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Analysis of Cocaine Analytes in Human Hair: Evaluation of Concentration Ratios in Different Hair Types, Cocaine Sources, Drug-User Populations, and Surface-Contaminated Specimens

FINAL REPORT

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ABSTRACT

Understanding the disposition of controlled substances in hair and the effects of environmental contamination on hair drug-testing results remains elusive. Current interpretation practices use a benzoylecgonine-to-cocaine (BE/COC) ratio of ≥0.05 to distinguish cocaine (COC) use from external contamination; however, ratios of other analytes, such as cocaethylene (CE) and norcocaine (NCOC), have not yet been fully investigated. This study's primary goal was to evaluate COC analyte concentrations and metabolite-to-parent drug concentration ratios in human hair to include hair type (e.g., color), COC source (e.g., pharmaceutical, street drug), and drug-environment conditions. This research evaluated the effects of COC composition, COC incorporation by ingestion and external contamination, and hair color (light and dark hair) on COC analytes and analyte-to-parent ratios found in hair. RTI International's[1] Center for Forensic Sciences evaluated hair obtained from commercial and professional sources and research facilities. Hair and refined illicit COC specimens were analyzed using liquid chromatography-tandem mass spectrometry. COC analyte concentrations and ratios for all drug-user populations agreed with concentrations previously reported by RTI and other researchers. Contamination studies with COC containing higher CE, BE, or NCOC concentrations resulted in significantly higher concentrations ($p = 0.0001$) of each of these drug compounds in the respective hair specimens. This indicates that the quantity of COC analyte found in illicit COC can affect the concentration of these compounds in the contaminated hair, although it may not be a linear relationship. BE/COC ratio increased significantly over time and could not be used reliably to identify COC-contaminated hair. Similar findings were observed when hair was contaminated with a COC hydrochloride (HCl) solution. Furthermore, criteria for distinguishing COC use from possible environmental exposure at realistic concentrations did not appear to be significantly improved by adding criteria that evaluated CE and NCOC to parent drug concentrations. In no instances did the cocaethylene-to-cocaine or the norcocaine-to-cocaine ratios result in confirmation rulings (e.g., COC positive or negative) different from that obtained by evaluating the BE concentration and the BE/COC ratio. The results also indicate that the COC cut-off concentrations and ratios currently used by many forensic drug-testing laboratories may not effectively discriminate between drug use and environmental exposure. Further research is needed to determine if using additional decision criteria, which may include a unique COC metabolite, wash criteria, or mathematical criteria that compare the presence of a drug in wash solutions to concentrations in hair, may be necessary to adequately and reliably identify external COC contamination.

[1] RTI is a trade name of Research Triangle Institute.

EXECUTIVE SUMMARY

Statement of the Problem

Detecting the compound cocaine (COC) in hair is not sufficient to identify drug use because hair shafts may be contaminated by COC released into the air during smoking, and contaminated hands can transfer COC powder residue from surrounding surfaces where use occurred (Stout et al., 2006b; Kidwell and Smith, 2007). For these reasons, other COC analytes and even parent drug-to-metabolite concentration ratios are evaluated in hair drug-testing programs to ensure that the hair test only identifies the illicit use of COC; however, the efficacy of established cut-off concentrations for COC in hair is still debated.

There are up to four COC analytes routinely investigated for hair drug testing. These analytes include the parent compound (COC), as well as benzoylecgonine (BE), cocaethylene (CE), and norcocaine (NCOC). COC is the most abundant analyte, followed by BE (<10%–50% of COC), CE (<20% of COC), and NCOC (<10% of COC). In addition to analyte concentrations, a benzoylecgonine-to-cocaine (BE/COC) ratio can be monitored with the understanding that a ratio of <0.05 suggests that the hair is contaminated with COC and should not be reported as positive for drug use (SAMHSA, 2004a). Ratios for other COC analytes have also been monitored with less success (Bourland et al., 2000; Ropero-Miller et al., 2002; Cairns et al., 2004a; SAMHSA, 2004a; Scheidweiler et al., 2005).

Research evaluating COC analyte concentrations in hair following external contamination and fortification with drug standard solutions is limited. Reported contamination studies have focused on COC and BE after contaminating hair with COC, most likely because these are the primary analytes in hair and because the CE and NCOC were largely believed to be metabolites of COC and not by-products of the manufacturing process. Therefore, researchers did not expect to find CE or NCOC if they exposed the hair to high-purity COC. More recent studies (Scheidweiler et al., 2005; Stout et al., 2006b) have demonstrated that these COC analytes can occur at appreciable concentrations in studies where high-purity COC hydrochloride (HCl) powder was administered subcutaneously under controlled dosing and where it was applied to the hair during a short exposure time followed by 10 weeks of daily hygienic treatment (i.e., shampooing).

Project Purpose and Goals

The purpose of this study was to investigate COC analyte concentrations in hair and determine if concentration ratios could be established to distinguish between hair from a COC user and hair from a non-user. RTI International (RTI) evaluated the effects of COC source (e.g., pharmaceutical, street drug), and specimen type (e.g., drug user in the street environment, drug user in the controlled research environment, external drug fortification, and hair surface contamination) on analyte concentrations and metabolite-to-parent drug concentration ratios found in human hair. COC source and specimen type were evaluated separately in hair externally contaminated with a dry COC HCl powder (e.g., surface contamination model) and hair that was fortified with the same COC materials dissolved in a fortification solution (effectively used as an alternate contamination model). RTI contracted with established forensic hair-testing laboratories to analyze hair specimens by liquid chromatography-tandem mass spectrometry (LC-MS/MS). RTI used research designs and methods that are applicable to the current proposed Mandatory

Guidelines and practices to determine the COC analyte concentrations in multiple drug-exposure situations. The research design and methods examined multiple potential effects in separate experiments, which included hair type, COC source, use pattern, laboratory fortification (i.e., spiking or drug incorporation methods), and environmental contamination. This study evaluated realistically expected situations to provide additional information about the potential differences in the COC concentration ratios observed. RTI has published the results of this study in the U.S. Drug Enforcement Administration's (DEA's) *Microgram Journal* and is currently in the process of submitting another manuscript to the *Journal of Analytical Toxicology* to achieve another project goal. RTI staff members have also presented the results at two annual Society of Forensic Toxicologists meetings.

Research Design and Methods

This study was designed to investigate COC analyte concentrations and their ratios in hair that was contaminated through drug ingestion, externally contaminated with a solid COC HCl powder (e.g., refined, illicit COC [street COC] and commercially available, pharmaceutical-grade COC), or externally fortified with a buffered solution of COC. The following are four stages of research grouped and discussed as sections in this report based on each experimental design, sample type and preparation, analysis procedures, findings, and conclusions:

- Stage I: Analysis of COC HCl powders
- Stage II: Analysis of hair from drug-user populations
- Stage III: Contamination of hair with COC HCl powders
- Stage IV: Fortification of hair specimens with COC HCl powders.

This experimental design was unique because some hair specimens were used in both Stages III and IV studies (e.g., externally incorporated COC protocols) and allowed for the direct comparison of analyte concentrations found after contaminating the hair with COC of varying purity (e.g., >99% COC, >1% CE and >10% BE, >5% NCOC). COC analyte concentration ratios were statistically evaluated to determine significant differences. All specimens were blinded prior to shipment to the testing laboratory, and drug-free blind control specimens were included with the study specimens. Sample types are listed in **Table ES-1** by the stage of the study, the number of specimens, and the type of analysis (e.g., singular, duplicate, or triplicate analysis).

Table ES-1. Number and Analysis of Specimens in the RTI Study

Stage of Study	Sample Description	Number of Specimens and Type of Analysis
Stage I	COC HCl powders	28 (singular and duplicate analysis)
Stage II	Drug-user hair with COC self-administered in a street environment (uncontrolled administration)	38 (singular and duplicate analysis)
Stage II	Drug-user hair from volunteers with COC administered in a clinical setting (controlled administration)	20 (singular analysis)

Stage of Study	Sample Description	Number of Specimens and Type of Analysis
Stage III	Drug-free hair surface contaminated with high-purity pharmaceutical-grade COC (i.e., <1.1% CE or NCOC) collected at 13 time points	26 (triplicate analysis)
Stage III	Drug-free hair surface contaminated with low-purity street COC (i.e., 1.4% CE and 10.1% BE) collected at 13 time points	26 (triplicate analysis)
Stage III	Drug-free hair surface contaminated with low-purity street COC with higher amount of NCOC (i.e., 8.7% NCOC) collected at 13 time points	26 (triplicate analysis)
Stage IV	Drug-free hair fortified with COC of varying purity in a solvent solution (used same COC HCl as Stage III)	5 (triplicate analysis)
Stage II–IV	Drug-free and drug-user hair as controls in study	31 (singular and triplicate analysis)
Stage IV	Decontamination wash fractions	36 (singular)
TOTAL		251 (~400 with replicate analysis)

When possible, replicate analysis was performed, and the average of the results was used for data analysis. Approximately 400 specimens were tested for COC analytes with LC-MS/MS analysis. RTI submitted both drug-free and spiked blind control specimens as a quality assurance measure to ensure that specimens were handled correctly by the laboratory.

Results

Stage I: Analysis of Cocaine Hydrochloride Powders

COC HCl powders available illicitly (i.e., on the street) have a wide range of purity and manufacturing by-products that may affect the incorporation of drug into hair and its subsequent detection by hair tests. For this reason, RTI evaluated multiple COC sources that would realistically represent illicit COC available for ingestion and environmental contamination.

The U.S. Department of Justice's DEA Special Testing and Research Laboratory performed an in-depth COC signature analysis, and the Armed Forces Institute of Pathology (AFIP) Laboratory completed a limited gas chromatography-mass spectrometry (GC-MS) analysis to determine the composition of 28 COC HCl powders. At the completion of Stage I, RTI successfully identified three COC HCl powders at varying purities (DEA: 65%–85%) that met our study design criteria and could be used for the remainder of the project. The AFIP Laboratory's results corroborated DEA's NCOC results; therefore, RTI used DEA's results to make the final selection of COC HCl powders to use in the contamination and fortification studies of this project. COC materials included one illicit COC HCl with BE at 10%, and CE at 1.4%, one illicit COC HCl with NCOC at 8.7%, and a U.S. Pharmacopeia material that was 98% pure with 1.1% CE. No base preparations of COC were used in this study.

As an additional unplanned project result, RTI compared DEA's signature analysis to an analysis on the time-of-flight direct analysis real time (TOF-DART) system (AccuTOF-DART™ manufactured by JEOL USA, Inc.). Because there was no sample preparation, RTI investigated

if the TOF-DART instrument could be used to complement traditional methods for determining compounds in seized materials. A total of nine COC analytes were identified by TOF-DART analysis. Anhydroecgonine methyl ester (AEME) and trans-cinnamoyl COC were easily detected in 23 of the 25 specimens. However, some COC analytes were difficult to identify (e.g., tropacocaine and truxilline isomers), whereas others (e.g., isomeric pair BE and NCOC) could not be distinctly determined because of their equal masses.

Stage II: Analysis of Hair from Drug-User Populations

Stage II compared COC analyte concentrations and ratios in the hair of various drug-user populations to the Division of Workplace Programs of the Substance Abuse and Mental Health Services Administration (SAMHSA) proposed Mandatory Guidelines criteria for federal workplace drug-testing programs and six other decision points of positive calls on confirmatory analytical results. The additional decision points were selected based on a review of the data and previously proposed criteria (Schaffer et al., 2007). Criteria 1 through 3 are the original criteria proposed in the Mandatory Guidelines. Criteria 4 and 5 evaluated the norcocaine-to-cocaine (NCOC/COC) ratios of ≥0.05 and ≥0.01, respectively. Criteria 6 through 9 evaluated the cocaethylene-to-cocaine (CE/COC) ratios of each of the following: ≥0.05, ≥0.02, ≥0.01, and ≥0.002. As a final evaluation, RTI compared the results of the drug-user populations in this study to the results of other drug-user populations using the same criteria (Cairns et al., 2004a; Bourland et al., 2000). These criteria are summarized in **Table ES-2**.

Table ES-2. Cocaine Analyte Concentrations (pg/mg) and Ratios Criteria for Determining Confirmation Results as Positive or Negative

Criteria 1 (BE criteria)	COC ≥500 and BE ≥50 and BE/COC ≥0.05
Criteria 2 (CE criteria)	COC ≥500 and CE ≥50
Criteria 3 (NCOC criteria)	COC ≥500 and NCOC ≥50
Criteria 4	COC ≥500 and NCOC ≥50 and NCOC/COC ≥0.05
Criteria 5	COC ≥500 and NCOC ≥50 and NCOC/COC ≥0.01
Criteria 6	COC ≥500 and CE ≥50 and CE/COC ≥0.05
Criteria 7	COC ≥500 and CE ≥50 and CE/COC ≥0.02
Criteria 8	COC ≥500 and CE ≥50 and CE/COC ≥0.01
Criteria 9	COC ≥500 and CE ≥50 and CE/COC ≥0.002

The federal criteria were selected for COC analyte concentrations and ratio criteria (Criteria 1 through 3) without the inclusion of any additional mathematical algorithms because these criteria are the only published criteria that, in the current state of hair testing, would be applicable to multiple laboratories.

For the drug-user population that self-administered COC in a street environment (i.e., street drug user [STREET], uncontrolled administration), only 50% of the population was positive for CE (i.e., 19 of 38 subjects). When the CE and NCOC criteria (Criteria 2 and 3) for each subject's results were compared to the BE criteria (Criteria 1), there was one subject who met these criteria, but did not meet the BE criteria. Conversely, there were four subjects who met the BE criteria (Criteria 1), but did not meet the NCOC criteria (Criteria 3). Therefore, the COC

confirmatory rate did not increase when COC analytes other than COC and BE were evaluated with the proposed cut-off concentrations (e.g., change in confirmation ruling for a subject).

If additional criteria for the CE/COC and NCOC/COC ratios were considered (e.g., ≥0.05, ≥0.02, ≥0.01, ≥0.002) for this drug-user population, the following conclusions were drawn. Although 27 of 38 STREET drug users had NCOC concentrations ≥50 pg/mg at a NCOC/COC ratio requirement of ≥0.05 (Criteria 4), all subjects tested negative with the inclusion of this additional decision point. If the NCOC/COC ratio was lowered to ≥0.01, 19 subjects would have confirmed positive by Criteria 5 (≥0.01); however, an additional 9 subjects met the NCOC cut-off concentrations of ≥50 pg/mg, but did not meet the NCOC/COC ratio of ≥0.01 (Criteria 1). Inclusion of a ≥0.05 or ≥0.01 NCOC/COC ratio did not yield confirmation results similar to the current proposed Mandatory Guidelines for BE (Criteria 1: 36 of 38 subjects [94.7%]) or NCOC (Criteria 3: 33 of 38 subjects [86.8%]). Both decision points for the NCOC/COC ratio were too high for this drug-user population, and confirmed positive, at the most, 24 of 38 subjects (63.2%). This reduced the confirmed positive rate in this drug-user population by more than a 30%.

Criteria 6 through 9 had decision points using the CE/COC ratio and yielded the following positives for COC use: ≥0.05 (3 of 38 subjects [7.9%]); ≥0.02 (8 of 38 subjects, [21.1%]); ≥0.01 (12 of 38 subjects [31.6%]); and ≥0.002 (17 of 38 subjects [44.7%]). The number of STREET drug users that met any of the CE/COC ratio criteria was much lower then the BE concentration and the BE/COC ratio criteria of the proposed Mandatory Guidelines. For the most part, the CE concentration requirement of ≥50 pg/mg is largely the determining factor (19 of 38 subjects [50%]) for confirmation rulings evaluating CE. Even a CE/COC ratio of ≥0.002 would not be equivalent to the proposed BE and CE criteria for the STREET drug-user population.

Hair specimens collected from clinically administered drug users (CLINICAL) (controlled administration) during an in-patient clinical study were procured from the National Institute on Drug Abuse's Intramural Research Program (NIDA's IRP) for analysis and inclusion in RTI's study. NIDA protocols for this clinical study were reviewed and accepted by NIDA's Institutional Review Board.

For the CLINICAL group, a smaller percentage of the population tested positive for CE (i.e., 6 out of 20 subjects). When the CE criteria (Criteria 2) for each subject's results was compared to the BE criteria (Criteria 1), there were three CLINICAL subjects who met the CE criteria, but did not meet the BE criteria. These same subjects also met the NCOC criteria, but did not meet the BE criteria. Conversely, there were four CLINICAL subjects who met the BE criteria (Criteria 1), but did not meet the NCOC criteria (Criteria 3). Therefore, there was no increase in the overall COC confirmatory rate when additional COC analytes beyond COC and BE were evaluated with the proposed cut-off concentrations (e.g., change in confirmation ruling for a subject) for the CLINICAL subjects.

If additional criteria for NCOC/COC and CE/COC ratios were considered (e.g., ratios of ≥0.05, ≥0.02, ≥0.01, ≥0.002) for the CLINICAL population, the conclusions were as follows: 3 subjects (15%) were positive at ≥0.05 NCOC/COC; 14 subjects (70%) were positive at ≥0.01 NCOC/COC; 0 subjects were positive at ≥0.05 and ≥0.02 CE/COC; 2 subjects (10%) were positive at ≥0.01; and 6 subjects were positive at ≥0.002 (the same 6 subjects as Criteria 2).

Overall, the NCOC criteria had equivalent positive calls to BE criteria, but there was a 40% reduction of positive calls using CE criteria for the CLINICAL drug-user group.

The CLINICAL drug-user population had a much smaller administered dose of COC compared to the other drug-user populations evaluated. As evidenced, the average BE concentration in hair was 7% of the COC concentration (434 pg/mg versus 6,171 pg/mg), the average CE concentration was 2% of the COC concentration (123 pg/mg versus 6,171 pg/mg), and the average NCOC concentration was 5% of the COC concentration (290 pg/mg versus 6,171 pg/mg).

Evaluation of the Cairns and colleagues (2004a) and Bourland and colleagues (2000) drug-user groups to the nine criteria yielded similar findings, so there was no significant advantage (<3% change in confirmation result) to evaluating additional COC analytes beyond COC and BE.

Stage III: Contamination of Hair with Cocaine Hydrochloride Powders

Stage III examined the ratios of analytes in hair after contaminating it with different source COC materials. The results of this study were consistent with what RTI has previously published for contaminating hair with pharmaceutical-grade COC (Stout et al., 2006b). All three COC sources resulted in significant quantities of COC on the hair and remaining on the hair over a 10-week period. As previously observed, there was a significant decline in the COC content over the course of the study.

In our study, the contamination with COC containing higher CE, BE, or NCOC concentrations resulted in significantly higher concentrations of each of these drug compounds in the respective hair specimens, indicating that the quantity of each of the drug compounds found in an illicit COC can affect the concentration of these compounds in the contaminated hair. As with COC, these compounds were resistant to removal by either hygienic treatment (i.e., shampooing) or laboratory decontamination. Additionally, exposure to the COC that contained CE at 1.4% of the COC material resulted in a maximum CE/COC ratio of 0.05, whereas the material containing 8% NCOC resulted in a maximum NCOC/COC ratio of 0.25. These results indicate that there may not be a direct relationship between the concentrations of CE and NCOC in hair and the concentration in the contaminating COC.

This study was also consistent with previous findings because the BE/COC ratio increased significantly over the course of the study period. In the recent study, the ratio exceeded the ≥0.05 point by Day 28 for those COC materials that contained less BE, instead of Day 21 as previously reported. For the illicit COC that had high CE and BE concentrations, this ratio increased after adding synthetic sweat and continued to rise over the course of the study. Hair specimens treated with the high NCOC illicit COC powder and the pharmaceutical-grade COC did not exhibit any decline in BE over the course of the study period.

A substantial number of analyzed specimens would have been determined as positive by most of the criteria applied. For the specimens exposed to COC that contained more CE, there were more specimens that would have resulted in positive calls. For these specimens, only the criteria, including a ≥0.05 CE/COC ratio, would have resulted in no positive results. At a ≥0.02 CE/COC ratio, there were 44% of the dark hair specimens and 33% of the light hair specimens that would have tested positive. For those specimens exposed to the high NCOC that contained COC, 33% of the light hair specimens and 92% of the dark hair specimens would have been

determined as positive by all of the criteria using NCOC. A more complex pattern was observed with BE criteria because BE appeared in the hair from all sources; therefore, varied amounts of NCOC, CE, and BE in the contaminating COC can substantially confound the use of ratios to discriminate contaminated hair specimens, even after using a laboratory's decontamination protocol.

It is important to note that RTI applied these criteria to hair specimens because a reference laboratory would apply them under the proposed Mandatory Guidelines. In other words, the reference laboratory would have had to analyze the specimens and apply the cut-off concentrations directly to these results. As Schaffer and colleagues (2007) have noted in several publications (Cairns et al., 2004a and b), they have applied various ratios of compounds and have used various mathematical calculations using the amounts of a drug found in the last wash solution. As noted by Kidwell and Smith (2007), this wash criteria has evolved over the years. The proposed Mandatory Guidelines (SAMHSA, 2004a) do not have a provision for the use of such criteria; therefore, we did not use any wash criteria during this analysis. RTI has retained the last wash solutions of all hair specimens to potentially conduct this analysis at a later date.

Developing ratios to discriminate contaminated hair is problematic because of the potentially variable CE, NCOC, and BE contents in illicit COC and the high inter-individual variability in the way an external drug interacts with the hair. The ≥0.05 CE/COC ratio in high illicit COC may have eliminated positive results in contaminated hair using COC materials with higher purity and less CE composition; however, illicit COC may contain higher concentrations of CE, potentially confounding even this ratio.

Stage IV: Fortification of Hair Specimens with Cocaine Hydrochloride Powders

Stage IV examined the ratios and concentrations of the COC analytes following the introduction of COC to the hair by fortification procedures (i.e., effectively an alternate contamination model). Results indicated that COC analyte concentrations in hair following fortification with COC HCl solutions were >50 pg/mg for NCOC, but not for BE and CE. Evaluation of these specimens using current proposed Mandatory Guidelines suggest that fortified specimens (e.g., external contamination through COC HCl solution) may not be differentiated from the hair of COC users who actually ingested COC.

Hair drug-testing laboratories could use additional steps to help differentiate COC contamination from actual ingestion, but none of these steps is routinely practiced by most laboratories. For example, a decontamination wash calculation can be applied against the COC analyte concentration for a more conservative interpretation of a hair concentration. Cairns and colleagues (2004a) suggested that the COC concentration in the final decontamination wash should be measured, multiplied by a factor of five, and then subtracted from the final COC concentration in the hair sample to estimate the amount of COC that would be further removed with additional decontamination washes. The Cairns decontamination procedure takes 3.75 hours to perform. Alternatively, Tsanaclis and Wicks (2008) suggested that a drug detected in a dried-down methanolic wash that was obtained rapidly and analyzed could be used to calculate a wash-to-hair (W/H) ratio. Evaluation of the fortification specimens with either of these decontamination wash calculations indicates that the specimens were externally contaminated. Hair drug-testing laboratories could also consider analysis of another truly metabolic product that does have a pathway for its presence as a by-product from the manufacturing process, but the abundance of such an analyte may be small in comparison to COC.

Overall Project Summary

After evaluating COC and COC analyte concentrations and ratios in user hair from various populations, contaminated hair with various sources of COC, and an alternate external application of COC to hair (fortification), the use of cut-off concentrations for any or all of the analytes would not be reliable to discriminate a user's hair from contaminated hair. The use of analyte ratios provides more information and some ability to discriminate user specimens from contaminated specimens; however, the use of CE and NCOC concentrations and ratios does not discriminate any more efficiently than does decision criteria using only BE and COC. All three analytes (i.e., CE, NCOC, and BE) can be present at varied concentrations in illicit COC as by-products of the manufacturing process, and as such, will confound the use of ratios to discriminate contamination from use. Contamination of hair with illicit COC materials that contain ≥1% to ≥10% (weight-to-volume) of CE, BE, and NCOC resulted in hair specimens that would not be discriminated from user hair by ratios or concentrations using the criteria applied. Even after decontaminating the hair, the application of concentration and ratio decision points does not adequately discriminate contamination from drug use.

In this study, RTI applied these decision criteria because laboratories would have to apply them under the current proposed federal Mandatory Guidelines. These guidelines do not have provisions for using additional decision criteria, which include wash criteria or those mathematical criteria that compare the presence of a drug in wash solutions to concentrations in hair. Published findings from Cairns and colleagues (2004a and b) and Tsanaclis and Wicks (2008), suggest some type of decision criteria is necessary to adequately and reliably identify contamination.

These results have implications for the proposed Mandatory Guidelines because the decision criteria, as proposed in this study, do not adequately discriminate contamination. This is of particular concern for individuals whose occupation (e.g., law enforcement) may put them in contact with large amounts of COC in their environment; therefore, a requirement for decontamination and further research are needed to determine the viability of comparative criteria using information from the decontamination.

1. INTRODUCTION

1.1 Background

For more than 30 years, hair has been used as a biological matrix to detect controlled substances, such as cocaine (COC), and to indicate drug use. Although conventional matrices, such as blood, oral fluids, and urine, document an individual's drug exposure for a period ranging from minutes to days, hair can extend the detection period from months to years, depending on the hair sampled and the collection process. Moreover, conventional matrices can be both physically and socially invasive to collect, they can require preservation through refrigeration or freezing, and, as in the case of urine, they may be susceptible to adulteration or substitution. In contrast, hair is easy to collect, the drug is relatively stable in the hair, and hair is difficult to adulterate or substitute.

Although hair testing has many applications, including death investigations, workplace drug testing, drug-facilitated crimes, and violation of probation or parole, there are still many issues that limit its widespread use. These issues include the absence of standardized techniques between laboratories, consistent proficiency-testing materials, a laboratory certification program, consistent results within and between laboratories, and easily identifiable drug analytes that discriminate between environmental contamination and drug use, as well as the presence of a potential bias of drug incorporation into the hair (i.e., hair color or ethnic differences) (Ropero-Miller, 2007a).

The potential "color bias" in hair testing arises from the ability of drugs to associate predominantly with melanin, which is a pigment in hair that contributes to its color. Drugs may associate with other hair proteins as well, but they are not usually as abundant as melanin. There are two types of melanin in hair: eumelanin, which is present at much greater quantities, and pheomelanin. Eumelanin contributes brown and black pigments (darker hair), whereas pheomelanin contributes yellow-red pigments (lighter hair). Drugs associate with eumelanin with a strong affinity. After ingesting or being exposed to the same amount of a drug, individuals who have a darker hair will have a greater amount of drug incorporated into their hair than individuals who have lighter hair (Claffey et al., 2001; Joseph et al., 1996; Stout and Ruth, 1999; Kronstrand and Scott, 2007; Stout, 2006b).

The potential "ethnic bias" in hair testing is the concept that hair testing may produce results from the same exposure that are disproportionately more positive or negative for one ethnic group. This may be a result because of the demonstrated affinity of many drugs for melanin and because more drug is present in hair that contains more eumelanin. However, the ethnic bias is more complex because the distinction between ethnic groups is not straightforward, and it is unclear whether some ethnic groups have consistently and significantly more eumelanin than other ethnic groups. Additionally, cultural differences in hygienic treatments, cosmetic treatments, and environmental exposure could also produce differences in the interaction of drugs with hair between groups. Therefore, ethnic bias is a potential phenomenon that is more complex than simply color of the hair. Both dark and light hair specimens were used in this study as a preliminary investigation of potential hair color effects on the study design; however, the sample populations were too small for a statistical comparison.

The degree to which a drug analyte is incorporated into hair is dependent on hair growth patterns and biological and environmental factors influencing its growth. For example, the drug

content in plucked hair is different from that found in shed hair because shed hair undergoes a resting period (no growth, or catagen phase) before falling out (Pichini et al., 1996). Factors that influence inter-individual growth rate of hair include age, gender, ethnicity, heredity, climate, health, injury and physical stress, as well as the anatomical site of hair growth (Hamilton et al., 1955; Hold, 1996; Robbins, 2002; Kronstrand and Scott, 2007).

1.2 Statement of the Problem

Detecting the parent compound, COC, in hair is not sufficient to identify drug use. COC is often smoked, and COC released into the air may coat the hair shaft, leading to environmental contamination. In addition, COC may be on the surfaces of areas where it was used and can be transferred to the hair by contaminated hands (Stout et al., 2006b; Kidwell and Smith, 2007). For this reason, other COC analytes, and even parent compound-to-metabolite ratios are evaluated in workplace drug-testing programs to ensure that the hair test only identifies illicit use of COC; however, the efficacy of established cut-off concentrations for COC in hair is still debated.

There are up to four COC analytes that are routinely investigated for workplace drug testing. These analytes include the parent compound (COC), as well as benzoylecgonine (BE), cocaethylene (CE), and norcocaine (NCOC). COC is the most abundant analyte followed by BE (10%–50% of COC), CE (<20% of COC), and NCOC (<10% of COC). Ratios for other COC analytes have also been monitored with less success (Bourland et al., 2000; Ropero-Miller et al., 2002; Cairns et al., 2004a; Scheidweiler et al., 2005; SAMHSA, 2004a).

Research that evaluates COC analyte concentrations in hair following external contamination and fortification with drug standard solutions has also been limited. Reported contamination studies have investigated COC and BE; however, the researchers did not report on other COC analytes, such as CE and NCOC. These investigations focused on COC and BE after contamination of hair with COC because these are the primary analytes in hair and because the CE and NCOC were considered to be metabolites of COC and not by-products of manufacture. Plus, the researchers did not expect to find these COC analytes when exposing the hair to high-purity COC. In this process, a benzoylecgonine-to-cocaine (BE/COC) ratio that is <0.05 suggests that the hair is contaminated with COC and should not be reported as positive for drug use (SAMHSA, 2004a).

More recent studies by Scheidweiler and colleagues (2005) and Stout and colleagues (2006b) have demonstrated that these COC analytes can occur at appreciable concentrations in studies where high-purity COC hydrochloride (HCl) was administered subcutaneously under controlled dosing and where it was applied to the hair during a short exposure time followed by 10 weeks of daily hygienic treatment, respectively.

More research is still needed to determine if current COC analyte concentrations and ratios that have been established to differentiate COC use from environmental exposure are adequate and if additional parent-to-metabolite ratio concentrations would assist in further discrimination.

1.3 Review of the Literature

1.3.1 Use of Hair Drug Testing

Forensic laboratories use hair as a complementary and alternative matrix to blood and urine in testing for controlled substances. The disposition of many controlled substances in hair is cited in the literature, and hair test results have been used as evidence in civil, criminal, and military courts of law for more than 20 years (Huestis, 1996). Controlled substances reported in hair include amphetamines, COC, opiates, cannabinoids, barbiturates, phencyclidine, and benzodiazepines (Ropero-Miller et al., 2000 and 2005). Hair attributes include its durability, its ability to indicate long-term drug use (weeks to years depending on length of hair), and its ease of collection and storage. Applications of hair drug testing include postmortem analyses, human performance testing, parole and drug treatment programs, workplace drug testing, and crime scene investigations, including drug-facilitated crimes. However, interpreting hair test results for controlled substances has been complicated by many issues, including potential hair color bias, the need for more sensitive analytical techniques, external contamination, and high individual variability due to many factors such as age, gender, hygiene, drug biotransformation and excretion, and hair growth rate (Cone, 1990; Stout and Ruth, 1999; Bourland et al., 2000; Ropero-Miller et al., 2000; Claffey et al., 2001; Cairns et al., 2004a; Ruth and Stout, 2004). For hair testing to be accepted in forensic toxicology applications, it is crucial that an individual who is environmentally exposed to a drug can be differentiated from a drug user.

1.3.2 Cocaine in Hair

Detecting only the parent compound, COC, in hair does not prove COC use. COC is highly concentrated in the hair of demonstrated COC users, with BE, CE, and NCOC in amounts ranging from undetectable to >40% COC (Cairns et al., 2004a).

Historically, one way of differentiating a drug user from a person who may be occupationally exposed to the drug (e.g., narcotics officer, pharmaceutical researcher) or one who may have unknowingly come into contact with a drug-contaminated surface has been to identify a unique in vivo metabolite. Although BE, CE, and NCOC are readily accepted as COC metabolites, these analytes are not exclusively produced through biotransformation within the body. All of these COC analytes have been reported as impurities in pharmaceutical-grade and STREET COC in the literature, including work performed in RTI International's (RTI's) Center for Forensic Sciences (CFS) Laboratory (Casale and Klein, 1993; Casale and Moore, 1994a and b; Moore et al., 1994; Casale et al., 2005a and b). Furthermore, BE can be derived from COC by non-enzymatic hydrolysis under basic conditions and, consequently, cannot be conclusively used as a biological marker for COC ingestion because it is derived by hydrolysis in the environment, which means that the presence of BE in the hair is due to COC degradation (may only be environmental exposure) and not metabolism. Scheidweiler and colleagues (2005) detected CE (C_{max}: 71 pg/mg–143 pg/mg), which is a COC metabolite formed by trans-esterification with ethanol, in human hair after controlling COC administration (doses: 75 mg/70 kg and 150 mg/70 kg) in a closed residence-research facility where ethanol was unavailable. It was unclear where the CE came from during this study, and it is important to determine if these analytes were present as impurities or metabolites. Another study by Ropero-Miller and colleagues (2002) used the same clinical study protocol and dosing scheme as Scheidweiler and colleagues (2005) but only reported COC concentrations as maximum total drug concentrations by taking the sum of the concentrations found in the hair and the combined wash fractions. The results were reported

this way to allow for the comparison of drug concentrations of nail scrapings collected at the same time to drug incorporation patterns of these keratinized matrices, which is the primary objective of this study. Ropero-Miller and colleagues (2002) said that other COC analytes were detected in initially collected specimens (anhydroecgonine methyl ester [AEME] and CE) and/or the first collection directly following drug administration (BE and NCOC). Generally, the combined concentration of all other COC analytes was <10% of the COC concentration for a given subject and collection, but in a few instances, the combined concentration of all other COC analytes was as much as 30% of the concentration of parent COC.

After extensive research, the U.S. Department of Health and Human Services (DHHS) proposed confirmatory test cut-off concentrations for COC analytes in hair, with the added stipulation that the parent drug compound must be present with at least one other COC analyte (Mangin, 1996). A BE/COC ratio of ≥0.05 was also specified. Due to limited data, ratios could not be determined for NCOC and CE. Other agencies, laboratories, and researchers have adopted similar practices (SOHT, 2004). **Table 1** summarizes established cut-off and threshold concentrations for COC in hair that have been published since 1998.

Table 1. Published Initial, Confirmatory, and Lower Limit Cut-Off Concentrations (pg/mg) for Cocaine in Hair

Agency or Organization	Testing Level	COC
Substance Abuse and Mental Health Services Administration (USA proposed 2004)	Initial	500
	Confirmatory	≥500 COC and ≥50 BE *and* BE/COC ≥0.05 **OR** ≥500 COC *and* ≥50 CE **OR** ≥500 COC *and* ≥50 NCOC
Society of Hair Testing	Initial	500
	Confirmatory	≥500 COC ≥50 metabolites
Gesellschaft fur Forensische und Toxikologische Chemie (Society of Toxicological and Forensic Chemistry) (Germany)	Initial	200
	Confirmatory	≥500 COC ≥100 metabolites
Societe Francaise de Toxicologie Analytique (French Society of Analytical Toxicology) (France)	Lower limit	500 COC, BE, and CE

Note: BE = benzoylecgonine; BE/COC = benzoylecgonine-to-cocaine ratio; CE = cocaethylene; COC = cocaine; NCOC = norcocaine; NCOC/COC = norcocaine-to-cocaine ratio

Table reprinted from Ropero-Miller, 2007a.

1.3.3 Cocaine Analyte Concentration and Ratio Studies in Drug-User Populations

There are a limited number of reports in the literature that actually provide data in the form of COC analyte concentrations by subject. Similarly, specific data for COC analyte concentration ratios are equally sparse (Cassani and Spieher, 1993; Kidwell, 1993; SOHT, 1997). The Society of Hair Testing (SOHT) was one of the first organizations to recommend that the presence of metabolites should be used for interpretive purposes and that metabolite-to-parent drug ratios should be calculated. SOHT (1997) also suggested that a BE/COC ratio >0.05 might indicate drug use. **Table 2** summarizes concentration and ratios for BE and COC reported in the literature. Two more reports (Cairns et al., 2004a; Bourland et al., 2000) are discussed separately in the following paragraphs because more detail and subjects are included in these cases.

Table 2. Review of the Literature for Reporting of Cocaine Analyte Concentrations and Ratios

Author (Year)	COC (ng/mg)	BE (ng/mg)	BE/COC	Other Information
(Kidwell, 1993) *n* = 5	2	1.9	0.950	Solid-probe heating direct sample introduction; tandem mass spectrometry; also reported ecgonine and ratio
	98	8.9	0.091	
	40	2.9	0.073	
	12	2.8	0.233	
	6.85	5.1	0.745	
(Gaillard and Pepin, 1998)	5.5	1.5	0.273	
(Romolo et al., 2003) *n* = 17	3.4	0.7	0.206	Gas chromatography-mass spectrometry
	25.1	3.3	0.131	
	2.2	ND	Not calculated	
	18.3	4.5	0.246	
	0.5	ND	Not calculated	
	2.6	1.1	0.423	
	21.3	6.1	0.286	
	>100	24.7	0.247	
	0.9	ND	Not calculated	
	>100	>100	Approximately >1.0	
	30.5	8.1	0.266	
	3.3	3.7	1.121	
	6.3	3.5	0.556	
	8.6	4.2	0.488	
	29.8	11.2	0.376	
	16.4	3.3	0.201	
	0.7	ND	Not calculated	
	21.7	7.6	0.350	
	8.2	2.1	0.256	

Note: BE = benzoylecgonine; BE/COC = bezoylecgonine-to-cocaine ratio; COC = cocaine; ND = not detected

One additional report from Cairns and colleagues (2004a) examined COC concentrations in two distinct populations: 75 confirmed drug users and more than 6,000 workplace drug-testing specimens. **Table 3** lists the distribution of COC concentrations in drug-user (confirmed positive urinalysis) and workplace populations (Cairns and colleagues [2004a] did not state total workplace population, they only confirmed positive hair-testing results at established limit of quantitation). Although 86.6% of the COC-containing hair from drug users had COC concentrations of 2,000 pg/mg and higher, 54.3% of the workplace population (individuals seeking employment or already gainfully employed) had similar concentrations. Additionally, the analyte profile for the workplace population showed the existence of BE levels >5% of the COC concentrations for most of the confirmed drug users, but in some individuals (4%–15% of those with COC $\geq$500 pg/mg), the BE was <5% of the COC values. Even in the drug-user population, 3%–5% of the COC-containing hairs did not contain BE at levels >5% of the COC concentration. For these individuals, their CE and/or NCOC concentrations would have tested positive using the proposed Mandatory Guidelines (SAMHSA, 2004a). Likewise, in the workplace population, more than half of the specimens contained CE >50 pg/mg hair, and approximately 78% of these specimens contained NCOC at >50 pg/mg hair.

Table 3. Distribution of Cocaine Concentrations in Drug-User and Workplace Populations Confirmed Positive by Hair Testing (Cairns et al., 2004a)

Cocaine in Hair (pg/mg)	Drug-User Population (%)	Workplace Population (%)
>20,000	39 (52)	725 (11.5)
10,000–20,000	9 (12)	633 (10.1)
5,000–9999	12 (16)	818 (13.0)
2,000–4,999	5 (6.6)	1,240 (19.7)
1,000–1,999	2 (2.7)	1,225 (19.5)
500–999	3 (4.0)	1,653 (26.3)
<500	5 (6.6)	Not reported
TOTAL	**75**	**6,294**

Table reprinted from Ropero-Miller, 2007a.

Cairns and colleagues (2004a) also reported all four COC analyte concentrations for each of the 75 confirmed drug users. These data are presented in **Table A-1** and are discussed in Sections 2.2.6 and 2.2.7. These data show the number of subjects with a detectable amount of COC analyte (i.e., above the limit of quantitation [LOQ]) and the pg/mg concentration range (mean and median) for each COC analyte as follows:

- 75 detectable results for COC at 30 to 227,000 pg/mg (mean: 43,038; median: 24,800)
- 73 detectable results for BE at not detected to 34,700 pg/mg (mean: 5,211; median: 2,380)
- 55 detectable results for CE at not detected to 12,790 pg/mg (mean: 1,218; median: 210)
- 70 detectable results for NCOC at not detected to 5,560 pg/mg (mean: 1,174; median: 810).

Many times, when the parent drug COC was detected, the other COC analytes were not detected in this drug-user population. For all COC analytes, the mean concentration was higher than the median, which indicates that the population distribution curves were skewed toward the left, or lower, concentrations. If the results from each subject were compared to the Substance Abuse and Mental Health Services Administration (SAMHSA) confirmatory cut-off concentrations (Table A-1), the number of subjects and the percentages of positive test results for this drug-user population were: 70 subjects positive for COC (93%), 69 subjects positive for BE (92%), 37 subjects positive for CE (49%), and 64 subjects positive for NCOC (85%). CE was the only analyte that appeared in two subjects at detectable concentrations but the concentrations were not high enough to confirm positive results by the proposed Mandatory Guidelines (SAMHSA, 2004a) (COC: 70 of 75, BE: 69 of 73, CE: 37of 55, and NCOC: 64 of 70).

In a second population study, Bourland and colleagues (2000) reported COC concentrations in 30 human head hair specimens. These specimens were randomly chosen production specimens that had previously been reported as positive by an enzyme-linked immunosorbent assay (ELISA) and gas chromatography-mass spectrometry (GC-MS); the data provided in this report were from re-analyzing these specimens. The COC analyte concentration ranges and means are listed in **Table 4**. Based on the proposed Mandatory Guidelines and SOHT Guidelines listed in Table 1, at least one subject (i.e., Subject X: COC 420 and BE 100) would have been reported as negative by SOHT Guidelines and two subjects (i.e., Subject E: COC 21,260 and BE 620; Subject X: COC 420 and BE 100) would have been reported negative for COC by the proposed Mandatory Guidelines. COC concentrations reported by Cairns and colleagues (2004a) and Bourland and colleagues (2000) are consistent (Tables A-1 and **A-2**); however, the smaller population studied by Bourland and colleagues had a lower percentage of subjects with concentrations <2,000 pg/mg (20% versus 45%).

Table 4. Cocaine Analyte Concentration Ranges and Means for 30 Human Head Hair Specimens Submitted for a Workplace Drug-Testing Program, with Method Limit of Detection and Limit of Quantitation (Bourland et al., 2000)

Analyte	Concentration Range (pg/mg)	Mean	LOD	LOQ	Percentage
COC	420 to 2,000 (*n* = 6) 2,001 to 35,500 (*n* = 24)	10,350	10	50	100
BE	70 to 4,710	1330	10	50	12.8
CE	<LOD to 10,870	1590	10	50	15.4
NCOC	<LOD to 1,580	260	10	50	2.5

Note: BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; NCOC = norcocaine; LOD = limit of detection; LOQ = limit of quantitation

Table reprinted from Ropero-Miller, 2007a.

The actual subject data for all four COC analyte concentrations of the 30 drug users was reported by Bourland and colleagues (2000) and are presented in Table A-2. The number of subjects with a detectable amount of COC analyte (i.e., greater than or equal to the LOQ), the pg/mg concentration range, and the mean and median for each COC analyte could be determined for the subject data and are summarized as the following:

- 30 detectable results for COC (97%) at 420 to 35,500 (mean: 10,348; median: 6,320) pg/mg
- 30 detectable results for BE (100%) at 70 to 4,710 (mean: 1,327; median: 705) pg/mg
- 19 detectable results for CE (60%) at not detected to 10,870 (mean: 1,590; median:
  - pg/mg
- 23 detectable results for NCOC (77%) at not detected to 1,580 (mean: 325; median:
  - pg/mg.

Although COC and BE were detected in all subjects, the other two COC analytes were not detected in 23% to 37% of this drug-user population. Again, the mean concentration was higher than the median for all COC analytes. For BE, 5 subjects who met the confirmatory cut-off concentration of 50 pg/mg did not meet the criteria of a BE/COC ratio of ≥0.05.

1.3.4 Preliminary RTI Studies

Preliminary RTI studies suggest that BE, CE, and NCOC may be deposited during in vitro surface contamination of the hair and can be detected at the current proposed DHHS cut-off concentrations for up to 10 weeks after applying COC. This recently published research by RTI's CFS Laboratory found that it was difficult to remove COC in hair after applying COC HCl powder to the hair surface, and, in some cases, it was difficult to discern contaminated hair from drug-user hair by the detection of metabolites (Stout et al., 2006b). Our work to date has corresponded with that of Romano and colleagues (2001). **Figure 1** shows data that RTI has produced for COC in contaminated hair that was subjected to different decontamination strategies.

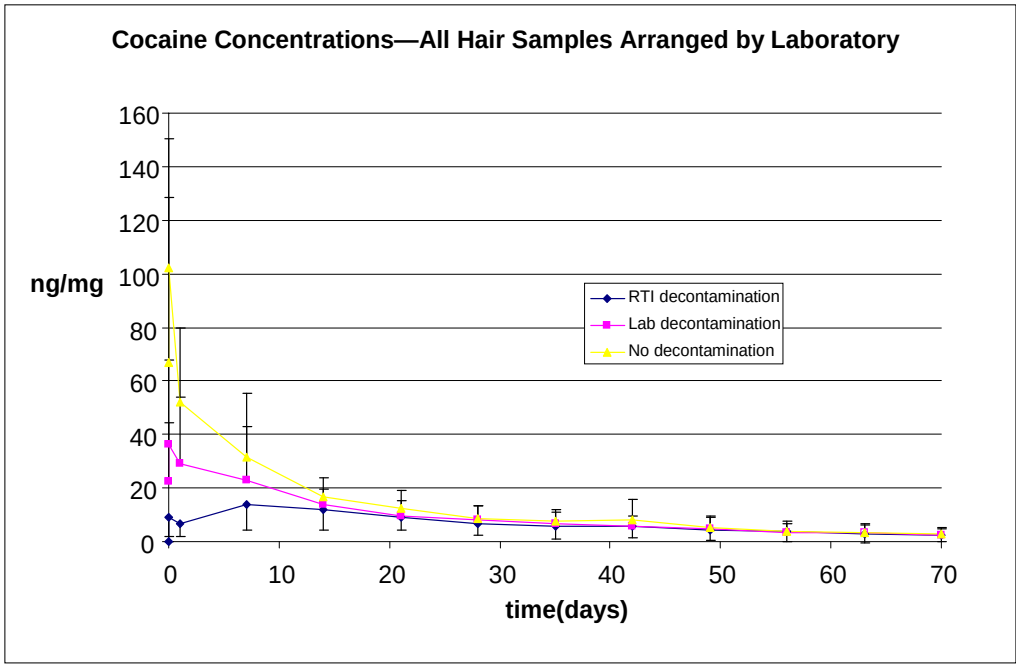


Figure 1. Data from RTI's investigations show COC concentrations in hair after contamination with 15 mg of COC hydrochloride powder distributed on hands. Hair specimens were decontaminated by RTI, decontaminated by testing laboratories, or not decontaminated. Error bars are 1 standard deviation, and each point represents the mean of 15 observations. The three lines are significantly different (*p* <.00001).

Significant COC concentrations were observed in hair specimens over the entire study period from a single contamination event.

Figure 2 presents the BE/COC ratio over the study period from hair decontaminated with an extensive buffer wash performed by RTI. The BE/COC ratio exhibited a significant increase over the study period (p <0.0001) and increased above the ratio of 0.05 proposed for hair testing in federal workplace drug-testing programs (SAMHSA, 2004b). There was no apparent relationship between the rate and the extent of ratio increase and hair color.

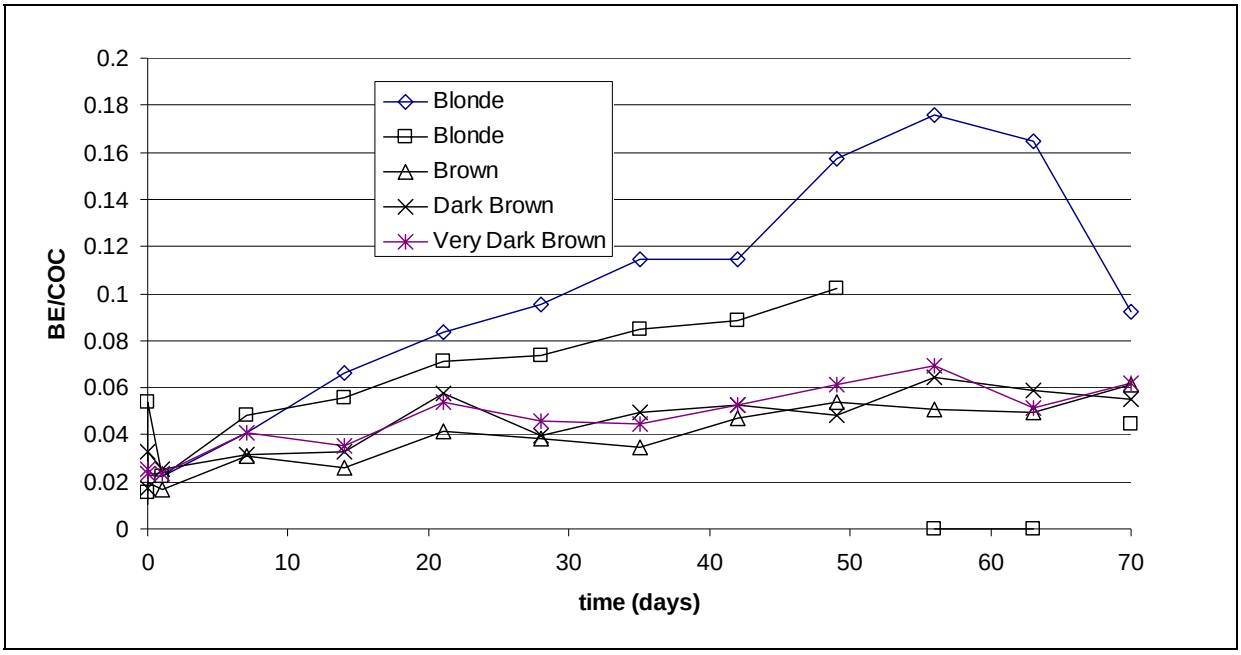


Figure 2. Benzoylecgonine-to-cocaine (BE/COC) ratio in hair over time in hair decontaminated with an extended aqueous buffer wash. Significant (p <0.0001) increases in the BE/COC ratio were observed during the 10-week period, with wide variability between hair types without any apparent relationship to hair color.

External contamination of hair with COC HCl powder resulted in the presence of COC, BE, CE, and, to a lesser extent, NCOC, which was resistant to removal over 10 weeks of model hygienic treatment and laboratory decontamination. The presence of trace quantities of CE and NCOC (<1%) in the COC used in the study confounded the use of ratios, cutoffs, and other mathematical criteria to distinguish a contaminated sample. This is likely to be a greater issue with illicit COC, which is reported to have up to 20 times the NCOC and 3 times the CE as the COC used in the study (Casale and Klein, 1993; Casale and Moore, 1994a and b; Moore et al., 1994; Casale et al., 2005a). 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, there did not appear to be any simple relationships between concentrations of COC, BE, CE, or NCOC with total melanin. RTI used the Schwarzkopf color scale (Schwarzkopf, 2001), which is employed by professional cosmetologists, to characterize the hair in this study and measured total melanin content using a digestion procedure and a spectrophotometric method modified from those developed by Kronstrand and colleagues (1999). Several researchers have associated higher melanin content with a proportional increase in drug incorporation (Joseph et al., 1996; Slawson et al., 1998).

Table 5 indicates that a range of melanin concentrations was used. These data suggest that the binding and retention of COC is a complex function of melanin and other hair characteristics.

Table 5. Hair Specimens—Hair Color (Based on the Schwarzkopf Color Scale), Subject Demographics, Texture Description of Hair, and Total Melanin in Hair (Kronstrand et al., 2001)

Schwarzkopf Color Scale (Modified by RTI)	Subject Demographics and Texture Description	Mean Total Melanin µg/mg (Standard Deviation)
Blonde 9.0	Caucasian female, thin strands	6.6 (5.4)[a]
Light brown 7.5	Caucasian female, thin strands, easily tangled	7.0 (4.5)[a]
Brown 6.5	Caucasian female, slight wave, smooth thick strands	31.1 (6.6)[b]
Dark brown 5.5	Caucasian female, slight wave, smooth thick strands	60.7 (10.5)[c]
Very dark brown 4.0	Asian female, thick fibers, straight and smooth strands	57.4 (6.2)[c]

[a, b, c] Indicates groups of specimens that were significantly different from one another when measured by a single factor analysis of variation (p <0.0001).

Contamination of the hair's surface may result in the incorporation of analytes into the hair without the addition of sweat. In RTI's study with COC, we observed that specimens decontaminated with an aggressive phosphate buffer procedure 1 hour after contamination and prior to contact with any moisture were completely decontaminated (no detectable COC or metabolites). Significantly more COC and metabolites were detected in specimens taken 1 hour after contamination and prior to contact with any moisture, and they were packaged for shipment to testing laboratories for decontamination. These results indicate that contaminating COC could only be removed from the hair for a short period of time by any means. The laboratories decontaminated the hair at least 5 days after the contamination event, and the drug was not removed by any of the decontamination procedures used in the study.

Adding moisture to the hair as artificial sweat markedly increased the concentrations of drug analytes in the hair. Wetting the hair only once resulted in significant COC and metabolites detectable in the hair after all decontamination procedures. After the drug analytes were absorbed into the hair, they were resistant to removal by shampooing and/or by current laboratory decontamination wash procedures used by other researchers and reported in the literature.

In summary, further investigation of COC analyte concentrations and metabolite-to-parent drug ratios is needed to determine if additional federal or laboratory-based guidelines can be established to correctly distinguish between a COC user and an individual who may have been unknowingly exposed to COC in the environment. Although several researchers have investigated COC analyte concentration ratios in a limited specimen type or sample size, no one group has simultaneously investigated COC analytes under varying factors, including hair type, COC source, use pattern, laboratory fortification, and environmental contamination, using the same sample preparation and instrumental analysis.

1.4 Rationale for the Research (Statement of Hypothesis)

The purpose of this study was to investigate COC analyte concentrations with respect to each other to determine if appropriate concentration ratios could be established to distinguish between COC user and non-user hair. RTI sought to evaluate metabolite-to-parent drug concentration ratios in human hair as affected by COC source to include pharmaceutical and street drugs, specimen type to include drug users in street environment, drug users in a controlled research environment, external drug fortification, and surface contaminated. RTI separately evaluated COC source and specimen type in hair externally contaminated with a dry COC HCl powder (e.g., surface contamination model) and also evaluated ratios obtained from fortifying hair with the same COC materials as used in external contamination. Although variables such as hair type (e.g., color, texture) were a part of the hair selection process because both light hair and dark hair were included in all stages of the study, statistical evaluation of the hair type differences could not be performed due to the small sample populations. We contracted with established forensic hair-testing laboratories to analyze hair specimens by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). RTI used research designs and methods that are applicable to the current proposed Mandatory Guidelines and practices to determine concentrations of four COC analytes (i.e., COC, BE, CE, NCOC) in multiple situations. The research design and methods evaluated as many situations as can be realistically expected to occur and provided additional information about the potential differences in the concentration ratios observed. In addition, RTI has published, and will continue to publish, the results in prominent forensic journals and has presented some findings at national meetings of leading forensic organizations.

2. RESEARCH DESIGN AND METHODS

This study was designed to investigate COC analyte concentrations and their ratios in hair that was contaminated through drug ingestion, externally contaminated with a solid COC HCl powder (e.g., refined, illicit COC and commercially available, pharmaceutical-grade COC), or externally fortified with a buffered solution of COC. The following are four stages of research that are grouped and discussed as sections in this report based on each experimental design, sample type and preparation, analysis procedures, findings, and conclusions:

- Stage I: Analysis of COC HCl powders
- Stage II: Analysis of hair from drug-user populations
- Stage III: Contamination of hair with COC HCl powders
- Stage IV: Fortification of hair specimens with COC HCl powders.

This experimental design was unique because some of these hairs (e.g., externally incorporated COC protocols of Stages III and IV) were introduced to the same COC of varying purities (e.g., >99% COC, >1% CE, >5% NCOC) at similar concentrations to investigate COC analyte concentration ratios obtained by different routes of externally introduced COC exposure. All analyses included the quantitation of COC, BE, CE, and NCOC by using LC-MS/MS hair-testing procedures. COC analyte concentration ratios were statistically evaluated to determine significant differences. All specimens were blinded prior to shipment to the laboratory, and drug-free blind control specimens were included with the study specimens.

More than 200 hair specimens were analyzed during this study. When possible, specimens were analyzed by replicate analysis and were averaged for data analysis. For example, for the contamination experiments, there were 257 total hair specimens (replicate analyses for some samples) analyzed plus 23 blind specimens. Therefore, approximately 400 hair specimens were tested for COC, BE, CE, and NCOC concentrations using 10-mg aliquots for LC-MS/MS analysis. RTI submitted both drug-free and spiked blind control specimens as a quality assurance measure to ensure that specimens were handled correctly by the laboratory.

The hair sample types that RTI analyzed and the approximate number for each are the following:

- Drug-user hair from the street environment (n = 38)
- Drug-user hair from subjects who were administered COC in a clinical setting (n = 20)
- Drug-free hair surface-contaminated with high-purity pharmaceutical-grade COC (i.e., insignificant amount [<1%] of CE or NCOC) collected at 13 time points (n = 26)
- Drug-free hair surface-contaminated with low-purity street COC with a higher amount of CE collected at 13 time points (n = 26)
- Drug-free hair surface-contaminated with low-purity street COC with a higher amount of NCOC collected at 13 time points (n = 26)
- Drug-free hair fortified with COC for proficiency testing at various concentrations similar to proposed cut-off concentration in Mandatory Guidelines for federal workplace drug testing (n = 20)

- Drug-free and drug-user hair as controls in study (n = 31)
- Decontamination wash fractions from the Stage IV fortification study (n = 36).

2.1 Stage I: Analysis of Cocaine Hydrochloride Powders

2.1.1 Experimental Design

Stage I of this research project focused on analyzing COC analytes in COC HCl powders, both refined illicit COC sources and commercially available pharmaceutical sources, to select an appropriate COC source to use in the COC surface-contamination study (Stage III) and the COC fortification study (Stage IV). RTI obtained street COC as 1-g sample of COC bricks seized by the U.S. Drug Enforcement Administration (DEA) and sent to RTI under contract to purify the COC for use by the National Institute on Drug Abuse (NIDA). RTI also obtained a 1-g sample from each seized COC HCl powder for this research.

Twenty-eight COC HCl powders were submitted to laboratories to analyze the powders' purity by using procedures commonly used for signature analysis (e.g., GC-MS) and other methods. This testing determined the concentrations of COC analytes, including CE, BE, CE, and NCOC. Other COC analytes, such as AEME, trans-cinnamoyl COC, tropacocaine, and truxilline isomers; manufacturing by-products, including solvents, such as methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK), and petroleum ether; and adulterants, such as lactose, mannitol, and caffeine, were also detected in the signature analysis process performed by DEA laboratories. Determining these cutting agents is routinely performed by DEA laboratories and can help law enforcement track high-level dealers of illicit substances and identify new local or national illicit manufacturing trends. **Figure 3** shows the chemical structures of all COC analytes determined during this stage of the study. (It is important to note that some analytes were determined in other stages as well, but this section determined all COC analytes to determine purity.)

After these specimens were correctly identified as COC specimens and their purity was determined by using traditional analytical procedures, most of these specimens were also analyzed by using a novel screening instrument as an additional study design. The purpose of the analysis was to determine if this novel time-of-flight direct analysis real time (TOF-DART) mass spectrometer using exact mass determination had the potential to greatly improve controlled substances screening in forensic laboratories (Laks et al., 2004; Song et al., 2004; Cody et al., 2005; Ojanpera et al., 2005). Although COC signature analyses have been routinely performed in many forensic laboratories, these laboratories could benefit from a rapid screening method to identify controlled substances (Ehleringer et al., 2000). Using the TOF-DART instrument in a procedure that would require minimal to no sample preparation was investigated to determine if this instrument could be developed and used to complement traditional methods for determining drug compounds in seized materials. If so, it would facilitate the tracking of drugs and other substances that are added to dilute or cut scheduled drugs to increase bulk and profit margins. So, as an adjunct project that was not originally proposed as part of this research, RTI directly compared COC signature analyses of crude illicit COC specimens with a new mass spectral technology that shows promise for identifying the controlled substances.

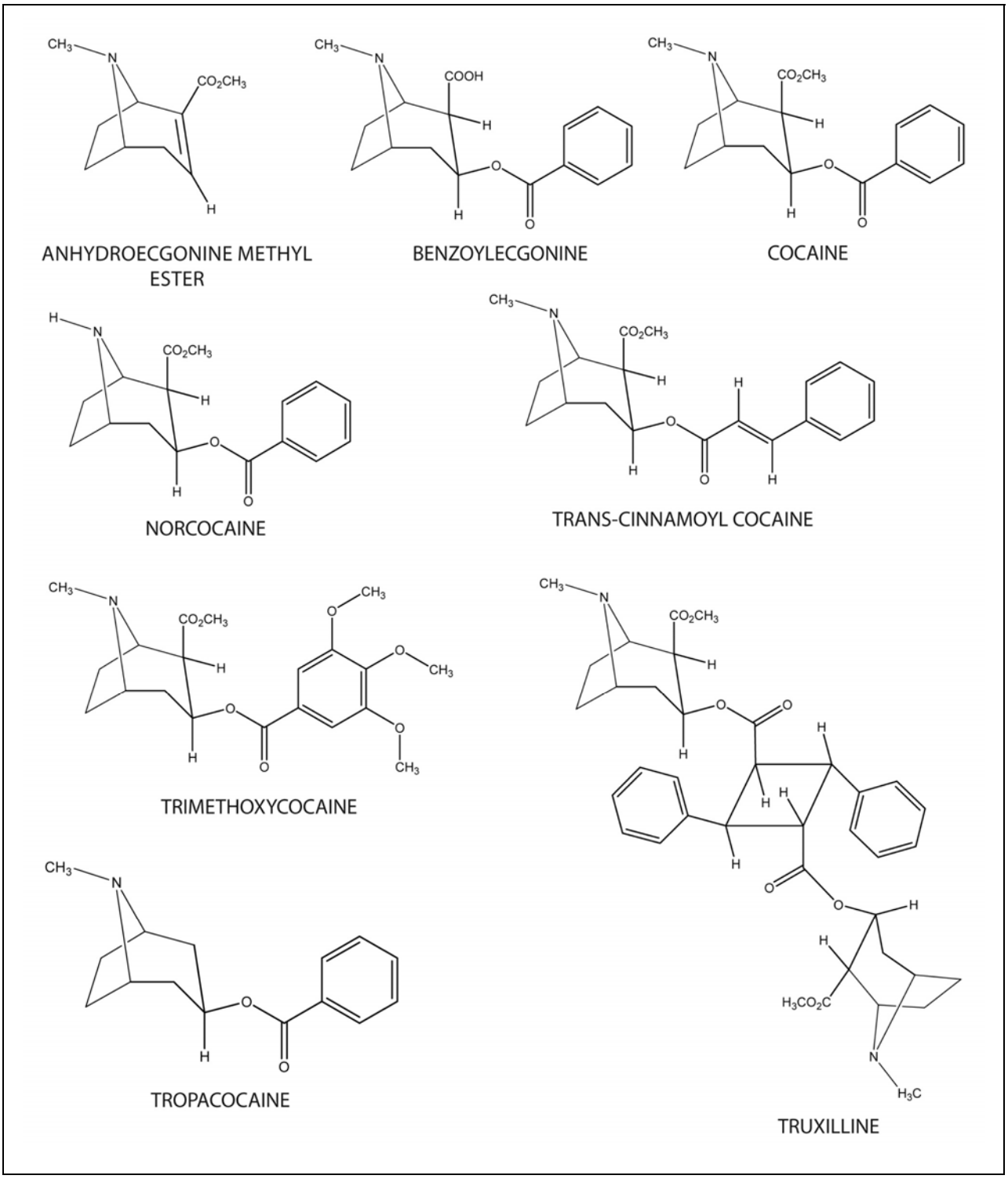


Figure 3. Chemical structures of cocaine analytes.

2.1.1.1 Powdered Cocaine Hydrochloride Specimens

There were two sources of powdered COC HCl specimens obtained for Stage I of this project. First, 25 illicit COC HCl powder specimens were obtained from NIDA's Division of Neuroscience and Behavioral Research. RTI obtained additional refined illicit COC powder from DEA laboratories when testing found that the specimens from NIDA did not have a CE concentration >1% as proposed in RTI's study design. DEA provided RTI with a COC sample from its inventory of illicit COC seizures after determining that the sample contained the appropriate CE concentration.

RTI also obtained a commercially available, pharmaceutical-grade COC HCl powder from the U.S. Pharmacopeia (USP). A certificate of analysis and further analytical testing by DEA laboratories confirmed that this COC source was of high purity (<1% of impurities).

2.1.2 Materials

COC analyte standards (i.e., COC, AEME, BE, CE, NCOC) were purchased as HCl salt (1 mg/mL) or base solutions of methanol (BE) or acetonitrile by Cerilliant Corp. Polyethylene glycol, which was used as the calibrating reagent for the TOF-DART instrument, was of reagent-grade quality and was obtained from Sigma-Aldrich Chemical Co. All other solvents and standards used by laboratories were of analytical grade and high purity.

2.1.3 Methods

For Stage I of this study, we used three protocols at three separate research facilities to identify components contained in the examined COC HCl powders. First, an in-depth COC signature analysis was performed by the U.S. Department of Justice's DEA Special Testing and Research Laboratory (henceforth referred to as the DEA Laboratory). Second, a limited GC-MS analysis was performed by the Armed Forces Institute of Pathology (henceforth referred to as the AFIP Laboratory). Third, a novel screening technique for controlled substances, such as COC, was performed by RTI in Research Triangle Park, NC. The results of Stage I of this study were published in DEA's *Microgram Journal* (Ropero-Miller, 2007b).

Cocaine Signature Analyses by DEA Laboratories

COC signature analyses were conducted by using GC-MS, capillary gas chromatography with electron capture, and flame ionization detection as reported by many researchers (Casale, 1991; Casale and Moore, 1994a and b; Morello and Meyers, 1995; Moore et al., 1996; Ehleringer et al., 2000). The GC-MS analysis was performed using an Agilent Technologies Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Technologies Model 6890 gas chromatograph. The MSD was operated in the electron ionization mode with an ionization potential of 70 eV, a scan range of 34–700 mass units, and at 1.34 scans per second. The gas chromatograph was fitted with a 30 m x 0.25 mm I.D. fused-silica capillary column coated with 0.25 μm DB-1 (J&W Scientific). The oven temperature was programmed as follows: initial temperature = 100°C; no hold, program rate, and 60°C per minute; final temperature = 300°C; with a final hold time of 5.67 minutes. The gas chromatograph injector was operated in the split mode (21.5:1) and at a temperature of 280°C. The auxiliary transfer line to the MSD was operated at 280°C.

Gas Chromatography-Mass Spectrometry by the AFIP Laboratory

COC concentrations in illicit COC specimens (>50%) are relatively high compared to other analytes (generally <5%); therefore, the specimens were tested separately using standard sample preparations and GC-MS analysis. AFIP used three separating GC-MS methods to analyze all four COC analytes.

TOF-DART Analysis by RTI Laboratories

RTI used the AccuTOF-DART (JEOL USA, Inc.) system to perform analyses, which were conducted using the positive mode on the DART ion source. COC HCl powders were introduced to the ion source as solid specimens (manual and autosampler introduction) and as methanolic solutions (autosampler introduction). The specimens were introduced into the ion source by dipping a glass probe into the sample, and then passing this through the stream. The AccuTOF-DART system was calibrated with polyethylene glycol before each sample run. When available, the monoisotopic ion plus the hydrogen ion (M + H) values of the COC analytes were verified using certified drug standard solutions.

The source was operated with a ring lens voltage of 5V, an orifice 1 voltage of 20V, and an orifice 2 voltage of 5V. The mass range was initially set from 60 to 1,000 daltons, but it was later reduced to a range of 60 to 700 daltons so more difficult compounds, such as alcohols, could be further evaluated. Electrodes 1 and 2 of the TOF-DART source were set to 150V and 350V, respectively, and the TOF-DART temperature was set to 300^{o}C. The detector was optimized at 2,200V.

2.1.3.1 Modifications to the Research Design and Rationale

After discussions, NIDA's Division of Neuroscience and Behavioral Research and collaborating laboratories mutually agreed to release 25 1-g specimens of COC HCl powders for this project. This number was larger than the originally proposed number of 20, but even with these representative COC HCl powder specimens, RTI was unable to find a COC HCl powder that contained a higher amount of CE (>1%), which was proposed. The DEA Special Testing and Research Laboratory (Dulles, VA) determined (through database and inventory searches) that they possessed a representative high CE sample and could release it to RTI for inclusion in this project.

Because the STREET COC was more realistic to use for this study, RTI decided that inclusion of one commercially available, pharmaceutical-grade COC was sufficient for meeting the study design objective. We obtained certified COC analyte reference materials from USP instead of Cerilliant Corp. as originally proposed. Furthermore, RTI was awarded a National Institute of Justice (NIJ) grant in 2006 (award number 2006-91774-NC-IJ), which is evaluating a novel analytical instrument for another forensic application. The AccuTOF-DART system has also recently shown applicability for identifying controlled substances. Because RTI possesses this instrumentation, we were able to include an additional study within Stage I to further evaluate these COC HCl specimens by using a novel screening technique for controlled substances identification.

Overall, RTI made minor modifications to the original study design proposed. With the current study design, RTI analyzed slightly more COC HCl powders than was originally proposed and still maintained and achieved the goals of this research.

2.1.4 Findings

The DEA Laboratory provided the primary results for the identification of the COC HCl powder specimens. Besides determining the relative concentration of all analytes investigated in this study to the parent COC concentration ratio, the comprehensive COC signature analysis performed by DEA Laboratory provides additional information that helps to characterize the purity, geographical origin, and manufacturing processes used to transform the COC plant materials into the COC HCl that is available illicitly on the street. For this study, the results of the COC signature analyses are presented as the COC analytes' characterization and purity and the presence of solvents and adulterants from the manufacturing process. A complete description of this process is outside the scope of this study but was described by Ehleringer and colleagues (2000).

The DEA COC signature analysis determines the relative amounts of the following COC analytes with respect to the parent COC: NCOC; CE; BE; AEME; trans-cinnamoyl COC; 3', 4', 5'-trimethoxycocaine; tropacocaines; and the truxilline isomers, among others. All specimens were positively identified as COC. The COC HCl powders had purity ranges from 65%–88.5%. BE concentrations ranged from 0.14%–1.1% (mean: 0.37%), and the NCOC concentrations ranged from trace up to 8.7% (mean: 0.9%). All specimens that contained BE also contained NCOC. All specimens contained only trace or non-detectable quantities of CE. Ethanol was in 14 of the 25 specimens (not reported for 3 specimens) with concentrations and ranged from 0.003%–0.12% (mean: 0.06%). **Table 6** summarizes the COC purity and the presence of each of the COC analytes that was detected in at least one of the submitted COC HCl powders.

Diluents and adulterants that were selected to be included in COC manufacturing processes and determined by the DEA Special Testing Laboratory's COC signature analysis were sodium chloride (NaCl), mannitol, caffeine, dimethylterephthlate, and lactose. Solvents determined to be included were isopropyl acetate, n-propyl acetate, petroleum ether, ethyl acetate, MEK, and MIBK. The base origin and HCl process for each COC HCl powder was determined as a Peruvian or Columbian manufacturing process. Table 6 shows the presence of each of these COC constituents in the COC HCl powders evaluated for this project.

Table 6. Details of the Content of the 25 NIDA Cocaine Specimens and the Three Cocaine Specimens Used in the Contamination and Fortification Experiments (Highlighted Yellow)

Count	Source	Purity	BE	NCOC	CE	Total cinnamoyls	Tropa-cocaine	Trimethoxy-cocaine	Truxillines	EtOH %	cis-cinnamoyl EEE %	trans-cinnamoyl EEE %
COC_HCl_1	NIDA	87.5	0.37	0.33	ND	2.3	0.10	0.18	6.6	0.08	ND	ND
COC_HCl_2	NIDA	86.9	0.42	0.28	ND	2.4	0.08	0.18	6.7	0.09	ND	ND
COC_HCl_3	NIDA	87.5	0.41	0.32	ND	2.3	0.12	0.18	7.1	0.09	ND	ND
COC_HCl_4	NIDA	85.1	0.31	0.06	0.0018	1.0	0.21	0.15	9.0	0.12	ND	ND
COC_HCl_5	NIDA	84.0	0.22	0.06	0.0017	1.0	0.33	0.14	9.6	0.11	ND	ND
COC_HCl_6	NIDA	79.6	0.24	0.05	ND	1.0	0.39	0.07	9.2	0.02	ND	ND
COC_HCl_7	NIDA	78.9	0.16	0.04	ND	1.1	0.38	0.08	9.4	0.01	ND	ND
COC_HCl_8	NIDA	80.9	0.27	0.04	ND	1.0	0.38	0.06	8.5	0.02	ND	ND
COC_HCl_9	NIDA	78.1	0.31	0.05	ND	0.9	0.44	0.07	9.1	0.02	ND	ND
COC_HCl_10	NIDA	82.4	0.24	Trace	ND	1.0	0.34	0.00	8.2	0.02	ND	ND
COC_HCl_11	NIDA	83.8	0.47	0.07	ND	8.2	0.06	0.18	5.8	ND	ND	ND
COC_HCl_12	NIDA	84.9	0.69	0.08	ND	5.6	0.06	0.20	5.6	ND	ND	ND
COC_HCl_13	NIDA	85.5	0.43	Trace	ND	6.4	0.04	0.12	5.9	ND	ND	ND
COC_HCl_14	NIDA	74.8	ND	ND	ND	6.5	0.19	0.22	9.9	ND	ND	ND
COC_HCl_15	NIDA	65.1	0.62	Trace	ND	5.4	0.10	0.10	10.1	0.04	ND	ND
COC_HCl_16	NIDA	74.6	ND	ND	ND	5.3	0.22	0.22	11.5	ND	ND	ND
COC_HCl_17	NIDA	87.3	ND	ND	0.0015	8.5	0.08	0.12	2.1	ND	0.0039	ND
COC_HCl_18	NIDA	71.2	ND	ND	ND	1.3	0.28	0.20	13.2	ND	ND	ND
COC_HCl_19	NIDA	79.6	1.1	0.04	ND	6.0	0.21	0.15	11.5	0.003	ND	ND
COC_HCl_20	NIDA	88.5	0.16	8.7	0.0031	0.2	0.08	0.34	3.3	0.06		
COC_HCl_21	NIDA	82.3	0.65	Trace	0.0019	6.0	0.33	0.10	7.7	0.09	ND	ND

Count	Source	Purity	BE	NCOC	CE	Total cinnamoyls	Tropa-cocaine	Trimethoxy-cocaine	Truxillines	EtOH %	cis-cinnamoyl EEE %	trans-cinnamoyl EEE %
COC_HCl_22	NIDA	85.2	0.29	2.0	0.0027	6.0	0.51	0.00	6.4	ND	ND	ND
COC_HCl_23	NIDA	84.8	0.14	1.4	0.012	6.6	0.25	0.08	7.3	ND	0.003	0.0027
COC_HCl_24	NIDA	87.8	0.19	0.65	ND	5.3	0.15	0.25	4.5	ND	ND	ND
COC_HCl_25	NIDA	79.0	0.17	1.8	ND	4.1	0.82	0.00	15.0	ND	ND	ND
COC_HCl_26	DEA[a]	93.3	NR	NR	2.30	NR	NR	NR	NR	NR	NR	NR
COC_HCl_27	DEA[b]	82.2	10.10	0.8	1.43	2.1	0.08	NR	NR	NR	NR	NR
COC_HCl_28	USP	98.9	Trace	NR	1.10	NR	NR	NR	NR	NR	NR	NR
	COUNT	25	21	21	10	25	25	25	25	14	2	1
	MIN Range	65.1	0.14	0.0	0.0015	0.2	0.04	0.00	2.1	0.00	0.0030	0.0027
	MAX Range	88.5	1.10	8.7	2.30	8.5	0.82	0.34	15.0	0.12	0.0039	0.0027
	Mean	81.8	0.37	0.9	2.06	3.8	0.25	0.14	8.1	0.06	0.0035	0.0027

Note: BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; DEA = U.S. Drug Enforcement Administration; EEE = ecgonine ethyl ester; EtOH = ethanol; NCOC= norcocaine; ND = not detected; NIDA = National Institute on Drug Abuse; NR = not reported; USP = U.S. Pharmacopeia

[a] The sample was enriched with CE prior to conversion to HCl, this was not used for the study.

[b] The sample contained CE at this amount when seized and was then converted to HCl by the Peruvian process by DEA.

The AFIP Laboratory performed a limited GC-MS analysis for 25 of the 28 COC HCl powders. The AFIP Laboratory procedure evaluated a limited number of COC analytes (did not evaluate BE); therefore, this procedure was only used to corroborate the primary analysis of the DEA Laboratory. Again, the AFIP Laboratory identified all submitted specimens as having COC and its analytes present at varying concentrations. The AFIP Laboratory identified 10 COC HCl powders that contained NCOC concentrations ranging from 0.1%–6.9% (mean: 1.3%). The AFIP Laboratory did not detect CE in any of the specimens, and although they detected AEME in concentrations ranging from 0.3%–0.8%, the results were not ultimately considered positive. The amounts of AEME determined at concentrations ranging from 0.5%–0.8% were lower than the 2% decision point for AEME to be considered as part of the illicit manufacturing artifact from the COC processing (as an acid hydrolysis product) and not an analytical artifact of the GC-MS analysis.

The results from the TOF-DART analyses were qualitatively compared to the results obtained by the AFIP and DEA laboratories. A total of nine COC analytes could be identified by AccuTOF-DART analysis (see **Table 7**). AEME and trans-cinnamoyl COC were easily detected in 23 out of the 25 specimens; however, some COC analytes were difficult to identify (e.g., tropacocaine and truxilline isomers), and others, such as isomeric pair BE and NCOC, could not be distinctly determined because of their equal masses.

In addition, some adulterants and solvents (e.g., dimethylterephthalate, MIBK, MEK) were minimally detected. **Table 8** summarizes these results.

Table 7. Comparison of TOF-DART Results with DEA Signature Analysis and AFIP Analysis of COC Materials[a]

Analytes	AccuTOF-DART (RTI Laboratory)	Cocaine Signature Analyses (DEA Laboratory)	GC/MS Analysis (AFIP Laboratory)
COC	25	25	25
BE[b]	25	21	NR
CE	ND	3	ND
NCOC[b]	25	21	7
AEME	23	ND	23
Trans-cinnamoyl COC	23	25	NR
3',4',5'-trimethoxy COC	ND	25	NR
Tropacocaine[c]	5	25	NR
Truxilline isomers[c]	7	25	NR

Note: AccuTOF-DART = time-of-flight direct analysis real time; AFIP = Armed Forces Institute of Pathology; AEME = anhydroecgonine methyl ester; BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; DEA = U.S. Drug Enforcement Administration; GC-MS = gas chromatography-mass spectrometry; NCOC= norcocaine; ND = not detected; NR = not reported

[a] The first 25 COC HCl samples appear in Table 6; the other 3 samples were analyzed only by the DEA Laboratory.

[b] Ions of these isomers (i.e., BE and NCOC) were indistinguishable by using the AccuTOF-DART system.

[c] These analytes were analyzed multiple times to verify the presence or absence of the analytes.

Table 8. Summary of Additional Solvent Characteristics from DEA's Cocaine Signature Analysis

Cocaine	Number of Specimens	Comments
Major diluents/adulterants	10	NaCl (5), mannitol (3), dimethylterephthlate (1), lactose (1)
Trace diluents	1	Caffeine
Solvent A	20	Isopropyl acetate (4), n-propyl acetate (9), petroleum ether (4), ethyl acetate (3)
Solvent B	11	MIBK (3), MEK (8)
Base origin	1 Peruvian and 25 Columbian	
HCl process	25 Columbian	

Note: HCl = hydrochloride; MIBK = methyl isobutyl ketone; MEK = methyl ethyl ketone; NaCl = sodium chloride

2.1.5 Conclusions

COC signature analyses performed by DEA laboratory and GC-MS performed by the AFIP Laboratory identified that 3 of the 16 COC HCl powder specimens sent for analysis met RTI's study design criteria for use in our contamination and fortification studies. For these studies, RTI required one highly pure COC with <1% impurities and two less pure COC HCl powder specimens with higher concentrations of other COC analytes (>1 CE and >2% NCOC and BE). These findings allowed the proposed studies to proceed with only minor modifications to the research design. The modified study design, which included three COC sources, was adequate for the evaluation of COC analyte concentrations and metabolite-to-COC ratios.

The TOF-DART instrument allowed for the rapid analysis of the 25 illicit COC specimens without the need for sample preparation. Although this direct analysis resulted in the rapid production of data, it also caused inconsistent results. The TOF-DART is a novel approach to forensic analysis; however, it could not detect many drug compounds that are used to trace a COC sample to its geographic origin. Forensic laboratories may want to use the TOF-DART as a rapid screening test for preliminary sample-to-sample comparison of the analytes that can be detected with this technology.

At the completion of Stage I, RTI successfully identified three COC HCl powders at varying purities (DEA: 65%–85%) that met our study design criteria and could be used in the remainder of the study. The AFIP Laboratory's results corroborated DEA's NCOC results; therefore, RTI used DEA's results to determine which COC HCl powders to use in the contamination and fortification studies of this project. COC HCl powders that are available on the street have a wide range of purity and manufacturing by-products that may affect the incorporation of the drug into hair and its subsequent detection by hair drug testing using the proposed Mandatory Guidelines. For this reason, RTI evaluated multiple COC sources that would represent realistic COC available for ingestion and environmental contamination for use in this project.

2.2 Stage II: Analysis of Hair from Drug-User Populations

2.2.1 Experimental Design

Stage II of this research project focused on analyzing COC analytes in hair from multiple drug-user populations to determine biomarker concentrations and the metabolite-to-parent concentration ratios. These data were statistically evaluated to determine the mean, median, and concentration range for each sample. In addition, drug-user populations' data were compared to confirmatory cut-off criteria of the proposed Mandatory Guidelines (SAMHSA, 2004a). When available, demographics and hair characterization were reported as part of this stage of the study. Before proceeding to other stages of this research, RTI evaluated COC analyte concentrations in hair by comparing the metabolic disposition of COC in head hair from two study populations. The study followed self-reported drug users (e.g., native environment) after ingesting the street drug and drug users who voluntarily participated in a 10-week in-patient NIDA study during which they were clinically administered COC in a double blind, placebo-controlled design. Participants provided written informed consent, and the protocol was approved by NIDA's Institutional Review Board.

It was important to evaluate drug-user populations as part of this research due to the limited availability of data reporting on all the COC analytes of interest. Furthermore, this parallel study design allowed for the evaluation of COC analyte disposition into the hair under controlled and non-controlled environments. COC analyte concentrations in the hair of drug users in a native environment are highly variable, and the dose is largely unknown, whereas COC analyte concentrations in hair following controlled drug administration allow researchers to investigate drug analyte distribution into human hair following a known dose. Therefore, a direct comparison of these populations by the same researchers who use the exact or similar analytical procedures was essential to the overall study design because this provided a foundation for understanding COC analyte concentrations and ratios in hair.

Tables 9 and **10** show the study design for Stage II based on dose setting (i.e., street environment versus clinical research). Table 9 shows hair samples from 38 drug users in a street environment (STREET drug user population) collection process that were obtained from a commercial source (27 subjects; Robert Fassio, Boston, MA) and hair collection from 11 subjects upon admission into an inpatient clinical research facility because these samples represented street use. Table 10 shows hair collected during controlled COC dosing from the same 11 subjects who were voluntarily enrolled in one of the two controlled dosage studies at an inpatient clinical research facility.

2.2.1.1 Street Drug-User Population

Human hair was voluntarily collected from self-reported drug users in their native environment; these specimens were purchased from a commercial source. All subjects provided informed consent and were compensated for their participation in the study. If known, demographic information (e.g., gender, age, and race) was obtained and reported to RTI with each hair specimen submission. Hair specimens were collected and analyzed from 38 drug users in this street environment. In addition, for purposes of this study, the head hair specimens collected from NIDA's Intramural Research Program (IRP) at the time of admittance and prior to controlled drug administration, which represented street drug use, were grouped and evaluated with this population. Head hair specimens from an additional 7 subjects were included in this

street drug-user population and all are referred to as STREET specimens, for a total of 38 subjects. Subject demographics, hair characterization, and the type of collection for the STREET population are listed in **Table 11.**

Table 9. Experimental Study Design and Hair Specimen Collection and Analysis Scheme for the STREET Population

Hair Specimen Collection																			
Subject Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Rep	1																			
	2																NA	NA	NA	NA
Subject Number		20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Rep	1																			
	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
TOTAL ANALYSES																				**53**

Note: NA = specimen unavailable; STREET = street drug user

Table 10. Experimental Study Design and Hair Specimen Collection and Analysis Scheme for Subjects Enrolled in NIDA's IRP Controlled Drug Administration Study in Which Cocaine Administration at Low and High Doses Occurred During a 9- to 10-Week Study Period (i.e., CLINICAL)

Hair Specimen Collection											
Subject Number	B	C	E	F	I	K	L	M	P	R	W
Low Dose (75 mg/70 kg)											
High Dose (150 mg/70 kg)					NA				NA		
TOTAL ANALYSES											**20**

Note: CLINICAL = clinically administered drug users; IRP = Intramural Research Program; NA = specimen unavailable; NIDA = National Institute on Drug Abuse

Table 11. Summary of Demographic Information for the STREET Drug-User Population

Subject	Race	Age	Sex	Hair Type	Schwarzkopf Scale (RTI Modified)	Location and Type of Hair Collection
STREET 1	C	43	F	Medium blonde/ dyed	8	Unspecified; street/native environment collection
STREET 2	C	20	F	Medium blonde	9	Unspecified; street/native environment collection
STREET 3	C	24	F	Medium blonde	7	Unspecified; street/native environment collection
STREET 4	C	42	M	Dark brown/ some gray	4	Unspecified; street/native environment collection
STREET 5	C	41	F	Reddish brown	6	Unspecified; street/native environment collection

Subject	Race	Age	Sex	Hair Type	Schwarzkopf Scale (RTI Modified)	Location and Type of Hair Collection
STREET 6	AA	36	F	Dark brown	3	Unspecified; street/native environment collection
STREET 7	C	24	F	Reddish brown/ dyed	6	Unspecified; street/native environment collection
STREET 8	C	52	M	Gray	10	Unspecified; street/native environment collection
STREET 9	C	44	M	Light brown	6	Unspecified; street/native environment collection
STREET 10	C	29	M	Dark brown	5	Unspecified; street/native environment collection
STREET 11	C	46	F	Medium brown	6	Unspecified; street/native environment collection
STREET 12	C	41	M	Dark brown	3	Unspecified; street/native environment collection
STREET 13	C	26	F	Blonde	8	Unspecified; street/native environment collection
STREET 14	C	48	M	Dark brown	5	Unspecified; street/native environment collection
STREET 15	UNK	UNK	UNK	Dark brown/ possibly colored	UNK	Unspecified; street/native environment collection
STREET 16	UNK	UNK	UNK	Light brown	5	Unspecified; street/native environment collection
STREET 17	UNK	UNK	UNK	Dark blonde	6	Unspecified; street/native environment collection
STREET 18	UNK	UNK	UNK	Dark blonde	6	Unspecified; street/native environment collection
STREET 19	UNK	UNK	UNK	Reddish brown	6.5	Unspecified; street/native environment collection
STREET 20	UNK	UNK	UNK	Dark blonde	6	Unspecified; street/native environment collection
STREET 21	UNK	UNK	UNK	Medium brown	4	Unspecified; street/native environment collection
STREET 22	UNK	UNK	UNK	Dark blonde	6	Unspecified; street/native environment collection
STREET 23	UNK	UNK	UNK	Ultra-light blonde	10	Unspecified; street/native environment collection
STREET 24	UNK	UNK	UNK	Medium blonde	7	Unspecified; street/native environment collection
STREET 25	UNK	UNK	UNK	Dark blonde	6	Unspecified; street/native environment collection
STREET 26	UNK	UNK	UNK	Light brown	5	Unspecified; street/native environment collection
STREET 27	UNK	UNK	UNK	Light brown	5	Unspecified; street/native environment collection
STREET 28	UNK	UNK	UNK	Light brown	5	Unspecified; street/native environment collection

Subject	Race	Age	Sex	Hair Type	Schwarzkopf Scale (RTI Modified)	Location and Type of Hair Collection
STREET 29	UNK	UNK	UNK	Dark brown	3	Unspecified; street/native environment collection
STREET 30	UNK	UNK	UNK	Light brown	5	Unspecified; street/native environment collection
STREET 31	UNK	UNK	UNK	Medium brown	4	Unspecified; street/native environment collection
STREET 32	C	34	M	UNK	UNK	AVH; admission to the clinical study
STREET 33	AA	39	M	Dark brown	3	Unspecified; admission to the clinical study
STREET 34	C	28	M	UNK	UNK	Unspecified; admission to the clinical study
STREET 35	AA	32	F	Dark brown	3	Unspecified; admission to the clinical study
STREET 36	AA	40	M	Dark brown	3	Unspecified; admission to the clinical study
STREET 37	AA	29	F	Dark brown	3	Unspecified; admission to the clinical study
STREET 38	C	26	M	UNK	UNK	Unspecified; admission to the clinical study

Note: STREET = street drug user; C = Caucasian; AA = African American; M = male; F = female; UNK = unknown; AVH = anterior vertex head hair

2.2.1.2 User Population—Clinically Administered Cocaine

Human hair collected during inpatient clinical studies was procured through collaboration with NIDA investigators. Participants in this study provided written informed consent, and the protocol was approved by NIDA's Institutional Review Board.

Detailed information about the participants and the study design was described in previous publications (Joseph et al., 1998; Huestis et al., 1999; Ropero-Miller et al., 2000; Kolbrich et al., 2003; Kacinko et al., 2005; Scheidweiler et al., 2005). Briefly, 11 healthy subjects voluntarily enrolled in a 9- to 10-week inpatient study conducted by the Chemistry and Drug Metabolism Section of the IRP, NIDA, and the National Institutes of Health. All subjects had a self-reported history of COC use and tested positive by urine drug tests. Medical and psychological evaluations were performed to verify each subject's health before participating in the study. All subjects provided informed consent and were compensated for their participation. Details of the subjects' demographics, head hair specimens, and dosages are provided in **Table 12.**

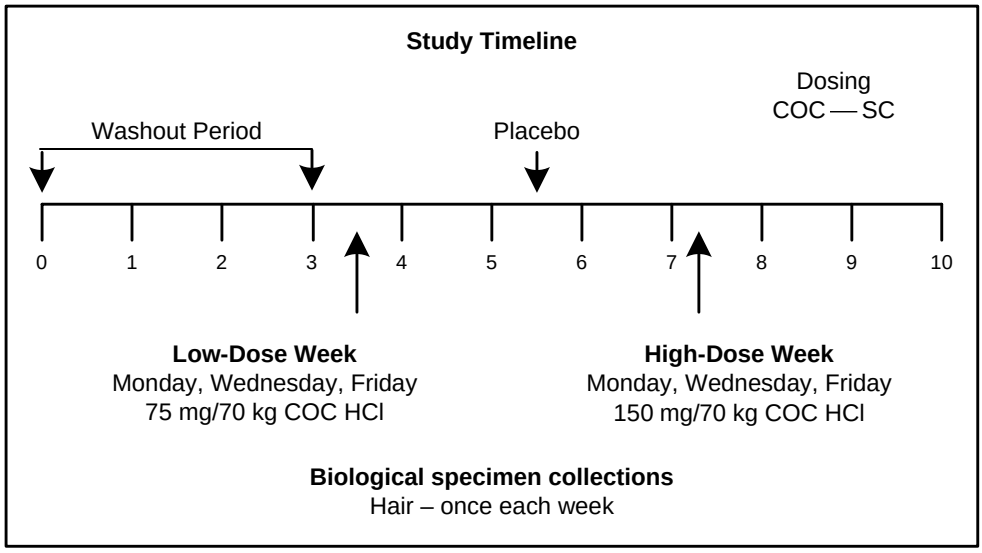
Table 12. Subjects Demographics and Hair Specimen Types in NIDA's IRP Controlled Drug Administration Study (i.e., Clinically Administered Drug-User Population)

Subject	Ethnicity	Age	Gender	Location Hair Collection	Time (Week)	Cocaine Dose (mg/70 kg)
B	C	34	M	AVH	Admission	UNK
				PVH	6	75 (low)
				FH	10	150 (high)
C	AA	39	M	Non-specific	Admission	UNK
C	AA	39	M	FH	6	75 (low)
C	AA	39	M	FH	10	150 (high)
E	C	28	M	Non-specific	Admission	UNK
E	C	28	M	PVH	6	75 (low)
E	C	28	M	PVH	10	150 (high)
F	AA	23	M	TH	6	75 (low)
F	AA	23	M	FH	10	150 (high)
I	AA	34	M	FH	6	75 (low)
K	AA	36	M	TH	6	75 (low)
K	AA	36	M	TH	10	150 (high)
L	AA	32	F	Non-specific	Admission	UNK
L	AA	32	F	PVH	6	75 (low)
L	AA	32	F	FH	10	150 (high)
M	AA	40	M	Non-specific	Admission	UNK
M	AA	40	M	TH	6	75 (low)
M	AA	40	M	TH	10	150 (high)
P	AA	29	F	Non-specific	Admission	UNK
P	AA	29	F	PVH	6	75 (low)
R	AA	35	F	AVH	6	75 (low)
R	AA	35	F	AVH	10	150 (high)
W	C	26	M	Non-specific	Admission	UNK
W	C	26	M	AVH	6	70
W	C	26	M	AVH	10	150 (high)

Note: AA = African American; AVH = anterior vertex head; C= Caucasian; F = female; FH = frontal head; IRP = Intramural Research Program; M = male; NIDA = National Institute on Drug Abuse; PVH = posterior vertex head; TH = temporal head; UNK = unknown

The time line for dosing and specimen collection is shown in **Figure 4**. Head hair was completely shaved the first day of the study (i.e., during admittance or Day 0), and weekly collection continued during the remainder of the study. Drugs were not administered during the first 3 weeks to allow time for all previously administered drugs, including COC, to be removed from the hair (i.e., the washout period). Hair was collected and analyzed during this period and

was determined to be mostly drug-free by the end of the washout period. Beginning in Week 4, subjects were administered a low dose of COC HCl powder (75 mg/70 kg) through subcutaneous injections on Monday, Wednesday, and Friday. Following low dosing, subjects were administered placebo doses subcutaneously during Weeks 7 and 8, observing the same daily schedule, with weekly head shaving. Beginning in Week 8, subjects were administered high doses of COC HCl (150 mg/70 kg) subcutaneously according to a similar dosing scheme. Specimen collection continued for 1 week after final dosing for follow up of drug elimination after high-dose administration. A total of 27 head hair specimens from 11 subjects were obtained from the specimen inventory of this NIDA IRP controlled drug administration study for inclusion in this research. Seven specimens that were collected at the time of admission were included in the STREET population because these were not collected under controlled drug administration and represented self-administration of street drugs (i.e., unknown COC dose). The remaining 20 head hair specimens from subjects were included as part of the controlled drug administration (clinically administered drug users [CLINICAL]) population.



COC = cocaine; HCl = hydrochloride salt; SC = subcutaneous
Adapted from Ropero-Miller et al., 2000

Figure 4. NIDA's IRP controlled drug administration study (i.e., clinically administered drug-user population) used the time line above for dosing and specimen collection.

2.2.2 Materials

Collection of Drug-User Hair Specimens by a Commercial Source—A commercial source pre-cleaned scissors with methanol, and then collected approximately 1 g of hair that was cut as close to the scalp as possible. After the specimens were collected, they were placed into sealed plastic bags at room temperature; any personally identifiable information was removed and shipped to RTI with limited demographic data when available.

Collection of Hair Specimens at NIDA's IRP During a Clinical Study—Hair was collected weekly by NIDA staff. For the first collection, cleaned grooming clippers were used to remove hair from different regions of the scalp (e.g., temporal, frontal, nape, posterior vertex, anterior vertex). For this study, hair was analyzed from the temporal, posterior vertex, and anterior vertex regions. Shaving cream and a straight-edge razor were used to remove remaining stubble, which was discarded. Hair from the first collection was stored in sealed plastic bags at

room temperature until it was finely cut with scissors and transferred to separate glass vessels for storage at −30ºC. For the remainder of the study, a cleaned electrical shaver was used to collect hair (approximately 2–3 mm in length) as close to the scalp as possible. Remaining stubble was removed and discarded with shaving cream and a straight-edge razor. Hair specimens only represented 1 week of growth; these pieces were weighed and analyzed for COC analytes. A portion of each specimen was sent to RTI for analysis.

Collection of Hair Specimens by RTI for Stage II of This Study—Human hair specimens were collected from three sources to meet all requirements for different variables and hair types. All specimens collected were human head hair that was cut with scissors from various regions of the scalp (e.g., frontal, temporal, posterior vertex, anterior vertex) and shaved with a cleaned electric razor close to the scalp (i.e., drug-user hair, clinical hair specimens after Week 1) or was not noted what was root end (drug-free hair, STREET population, and some CLINICAL population hair specimens before Week 1).

Drug-free hair was collected for use as blind negative analytical controls for Stage II of this research. Professional cosmetologists collected drug-free dark and light hair samples, which were not chemically treated (e.g., straightened, permanent waved, colored), during the normal process of a hair cut. If available, cosmetologists also collected demographic information (e.g., gender, age, ethnicity) to submit to RTI with hair specimens. The sources of these data are not publicly available. RTI collected information and recorded it in such a manner that human subjects cannot be identified, directly or through identifiers linked to the subjects. All drug-free hair specimens were analyzed for analytes of interest before inclusion in this study. Drug-free hair was used as a negative control specimen and was shipped in a blinded manner, appearing as actual study specimens, to the Immunalysis Corp. for quality assurance purposes. This drug-free hair was also used as the precursor for the contaminated hair specimens and the fortified proficiency specimens detailed in Stages III and IV of this research.

Preparation of Drug-User Hair Specimens for Shipment to the Reference Hair-Testing Laboratory—Before shipping hair specimens to the reference laboratory, all drug-user hair specimens were subjected to a decontamination procedure as described in Section 2.2.3.1. All specimens were labeled with unique identifiers that could not be linked to the type of hair specimen being submitted for analysis. Approximately 100 specimens were analyzed for Stage II of this study.

2.2.3 Methods

2.2.3.1 Hair Characterization and Decontamination Procedures

Characterization of Hair Specimens—The Schwarzkopf color scale, which is used by professional cosmetologists to categorize hair color, was modified by RTI (**Table 13**) to determine hair color (1 = black, up to 10 = light blonde and gray) samples during this study. One person visually and physically determined all hair specimens by hand, and another individual confirmed these determinations separately (Schwarzkopf, 2001). Prior to fortification, the hair was received into the inventory, weighed, and visually evaluated for color using the Schwarzkopf scale modified by RTI.

Table 13. RTI's Color Scale Based on the Schwarzkopf Scale

Hair Color	Schwarzkopf Scale
Black	1
Dark brown	2 through 4
Brown	5 and 6
Light brown	7
Medium blonde	8 and 9
Light blonde and gray	10

Decontamination of Hair Specimens—Prior to sending the specimens to reference hair-testing laboratories, RTI decontaminated the hair by using a protocol published by Cairns and colleagues (2004b), which employed the use of an extended aqueous buffer wash. Following the receipt procedure, including characterization, each 50 mg–100 mg hair specimen was shaken vigorously at 120 rpm at 37°C for 15 minutes in 20 mL of isopropanol. Hair specimens were then shaken at 120 rpm in sufficient 0.01 M phosphate buffer with 0.01% bovine serum albumin (pH 6) for 30 minutes at 37°C for three 30-minute intervals and two 60-minute intervals, and the phosphate buffer was replaced after each wash step. 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-minute buffer washes using the same conditions. Specimens were allowed to dry overnight before aliquoting and preparing them for shipment to the reference laboratories.

2.2.3.2 Data Collection and Statistical Analysis

All statistical analyses were performed using SAS (version 9.1.3) or Microsoft Excel. For Stage II comparisons, a one-way analysis of variation or student t-test was used. Specimens were analyzed in singular or in replicate as presented in Tables 9 and 10.

2.2.4 Analytical Procedures

All specimens were submitted to Immunalysis Corp. for Stage II analyses by established standard operating procedures for performing hair drug testing. This laboratory quantified COC analytes in the hair specimens and was compensated for performing the analytical work. Appropriate digestion methods for hair were selected to maintain all COC analyte concentrations with minimal COC hydrolysis to BE, which can be more labile under many hair-digestion methods. Matrix-matched quality controls were included in the analysis to monitor for hydrolysis, with ≤5% considered acceptable.

Liquid Chromatography-Tandem Mass Spectrometry—LC-MS/MS was used for hair testing according to previously published and peer-reviewed methods (Moore et al., 2007). Quantitative analytical procedures for determining COC, BE, CE, and NCOC in hair were performed on an Agilent Technologies 1200 Series liquid chromatograph pump coupled to a 6410 triple quadrupole mass spectrometer that was operated in positive APCI mode. For confirmation, two transitions were monitored, and, in some cases, one ion ratio was determined and found to be acceptable if it was within 20% of the ratio performance of known calibration standards. **Figure 5** and **Table 14** show representative LC-MS/MS chromatography and validation statistics for the primary reference laboratory's (Immunalysis Corp.) method used for hair analysis for this study.

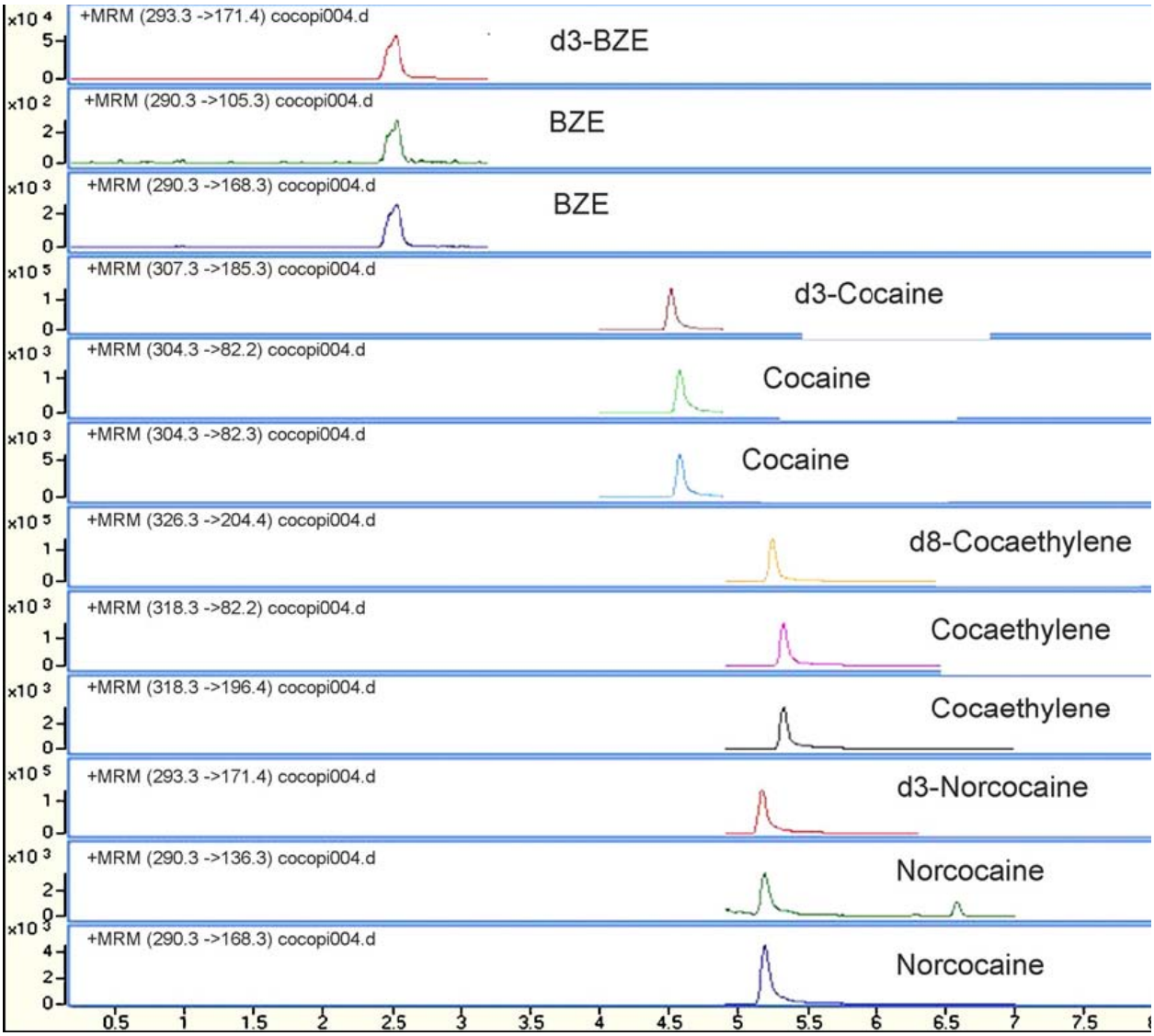


Figure 5. LC-MS/MS chromatography performance from Immunalysis Corp. for a standard material at 50 pg/mg.

Table 14. Validation Statistics for the Primary Reference Laboratory's (Immunalysis Corp.) Method Used for Hair Analysis and Reported to RTI for This Research

LC-MS/MS Operating in APCI Mode	COC	BE	CE	NCOC
Limit of detection (pg/mg)	25	25	25	25
Limit of quantification (pg/mg)	50	50	50	50
Limit of linearity (pg/mg)	10,000	10,000	10,000	10,000
Accuracy; *n* value	5	5	5	5
50 pg/mg target; % accuracy	99.9	101.7	99.3	108.0

LC-MS/MS Operating in APCI Mode	COC	BE	CE	NCOC
100 pg/mg target; % accuracy	101.4	93.7	92.5	88.4
200 pg/mg target; % accuracy	94.5	94.3	88.4	86.1
Within run imprecision data; *n* value	5	5	5	5
Target concentration 1 (pg/mg)	100	100	100	100
Coefficient of variation (% CV)	1.3	8.1	0.8	0.4
Between run imprecision data; *n* value	10	10	10	10
Target concentration 1 (pg/mg)	100	100	100	100
Coefficient of variation (% CV)	4.8	9.2	15.7	12.6
% recovery data; *n* value	3	3	NR	NR
% Recovery	82.7	93.8	NR	NR

Note: APCI = atmospheric pressure chemical ionization; BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NCOC= norcocaine; NR = not reported

2.2.5 Modifications to the Research Design and Rationale

The specimen number in some population groups may have changed from those originally proposed, but the overall number of specimens analyzed for COC analyte concentrations and ratios was maintained and statistical analysis procedures were chosen appropriately for the final population. For example, the proposed number from the CLINICAL population was approximated to be 40 specimens from two separate clinical studies. Ultimately, 20 specimens were collected for this population for which replicate analyses were not performed. Hair from COC-using pregnant women (n = ~20) were not available and were not included in this study. However, 10 subjects proposed for the STREET population were included, and a total of 38 were included in the final study. Head hair from approximately 100 subjects was proposed for this study, and specimens from 185 subjects were actually included. A total of 420 specimens, including controls and replicate analyses, were originally proposed for the evaluation of COC analyte concentration and ratios in hair, and approximately 400 were ultimately analyzed.

2.2.6 Findings

An LC-MS/MS method for COC analytes was used for analyzing head hair from two drug-user populations. Results are reported for 38 hair specimens from the STREET group and 20 from the CLINICAL.

The STREET population was identified from hair specimens collected after self-reported street use by the volunteers. The mean, median, and concentration range were determined for each COC analyte. Data for analytes above the LOQ appear in **Table A-3** and are summarized as follows:

- 38 detectable results for COC at 159 to 218,276 (mean: 27,889; median: 12,470) pg/mg
- 38 detectable results for BE at 65 to 67,253 (mean: 8,132; median: 1,746) pg/mg

- 20 detectable results for CE at not detected to 5,003 (mean: 901; median: 436) pg/mg
- 32 detectable results for NCOC at not detected to 1,810 (mean: 345; median: 253) pg/mg.

Although the parent drug, COC, and BE were detected in all subjects, the other two COC analytes were not detected in 16% (NCOC) to 47% (CE) of the STREET population. Mean concentrations were higher than the median for all analytes. Comparing each subject's results to the SAMHSA confirmatory cut-off concentrations (Table A-3) shows that the number of subjects and percentages of positive results for the STREET population were as follows: 37 subjects tested positive for COC (97%), 38 for BE (100%); 18 for CE (47%), and 27 for NCOC (71%). For BE, 1 subject (STREET 35) met the confirmatory cut-off concentration of 50 pg/mg, but did not meet the criteria of a BE/COC ratio of ≥0.05.

To compare COC analyte concentrations and metabolite-to-parent drug concentration ratios, nine decision points of positive calls on confirmatory analytical results (**Table 15**) were applied to the STREET and CLINICAL populations of this study. Criteria 1 through 3 are the originally proposed criteria of the Mandatory Guidelines. The additional decision points were selected based on a review of the data and previously proposed criteria (Schaffer et al., 2007). Criteria 4 and 5 evaluate the norcocaine-to-cocaine (NCOC/COC) ratios of ≥0.05 and ≥0.01, respectively. Criteria 6 through 9 evaluate the cocaethylene-to-cocaine (CE/COC) ratios of ≥0.05, ≥0.02, ≥0.01, and ≥0.002, respectively. Criteria 1 through 3 were included in all subject data tables as an example of findings; additional criteria were not included for brevity. Results of COC by using LC-MS/MS for the STREET population are shown in **Tables A-3** and **A-4**.

Table 15. Number of Positive Calls and Percentages for Evaluated Cocaine (COC) Analyte Criteria for the Drug-User Population with Self-Administered COC in a Street Environment (STREET Group; Uncontrolled COC Administration; *n* = 38)

Drug-User Population: Street Environment (Uncontrolled Administration): STREET		Number of Positive Calls	Percentage
Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05	36	94.7
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg	19	50.0
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg	33	86.8
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05	0	0.0
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01	24	63.2
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05	3	7.9
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02	8	21.1
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01	12	31.6
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002	17	44.7

Note: BE = benzoylecgonine; BE/COC = benzoylecgonine-to-cocaine ratio; CE = cocaethylene; CE/COC = cocaethylene-to-cocaine ratio; COC = cocaine; NCOC= norcocaine; NCOC/COC = norcocaine-to-cocaine ratio; STREET = street drug user

For the STREET group, only 50% of the population was positive for CE (Criteria 2: 19 of 38 subjects were positive). When the CE criteria (Criteria 2) for each subject's results was compared to the BE criteria (Criteria 1), there was one subject (i.e., STREET 35) that met the CE criteria, but did not meet the BE criteria. This same subject (i.e., STREET 35) also met the NCOC criteria, but did not meet the BE criteria. Conversely, there were four subjects (i.e., STREET 2, 5, 11, 30) that met the BE criteria (Criteria 1), but not the NCOC criteria. Therefore, there was not an increase in the COC confirmatory rate when additional COC analytes beyond COC and BE were evaluated with the proposed cut-off concentrations (e.g., change in confirmation ruling for a subject).

If additional criteria for the NCOC/COC and CE/COC ratios were considered (e.g., ≥0.05 or ≥0.01, ≥0.002) for the STREET population, the following conclusions can be drawn (Table A-4). At an NCOC/COC ratio requirement of ≥0.05 (Criteria 4), all subjects tested negative with the inclusion of this additional decision point. Nineteen subjects met the NCOC cut-off concentrations of ≥50 pg/mg, and an additional 9 subjects (i.e., STREET 7, 16, 17, 19, 23, 24, 25, 32, 34) also had a NCOC/COC ratio of ≤0.01 (Criteria 5). Inclusion of a ≥0.05 or a ≥0.01 NCOC/COC ratio did not give similar confirmation rulings to the current proposed Mandatory Guidelines for BE (Criteria 1: 36 of 38 subjects, [94.7%]) or NCOC (Criteria 3: 33 of 38 subjects, [86.8%]). Both evaluated decision points of an NCOC/COC ratio were too high for the STREET population and confirmed, at most, 24 of 38 subjects (63.2%), which is more than a 30% reduction of positive calls for this drug-user group.

Criteria 6 through 9 had decision points of the CE/COC ratio and gave the following positive calls or confirmation rulings: ≥0.05 (3 of 38 subjects [7.9%]), ≥0.02 (8 of 38 subjects, [21.1%]), ≥0.01 (12 of 38 subjects, [31.6%]), and ≥0.002 (17 of 38 subjects [44.7%]). None of the CE/COC ratio criteria were similar to the proposed Mandatory Guidelines for the BE concentration and the BE/COC ratio. For the most part, the CE concentration requirement of ≥50 pg/mg is largely the determining factor (19 of 38 subjects [50%]). Even a CE/COC ratio of ≥0.002 would not be equivalent to the proposed BE and CE criteria for the STREET population (Table A-4).

For the STREET group, the BE concentration in hair was 29% of the COC concentration (8,132 pg/mg versus 27,889 pg/mg), the CE was 3% of the COC concentration (901 pg/mg versus 27,889 pg/mg), and NCOC was 1% of the COC concentration (345 pg/mg versus 27,889 pg/mg).

Results from a second drug-user population (i.e., CLINICAL) in Stage II of this study were included to evaluate COC analytes in hair after controlled COC administration. Hair specimens were collected during Week 6 and Week 10, the weeks determined to be the peak concentration of COC in hair after low and high dosing, respectively. RTI compared these results to the STREET group and others from literature below (i.e., Cairns et al., 2004a; Bourland et al., 2000). COC analyte mean, median, and concentration ranges (pg/mg) for each COC analyte were determined for the CLINICAL subjects and the above the LOQ counts are summarized as follows:

- 20 detectable results for COC at 725 to 32,786 (mean: 6,171; median: 4,141) pg/mg
- 17 detectable results for BE at not detected to 1,501 (mean: 434; median: 336) pg/mg
- 7 detectable results for CE at not detected to 397 (mean: 123; median: 75) pg/mg

- 14 detectable results for NCOC at not detected to 2,075 (mean: 290; median: 142) pg/mg.

COC analyte concentrations in all of the CLINICAL subjects were substantially lower than the other drug-user populations evaluated. Although COC was detected in the hair of all of the CLINICAL subjects, NCOC was the next most detected COC analyte (70%) followed by BE (57%) and CE (35%). Again, the mean concentration was higher than the median for all COC analytes. Comparing the results from each subject to the SAMHSA confirmatory cut-off concentrations (**Table A-5** shows that the number of subjects and the percentages of positive results for the CLINICAL population receiving known doses of COC, based on concentrations alone, were as follows: 20 subjects tested positive for COC (100%), 14 subjects tested positive for BE (70%), 6 subjects tested positive for CE (30%), and 14 subjects tested positive for NCOC (70%). For BE, three subjects (i.e., CLINICAL 2, 17, 20) met the confirmatory cut-off concentration of ≥50 pg/mg, but did not meet the criteria of a BE/COC ratio of ≥0.05. Results of testing for COC by using LC-MS/MS for the CLINICAL population are shown in Table A-5.

For the CLINICAL group, a smaller percentage of the population tested positive for CE (Criteria 2: 6 out of 20 subjects [30%]). When the CE criteria (Criteria 2) for each subject's results was compared to the BE criteria (Criteria 1), there were three subjects (i.e., CLINICAL 2, 17, 20) who met the CE criteria, but did not meet the BE criteria. These same subjects also met the NCOC criteria, but did not meet the BE criteria. Conversely, there were four subjects (i.e., CLINICAL 1, 7, 11, 12) who met the BE criteria (Criteria 1), but did not meet the NCOC criteria. Therefore, there was not an overall increase in the COC confirmatory rate when additional COC analytes beyond COC and BE were evaluated with the proposed cut-off concentrations (e.g., change in confirmation ruling for a subject) for the CLINICAL subjects. **Table 16** shows the criteria used for decision points of positive calls on analytical results for CLINICAL subjects.

Table 16. Number of Positive Calls and Percentages for Evaluated COC Analyte Criteria for the Drug-User Population with Controlled COC Administration in the NIDA IRP Clinical Research Facility (CLINICAL Group; *n* = 20)

Drug-User Population: NIDA IRP Study Subjects (Controlled Clinical Administration): CLINICAL		Number of Positive Calls	Percentage
Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05	14	70.0
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg	6	30.0
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg	14	70.0
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05	3	15.0
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01	14	70.0
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05	0	0.0
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02	0	0.0

Drug-User Population: NIDA IRP Study Subjects (Controlled Clinical Administration): CLINICAL		Number of Positive Calls	Percentage
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01	2	10.0
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002	6	30.0

Note: CE = cocaethylene; CE/COC = cocaethylene-to-cocaine ratio; CLINICAL = clinically administered drug user; COC = cocaine; NCOC= norcocaine; NCOC/COC = norcocaine-to-cocaine ratio

If additional criteria for NCOC/COC and CE/COC ratios were considered (e.g., ratios of ≥0.05 or ≥0.01, ≥0.002) for the CLINICAL population (**Table A-6**), the conclusions are as follows: at an NCOC/COC ratio requirement of ≥0.05 (Criteria 4), three subjects (15%) tested positive. An NCOC/COC ratio of ≥0.01 indicated that 14 subjects would have confirmed positive by Criteria 5, which is equivalent to Criteria 3 (i.e., NCOC cut-off concentrations of ≥50 pg/mg). For the CLINICAL population, an NCOC/COC ratio was also equivalent to the BE (Criteria 1) positive call. The CLINICAL population most likely had a much smaller administered dose of COC compared to dosages commonly ingested by drug-user populations in a street environment. Self-administered doses by most common routes (i.e., nasal insufflation or intravenous injection of the hydrochloride salt or smoking of the free base form) range from 10 mg–120 mg; commonly multiple doses are taken in a very short time period by chronic users. Doses of 750 mg to 2,000 mg have been reportedly ingested by chronic cocaine users on a given day of drug use (Baselt, 2008). Therefore, the dose and the amounts of hair collected were lower than routine self-administered doses and could have affected the COC analyte disposition into hair. As evidence by the average concentrations, BE concentration in hair was 7% of the COC concentration (434 pg/mg versus 6,171 pg/mg), the CE was 2% of the COC concentration (123 pg/mg versus 6,171 pg/mg), and the NCOC was 5% of the COC concentration (290 pg/mg versus 6,171 pg/mg).

Criteria 6 and 7, decision points of the CE/COC ratios of ≥0.05 and ≥0.02, had no positive calls or confirmation rulings, and ≥0.01 yielded 2 of 20 positive subjects (10%) in Criteria 8. For the ≥0.002 CE/COC requirement (Criteria 9), the same 6 subjects who had CE at ≥50 pg/mg were positive by this additional ratio criteria (30%), but this was still a 40% reduction of positive calls using CE instead of BE criteria.

Finally, statistical evaluation of these drug-user groups, along with other data reported in the literature, was performed. **Table 17** shows that the CLINICAL specimens have significantly lower COC and BE concentrations than the STREET specimens, as well as the results of two additional drug-user populations reported in the peer-reviewed literature by Cairns and colleagues (2004a) and Bourland and colleagues (2000) and the Cairns' reported specimens. This is consistent with the likely lower dosages than self-reported users are likely using. There were no significant differences in the CE concentrations between any of the groups. For NCOC, only the results reported by Cairns and colleagues were significantly higher than the other groups.

Table 17. Analysis of Variance Comparison of the Four Groups of Users

	Average pg/mg				Ratios		
Groups	**COC**	**BE**	**CE**	**NCOC**	**BE/ COC**	**CE/ COC**	**NCOC/ COC**
NIDA–IRP specimens (CLINICAL)	6,171	434	123	290	0.070	0.010	0.034
Self-reported street users (STREET)	27,889	8,132	901	345	0.374	0.046	0.015
Results reported by Cairns and colleagues (2004a)	43,038	5,353	1,218	1,174	0.128	0.040	0.027
Results reported by Bourland and colleagues (2000)	10,348	1,327	1,590	325	0.160	0.133	0.039
Analysis of variance results for each drug or ratio							
F values	6.67	4.73	0.75	8.68	26.54	5.82	7.64
P values	0.0003	0.0035	0.526	<0.0001	<0.0001	0.0011	<0.0001
Yellow highlighted values are significantly different from blue highlighted values, but are not significantly different from each other.							
Blue highlighted values are significantly different from orange highlighted values, but are not significantly different from each other.							
Green highlighted values are significantly different from the other values, and all other values are not significantly different from each other.							

Note: BE = benzoylecgonine; BE/COC = benzoylecgonine-to-cocaine ratio; CE = cocaethylene; CE/COC = cocaethylene-to-cocaine ratio; CLINICAL = clinically administered drug user; COC = cocaine; IRP = Intramural Research Program; NCOC = norcocaine; NCOC/COC = norcocaine-to-cocaine ratio; NIDA = National Institute on Drug Abuse; STREET = street drug user

CE results were not significantly different for the comparison of all drug-user groups. These CE results suggest that the presence and concentration of CE is highly variable and does not relate well to the amount of COC exposure. This high variability is supported by the lack of any significant difference between the CLINICAL specimens and self-reported users (i.e., other three drug-user populations) even with a 10-fold difference in the mean.

2.2.7 Conclusions

RTI obtained 38 drug-user hair specimens after COC use occurring in a native street environment where the dose was unknown (i.e., uncontrolled COC administration), and these specimens were analyzed in the first drug-user population (STREET). Seven hair specimens collected for NIDA's IRP study at the time of admission were also included in this group because this head hair represented the same uncontrolled COC administration or street environment.

RTI results were further compared to two drug-user groups previously reported in the literature by Cairns and colleagues (2004a) and Bourland and colleagues (2000). **Table 18** reports the COC concentrations and ratios for these two drug-user populations.

A review and evaluation by the same COC analyte criteria was performed on the Cairns and colleagues data. For BE, two subjects (i.e., Subjects 69 and 70) met the criteria of a BE/COC ratio of ≥0.05, but the BE concentration was not high enough for the subjects to confirm positive for COC. Furthermore, when the CE criteria (Criteria 2) for each subject's results was compared to the BE criteria (Criteria 1), 31 subjects met the BE criteria, but did not meet the CE criteria. Similarly, when the NCOC criteria (Criteria 3) for each subject's results were compared to the BE criteria (Criteria 1), four subjects (i.e., Subjects 52, 7, 57, 54) met the BE criteria, but did not

meet the NCOC criteria. Conversely, there was one subject (i.e., Subject 32) who met the CE and NCOC criteria, but did not meet the BE criteria (Criteria 1). Therefore, for Cairns' drug-user population, there was no significant advantage (<3%) to evaluate additional COC analytes beyond COC and BE. If additional criteria for CE/COC and NCOC/COC ratios were considered (e.g., ratio of ≥0.05 for CE/COC or ≥0.01 for NCOC/COC), further conclusions were evident. An NCOC/COC ratio of ≥0.01 would give similar confirmed positive results as the BE criteria (64 of 75 subjects [85.3%] compared to 66 of 75 subjects [88.0%]). If the NCOC/COC ratio was set higher to ≥0.05, the number of positive calls was greatly reduced (4 of 75 subjects [5.3%]).

RTI also evaluated the Cairns' drug-user population using the decision points using a CE/COC ratio (Criteria 6 through 9). In contrast, none of the decision points for the CE/COC ratio was comparable to the proposed BE criteria, including a BE/COC ratio (Criteria 1). Results for Cairns' drug user-population using CE/COC decision points were as follows: ≥0.05: 11 of 75 subjects (14.7%), ≥0.02: 16 of 75 subjects (21.3%), ≥0.01: 22 of 75 subjects (29.3%), and ≥0.002: 33 of 75 subjects (44.0%). These decision points were expected because only 36 of the 75 subjects (48%) had a CE concentration of ≥50 pg/mg. Hence, a CE/COC ratio of ≥0.002 would not confirm as many positive results as the currently proposed BE/COC ratio criteria.

The nine decision points for defining a positive test by confirmatory analytical results (Table 17) were also applied to the data from Bourland and colleagues (2000). When the CE criteria (Criteria 2) for each subject's results was compared to the BE criteria (Criteria 1), Subject P (3.3%) met the CE criteria, but did not meet the BE criteria. When the NCOC criteria (Criteria 3) for each subject's results was compared to the BE criteria (Criteria 1), four subjects (i.e., Subjects C, P, V, U), or 13%, met the NCOC criteria, but did not meet the BE criteria. Conversely, there were five subjects (i.e., Subjects B, G, H, I, R) who met the BE criteria (Criteria 1), but did not meet the NCOC criteria. Unlike data from Cairns and colleagues (2004a), for Bourland's drug-user population, there was nearly a 15% increase in COC confirmatory rate when additional COC analytes beyond COC and BE were evaluated with the proposed cut-off concentrations; however, this was in agreement with the workplace population of more than 6,000 subjects reported by Cairns and colleagues (2004a) in the same study.

If additional criteria for CE/COC and NCOC/COC ratios were considered (e.g., ratio of ≥0.05, ≥0.01, or ≥0.002) for the drug-user population studied by Bourland and colleagues (2000), the conclusions are as follows: an NCOC/COC ratio of ≥0.01 indicated that there were three subjects (i.e., Subjects C, V, Y) who would have tested positive by the NCOC/COC ratio, whereas five subjects (i.e., Subjects B, G, H, I, R) would not test positive because these subjects had undetectable NCOC concentrations. A decision point of an NCOC/COC ratio of ≥0.05 was too high for this drug-user population, only confirming 6 of 30 subjects (20%). An NCOC/COC ratio of ≥0.01 (21 of 30 subjects [70%]) would be required to meet a similar positive confirmation rate as the proposed BE (24 of 30 subjects [80%]) and NCOC criteria (23 of 30 subjects [76.7%]). Similarly, a CE/COC ratio of ≥0.05 would be too high (12 of 30 [40%]) to confirm positive results because of the BE and CE criteria (24 of 30 subjects [80.0%] and 18 of 30 subjects [60.0%]). Decision points of the CE/COC ratios of ≥0.02 (16 of 30 subjects [53.3%]); ≥0.01 (17 of 30 subjects [56.7%]); and ≥0.002 (18 of 30 subjects [60.0%]) were equivalent to the proposed BE and CE criteria.

For direct comparison, these drug-user populations were evaluated using the nine decision points used to evaluate RTI drug-user groups (**Table 19**). These data show that the proposed CE and

NCOC cut-off concentrations of ≥50 pg/mg (Criteria 2 and 3) do not identify as many subjects as the proposed BE criteria, although NCOC is within a few percentage points of BE data for both groups. Furthermore, a NCOC/COC ratio of ≥0.01 was comparable to the BE/COC ratio for one group (Cairns) but not for the other (Bourland). For a CE/COC ratio, even a ≥0.002 was not comparable to the BE/COC ratio. This is attributed primarily to no to low CE concentrations in these drug populations. Requiring a CE/COC ratio as low as ≤0.002 would not be practical to most forensic drug testing applications. This is additionally supported by the highly variable CE results from the four groups in which there was no statistically significant difference between the groups even though there was a 10-fold difference in the mean CE concentrations between the clinical group and some of the self-reported users.

Thus, evaluating the COC analyte concentrations in four separate drug-user populations, in which COC was administered in both controlled and uncontrolled environments, suggests that using CE and NCOC and their ratios to parent COC do not identify drug-user populations more effectively than COC and BE criteria in the proposed Mandatory Guidelines for hair drug testing. RTI's investigation of nine criteria yielded similar findings for these four drug-user populations (there was no significant advantage in confirmation result when evaluating additional COC analytes beyond COC and BE). As discussed in Section 3, using analyte ratios provides more information and some ability to discriminate user specimens from contaminated specimens; however, using CE and NCOC concentrations and ratios does not discriminate any more efficiently than does decision criteria using only BE and COC.

Table 18. Number of Positive Calls and Percentages for Evaluated COC Analyte Criteria for the Drug-User Population with Uncontrolled, Self-Administered COC in a Street Environment Reported by Cairns and Colleagues (2004a) and Bourland and Colleagues (2000)

Drug-User Populations: Literature Review		(Cairns et al., 2004a) n = 75		(Bourland et al., 2000) n = 30	
		Number of Positive Calls	Percentage	Number of Positive Calls	Percentage
Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05	66	88.0	24	80.0
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg	36	48.0	18	60.0
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg	64	85.3	23	76.7
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05	4	5.3	6	20.0
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01	64	85.3	21	70.0
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05	11	14.7	12	40.0
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02	16	21.3	16	53.3
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01	22	29.3	17	56.7

Drug-User Populations: Literature Review		(Cairns et al., 2004a) n = 75		(Bourland et al., 2000) n = 30	
		Number of Positive Calls	Percentage	Number of Positive Calls	Percentage
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002	33	44.0	18	60.0

Note: BE = benzoylecgonine; BE/COC = benzoylecgonine-to-cocaine ratio; CE = cocaethylene; CE/COC = cocaethylene-to-cocaine ratio; COC = cocaine; NCOC = norcocaine; NCOC/COC = norcocaine-to-cocaine ratio

Table 19. Cocaine Analyte Concentrations and Ratios Criteria for Determining Confirmation Results as Positive or Negative

Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002

Note: BE = benzoylecgonine; BE/COC = benzoylecgonine-to-cocaine ratio; CE = cocaethylene; CE/COC = cocaethylene-to-cocaine ratio; COC = cocaine; NCOC = norcocaine; NCOC/COC = norcocaine-to-cocaine ratio

2.3 Stage III: Contamination of Hair with Refined Illicit COC Specimens

2.3.1 Experimental Design

The purpose of the hair contamination was to investigate the ratios and dynamics of the drug detected in hair after known contamination. After the hair was contaminated, RTI wanted to determine if the most effective decontamination protocol that was previously examined could reliably differentiate contaminated hair from the other sources of drug-containing hair examined in the study. Additionally, because RTI previously reported that BE ratios with COC in hair may not distinguish contaminated hair from drug user hair based on the proposed Mandatory Guidelines, and that illicit COC may contain varying and high quantities of NCOC and CE as by-products of manufacture, we wanted to determine what ratios of these compounds would be observed in known contaminated hair. RTI used three different sources of COC (i.e., two illicit COC materials and one USP pharmaceutical-grade COC), which provided a range of BE, CE, and NCOC concentrations. Both dark and light hair specimens were used in the contamination study as a preliminary investigation of potential hair color effects on the study design.

RTI used a contamination model that was previously published for this experiment with approximately one-half of the quantity of COC per gram of hair. Although this quantity of COC has been criticized by Schaffer and colleagues (2007) as being too high, little information is available on the amount of a drug that exists on surfaces where drug use or handling has

occurred. Some research has addressed the potential surface contamination of currency (Jenkins, 2001), the potential exposure of police based on a self-report (Mieczkowski and Lersch, 2002), and the exposure of personnel handling large quantities of a drug as determined by personal breathing space measurements and urine assays (Stout et al., 2006a). However, these studies do not provide sufficient evidence about a given drug's potential surface exposure amounts.

More pertinent studies have been conducted, but they are not in the peer-reviewed literature. The DEA, in conjunction with the National Jewish Medical Center in Denver, CO, conducted several studies to estimate the exposure potential of workers who process and clean-up sites where methamphetamines have been manufactured or smoked (Martyny et al., 2005). Although the main goal of these studies was to estimate the occupational hazards faced by these workers, the investigators collected surface specimens from known methamphetamine cook sites, and they also conducted controlled methamphetamine cooks (preparation of methamphetamine from chemical precursors in a clandestine environment or STREET production) to verify the numbers that were obtained from illicit sites. Martyny and colleagues (2005) reported the following:

> Methamphetamine contamination of buildings employed in the manufacture of methamphetamines has been a common finding during all of our test cooks and in all methamphetamine laboratories that we have investigated. Even labs that had been shutdown several months prior to testing still had high contamination levels of methamphetamines present on many surfaces within the building with specimens as high as 16,000 μg/sample and most specimens over 25 μg/100 cm^2.

Martyny and colleagues (2005) also sampled personal protective equipment on DEA personnel who prepared the methamphetamines before and after decontamination and found that wet decontamination procedures may move contamination onto the individual's body. Specimens taken after the personnel were decontaminated revealed that levels of methamphetamines were still present on the personal protective equipment and on their hands. Carpeting outside the cook area sites were also sampled up to 20 feet away, and substantial amounts of methamphetamines (up to 12.4 μg/100 cm^2) were found, indicating that methamphetamines could easily be tracked away from the cook site. These preliminary findings for methamphetamines demonstrate that there is a significant contamination potential in illicit drug use sites. Obviously, the manufacture methods for COC and methamphetamines are different, but the extensive contamination potential with methamphetamine suggests that COC surface contamination may be extensive, as well in locations where use or handling of COC has occurred.

It is crucial to the legal acceptance of hair-testing results that it be possible to differentiate environmentally exposed individuals from drug users, given current analytical technologies and results interpretation.

For the contaminated hair experiments, a cosmetologist obtained two samples: one brunette hair specimen from a 24-year-old female subject and a blonde specimen from a 13-year-old female subject. The cosmetologist reported that neither subject's hair was chemically treated or exhibited signs of any chemical treatments. These hair specimens had been previously rinsed three times with distilled water to remove any styling material residues, and then the specimens were stored dry and protected from light.

From these two specimens, 8-g portions were weighed for each of the surface contamination treatments. Three separate COC materials were used to contaminate the hair as indicated in **Figure 6**. So, for each COC material, dark and light hair specimens (one each) were contaminated for a total of six contaminated hair specimens. The use of hair samples such as these has been criticized by Schaffer and colleagues (2007) as not representative of their preferred method of hair sampling which is to use the first 1.5 inches of hair from the scalp. We have continued to use the large hair samples from donors to provide more homogenous hair materials from one individual, thus simplifying inter-individual variation for this study. Also, although Schaffer and colleagues may be able to routinely obtain such collections from the scalp, other laboratories conduct hair testing and there are no standardized methods of hair collection, so end-strand and mid-shaft samples may be representative of what some laboratories obtain.

The design of the experiment was adapted from Stout and colleagues (2006b), and it was a three-way cross-design with sub-sampling. The factors investigated were time, hair type, and COC source. Specimens were removed at 13 time points with respect to contamination (i.e., pre-contamination, 1 hour, 6 hours, 27 hours, and weekly) during a 70-day period (Figure 6).

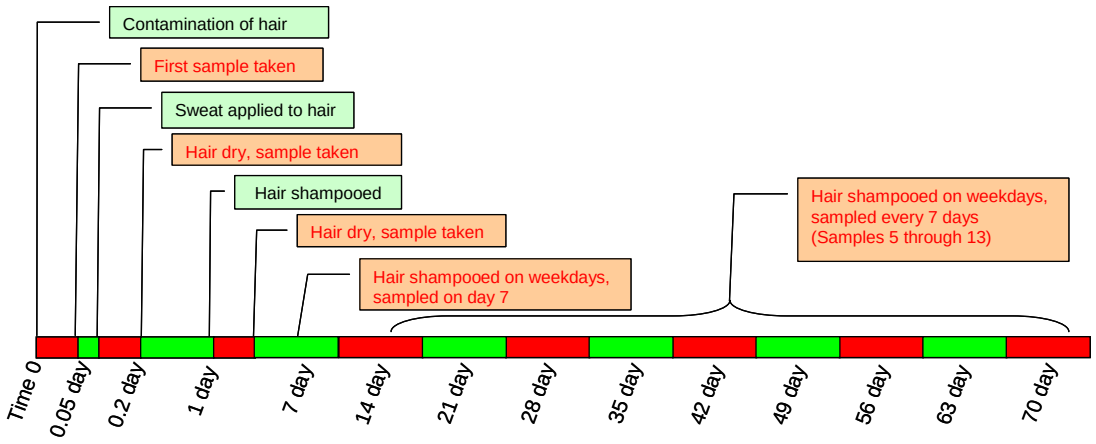


Figure 6. The sampling design, including contamination, sweat treatment, and sampling, that was used in this study.

All hair types were contaminated with COC, subjected to a treatment with synthetic sweat after 1 hour, and shampooed each weekday evening (i.e., Monday through Friday) during the 70-day period. Hair was collected before contamination; after contamination, but prior to the sweat application; approximately 6 hours post-contamination (i.e., approximately 4 hours after sweat application followed by a drying period); and then weekly for 10 weeks. Weekly specimens were collected on Monday afternoons following a 4-hour drying period in the morning. At all time points, the hair was analyzed, and quantitative results were obtained for COC, BE, CE, and NCOC.

All specimens were submitted to Immunalysis Corp. for analysis in a randomized blinded manner, with both positive and negative control materials. This reference laboratory conducted all of the quantitative analyses for the study and was compensated for performing the analytical work.

Other than sample extraction and instrumental analysis performed by the reference laboratory, all other protocols were performed at RTI facilities in laboratory space that was not previously used for handling COC. All laboratory equipment and bench spaces were thoroughly

cleaned with water and methanol and dried prior to the study, and bench-top blotter paper covers were regularly changed throughout the study period.

COC materials were weighed and reported in **Table 20**. The weight targets were determined to provide a quantity that could be reliably weighed and handled. The amount of hair material limited the size of the hair specimen available for use; the weight determination was roughly equivalent to previous study designs (Stout et al., 2006a). RTI targeted an approximately 8-mg COC equivalence for an 8-g specimen of hair. Previously, RTI had used a 12-g specimen of hair with 15 g of COC or 1.25 mg COC/g of hair. In this study, RTI used 1.33 mg COC/g of hair.

Table 20. Purities and Quantities Used of Each COC Material

Source (COC Type in Appendix)	Purity of COC (%)	Weight Used	COC Equivalency
NIDA High NCOC (NCOC)	88.5	10 mg	Approximately 8 mg
USP COC (PHARM)	98.9	8 mg	Approximately 8 mg
DEA High CE and BE (CE)	82.2	11 mg	Approximately 8 mg

Note : BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; NCOC= norcocaine; NIDA = National Institute on Drug Abuse; USP = U.S. Pharmacopeia; DEA = Drug Enforcement Administration

Portions of COC were weighed for each of the hair specimens (i.e., light and dark hair specimens [one each] for each COC source). Gloved hands were misted with a synthetic sweat solution (described further below) and rubbed together until dry to reduce the static effect of the gloves. The weighed COC was then applied to the gloved hands and rubbed until the COC was no longer visible on the palms. At this point, the hair was gently handled with gloved hands for 5 minutes to distribute the COC throughout the hair. The hair specimens were then allowed to sit at ambient conditions for 1 hour, at which time the first specimens were taken (Figure 6, 0.05-day aliquot).

Synthetic Sweat Treatment

One hour post-contamination, but after the first specimens were taken, the individual hair specimens were treated with a synthetic sweat solution, consisting of 65 mM NaCl (OmniPur), 5 mM KCl (Mallinckrodt, Inc.), 9 mM sodium lactate (FLUKA), and 22 mM urea (J.T. Baker, Inc.) as described by Cairns and colleagues (2004b). The mixture was modified with the addition of 30 μL of olive oil per 100 mL of synthetic sweat solution to mimic human body oils.

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

Daily Shampoo Treatment

The hair specimens were shampooed in the mornings, Mondays through Fridays, for 10 weeks. The hair was wet with warm tap water; shampooed for approximately 1 minute unbound in gloved hands using approximately 1 mL of Pert Plus with conditioner (Procter & Gamble), which was selected to reduce hair tangling during the process; and then thoroughly rinsed in warm tap water. The hair samples were blotted dry using clean blotter paper, and the hair was allowed to dry at ambient conditions. Hair was completely dry by inspection after 3 hours.

Hair Sampling

At each sampling time point, approximately 240 mg of hair was removed from each hair specimen. This was cut into approximately 1-cm pieces and thoroughly mixed. This mixture was divided into two 120-mg portions: one portion was decontaminated and the other was retained. After decontamination, the 120-mg portion was divided into three approximately 40-mg portions to send to the analytical laboratory. The hair decontaminated at RTI was decontaminated as the entire 120-mg aliquot, and then subdivided for submission to the reference laboratory for analysis following decontamination.

RTI used the extended buffer decontamination protocol previously described by Cairns and colleagues (2004b). In brief, the 120-mg hair specimen was shaken vigorously at 120 rpm at 37°C for 15 minutes in 20 mL of isopropanol. Then the hair specimen was shaken at 120 rpm in 20 mL 0.01 M pH 6 phosphate buffer with 0.01% bovine serum albumin for 30 minutes 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 (i.e., bumped at the ends). This was repeated two more times, followed by two 60-minute buffer washes using the same conditions. The hair aliquots were allowed to air dry prior to shipping.

The final decontamination wash solutions were retained and placed in the freezer for future potential analyses.

Preparation of Specimens for Analysis

All hair specimens were weighed, packaged in aluminum foil, sealed in individual plastic bags, and sent by overnight carrier to the analytical reference laboratories. Positive and negative control specimens were randomly inserted with each shipment.

Controls were included in submissions so that overall there was an 8.9% blind control rate (i.e., 23 blinds in 257 specimens) in the specimens submitted to the reference laboratory. Control materials were inserted in a randomized blinded manner and were submitted with specimens to the analytical reference laboratory each week throughout the study.

Negative control specimens were prepared from each of the hair samples prior to COC exposure. Portions of one of the hair specimens used in the study were used as negative controls several times throughout the study, so that multiple determinations were made on each negative hair specimen by the reference laboratory. Control materials were packaged in a randomized blinded manner, similarly to how they were packaged for the human study specimens shipped to the reference laboratories.

Positive control specimens consisted of control hair preparations manufactured in association with RTI's efforts in the National Laboratory Certification Program (NLCP) Pilot Performance Testing for hair-testing laboratories conducted under contract to SAMHSA and DHHS. Two different target concentrations in these control materials were used throughout the study.

Analysis Procedures

Immunalysis Corp. analyzed the specimens by using LC-MS/MS as described in Section 2.2.1. Data were tabulated in Microsoft Excel, graphs were produced using Microsoft Excel, and statistical analyses were performed using SAS software. Statistical analyses were conducted to

evaluate the five outcomes of interest (i.e., COC, CE, BE, NCOC, and BE/COC ratio). For each of these measured outcomes RTI sought to determine the following:

- If there was a significant difference among the three COC types (i.e., pharmaceutical, high CE and BE, and high NCOC) over time (days), RTI fit the linear and quadratic components of time, as well as their interactions with COC type.
- If there was a significant overall effect of COC type, then this was averaged over time.
- If there was a significant interaction between hair type (i.e., light, dark) and COC type, then this was averaged over time.

There were three replicate analyses within each COC type by hair-per-day combination. A repeated measures model with repeated measures over time in days was fit to these data. The initial linear mixed model included the effects of the following:

- Day: linear
- Day: quadratic
- COC type (i.e., pharmaceutical, NCOC, CE, BE)
- COC type by day linear
- COC type by day quadratic
- Hair type (i.e., light, dark)
- COC type by hair type.

For those treatments in which there was a statistically significant COC type*hair type interaction ($p < 0.05$), separate models were fit for each hair type (i.e., light, dark), with each model containing the following effects:

- Day: linear
- Day: quadratic
- COC type
- COC type by day linear
- COC type by day quadratic.

From this reduced model, RTI determined the final estimates of the effects of time (i.e., linear, quadratic), the COC type followed up with the Tukey test for multiple pair-wise comparisons among the three groups, and their interactions.

Modifications to the Research Design and Rationale

In the original proposal, RTI planned to use five COC types: two consisting of high-purity pharmaceutical-grade COC from two separate sources and three consisting of street COC with varying amounts of CE or NCOC (i.e., a higher amount of CE, a higher amount of NCOC, or an insignificant amount of either of these analytes). After analyzing 25 illicit COC materials and discussions with the DEA Special Testing Laboratory, RTI compromised on three COC types that accomplished the same goal of exposure to minimal concentrations of CE, BE, and NCOC and varying COC purity based on the higher concentrations of these analyte compounds.

Because RTI was most interested in actual seized COC materials to be most representative of the possibilities that actually exist on the street, we were limited as to the availability of materials. The three COC materials detailed in Table 6 and Table 20 included a pharmaceutical-grade COC with minimal CE, BE, and NCOC (the PHARM group); one illicit COC with high NCOC (the NCOC group) and minimal CE and BE; and one illicit COC with high CE and BE, but minimal NCOC (the CE group).

RTI also originally proposed using six different hair types for fewer time points, but we were unable to obtain subjects who had quality hair materials in large enough quantities to conduct the study. Instead, we used one large sample of light hair, one large sample of dark hair, and more time points and replication. This provided us with a better estimate of laboratory variability and more homogenous results between COC materials. The purpose of the study was to evaluate the effects of the COC source on the ratios of these compounds in hair and not the effect of individual hair materials on these ratios. Therefore, RTI believed that the design of fewer individual hair types, but the same hair material used between the drug sources, produced more directly comparable results than hair results from different individuals used with each drug material.

Lastly, as the analysis of drug concentrations were conducted, the early time points, especially for the pharmaceutical-grade COC material, had high concentrations of COC that exceeded the linearity of the reference laboratory's assay. Later, hair specimens were diluted by the reference laboratory to more accurately estimate these high concentrations. Due to the small samples size of the specimens produced, RTI was not able in all cases to go back to the hair specimens to re-analyze them with dilutions to be within the linear range. Using the diluted values as a guide, RTI estimated values for these highly concentrated specimens to use in the statistical modeling. As shown in Table 20, these values likely resulted in an underestimation of the amount of COC in the hair specimens. We believe the estimates were reasonable based on the subsequently diluted specimens, and that they provided a conservative view of the potential results.

Findings

For COC in contaminated light and dark hair specimens, there were significant effects of time on COC levels (i.e., linear and/or quadratic components [$p = 0.0001$]); however, the COC types differed significantly in their linear trends over time. For the light hair type, the high CE group showed the steepest declines; for the dark hair type, the pharmaceutical COC-treated (PHARM) group had the steepest declines over time. The groups with the steepest declines were also the groups with the highest maximum COC levels over time (**Figures 7** and **8**). For all of the figures, the points on the graphs represent the mean, and the error bars represent ±1 standard deviation.

For both light and dark hair types, the overall effects of the COC type adjusted for time was statistically significant. For dark hair, the PHARM group had significantly higher COC levels ($p = 0.0001$) than both the CE and NCOC groups; for light hair, the CE group had significantly higher COC levels ($p = 0.0001$) than both the PHARM and NCOC groups.

For CE in contaminated light and dark hair specimens, there were significant effects of time on response levels (i.e., linear and/or quadratic components [$p = 0.0001$]); however, the COC types differed significantly in their linear trends over time ($p = 0.0001$). For both hair types, NCOC had values of zero for all replicates over time (i.e., slope = 0). For dark hair, the

PHARM and CE groups had statistically equivalent linear declines over time; for light hair, the CE group showed a significantly steeper decline than the PHARM group partly because the CE group reached a higher maximum value over time than the PHARM group.

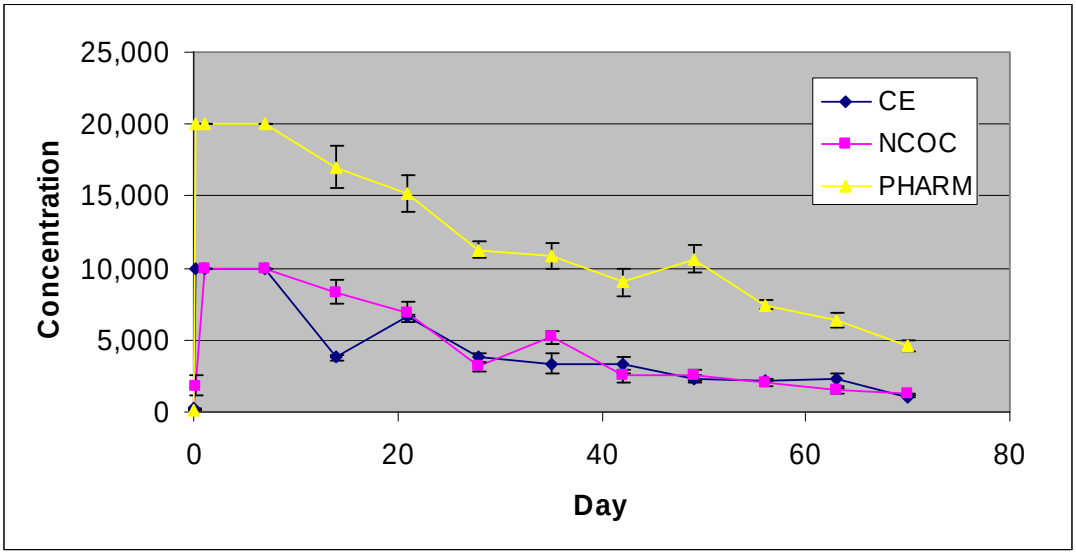


Figure 7. The average COC (pg/mg) for dark hair type over the study period.

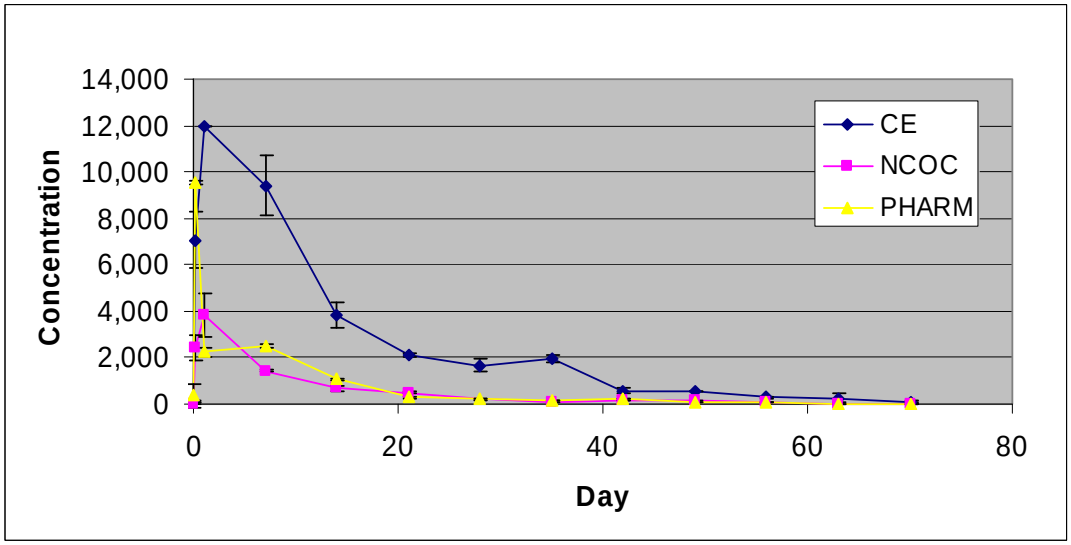


Figure 8. The average COC (pg/mg) for light hair type over the study period.

For both hair types, the overall effect of the COC type adjusted for time was statistically significant ($p = 0.0001$). For dark hair (**Figure 9**), the PHARM group had significantly higher response levels than the CE group, and both groups were significantly higher than the NCOC group, which had a zero response level.

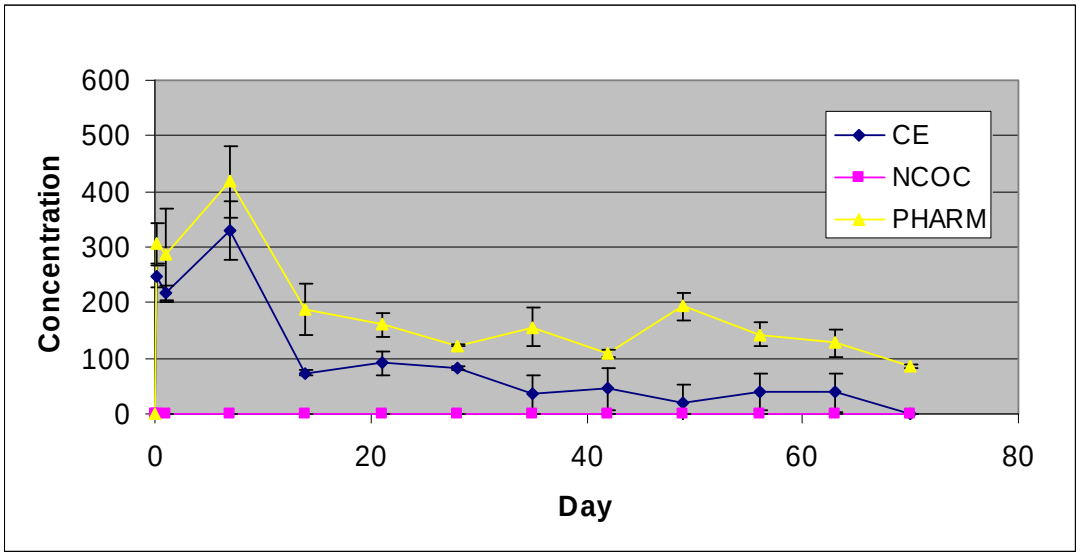


Figure 9. The average CE (pg/mg) for dark hair type over the study period.

For light hair (**Figure 10**), the CE group had significantly higher response levels than the PHARM group, and both groups were significantly higher than the NCOC group, which had zero response level.

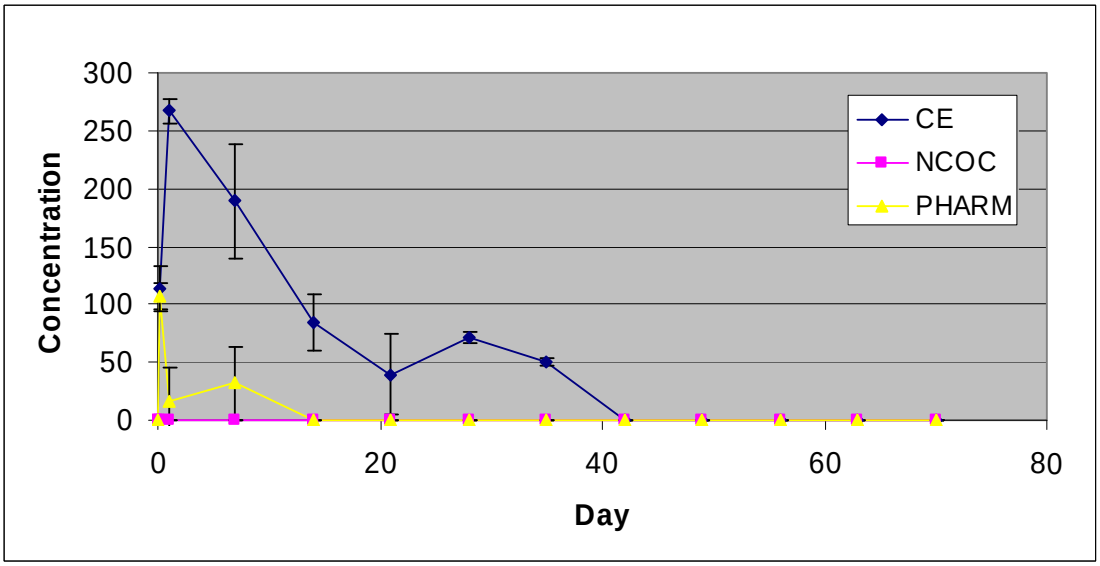


Figure 10. The average CE (pg/mg) for light hair type over the study period.

For BE in contaminated dark and light hair (**Figures 11** and **12**, respectively), the COC groups differed significantly in their linear trends over time. Only the CE group showed significant (p <0.05) linear declines in BE over time. The slopes for the PHARM and NCOC groups in the light hair were not significantly different from zero (i.e., their time profiles were basically flat as shown in Figure 11).

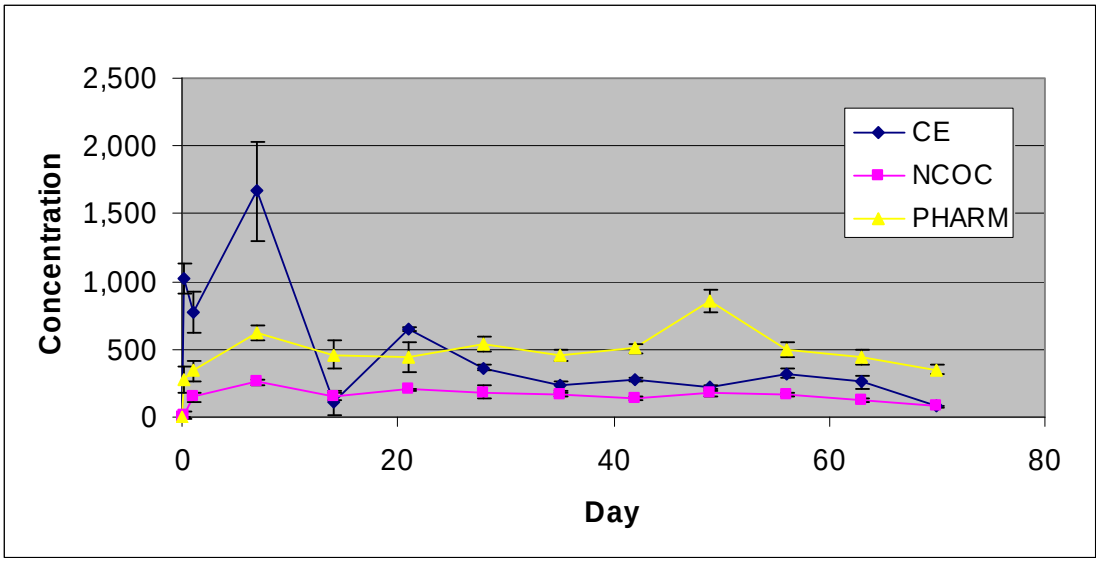


Figure 11. The average BE (pg/mg) for dark hair type over the study period.

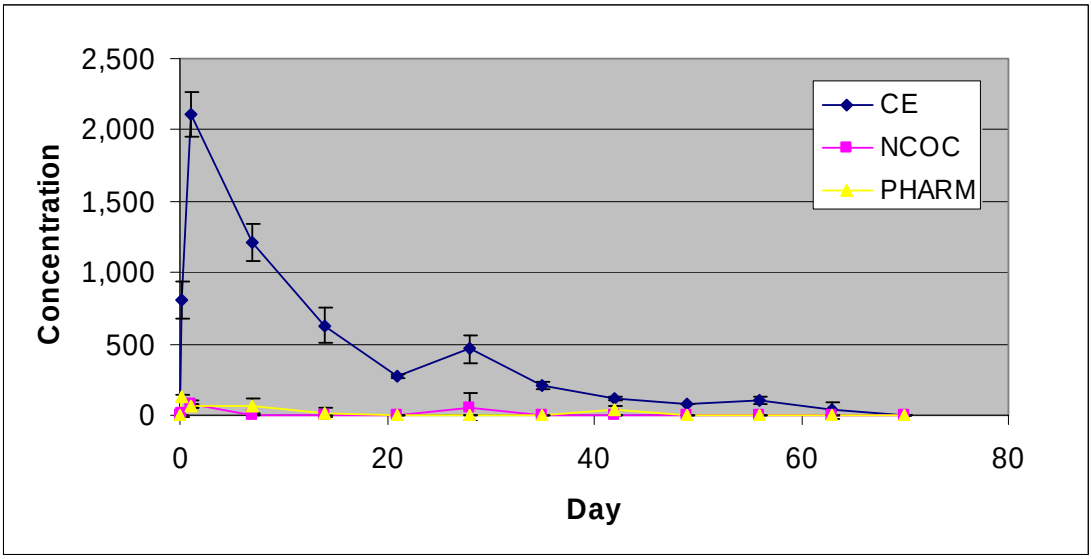


Figure 12. The average BE (pg/mg) for light hair type over the study period.

For both hair types, the overall effects of the COC type adjusted for time was statistically significant. For dark hair, the PHARM and CE groups had significantly higher BE levels than the NCOC group. Although the PHARM group did not exhibit any linear decline in BE over time, its average value was not significantly different from that of the CE group. For light hair, the CE group had significantly higher BE levels than both the PHARM and the NCOC groups, both of which exhibited low BE values over time.

For the BE/COC ratio, the light and dark hair types differed in their COC group linear trends, as well as the size of the COC group effects. For the dark hair type, all groups exhibited significant positive linear trends in the response ratio over time, most consistently in the PHARM and NCOC groups. However, the CE group maintained significantly higher average BE/COC ratios over time compared to both the PHARM and NCOC groups (**Figure 13**).

For the light hair type (**Figure 14**), days 63 and 70 were removed from the analysis because of all the missing values in two of the groups (i.e., denominator of the ratio was zero for all observations at these time points). Only the CE group exhibited a significant linear increase in the response ratios over time. In addition, the overall effect of the COC type adjusted for time was statistically significant (p = 0.0001). The CE group had significantly higher response ratio levels than the PHARM and NCOC groups, and this effect was more pronounced in the light hair versus dark hair types (Figures 13 and 14).

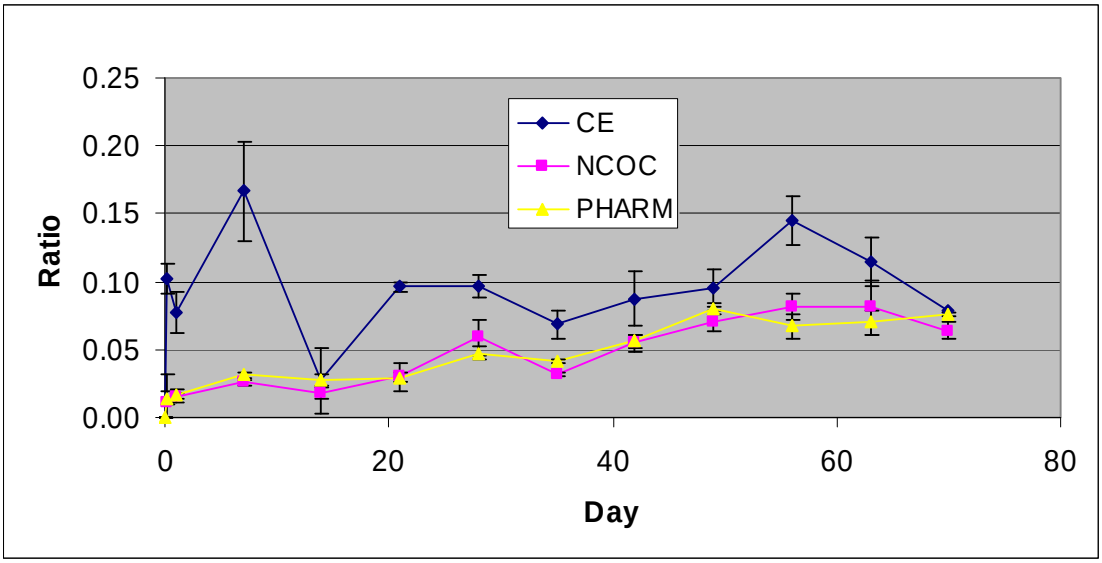


Figure 13. The average BE/COC ratio over the study period for the dark hair type.

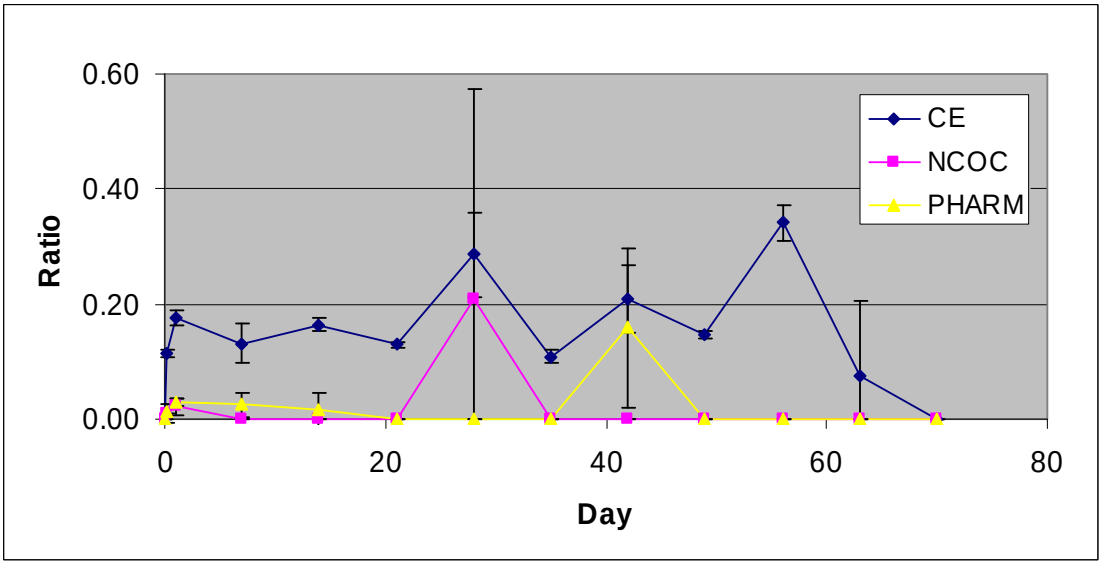


Figure 14. The average BE/COC ratio over the study period for the light hair type.

For NCOC in contaminated light and dark hair, the COC groups differed significantly in their linear trends over time. Only the NCOC group showed significant (p <0.05) linear declines in the response over time. The slopes for the PHARM and CE groups were not significantly different from zero (i.e., their time profiles were basically flat). The significant interaction

between COC type and hair type was due to the significantly larger linear decline in the NCOC group for dark versus light hair types (**Figures 15** and **16**).

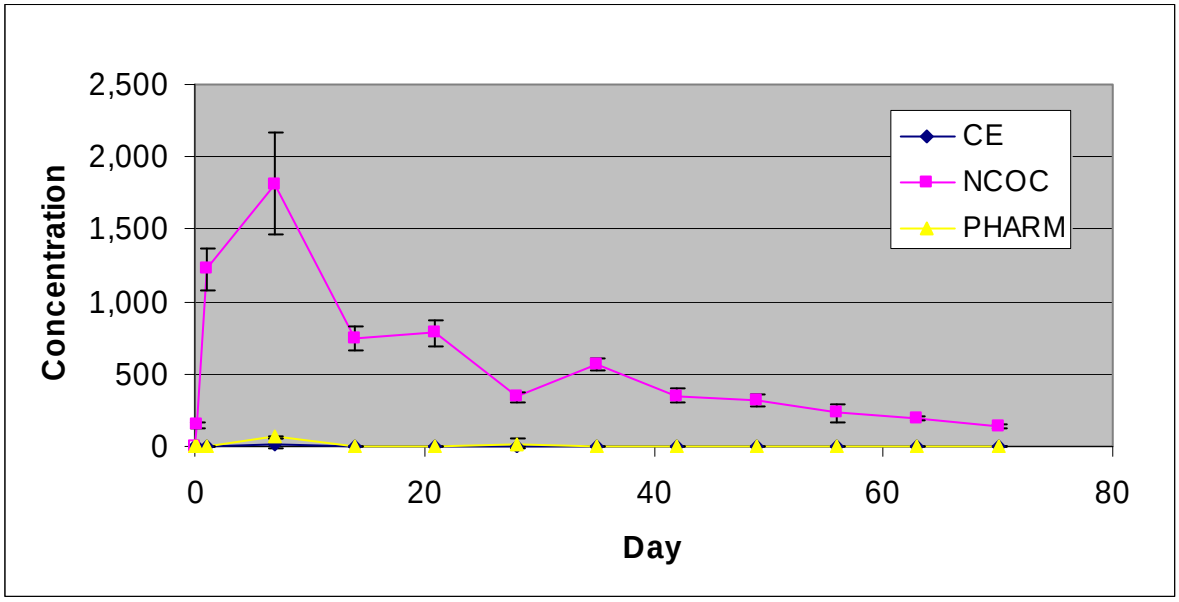


Figure 15. The average NCOC (pg/mg) for dark hair type over the study period.

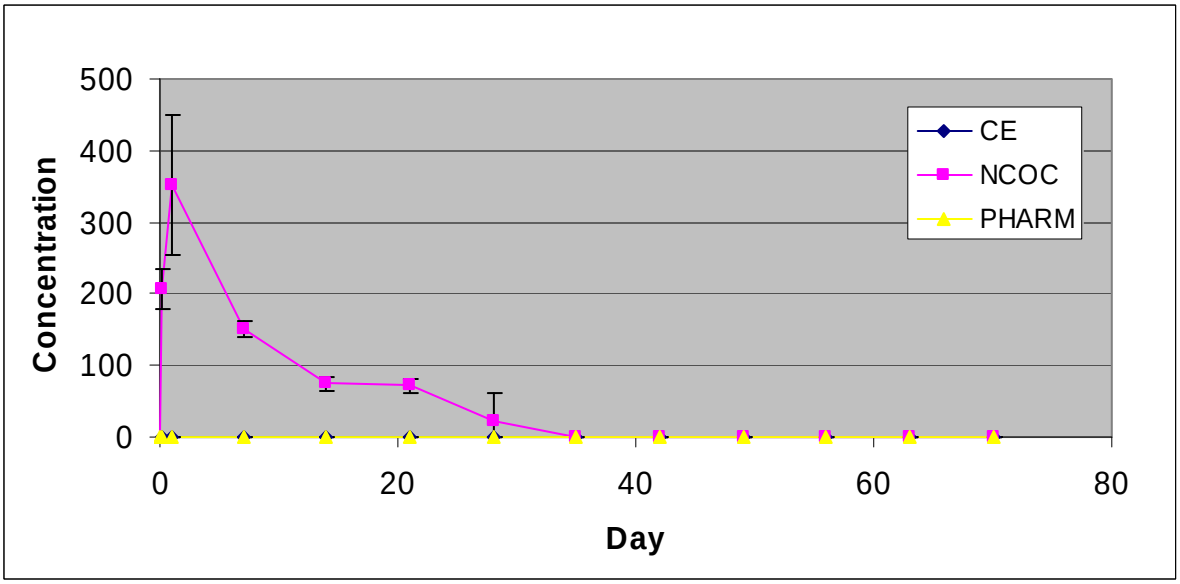


Figure 16. The average NCOC (pg/mg) for light hair type over the study period.

For both hair types, the overall effects of the COC type adjusted for time was statistically significant (p = 0.0001). The NCOC group had significantly higher response levels than the PHARM and CE groups, and the effect was most pronounced for dark hair types.

The analytical results were also compared against several decision criteria, as were the other hair specimens in the study. These criteria are listed in **Table 21**. A full listing of the specimen data results is included as **Table A-7**; the first three criteria are listed in this table

because they are the criteria in the proposed Mandatory Guidelines. **Table A-8** summarizes the counts and relative percentages of analyses that would result in a positive call by the given criteria. All analyses were treated separately, so the percentages are relative to the total number of analyses conducted for that treatment group, including replicate analyses.

Table 21. Cocaine Analyte Concentrations and Ratios Criteria for Determining Confirmation Results as Positive or Negative

Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002

BE = benzoylecgonine; BE/COC = benzoylecgonine-to-cocaine ratio; CE = cocaethylene; CE/COC = cocaethylene-to-cocaine ratio; COC = cocaine; NCOC= norcocaine; NCOC/COC = norcocaine-to-cocaine ratio

For Criteria 1, there appeared to be a difference over time in the number of specimens that would have resulted in a positive call. Criteria 1 are presented separately below because there appeared to be more of a change over time in the results than for the other criteria. **Figures 17** and **18** present the number of replicates that would have been called positive from each treatment group for light and dark hair specimens. It is important to note the increase in the number of positive calls for this criteria, depending on the BE/COC ratio, and that the light hair was only positive by this criteria when exposed to the high BE and CE.

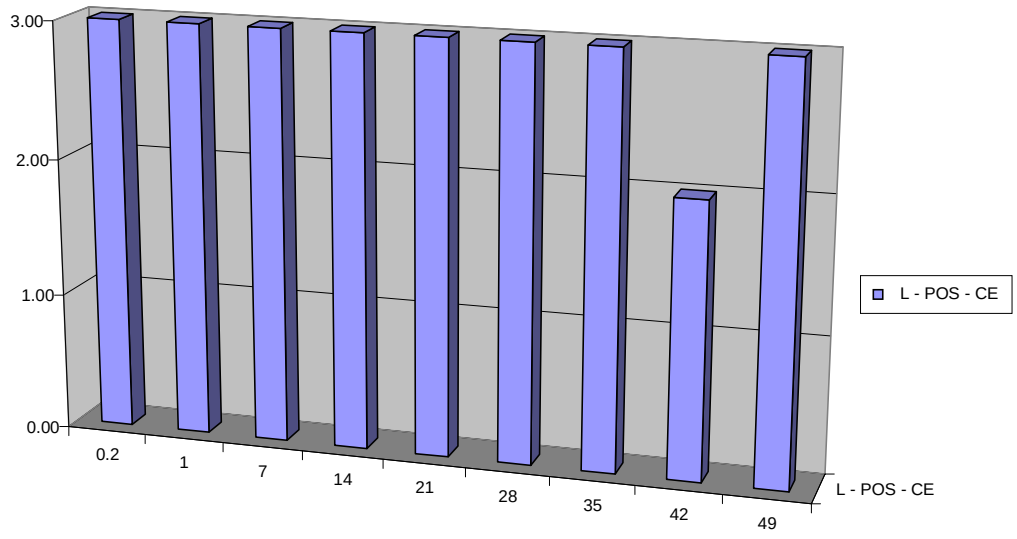


Figure 17. RTI used Criteria 1 (i.e., BE and BE/COC ratio criteria) during specimen analyses of the light hair to determine that it was positive. Only those light hair specimens exposed to the high CE and BE COC material were positive by this criteria over the study period. Specimens beyond 49 days were not positive.

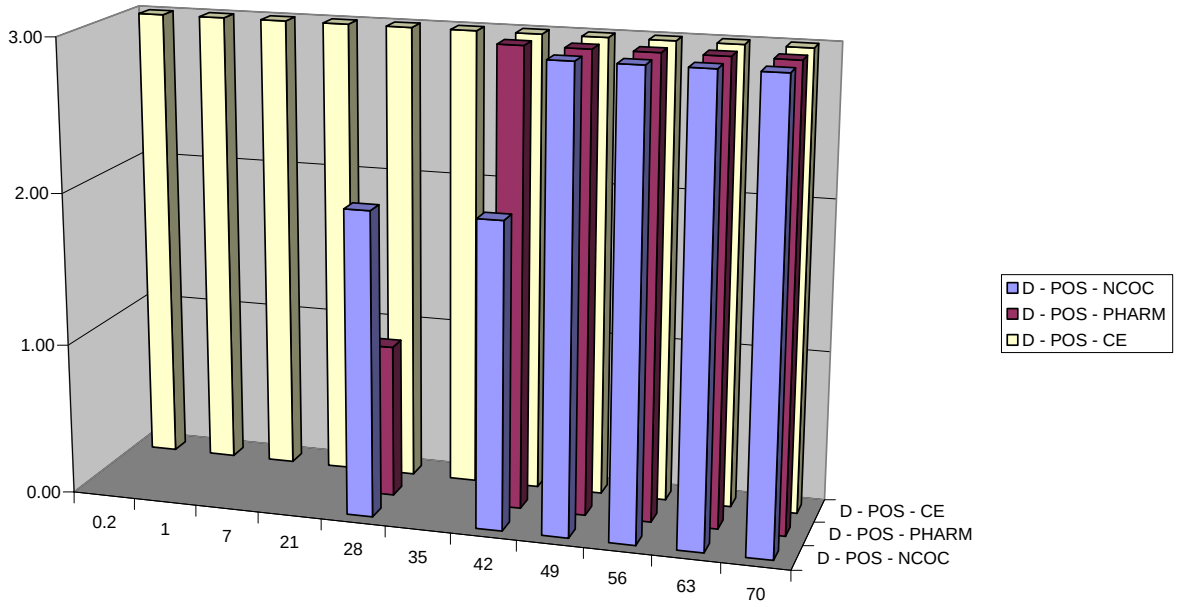


Figure 18. Number of specimen analyses of the dark hair determined to be positive by Criteria 1 (i.e., BE and BE/COC ratio criteria). All dark hair specimens after exposure to sweat and the high BE and CE containing COC were positive by using this criteria. Dark hair exposed to the other COC materials exhibited an increase in the number of positive calls over time.

2.3.2 Conclusions

The results of this study are consistent with what RTI has previously published for contaminating hair with pharmaceutical-grade COC (Stout et al., 2006b). All three COC sources resulted in significant quantities of COC being present on the hair and remaining there over the course of 10 weeks. This COC was resistant to removal by hygienic treatment or by laboratory decontamination. As was previously observed, there was a significant decline in the content of COC over the course of the study.

In our study, contaminating the hair with COC that contained higher CE, BE, or NCOC concentrations resulted in significantly higher concentrations of each of these drug compounds in the respective hair specimens. This indicates that the quantity of the drug compounds found in illicit COC can affect their concentrations in the contaminated hair. As with COC, these compounds were resistant to removal by either hygienic treatment or laboratory decontamination procedures. In addition, exposure to the COC containing CE at 1.4% of the COC material resulted in a maximum CE/COC ratio of 0.048, whereas the material containing 8% NCOC resulted in a maximum NCOC/COC ratio of 0.25. These ratios indicate that there may not be a direct relationship between the concentration of CE and NCOC in hair and the concentration in the contaminating COC.

This study was also consistent with previous findings because the BE/COC ratio increased significantly over the course of the study period. In the recent study, the ratio exceeded ≥0.05 by Day 28 for those COC materials that contained less BE, instead of Day 21 as previously reported. For the illicit COC that had high BE and CE concentrations, this ratio increased after adding synthetic sweat and continued to rise over the course of the study. Hair specimens treated with the high NCOC illicit COC powder and the pharmaceutical-grade COC did not exhibit any decline in BE over the course of the study period.

As shown in Table A-6, a substantial number of analyzed specimens would have been determined as positive by most of the criteria applied. For the specimens exposed to COC that contained more CE, there were more specimens that would have resulted in positive calls. For these specimens, only the criteria, including a ≥0.05 CE/COC ratio, would have resulted in no positive results. Using the ≥0.02 CE/COC criteria, there were 44% of the dark hair specimens and 33% of the light hair specimens that would have had tested positive. For those specimens exposed to the high NCOC that contained COC, 33% of the light hair specimens and 92% of the dark hair specimens would have been determined as positive by all of the criteria using NCOC. A more complex pattern was observed with BE criteria because BE appeared in the hair from all sources; therefore, varied amounts of NCOC, CE, and BE in the contaminating COC can substantially confound the use of ratios to discriminate contaminated hair specimens, even after using a laboratory's decontamination procedure.

It is important to note that RTI applied these criteria to hair specimens because a reference laboratory would apply them under the proposed federal Mandatory Guidelines. In other words, the reference laboratory would have had to analyze the specimens and apply the cutoffs directly to these results. As Schaffer and colleagues (2007) have noted in several publications (Cairns et al., 2004a and b), they have applied various ratios of compounds and have used various mathematical calculations using the amounts of a drug found in the last wash solution. As noted by Kidwell and Smith (2007), this wash criteria has evolved over the years. The proposed Mandatory Guidelines (SAMHSA, 2004a) do not have a provision for the use of such criteria; therefore, we did not use any wash criteria in this analysis. RTI has retained the last wash solutions of all hair specimens to potentially conduct this analysis at a later date.

As noted by Schaffer and colleagues (2007) in a letter to the editor in the *Journal of Analytical Toxicology* about RTI's previous research, this was an in vitro model, and it has limitations because of its use. However, this model allows for exposures and controls that would not be possible in an in vivo model. Schaffer and colleagues have also criticized that the quantity of COC used in this in vivo model is unrealistic because it is not clearly understood how much COC may be on a surface that is touched by law enforcement or others. Some research with methamphetamine cook houses suggests that high surface contamination may be possible. Although this quantity of COC we used may be too large for some scenarios, it may be too small to be representative of other scenarios. In this study, the amount of COC used in the surface contamination model was approximately half of the concentration used in our previously reported study (Stout et al., 2006b).

This study also addresses the criticism that CE and NCOC are not in COC in the United States. RTI used illicit COC materials seized in the United States that contained higher relative concentrations of NCOC and CE in them.

With the potentially variable CE, NCOC, and BE content of illicit COC and high inter-individual variability in the way an external drug associates with the hair, developing ratios to discriminate contaminated hair is problematic. Ratios such as ≥0.05 CE/COC in this instance may have eliminated positive results in contaminated hair using these COC materials; however, illicit COC may contain higher concentrations of CE, potentially confounding even this ratio.

2.4 Stage IV: Fortification of Hair Samples with Cocaine Hydrochloride in Solution

Stage IV of this research project investigated the dynamics of incorporating COC into hair and detecting the ratios of COC analytes in hair after soaking it in a COC HCl solution. Although this study model does not represent realistic contamination of hair in vivo, it does represent in vitro conditions used to prepare hair proficiency and calibrator and control samples in a forensic laboratory setting. This fortification study was conducted in parallel with the contamination study in Stage III, using the same COC sources and hair types, which allowed RTI to evaluate the COC-contaminated hair in different environments.

2.4.1 Experimental Design

Hair-drug fortification processes are used by laboratories to prepare quality control samples (e.g., matrix-matched calibrator or proficiency samples) that allow researchers to assess analytical processes and an instrument's performance. Fortification protocols are used by laboratories to produce hair samples that are thought to mimic the processes through which a contaminating drug is incorporated into hair. The incorporation of drug into hair by any external mechanism is not like the incorporation from ingestion; however, externally incorporated drug may be useful if its characteristics are similar enough to ingested samples, and they are more controlled in nature allowing for more standardization.

In the fortification model, hair was placed in a COC HCl solution, which incorporates some of the drug into the hair. After the hair was fortified with COC to achieve a target concentration several orders of magnitude higher than the proposed confirmatory cut-off concentrations, an effective decontamination protocol that was previously examined by RTI and other researchers was used to remove weakly associated compounds. These hair samples were then submitted in a blinded manner to a reference laboratory for analysis of COC analyte concentrations. Results were evaluated to determine if hair contaminated by soaking it in the COC HCl solution yielded information that differed from hair contaminated with powdered COC HCl. Results were also evaluated to determine if hair contaminated in this liquid environment (e.g., solvent-containing drug) could be reliably identified as contaminated hair. Primarily, this study investigated whether BE/COC ratios in contaminated hair were distinguishable from those ratios found in hair after COC ingestion. Furthermore, the study determined the presence of other COC analytes, such as CE and NCOC, in the hair from the fortification process, which was performed by RTI (Ropero-Miller et al., 2005), and whether the CE/COC and NCOC/COC ratios could be used to distinguish a contaminated hair sample from a non-contaminated hair sample.

Several COC sources were investigated in this fortification study (i.e., two illicit COC materials and one USP pharmaceutical-grade COC) to provide a range of BE, CE, and NCOC concentrations.

2.4.2 Materials

Hair—Non-chemically treated hair was purchased from a cosmetologist. When the hair was collected, the cosmetologist documented demographic information from the volunteer and determined the condition of the hair. The volunteer was a 24-year-old Caucasian female whose hair was straight and determined to be healthy (e.g., not visually damaged, cuticle intact). To protect the volunteer's privacy, her identity was blinded to RTI. This hair was used for all COC fortification protocols during Stages III and IV of this study.

Cocaine Hydrochloride—Two street COC samples were obtained from seized materials, and one COC HCl was purchased from USP as detailed in Stage I of this study. The samples used in the fortification study included a NIDA sample COC_HCl_20 (88.5% COC and 8.7% NCOC), a DEA sample COC_HCl_27 (82.2% COC, 1.43% CE, 0.8% NCOC, and 10.1% BE), and a USP sample (98.9% COC and 1.1% CE). These COC samples were selected for the fortification study because they contained a significant amount of analytes that are considered metabolites of COC but can be by-products of a licit or illicit manufacturing process as proven in Stage I of this study. These COC HCl samples were also used in the contamination studies for the Stage III study.

Reagents and Laboratory Supplies—Sodium chloride (American Chemical Society grade), sodium phosphate monobasic (analytical grade), and isopropanol (high-performance liquid chromatography grade) were purchased from VWR International. Bovine serum albumin (minimum 96%) was purchased from Sigma-Aldrich. The shaker (Eberbach Model 600), two water baths (Boekel Grant PB-600), a pH meter (Mettler Toledo), an analytical balance (Mettler Toledo Model AX 105), amber storage jars (Qorpac), and other laboratory supplies were purchased from VWR International. Prior to use in the study, the pH meter was validated over a range from 2.00 to 12.45. The instrument was calibrated daily before use, and appropriate controls were analyzed concurrently with the fortification solutions.

2.4.3 Methods

2.4.3.1 Hair Characterization

The hair was received into inventory, weighed, and visually evaluated for color using the Schwarzkopf scale modified by RTI. The volunteer's hair was determined to be brown by visual observation and have a Schwarzkopf color of 6.0. Before beginning the fortification protocol, hair samples were washed three times with distilled water for 15 minutes to remove hygienic residues (e.g., shampoo, conditioner, styling products). The hair was thoroughly dried, and an aliquot was sent to the reference laboratory for analysis. Radioimmunoassay analysis indicated that the hair was negative for COC analytes. The hair sample was then divided into 5-g aliquots for the Stage IV study, and each portion was placed in a plastic zippered bag to protect it from environmental drug exposure.

2.4.3.2 Hair Fortification

Five fortification studies were conducted using different COC HCl powders. Three different COC HCl powders were used to fortify hair at a target of 1,500 pg/mg of COC, and the same two refined illicit COC HCl powders were also used to fortify two more hair sample at a target of 5,000 pg/mg. The target concentrations for COC were selected based on the confirmatory cutoff (COC at a target of 500 pg/mg) in the proposed Mandatory Guidelines (SAMHSA, 2004a). The lower concentration sample was targeted at three times (at a target of 1,500 pg/mg of COC) the proposed federal cutoff, and the higher sample was targeted to have a concentration of 10 times (i.e., 5,000 pg/mg COC) the proposed federal cutoff. The expected amounts of the COC impurities were determined based on the target values.

The same COC equivalency used in the external surface contamination study of Stage II of this study were also used for the external drug fortification of Stage IV. As shown in **Table 22**, a 35.86-mg portion of the DEA COC (82.2%) was weighed out for the first two samples. The COC HCl was dissolved an aqueous solution (pH 6) and diluted to the target concentrations of

the final fortification solutions. Similarly, solutions containing a 49.90-mg portion of the NIDA COC (88.5%) and a 37.75-mg USP (98.9%) were prepared and diluted to target concentrations.

Table 22. Purities and Quantities Used of Each COC Material

Source (COC Type in Appendix)	Purity of COC (%)	Weight Used	COC Equivalency
NIDA High NCOC (NCOC)	88.5	49.90 mg	Approximately 8 mg
USP COC (PHARM)	98.9	37.75 mg	Approximately 8 mg
DEA High CE and BE (CE)	82.2	35.86 mg	Approximately 8 mg

Note : BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; NCOC= norcocaine; NIDA = National Institute on Drug Abuse; USP = U.S. Pharmacopeia; DEA = Drug Enforcement Administration

Hair aliquots (5 g) were placed in clean, pre-labeled amber jars; a fortification solution was added until the hair was covered; and then each jar was capped to prevent external contamination. The jars that contained the fortification solutions were oscillated on a shaker for a period of time based on RTI protocols and target COC concentrations; generally 3 to 5 days. The entire protocol was performed under ambient conditions.

During the fortification process, aliquots of the hair were removed to monitor the incorporation of the analytes in the hair. Concurrently, an aliquot of the solution was removed to monitor pH. After each hair aliquot was removed, the specimen was immediately washed using an isopropanol/extended buffer wash procedure at 37ºC as previously discussed in Stages II and III.

After the fortification process was complete, a 20-mL aliquot of solution was removed and placed in the freezer (−20ºC) for future analysis. The remaining solution was decanted from the sample, and then the hair specimen was washed at 37ºC with an isopropanol/extended buffer wash procedure as previously described in this study. Each wash solution was collected in a scintillation vial, capped tightly, and stored in the freezer for future analysis.

Each hair sample was wrapped in filter paper and dried at ambient temperature overnight. After the sample was completely dried, 100-mg aliquots were collected for analysis by the reference laboratory (three replicates). The hair was weighed into a tarred, pre-labeled scintillation vial, capped with a foil-lined screw cap, and placed in an individual plastic zippered bag. At the same time, the vials that contained the final wash solutions were removed from the freezer and each one was placed in an individual plastic zippered bag. Both the hair aliquots and the wash solution vials were then placed into a secondary bag and sent overnight to the reference laboratory for analysis.

2.4.3.3 Hair Analysis by Using LC-MS/MS

Immunalysis Corp. served as the reference laboratory for the Stage IV study. Triplicate analyses of hair samples were performed by using LC-MS/MS, as detailed in Stage II of this study. The limit of detection was 25 pg/mg and the limit of quantitation was 50 pg/mg for all COC analytes.

2.4.3.4 Modifications to the Research Design and Rationale

There were no modifications to the research design and rational of Stage IV of this study.

2.4.4 Findings

The fortification studies incorporated COC analytes into the hair specimens, and despite multiple, extended decontamination washes, all of the incorporated analytes from the hair could not be removed from the hair. The amount of the COC analytes incorporated into the hair by this protocol was concentration dependent. Even though higher solvent COC analyte concentrations provided increased concentrations in the fortified hair, a direct linear relationship was not evident. **Table 23** lists the COC analytes present in each COC sample and their percentage of composition, the target concentration for each fortification protocol, the average COC analyte concentrations found in each hair after being fortified with a solution of COC HCl powder, and the relationship (ratio) of the amount of each COC analyte found in the hair to the amount of COC in the hair.

Table 23. Percentage of Composition of Cocaine Analytes and Analyte Ratios for Cocaine HCl Powders Used in the Fortification Study

Sample	COC	BE	CE	NCOC	BE/COC	CE/COC	NCOC/COC
COC-HCl_27 (DEA)	**82.20%**	**10.10%**	**1.43%**	**0.80%**			
TARGET (pg/mg)	5,000	186	143	ND			
Replicate #1	3,506	166	99	NEG	0.047	0.028	0
Replicate #2	3,467	179	117	NEG	0.052	0.034	0
Replicate #3	4,645	169	92	NEG	0.036	0.020	0
AVERAGE	3,873	171	103	NEG	0.044	0.027	0
TARGET (pg/mg)	1,500	56	43	ND			
Replicate #1	1,415	49	35	NEG	0.035	0.025	0
Replicate #2	1,416	44	42	NEG	0.031	0.030	0
Replicate #3	1,391	48	40	NEG	0.035	0.029	0
AVERAGE	1,407	47	39	NEG	0.033	0.028	0
COC-HCl_20 (NIDA)	**88.5%**	**0.16%**	**<0.01%**	**8.70%**			
TARGET (pg/mg)	5,000	ND	ND	1,206			
Replicate #1	4,409	123	NEG	1,113	0.028	0	0.252
Replicate #2	4,146	129	NEG	950	0.031	0	0.229
Replicate #3	3,921	133	NEG	938	0.034	0	0.239
AVERAGE	4,159	128	NEG	1,000	0.031	0	0.241
TARGET (pg/mg)	1,500	ND	ND	1206			
Replicate #1	1,460	34	NEG	335	0.023	0	0.229
Replicate #2	1,237	41	NEG	256	0.033	0	0.207
Replicate #3	1,146	43	NEG	323	0.038	0	0.282
AVERAGE	1,281	39	NEG	305	0.031	0	0.238
COC-HCl_28 (USP)	**98.9%**	**<0.01%**	**1.10%**	**NR**			
TARGET (pg/mg)	1,500	ND	145	ND			
AVERAGE (n = 72)	2,212	85	57	NEG	0.038	0.025	0

BE = benzoylecgonine; BE/COC = benzoylecgonine-to-cocaine ratio; CE = cocaethylene; CE/COC = cocaethylene-to-cocaine ratio; COC = cocaine; DEA = U.S. Drug Enforcement Administration; NCOC= norcocaine; NCOC/COC = norcocaine-to-cocaine ratio; ND = none detected; NEG = negative; NIDA = National Institute on Drug Abuse; NR = not reported; USP = U.S. Pharmacopeia

When a COC analyte concentration was ≥1% in the starting COC HCl powder, dissolved into the solution, and introduced to the hair, then that analyte's concentration was detected in the hair using this fortification design. Evaluating the COC analyte concentrations in these fortified hair samples (e.g., surface contaminated by emersion in an external fortification solution) by the proposed Mandatory Guidelines demonstrated several findings. First, although BE concentrations were ≥50 pg/mg in fortified hair samples contaminated with a COC HCl solution that contained approximately 10%, the BE/COC ratio was not ≥0.05. Second, CE in concentrations up to 1.5% of the COC HCl powder, which can be found on the street or commercially, resulted in CE concentrations ≥50 pg/mg in fortified hair samples that were contaminated at a target concentration of 5,000 pg/mg (10 times the confirmation cut-off concentration of 500 pg/mg COC); however, the CE/COC ratio was not ≥0.05. A CE/COC ratio of 0.03 was evident, and this exceeded the CE/COC ratios found in the drug-user populations investigated in Stage II of this study. Finally, the NCOC concentrations in the hair specimens were higher in all analytes other than COC. Concentrations of NCOC found in hair fortified with the COC HCl powder that contained 8.7% NCOC averaged 1,000 pg/mg and 305 pg/mg (Table 23). The NCOC/COC ratio was approximately 0.25 for both samples. Again, these NCOC/COC ratios were higher than the drug-user populations.

A comparison of the ratios obtained from the dry contaminated hair and the hair fortified with solution showed that the numbers analyzed were too small to allow for statistical evaluation. It is interesting to note that the two target concentrations used in the fortification experiment with the two illicit COC samples resulted in the same ratios for CE/COC (0.027 for both low and high targets) and NCOC/COC (0.240 for both low and high targets) for each of the materials. The BE/COC ratio was similar after fortification from both illicit COC materials (0.039 from the high BE and CE and 0.031 from the high NCOC materials).

Additionally, when these ratios are compared to those from the dry, surface contamination experiments (Stage III), the BE/COC ratio was roughly equivalent to the contamination samples at Days 7–21; half of that concentration was found for the contamination samples (0.06) by the end of the 70-day study period. The CE/COC ratios are roughly equivalent (approximately 0.02) between the two external routes.

2.4.5 Conclusions

COC analyte concentrations in hair following contamination with COC HCl solutions were at concentrations that would be ruled as a positive result for NCOC, but not BE and CE. If these samples were evaluated by a forensic laboratory using current proposed Mandatory Guidelines, some of these results (both concentrations and ratios) suggest that fortified samples (e.g., external contamination through COC HCl solution) may not be differentiated from hair of COC users who actually ingested COC. Because a fortification process can be used to make hair reference materials for use as a calibrator and control or as proficiency samples, there is no way to identify these hair samples as suspected of external contamination given proposed cut-off concentrations and ratio criteria for COC analytes.

Laboratories could use additional steps to help differentiate COC contamination from actual ingestion, but none of these steps are routinely practiced by most laboratories. For example, a decontamination wash calculation can be applied against the COC analyte concentration for a more conservative interpretation of a hair concentration. Cairns and colleagues (2004a) suggest that the COC concentration in the final decontamination wash should

be measured, multiplied by a factor of five, and then subtracted from the final COC concentration in the hair sample to estimate the amount of COC that would be further removed with additional decontamination washes. The Cairns decontamination procedure takes 3.75 hours to perform, and additional analyses are required. Alternatively, Tsanaclis and Wicks (2008) suggested that a drug detected in a dried-down methanolic wash that was obtained rapidly and analyzed could be used to calculate a Wash (W)-to-Hair (H) ratio. The researchers propose that a W/H ratio ≤ 0.1 would indicate drug use as opposed to environmental contamination, a W/H ratio ≥ 0.1 but ≤ 0.5 would likely indicate possible use with potential for simultaneous external contamination, and a W/H ≥ 0.5 would indicate external contamination. Evaluation of the fortification specimens with either of these decontamination wash calculations indicates that the samples were externally contaminated. Another step that laboratories could consider would be to evaluate other COC analytes that are truly metabolic products and that do not have a pathway for their presence as by-products of manufacture; however, the abundance of these analytes are small in comparison to the parent compound (COC) and would probably pose similar difficulties as CE.

3. CONCLUSIONS

3.1. Discussion of Findings

After evaluating COC and COC analyte concentrations and ratios in drug-user hair from various populations, dry contaminated hair with various COC sources, and an alternate external application of COC to hair (fortification), the use of cut-off concentrations for any or all of the COC analytes would not be reliable to discriminate a drug-user's hair from dry contaminated hair. Using COC analyte ratios provides more information and some ability to discriminate drug-user specimens from contaminated specimens; however, using CE and NCOC concentrations and ratios are not any more efficient in discriminating between the two specimens than is using only BE and COC decision criteria. All three analytes (i.e., CE, NCOC, and BE) can be present at varied concentrations in illicit COC as by-products of the manufacturing process, and as such, will confound the use of ratios to discriminate contamination from use. Contaminating hair with illicit COC materials that contain approximately 1% to 10% of CE, BE, and NCOC resulted in hair specimens that would not be discriminated from drug-user hair by ratios or concentrations. Even after decontaminating the hair, the application of concentration and ratio decision points does not adequately discriminate contamination from drug use.

In this study, RTI applied some of these decision criteria because laboratories would have to apply them under the current proposed Mandatory Guidelines. These guidelines do not have provisions for using additional decision criteria, which include wash criteria or those mathematical criteria that compare the presence of a drug in wash solutions to concentrations in the hair being tested. Consistent with what Cairns and colleagues (2004a and b) and Tsanaclis and Wicks (2008) have previously published, some type of decision criteria may be necessary to adequately and reliably identify contamination.

These results have implications for the proposed federal Mandatory Guidelines because the decision criteria, as proposed in this study, do not adequately discriminate contamination. This is of particular concern for those individuals whose occupation (e.g., law enforcement) may put them in contact with large amounts of COC in their environment; therefore, a requirement for decontamination and further research are needed to determine the viability of comparative criteria using information from the decontamination. Alternatively, investigations are necessary to determine if other unique COC metabolites can be identified in hair testing and the extent of the effects of environmental exposure on these metabolites.

3.1.1 Implications for Policy and Practice

The work performed under this study will directly affect the use of hair testing in a variety of investigatory applications, including drug-related criminal cases, workplace drug testing, and other legal arenas, such as child custody, parole, and probation.

The results of this study will influence how hair-testing results are interpreted and could significantly impact whether national agencies use hair testing in their drug-free workplace programs. These results may also directly affect policy implementation for drug-facilitated crimes (e.g., drug-facilitated sexual assault, homicide by drug poisoning, child abuse by drug poisoning), workplace drug testing, and other uses for hair testing, including parole and rehabilitation compliance, by determining what decision criteria should be enforced for these COC analytes and concentration ratios.

Likely the most significant impact of this research will be for the federally regulated workplace drug testing program because the current proposed Mandatory Guidelines only have provisions for the use of cut-off concentrations and the BE/COC ratio. These results suggest these criteria are not sufficient to discriminate potential environmental contamination from usage of COC. Although it is unlikely that widespread contamination of hair is an issue, the federal workplace drug-testing program includes individuals who may have exposure to high drug concentrations because of their jobs. Therefore, the proposed Mandatory Guidelines for hair testing may need to be amended by adding an additional specimen preparatory step for COC analysis that would include a requirement for a laboratory to decontaminate hair specimens prior to analysis. Furthermore, potential criteria to evaluate the decontamination solutions in relation to the hair concentrations may be necessary unless the presence of other unique COC metabolites in hair can be reliably established.

3.1.2 Implications for Further Research

These results suggest the need for a decontamination strategy to be used by laboratories that conduct hair testing to detect COC. The most effective decontamination strategy remains debated. Further work to examine the efficacy of decontamination procedures is essential, and it would include not only the use of decontamination strategies, but also the evaluation of the hair-drug concentrations in comparison to the drug detected in the decontamination solvents. The criteria for both known contaminated and drug-user hair specimens subjected to these decontamination procedures also needs to be evaluated.

The extent of surface contamination is poorly understood for environments where exposures may occur such as for law enforcement who work in areas where there is known drug usage. Better estimation of the extent of surface contamination and the quantities of drug that could be realistically transferred to hair is a central component to understanding if contamination is a problem. This information is also key to resolving if in vitro contamination models are using quantities of COC that are too low or too high to be realistic.

Other drugs, including heroin and methamphetamines, as well as other opioids, may also cause contamination problems for hair because drug use sometimes occurs by crushing the tablets and snorting the powder. Limited research is available on the potential impact of transferred contamination and its impact on analysis of specific drugs. These COC results do not necessarily imply that all drugs will interact with hair so strongly and be so difficult to discriminate, but they do indicate that there is significant possibility of a similar potential problem for other drugs.

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5. DISSEMINATION OF RESEARCH FINDINGS

The research findings of this project have been presented at the following annual meetings:

1) Miller, J.D.R., E.J. Minden, N.D. Bynum, P.R. Stout, J.F. Casale, I. Kim, J. Runkle, M. Past, and B.D. Paul. 2008. Signature Analysis of 25 Illicit Cocaine Samples and a Comparison to Analysis by AccuTOF-DART. American Academy of Forensic Sciences, Washington, DC. February 18–22.

2) Ropero-Miller, J.D., and P. Stout. 2007. NIJ Grantees' Meeting: RTI International Forensic Research Programs. American Academy of Forensic Sciences, San Antonio, TX. February 19–24.

3) Ropero-Miller, J.D, M.A. Huestis, E.J. Cone, J.M. Mitchell, M.R. Baylor, M.A. Meaders, and P.R. Stout. 2008. Cocaine Analytes in Human Hair I: Evaluation of Concentration Ratios in Drug User Populations. Presented at the Annual SOFT Meeting 2008, Phoenix, AZ. October 27–31.

4) Ropero-Miller, J.D, M.A. Huestis, E.J. Cone, J.M. Mitchell, M.R. Baylor, M.A. Meaders, and P.R. Stout. 2008. Cocaine Analytes in Human Hair II: Evaluation of Concentration Ratios in Different Cocaine Sources and Surface-Contaminated Specimens. Presented at the Annual SOFT Meeting 2008, Phoenix, AZ. October 27–31.

In addition, RTI has made the dissemination of these research findings a priority goal of this project. The following publications and their status are included:

1) Published Manuscript:

   Ropero-Miller, J.D., P.R. Stout, N.D. Bynum, and J.F. Casale. 2007. Comparison of the novel direct analysis in real time time-of-flight mass spectrometry (AccuTOF-DART™) and signature analysis for the identification of constituents of refined illicit cocaine. *Drug Enforcement Administration Microgram Journal 5*(1–4):34–40.

2) Planned Submission of Manuscripts:

   Ropero-Miller, J.D., P.R. Stout, M.R Baylor, and J.M. Mitchell. 2008. External contamination of hair with cocaine II: Evaluation of external cocaine contamination using cocaine sources of varying purity. *Journal of Analytical Toxicology*. March.

   Ropero-Miller, J.D., P.R. Stout, and M.H. Huestis. 2008. Analysis of cocaine analytes in human hair: Evaluation of concentration ratios in different drug-user populations of both controlled and uncontrolled administration. *The Journal of Pharmacology and Experimental Therapeutics*.

Appendix A
Detailed Data of User Populations and Contaminated Hair

Table A-1. COC Analyte Concentrations Results and Ratios by LC-MS/MS for the Cairns and Colleagues (2004a) Drug-User Population

Subject	COC	BE	BE/COC Ratio	CE[a]	CE/COC Ratio[a]	NCOC[a]	NCOC/COC Ratio	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
1	227,000	24,900	0.110	30	0.000	3,330	0.015	POS	NEG	POS
16	185,200	14,400	0.078	1,220	0.007	5,560	0.030	POS	POS	POS
28	181,400	18,600	0.103	40	0.000	5,340	0.029	POS	NEG	POS
27	172,500	18,500	0.107	2,420	0.014	3,740	0.022	POS	POS	POS
18	157,400	17,700	0.112	10	0.000	5,010	0.032	POS	NEG	POS
13	149,800	17,000	0.113	12,430	0.083	3,090	0.021	POS	POS	POS
20	114,400	10,100	0.088	2,130	0.019	4,100	0.036	POS	POS	POS
31	104,500	11,200	0.107	970	0.009	2,030	0.019	POS	POS	POS
47	97,700	11,100	0.114	1,720	0.018	1,920	0.020	POS	POS	POS
19	91,200	17,000	0.186	10	0.000	2,120	0.023	POS	NEG	POS
53	89,900	9,240	0.103	10	0.000	2,900	0.032	POS	NEG	POS
29	89,500	8,560	0.096	1,200	0.013	1,810	0.020	POS	POS	POS
11	88,000	16,900	0.192	570	0.006	1,570	0.018	POS	POS	POS
9	83,100	1,250	0.015	10	0.000	1,520	0.018	NEG	NEG	POS
30	82,300	13,500	0.164	5,080	0.062	1,540	0.019	POS	POS	POS
6	79,400	5,770	0.073	570	0.007	1,560	0.020	POS	POS	POS
12	68,000	34,700	0.510	430	0.006	2,040	0.030	POS	POS	POS
10	66,500	8,330	0.125	480	0.007	2,440	0.037	POS	POS	POS
17	61,300	8,340	0.136	10	0.000	1,390	0.023	POS	NEG	POS
26	59,900	6,090	0.102	2,920	0.049	3,040	0.051	POS	POS	POS
14	58,900	8,760	0.149	1,760	0.030	980	0.017	POS	POS	POS
8	58,800	4,660	0.079			1,330	0.023	POS	NEG	POS
21	55,000	3,430	0.062	780	0.014	1,590	0.029	POS	POS	POS
72	53,400	7,880	0.148	30	0.001	1,110	0.021	POS	NEG	POS
33	45,500	8,310	0.183	460	0.010	1,110	0.024	POS	POS	POS
45	44,700	5,160	0.115	160	0.004	1,030	0.023	POS	POS	POS
40	44,000	4,760	0.108			1,180	0.027	POS	NEG	POS
37	40,400	4,580	0.113	80	0.002	1,140	0.028	POS	POS	POS
25	39,500	3,220	0.082	210	0.005	780	0.020	POS	POS	POS

Subject	COC	BE	BE/COC Ratio	CE[a]	CE/COC Ratio[a]	NCOC[a]	NCOC/COC Ratio	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
24	36,600	6,540	0.179			900	0.025	POS	NEG	POS
71	36,500	5,190	0.142	4,500	0.123	910	0.025	POS	POS	POS
41	34,400	2,110	0.061	60	0.002	840	0.024	POS	POS	POS
73	34,100	4,610	0.135	40	0.001	1,520	0.045	POS	NEG	POS
67	32,800	2,150	0.066	880	0.027	640	0.020	POS	POS	POS
22	29,300	5,310	0.181	12,790	0.437	640	0.022	POS	POS	POS
34	26,900	3,120	0.116	1,530	0.057	620	0.023	POS	POS	POS
5	26,300	2,380	0.090	40	0.002	550	0.021	POS	NEG	POS
58	24,800	2,680	0.108	140	0.006	1,090	0.044	POS	POS	POS
63	24,100	2,260	0.094	3,010	0.125	500	0.021	POS	POS	POS
59	20,000	3,200	0.160	30	0.002	1,180	0.059	POS	NEG	POS
68	15,300	950	0.062	2,640	0.173	290	0.019	POS	POS	POS
43	15,200	1,800	0.118	120	0.008	450	0.030	POS	POS	POS
35	15,200	880	0.058	40	0.003	330	0.022	POS	NEG	POS
50	14,500	2,400	0.166	50	0.003	1,000	0.069	POS	POS	POS
42	13,300	1,810	0.136	1,410	0.106	540	0.041	POS	POS	POS
46	13,000	1,900	0.146	110	0.008	380	0.029	POS	POS	POS
15	12,000	1,140	0.095			530	0.044	POS	NEG	POS
4	11,600	2,520	0.217			360	0.031	POS	NEG	POS
61	9,100	660	0.073	40	0.004	120	0.013	POS	NEG	POS
64	8,500	800	0.094	290	0.034	190	0.022	POS	POS	POS
32	8,000	340	0.043	450	0.056	200	0.025	NEG	POS	POS
36	7,400	640	0.086	780	0.105	170	0.023	POS	POS	POS
60	6,500	910	0.140	2,030	0.312	140	0.022	POS	POS	POS
23	6,400	740	0.116			330	0.052	POS	NEG	POS
38	6,000	820	0.137			180	0.030	POS	NEG	POS
65	5,600	2,260	0.404	10	0.002	180	0.032	POS	NEG	POS
51	5,470	1,100	0.201			110	0.020	POS	NEG	POS
39	5,400	610	0.113			150	0.028	POS	NEG	POS
49	5,370	830	0.155	40	0.007	120	0.022	POS	NEG	POS

Subject	COC	BE	BE/COC Ratio	CE[a]	CE/COC Ratio[a]	NCOC[a]	NCOC/COC Ratio	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
55	5,320	940	0.177	110	0.021	120	0.023	POS	POS	POS
2	4,500	370	0.082			110	0.024	POS	NEG	POS
62	4,000	330	0.083			90	0.023	POS	NEG	POS
56	3,570	1,180	0.331			130	0.036	POS	NEG	POS
3	3,300	580	0.176	10	0.003	150	0.045	POS	NEG	POS
52	2,030	160	0.079			40	0.020	POS	NEG	NEG
7	1,870	140	0.075	30	0.016		0.000	POS	NEG	NEG
57	1,130	150	0.133			10	0.009	POS	NEG	NEG
54	860	110	0.128			30	0.035	POS	NEG	NEG
48	660	30	0.045					NEG	NEG	NEG
70	650	40	0.062			10	0.015	NEG	NEG	NEG
66	400	100	0.250	50	0.125	20	0.050	NEG	NEG	NEG
69	370	40	0.108	20	0.054	10	0.027	NEG	NEG	NEG
44	290	20	0.069					NEG	NEG	NEG
75	50	0	0.000					NEG	NEG	NEG
74	30	0	0.000					NEG	NEG	NEG
Mean	43,038	5,211	0.125	1,218	0.040	1,174	0.027			
Median	24,800	2,380	0.112	210	0.008	810	0.023			
MIN Range	0	0	0.000	0	0.000	0	0.000			
MAX Range	227,000	34,700	0.510	12,790	0.437	5,560	0.069			
Above LOQ Count [b]	75	73	73	55	55	70	70			
Total Positive[c]	70	69	70	37		64				
% Positive by confirmatory cutoff[d]	93	92	93	49		85				

Note: COC = cocaine; BE = benzoylecgonine; CE = cocaethylene; NCOC = norcocaine; POS = positive; NEG = negative

[a] A blank cell is indicated when an analyte was not detected at the reported limit of quantitation.

[b], [c], [d]: The number of the subjects with a detectable concentration greater than the limit of quantitation (above LOQ count), the number of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (total positive); and the percentage of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (% positive).

Table A-2. Distribution of COC Concentrations in Drug-User Populations That Screened Positive by Urine Testing (Bourland et al., 2000)

Subject	COC	BE	BE/COC Ratio	CE[a]	CE/COC Ratio[a]	NCOC[a]	NCOC/COC Ratio[a]	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
O	35,500	3,840	0.108	10,870	0.306	700	0.020	POS	POS	POS
AA	35,300	3,150	0.089	140	0.004	1,580	0.045	POS	POS	POS
U	31,210	660	0.021	30	0.001	80	0.003	NEG	NEG	POS
Q	25,440	3,180	0.125	9,070	0.357	420	0.017	POS	POS	POS
A	21,780	4,260	0.196	630	0.029	520	0.024	POS	POS	POS
E	21,260	620	0.029					NEG	NEG	NEG
D	19,350	4,650	0.240	1,380	0.071	450	0.023	POS	POS	POS
K	19,070	4,710	0.247	830	0.044	280	0.015	POS	POS	POS
J	11,590	1,710	0.148	1,680	0.145	630	0.054	POS	POS	POS
Y	10,490	1,040	0.099			420	0.040	POS	NEG	POS
S	10,430	2,080	0.199	740	0.071	310	0.030	POS	POS	POS
T	8,760	1,190	0.136	1,830	0.209	220	0.025	POS	POS	POS
W	7,330	870	0.119	80	0.011	80	0.011	POS	POS	POS
B	6,920	690	0.100					POS	NEG	NEG
N	6,630	1,550	0.234	440	0.066	350	0.053	POS	POS	POS
P	6,010	270	0.045	130	0.022	50	0.008	NEG	POS	POS
CC	5,370	510	0.095	220	0.041	80	0.015	POS	POS	POS
C	3,330	160	0.048			60	0.018	NEG	NEG	POS
Z	3,320	250	0.075	310	0.093	170	0.051	POS	POS	POS
I	3,280	200	0.061					POS	NEG	NEG
BB	3,250	1,090	0.335			660	0.203	POS	NEG	POS
V	3,050	70	0.023			50	0.016	NEG	NEG	POS
DD	2,100	1,220	0.581			90	0.043	POS	NEG	POS
G	2,100	150	0.071	840	0.400			POS	POS	NEG
R	1,770	130	0.073	220	0.124			POS	POS	NEG
M	1,690	380	0.225	610	0.361	60	0.036	POS	POS	POS
F	1,560	720	0.462			160	0.103	POS	NEG	POS
H	1,250	70	0.056					POS	NEG	NEG
L	890	280	0.315	160	0.180	50	0.056	POS	POS	POS

Subject	COC	BE	BE/COC Ratio	CE[a]	CE/COC Ratio[a]	NCOC[a]	NCOC/COC Ratio[a]	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
X	420	100	0.238					NEG	NEG	NEG
Mean	10,348	1,327	0.160	1,590	0.133	325	0.039			
Median	6,320	705	0.113	610	0.071	220	0.025			
MIN Range	420	70	0.021	30	0.001	50	0.003			
MAX Range	35,500	4,710	0.581	10,870	0.400	1,580	0.203			
Above LOQ Count [b]	30	30	30	19	19	23	23			
Total Positive[c]	29	30	25	18		23				
% Positive by Confirmatory Cutoff[d]	97	100	83	60		77				

Note: COC = cocaine; BE = benzoylecgonine; CE = cocaethylene; NCOC = norcocaine; POS = positive; NEG = negative

[a] A blank cell is indicated when a COC analyte was not detected at the reported limit of quantitation.

[b, c, d] The number of the subjects with a detectable concentration greater than the limit of quantitation (above LOQ count), the number of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (total positive); and the percentage of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (% positive).

Table A-3. Results of COC Analyte Concentrations and Ratios by LC-MS/MS for the STREET Population

Specimen ID	COC	BE	BE/COC	CE[a]	CE/COC	NCOC[a]	NCOC/COC	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
STREET 1	4,375	2,824	0.646	157	0.036	68	0.015	POS	POS	POS
STREET 2	3,794	1,266	0.334	54	0.014	23	0.006	POS	POS	**NEG**
STREET 3	23,681	8,832	0.373	1,109	0.047	258	0.011	POS	POS	POS
STREET 4	38,474	8,542	0.222	5,003	0.130	745	0.019	POS	POS	POS
STREET 5	884	734	0.831					POS	**NEG**	**NEG**
STREET 6	7,867	1,035	0.131			86	0.011	POS	**NEG**	POS
STREET 7	60,587	37,942	0.626			390	0.006	POS	**NEG**	POS
STREET 8	5,148	4,070	0.791			62	0.012	POS	**NEG**	POS
STREET 9	37,959	12,342	0.325	227	0.006	568	0.015	POS	POS	POS
STREET 10	15,717	3,049	0.194	1,098	0.070	175	0.011	POS	POS	POS
STREET 11	1,174	461	0.393					POS	**NEG**	**NEG**
STREET 12	7,609	1,068	0.140			259	0.034	POS	**NEG**	POS
STREET 13	3021	1,355	0.449	56	0.019	69	0.023	POS	POS	POS
STREET 14	159	65	0.408					NEG	NEG	NEG
STREET 15	4,055	893	0.220			85	0.021	POS	**NEG**	POS
STREET 16	2,146	1,318	0.614					POS	POS	POS
STREET 17	218,276	62,313	0.285	57	0.000	1,124	0.005	POS	POS	POS
STREET 18	6,035	4,153	0.688	79	0.013	107	0.018	POS	POS	POS
STREET 19	4,581	1,617	0.353					POS	**NEG**	POS
STREET 20	4,622	1,352	0.293			81	0.018	POS	**NEG**	POS
STREET 21	19,404	8,749	0.451			199	0.010	POS	**NEG**	POS
STREET 22	90,460	67,253	0.743	249	0.003	1,160	0.013	POS	POS	POS
STREET 23	7,940	1,473	0.186			63	0.008	POS	**NEG**	POS
STREET 24	143,604	27,156	0.189	802	0.006	713	0.005	POS	POS	POS
STREET 25	1,682	642	0.382					POS	**NEG**	POS
STREET 26	11,747	13,858	1.180			199	0.017	POS	**NEG**	POS
STREET 27	16,046	13,748	0.857			282	0.018	POS	**NEG**	POS
STREET 28	3,665	1,457	0.398			59	0.016	POS	**NEG**	POS
STREET 29	10,321	2,903	0.281	35	0.003	265	0.026	POS	**NEG**	POS
STREET 30	2,074	786	0.379			47	0.023	POS	**NEG**	**NEG**
STREET 31	3,682	1,048	0.285	138	0.037	57	0.015	POS	POS	POS
STREET 32	14,256	1,316	0.092	28	0.002	107	0.008	POS	**NEG**	POS
STREET 33	12,470	703	0.056	623	0.050	309	0.025	POS	POS	POS
STREET 34	16,325	1,627	0.100	91	0.006	107	0.007	POS	POS	POS
STREET 35	164,127	4,670	0.028	1,912	0.012	1,810	0.011	NEG	POS	POS
STREET 36	38,059	2,242	0.059	144	0.004	826	0.022	POS	POS	POS
STREET 37	10,640	1,746	0.164	4,443	0.418	253	0.024	POS	POS	POS
STREET 38	43,126	2,420	0.056	1,716	0.040	473	0.011	POS	POS	POS

Specimen ID	COC	BE	BE/COC	CE[a]	CE/COC	NCOC[a]	NCOC/COC	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
Mean	27,889	8,132	0.374	901	0.046	345	0.015			
Median	12,470	1,746	0.285	436	0.009	253	0.015			
MIN Range	159	65	0.028	0	0.000	0	0.005			
MAX Range	218,276	67,253	1.180	5,003	0.418	1,810	0.034			
Above LOQ Count[b]	38	38	38	20	20	32	32			
Total Positive[c]	37	38	37	18		27				
% Positive by Confirmatory Cutoff[d]	97	100	97	47		71				

Note: BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; NCOC= norcocaine; NEG = negative; POS = positive

Note: Bolded results for Criteria 2 or 3 (CE and NCOC, respectively) indicate a change from Criteria 1 (BE criteria).

[a] A blank cell is indicated when a COC analyte was not detected at the reported limit of quantitation.

[b,c,d] The number of the subjects with a detectable concentration greater than the limit of quantitation (above LOQ count), the number of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (total positive); and the percentage of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (% positive).

Table A-4. Results of Additional COC Analyte Ratios Criteria by LC-MS/MS for the STREET Population

Sample ID	Criteria 4 (0.05 NCOC Ratio)	Criteria 5 (0.01 NCOC Ratio)	Criteria 6 (0.05 CE Ratio)	Criteria 7 (0.02 CE Ratio)	Criteria 8 (0.01CE Ratio)	Criteria 9 (0.002 CE Ratio)
STREET 1	**NEG**	POS	**NEG**	POS	POS	POS
STREET 2	**NEG**	**NEG**	**NEG**	**NEG**	POS	POS
STREET 3	**NEG**	POS	**NEG**	POS	POS	POS
STREET 4	**NEG**	POS	POS	POS	POS	POS
STREET 5	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 6	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 7	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 8	**NEG**	POS	**NEG**	**NEG**	**NEG**	NEG
STREET 9	**NEG**	POS	**NEG**	**NEG**	**NEG**	POS
STREET 10	**NEG**	POS	POS	POS	POS	POS
STREET 11	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 12	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**

Sample ID	Criteria 4 (0.05 NCOC Ratio)	Criteria 5 (0.01 NCOC Ratio)	Criteria 6 (0.05 CE Ratio)	Criteria 7 (0.02 CE Ratio)	Criteria 8 (0.01CE Ratio)	Criteria 9 (0.002 CE Ratio)
STREET 13	**NEG**	POS	**NEG**	**NEG**	POS	POS
STREET 14	NEG	**NEG**	NEG	NEG	NEG	NEG
STREET 15	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 16	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 17	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 18	**NEG**	POS	**NEG**	**NEG**	POS	POS
STREET 19	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 20	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 21	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 22	**NEG**	POS	**NEG**	**NEG**	**NEG**	POS
STREET 23	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 24	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	POS
STREET 25	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 26	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 27	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 28	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 29	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
STREET 30	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 31	**NEG**	POS	**NEG**	POS	POS	POS
STREET 32	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
STREET 33	**NEG**	POS	**NEG**	POS	POS	POS
STREET 34	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	POS
STREET 35	NEG	**POS**	NEG	**NEG**	POS	POS
STREET 36	**NEG**	POS	**NEG**	**NEG**	**NEG**	POS
STREET 37	**NEG**	POS	POS	POS	POS	POS
STREET 38	**NEG**	POS	**NEG**	POS	POS	POS
Total Positive[a]	0	24	3	8	12	17
% Positive by Confirmatory Cutoff[b]	0	63	8	21	32	45

Note: BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; NCOC= norcocaine; NEG = negative; POS = positive

Note: Bolded results for Criteria 4 through 9 indicate a change from Criteria 1 (BE criteria).

[a,b,] The number of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (total positive); and the percentage of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (% positive).

Table A-5. Results of COC Analyte Concentrations and Ratios by LC-MS/MS for the CLINICAL Population

Specimen ID	COC	BE	BE/COC	CE	CE/COC	NCOC	NCOC/COC	Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
CLINICAL 1	4,421	340	0.077					POS	**NEG**	**NEG**
CLINICAL 2	9,958	484	0.049	134	0.013	213	0.021	NEG	**POS**	**POS**
CLINICAL 3	3,771	244	0.065			147	0.039	POS	**NEG**	POS
CLINICAL 4	1,631					98	0.060	NEG	NEG	**POS**
CLINICAL 5	1,054							NEG	NEG	NEG
CLINICAL 6	2,619	194	0.074			137	0.052	POS	**NEG**	POS
CLINICAL 7	4,195	269	0.064					POS	**NEG**	**NEG**
CLINICAL 8	725							NEG	NEG	NEG
CLINICAL 9	1,098	81	0.074			50	0.046	POS	**NEG**	POS
CLINICAL 10	7,736	477	0.062	75	0.010	280	0.036	POS	POS	POS
CLINICAL 11	2,949	231	0.078					POS	**NEG**	**NEG**
CLINICAL 12	4,086	336	0.082					POS	**NEG**	**NEG**
CLINICAL 13	8,396	691	0.082	64	0.008	129	0.015	POS	POS	POS
CLINICAL 14	3,431	297	0.087			60	0.017	POS	**NEG**	POS
CLINICAL 15	6,421	491	0.076			186	0.029	POS	**NEG**	POS
CLINICAL 16	12,161	796	0.065	78	0.006	267	0.022	POS	POS	POS
CLINICAL 17	32,786	1,501	0.046	397	0.012	2,075	0.063	NEG	**POS**	**POS**
CLINICAL 18	4,323	487	0.113			101	0.023	POS	**NEG**	POS
CLINICAL 19	2,970	175	0.059	40	0.013	88	0.030	POS	**NEG**	POS
CLINICAL 20	8,694	279	0.032	74	0.009	225	0.026	NEG	**POS**	**POS**
Mean	6,171	434	0.070	123	0.010	290	0.034			
Median	4,141	336	0.074	75	0.010	142	0.029			
MIN Range	725	ND	0.032	ND	0.006	ND	0.015			
MAX Range	32,786	1,501	0.113	397	0.013	2,075	0.063			
Above LOQ Count[b]	20	17	17	7	7	14	14			
Total Positive[c]	20	17	14	6		13				
% Positive by Confirmatory Cutoff[d]	100	85	70	30		65				

Note: BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; NCOC= norcocaine; ND = not detected; NEG = negative; POS = positive

Note: Bolded results for Criteria 2 or 3 (CE and NCOC, respectively) indicate a change from Criteria 1 (BE criteria).

[b,c,d] The number of the subjects with a detectable concentration greater than the limit of quantitation (above LOQ count), the number of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (total positive); and the percentage of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (% positive).

Table A-6. Results of Additional COC Analyte Ratios Criteria by LC-MS/MS for the CLINICAL Population

Sample ID	Criteria 4 (0.05 NCOC Ratio)	Criteria 5 (0.01 NCOC Ratio)	Criteria 6 (0.05 CE Ratio)	Criteria 7 (0.02 CE Ratio)	Criteria 8 (0.01 CE Ratio)	Criteria 9 (0.002 CE Ratio)
CLINICAL 1	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 2	NEG	**POS**	NEG	NEG	**POS**	POS
CLINICAL 3	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 4	**POS**	**POS**	**NEG**	NEG	NEG	NEG
CLINICAL 5	NEG	NEG	NEG	NEG	NEG	NEG
CLINICAL 6	POS	POS	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 7	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 8	NEG	NEG	NEG	NEG	NEG	NEG
CLINICAL 9	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 10	**NEG**	POS	**NEG**	**NEG**	**NEG**	**POS**
CLINICAL 11	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 12	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 13	**NEG**	POS	**NEG**	**NEG**	**NEG**	**POS**
CLINICAL 14	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 15	NEG	POS	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 16	**NEG**	POS	**NEG**	**NEG**	**NEG**	**POS**
CLINICAL 17	**POS**	**POS**	**NEG**	**NEG**	POS	**POS**
CLINICAL 18	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 19	**NEG**	POS	**NEG**	**NEG**	**NEG**	**NEG**
CLINICAL 20	NEG	**POS**	NEG	NEG	NEG	POS
Total Positive[a]	3	14	0	0	2	6
% Positive by Confirmatory Cutoff[b]	15	70	0	0	10	30

Note: BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; NCOC= norcocaine; NEG = negative; POS = positive

Note: Bolded results for Criteria 4 through 9 indicate a change from Criteria 1 (BE criteria).

[a,b,] The number of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (total positive); and the percentage of the subjects positive using the confirmatory cutoff or criteria of the proposed Mandatory Guidelines (% positive).

Table A-7. A Complete Listing of the Analytical Results of Contaminated Hair Specimens, the Ratios of Each of the Drugs, and the First Three Criteria Results (i.e., Proposed Federal Regulation Cutoffs)

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-6-1	Before sweat, after contamination	CE	D	3	159	0	0	0	0.00	0.00	0.00			
11367-76-6-1	Before sweat, after contamination	CE	D	2	161	0	0	0	0.00	0.00	0.00			
11367-76-6-1	Before sweat, after contamination	CE	D	1	260	0	0	0	0.00	0.00	0.00			
11367-76-6-2	After sweat, after contamination	CE	D	3	10,000	0	234	894	0.00	0.02	0.09	POS	POS	
11367-76-6-2	After sweat, after contamination	CE	D	1	10,000	0	272	1,048	0.00	0.03	0.10	POS	POS	
11367-76-6-2	After sweat, after contamination	CE	D	2	10,000	0	238	1,110	0.00	0.02	0.11	POS	POS	
11367-76-6-3	Before shampoo, 24 hours	CE	D	2	10,000	0	202	671	0.00	0.02	0.07	POS	POS	
11367-76-6-3	Before shampoo, 24 hours	CE	D	1	10,000	0	231	702	0.00	0.02	0.07	POS	POS	
11367-76-6-3	Before shampoo, 24 hours	CE	D	3	10,000	0	218	944	0.00	0.02	0.09	POS	POS	
11367-76-6-4	Day 7	CE	D	3	10,000	0	321	1,254	0.00	0.03	0.13	POS	POS	
11367-76-6-4	Day 7	CE	D	2	10,000	0	283	1,796	0.00	0.03	0.18	POS	POS	
11367-76-6-4	Day 7	CE	D	1	10,000	58	386	1,950	0.01	0.04	0.20	POS	POS	POS
11367-76-6-5	Day 14	CE	D	2	3,965	0	78	146	0.00	0.02	0.04		POS	
11367-76-6-5	Day 14	CE	D	1	3,857	0	74	174	0.00	0.02	0.05		POS	
11367-76-6-5	Day 14	CE	D	3	3,580	0	68	0	0.00	0.02	0.00		POS	
11367-76-6-6	Day 21	CE	D	3	6,810	0	96	632	0.00	0.01	0.09	POS	POS	
11367-76-6-6	Day 21	CE	D	1	6,643	0	68	645	0.00	0.01	0.10	POS	POS	
11367-76-6-6	Day 21	CE	D	2	6,612	0	109	659	0.00	0.02	0.10	POS	POS	

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-6-7	Day 28	CE	D	2	3,955	0	85	351	0.00	0.02	0.09	POS	POS	
11367-76-6-7	Day 28	CE	D	1	3,396	0	84	358	0.00	0.02	0.11	POS	POS	
11367-76-6-7	Day 28	CE	D	3	4,007	0	77	389	0.00	0.02	0.10	POS	POS	
11367-76-6-8	Day 35	CE	D	3	3,334	0	69	207	0.00	0.02	0.06	POS	POS	
11367-76-6-8	Day 35	CE	D	1	2,642	0	0	213	0.00	0.00	0.08	POS		
11367-76-6-8	Day 35	CE	D	2	4,164	0	35	266	0.00	0.01	0.06	POS		
11367-76-6-9	Day 42	CE	D	2	3,682	0	70	260	0.00	0.02	0.07	POS	POS	
11367-76-6-9	Day 42	CE	D	3	3,517	0	65	288	0.00	0.02	0.08	POS	POS	
11367-76-6-9	Day 42	CE	D	1	2,628	0	0	288	0.00	0.00	0.11	POS		
11367-76-6-10	Day 49	CE	D	1	2,616	0	0	210	0.00	0.00	0.08	POS		
11367-76-6-10	Day 49	CE	D	3	2,129	0	*0*	224	0.00	0.00	0.11	POS		
11367-76-6-10	Day 49	CE	D	2	2,314	0	57	232	0.00	0.02	0.10	POS	POS	
11367-76-6-11	Day 56	CE	D	2	2,248	0	57	295	0.00	0.03	0.13	POS	POS	
11367-76-6-11	Day 56	CE	D	3	2,256	0	0	312	0.00	0.00	0.14	POS		
11367-76-6-11	Day 56	CE	D	1	2,182	0	58	360	0.00	0.03	0.16	POS	POS	
11367-76-6-12	Day 63	CE	D	1	1,966	0	0	202	0.00	0.00	0.10	POS		
11367-76-6-12	Day 63	CE	D	3	2,085	0	51	281	0.00	0.02	0.13	POS	POS	
11367-76-6-12	Day 63	CE	D	2	2,802	0	65	295	0.00	0.02	0.11	POS	POS	
11367-76-6-13	Day 70	CE	D	1	1,057	0	0	68	0.00	0.00	0.06	POS		
11367-76-6-13	Day 70	CE	D	3	956	0	0	81	0.00	0.00	0.08	POS		
11367-76-6-13	Day 70	CE	D	2	1,122	0	0	98	0.00	0.00	0.09	POS		
11367-76-5-1	Before sweat, after contamination	CE	L	2	97	0	0	0	0.00	0.00	0.00			
11367-76-5-1	Before sweat, after contamination	CE	L	1	104	0	0	0	0.00	0.00	0.00			

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-5-1	Before sweat, after contamination	CE	L	3	154	0	0	0	0.00	0.00	0.00			
11367-76-5-2	After sweat, after contamination	CE	L	2	5,674	0	93	683	0.00	0.02	0.12	POS	POS	
11367-76-5-2	After sweat, after contamination	CE	L	3	7,559	0	119	799	0.00	0.02	0.11	POS	POS	
11367-76-5-2	After sweat, after contamination	CE	L	1	7,998	0	129	937	0.00	0.02	0.12	POS	POS	
11367-76-5-3	Before shampoo, 24 hours	CE	L	1	12,000	0	274	1,983	0.00	0.02	0.17	POS	POS	
11367-76-5-3	Before shampoo, 24 hours	CE	L	2	12,000	0	255	2,052	0.00	0.02	0.17	POS	POS	
11367-76-5-3	Before shampoo, 24 hours	CE	L	3	12,000	0	272	2,292	0.00	0.02	0.19	POS	POS	
11367-76-5-4	Day 7	CE	L	3	10,228	0	167	1,072	0.00	0.02	0.10	POS	POS	
11367-76-5-4	Day 7	CE	L	1	10,101	0	246	1,212	0.00	0.02	0.12	POS	POS	
11367-76-5-4	Day 7	CE	L	2	7,900	0	155	1,335	0.00	0.02	0.17	POS	POS	
11367-76-5-5	Day 14	CE	L	3	3,322	0	72	553	0.00	0.02	0.17	POS	POS	
11367-76-5-5	Day 14	CE	L	2	3,705	0	68	564	0.00	0.02	0.15	POS	POS	
11367-76-5-5	Day 14	CE	L	1	4,405	0	112	769	0.00	0.03	0.17	POS	POS	
11367-56-5-6	Day 21	CE	L	3	2,110	0	0	261	0.00	0.00	0.12	POS		
11367-76-5-6	Day 21	CE	L	1	2,034	0	68	270	0.00	0.03	0.13	POS	POS	
11367-76-5-6	Day 21	CE	L	2	2,207	0	51	290	0.00	0.02	0.13	POS	POS	
11367-76-5-7	Day 28	CE	L	2	1,766	0	73	367	0.00	0.04	0.21	POS	POS	
11367-76-5-7	Day 28	CE	L	3	1,335	0	65	473	0.00	0.05	0.35	POS	POS	
11367-76-5-7	Day 28	CE	L	1	1,907	0	74	561	0.00	0.04	0.29	POS	POS	
11367-76-5-8	Day 35	CE	L	2	1,811	0	51	185	0.00	0.03	0.10	POS	POS	
11367-76-5-8	Day 35	CE	L	1	2,111	0	47	215	0.00	0.02	0.10	POS		

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-5-8	Day 35	CE	L	3	1,954	0	53	238	0.00	0.03	0.12	POS	POS	
11367-76-5-9	Day 42	CE	L	2	644	0	0	106	0.00	0.00	0.16	POS		
11367-76-5-9	Day 42	CE	L	3	633	0	0	120	0.00	0.00	0.19	POS		
11367-76-5-9	Day 42	CE	L	1	449	0	0	124	0.00	0.00	0.28			
11367-76-5-10	Day 49	CE	L	3	539	0	0	75	0.00	0.00	0.14	POS		
11367-76-5-10	Day 49	CE	L	2	508	0	0	76	0.00	0.00	0.15	POS		
11367-76-5-10	Day 49	CE	L	1	536	0	0	80	0.00	0.00	0.15	POS		
11367-76-5-11	Day 56	CE	L	2	230	0	0	70	0.00	0.00	0.30			
11367-76-5-11	Day 56	CE	L	3	303	0	0	109	0.00	0.00	0.36			
11367-76-5-11	Day 56	CE	L	1	326	0	0	117	0.00	0.00	0.36			
11367-76-5-12	Day 63	CE	L	3	466	0	0	105	0.00	0.00	0.23			
11367-76-5-12	Day 63	CE	L	1	164	0	0	0	0.00	0.00	0.00			
11367-76-5-12	Day 63	CE	L	2	189	0	0	0	0.00	0.00	0.00			
11367-76-5-13	Day 70	CE	L	1	94	0	0	0	0.00	0.00	0.00			
11367-76-5-13	Day 70	CE	L	2	106	0	0	0	0.00	0.00	0.00			
11367-76-5-13	Day 70	CE	L	3	119	0	0	0	0.00	0.00	0.00			
11367-76-2-1	Before sweat, after contamination	NCOC	D	2	87	0	0	0	0.00	0.00	0.00			
11367-76-2-1	Before sweat, after contamination	NCOC	D	1	100	0	0	0	0.00	0.00	0.00			
11367-76-2-1	Before sweat, after contamination	NCOC	D	3	101	0	0	0	0.00	0.00	0.00			
11367-76-2-2	After sweat, after contamination	NCOC	D	2	1,442	142	0	50	0.10	0.00	0.03			POS
11367-76-2-2	After sweat, after contamination	NCOC	D	3	2,644	164	0	0	0.06	0.00	0.00			POS
11367-76-2-2	After sweat, after contamination	NCOC	D	1	1,390	134	0	0	0.10	0.00	0.00			POS

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-2-3	Before shampoo, 24 hours	NCOC	D	1	10,000	1,051	0	117	0.11	0.00	0.01			POS
11367-76-2-3	Before shampoo, 24 hours	NCOC	D	2	10,000	1,281	0	130	0.13	0.00	0.01			POS
11367-76-2-3	Before shampoo, 24 hours	NCOC	D	3	10,000	1,336	0	192	0.13	0.00	0.02			POS
11367-76-2-4	Day 7	NCOC	D	1	10,000	1,409	0	234	0.14	0.00	0.02			POS
11367-76-2-4	Day 7	NCOC	D	3	10,000	2,032	0	265	0.20	0.00	0.03			POS
11367-76-2-4	Day 7	NCOC	D	2	10,000	1,998	0	268	0.20	0.00	0.03			POS
11367-76-2-5	Day 14	NCOC	D	1	8,123	710	0	121	0.09	0.00	0.01			POS
11367-76-2-5	Day 14	NCOC	D	2	9,298	843	0	158	0.09	0.00	0.02			POS
11367-76-2-5	Day 14	NCOC	D	3	7,594	684	0	182	0.09	0.00	0.02			POS
11367-76-2-6	Day 21	NCOC	D	1	7,707	736	0	201	0.10	0.00	0.03			POS
11367-76-2-6	Day 21	NCOC	D	3	6,211	726	0	202	0.12	0.00	0.03			POS
11367-76-2-6	Day 21	NCOC	D	2	6,895	890	0	213	0.13	0.00	0.03			POS
11367-76-2-7	Day 28	NCOC	D	2	2,714	309	0	158	0.11	0.00	0.06	POS		POS
11367-76-2-7	Day 28	NCOC	D	1	3,422	338	0	158	0.10	0.00	0.05			POS
11367-76-2-7	Day 28	NCOC	D	3	3,261	369	0	235	0.11	0.00	0.07	POS		POS
11367-76-2-8	Day 35	NCOC	D	1	4,686	520	0	148	0.11	0.00	0.03			POS
11367-76-2-8	Day 35	NCOC	D	3	5,385	574	0	161	0.11	0.00	0.03			POS
11367-76-2-8	Day 35	NCOC	D	2	5,432	600	0	180	0.11	0.00	0.03			POS
11367-76-2-9	Day 42	NCOC	D	2	2,348	384	0	131	0.16	0.00	0.06	POS		POS
11367-76-2-9	Day 42	NCOC	D	3	2,229	293	0	134	0.13	0.00	0.06	POS		POS
11367-76-2-9	Day 42	NCOC	D	1	3,036	377	0	147	0.12	0.00	0.05			POS
11367-76-2-10	Day 49	NCOC	D	1	2,165	272	0	153	0.13	0.00	0.07	POS		POS
11367-76-2-10	Day 49	NCOC	D	3	2,960	340	0	187	0.11	0.00	0.06	POS		POS

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-2-10	Day 49	NCOC	D	2	2,372	344	0	188	0.15	0.00	0.08	POS		POS
11367-76-2-11	Day 56	NCOC	D	1	2,200	286	0	158	0.13	0.00	0.07	POS		POS
11367-76-2-11	Day 56	NCOC	D	3	1,808	171	0	163	0.09	0.00	0.09	POS		POS
11367-76-2-11	Day 56	NCOC	D	2	2,112	229	0	174	0.11	0.00	0.08	POS		POS
11367-76-2-12	Day 63	NCOC	D	1	1,552	174	0	107	0.11	0.00	0.07	POS		POS
11367-76-2-12	Day 63	NCOC	D	3	1,613	198	0	114	0.12	0.00	0.07	POS		POS
11367-76-2-12	Day 63	NCOC	D	2	1,279	192	0	133	0.15	0.00	0.10	POS		POS
11367-76-2-13	Day 70	NCOC	D	3	1,269	136	0	73	0.11	0.00	0.06	POS		POS
11367-76-2-13	Day 70	NCOC	D	2	1,140	123	0	79	0.11	0.00	0.07	POS		POS
11367-76-2-13	Day 70	NCOC	D	1	1,287	149	0	84	0.12	0.00	0.07	POS		POS
11367-76-1-1	Before sweat, after contamination	NCOC	L	1	102	0	0	0	0.00	0.00	0.00			
11367-76-1-1	Before sweat, after contamination	NCOC	L	2	0	0	0	0	0.00	0.00	0.00			
11367-76-1-1	Before sweat, after contamination	NCOC	L	3	0	0	0	0	0.00	0.00	0.00			
11367-76-1-2	After sweat, after contamination	NCOC	L	1	1,871	184	0	54	0.10	0.00	0.03			POS
11367-76-1-2	After sweat, after contamination	NCOC	L	2	2,436	238	0	0	0.10	0.00	0.00			POS
11367-76-1-2	After sweat, after contamination	NCOC	L	3	2,920	198	0	0	0.07	0.00	0.00			POS
11367-76-1-3	Before shampoo, 24 hours	NCOC	L	3	4,017	412	0	60	0.10	0.00	0.01			POS
11367-76-1-3	Before shampoo, 24 hours	NCOC	L	2	4,677	404	0	62	0.09	0.00	0.01			POS
11367-76-1-3	Before shampoo, 24 hours	NCOC	L	1	2,800	239	0	111	0.09	0.00	0.04			POS
11367-76-1-4	Day 7	NCOC	L	2	1,420	159	0	0	0.11	0.00	0.00			POS
11367-76-1-4	Day 7	NCOC	L	1	1,459	156	0	0	0.11	0.00	0.00			POS

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-1-4	Day 7	NCOC	L	3	1,393	138	0	0	0.10	0.00	0.00			POS
11367-76-1-5	Day 14	NCOC	L	2	642	73	0	0	0.11	0.00	0.00			POS
11367-76-1-5	Day 14	NCOC	L	1	583	66	0	0	0.11	0.00	0.00			POS
11367-76-1-5	Day 14	NCOC	L	3	787	84	0	0	0.11	0.00	0.00			POS
11367-76-1-6	Day 21	NCOC	L	3	473	79	0	0	0.17	0.00	0.00			
11367-76-1-6	Day 21	NCOC	L	2	534	62	0	0	0.12	0.00	0.00			POS
11367-76-1-6	Day 21	NCOC	L	1	498	73	0	0	0.15	0.00	0.00			
11367-76-1-7	Day 28	NCOC	L	2	265	66	0	167	0.25	0.00	0.63			
11367-76-1-7	Day 28	NCOC	L	1	213	0	0	0	0.00	0.00	0.00			
11367-76-1-7	Day 28	NCOC	L	3	224	0	0	0	0.00	0.00	0.00			
11367-76-1-8	Day 35	NCOC	L	1	85	0	0	0	0.00	0.00	0.00			
11367-76-1-8	Day 35	NCOC	L	2	96	0	0	0	0.00	0.00	0.00			
11367-76-1-8	Day 35	NCOC	L	3	111	0	0	0	0.00	0.00	0.00			
11367-76-1-9	Day 42	NCOC	L	3	142	0	0	0	0.00	0.00	0.00			
11367-76-1-9	Day 42	NCOC	L	2	159	0	0	0	0.00	0.00	0.00			
11367-76-1-9	Day 42	NCOC	L	1	162	0	0	0	0.00	0.00	0.00			
11367-76-1-10	Day 49	NCOC	L	1	101	0	0	0	0.00	0.00	0.00			
11367-76-1-10	Day 49	NCOC	L	2	125	0	0	0	0.00	0.00	0.00			
11367-76-1-10	Day 49	NCOC	L	3	130	0	0	0	0.00	0.00	0.00			
11367-76-1-11	Day 56	NCOC	L	3	60	0	0	0	0.00	0.00	0.00			
11367-76-1-11	Day 56	NCOC	L	1	66	0	0	0	0.00	0.00	0.00			
11367-76-1-11	Day 56	NCOC	L	2	68	0	0	0	0.00	0.00	0.00			
11367-76-1-12	Day 63	NCOC	L	1	0	0	0	0	0.00	0.00	0.00			
11367-76-1-12	Day 63	NCOC	L	2	0	0	0	0	0.00	0.00	0.00			

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-1-12	Day 63	NCOC	L	3	0	0	0	0	0.00	0.00	0.00			
11367-76-1-13	Day 70	NCOC	L	1	0	0	0	0	0.00	0.00	0.00			
11367-76-1-13	Day 70	NCOC	L	2	0	0	0	0	0.00	0.00	0.00			
11367-76-1-13	Day 70	NCOC	L	3	0	0	0	0	0.00	0.00	0.00			
11367-76-4-1	Before sweat, after contamination	PHARM	D	2	77	0	0	0	0.00	0.00	0.00			
11367-76-4-1	Before sweat, after contamination	PHARM	D	3	124	0	0	0	0.00	0.00	0.00			
11367-76-4-1	Before sweat, after contamination	PHARM	D	1	133	0	0	0	0.00	0.00	0.00			
11367-76-4-2	After sweat, after contamination	PHARM	D	3	20,000	0	298	204	0.00	0.01	0.01		POS	
11367-76-4-2	After sweat, after contamination	PHARM	D	2	20,000	0	346	244	0.00	0.02	0.01		POS	
11367-76-4-2	After sweat, after contamination	PHARM	D	1	20,000	0	273	392	0.00	0.01	0.02		POS	
11367-76-4-3	Before shampoo, 24 hours	PHARM	D	2	20,000	0	382	281	0.00	0.02	0.01		POS	
11367-76-4-3	Before shampoo, 24 hours	PHARM	D	1	20,000	0	234	320	0.00	0.01	0.02		POS	
11367-76-4-3	Before shampoo, 24 hours	PHARM	D	3	20,000	0	244	419	0.00	0.01	0.02		POS	
11367-76-4-4	Day 7	PHARM	D	1	20,000	72	419	578	0.00	0.02	0.03		POS	POS
11367-76-4-4	Day 7	PHARM	D	3	20,000	70	482	615	0.00	0.02	0.03		POS	POS
11367-76-4-4	Day 7	PHARM	D	2	20,000	52	351	680	0.00	0.02	0.03		POS	POS
11367-76-4-5	Day 14	PHARM	D	1	15,952	0	159	346	0.00	0.01	0.02		POS	
11367-76-4-5	Day 14	PHARM	D	3	18,647	0	240	516	0.00	0.01	0.03		POS	
11367-76-4-5	Day 14	PHARM	D	2	16,391	0	162	525	0.00	0.01	0.03		POS	
11367-76-4-6	Day 21	PHARM	D	3	16,453	0	160	364	0.00	0.01	0.02		POS	
11367-76-4-6	Day 21	PHARM	D	2	15,158	0	182	396	0.00	0.01	0.03		POS	

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-4-6	Day 21	PHARM	D	1	13,890	0	138	567	0.00	0.01	0.04		POS	
11367-76-4-7	Day 28	PHARM	D	1	10,696	0	121	490	0.00	0.01	0.05		POS	
11367-76-4-7	Day 28	PHARM	D	3	11,784	56	122	513	0.00	0.01	0.04		POS	POS
11367-76-4-7	Day 28	PHARM	D	2	11,367	0	125	596	0.00	0.01	0.05	POS	POS	
11367-76-4-8	Day 35	PHARM	D	2	9,874	0	135	408	0.00	0.01	0.04		POS	
11367-76-4-8	Day 35	PHARM	D	1	11,162	0	197	453	0.00	0.02	0.04		POS	
11367-76-4-8	Day 35	PHARM	D	3	11,459	0	136	498	0.00	0.01	0.04		POS	
11367-76-4-9	Day 42	PHARM	D	2	9,231	0	117	471	0.00	0.01	0.05	POS	POS	
11367-76-4-9	Day 42	PHARM	D	1	8,005	0	103	492	0.00	0.01	0.06	POS	POS	
11367-76-4-9	Day 42	PHARM	D	3	9,835	0	108	550	0.00	0.01	0.06	POS	POS	
11367-76-4-10	Day 49	PHARM	D	1	10,016	0	179	773	0.00	0.02	0.08	POS	POS	
11367-76-4-10	Day 49	PHARM	D	2	10,075	0	177	859	0.00	0.02	0.09	POS	POS	
11367-76-4-10	Day 49	PHARM	D	3	11,733	0	223	931	0.00	0.02	0.08	POS	POS	
11367-76-4-11	Day 56	PHARM	D	2	7,268	0	138	452	0.00	0.02	0.06	POS	POS	
11367-76-4-11	Day 56	PHARM	D	1	7,773	0	165	482	0.00	0.02	0.06	POS	POS	
11367-76-4-11	Day 56	PHARM	D	3	7,278	0	123	564	0.00	0.02	0.08	POS	POS	
11367-76-4-12	Day 63	PHARM	D	3	5,852	0	125	395	0.00	0.02	0.07	POS	POS	
11367-76-4-12	Day 63	PHARM	D	2	6,896	0	154	433	0.00	0.02	0.06	POS	POS	
11367-76-4-12	Day 63	PHARM	D	1	6,351	0	102	502	0.00	0.02	0.08	POS	POS	
11367-76-4-13	Day 70	PHARM	D	2	4,352	0	85	325	0.00	0.02	0.07	POS	POS	
11367-76-4-13	Day 70	PHARM	D	3	4,436	0	88	341	0.00	0.02	0.08	POS	POS	
11367-76-4-13	Day 70	PHARM	D	1	5,069	0	82	385	0.00	0.02	0.08	POS	POS	
11367-76-3-1	Before sweat, after contamination	PHARM	L	3	51	0	0	0	0.00	0.00	0.00			

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-3-1	Before sweat, after contamination	PHARM	L	1	71	0	0	0	0.00	0.00	0.00			
11367-76-3-1	Before sweat, after contamination	PHARM	L	2	936	0	0	0	0.00	0.00	0.00			
11367-76-3-2	After sweat, after contamination	PHARM	L	1	9,614	0	120	109	0.00	0.01	0.01		POS	
11367-76-3-2	After sweat, after contamination	PHARM	L	3	9,484	0	103	122	0.00	0.01	0.01		POS	
11367-76-3-2	After sweat, after contamination	PHARM	L	2	9,487	0	97	152	0.00	0.01	0.02		POS	
11367-76-3-3	Before shampoo, 24 hours	PHARM	L	3	2,404	0	50	57	0.00	0.02	0.02		POS	
11367-76-3-3	Before shampoo, 24 hours	PHARM	L	2	2,250	0	0	67	0.00	0.00	0.03			
11367-76-3-3	Before shampoo, 24 hours	PHARM	L	1	2,045	0	0	72	0.00	0.00	0.04			
11367-76-3-4	Day 7	PHARM	L	1	2,478	0	33	90	0.00	0.01	0.04			
11367-76-3-4	Day 7	PHARM	L	2	2,592	0	63	97	0.00	0.02	0.04		POS	
11367-76-3-4	Day 7	PHARM	L	3	2,412	0	0	0	0.00	0.00	0.00			
11367-76-3-5	Day 14	PHARM	L	1	1,057	0	0	52	0.00	0.00	0.05			
11367-76-3-5	Day 14	PHARM	L	2	1,078	0	0	0	0.00	0.00	0.00			
11367-76-3-5	Day 14	PHARM	L	3	1,039	0	0	0	0.00	0.00	0.00			
11367-76-3-6	Day 21	PHARM	L	3	265	0	0	0	0.00	0.00	0.00			
11367-76-3-6	Day 21	PHARM	L	2	338	0	0	0	0.00	0.00	0.00			
11367-76-3-6	Day 21	PHARM	L	1	325	0	0	0	0.00	0.00	0.00			
11367-76-3-7	Day 28	PHARM	L	2	184	0	0	0	0.00	0.00	0.00			
11367-76-3-7	Day 28	PHARM	L	1	197	0	0	0	0.00	0.00	0.00			
11367-76-3-7	Day 28	PHARM	L	3	222	0	0	0	0.00	0.00	0.00			
11367-76-3-8	Day 35	PHARM	L	1	170	0	0	0	0.00	0.00	0.00			

Specimen Number	Specimen Note	COC Type	Hair Type	Replicate	pg/mg				Drug Ratios			Criteria 1 (BE Criteria)	Criteria 2 (CE Criteria)	Criteria 3 (NCOC Criteria)
					COC	NCOC	CE	BE	NCOC/ COC	CE/ COC	BE/ COC			
11367-76-3-8	Day 35	PHARM	L	3	173	0	0	0	0.00	0.00	0.00			
11367-76-3-8	Day 35	PHARM	L	2	185	0	0	0	0.00	0.00	0.00			
11367-76-3-9	Day 42	PHARM	L	3	239	0	0	51	0.00	0.00	0.21			
11367-76-3-9	Day 42	PHARM	L	1	202	0	0	53	0.00	0.00	0.26			
11367-76-3-9	Day 42	PHARM	L	2	220	0	0	0	0.00	0.00	0.00			
11367-76-3-10	Day 49	PHARM	L	2	60	0	0	0	0.00	0.00	0.00			
11367-76-3-10	Day 49	PHARM	L	1	72	0	0	0	0.00	0.00	0.00			
11367-76-3-10	Day 49	PHARM	L	3	84	0	0	0	0.00	0.00	0.00			
11367-76-3-11	Day 56	PHARM	L	1	54	0	0	0	0.00	0.00	0.00			
11367-76-3-11	Day 56	PHARM	L	2	54	0	0	0	0.00	0.00	0.00			
11367-76-3-11	Day 56	PHARM	L	3	62	0	0	0	0.00	0.00	0.00			
11367-76-2-12	Day 63	PHARM	L	3	0	0	0	0	0.00	0.00	0.00			
11367-76-3-12	Day 63	PHARM	L	1	0	0	0	0	0.00	0.00	0.00			
11367-76-3-12	Day 63	PHARM	L	2	0	0	0	0	0.00	0.00	0.00			
11367-76-3-13	Day 70	PHARM	L	1	0	0	0	0	0.00	0.00	0.00			
11367-76-3-13	Day 70	PHARM	L	2	0	0	0	0	0.00	0.00	0.00			
11367-76-3-13	Day 70	PHARM	L	3	0	0	0	0	0.00	0.00	0.00			

Note: BE = benzoylecgonine; CE = cocaethylene; COC = cocaine; D = dark; L = light; NCOC= norcocaine; PHARM = pharmaceutical-grade COC with minimal CE, BE, and NCOC (Source- US Pharmacopeia); CE = COC with high CE and BE, but minimal NCOC (Source- Drug Enforcement Administration); NCOC = COC with high NCOC and minimal CE and BE (Source- National Institute on Drug Abuse); POS = positive

Note: A blank cell for Criteria 1 through 3 indicates when Criteria results were negative for a sample.

Table A-8. Number and Percentages of Analyzed Specimens That Would Have Been Determined As Positive by Each of the Criteria

COC Type	Hair Type	Criteria 2, CE Criteria Number (%)	Criteria 3, NCOC Criteria Number (%)	Criteria 4, 0.05 NCOC Ratio Number (%)	Criteria 5, 0.01 NCOC Ratio Number (%)	Criteria 6, 0.05 CE Ratio Number (%)	Criteria 7, 0.02 CE Ratio Number (%)	Criteria 8, 0.01 CE Ratio Number (%)	Criteria 9, 0.002 CE Ratio Number (%)
High CE and BE	Light	19 (49)	0 (0)	0 (0)	0 (0)	0 (0)	13 (33)	19 (49)	19 (49)
High CE and BE	Dark	26 (67)	1 (3)	0 (0)	0 (0)	0 (0)	17 (44)	26 (67)	26 (67)
Pharmaceutical	Light	5 (13)	0 (0)	0 (0)	0 (0)	0 (0)	2 (5)	5 (13)	5 (13)
Pharmaceutical	Dark	36 (92)	4 (10)	0 (0)	0 (0)	0 (0)	5 (13)	32 (82)	36 (92)
High NCOC	Light	0 (0)	13 (33)	13 (33)	13 (33)	0 (0)	0 (0)	0 (0)	0 (0)
High NCOC	Dark	0 (0)	36 (92)	36 (92)	36 (92)	0 (0)	0 (0)	0 (0)	0 (0)

Note: CE = cocaethylene; BE = benzoylecgonine; NCOC = norcocaine