Time Course of Nicotine and Cotinine Incorporation into Samples of Nonsmokers’ Beard Hair Following a Single Dose of Nicotine Polacrilex

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Abstract

Hair nicotine and cotinine have been proposed as longer-term markers of exposure to secondhand smoke. In this study, we evaluated the rate and extent of nicotine and cotinine deposition into beard hair among six male nonsmokers following a single exposure to 4 mg of nicotine in Nicorette® (nicotine polacrilex) gum. We collected beard hair samples daily for 12 days following exposure and urine samples for 6 days after exposure. Using liquid chromatographic–tandem mass spectrometric analysis, we found that both nicotine and cotinine could be detected in beard samples within 24 h of the exposure and reached a maximum of about 71 pg nicotine and 47 pg cotinine/mg hair, respectively, within 1–2 days, followed by a gradual decline. Compared to beard hair concentrations, nicotine, cotinine, and hydroxycotinine were excreted in urine at much higher levels and also peaked on the day after exposure (mean ± SD urine cotinine = 300 ± 183 ng/mL). Our results confirmed that both nicotine and cotinine can be measured in beard hair samples following a single dose of nicotine. However, both the time-course and extent of deposition of these analytes in beard hair in this study differed from the results reported previously from a similar evaluation.

Introduction

Secondhand smoke (SHS) is a mixture of sidestream smoke emitted from the smoldering tip of a cigarette between puffs and mainstream smoke that has been exhaled by the smoker. This mixture may then undergo further dilution and aging, forming a complex and protein substance containing more than 4000 chemicals including many important carcinogens and other toxins. Nonsmokers may be exposed to SHS at home, in the workplace or in various public places such as bars, restaurants, and other gathering areas. Significant SHS exposure is associated with an increased risk for many illnesses including asthma, lower respiratory tract infections, lung cancer, and heart disease (1,2). As noted in a recent Surgeon General’s Report (1), no level of exposure to SHS is without risk.

Nonsmokers may differ in their awareness of exposure to SHS, and as a result, using specific biomarkers of exposure can be important for objectively monitoring the extent and type of exposures that might occur. Currently, there is general agreement that the measurement of cotinine, the primary proximate metabolite of nicotine, in various matrices including serum, urine, and saliva represents a sensitive, specific, and reliable marker of exposure to SHS, and it has become the preferred biomarker for this purpose (1,3–5). Because the elimination half-life of cotinine is significantly longer than nicotine providing a longer effective window of detection, cotinine is normally measured rather than nicotine itself. However, the half-life of cotinine, although longer than that of nicotine, averages only about 16–18 h in most cases; thus, cotinine measurements in serum or urine are able to detect only recent exposures, over the past few days at most.

Identification of a marker that could represent longer-term exposures and reliably integrate such exposures (e.g., over a period of weeks or even months, rather than just over the previous few days) is of interest. One possibility that has been suggested is the use of hair as the sample. Approximately 25 years ago, Ishiyama et al. (6) first proposed using scalp hair for nicotine analyses, and this matrix has been evaluated extensively since then. The essential concept is that circulating nicotine (and its metabolite cotinine) is taken up by the hair root and deposited into the growing hair shaft over time. Once nicotine is deposited into the hair shaft it presumably becomes fixed and remains stable thereafter. Because scalp hair report-
edly grows about 1 cm/month on average (7,8), the analysis of the proximal 3-cm segment of hair should represent, in theory, the aggregated exposure of an individual to SHS over the past three months. If so, this would represent integrated exposure information that covers a much longer time period than what is accessible through cotinine measurements in other matrices such as blood.

There is good evidence that scalp hair nicotine and cotinine measurements can be used to detect both active tobacco use and SHS exposure among non-smokers. In hair, unlike other matrices such as serum or urine, nicotine is the analyte at higher concentration rather than cotinine, and most studies have reported hair nicotine measurements, although cotinine is also measurable and has been used for some studies, particularly those based on immunoassays. Many investigators during the past several years have measured scalp hair nicotine or cotinine, and sometimes both, and they have consistently shown higher concentrations in smokers than in non-smokers, as well as higher concentrations in non-smokers with known significant exposure to SHS than in non-smokers with no known exposure (9–15). Furthermore, several studies have found similar SHS exposure estimates when hair nicotine or cotinine analyses were compared with cotinine assays in urine (12,16,17).

Nicotine has generally been found to be the predominant analyte in scalp hair samples, but in a prior evaluation of the course for nicotine and cotinine incorporation into beard hair following a dose of nicotine polacrilex gum, only cotinine could be detected; no nicotine incorporation was observed (18). Prior tobacco exposure studies have consistently demonstrated the presence and indeed the predominance of nicotine compared to cotinine in hair. Thus, unless the deposition of nicotine into beard hair is fundamentally different than that observed with other types of hair, the findings by Gwent et al. (18) seem to be in conflict with the systemic deposition of nicotine into human hair. Consequently, in this study we have re-investigated the time course for nicotine and cotinine incorporation into beard hair following a single nicotine polacrilex dose in non-smoker volunteers, to better understand the nature and reliability of hair nicotine and cotinine assays as an indicator of SHS exposure.

Materials and Methods

Standards and reagents

Native (i.e., unlabeled) nicotine and cotinine were purchased from Sigma-Aldrich (St. Louis, MO). We obtained trans-3'-hydroxycotinine, norcotinine, nicotine-N-oxide, cotinine-N-oxide, and norcotinine from Toronto Research Chemicals (Toronto, ON, Canada). We purchased N-methyl-d9-nicotine and N-methyl-d3-cotinine, which were used as internal standards, from either Cambridge Isotopes Laboratories (Andover, MA), or from Toronto Research Chemicals; N-methyl-d9-trans-3'-hydroxycotinine and the remaining labeled analytes were also from Toronto Research Chemicals. The purity of standards was nominally about 98% in each case. We obtained ammonium acetate (99.999%) from Sigma-Aldrich; potassium hydroxide (85–90% reagent) from Fisher Scientific (Norcross, GA); and 0.25 mM methanolic HCl from Supelco (Bellefonte, PA). We purchased high-purity water from Telia (Fairfield, OH) and the remaining solvents from Burdick and Jackson Labs (distributed by Baxter, Stone Mountain, GA). All solvents and gases were of the highest purity available.

Hair analysis

We analyzed beard hair samples by a liquid chromatography-atmospheric pressure ionization-tandem mass spectrometry (LC–API-MS–MS) method. Briefly, each sample was collected from the electric shaver and weighed. Typically, we took a 25-mg sample for analysis; to this sample we added the labeled nicotine and cotinine internal standards (5 ng nicotine and 1.25 ng cotinine) in 100 μL of water in a glass vial, followed by 900 μL of 5 N KOH. We capped and rotated the vials overnight at room temperature (23°C) to digest the hair, after which the contents were transferred to precleaned 13 × 100-mm glass tubes and 2 mL methylene chloride was added. The tubes were capped and rotated for 5 min at 60 rpm and then centrifuged at 3000 × g for 5 min to achieve phase separation. We transferred the methylene chloride to a clean tube, added 1 mL of 0.1 N HCl, and mixed the contents again for 5 min. After recovering the top (aqueous) layer, 20 μL of 10 N NaOH was added and mixed, and the samples were placed on pre-washed Chem Elute columns (Varian, Harbor City, CA) that we eluted three times with 2-mL aliquots of methylene chloride. After adding 0.5 mL of 0.25 mM methanolic HCl as a keeper, we concentrated the combined eluants on a Savant AES 2010 vacuum evaporator without heating. The residue was recovered in 20 μL of water for analysis.

We analyzed samples on a Shimadzu LC-10AD high-performance liquid chromatograph (HPLC) interfaced to an Applied Biosystems API 4000 triple-quadrupole MS, using the heated nebulizer inlet. Separations involved gradient elution of the two analytes on a Varian Pursuit XRs 3 μm C-18 150 × 2.0-mm column which was eluted at a flow rate of 0.4 mL/min. The initial mobile phase was 15% solvent B (acetonitrile) in solvent A (0.77 g/L ammonium acetate at pH 9.2), and this was increased to 100% acetonitrile over 8 min. Nicotine and cotinine eluted in this system at about 6.5 and 2.9 min, respectively. Dwell times were set to 250 ms and collision energies (28 to 35 V) were optimized for each transition. Transition ions of m/z 163.1/117.0 and 163.1/130.0 were used for nicotine; cotinine transitions were monitored at m/z 177.0/80.0 and 177.0/98.0. In both cases, the initial ion was used for primary quantitation. The internal standard transitions for nicotine and cotinine were m/z 166.1/130.1 and 180.0/80.0, respectively. Concentrations were determined by evaluating the recorded area ratios in comparison with a standard curve which was included with each analytical run. Thirteen calibration standards were included in each series ranging from 0 to 12 ng/ng internal standard (nicotine) and from 0 to 8 ng/ng internal standard (cotinine). Calculations were made online with Analyst vers. 1.4.2 by using weighted (1/x) regression. A water blank and aliquots of control hair samples were included in each analytical run for
quality control purposes, and all results are from runs confirmed to be in statistical control. The estimated detection limits for nicotine and cotinine were 22.5 and 1.6 pg/mg hair, respectively, based on a 25-mg hair sample. Detection limits were estimated as the mean plus 3 times the SD of the blank observed over all runs for each analyte. Several quality control “pools” of hair samples were used with nicotine concentrations ranging from 114 pg to 5.4 ng/mg hair, and cotinine concentrations from 3 to 350 pg/mg hair. Long-term precision ranged from CVs of 8 to 28.7%, depending on concentrations. Accuracy estimates with fortified samples ranged from 92 to 99%.

Urine analysis
Nicotine, cotinine, trans-3′-hydroxycotinine, and four minor nicotine metabolites (nornicotine, nicotine-N-oxide, cotinine-N-oxide, and norcotinine) were also analyzed by LC–API-MS–MS using a modification of a method previously described (19). Briefly, the urine aliquot (typically 500 μL) was fortified with an aliquot of the seven internal standards in acidified water, the pH was adjusted to 5.1, and the sample was hydrolyzed with β-glucuronidase in an overnight incubation at 37°C. The samples were then processed on prewashed Chem Elute columns eluted with methylene chloride, and with methylene chloride/ethanol mixtures. The extracts were concentrated and recovered in 20 μL of water for analysis.

Samples were analyzed on a Shimadzu HPLC system interfaced to an Applied Biosystems API 4000 tandem MS by using the TurboIonSpray interface. A Phenomenex Gemini-NX C18 column at 40°C was used for the separations, and analytes were eluted with a gradient of 5 to 30% acetonitrile in 6.5 mM ammonium acetate, pH 10.5. The appropriate transition ions for all analytes and their internal standards were monitored using multiple reaction monitoring. Two transition ions were monitored for each native analyte, and one for each internal standard. Dwell times were set to 100–150 ms, and the ion potentials and collision energies were optimized for each transition. The system was calibrated with a 14-point standards mixture analyzed with each group of samples. The data were integrated online using Scienc Analyst software with weighted (1/x) regression relative to standards included with each run. Further data processing was completed using SAS 9.1. Limits of detection were monitored as described and ranged from 0.10 ng/mL (hydroxycotinine) to 1.28 ng/mL (nicotine-N-oxide). Precision for each analyte was monitored at three concentration levels from 10 to 100 ng/mL and was in the range of 2–6% in all cases except for nicotine-N-oxide in the lowest concentration sample, which had a CV of 10.2%. Estimated accuracies were all within ±10% except for nornicotine with an estimated bias of –11.3%. A blank and quality control urine samples were included with each series of analyses, and all reported results were from runs found to be in statistical control for that analyte.

Study participants
Eight male subjects ages 23–64 years initially volunteered to participate in this study. The nonsmoker status of each subject was confirmed before enrollment in the study from an initial salivary cotinine assay. During the course of the study, two participants were found to have relatively light beard growth and could not generate a sufficient weight of whiskers each day as required for this work. Therefore, only six subjects were included in the final study. One participant was Hispanic, and the remaining five were white. To facilitate the required daily interaction with each subject, and to help assure compliance with the protocol, we recruited participants locally, primarily from the Centers for Disease Control and Prevention. All participants were healthy nonsmokers with no known exposure to SHS in their daily lives. Each participant reviewed the protocol and signed an informed consent form before entering the study, which was approved by an Institutional Review Board at the Centers for Disease Control and Prevention. Subjects received nominal monetary compensation for their time and inconvenience in participating in this study.

Dosing protocol
Participants were scheduled individually. On the initial morning, they shaved using the new shaver provided (Philips Norelco rechargeable 7310XL cordless razor), and an initial urine sample was collected. The whiskers were recovered from the shaver by tapping the opened razor over weighing paper followed by use of a disposable brush. The whiskers and urine sample were then analyzed to provide the initial (pre-exposure) day 1 values. Each participant then chewed one piece of Nicorette (nicotine polacrilex) gum containing 4 mg of nicotine, following the manufacturer’s directions (20). This involved slowly chewing the gum several times until a slight tingling was detected, “parking” the gum between the cheek and gum for 1 min, and then repeating the process until no further tingling or peppery taste was noted. The entire process typically involved chewing the gum for about 30 min. Participants were asked not to eat or drink for at least 30 min before exposure to the gum. No post-exposure restrictions were imposed other than strict avoidance of any secondhand smoke to the extent possible during the three-week period of sample collections.

Each subject shaved again the following day with a pre-cleaned razor, and another morning urine sample was collected. New razors were provided for each participant, and razors were cleaned after each use after collecting the whiskers by the use of compressed air. In addition, two participants collected 24-h urine samples for the first day following dosing. In those two cases, each void was retained over the initial 24-h period. However, the initial exposure for those two subjects at day 2 was based only on the sample obtained as the first morning void on the second day. Urine samples were collected for a total of 6 days in all cases, and beard hair samples were recovered for 12 consecutive days. All urine samples were stored on ice and transported into the laboratory each day. Subjects took nicorette with the match of air on weekends and collected samples as usual on Saturday and Sunday. They returned the shavers to the laboratory the following Monday, and the whiskers were recovered for analysis. The final urine sample was typically collected on a Saturday and stored frozen until it could be brought into the laboratory the following Monday.
A final sample of scalp hair was collected from all but one participant at 21 days after exposure. One subject had insufficient scalp hair for this purpose. This sample was collected by using precleaned, stainless-steel scissors to cut a sample of hair as closely as possible to the scalp from the posterior vertex region at the back of the head. This sample was then processed in the same manner as described for beard hair analysis.

Results

Results for nicotine and cotinine measured in beard hair samples obtained each day, and for cotinine in urine samples, are summarized in Table I. We required at least 15 mg, and preferably 25 mg of hair for each analysis to maintain adequate sensitivity. The mean daily weight of beard hair collected from the six participants was 65.7 mg (SD = 13.5; n = 70). There was substantial variability among the individual subjects as indicated by the standard deviations listed in Table I. Figure 1 shows the mean concentrations for both nicotine and cotinine in beard hair samples during the 12-day period after dosing with nicotine, and Figure 2 shows the daily response in urine for cotinine, nicotine, and 3'-hydroxycotinine.

Twenty-four-hour urine samples were collected from two individuals following dosing with Nicorette gum to provide a more detailed urinary exposure pattern. We analyzed aliquots of these samples for nicotine and six of its metabolites. Table II summarizes the recovery of nicotine and its metabolites during the 24-h period after dosing. These analytes, expressed in terms of nicotine equivalents (i.e., normalized to the formula weight of nicotine), accounted for about one-third of the expected dose of absorbed nicotine within the initial 24-h period assuming approximately 2.5 mg of systemically available nicotine from this gum, as reported by Choi et al. (21).

In these analyses, we did not wash the whiskers before digestion and analysis. However, following assays of the individual samples, we pooled some of the remaining hair from several early (day 2 and 3) samples and reanalyzed aliquots both before and after washing. This was accomplished by placing the aliquot on filter paper (Whatman #1) and washing three times with methylene chloride. Nicotine and cotinine in the pooled, unwashed aliquots averaged 64.4 and 41.3 pg/mg, respectively. The mean concentrations after washing (63.4 and 44.3 pg/mg, respectively) were essentially identical, consistent with the absence of external contamination in these samples, although the low concentrations observed throughout this study made detection of small differences difficult.

### Table I. Mean Analyte Concentrations by Day*

<table>
<thead>
<tr>
<th>Day</th>
<th>Beard Nicotine Mean (SD) (pg/mg)</th>
<th>Beard Cotinine Mean (SD) (pg/mg)</th>
<th>Urine Cotinine Mean (SD) (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1‡</td>
<td>10.3 (10.3)</td>
<td>1.6 (2.0)</td>
<td>2.8 (5.8)</td>
</tr>
<tr>
<td>2</td>
<td>70.8 (33.1)</td>
<td>47.4 (34.0)</td>
<td>299.6 (182.8)</td>
</tr>
<tr>
<td>3</td>
<td>53.7 (16.4)</td>
<td>38.1 (13.8)</td>
<td>130.6 (73.5)</td>
</tr>
<tr>
<td>4</td>
<td>50.6 (31.3)</td>
<td>20.7 (7.8)</td>
<td>53.0 (22.1)</td>
</tr>
<tr>
<td>5</td>
<td>27.7 (14.3)</td>
<td>9.6 (4.2)</td>
<td>26.9 (17.7)</td>
</tr>
<tr>
<td>6</td>
<td>20.0 (5.6)</td>
<td>6.1 (2.4)</td>
<td>6.8 (5.1)</td>
</tr>
<tr>
<td>7</td>
<td>20.9 (25.5)</td>
<td>4.4 (2.1)</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>11.8 (12.9)</td>
<td>2.7 (1.7)</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>14.0 (13.6)</td>
<td>2.4 (1.4)</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>6.9 (10.2)</td>
<td>1.5 (1.2)</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>1.4 (49.6)</td>
<td>1.5 (0.7)</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>9.1 (8.4)</td>
<td>1.3 (0.8)</td>
<td>–</td>
</tr>
</tbody>
</table>

*All beard hair values are the mean from six individuals except for days 7 and 8, when each had one missing value. The urine values are from the same six individuals with no missing values.

‡ Day 1 is the pre-exposure value measured immediately before dosing with the Nicorette gum.
We also collected hair samples from the posterior vertex of the scalp from 5 subjects 21 days after their exposure. The samples were cut adjacent to the scalp and divided into two (proximal and distal) segments of 1.5 cm each. Each segment would be expected to correspond to about six weeks of hair growth. The single exposure to nicotine occurring three weeks earlier would be expected to be measurable only in the proximal hair segment, but we were unable to detect any significant difference in either nicotine or cotinine concentrations between the two segments in the participants in this study. All results were consistent with only background levels of exposure over time, suggesting that the single 4-mg nicotine exposure on day 1 did not provide a large enough dose to be detected in a time-integrated hair sample in this manner.

Discussion

The results of this study indicate that systemic nicotine, arising from a single oral dose, leads to the deposition of both nicotine and cotinine in beard hair samples beginning within 24 h of dosing. Most deposition of both nicotine and cotinine occurred soon after exposure, and the concentration of nicotine in beard hair was consistently greater than that of cotinine at all time points. The concentration of nicotine per mg of hair was approximately 1.5 times that of cotinine during the first 2 days after exposure, and the nicotine/cotinine ratio further increased continuously thereafter. During the 12 days of collection we found that approximately 55% of the nicotine and 78% of the cotinine recovered overall in beard hair was present in samples collected in the first 3 days after exposure. As expected, the concentrations of nicotine and cotinine (and hydroxycotinine) were far greater in urine than in the hair samples, with cotinine concentrations averaging almost 300 ng/mL at the peak on day 2. As is typically found in matrices other than hair, cotinine in urine was consistently higher in concentration than nicotine throughout the six-day collection period.

The peak concentration of both nicotine and cotinine in beard hair samples occurred the day after dosing. After day 3, cotinine deposition declined rapidly and in a smooth manner, whereas the decline in nicotine was neither as rapid nor as consistent. This more extended deposition of nicotine following the single oral dose may reflect the dispersion of nicotine into tissues from which it may be gradually released over time. The relatively large peak for mean nicotine occurring at day 11 was mainly the result of one unusually high sample; if that sample had been excluded, the mean concentration for nicotine on day 11 would have been approximately 11.6 pg/mg hair. The relatively high concentration in that person’s sample was confirmed with reanalysis, although no explanation for the anomalous result was obtained. One likely possibility could be the external contamination of that sample either during collection or initial processing.

Dosing with Nicorette gum should have assured that only systemic exposure to nicotine would occur; however, we cannot exclude the role of sweat and/or sebum in depositing nicotine (and cotinine) on the exterior of the emerging hair samples. Typically, the immediate deposition of analyte in hair samples collected within 24–48 h after dosing has been associated with hair contamination by sweat (22,23), and we found very little delay in deposition in this study; with the mean concentrations of both nicotine and cotinine reaching a peak within 1–2 days after dosing. Thus, it is possible that deposition of nicotine and cotinine from sweat onto the growing hair shaft was a contributing mechanism. However, washing the collected beard hair samples did not lead to a detectable decrease in either nicotine or cotinine as would be expected if external contamination with sweat was a major mechanism for exposure.

Another possible source of interference could be the contamination of beard samples with skin shavings collected with the whiskers. The collection protocol we used for whiskers is essentially the same as that used in several previous studies of drug and metabolite incorporation into beard hair. With this method, it is reasonable to expect that some contamination of the whiskers with skin cells could occur, and the analyte content of these cells might also contribute to the amounts measured, which might also contribute to the earlier response pattern we observed. However, washing of the collected hair samples did not decrease the recoveries, which might be expected if more labile skin cells had been major contributors to the measured amounts, and contribution of skin cells is unlikely to explain the response difference.

Table II. Nicotine Metabolites Measured in Two 24-h Urine Samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Subject 1 (ng/mL)</th>
<th>Subject 2 (ng/mL)</th>
<th>Mean (ng/mL)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>84.7</td>
<td>48.1</td>
<td>66.4</td>
<td>13.9</td>
</tr>
<tr>
<td>Cotinine</td>
<td>200.9</td>
<td>136.9</td>
<td>168.9</td>
<td>35.3</td>
</tr>
<tr>
<td>3-Hydroxycotinine</td>
<td>196.0</td>
<td>185.2</td>
<td>190.6</td>
<td>39.8</td>
</tr>
<tr>
<td>Nornicotine</td>
<td>5.4</td>
<td>4.0</td>
<td>4.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Nicotine-N-oxide</td>
<td>31.8</td>
<td>26.3</td>
<td>29.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Cotinine-N-oxide</td>
<td>19.2</td>
<td>13.0</td>
<td>16.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Norcotinine</td>
<td>3.6</td>
<td>3.3</td>
<td>3.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Total nicotine equivalents</td>
<td>541.6</td>
<td>416.8</td>
<td>479.2</td>
<td></td>
</tr>
<tr>
<td>Total volume (mL)</td>
<td>1840</td>
<td>1820</td>
<td>1830</td>
<td></td>
</tr>
</tbody>
</table>

Mean 24-h nicotine equivalents 877 μg
between our results and those of Gwent et al. (18) because both studies apparently used the same approach for sample collection.

Our results concerning the deposition of cotinine into hair differ from the prior report of Gwent et al. (18) in several respects. For example, the maximum concentration of cotinine we measured (ca. 47 pg/mg hair) was far less than the maximum reported by Gwent et al. (> 1.5 ng/mg) following a similar dosage scheme. Furthermore, Gwent et al. found no cotinine (or nicotine) for the first 2 days after dosing, followed by a gradual rise of cotinine in beard hair during the next 3 days to a peak at 5 days post-exposure. By contrast, we found a much faster response with a substantial increase in both nicotine and cotinine within the first 24 h after exposure, and a maximum concentration within 1–2 days after the exposure. Most significantly, Gwent et al. (18) found no nicotine in beard hair at any time after exposure; this finding was an important impetus for conducting the present study because the earlier finding seemed inconsistent with hair analyses in general in which nicotine has been the predominant metabolite. However, in our study we found that nicotine and cotinine deposition followed a similar trend, and nicotine was detectable at all time points after the initial oral exposure. In all cases, nicotine was the predominant analyte in the sample.

Although the individual patterns of deposition varied substantially among the six participants in our study, the mean pattern was that of early deposition as shown in Figure 1. The reasons for the more rapid time course among our subjects and the much lower extent of cotinine incorporation into hair as compared to the results of Gwent et al. (18) are not clear. However, with respect to the absence of hair nicotine in the study of Gwent et al. (18), it is possible that the method they used to extract and recover nicotine and cotinine from digested beard samples may have led to losses of nicotine because of its volatility. Nicotine is much more volatile than cotinine, and taking methylene chloride extracts to dryness under a stream of nitrogen, as used by Gwent et al. (18), will typically result in substantial losses of nicotine unless a suitable keeper is included. Although the assay by Gwent et al. (18) were by gas chromatography–MS, they used biperidine as the sole internal standard rather than isotopically labeled analytes, and consequently the loss of nicotine in the extracts might not have been apparent.

Finally, we could not detect any difference between proximal and distal 1.5 cm hair segments collected from five of the participants (one subject had very short hair and could not be assayed) 21 days after the single exposure. Given the low concentrations of both nicotine and cotinine we measured in beard hair, this failure to detect exposure in the proximal scalp hair segments was likely due to signal dilution in the samples (e.g., from either the limited initial deposition into growing hair shafts, or from possible diffusion of the analyte over time).

However, these results do suggest that the sensitivity of hair analyses to rare exposures, even of a reasonably substantial nature such as this one, may be limited. Overall, our results are in agreement with the many prior findings of the incorporation of both nicotine and cotinine into hair samples following exposure to nicotine, and our results are at least consistent with the concept that systemic nicotine and cotinine are deposited internally within the beard hair shaft during growth. However, the sensitivity of the beard analysis was relatively low, and we could not detect a single, relatively high-exposure event three weeks later by use of standard scalp hair analysis. Further studies are needed to confirm the nature of nicotine (and cotinine) measured in hair following atmospheric exposures to tobacco smoke. In particular, there are notable differences between our findings and the findings of Gwent et al. (18) concerning both the rate and the extent of nicotine and cotinine incorporation into beard hair samples following a single oral dose of nicotine polacrilex. Further work to resolve such differences may require a larger number of participants because of the variable responses that may occur following a single, uniform exposure.

Acknowledgments

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