External Contamination of Hair with Cocaine: Evaluation of External Cocaine Contamination and Development of Performance-Testing Materials*

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Abstract

The National Laboratory Certification Program undertook an evaluation of the dynamics of external contamination of hair with cocaine (COC) while developing performance testing materials for Federal Drug-Free Workplace Programs. This characterization was necessary to develop performance materials that could evaluate the efficacy of hair testing industry's decontamination procedures. Hair locks (blonde to dark brown/black) from five different individuals were contaminated with cocaine HCl. Hair locks were then treated with a synthetic sweat solution and hygienic treatments to model real-life conditions. Hair locks were shampooed daily (Monday through Friday) for 10 weeks, and samples of the hair locks were analyzed for COC, benzoylecgonine (BE), cocaethylene (CE), and norcocaine (NCOC). Three commercial analytical laboratories analyzed samples under three protocols: no decontamination procedure, individual laboratory decontamination, or decontamination by an extended buffer procedure at RTI International. Results indicated substantial and persistent association of all four compounds with all hair types. Hair that was not decontaminated had significantly greater quantities of COC and BE than did hair that was decontaminated. The only hair samples below detection limits for all four compounds were those decontaminated 1 h after contamination. Additionally, BE/COC ratios increased significantly over the 10-week study (regardless of decontamination treatment). From 21 days postcontamination until the end of the study, the mean BE/COC ratio for all hair types exceeded 0.05, the proposed Federal Mandatory Guidelines requirement. The largest variability in results was observed for samples decontaminated by participant laboratories. This suggests that current laboratory decontamination strategies will increase variability of performance testing sample results. None of the decontamination strategies used in the study were effective at removing all contamination, and some of the contaminated hair in this study would have been reported as positive for cocaine use based on the proposed Federal Mandatory Guidelines.

Introduction

In 2000, RTI International was directed by the Division of Workplace Programs, Center for Substance Abuse Prevention (CSAP), Substance Abuse and Mental Health Services Administration (SAMHSA), Department of Health and Human Services (HHS), to conduct a pilot hair performance testing (PT) under the National Laboratory Certification Program (NLCP). The purpose of this pilot was to develop quality assurance testing materials in support of anticipated changes in Federal Drug-Free Workplace testing programs. Initially, participating laboratories were directed to test the PT samples using their complete procedures including their decontamination wash procedures prior to confirmation. The large variability in the reported quantitative results required the program to limit procedural variables. The most obvious contributor to variability, individual laboratory decontamination procedures, was discontinued in favor of a uniform decontamination procedure as part of the manufacturing process. Further testing of samples has found continued variability in results both between and within laboratories, but this can no longer be attributed to differences between decontamination procedures (1). However, the need to assess the effectiveness of decontamination procedures continues to remain a PT objective. In RTI's efforts to refine and develop performance testing materials to assess laboratory performance, we have sought to evaluate the dynamics of external contamination to produce a means to test the efficacy of decontamination procedures.

For the past two decades, researchers and scientists have investigated and employed hair testing for drugs of abuse as a complementary and alternate matrix to blood and urine. Results from hair tests have been used in clinical, forensic, and epidemiological studies; historical research; and courts of law. Testing for drugs in hair has evolved to the point that the identity of the drug found is less of an issue than the explanation of its origin. The risk for environmental contamination alone to produce a positive drug hair test result is not clear. All mechanisms by which drug is incorporated into hair are not fully understood. Drug incorporation into hair can occur...

* This paper was developed (in part) under contract number 277-2003-00044 from the Substance Abuse and Mental Health Services Administration (SAMHSA), U.S. Department of Health and Human Services (HHS). The views, policies, and opinions expressed are those of the authors and do not reflect those of SAMHSA or HHS.

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* RTI International is a trade name of Research Triangle Institute.
substances such as lipids, oils, and cosmetics, as well as exogenous analytes (e.g., drug) possibly coating the hair. Laboratories have developed decontamination procedures to remove potentially interfering content, including drug residues, contaminated surfaces, and vaporized drug (2-5). Each of these mechanisms is affected by the chemical and physiological composition of the hair matrix.

The functional groups (e.g., carboxyl and phenolic groups) of many hair proteins promote cation-exchange activity between the hair proteins and small ionic molecules such as drug (6). Hydrophobic and hydrophilic interactions between hair and drugs have been demonstrated in vivo (7). These interactions result in ionic bonding between hair and the retained drugs (8). Claffey and Ruth (9) and Dehn et al. (10), using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, reported evidence of covalent adduct formation of amphetamine and nicotine/cotinine with melanin intermediates during in vitro melanogenesis. The relative contribution to drug retention in hair by each of these interactions is poorly defined.

The issue of environmental contamination is further confounded by evidence that incorporation rates of drugs vary in hair with different melanin and protein content (5,11). The relative proportion of melanin associated protein can vary as much as 40% (11). Similarly, Chinese black hair has an average melanin content of 3%, and Scandinavian blonde hair contains 0.07% melanin (12). Thus, it is possible that the amount of drug incorporated into hair, either by environmental contamination or ingestion may vary with the protein and melanin content further complicating the development of a clear understanding of a hair test result.

To minimize the impact of environmental contamination issues, laboratories have developed decontamination procedures to remove putative surface contaminants. A variety of decontamination schemes are used to remove potentially interfering substances such as lipids, oils, and cosmetics, as well as exogenous analytes (e.g., drug) possibly coating the hair surface from environmental exposure. Researchers have investigated organic solvents, aqueous buffers, water, soaps, and combinations of these for decontamination wash procedures (13). These procedures can take from minutes to hours to perform. The extent to which hair can be decontaminated depends on factors governing penetration of the drug into the hair matrix such as cosmetic treatment of hair and the chemical and physical properties of the drug analyte (14-16).

Regardless of the decontamination procedure employed, the efficacy of decontamination washes is debatable. Some researchers believe a rapid aqueous or organic solvent wash is sufficient (13). Other investigators have proposed a multi-step decontamination procedure including a “wash kinetic criteria” to properly distinguish passive exposure and active ingestion of a drug by comparing drug in the wash fractions with the drug extracted from the hair matrix (17). Researchers who have demonstrated that drug residue remains associated with the hair even after extensive washing procedures have questioned the efficacy of wash procedures (18,19).

Several groups investigated the efficiency of removing cocaine (COC) from hair after external application. A review of the literature indicates that aggressive washing techniques can remove COC from hair 1 h after it has been applied to the hair either as a solution or as a powder (18,20). However, beyond an hour, one group reported wash procedures were unable to remove all of the COC in the hair up to 10 weeks post-application (18). Cairns et al. (17), utilized a solution of COC in chloroform to contaminate hair samples, arguing this more closely approximated surface contamination of hair. These investigators reported that following several shampoo wash-dry cycles, the external contamination could be differentiated by an extensive wash and application of a mathematical factor similar to that used in two previous publications. The article also stressed differences in the wash procedures utilized by their group and Romano et al. (18) as an explanation for why the results differed between the two groups.

Our laboratory investigated the issue of environmental contamination of hair with COC using a protocol similar to that of Cairns et al. (17) and Romano et al. (18). The hair samples were sent for analysis to three laboratories. Briefly, COC HCl was externally applied to different hair types and the concentrations of COC analytes determined over a 10-week period during which the hair was subjected to regular shampooing.

**Experimental**

**Experimental design**

The design of the experiment was as a three-way, crossed design, with sub-sampling. The factors investigated were time, hair-type, and decontamination protocol. Samples were removed at 14 time points with respect to contamination (pre-contamination, 1 h, 6 h, 27 h, weekly) during a 70-day period (Figure 1). Hair-type consisted of 5 hair locks of various colors and types (Table I). Decontamination protocols comprised three strategies: 1. no decontamination; 2. a decontamination by the analytical laboratory using the laboratory’s standard protocol; and 3. a decontamination protocol at RTI prior to submission to analytical laboratories.

![Figure 1. Schematic representation of the study design indicating major treatment or sampling events.](image-url)
All hair types were contaminated with cocaine, subjected to a treatment with synthetic sweat after 1 h, and shampooed each weekday evening (Monday through Friday) for 10 weeks. Hair was collected before contamination, after contamination but prior to the sweat application, approximately 6 h post-contamination (approximately 4 h after sweat application followed by a drying period), and then weekly for 10 weeks. Weekly samples were collected on Thursday mornings following overnight drying. A schematic of the sampling design is presented in Figure 1. At all points, hair was analyzed and quantitative results were obtained for COC, benzoylecgonine (BE), cocaethylene (CE), and norcocaine (NCOC).

All samples were submitted to analytical laboratories in a randomized blinded fashion with both positive and negative control materials. The three analytical laboratories were solicited by RTI prior to the study. These laboratories were provided the study protocol and provided the opportunity to comment on the study design prior to agreeing to participate. The laboratories conducted all quantitative analyses for the study and were compensated for their analytical work.

All other protocols were performed at RTI facilities, in laboratory space not previously used for handling COC. All laboratory equipment and bench spaces were thoroughly cleaned with alcohol and dried prior to the study, and bench-top blotter paper covers were changed regularly throughout the study period.

Hair selection

Hair used in this study was obtained from hair donations collected by a professional stylist. All hair donations were from young females and had not been subjected to cosmetic treatments such as bleaching, coloring, or permanent wave. Prior to the study, all hair samples were analyzed and determined not to contain detectable concentrations of drugs of abuse including cocaine. Samples were selected to cover a range of colors from blonde to black. The study was designed to provide an estimate of interindividual variation but did not include sufficient samples to determine differences between ethnic groups or hair color with statistical significance. Table I provides descriptions of each of the hair types used including hair color based on the Schwarzkopf scale (21). Melanin content was estimated as described.

Hair samples were also evaluated by scanning electron microscopy to determine if any visibly obvious differences in the cuticle were present. Hair samples were also examined for excessive wear indications such as missing or grossly broken cuticle. Hair samples were prepared as described in Stout et al. (22). All hair locks were judged to be substantially similar in the extent of cuticle damage prior to use in the study.

Twelve grams of each of the five hair types were used in the study. The locks were maintained loose and were stored loose throughout the study on clean laboratory blotter paper covered with clean filter papers. Hair samples were stored at ambient laboratory conditions of approximately 25°C and approximately 50% relative humidity (RH) with fluorescent lighting.

Total melanin measurement

Hair samples were analyzed for melanin content by a method modified from Kronstrand et al. (23). Pulverized hair (10 mg, 5 replicates) from each hair type was heated at 90°C for 16 h in Solvable™ (PerkinElmer, Rigaweg, The Netherlands), a commercially available aqueous solubalizer. Solvable was selected to replace the Soluene-350 used by Kronstrand et al. (23) for laboratory safety reasons. Absorption was then measured at 500 nm using a Gilford Instruments model 260 spectrometer (Oberlin, OH). An eight-point standard curve was constructed using sepia melanin (Sigma Aldrich) concentrations from 5 to 1000 µg/mL. A linear curve was obtained over this range \( (r^2 = 0.9998) \) and this curve was used to calculate the total melanin concentration in the hair samples. Results are presented in Table I. A single-factor ANOVA was used to compare the five groups (Excel 2003, Seattle, WA). Melanin concentrations were significantly different \( (P < 0.0001) \). Hair samples 1 and 2 had significantly less melanin than hair sample 3, which had significantly less than hair samples 4 and 5. Concentrations were consistent with those reported in Scheidweiler et al. (24).

Cocaine contamination

A separate determination of the purity of the COC HCl used in the study was conducted by one of the analytical laboratories. The COC was submitted to the laboratory as a solution in acetonitrile. The COC was determined to have approximately 0.6% CE and approximately 0.1% NCOC.

One 15-mg portion of COC HCl (Malinckrodt, Paris, KY) was weighed out for each of the 5 hair locks. Gloved hands were misted with the synthetic sweat solution described and rubbed together until dry. This reduced static effects from the gloves. The weighed COC was then applied to the gloved hands and rubbed until the COC was no longer visible on the palmer surfaces. At this point, the hair was handled with gloved hands.

<table>
<thead>
<tr>
<th>Hair Color*</th>
<th>Subject Demographics and Texture Description</th>
<th>Mean Total Melanin (µg/mg) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Blonde 9.0</td>
<td>Caucasian female, thin strands</td>
<td>6.6 (5.4)*</td>
</tr>
<tr>
<td>2 Light brown 7.5</td>
<td>Caucasian female, thin strands, easily tangled</td>
<td>7.0 (4.5)*</td>
</tr>
<tr>
<td>3 Brown 6.5</td>
<td>Caucasian female, slight wave, smooth, thick strands</td>
<td>31.1 (6.6)*</td>
</tr>
<tr>
<td>4 Dark brown 5.5</td>
<td>Caucasian female, slight wave, smooth, thick strands</td>
<td>60.7 (10.5)*</td>
</tr>
<tr>
<td>5 Very dark brown 4.0</td>
<td>Asian female, thick, straight, and smooth strands</td>
<td>57.4 (6.2)*</td>
</tr>
</tbody>
</table>

* Color is based on the Schwarzkopf scale (21).
\( 1, 2, 3, 4, 5 \) Indicates groups of samples that were significantly different from the other groups different by a single-factor ANOVA \( (p < 0.01) \).
Figure 2. Comparison of three separate decontamination strategies. Points represent the mean of 15 observations (all hair types and all laboratories grouped together): cocaine concentrations (A), BE concentrations (B), and CE concentrations (C). Error bars are 1 SD. Decreasing trends are significant (p = 0.0001), and treatments are significantly different (p = 0.0001). Inset panels present data for pre-contamination (time 0), 1 h post-contamination, 6 h post-contamination (post sweat application), and 24 h post-contamination after the first shampooing.

Sweat treatment
One hour post-contamination but after the first samples were taken, the individual hair locks were treated with a synthetic sweat solution. This solution consisted of 65mM NaCl (OmniPur), 5mM KCl (Mainckrodt), 9mM sodium lactate (Fluka), and 22mM urea (J.T. Baker) as described by Cairns et al. (17). The mixture was modified with the addition of 30 µL of olive oil per 100 mL of solution to mimic body oils.

Sweat was applied to the entire hair lock using a sprayer. Hair was saturated with the solution to the point of runoff. The hair was allowed to dry on blotter paper at ambient conditions and was completely dry by inspection within 3 h of the treatment.

Daily shampoo treatment
Hair locks were shampooed in the evenings, Mondays through Fridays for 10 weeks. They were wrapped in a gauze wrapping and wet with warm tap water as described in Schaffer et al. (25). Approximately 1 mL of baby shampoo (Johnson and Johnson, New Brunswick, NJ) was applied to the hair, and the hair was massaged for approximately 1 min. The hair was then thoroughly rinsed in warm tap water. The lock was blotted dry using clean blotter paper, and the hair was allowed to dry at ambient conditions. Hair was completely dry by inspection after 3 h.

Hair sampling
At each sampling time point, approximately 400 mg of hair was removed from each hair lock. This was cut into approximately 1-cm pieces and thoroughly mixed. This mixture was divided into three 120-mg portions for each of the three decontamination protocols. Each of these portions was then divided into approximately 40-mg aliquots to send to each analytical laboratory. The hair decontaminated at RTI International was decontaminated as the entire 120-mg aliquot, then subdivided for submission to laboratories for analysis following decontamination.

Decontamination procedures
Samples requiring decontamination prior to analysis were clearly identified by RTI instructions to the laboratories. Blind control materials were included to ensure that a pos-
itive and negative control would be analyzed with samples in all decontamination protocols.

RTI utilized the extended buffer decontamination protocol previously described by Cairns et al. (17). In brief, 120-mg hair samples were shaken vigorously at 120 rpm at 37°C for 15 min in 20 mL isopropanol. Then hair samples were shaken at 120 rpm in 20 mL 0.01M phosphate buffer/0.01% bovine serum albumin (BSA), pH 6 for 30 min at 37°C. The shaker was configured so that the sample tubes traveled a short distance and experienced an abrupt change in direction at the ends of the shake cycle ("bumped" at the ends). This was repeated two more times, followed by two 60-min buffer washes using the same conditions. The hair aliquots were allowed to air dry prior to shipping.

The analytical laboratories all used different decontamination procedures. One laboratory used an extensive aqueous buffer wash. The second laboratory used a brief methanol wash. The third laboratory used two sequential brief washes in methanol.

Preparation of samples for analysis

All hair samples submitted to analytical laboratories were weighed, packaged in foil, sealed in individual plastic bags, and sent by overnight carrier to the laboratories. Negative and positive control samples were randomly inserted with each shipment.

Negative control materials were prepared from each of the five hair locks prior to cocaine exposure. Portions of each hair lock were used as negative controls several times throughout the study so that multiple determinations were made on each negative hair by all laboratories. Control materials were packaged similarly to study samples so as to be blind to the laboratories.

Positive control materials consisted of hair from both known drug users and control hair preparations manufactured in association with RTI's efforts in the NLCP Pilot PT for hair testing laboratories conducted under contract to SAMHSA, HHS. Several different target concentrations in these materials were used throughout the study.

Controls were included in submissions such that at least two negative and two positive controls were sent to each laboratory with each submission. Control materials were inserted in a randomized blinded fashion and submitted with samples to analytical laboratories on a weekly basis throughout the study.

Statistical analysis

All statistical analyses were performed using SAS (version 9.1.3, Cary, NC). Comparisons of the effects of time, decontamination method, and their interactions were accomplished using linear mixed models (PROC MIXED). Results from the three laboratories were treated as replicate analyses, yielding 15 clusters, 3 per hair type. For the first analysis,
a repeated measures model with doubly repeated measures (two within-cluster covariates, time, decontamination method, and their interaction) was fit to these data. The correlation across decontamination methods was accommodated by fitting a random intercept model to decontamination methods within person, and compound symmetry was used to describe the correlation of observations across time within decontamination methods. Tukey's method was used to adjust for multiple comparisons among decontamination levels. Statements of significance related to Figures 2 and 3 are derived from this model.

A second analysis was conducted comparing hair types (separately by decontamination procedure). Laboratory results were treated as replicate analyses, resulting in 15 clusters, 3 per hair-type. A repeated measures model with one repeated factor (time) and one between cluster covariate (hair-type) was fit. Compound symmetry was used to describe the pair-wise correlation of observations over time. Tukey’s method was used to adjust for multiple comparisons among hair types. Statements of significance related to Figures 4–7 are derived from this model.

Results

All three laboratories reported results on all hair samples (no missing results). Also, all control materials submitted to the laboratories were analyzed and the reported results were consistent with expected performance. All five hair types had no detectable COC, BE, CE, or NCOC prior to contamination. NCOC results are not presented in the figures as most results were below limits of quantitation. All reported results are above laboratory limits of quantitation. Table II lists the limits of quantitation for the three analytical laboratories.

Figure 2 presents the mean results of the five hair types analyzed by the three laboratories over time by decontamination strategy. Error bars on all charts represent one standard deviation (SD). For all three frames in Figure 2, each point represents the mean of five hair samples with one determination by each analytical laboratory (n = 15). Only hair that was decontaminated at RTI at 1 h post-contamination prior to sweat treatment and shampooing had no detectable COC, BE, and CE than decontaminated hair samples (p = 0.0001) until approximately day 21 of the study period. Hair decontaminated at the analytical laboratories contained significantly more COC, BE, and CE than hair decontaminated at RTI (p = 0.0001) until approximately day 21 of the study period.

Concentrations of COC, BE, and CE in all decontamination protocols increased after the application of artificial sweat. This was followed by a significant linear decline over the 10-week sampling period (p = 0.0001).
Throughout the 10-week period, COC was detected in all hair samples and some hair samples still had detectable BE and CE. All results were highly variable as indicated by the error bars. Figure 3 presents mean results by hair type for the hair samples that were decontaminated by the analytical laboratories. Each point is the mean of the three analytical laboratory results, and error bars represent one SD. Results in Figure 3 for COC, BE and CE in each of the hair types are consistent with Figure 2. A significant linear decrease in COC ($p = 0.0001$), BE ($p = 0.0001$), and CE ($p = 0.0001$) concentrations over time was evident. Interestingly, at day 7, hair type 1 (blonde hair) had significantly higher concentrations of COC than hair types 3 (brown, $p = 0.0005$) and 5 (dark brown, $p = 0.0136$). By day 21, the differences were small or insignificant. BE was not detectable in blonde hair type 1 at days 56 and 63 (indicated by zero points).

There was no apparent simple relationship between concentration and hair color. For example, after day 7, hair type 4 (dark brown) had significantly greater concentrations of COC than all other hair types ($p = 0.0001$). However, hair types 4 (dark brown) and 5 (very dark brown) had similar melanin contents but significantly different ($p = 0.0001$) cocaine concentrations. Again there was large variability in results between laboratories.

Figure 4 presents results separated by hair type for the hair samples that were decontaminated at RTI (decontamination not performed by the analytical laboratories). Results presented in Figure 4 indicate a pattern similar to that found in Figures 2 and 3 characterized by a sharp increase in concentration after the sweat treatment and a significant, linear decrease over remainder of the study period for all hair types ($p = 0.0001$). In Figure 4A, hair type 4 (dark brown) had significantly higher concentrations of COC than all other hair types ($p = 0.0001$). In Figure 4B, hair type 4 had significantly greater BE concentrations than hair types 2 and 3 ($p = 0.0001$ and $p = 0.0001$, respectively). In Figure 4C, hair type 4 had greater concentrations of CE but not significantly at all time points. COC concentrations were the lowest in hair type 2 (light brown). Notably, the variance in results was considerably less as indicated by the error bars than the variance observed in Figures 2 and 3.

Figure 5 presents the mean ratio of BE/COC concentrations for all hair types analyzed by all three analytical laboratories over the 10-week period. Error bars were omitted from these data and %CV for all points was approximately 50%. There were no significant differences between the three decontamination strategies ($p = 0.5919$). The BE/COC ratio rose significantly in a linear fashion ($p = 0.0001$) over the test period indicating an increased concentration of BE relative to COC over time during the study. The mean BE/COC ratio exceeded 5% by day 21 in all hair types.

### Table II. Limits of Quantitation for the Three Analytical Laboratories

<table>
<thead>
<tr>
<th></th>
<th>COC</th>
<th>BE</th>
<th>CE</th>
<th>NCOC</th>
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<tbody>
<tr>
<td>Lab 1</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Lab 2</td>
<td>100</td>
<td>20</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Lab 3</td>
<td>60</td>
<td>15</td>
<td>15</td>
<td>10</td>
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</table>
Figure 6 presents mean BE/COC ratios by hair types subjected to each individual laboratory's decontamination protocol (each point is the mean of the three analytical laboratory results). Hair type 1 (blonde) had significantly faster and higher increases in the BE/COC ratio over the study period than did all the darker hair samples (when compared to type 2 \( p = 0.0150 \), to type 3 \( p = 0.0018 \), to type 4 \( p = 0.0103 \), and to type 5 \( p = 0.0339 \)). The trend for all hair samples was significant, positive, and linear \( (p = 0.0001) \) over the study period.

Figure 7 presents mean BE/COC ratios for each of the five hair types subjected to decontamination at RTI (mean of the reported determinations). A significant linear increase in the BE/COC ratio was evident \( (p = 0.0001) \). Hair type 1 (blonde) had a significantly greater increase than hair types 3 \( (p = 0.0069) \), 4 \( (p = 0.0014) \), and 5 \( (p = 0.0022) \). The pattern was very similar to that observed in Figure 6. Again, there was no apparent simple relation between hair color and concentration, at least within the hair types tested in the study. The zero points are due to BE not being detected by any laboratory at those time points; thus, the ratio was zero. For hair type 2 at 70 days, the high ratio is due to a very low reported BE result.

Individual hair type results (concentrations and BE/COC ratios) that were not decontaminated are not presented, as there were no additional patterns and the results were consistent with the overall results.

The application of the proposed Federal Mandatory Guideline criteria \((26)\) for designating a hair specimen as positive or negative to the data obtained in this study are presented in Table III. When the proposed criteria of COC greater than or equal to 500 pg/mg, BE greater than or equal to 50 pg/mg, and BE/COC ratio greater than or equal to 0.05 were used to designate an analytical result as positive, 235 of the 585 total analytical results for the contaminated hair aliquots would have been called positive, including those samples that had no decontamination performed. Of the 390 samples that were decontaminated, 182 samples met the proposed criteria to be called positive. For all hair types, there were samples that would have been called positive by at least one analytical laboratory for almost the entire study period.

Table IV presents the results of applying the alternative proposed federal cutoff of COC greater than or equal to 500 pg/mg and CE or NCOC greater than or equal to 50 pg/mg. This resulted in a total of 303 samples of the 585 total analyzed samples that would have been called positive including samples that were not decontaminated. Of the 390 samples that were decontaminated, 182 samples met the criteria to be called positive. Thirty-four samples had NCOC greater than 50 pg/mg including samples that were not decontaminated. Of those samples that were decontaminated, 20 samples contained detectable NCOC, and 13 had NCOC greater than 50 pg/mg. All samples containing NCOC also had CE greater than 50 pg/mg.

**Discussion**

The results obtained in this study generally confirm the results of Romano et al. \((18)\), who found that COC deposited from the hands remained present on the hair in substantial quantities even after 10 weeks of hygienic treatment and subtraction of hair samples to decontamination procedures. The measured concentrations in this study were generally on the same order of magnitude in concentration of COC with Romano et al. \((18)\), these results were consistent with either an increase in the BE concentration developing in situ overtime in the hair, and/or a differential in the rate at which BE and COC were removed from the hair by shampooing and/or decontamination procedures. Nakahara et al. \((27)\) reported that BE in hair was due to hydrolysis of COC deposited in hair, and Wang and Cone \((13)\) found that BE could be deposited into hair from the environment. Cairns et al. \((17)\) and Schaeffer et al. \((28)\)
also indicated that BE can form on the hair from parent drug by non-metabolic processes. The analytical laboratories all analyzed controls to determine the potential contribution of analytical production of BE and corrected for any observed analytical artifact.

The statistically significant increase in the BE/COC ratios shown in Figures 5–7, although not specific proof of in vitro formation, was consistent with the referenced reports of BE formation in vitro as well as our experience with manufactured hair testing performance samples stored at room temperature. As Table III demonstrates, this formation resulted in numerous samples being called positive when the proposed federal cutoff criteria of BE/COC greater than or equal to 0.05 and COC of 500 pg/mg or greater were applied. By the COC and the COC/BE criteria, 85 or 44% of the 195 non-decontaminated samples would have been reported positive. By the same criteria, 148 or 38% of the 390 decontaminated samples were positive. The wide differences in the rates at which the BE/COC ratios increased in the five hair samples (Figures 6 and 7), further complicates the development of rules to decrease the number of potential false negatives and false positives.

Additionally, the ratios of metabolites may be influenced by the source of contaminating COC. Illicit COC is known to have widely varying and numerous contaminants and by-products of manufacture, including NCOC, CE, and BE (29). The relative concentrations of these compounds in illicit COC are variable. Bourland et al. (30) reported that 22 “street” COC samples had BE concentrations ranging from 0.2 to 1.9%, EME from 0.5 to 13.7%, no detected CE, and NCOC up to 1.5%. Other investigators report that NCOC ranges up to 5% and CE up to 2% (31). It is known that most, if not all, pharmaceutical COC has some CE present (32).

The COC used in this study was confirmed to have 0.6% CE present in it; 0.6% CE in the COC used in this study resulted in 303 positive samples (including those that were not decontaminated) or 51% of all tested aliquots being positive by the proposed federal cutoff of 500 pg/mg COC and 50 pg/mg CE. Although the number of samples designated as positive by the BE/COC criteria was 235 (including those that were not decontaminated or 40% of all tested aliquots) (Table IV). Of the 195 samples that were not decontaminated, 100% had concentrations of COC above laboratory quantitation limits. By the COC-CE criteria, 121, or 62%, of the non-decontaminated samples would have been reported positive.

Of the 390 samples that were decontaminated, 379 samples had concentrations of COC above laboratory quantitation limits. By the COC-CE criteria, 182, or 46%, of the decontaminated samples were positive. Intuitively, the number of samples designated as positive by the COC and CE criteria would increase as the CE concentration in licit or illicit COC increases. Thus, CE would not be a reliable marker of ingestion at this cutoff because hygienic treatment and laboratory decontamination in this study did not adequately remove it from hair after contamination.

The COC used in this study also had approximately 0.1% NCOC present. Forty-seven (including samples that were not decontaminated) of the hair samples had detectable NCOC reported from at least one analytical laboratory. Of those samples, 20 decontaminated samples had detectable NCOC, and 13 had NCOC greater than 50 pg/mg. Again, with reported illicit COC containing 10–50 times more NCOC, NCOC could be present in the hair because of environmental contamination rather than metabolism. The levels of NCOC observed in this study were too low to be conclusive and require additional studies to determine the potential for this to confound interpretation.

Additionally, wide interindividual variation in the uptake and retention of the analytes in this study, as well as the variations in rates at which the BE/COC ratios increased, did not appear to be simply related to measured melamin content. Blonde hair type 1 had significantly higher BE/COC ratios that rose faster over the study period than did darker hair samples. Hair type 4 (brown), with significantly higher total melamin than hair types 1, 2, or 3, was found to have higher concentrations of COC over much of the study period (Figures 6 and 7) even after decontamination. The sample size in this study was too small to extrapolate these results to the larger population, but the results are suggestive that binding and retention of analytes after environmental contamination is more complex than interaction with melamin alone.

Only hair samples that were decontaminated at RTI almost immediately after sampling at the 1 h post-contamination time point (prior to sweat application) had no detectable COC, BE, CE, or NCOC. This was consistent with the findings of Romano et al. (18) and other authors who appear to have decontaminated the hair very soon after contaminating the hair (17, 20, 28, 33, 34). This result is of particular note when compared to hair samples from the same time point (1 h post-contamination prior to sweat application or hygienic washing) submitted to the laboratories. These samples were analyzed by all three laboratories. All laboratories reported significant quantities of COC and some reported small quantities of BE, CE, and NCOC after the individual laboratories had decontaminated these 1 h post-contamination samples.

These hair samples were decontaminated between 5 and 30 days after the contamination event. Thus, in the period between 1 h and 5 days after the contamination event, the analytes became resistant to removal from the hair. This hair was not exposed to either artificial sweat or shampoo.

Hair is a dynamic material of which water is an integral part. In light of the results obtained for the hair samples before they were wet with artificial sweat, it is possible that changes in humidity throughout shipping and storage aided the migration of COC from the surface into the hair matrix with the resulting incorporation being resistant to removal. This phenomenon merits further study and, if confirmed, would further confound discriminating drug positives due to ingestion from those due to environmental contamination.

Although the results of this study were consistent with Romano et al. (18), Wang and Cone (13), and Welch et al. (19), who all found that external contamination was resistant to removal by decontamination strategies, other authors have had different results. Cairns et al. (17) sought specifically to examine the results of Romano et al. (18). They concluded that an extensive buffer wash procedure and the application of a “Wash Criterion” would differentiate contaminated and non-contaminated samples. They attributed the differences in their study from the re-
and retention of drugs is a complex function of melanin and NCOC with total melanin suggesting that the in vitro binding be any simple relation of concentrations of COC, BE, CE, or sampling of hair types used in the study, there did not appear to study confounded the use of ratios, cutoffs, and other mathematical criteria to distinguish a contaminated sample. This likely will be a greater issue with illicit COC, which has been reported to have up to 20 times the NCOC and 3 times the CE as evidenced by a significant linear increase in BE/COC ratios over the study period. Within the small sampling of hair types used in the study, there did not appear to be any simple relation of concentrations of COC, BE, CE, or NCOC with total melanin suggesting that the in vitro binding and retention of drugs is a complex function of melanin and other hair components. Contamination of the surface of hair may result in the incorporation of analytes into the hair without wetting the hair. The addition of moisture to the hair as artificial sweat markedly increased the concentrations of drug in the hair. Once the analytes were absorbed into the hair, they were resistant to removal by shampooing the hair and/or current laboratory decontamination wash procedures.

Conclusions

It appears that it will be difficult to develop hair PT samples that will demonstrate that all cocaine analytes applied to hair by dry transfer can be removed from hair by current decontamination procedures. Also, the large variability in results from samples decontaminated by laboratories using different decontamination strategies suggests that reinstating the use of these strategies will increase the variability in the current pilot PT program. In addition, the results from this study may have significant impact on further federal policy decisions since these results in general confirmed the findings of Romano et al. (18). Externally contamination of hair with powdered COC HCl resulted in the presence of COC, BE, CE, and, to a lesser extent, NCOC that was resistant to removal over 10 weeks of model hygienic treatment and laboratory decontamination. The presence of trace quantities of CE and NCOC in the COC used in the study confounded the use of ratios, cutoffs, and other mathematical criteria to distinguish a contaminated sample. This likely will be a greater issue with illicit COC, which has been reported to have up to 20 times the NCOC and 3 times the CE as the COC used in the study. BE also appeared to increase in comparison to COC as evidenced by a significant linear increase in BE/COC ratios over the study period. Within the small sampling of hair types used in the study, there did not appear to be any simple relation of concentrations of COC, BE, CE, or NCOC with total melanin suggesting that the in vitro binding and retention of drugs is a complex function of melanin and other hair components. Contamination of the surface of hair...


