366 ng/cigarette, with 16 out of 17 brands containing these constituents at levels higher than 100 ng/cigarette.[18] The range of PAH levels measured in this study was similar to that reported for US cigarettes,[2,24,25] with 93% of the tested brands containing BaP at levels below 30 ng/cigarette (Figure 1B). These results are in agreement with the limited available data for Chinese cigarettes.[26] On the other hand, these results are in contrast with the general expectation that lower levels of TSNA in cigarette smoke are necessarily accompanied by increases in PAH levels, which is based in part on the contrasting effect of nitrate content in tobacco on TSNA formation and PAH pyrosynthesis in smoke Nitrate is the source of nitrosating species that react with tobacco alkaloids producing TSNA, and it has been shown that TSNA levels in tobacco products depend on tobacco nitrate content.[23,27,28] At the same time, higher nitrate content generates higher amounts of nitrogen oxides during tobacco combustion, and these oxides ‘capture’ and neutralize some radicals that otherwise could form PAH.[29] Indeed, it has been previously reported that various cigarette brands generally delivered increased amounts of PAH as TSNA levels decreased.[17] However, brand-by-brand examination of an international sample of cigarettes for which an overall negative correlation between TSNA and PAH was observed shows that many individual brands do not follow this pattern.[17] In the present study, TSNA levels in both tobacco filler and cigarette smoke correlated with nitrate levels in tobacco (Table 4). We also observed a slight negative correlation between tobacco nitrate levels and the smoke PAH yields (Table 4). However, there was no significant relationship between BaP and the sum of NNN and NNK in the smoke (Figure 2). These findings suggest that TSNA levels in cigarette smoke can be reduced without necessarily increasing PAH levels in the smoke of the same cigarettes. Furthermore, TSNA levels in cigarette smoke strongly correlated with those in the tobacco filler, consistent with previous findings that the levels of preformed TSNA in tobacco determine yields in smoke.[2,16,18,30] Together, these observations support the importance of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

tobacco processing and blending approaches which could be modified to reduce smoke TSNA exposures.[31]

The results of this study help to provide insights into the contribution of cigarette smoke TSNA and PAH content to the biomarker-assessed exposure to these carcinogens in smokers in the Shanghai Cohort Study. For instance, our previous research showed that urinary levels of TSNA biomarkers were lower, and those of PAH higher, in the smokers from the Shanghai Cohort Study as compared to the levels of corresponding biomarkers typically reported for U.S. smokers. Urinary total NNN averaged 0.06 pmol/mg creatinine[14] and total NNAL averaged 0.20 pmol/mg creatinine[12] in the Shanghai Cohort Study smokers, while in U.S. smokers these levels are 0.14 pmol/mg creatinine[32] and approximately 1.0−1.5 pmol/mg creatinine,[33,34] respectively. More than 95% of smokers in the Shanghai Cohort Study smoked cigarette brands containing the sum of NNN and NNK at levels below 30 ng/cigarette (see Tables 1 and 2). Taken together, our findings suggest that lower NNN and NNK levels in mainstream smoke of cigarette brands used by smokers in the Shanghai Cohort Study are most likely responsible for the lower urinary levels of total NNN and total NNAL in these smokers as compared to the levels typically measured in U.S. smokers. This is in agreement with a previous report showing that smokers of cigarettes with lower NNK content have in their urine lower levels of NNAL as compared to smokers of high-NNK cigarettes.[35] In contrast, urinary PheT in the Shanghai Cohort Study averaged 28.1 pmol/mg creatinine (95% confidence interval, 26.7−29.5),[12] or approximately 10-fold higher than the levels of this biomarker in U.S. smokers (ranging from 3.7 to 5 pmol/mg creatinine).[36–38] Given that smoke PAH levels measured in this study are similar to those found in the U.S. cigarettes, and that PAH are ubiquitous environmental contaminants, it is likely that exposures from other sources, for instance air pollution, diet, or occupational exposures contributed to the high levels of PAH exposure in the Shanghai Cohort Study smokers. In support of this hypothesis, considerably higher levels of PheT were also observed in Chinese non-smokers as compared to non-smokers from the US.[39]

In summary, we analyzed TSNA and PAH in cigarette brands that were used by smokers in the Shanghai Cohort Study. Our findings support the role of NNN and NNK content in cigarette smoke as an important factor influencing the exposures to these carcinogens in smokers. The results of PAH analyses suggest that the high levels of PAH biomarkers measured in the Shanghai Cohort Study are substantially affected by factors other than the levels of these constituents in Chinese cigarettes. While these findings do not undermine the importance of the association between urinary PheT and lung cancer risk in the Shanghai Cohort smokers, further research is needed to understand the major factors affecting PAH intake and the subsequent risk of lung cancer in this cohort.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Ms. Yue-Li Wang of the Shanghai Cancer Institute and Dr. Bin Ma for help with purchasing and characterizing cigarettes, Dr. Peter Villalta for help with mass-spectrometry, and Dr. Linda VonWeymarn for help with nicotine assay. We also thank Bob Carlson for editorial assistance. This study was supported by grants no. CA-129534, CA-144034, and CA-81301 from the National Cancer Institute, and startup funds to IS from the Masonic Cancer Center.

Reference List

1. Hecht SS. Tobacco carcinogens, their biomarkers, and tobacco-induced cancer. Nature Rev Cancer. 2003; 3:733–744. [PubMed: 14570033]
2. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 83. Lyon, FR: IARC; 2004. International Agency for Research on Cancer, Tobacco Smoke and Involuntary Smoking.
3. Ding YS, Ashley DL, Watson CH. Determination of 10 carcinogenic polycyclic aromatic hydrocarbons in mainstream cigarette smoke. J Agric Food Chem. 2007; 55:5966–5973. [PubMed: 17602652]
4. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 92. Lyon, FR: IARC; 2010. International Agency for Research on Cancer, Some Non-Heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures; p. 35-818.
5. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific *N*-nitrosamines. Chem Res Toxicol. 1998; 11:559–603. [PubMed: 9625726]
6. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 89. Lyon, FR: IARC; 2007. International Agency for Research on Cancer, Smokeless tobacco and some tobacco-specific *N*-nitrosamines.
7. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene. 2002; 21:7435–7451. [PubMed: 12379884]
8. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst. 1999; 91:1194–1210. [PubMed: 10413421]
9. Hecht SS, Murphy SE, Stepanov I, Nelson HH, Yuan JM. Tobacco smoke biomarkers and cancer risk among male smokers in the Shanghai Cohort Study. Cancer Lett. 2012; 334:34–38. [PubMed: 22824243]
10. Yuan JM, Koh WP, Murphy SE, Fan Y, Wang R, Carmella SG, Han S, Wickham K, Gao YT, Yu MC, Hecht SS. Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. Cancer Res. 2009; 69:2990–2995. [PubMed: 19318550]
11. Church TR, Anderson KE, Caporaso NE, Geisser MS, Le CT, Zhang Y, Benoit AR, Carmella SG, Hecht SS. A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. Cancer Epidemiol Biomarkers Prev. 2009; 18(1):260–266. [PubMed: 19124507]
12. Yuan JM, Gao YT, Murphy SE, Carmella SG, Wang R, Zhong Y, Moy KA, Davis AB, Tao L, Chen M, Han S, Nelson HH, et al. Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. Cancer Res. 2011; 71(21): 6749–6757. [PubMed: 22028322]
13. Hecht SS, Carmella SG, Villalta PW, Hochalter JB. Analysis of phenanthrene and benzo[a]pyrene tetraol enantiomers in human urine: relevance to the bay region diol epoxide hypothesis of benzo[a]pyrene carcinogenesis and to biomarker studies. Chem Res Toxicol. 2010; 23:900–908. [PubMed: 20369855]
14. Yuan JM, Knezevich AD, Wang R, Gao YT, Hecht SS, Stepanov I. Urinary levels of the tobacco-specific carcinogen N'-nitrosonornicotine and its glucuronide are strongly associated with esophageal cancer risk in smokers. Carcinogenesis. 2011; 32(9):1366–1371. [PubMed: 21734256]
15. Xiong W, Hou H, Jiang X, Tang G, Hu Q. Simultaneous determination of four tobacco-specific *N*-nitrosamines in mainstream smoke for Chinese Virginia cigarettes by liquid chromatography-tandem mass spectrometry and validation under ISO and "Canadian intense" machine smoking regimes. Anal Chim Acta. 2010; 674(1):71–78. [PubMed: 20638502]
16. 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. [PubMed: 16085512]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

17. Ding YS, Yan XJ, Jain RB, Lopp E, Tavakoli A, Polzin GM, Stanfill SB, Ashley DL, Watson CH. Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from U.S. brand and non-U.S. brand cigarettes. Environ Sci Technol. 2006; 40(4):1133–1138. [PubMed: 16572766]

18. Stepanov I, Knezevich A, Zhang L, Watson CH, Hatsukami DK, Hecht SS. Carcinogenic tobacco-specific N-nitrosamines in US cigarettes: three decades of remarkable neglect by the tobacco industry. Tobacco Control. 2012; 21:44–48. [PubMed: 21602537]

19. Stepanov I, Villalta PW, Knezevich A, Jensen J, Hatsukami DK, Hecht SS. Analysis of 23 polycyclic aromatic hydrocarbons in smokeless tobacco by gas chromatography-mass spectrometry. Chem Res Toxicol. 2010; 23:66–73. [PubMed: 19860436]

20. Murphy SE, Villalta P, Ho SW, von Weymarn LB. Analysis of [3',3'-d(2)]-nicotine and [3',3'-d(2)]-cotinine by capillary liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 857:1–8.

21. Stepanov I, Hecht SS, Ramakrishnan S, Gupta PC. Tobacco-specific nitrosamines in smokeless tobacco products marketed in India. Intl J Cancer. 2005; 116:16–19.

22. Stepanov I, Jensen J, Hatsukami D, Hecht SS. New and traditional smokeless tobacco: comparison of toxicant and carcinogen levels. Nicotine Tob Res. 2008; 10(12):1773–1782. [PubMed: 19023828]

23. Hoffmann D, Adams JD, Brunnemann KD, Hecht SS. Assessment of tobacco-specific N-nitrosamines in tobacco products. Cancer Res. 1979; 39:2505–2509. [PubMed: 445452]

24. Borgerding, MF.; Bodnar, JA.; Wingate, DE. A Research Study Conducted after Consultation with the Massachusetts. Department of Public Health; 2000. The 1999 Massachusetts Benchmark Study - Final report. [http://www.brownandwilliamson.com/APPS/PDF/Final_Report_1999_Mass_Benchmark_Study pdf]

25. Counts ME, Hsu FS, Laffoon SW, Dwyer RW, Cox RH. Mainstream smoke constituent yields and predicting relationships from a worldwide market sample of cigarette brands: ISO smoking conditions. Regul Toxicol Pharmacol. 2004; 39:111–134. [PubMed: 15041144]

26. Akpan V, Huang S, Lodovici M, Dolara P. High levels of carcinogenic polycyclic aromatic hydrocarbons (PAH) in 20 brands of Chinese cigarettes. J Appl Toxicol. 2006; 26(6):480–483. [PubMed: 17080399]

27. Hoffmann, D.; Hecht, SS.; Ornaf, RM.; Wynder, EL.; Tso, TC. Nitrosonornicotine: presence in tobacco, formation and carcinogenicity. In: Walker, EA.; Bogovski, P.; Griciute, L., editors. Environmental N-Nitroso Compounds: Analysis and Formation. Lyon, France: International Agency for Research on Cancer; 1976. p. 307-320.

28. Hecht SS, Chen CB, Dong M, Ornaf RM, Hoffmann D, Tso TC. Studies on non-volatile nitrosamines in tobacco. Beitr Tabakforsch Int. 1977; 9:1–6.

29. Ding YS, Zhang L, Jain RB, Jain N, Wang RY, Ashley DL, Watson CH. Levels of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons in mainstream smoke from different tobacco varieties. Cancer Epidemiol Biomarkers Prev. 2008; 17(12):3366–3371. [PubMed: 19064552]

30. Fischer S, Spiegelhalder B, Eisenbarth J, Preussmann R. Investigations on the origin of tobacco-specific nitrosamines in mainstream smoke of cigarettes. Carcinogenesis. 1990; 11:723–730. [PubMed: 2335004]

31. Hecht SS. It is time to regulate carcinogenic tobacco-specific nitrosamines in cigarette tobacco. Cancer Prevention Research. 2014; 7:639–647. [PubMed: 24806664]

32. Stepanov I, Carmella SG, Briggs A, Hertsgaard L, Lindgren B, Hatsukami D, Hecht SS. Presence of the carcinogen *N*-nitrosonornicotine in the urine of some users of oral nicotine replacement therapy products. Cancer Res. 2009; 69(21):8236–8240. [PubMed: 19843845]

33. Hatsukami DK, Kotlyar M, Hertsgaard LA, Zhang Y, Carmella SG, Jensen JA, Shields PG, Murphy SE, Stepanov I, Hecht SS. Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation. Addiction. 2010; 105:343–355. [PubMed: 20078491]

34. Kotlyar M, Hertsgaard LA, Lindgren BR, Jensen JA, Carmella SG, Stepanov I, Murphy SE, Hecht SS, Hatsukami DK. Effect of oral snus and medicinal nicotine in smokers on toxicant exposure and withdrawal symptoms: a feasibility study. Cancer Epidemiol Biomarkers & Prev. 2011; 20(1): 91–100.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

35. Ashley DL, O'Connor RJ, Bernert JT, Watson CH, Polzin GM, Jain RB, Hammond D, Hatsukami DK, Giovino GA, Cummings KM, McNeill A, Shahab L, et al. Effect of differing levels of tobacco-specific nitrosamines in cigarette smoke on the levels of biomarkers in smokers. Cancer Epidemiol Biomarkers & Prev. 2010; 19(6):1389–1398.

36. Hecht SS, Chen M, Yagi H, Jerina DM, Carmella SG. *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. Cancer Epidemiol Biomarkers & Prev. 2003; 12:1501–1508.

37. Hecht SS, Chen M, Yoder A, Jensen J, Hatsukami D, Le C, Carmella SG. Longitudinal study of urinary phenanthrene metabolite ratios: effect of smoking on the diol epoxide pathway. Cancer Epidemiol Biomarkers & Prev. 2005; 14(12):2969–2974.

38. Hecht SS, Carmella SG, Yoder A, Chen M, Li Z, Le C, Jensen J, Hatsukami DK. Comparison of polymorphisms in genes involved in polycyclic aromatic hydrocarbon metabolism with urinary phenanthrene metabolite ratios in smokers. Cancer Epidemiol Biomarkers & Prev. 2006; 15:1805–1811.

39. Kensler TW, Ng D, Carmella SG, Chen M, Jacobson LP, Munoz A, Egner PA, Chen JG, Qian GS, Chen TY, Fahey JW, Talalay P, et al. Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China. Carcinogenesis. 2012; 33(1):101–107. [PubMed: 22045030]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Novelty and Impact

Biomarker-assessed levels of exposure to carcinogenic tobacco-specific *N*-nitrosamines (TSNA) and polycyclic aromatic hydrocarbons (PAH) have been associated with the risk of lung cancer in smokers from the Shanghai Cohort Study. Understanding the factors contributing to these exposures could provide critical insights for the development of preventive measures. We examined the levels of TSNA and PAH in Chinese cigarette brands that were smoked by the Shanghai Cohort Study participants. This is the first study to characterize multiple constituents in both the smoke and the tobacco filler of a wide range of Chinese cigarette brands. The results indicate that smoke TSNA content play an important role in smokers' exposures to these constituents, while additional sources of exposure most likely contributed significantly to PAH intake among the Shanghai Cohort Study smokers.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

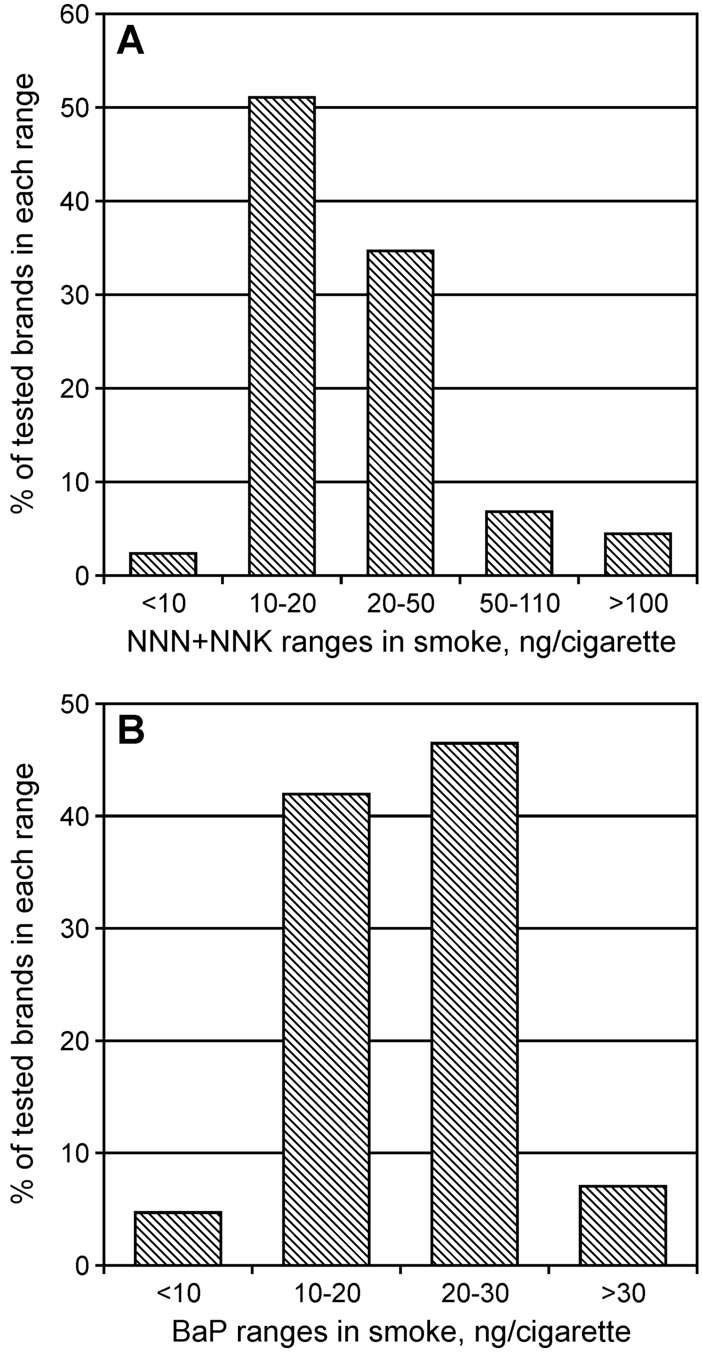

Figure 1.

Distribution of carcinogenic constituents in the smoke of Chinese cigarettes analyzed in this study: **A**, tobacco-specific *N*-nitrosamines; **B**, benzo[*a*]pyrene.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

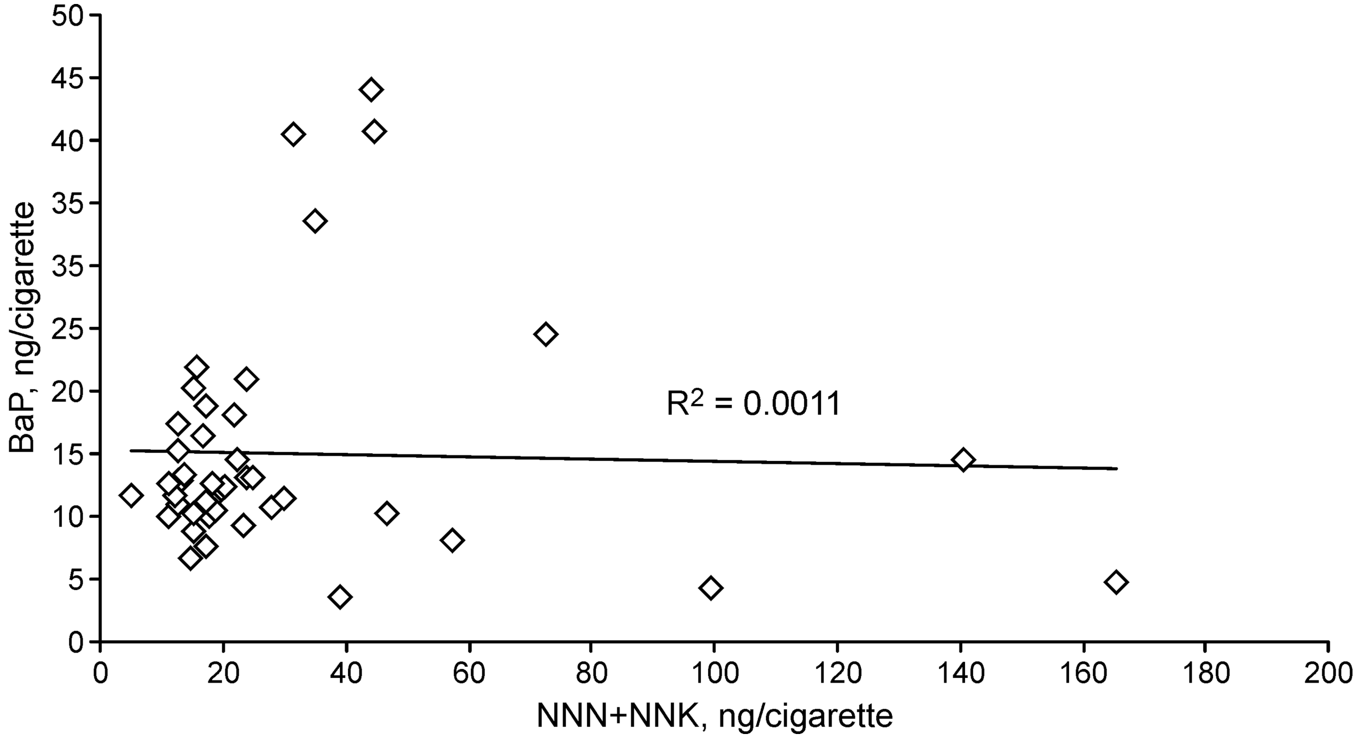

Figure 2.
Relationship between levels of tobacco-specific *N*-nitrosamines and benzo[*a*]pyrene in the smoke of cigarettes analyzed in this study.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Cigarette brands analyzed in this study and frequency of their use by the Shanghai Cohort Study smokers.

No.	Brand name in Chinese	Brand name in English (pack type and cigarette size[a])	Additional descriptors	Frequency of smokers in Shanghai Cohort Study (%) (n = 1356)
1	红双喜	Double Happiness (HP, KS)	red/pink pack	858 (63.27)
2	红双喜	Double Happiness (HP, KS)	red/silver pack	
3	绿双喜	Double Happiness (HP, KS)	green pack	0
4	大前门	DAQIANMEN (HP, KS)	silver pack	102 (7.52)
5	牡丹	Peony (HP, KS)	red pack	30 (2.21)
6	上海牌－金色红双喜	Double Happiness (HP, KS)	gold pack	150 (11.06)
7	中华	Chunghwa (HP, KS)	red pack	55 (4.06)
8	三五牌	555-Gold Pearl (HP, KS)	white pack	14 (1.03)
9	红梅	Hongmei (HP, KS)	orange pack	11 (0.81)
10	利群(红)	Ligun-Virginia type (HP, KS)	silver pack	52 (3.83)
11	利群(蓝)	Ligun-Virginia type (SP, KS)	gold pack	
12	哈德门	Hatamen (HP, KS)	gold pack	5 (0.37)
13	中南海(蓝)	Jhonqnanhai (HP, KS)	blue pack	14 (1.03)
14	中南海(白)	Jhonqnanhai/Five (HP, KS)	white pack	
15	红河(红)	Honghe (HP, KS)	red/gold pack	4 (0.29)
16	红河(白)	Honghe (HP, KS)	white/red pack	
17	黄山(红)	HuangShan (SP, KS)	red/gold pack	9 (0.66)
18	黄山(棕)	HuangShan (SP, KS)	black/red pack	
19	玉溪(红)	Yuxi (SP, KS)	red dot pack	7 (0.52)
20	玉溪(棕)	Yuxi (SP, KS)	bronze pack	
21	红塔山	Hongtashan (HP, KS)	white pack	4 (0.29)
22	黄果树	Huangguoshu (HP, KS)	red pack	9 (0.66)
23	白沙(白)	Baisha (HP, KS)	white pack	4 (0.29)
24	白沙(棕)	Baisha (SP, KS)	gold pack	
25	云烟(灰)	Yun Yan/Win (HP, 95mm)	silver pack	8 (0.59)
26	云烟(红)	YunYan (HP, KS)	black/red pack	
27	泰山	Taishan (SP, KS)	rose-gold pack	1 (0.07)
28	猴王	Houwang (HP, KS)	gold pack	1 (0.07)
29	大红鹰	Dohongying (HP, KS)	pink/maroon pack	3 (0.22)
30	甲天下	Fiatianxia (HP, KS)	pink stripe pack	2 (0.15)
31	五牛	Five Bulls (HP, KS)	gold pack	1 (0.07)
32	南京(红)	NanJing (HP, KS)	red pack	2 (0.15)
33	南京(绿)	NanJing (HP, KS)	green pack	
34	熊猫	Panda (HP, KS)	orange pack	1 (0.07)
35	七星	Mild Seven-Sky blue (HP, KS)	blue pack	2 (0.15)

No.	Brand name in Chinese	Brand name in English (pack type and cigarette size[a])	Additional descriptors	Frequency of smokers in Shanghai Cohort Study (%) (n = 1356)
36	红旗渠	Hongqiqu (HP, KS)	red/gold pack	1 (0.07)
37	红旗渠	Hongqiqu (SP, KS)	red pack	
38	韩国 ESSE	Esse Blue (HP, SS, 100mm)	white/blue pack	2 (0.15)
39	黄金叶	Goldenleaf (HP, KS)	gold pack	1 (0.07)
40	芙蓉王	Furongwang (HP, KS)	gold pack	1 (0.07)
41	人民大金星	RenminDanuitang (HP, KS)	red pack	1 (0.07)
42	大卫杜夫(silver)	Davidoff Neon silver (HP, KS)	silver pack	1 (0.07)
43	大卫杜夫(supreme)	Davidoff supreme (HP, 95mm)	red pack	

[a]HP – hard pack; SP – soft pack; KS – king size; SS – super-slim

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Levels of nicotine, polycyclic aromatic hydrocarbons, and tobacco-specific *N*-nitrosamines in the smoke of Chinese cigarettes analyzed in this study.

No.	Nicotine, mg/cigarette	ng/cigarette					
		Phenanthrene	Pyrene	BaP	NNN	NNK	Total TSNA[a]
1	0.86	57	18	7	7.3	7.4	35.8
2	0.98	111	32	13	9.9	13.6	58.7
3	0.96	101	30	12	7.6	11.5	50.4
4	0.83	76	27	11	10.7	16.8	69.3
5	0.94	62	26	10	29.2	17.2	133.9
6	0.71	84	28	10	6.3	11.4	40.7
7	0.72	101	31	12	6.0	13.6	40.6
8	0.70	98	33	12	17.2	12.6	88.4
9	0.86	126	38	15	8.9	13.2	52.6
10	0.94	103	29	12	6.8	13.5	45.2
11	0.97	83	26	10	6.9	11.8	40.4
12	0.75	105	28	13	12.8	12.2	57.8
13	0.31	48	16	5	135.3	30.0	261.1
14	0.31	40	14	4	77.8	21.5	160.4
15	0.74	82	25	10	3.7	7.5	30.6
16	0.85	83	24	9	5.2	10.0	40.5
17	0.80	97	29	13	6.2	6.7	37.7
18	0.96	68	20	8	7.7	9.5	42.0
19	0.73	105	30	12	1.8	3.3	10.3
20	0.83	91	28	11	4.1	8.5	31.0
21	0.71	129	34	15	3.3	9.3	27.2
22	0.80	76	23	9	9.1	14.1	62.0
23	0.83	96	27	13	5.2	12.7	35.4
24	0.76	100	29	13	4.5	9.2	32.5
25	0.63	90	25	12	3.0	9.0	26.2

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

No.	Nicotine, mg/cigarette	ng/cigarette					
		Phenanthrene	Pyrene	BaP	NNN	NNK	Total TSNA[a]
26	0.95	76	28	11	6.3	10.8	40.3
27	0.59	122	40	16	5.7	10.9	41.2
28	0.95	139	54	20	5.5	9.8	36.2
29	1.94	274	88	41	25.8	18.7	158.2
30	1.54	284	80	34	18.4	16.7	113.7
31	1.16	307	91	44	25.8	18.1	157.7
32	1.66	297	82	40	13.5	17.8	61.4
33	0.85	157	54	21	11.2	12.3	62.8
34	0.87	193	62	25	43.7	28.7	170.3
35	0.42	107	33	13	3.0	8.2	20.5
36	0.82	84	26	10	7.6	7.6	39.3
37	0.61	187	52	22	8.2	7.2	52.6
38	0.40	67	24	8	40.1	17.2	115.7
39	0.79	165	49	18	7.9	13.7	52.9
40	0.93	145	45	17	3.6	8.7	24.9
41	0.98	143	46	19	5.7	11.2	39.6
42	0.53	34	11	4	17.4	21.5	77.4
43	0.72	108	40	15	76.5	63.9	259.1
Average	**0.84**	**119**	**37**	**15**	**16.8**	**14.2**	**70.6**
SD	**0.31**	**66**	**19**	**9**	**25.1**	**9.5**	**58.8**

[a]Total TSNA, sum of the four TSNA analyzed in this study: NNN, NNK, NAT, and NAB.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 3

Levels of nicotine, nitrate, nitrite, and tobacco-specific *N*-nitrosamines in tobacco filler of Chinese cigarettes analyzed in this study.

No.	Moisture, %	mg/g (wet weight)			ug/g (wet weight)		
		Nicotine	Nitrite	Nitrate	NNN	NNK	Total TSNA[b]
1	14.1	11.9	0.004	1.0	0.054	0.058	0.266
2	13.8	15.9	0.021	3.3	0.070	0.073	0.319
3	12.7	7.9	< 0.001	4.7	0.073	0.060	0.300
4	15.1	10.0	0.016	7.0	0.066	0.080	0.337
5	16.9	7.5	0.050	32.8	0.237	0.110	0.82
6	16.4	10.9	0.019	3.3	0.076	0.132	0.422
7	15.6	13.1	< 0.001	0.9	0.054	0.060	0.258
8	15.7	12.4	< 0.001	8.2	0.282	0.127	0.842
9	15.2	15.5	0.014	1.9	0.089	0.058	0.298
10	12.7	16.3	0.009	0.4	0.097	0.066	0.353
11	13.6	12.8	0.007	1.2	0.090	0.072	0.336
12	14.7	13.4	< 0.001	2.9	0.113	0.047	0.356
13	14.7	9.6	0.009	13.3	1.95	0.171	2.93
14	15.0	10.3	0.016	14.4	4.67	0.286	6.38
15	13.7	13.2	0.011	1.8	0.043	0.071	0.310
16	14.1	13.3	0.011	1.3	0.036	0.040	0.232
17	14.8	15.5	0.030	5.5	0.099	0.060	0.326
18	16.6	17.4	0.027	0.9	0.118	0.059	0.341
19	15.9	14.9	0.069	1.1	0.035	0.053	0.183
20	15.4	15.9	0.019	2.3	0.041	0.071	0.228
21	15.6	16.3	0.027	1.4	0.040	0.042	0.195
22	19.0	16.4	< 0.001	5.8	0.063	0.059	0.252
23	16.4	23.2	0.012	0.8	0.052	0.055	0.229
24	14.7	17.8	0.010	4.1	0.081	0.049	0.274
25	13.1	23.3	0.011	3.3	0.027	0.048	0.167

No.	Moisture, %	mg/g (wet weight)			ug/g (wet weight)		
		Nicotine	Nitrite	Nitrate	NNN	NNK	Total TSNA[b]
26	13.4	23.0	0.046	0.9	0.058	0.064	0.238
27	13.3	15.5	0.023	2.4	0.076	0.054	0.267
28	12.7	19.1	0.043	3.4	0.110	0.079	0.40
29	13.9	17.5	0.022	1.3	0.060	0.041	0.187
30	14.1	16.8	0.036	6.4	0.132	0.059	0.405
31	14.3	15.9	0.026	8.4	0.099	0.057	0.370
32	12.2	21.1	0.032	4.1	0.082	0.060	0.248
33	15.9	15.9	< 0.001	3.1	0.131	0.087	0.418
34	13.5	18.7	< 0.001	0.2	0.020	0.037	0.103
35	15.8	18.6	0.084	8.3	0.586	0.343	1.51
36	14.0	16.3	0.033	1.6	0.083	0.074	0.285
37	15.6	14.1	0.031	2.6	0.072	0.032	0.201
38	15.4	17.4	0.099	12.6	0.752	0.189	1.40
39	13.9	21.6	0.052	4.1	0.067	0.057	0.234
40	13.4	17.5	0.031	0.5	0.062	0.051	0.25
41	13.6	16.3	0.018	3.0	0.098	0.064	0.35
42	21.4	17.8	0.019	1.4	0.554	0.322	1.81
43	12.9	14.2	0.029	5.9	1.10	1.35	4.07
Average	**14.8**	**15.7**	**0.024**	**4.5**	**0.276**	**0.088**	**0.61**
SD	**1.7**	**3.8**	**0.022**	**5.6**	**0.756**	**0.071**	**1.04**

[a]Below the limit of detection for nitrite assay (0.001 μg/g tobacco).

[b]Total TSNA, sum of the four TSNA analyzed in this study: NNN, NNK, NAT, and NAB.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 4

Relationship between constituents in tobacco filler and smoke

		tobacco filler						cigarette smoke					
		nicotine	**nitrite**	**nitrate**	**NNN**	**NNK**	**Total TSNA**[a]	**nicotine**	**phenanthrene**	**pyrene**	**BaP**	**NNN**	**NNK**
tobacco filler	Nitrite	r = 0.162 *P* = 0.23											
	Nitrate	−0.441 0.0007	0.356 0.007										
	NNN	−0.289 0.03	0.038 0.78	0.471 0.0002									
	NNK	−0.099 0.47	0.176 0.19	0.257 0.06	0.449 0.0005								
	Total TSNA[a]	−0.280 0.037	0.095 0.49	0.488 0.0001	0.944 <0.0001	0.711 <0.0001							
cigarette smoke	Nicotine	0.240 0.075	−0.124 0.36	−0.210 0.12	−0.413 0.0016	−0.227 0.093	−0.413 0.0016						
	Phenanthrene	0.353 0.0076	0.037 0.79	−0.184 0.17	−0.276 0.039	−0.148 0.28	−0.285 0.033	0.731 <0.0001					
	Pyrene	0.338 0.011	0.092 0.50	−0.136 0.32	−0.265 0.048	−0.106 0.44	−0.263 0.050	0.725 <0.0001	0.972 <0.0001				
	BaP	0.317 0.017	0.029 0.83	−0.144 0.29	−0.267 0.047	−0.138 0.31	−0.272 0.043	0.749 <0.0001	0.958 <0.0001	0.965 <0.0001			
	NNN	−0.291 0.029	0.065 0.63	0.523 <0.0001	0.746 <0.0001	0.581 <0.0001	0.806 <0.0001	−0.259 0.054	−0.123 0.37	−0.099 0.47	−0.103 0.45		
	NNK	−0.109 0.42	0.097 0.48	0.347 0.009	0.490 0.0001	0.829 <0.0001	0.695 <0.0001	−0.049 0.72	0.029 0.83	0.055 0.69	0.048 0.73	0.807 <0.0001	
	Total TSNA[a]	−0.233 0.084	0.123 0.37	0.513 <0.0001	0.586 <0.0001	0.633 <0.0001	0.707 <0.0001	−0.041 0.76	0.067 0.62	0.098 0.47	0.104 0.44	0.922 <0.0001	0.912 <0.0001

[a]Total TSNA, sum of the four TSNA analyzed in this study: NNN, NNK, NAT, and NAB.