

# Relationship of Human Toenail Nicotine, Cotinine, and 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanol to Levels of These Biomarkers in Plasma and Urine

Irina Stepanov,<sup>1</sup> Stephen S. Hecht,<sup>1</sup> Bruce Lindgren,<sup>1</sup> Peyton Jacob III,<sup>2</sup> Margaret Wilson,<sup>2</sup> and Neal L. Benowitz<sup>2</sup>

<sup>1</sup>The Cancer Center, University of Minnesota, Minneapolis, Minnesota and <sup>2</sup>Division of Clinical Pharmacology and Experimental Therapeutics, University of California, San Francisco, California

## Abstract

Recently, we developed sensitive and quantitative methods for analysis of the biomarkers of tobacco smoke exposure nicotine, cotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human toenails. In this study, we further evaluated the newly developed toenail biomarkers by investigating their relationship to demographic factors, reported exposure, plasma nicotine, cotinine, and *trans*-3'-hydroxycotinine, and urinary NNAL. Toenails of 105 smokers, mean age 38.9 years (range, 19-68), were analyzed. Fifty-five (53.4%) were male, with approximately equal numbers of Whites and African-Americans. The average number of cigarettes smoked per day was 18 (range, 5-50). There was no effect of age or gender on the toenail

biomarkers. Toenail NNAL was higher in White than in African-American participants ( $P = 0.019$ ). Toenail nicotine and toenail cotinine correlated significantly with cigarettes smoked per day ( $r = 0.24$ ;  $P = 0.015$  and  $r = 0.26$ ;  $P = 0.009$ , respectively). Toenail nicotine correlated with plasma nicotine ( $r = 0.39$ ;  $P < 0.001$ ); toenail cotinine correlated with plasma cotinine ( $r = 0.45$ ;  $P < 0.001$ ) and plasma *trans*-3'-hydroxycotinine ( $r = 0.30$ ;  $P = 0.008$ ); and toenail NNAL correlated with urine NNAL ( $r = 0.53$ ;  $P = 0.005$ ). The results of this study provide essential validation data for the use of toenail biomarkers in investigations of the role of chronic tobacco smoke exposure in human cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(7):1382-6)

## Introduction

Cigarette smoking causes 90% of lung cancer cases (1) and is responsible for 30% of all cancer deaths in developed countries (2, 3). Exposure to environmental tobacco smoke is also recognized as a causative factor for cancer of the lung in humans (1).

Biomarkers of chronic tobacco smoke exposure help to increase our understanding of tobacco-related cancer mechanisms and to develop preventive measures. Systemic exposure to cigarette smoke is commonly monitored by measuring nicotine and its major metabolite cotinine in urine, saliva, blood, and hair (4-12). In some studies, *trans*-3'-hydroxycotinine, the major metabolite of cotinine, is used along with nicotine and cotinine to estimate total nicotine uptake and exposure (13-15). However, nicotine, cotinine, and *trans*-3'-hydroxycotinine are not carcinogenic. A tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, is the most prevalent systemic lung carcinogen in tobacco products (16, 17). Quantitatively significant metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and its *O*- and *N*-glucuronides (NNAL-Glucs), referred to as total NNAL, are the most extensively used biomarkers of tobacco-specific carcinogen uptake and are important in understanding of tobacco-related cancer mechanisms (18, 19). Total NNAL has been quantified in the urine and plasma of smokers and

smokeless tobacco users and in the urine of nonsmokers exposed to environmental tobacco smoke (19-25).

Most investigations show a significant positive correlation between urinary cotinine and total NNAL (19), indicating that both of these compounds are biomarkers of tobacco toxicant exposure. Measurement of urinary cotinine and total NNAL is a common approach and has several advantages, but a disadvantage of urinary biomarkers is their transient nature (19). Thus, the distribution half-life of urinary total NNAL is 3 to 4 days (23), and cotinine has a half-life of only ~15 to 17 h (26).

Recently, we developed sensitive and quantitative methods for analysis of nicotine, cotinine, and NNAL in human toenails (27). Measurement of toenail biomarkers has certain advantages, including ease of collection and storage and potential evaluation of cumulative exposure over a relatively long period due to the slow (~0.1 cm/mo) growth of toenails (28). Another important advantage of toenail nicotine, cotinine, and NNAL measurements is the seemingly indefinite stability of the collected sample caused by incorporation of these biomarkers into the keratinic matrix of the nail.

Although our previous study provided accurate and sensitive methods for analysis of nicotine, cotinine, and NNAL in human toenails, there is a need for their validation as biomarkers of human chronic exposure to tobacco smoke. The criteria for evaluation of a new biomarker include the relationship between the level of exposure and the amount of the biomarker in a biological sample; relationship between the new biomarker and one that is already proven and widely accepted; effects of gender, age, and race on the biomarker level; longitudinal intrasubject reliability; and kinetics of biomarker elimination from the biological sample after exposure is stopped. In this study, we evaluated the newly developed toenail biomarkers by investigating their relationship to demographic factors, reported exposure, plasma nicotine, cotinine, and *trans*-3'-hydroxycotinine, and urinary total NNAL. Structures of the biomarkers analyzed here are shown in Fig. 1.

Received 2/14/07; revised 3/19/07; accepted 4/17/07.

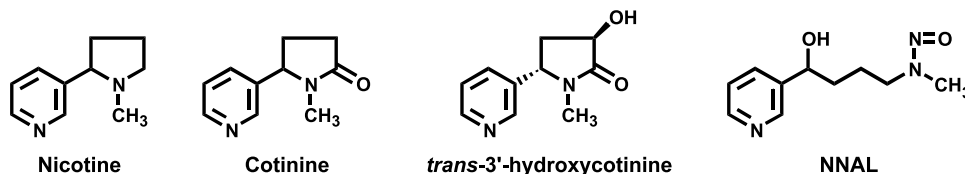
**Grant support:** National Cancer Institute USPHS grants CA-81301 and CA-78603, National Institute on Drug Abuse grants DA 02277 and DA 12393, National Center for Research Resources grant RR024131, and Flight Attendants Medical Research Institute grant.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Irina Stepanov, The Cancer Center, University of Minnesota, Mayo Mail Code 806, 420 Delaware Street Southeast, Minneapolis, MN 55455. Phone: 612-624-4998; Fax: 612-626-5135. E-mail: stepa011@umn.edu

Copyright © 2007 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-07-0145



**Figure 1.** Structures of compounds analyzed in human toenails.

## Materials and Methods

**Caution.** NNAL is carcinogenic and mutagenic and should be handled with extreme care, using appropriate protective clothing and ventilation at all times.

**Chemicals and Enzymes.** NNAL was purchased from Toronto Research Chemicals, Inc. [Pyridine-D<sub>4</sub>]NNAL was synthesized from [pyridine-D<sub>4</sub>]ethyl nicotinate (Cambridge Isotope Laboratories) as described previously (29, 30). [<sup>13</sup>C<sub>6</sub>]NNAL was synthesized by NaBH<sub>4</sub> reduction of [<sup>13</sup>C<sub>6</sub>]4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Cambridge Isotope Laboratories). Nicotine, [CD<sub>3</sub>]nicotine, cotinine, and [CD<sub>3</sub>]cotinine were purchased from Sigma Chemical Co., and *trans*-3'-hydroxycotinine was purchased from Toronto Research Chemicals.

### Analyses

*Nicotine, Cotinine, and NNAL in Toenails.* Nicotine, cotinine, and NNAL in toenail clippings were analyzed as described previously (27). Because the analysis involves NaOH digestion, *N*-glucuronides of these compounds would be converted to their aglycones. Further experiments with β-glucuronidase indicated that NNAL-*O*-Gluc was not present in human toenails (27). Therefore, the values reported in this article are total nicotine, cotinine, and NNAL, but it is likely that these analytes in toenails are mainly in their nonconjugated form.

*Nicotine, Cotinine, and 3'-Hydroxycotinine in Plasma.* Concentrations of cotinine, and *trans*-3'-hydroxycotinine in plasma were determined by liquid chromatography-tandem mass spectrometry as described previously (31). Plasma nicotine concentration was measured by gas chromatography with nitrogen phosphorus detection as described previously (32). Values are free (nonconjugated) nicotine, cotinine, and 3'-hydroxycotinine.

*NNAL in Urine.* To measure total urinary NNAL, 10,000 units of *Helix pomatia* β-glucuronidase were added to 5 mL aliquots of urine, and the sample was incubated for 48 h at 37 °C and further worked up and analyzed as described elsewhere (33).

**Subjects.** The subjects were regular smokers who had smoked for at least 1 year and who were healthy by self-report. Users of smokeless tobacco, pipes, cigars, or nicotine medications were excluded, as were people who used medications other than vitamins, hormones, and over the counter analgesics.

Seventy-nine smokers with available toenail samples were selected from subjects being recruited in San Francisco for a larger study of the racial differences in smoking behaviors and

tobacco smoking biomarkers. The subjects were recruited by newspaper advertisements, notices posted in local colleges, community centers and other public places, and via the internet (Craig's list and our Web site). The study protocol was approved by the Institutional Review Board at the University of California San Francisco. Eligible subjects were asked to come to the General Clinical Research Center at San Francisco General Hospital Medical Center, having smoked their cigarettes in their usual way before the study visit. Written consent was obtained, and subjects were asked to complete questionnaires that included demographic and smoking history questions. A blood sample, urine sample, and toenail clippings were collected.

To improve the statistical power of the results, the sample size was increased by recruiting of another 26 active smokers from several smoking studies conducted at the Transdisciplinary Tobacco Use Research Center (Minneapolis, MN). The entrance criteria of these studies required subjects to smoke at least 10 cigarettes per day for at least 1 year. Subjects were offered the opportunity to participate in this addendum study for additional payment. All studies were approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee.

**Statistical Analyses.** The distribution of each biomarker was assessed and log transformation was used if needed. In addition to the summary statistics, the analyses included the Pearson correlation, the two-sample *t* test, and multiple regression to determine predictive models for the biomarkers. All of the statistical analyses were carried out using SAS version 9.1 (SAS Institute, Inc.). *P* values <0.05 were considered statistically significant.

## Results

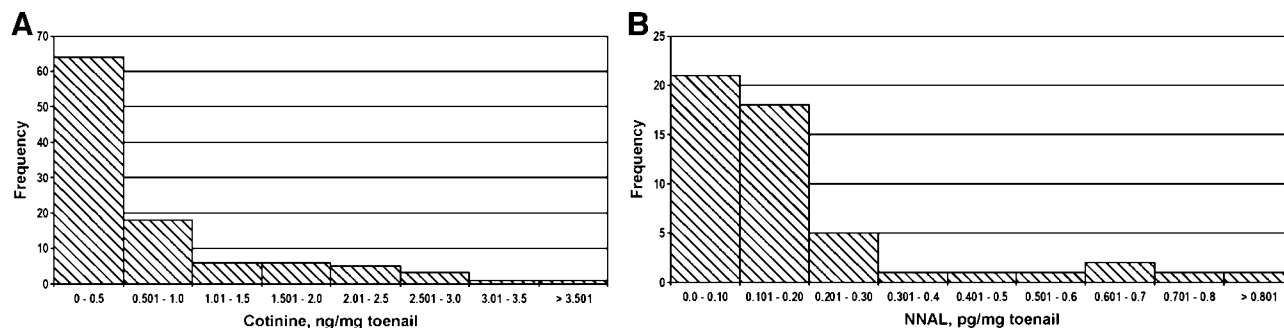
There were 105 participants in the study. Their demographic characteristics and smoking and biomarker data are summarized in Table 1.

A total of 104 participants provided age information and 103 gender and race information. The mean age was 38.9 years (SD, 11.7 years); 55 (53.4%) were male and 51 (49.5%) were White. Data for number of cigarettes smoked per day (CPD) were available for 104 participants and ranged from 5 to 50 with a mean of 18 (SD, 8.4).

Data on plasma nicotine, cotinine, and *trans*-3'-hydroxycotinine were available for 78 subjects; data on urinary total NNAL were available for 71 subjects. Toenail samples from all

**Table 1. Characteristics of the study participants and biomarker levels in their toenails, plasma, and urine**

Characteristic/biomarker	<i>n</i>	Frequency	Mean (SD)	Range
Age (y)	104	—	38.9 (11.7)	19-68
Gender (M/F)	103	55:48	—	—
Race (White/African-American)	103	51:52	—	—
CPD	104	—	18 (8.4)	5-50
Toenail nicotine (ng/mg)	105	—	5.44 (6.41)	0.01-33.18
Toenail cotinine (ng/mg)	105	—	0.67 (0.79)	0.01-3.87
Toenail NNAL (pg/mg)	51	—	0.18 (0.22)	0.01-1.10
Plasma nicotine (ng/mL)	78	—	9.56 (7.74)	0.00-35.40
Plasma cotinine (ng/mL)	78	—	198 (134)	0-580
Plasma <i>trans</i> -3'-hydroxycotinine (ng/mL)	78	—	69.5 (53.1)	0-251.8
Urine NNAL (ng/mL)	71	—	0.24 (0.26)	0.01-1.37



**Figure 2.** Frequency distribution of cotinine and NNAL in toenails of smokers. **A.** Toenail cotinine distribution in 105 smokers. **B.** Toenail NNAL distribution in 51 smokers.

105 subjects were analyzed for nicotine and cotinine. Because of the limited size of a toenail sample, only 51 samples were analyzed for NNAL. Frequency histograms of toenail NNAL and toenail cotinine are shown in Fig. 2. The overall mean toenail biomarker levels were 5.44 ng nicotine/mg toenail (SD, 6.41), 0.67 ng cotinine/mg toenail (SD, 0.79), and 0.18 pg NNAL/mg toenail (SD, 0.22; Table 1). Mean levels of the other biomarkers were as follows: 9.56 ng nicotine/mL plasma (SD, 7.74), 198 ng cotinine/mL plasma (SD, 134), 69.5 ng *trans*-3'-hydroxycotinine/mL plasma (SD, 53.1), and 0.24 ng total NNAL/mL urine (SD, 0.26).

Toenail NNAL correlated with toenail nicotine ( $r = 0.68$ ;  $P < 0.001$ ) and toenail cotinine ( $r = 0.69$ ;  $P < 0.001$ ). There were weak but significant correlations of CPD with toenail nicotine ( $r = 0.24$ ;  $P = 0.015$ ) and cotinine ( $r = 0.26$ ;  $P = 0.009$ ) but not NNAL ( $r = 0.25$ ;  $P = 0.076$ ).

Relationships among the biomarkers are summarized in Table 2. Toenail nicotine and cotinine correlated with all other biomarkers. Toenail NNAL correlated with plasma cotinine and urinary total NNAL but not plasma nicotine or *trans*-3'-hydroxycotinine. Based on the regression analyses, the inclusion of demographic factors and reported exposure did not significantly alter the correlation between biomarkers.

Toenail biomarkers were standardized by CPD to investigate the effects of age and gender. There were no significant

relationships. Toenail NNAL/CPD was higher in White than in African-American participants ( $P = 0.019$ ), but there was no relationship of race to toenail nicotine/CPD or cotinine/CPD (Table 3). Controlling for age and gender did not change the effect of race on the biomarkers.

## Discussion

Measurement of nicotine, cotinine, and NNAL in human toenails is a promising new tool for investigating the role of chronic tobacco smoke exposure in human cancer. The purpose of our study was to provide validation data for these recently developed biomarkers. We investigated the relationship of toenail nicotine, cotinine, and NNAL levels to CPD as reported by the study participants. We also studied the relationship between toenail biomarkers and plasma concentrations of nicotine, cotinine, *trans*-3'-hydroxycotinine, and urinary total NNAL, as well as the effects of age, gender, and race on toenail biomarker levels.

The overall mean values and the ranges of toenail biomarker levels observed here are in good agreement with those obtained in our previous study (27). The correlation of toenail NNAL with toenail cotinine observed in this study ( $r = 0.69$ ) was also similar to that reported previously ( $r = 0.77$ ; ref. 27).

**Table 2. Relationships among biomarkers analyzed in this study**

	Toenail nicotine	Toenail cotinine	Toenail NNAL	Plasma nicotine	Plasma cotinine	Plasma 3HC
<b>Toenail cotinine</b>						
Pearson correlation coefficient	0.43					
<i>P</i> value	<0.001					
No. samples	105					
<b>Toenail NNAL</b>						
Pearson correlation coefficient	0.68	0.69				
<i>P</i> value	<0.001	<0.001				
No. samples	51	51				
<b>Plasma nicotine</b>						
Pearson correlation coefficient	0.39	0.26	0.22			
<i>P</i> value	<0.001	0.021	0.252			
No. samples	78	78	29			
<b>Plasma cotinine</b>						
Pearson correlation coefficient	0.54	0.45	0.37	0.86		
<i>P</i> value	<0.001	<0.001	0.050	<0.001		
No. samples	78	78	29	78		
<b>Plasma 3HC</b>						
Pearson correlation coefficient	0.39	0.30	0.24	0.55	0.67	
<i>P</i> value	<0.001	0.008	0.220	<0.001	<0.001	
No. samples	78	78	29	78	78	
<b>Urine NNAL</b>						
Pearson correlation coefficient	0.42	0.43	0.53	0.35	0.53	0.40
<i>P</i> value	<0.001	<0.001	0.005	0.003	<0.001	<0.001
No. samples	71	71	27	70	70	70

Abbreviation: 3HC, *trans*-3'-hydroxycotinine.

The major potential use of toenail biomarkers is to estimate cumulative carcinogen dose from chronic exposure to tobacco smoke. CPD was found to positively correlate with toenail nicotine ( $r = 0.24$ ) and cotinine ( $r = 0.26$ ); however, these correlations were lower than that reported by Al-Delaimy et al. (34) for CPD and toenail nicotine ( $r = 0.85$ ). Several other studies that compared self-reported CPD with nicotine levels in another keratinic matrix, hair, did not produce consistent results. Some of them reported significant associations, whereas others found no association between CPD and hair nicotine (reviewed in ref. 35). In our study, correlation of CPD with toenail NNAL was not statistically significant, unlike the reported significant correlation between cigarette consumption and urinary NNAL ( $r = 0.48$ ;  $P < 0.0001$ ; ref. 36); however, the latter study had a much larger sample size. Considerable individual variability in toenail biomarker levels was observed at all levels of smoking, similar to that observed for urinary biomarkers (36). The CPD data were based on consumption at the time of blood and urine sampling, whereas the toenail levels reflect exposure over several months before the time of sampling. Possible lack of accuracy in self-reported CPD, the relatively small number of samples analyzed for NNAL, and the small number of low-level and high-level smokers recruited for this study limit our ability to make definitive conclusions about the relationship between CPD and toenail biomarkers.

Plasma nicotine and cotinine, and urinary total NNAL are well established biomarkers of nicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone uptake in smokers, smokeless tobacco users, and nonsmokers exposed to environmental tobacco smoke. Our results show significant positive correlations of toenail nicotine or cotinine with plasma nicotine, cotinine, and *trans*-3'-hydroxycotinine and of toenail NNAL with plasma cotinine and urinary total NNAL (Table 2). These findings indicate that toenail nicotine, cotinine, and NNAL promise to be valid and reliable biomarkers of human chronic exposure to nicotine and NNAL.

Age, gender, race, differences in smoking behavior, cigarette preferences, and metabolic processes may also influence toenail biomarker levels in smokers. Because in general male smokers smoke more CPD than do female smokers (37, 38) and Whites smoke more CPD than do Blacks (38, 39), we normalized toenail biomarkers by CPD before comparing them by age, gender, and race. There was no significant effect of age or gender on toenail biomarker levels, although some studies reported that male smokers have higher cotinine concentrations than female smokers (40). In addition, in our study, there was no relationship of race to toenail cotinine, although toenail NNAL was higher in White than in African-American participants (Table 3). These results disagree with earlier published data that show that Whites have lower plasma cotinine concentrations (41) and urinary cotinine and NNAL levels (42) than do Black smokers. Small sample size in this study might be responsible for this outcome.

There are several limitations to this study. As mentioned previously, toenail biomarker levels reflect exposure over several months, whereas the number of CPD and plasma and urine biomarker levels reflect exposure at one point in time. Smokers with low and high CPD were underrepresented;

therefore, we could not make definitive conclusions about the relationship between the level of exposure and toenail biomarkers. In addition, only about half of all toenail samples were analyzed for NNAL, which reduced the statistical power of our analysis. Another disadvantage consists in single determinations of plasma and urinary biomarkers because significant intraindividual differences in urinary biomarkers were observed in some studies (43), suggesting that a single determination of biomarker level may not be optimal.

In summary, we report here the first study that provides essential validation data for the use of toenail nicotine, cotinine, and NNAL as biomarkers in investigations of the role of chronic tobacco smoke exposure in human cancer.

## Acknowledgments

We thank Dr. Delia Dempsey for medical oversight, Sandra Tinetti for assistance in the clinical study, Duan Minjang and Lita Ramos for analyses of plasma and urine samples, Rachel Feuer and Brad Lieberman for technical assistance, Dr. Faith Allen for data management, and Bob Carlson for editorial assistance.

## References

- IARC. Tobacco smoke and involuntary smoking. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon (France): IARC; 2004.
- World Health Organization. Tobacco or health: a global status report. Geneva: WHO; 1997. pp. 10–48.
- Peto R, Lopez AD, Boreham J, et al. Mortality from smoking worldwide. *Br Med Bull* 1996;52:12–21.
- Benowitz NL, Jacob P, Fong I, Gupta S. Nicotine metabolic profile in man: comparison of cigarette smoking and transdermal nicotine. *J Pharmacol Exp Ther* 1994;268:296–303.
- Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev* 1996;18:188–204.
- Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev* 2005;57:79–115.
- DeLeon J, Diaz FJ, Rogers T, et al. Total cotinine in plasma: a stable biomarker for exposure to tobacco smoke. *J Clin Psychopharmacol* 2002;22:496–501.
- Jarvis MJ, Primatesta P, Erens B, et al. Measuring nicotine intake in population surveys: comparability of saliva cotinine and plasma cotinine estimates. *Nicotine Tob Res* 2003;5:349–55.
- Heinrich J, Hoelscher B, Seiwert M, et al. Nicotine and cotinine in adults' urine: the German environmental survey 1998. *J Expo Anal Environ Epidemiol* 2005;15:74–80.
- Jacqz-Aigrain E, Zhang D, Maillard G, et al. Maternal smoking during pregnancy and nicotine and cotinine concentrations in maternal and neonatal hair. *Br J Obstet Gynaecol* 2002;109:909–11.
- Chan D, Caprara D, Blanchette P, et al. Recent developments in meconium and hair testing methods from the confirmation of gestational exposures to alcohol and tobacco smoke. *Clin Biochem* 2004;37:429–38.
- Al-Delaimy WK, Crane J, Woodward A. Passive smoking in children: effect of avoidance strategies at home as measured by hair nicotine levels. *Arch Environ Health* 2001;56:117–22.
- Matt GE, Quintana PJE, Liles S, et al. Evaluation of urinary *trans*-3'-hydroxycotinine as a biomarker of children's environmental tobacco smoke exposure. *Biomarkers* 2006;11:507–23.
- St. Charles FK, Krautter GR, Dixon M, Mariner DC. A comparison of nicotine dose estimates in smokers between filter analysis, salivary cotinine, and urinary excretion of nicotine metabolites. *Psychopharmacologia* 2006;189:345–54.
- Kim I, Darwin WD, Huestis MA. Simultaneous determination of nicotine, cotinine, norcotinine, and *trans*-3'-hydroxycotinine in human oral fluid using solid phase extraction and gas chromatography-mass spectrometry. *J Chromatogr B* 2005;814:233–40.
- Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. *Lancet Oncol* 2002;3:461–9.
- Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 1999;91:1194–210.
- Hecht SS. Tobacco carcinogens, their biomarkers, and tobacco-induced cancer. *Nat Rev Cancer* 2003;3:733–44.
- Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis* 2002;23:907–22.
- Carmella SG, Akerkar S, Richie JP, Jr., et al. Intraindividual and interindividual differences in metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers' urine. *Cancer Epidemiol Biomarkers Prev* 1995;4:635–42.
- Anderson KE, Carmella SG, Ming Ye, et al. Metabolites of a tobacco-specific lung carcinogen in nonsmoking women exposed to environmental tobacco smoke in their homes. *J Natl Cancer Inst* 2001;93:378–81.

**Table 3. Comparison of toenail biomarkers by race**

Biomarker	Race	n	Mean (SD)	P
Toenail nicotine	African-American	52	5.77 (7.71)	0.504
	White	51	5.21 (4.92)	
Toenail cotinine	African-American	52	0.79 (0.88)	0.351
	White	51	0.58 (0.67)	
Toenail NNAL	African-American	33	0.14 (0.17)	0.019
	White	17	0.27 (0.28)	

22. Hecht SS, Ming Ye, Carmella SG, et al. Metabolites of a tobacco-specific lung carcinogen in the urine of elementary school-aged children. *Cancer Epidemiol Biomarkers Prev* 2001;10:1109–16.
23. Hecht SS, Carmella SG, Chen M, et al. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. *Cancer Res* 1999; 59:590–96.
24. Hecht SS, Carmella SG, Ye M, et al. Quantitation of metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone after cessation of smokeless tobacco use. *Cancer Res* 2002;62:129–34.
25. Carmella SG, Han S, Villalta PW, et al. Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers' blood. *Cancer Epidemiol Biomarkers Prev* 2005;14:2669–72.
26. Benowitz NL, Jacob P III. Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clin Pharmacol Ther* 1994;56:483–93.
27. Stepanov I, Feuer R, Jensen J, et al. Mass spectrometric quantitation of nicotine, cotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in human toenails. *Cancer Epidemiol Biomarkers Prev* 2006;15:2378–83.
28. Palmeri A, Pichini S, Pacifici R, et al. Drugs in nails. Physiology, pharmacokinetics, and forensic toxicology. *Clin Pharmacokinet* 2000;38:95–110.
29. Hecht SS, Lin D, Castonguay A. Effects of  $\alpha$ -deuterium substitution on the mutagenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). *Carcinogenesis* 1983;4:305–10.
30. Hecht SS, Young R, Chen CB. Metabolism in the F344 rat of 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen. *Cancer Res* 1980;40:4144–50.
31. Dempsey D, Tutka P, Jacob P III, et al. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin Pharmacol Ther* 2004;76: 64–72.
32. Jacob P III, Wilson M, Benowitz NL. Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. *J Chromatogr* 1981;222:61–70.
33. Benowitz NL, Jacob P III, Bernert JT, et al. Carcinogen exposure during short-term switching from regular to "light" cigarettes. *Cancer Epidemiol Biomarkers Prev* 2005;14:1376–83.
34. Al-Delaimy WK, Mahoney GN, Speizer FE, et al. Toenail nicotine levels as a biomarker of tobacco smoke exposure. *Cancer Epidemiol Biomarkers Prev* 2002;11:1400–04.
35. Al-Delaimy WK. Hair as biomarker for exposure to tobacco smoke. *Tob Control* 2002;11:176–82.
36. Joseph AM, Hecht SS, Murphy SE, et al. Relationships between cigarette consumption and biomarkers of tobacco toxin exposure. *Cancer Epidemiol Biomarkers Prev* 2005;14:2963–68.
37. Thun MJ, Heath CW. Changes in mortality from smoking in two American Cancer Society prospective studies since 1959. *Prev Med* 1997; 26:422–6.
38. Kandel DB, Chen K. Extent of smoking and nicotine dependence in the United States: 1991–1993. *Nicotine Tob Res* 2000;2:263–74.
39. Murray RP, Connett JE, Buist AS, et al. Experience of Black participants in the Lung Health Study smoking cessation intervention program. *Nicotine Tob Res* 2001;3:375–82.
40. Etter JF, Duc TV, Perneger TV. Saliva cotinine levels in smokers and nonsmokers. *Am J Epidemiol* 2000;151:251–8.
41. Caraballo RS, Giovino GA, Pechacek TF, et al. Racial and ethnic differences in serum cotinine levels of cigarette smokers: Third National Health and Nutrition Examination Survey, 1988–1991. *JAMA* 1998;280: 135–9.
42. Muscat JE, Djordjevic MV, Colosimo S, et al. Racial differences in exposure and glucuronidation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). *Cancer* 2005;103:1420–6.
43. Murphy SE, Link KA, Jensen J, et al. A comparison of urinary biomarkers of tobacco and carcinogen exposure in smokers. *Cancer Epidemiol Biomarkers Prev* 2004;13:1617–23.

## Relationship of Human Toenail Nicotine, Cotinine, and 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanol to Levels of These Biomarkers in Plasma and Urine

Irina Stepanov, Stephen S. Hecht, Bruce Lindgren, et al.

*Cancer Epidemiol Biomarkers Prev* 2007;16:1382-1386.

**Updated version** Access the most recent version of this article at:  
<http://cebp.aacrjournals.org/content/16/7/1382>

**Cited articles** This article cites 41 articles, 14 of which you can access for free at:  
<http://cebp.aacrjournals.org/content/16/7/1382.full#ref-list-1>

**Citing articles** This article has been cited by 6 HighWire-hosted articles. Access the articles at:  
<http://cebp.aacrjournals.org/content/16/7/1382.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://cebp.aacrjournals.org/content/16/7/1382>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.