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Article

Analysis of Extensively Washed Hair from Cocaine Users and Drug Chemists to Establish New Reporting Criteria

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Samples from a self-proclaimed cocaine (COC) user, from 19 drug users (postmortem) and from 27 drug chemists were extensively washed and analyzed for COC, benzoylecgonine, norcocaine (NC), cocaethylene (CE) and aryl hydroxycocaines by liquid chromatography–tandem mass spectrometry. Published wash criteria and cutoffs were applied to the results. Additionally, the data were used to formulate new reporting criteria and interpretation guidelines for forensic casework. Applying the wash and reporting criteria, hair that was externally contaminated with COC was distinguished from hair collected from individuals known to have consumed COC. In addition, CE, NC and hydroxycocaine metabolites were only present in COC users' hair and not in drug chemists' hair. When properly applied, the use of an extended wash, along with the reporting criteria defined here, will exclude false-positive results from environmental contact with COC.

Introduction

Hair has proven to be a useful forensic toxicology specimen. Its testing may be helpful to corroborate other toxicological evidence. Segmental hair analysis may be performed to document historical exposure to a drug or poison. A positive result for a controlled substance in the hair of a toddler may prove informative to the court system if trying to determine an appropriate custody situation. Although hair testing cannot be used to prove impairment, positive results indicate exposure. A current debate in the area of hair testing is whether or not positive hair results are proof of actual drug use. This debate often centers around cocaine (COC), likely because of studies that have demonstrated COC is prevalent in innocuous sources such as on currency (1, 2).

When hair testing results are intended to suggest consumption of a drug, or when concentrations of drugs in hair are to be interpreted, it is very important to remove any external contamination by washing the hair (3). One common source of external contamination is secondhand smoke, which is of concern when testing hair for COC. Another source of contamination is exposure to sweat containing drugs and metabolites. This latter source is particularly important to remove if segmental analysis is going to be performed to estimate the time of drug exposure or to compare periods of time of drug exposure.

Despite years of research, there are no standard practices to decontaminate hair samples prior to analysis (3). Published studies have ranged from no washing (4–6) to a combination of shampooing and solvent rinsing (7, 8) to a series of washes with both organic solvents and aqueous buffers (9–13). A number of recent studies have examined the effectiveness of various washing protocols at removing COC deposited onto the exterior of COC-free hair. Schaffer *et al.* (11) effectively removed ~90% of COC contamination utilizing one *n*-propanol wash followed by three 30 min phosphate buffer washes and two 60 min phosphate buffer washes. Romano *et al.* had four drug-free volunteers rub 10 mg of COC hydrochloride into their hands for 5 min and then into their hair from roots to the ends. Over the next 10 weeks, hair was collected from these volunteers. The collected samples were then subjected to either three methylene chloride washes or one ethanol wash and eight phosphate buffer washes (14). COC concentrations in the hair samples ranged from 30 pg/mg to 18 ng/mg, and benzoylecgonine (BE) was detectable in many of the specimens after 1 month. Stout *et al.* (15) rubbed 15 mg COC hydrochloride into five hair specimens (12 g each), subjected the specimens to a sweat solution, shampooed the hair daily and sampled the hair at various intervals over 70 days. Specimens were analyzed (i) without washing, (ii) after decontamination at the primary research site or (iii) after decontamination by the three participating laboratories. Even with an extensive wash at the primary research site, most of the samples remained positive for COC for several weeks. However, after an extended wash, the application of wash kinetics calculation and evaluation of the results, these samples were interpreted as negative for COC (16, 17). Ropero-Miller *et al.* (18) soaked two hair specimens in COC solutions containing varying concentrations of COC metabolites, subjected them to a simulated sweat treatment and then shampooed the hair specimens daily for a period of 70 days. The hair specimens were then subjected to an extended buffer wash prior to analysis. COC concentrations as high as 20 ng/mg were detected in the samples, with some significant findings of cocaethylene (CE) and norcocaine (NC), as well.

It is unclear whether these COC contamination studies are realistic. The Romano study had volunteers rub 10 mg of COC into their own hair (14). The Stout study rubbed 15 mg of COC hydrochloride into 12 g of hair (15). Similarly, the Ropero-Miller study contaminated hair with 1 mg of COC per gram of hair (18). The results of these studies suggested that positive results could be obtained from contaminated hair samples, even when using the proposed 2004 guidelines for interpretation. Without a justifiable interpretation of positive COC findings in hair, the FBI Laboratory suspended COC analyses in hair in 2009 until more research could be performed to determine the best approach to differentiate positive COC findings in hair samples as contamination versus ingestion (19).

Others have evaluated the effectiveness of hair decontamination procedures by testing hair samples collected from COC users and nonusers who are exposed to COC routinely as part of their profession. In 1997, Mieczkowski (10) reported on the testing of hair from 36 narcotics officers and 4 evidence clerks who routinely handled COC seizures. One of the subjects in this study had a concentration of COC >500 pg/mg; however, this result may be explained as he was an undercover officer who would taste COC during a transaction. Limited washing was conducted; however, wash kinetics calculations were not

applied to this sample and it was not reported if any metabolites were identified.

Another issue currently debated in the testing of hair for COC is the criteria used to report a hair sample as 'positive' for COC. The Society of Hair Testing recommends COC concentrations ≥500 pg/mg, and BE, ecgonine methyl ester (EME), CE or NC ≥50 pg/mg to report a hair sample as positive for COC (3). Likewise, in 2004, proposed guidelines were published in the US Federal Register that also suggested a minimum limit of 500 pg/mg of COC (20). These guidelines also required that a second criterion be met. Specifically, the BE/COC ratio must be ≥0.05, or there must be CE or NC ≥50 pg/mg. It has been suggested that because BE, NC and CE may be present in street COC samples, they too may be a source of external contamination of hair, and that their presence does not prove ingestion (4, 15, 21, 22).

COC is extensively metabolized. Although BE, EME, CE and NC are considered the major metabolites of COC, other minor COC metabolites have been elucidated. *Para*- and *meta*-hydroxycocaine (*p*-OH-COC and *m*-OH-COC, respectively) have been identified in blood specimens from those administered COC (23) as well as from a COC user (24). The ratio of *p*-OH-COC and *m*-OH-COC to COC in blood in Kolbrich's study ranged from 0.01 to 0.11. *m*-OH-COC has also been identified in the bile of a COC overdose (25) and in the urine of COC users (26). However, some hydroxycocaines have also been measured in seized COC samples on the order of 0.01% and are likely trace coca leaf alkaloids (27, 28).

This study examined hair specimens from 20 drug users and 27 drug chemists for COC and 6 COC metabolites. The goal of these experiments was to determine whether COC users could be reliably differentiated from those exposed to COC in the course of their daily work. Results of these experiments were used to develop our laboratory's criteria for reporting results of hair analyses for COC.

Experimental

Materials

Certified reference standards (traceable to NIST) of COC, COC-d_3, NC, NC-d_3, CE, CE-d_3, BE and BE-d8 were purchased from Cerilliant (Round Rock, TX, USA). Reference standards (not traceable to NIST) of *ortho*-hydroxycocaine (*o*-OH-COC), *m*-OH-COC, *p*-OH-COC and *m*-OH-COC-d_3 were purchased from Elsohly Laboratories, Inc. (Oxford, MS, USA). The drugs and metabolites used for interference studies were purchased from Cerilliant (Round Rock, TX, USA), Grace Laboratories (Deerfield, IL, USA) and Sigma-Aldrich (St Louis, MO, USA) or donated by Hoffmann La Roche Inc. and Pfizer, Inc. (Table I).

Reagents

Bis-Tris buffer, bovine serum albumin (≥96%), dithiothreitol (99%), proteinase K (≥30 units/mg from tritirachium albumin), sodium cholate hydrate (>99%), sodium phosphate monobasic (reagent plus ≥99%) and formic acid (≥98%) were purchased from Sigma-Aldrich. Hydrochloric acid (ACS grade), potassium hydroxide (reagent grade), ammonium hydroxide (certified ACS plus), *n*-propanol (HPLC grade), methanol (HPLC grade and Optima grade), methylene chloride (HPLC grade), water (optima LC–MS grade) and acetonitrile (Optima grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Isolute HCX SPE cartridges (130 mg, 10 mL) were purchased from Biotage (Charlotte, NC, USA).

Table I
Potential Interferences Tested

	Drug names
Solution #1	Alprazolam, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam, temazepam
Solution #2	Ephedrine, amphetamine, methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, methylenedioxy-*N*-ethylamphetamine
Solution #3	THC, THC-OH, THC-COOH
Solution #4	Codeine, hydromorphone, hydrocodone, oxymorphone, oxycodone, morphine, normorphine, norcodeine, dihydrocodeine, dihydromorphone, 6-acetylmorphine
Solution #5	Benzocaine, bupivacaine, dicyclomine, lidocaine, mepivacaine, mexiletine, prilocaine, procaine, monoethylglycinexylidide (MEGX), scopolamine, atropine, flumazenil, tetrabenzine
Solution #6	Caffeine, cetirizine, cotinine, cyclizine, diltiazem, hydroxyzine, levamisole, norchlorcyclizine, nicotine
Solution #7	Cocaine-*N*-oxide

Hair samples

The FBI Institutional Review Board granted exemptions for the anonymous submission of hair samples to the FBI Laboratory. Negative control head hair was collected from anonymous drug-free donors at the FBI Laboratory ($n = 18$) for method validation. Negative sources were mixed for use as calibrators and controls after verifying that each individual source was negative. An antemortem head hair sample from a self-proclaimed COC user (2011) and postmortem head hair from documented drug users (1992–1994, 2011) were collected during routine investigations. Drug chemists actively working with COC for 0.5–28 years ($n = 27$) voluntarily donated their head hair to the FBI Laboratory from 2010 to 2013 from various laboratories throughout the USA. All hair samples were stored in envelopes at ambient temperature in the dark until analysis.

Methods

If the length and manner of collection permitted, hair samples were sectioned into 2-cm segments. Additionally, specimens were analyzed in duplicate when the sample size was sufficient. The hair samples were washed according to previously published methods (13). Briefly, 12-mg samples (up to 4 cm in length) were weighed into 12 × 75 mm test tubes. *n*-Propanol was added to each sample at a volume of 2 mL/10 mg of hair. Samples were immediately vortexed and placed into a 37°C oscillating (120 shakes/min) water bath for 15 min, and then the *n*-propanol was removed. Hair wash buffer (0.01 M phosphate buffer with 0.1% BSA; pH 6) was added to each sample at a volume of 2 mL/10 mg of hair. Samples were immediately vortexed and placed back into the 37°C oscillating water bath for a 30 min cycle and the phosphate buffer was discarded to waste. This was repeated two more times for 30-min cycles and then two times for 60-min cycles (a total of five washes with the hair wash buffer). The final phosphate buffer wash was transferred into a 2-mL polypropylene microcentrifuge tube and immediately frozen at −20°C overnight. A hydrolysis wash sample (negative buffer solution fortified with COC at 480 pg/mL) was also prepared and

immediately frozen. The hair wash buffer solution was also used to prepare calibrators and controls. One milliliter and 0.1 mL aliquots from each sample's final wash buffer were extracted using Isolute SPE columns. Final wash extracts were reconstituted in 100 μL of water–acetonitrile–formic acid (90:10:0.1).

The washed hair samples were next digested at pH 5.5 according to previously published methods (9, 11). A hair digest solution (proteinase-K, dithiothreitol, detergent and Bis-Tris buffer) was added to each sample at 1 mL/10 mg of hair. A hydrolysis digest sample (Hair Digest Solution fortified with COC at 1,000 pg/mL) was also prepared. Samples were immediately vortexed and placed into a 37°C oscillating water bath overnight. One milliliter and 0.1 mL aliquots from each sample digest were also extracted using Isolute SPE columns.

Instrumentation

The LC consisted of a Spark Holland Symbiosis™ system with the following components: two HPLC pumps, autosampler, refrigerated storage compartment (15°C), solvent mixing system and degasser. Chromatographic separation of COC, NC, CE and BE was achieved using a Waters Xbridge® 5 μm octadecyl (C8) analytical column (Franklin, MA, USA; 2.1 × 50 mm) maintained at an ambient temperature. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient program started at 85% A held for 2.5 min, ramped linearly to 0% A at 10 min and held at 0% for an additional 2 min, for a total run time of 12 min. The column was re-equilibrated at 85% A for 5 min prior to each injection. The flow rate was set to 0.3 mL/min with injection volumes of 25 μL for both the washes and digests.

The separation of the hydroxycocaine metabolites was performed in a separate analytical run. Chromatographic separation for *p*-OH-COC, *m*-OH-COC and *o*-OH-COC was achieved using a Phenomenex Gemini® Phenyl 5 μm phenyl (C6) analytical column (Torrance, CA, USA; 3.0 × 50 mm) maintained at the ambient temperature. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient program started at 91% A held for 4.5 min, ramped linearly to 45% A at 5.5 min and was held at 0% A for an additional 3.5 min, for a total run time of 9 min. The column was re-equilibrated at 85% A for 10 min prior to each injection. The flow rate was set to 0.3 mL/min with injection volumes of 50 μL for both the washes and digests.

ESI mass spectrometry analysis was performed on an AB Sciex QTRAP® 4000 linear ion trap quadrupole mass spectrometer in positive ionization mode. Multiple reaction monitoring (MRM) was utilized with four transitions each for COC, NC, CE, BE, *p*-OH-COC, *m*-OH-COC and *o*-OH-COC and two transitions each for the COC-d_3, NC-d_3, CE-d_3, BE-d_3 and *m*-OH-COC-d_3 internal standards (Table II).

Analyst® software version 1.4.2 was used to process the data. Samples were considered positive for an analyte when two ion ratios were within 25% (relative) for ion ratios >40% or within 10% (absolute) for ion ratios ≤40% of those for the reference material. In addition, the retention times were required to match within 5% of the reference material.

Validation

Validation experiments were performed for COC, BE, NC and CE in both hair matrix and the wash matrix. Validation was also performed for the hydroxycocaines; however, because the hydroxycocaine source reference material was not NIST traceable and is only presently available from one company, the results for these metabolites were only qualitatively reported. The validation experiments were performed according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines as well as internal requirements (29). The following performance characteristics were evaluated: interferences, ionization suppression/enhancement (matrix effect), carryover, limit of detection (LOD), limit of quantitation (LOQ), bias and precision (Tables III and IV). The LOD and LOQ were experimentally determined by analyzing five sets of calibrators and controls. The LOD was chosen as the lowest value that gave adequate signal to noise (>10) for at least three MRM transitions, whereas the LOQ was chosen as the lowest calibrator that could be determined with accuracy (within ± 20%). For some compounds, the LOD and LOQ were administratively set as the lowest calibrator.

Table II
Mass Spectrometer Operating Parameters

Analyte	Q1 mass[a]	Q3 mass[b]	Dwell time (ms)	DP[c] (v)	CE[d] (v)	CXP[e] (v)
COC						
BE	290	168, 105, 91, 122	50	71	29, 43, 57, 43	12, 16, 14, 20
BE-d_8	298	171, 110	50	51	29, 43	10, 6
COC	304	182, 105, 91, 150	50	71	29, 47, 61, 35	14, 16, 14, 24
COC-d_3	307	185, 105	50	62	27, 49,	12, 16
CE	318	196, 105, 150, 91	50	71	33, 49, 39, 67	14, 6, 8, 4
CE-d_3	321	199,105	50	51	27, 47	16, 16
NC	290	168, 136, 108, 105	50	56	25, 37, 41, 39	12, 10, 6, 18
NC-d_3	293	171, 136	50	46	25, 33	30, 20
Hydroxycocaines						
o-OH-COC	320	200, 182, 121, 93	100	46	23, 41, 47, 63	34, 32, 8, 14
m-OH-COC	320	182, 121, 93, 91	100	61	31, 47, 57, 69	28, 18, 14, 4
m-OH-COC-d_3	323	185, 93	100	56	29, 61	28, 14
p-OH-COC	320	182, 121, 200, 93	100	41	27, 41, 33, 59	14, 8, 12, 12

[a]Quadrupole 1.
[b]Quadrupole 3.
[c]Declustering potential.
[d]Collision energy.
[e]Collision exit potential.

Curves for the hair washes were evaluated for all seven analytes from 60 to 840 pg/mL, which is equivalent to 10–140 pg/mg based on a 12-mg sample size. However, the data for BE were only qualitatively acceptable for the wash samples due to poor recovery and inaccuracy. Curves for the hair digests were also evaluated for all seven analytes (COC: 250–7,500 pg/mg; BE, NC and CE: 25–750 pg/mg). Interferences were evaluated by mixing hair wash and hair digest extracts with various drugs and metabolites (Table I) at concentrations of 10 ng/mL (~20 ng/mg) and 50 ng/mL (~100 ng/mg).

Application of extended wash kinetics calculation

For this study, we evaluated results based on both the amount of COC measured in the hair sample (COC_{hair}) and the final COC hair concentration (COC_{final}) after compensating for COC in the last wash (COC_{wash}). We adopted the calculations in one publication (12), which measures the amount of COC in the final wash (COC_{wash}), multiplies that concentration by 5 (as a deliberate overestimation of five additional 1-h washes) and subtracts this value from the amount of COC measured in the hair sample (COC_{hair}).

$$COC_{final} = COC_{hair} - (5 \times COC_{wash}).$$

These wash kinetic calculations were performed with the quantitative results obtained from the tested samples.

Results

Validation results

COC and metabolites were not detected in any of the anonymous drug-free donors from the Federal Bureau of Investigation (FBI) Laboratory. Additionally, none of the drugs and metabolites tested interfered with the detection or quantitation of COC and metabolites. Negative hair washes and negative hair digests spiked with the internal standards demonstrated good recovery of all internal standards and no interference with any of the seven non-deuterated compounds. No hydrolysis of COC to BE was observed in any of the COC hydrolysis controls. Other validation results are summarized in Tables III and IV.

Results of hair from a COC user (antemortem)

Six 2-cm segments were analyzed in duplicate. Segment #1 was the most proximal segment, and Segment #6 was the most distal segment tested. All washes of the hair from the self-proclaimed COC user contained COC and BE, but no other COC metabolites were detected. Segments #1 through #3 hair digests were positive for COC ranging from 1,300 to 4,270 pg/mg, whereas Segments #4 through #6 hair digests were negative for COC using a cutoff of ≥500 pg/mg. After the application of the cocaine extended wash kinetics calculation, Segments #1 through #3 remained positive, as the COC_{final} ranged from 1,040 to 3,730 pg/mg. Additionally, the following COC metabolites were identified in Segments #1 and #2: BE >1,000 pg/mg, CE >200 pg/mg and NC >100 pg/mg, and *p*-OH-COC and *m*-OH-COC were also positive. In Segment #3, the following metabolites were identified: BE >1000 pg/mg and CE >100 pg/mg, and both *p*-OH-COC and *m*-OH-COC were positive (Table V).

Results of hair from 19 drug users (postmortem)

Eighteen of the final washes contained COC. Six of the 19 washes contained CE, 3 contained NC and 10 contained BE. No hydroxycocaine metabolites were detected in any of the hair washes. Nine of the hair digests contained COC_{final} <500 pg/mg. The remaining 10 hair digests were as follows: COC_{final} (2,730–

Table III
Validation Parameters for Method Used to Analyze Hair Washes

	COC	BE	NC	CE	*p*-OH-COC	*m*-OH-COC	*o*-OH-COC
Ionization suppression/enhancement	96–99%	75–80%	97–98%	96–100%	96–97%	99–100%	70–80%
LOD	Set at 60 pg/mL	120 pg/mL	Set at 60 pg/mL	Set at 60 pg/mL	120 pg/mL	120 pg/mL	120 pg/mL
LOQ	Set at 60 pg/mL		Set at 60 pg/mL	Set at 60 pg/mL			
Linearity	60–840 pg/mL		60–840 pg/mL	60–840 pg/mL			
Bias	1.6 to 6.3%		3.6 to 8.1%	5.3 to 9.4%			
Repeatability	6.3 to 13.3%		6.7 to 15.1%	6.3 to 14.8%			
Intermediate precision	7.0 to 15.0%		7.7 to 16.7%	7.5 to 15.4%			

Table IV
Validation Parameters for Method Used to Analyze Hair Digests

	COC	BE	NC	CE	*p*-OH-COC	*m*-OH-COC	*o*-OH-COC
Ionization suppression/enhancement	77–91%	54–82%	49–72%	52–78%	61–83%	50–70%	45–61%
Carryover	None after 10,000 pg/mg	None after 1,000 pg/mg	Trace after 1,000 pg/mg[a]	None after 1,000 pg/mg	None after 160 pg/mg	None after 160 pg/mg	None after 160 pg/mg
LOD	Set at 250 pg/mg	100 pg/mg	25 pg/mg	Set at 25 pg/mg	5 pg/mg	5 pg/mg	5 pg/mg
LOQ	Set at 250 pg/mg	100 pg/mg	100 pg/mg	Set at 25 pg/mg			
Linearity	250–7,500 pg/mg	100–750 pg/mg	100–750 pg/mg	25–750 pg/mg			
Bias	1.3 to 10.9%	−14.5 to 4.7%	7.9 to 16.5%	7.8 to 18.2%			
Repeatability	2.8 to 4.0%	5.7 to 14.3%	2.8 to 4.5%	4.4 to 5.5%			
Intermediate precision	4.0 to 6.4%	5.7 to 15.1%	3.3 to 5.9%	4.4 to 6.6%			

[a]Trace: <0.2% carryover noted in two out of six samples after 1,000 pg/mg.

39,600 pg/mg), NC (123–1,070 pg/mg) and CE (67–5,190 pg/mg). BE was not quantifiable (instrumental issue) in samples 1–9 but ranged from 277 to 15,550 pg/mg in samples 10–19. Thirteen were positive for *p*-OH-COC, 14 were positive for *m*-OH-COC and 6 were positive for *o*-OH-COC (Table VI).

Results of hair from 27 drug chemists

Eleven of the hair washes contained COC and four contained BE, but none of the other five COC metabolites. Eight of the hair digests contained COC; four hair samples ≥500 pg/mg and four ≥250 pg/mg, but <500 pg/mg. Eight of the drug chemists' hair contained BE ≥100 pg/mg, and BE/COC ranged from 0.24 to 0.73. None of the drug chemists' hair digests contained NC, CE or any of the hydroxycocaine metabolites. Once the cocaine extended wash kinetics calculation was applied, the four with COC ≥500 pg/mg had COC_{final} <500 pg/mg. However, if the cocaine extended wash kinetics calculation was not applied and either the cutoff of BE ≥100 pg/mg or the BE/COC ≥0.05 was utilized as per the proposed 2004 guidelines, four of the drug chemists may have been reported as either a COC user or chronically exposed to COC (Table VII).

Discussion

BE concentrations have been shown to increase in hair samples with time after external contamination with COC (7, 14, 15). In Cone's study (30), as well as the results reported here, BE has been detected in hair washes from suspected COC users after decontamination (Tables V and VI). Based upon these external contamination studies and our results, there appears to be little

Table V
Segmental Analysis of Hair From a Self-Proclaimed COC User

	Wash comments	COC washes (pg/mL)	COC washes (pg/mg)	5× wash	Hair COC (pg/mg)	$COC_{corrected}$[a] Hair—5× wash	Hair BE (pg/mg)	Hair CE (pg/mg)	Hair NC (pg/mg)	Hair OH-COC metabolites			Reporting results
										Para	*Meta*	*Ortho*	
Seg 1A	BE	514	86	428	3,610	3,180	2,210	248	102	Pos	Pos	ND	PCCU
Seg 1B	BE	473	79	394	3,250	2,850	1,860	245	101	Pos	Pos	ND	PCCU
Seg 2A	BE	601	100	501	3,880	3,370	2,420	291	107	Pos	Pos	ND	PCCU
Seg 2B	BE	637	106	531	4,270	3,730	2,500	331	123	Pos	Pos	ND	PCCU
Seg 3A	BE	305	51	254	1,300	1,040	1,080	124	ND	Pos	Pos	ND	PCCU
Seg 3B	BE	449	75	374	1,840	1,460	1,300	172	ND	Pos	Pos	ND	PCCU
Seg 4A	BE	247	41	206	<500	<500	678	<50	ND	ND	ND	ND	Negative
Seg 4B	BE	187	31	156	<500	<500	639	<50	ND	ND	ND	ND	Negative
Seg 5A	BE	172	29	143	ND	ND	492	ND	ND	ND	ND	ND	Negative
Seg 5B	BE	125	21	104	ND	ND	450	ND	ND	ND	ND	ND	Negative
Seg 6A	BE	143	24	119	ND	ND	343	ND	ND	ND	ND	ND	Negative
Seg 6B	BE	146	24	122	ND	ND	408	ND	ND	ND	ND	ND	Negative

PCCU, positive chronic cocaine user; ND, not detected.
[a]Value corrected to not more than three significant figures.

Table VI
Postmortem Hair Analysis of Drug Users' Hair

PM history	Subject[a]	Wash comments	COC washes (pg/mL)	COC washes (pg/mg)	5× wash	Hair COC (pg/mg)	$COC_{corrected}$[a] Hair—5× wash	Hair BE (pg/mg)	Hair CE (pg/mg)	Hair NC (pg/mg)	Hair OH-COC metabolites			Reporting results
											Para	*Meta*	Ortho	
PM COC present	1	CE	424	71	353	4,900	4,540	n/a	902	123	Pos	Pos	Pos	PCCU
PM COC present	2	CE	**18,200**	3,030	15,100	41,200	26,100	n/a	4,130	711	Pos	Pos	Pos	PCCU
Hx Drug user, no labs	3		171	29	143	<500	<500	n/a	ND	ND	ND	ND	ND	Negative
Hx COC use	4		1,350	225	1,120	13,200	12,000	n/a	607	725	Pos	Pos	Pos	PCCU
Hx Drug user, no labs	5		175	29	146	<500	<500	n/a	ND	ND	ND	ND	ND	Negative
Hx Drug user, no labs	6		82	14	69	510	<500	n/a	ND	ND	ND	ND	ND	Negative, EC
Hx Drug user, no labs	7		ND	n/a	n/a	ND	n/a	n/a	ND	ND	ND	ND	ND	Negative
Hx COC use	8	CE	2,400	400	2,000	5,860	3,860	n/a	937	186	Pos	Pos	Pos	PCCU
Hx COC use	9		6,540	1,090	5,450	10,300	4,850	n/a	67	158	Pos	Pos	Pos	PCCU
B:COC/BE, U:COC/MET	10	BE and CE	1,210	202	1,000	1,030	<500	642	365	ND	ND	Pos	ND	Negative, EC
B:BE, U:COC/BE	11	BE	4,620	770	3,850	11,500	7,650	2,150	358	114	Pos	Pos	ND	PCCU
Hosp: urine pos, (died 6 days later)	12	BE and NC	**11,200**	1,860	9,300	2,950	<500	540	ND	ND	Pos	Pos	ND	EC, PCCU
B: BE	13	BE and NC	**11,600**	1,930	9,650	8,540	<500	2,710	93	230	Pos	Pos	Pos	EC, PCCU
B:COC/BE, U:COC/MET	14	BE	220	37	185	3,700	3,515	1,480	ND	ND	Pos	Pos	ND	PCCU
B:BE, U:COC/MET	15	BE	483	81	403	330	<500	277	ND	ND	ND	ND	ND	Negative
Hosp: urine pos, (died 10 days later)	16	BE and CE	1,680	280	1,400	41,000	39,600	**15,500**	5,190	1,070	Pos	Pos	ND	PCCU
B:BE, U:COC/MET	17	BE and NC	**44,800**	7,460	37,300	29,100	<500	**13,500**	ND	446	Pos	Pos	ND	EC, PCCU
B:BE, U:COC/MET	18	BE and CE	2,970	490	2,450	5,180	2,730	1,710	262	ND	Pos	Pos	ND	PCCU
B:BE, U:COC/BE	19	BE	6,150	1,020	5,100	14,000	8,900	1,940	126	113	Pos	Pos	ND	PCCU

Bold numbers = above curve.
EC, externally contaminated; PCCU, positive chronic cocaine user; n/a = not applicable; ND, not detected.
[a]Value corrected to not more than three significant figures.

utility in measuring BE or BE/COC ratios in hair. However, our results suggest that the presence of NC, CE and aryl hydroxycocaine metabolites in hair samples provides positive interpretative value.

A closer look at hydroxycocaine impurities reveal that at least five of these hydroxycocaines are tropane alkaloids with a hydroxy substituent on the tropane ring (six or seven *exo*/*endo*-hydroxycocaines) or on the aromatic substituent at C-3 (*meta*-hydroxybenzoylecgonine methyl ester or *m*-OH-COC). Although *m*-OH-COC has been identified as a COC impurity, *p*-OH-COC and *o*-OH-COC have not (31). While others have reported on a method used to identify *p*-OH-COC and *o*-OH-COC metabolites in hair (16, 32), to our knowledge, this is the first time *o*-OH−COC has been reported in a scientific paper.

Based on published studies and recommendations, as well as our in-house studies, we have established the following criteria for reporting the interpretation of COC and metabolites in hair (Figure 1):

- If COC_{hair} <500 pg/mg, the sample is reported as *negative*.
- If COC_{hair} ≥500 pg/mg, but the COC_{final} <500 pg/mg and two of three OH-COC metabolites are not identified in the hair sample, the sample is reported as *negative but externally contaminated*.
- If COC_{hair} ≥500 pg/mg, but the COC_{final} <500 pg/mg and two of three OH-COC metabolites are identified in the hair sample, the sample is reported as *positive, consistent with chronic use, but contaminated*.
- If COC_{final} ≥500 pg/mg, but no metabolites are identified, the sample is reported as *contaminated with COC*.
- If COC_{final} ≥500 pg/mg, and either NC and/or CE are identified, but no OH-COC metabolites are identified, the sample is reported as *positive, consistent with COC exposure*.
- If COC_{final} ≥500 pg/mg and two of the OH-COC metabolites are identified, the sample is reported as *positive, consistent with chronic COC use*.
- If the amount of COC in the last wash is either above the curve (0.1 mL aliquot) or 10 times above the curve (1 mL aliquot),

Table VII
Analysis of Drug Chemists' Hair (Chronic Occupational Exposure)

Chemist	Years	Reported acute exposures (case work info)	Samples	COC washes (pg/mL)	COC washes (pg/mg)	5× wash	Hair COC (pg/mg)	$COC_{corrected}$[c] Hair—5× wash	Hair BE (pg/mg)	Hair BE/COC	Reporting results
0[a]	<0.2[a]	2 h/d × 2 d, envir only	Dup								
1	7	2-day processing	Dup	**870**	145	725	784	<500	194	0.25	EC
				887	148	739	787	<500	190	0.24	EC
2	20	(2 cm seg × 4)	4 seg	410	68	342	613	<500	447	0.73	EC
				139	23	116	ND	n/a	300	n/a	Negative
				160	27	133	ND	n/a	198	n/a	Negative
				178	30	148	ND	n/a	199	n/a	Negative
3	12	(2 cm seg × 4)	4 seg	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
4	17	Two-day processing, few cases/year	Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
5	7	Two hours processing prior day	Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
6	23		Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
7	6	Routine small >20 + g	Dup	79	13	66	372	<500	110	n/a	Negative
				98	16	82	375	<500	111	n/a	Negative
8	25		Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
9	16		Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
10	6	Few COC cases last 2–3 months	Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
11	12	2 days ago many small rocks <1 g	1	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
12	5	1 year ago bulk > 10 kg	1	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
13	5	1 day <1 g	1	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
14	11	Multiple small cases ~3 weeks ago	1	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
15	0.5	1 day prior multiple small cases	1	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
16	1	1 day, crack case	1	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
17	6	1 day prior, crack case	1	107	18	89	508	<500	299	0.59	EC
18	10	1 day prior: powder and crack	Dup	85	14	71	376	<500	107	*n/a*	Negative
				78	13	65	384	<500	107	*n/a*	Negative
19	8	3 days prior: powder and crack	Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
20	7	3 days prior: powder and crack	Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
21	12	1 day: crack, meth, heroin cases	Dup	88	15	73	ND	n/a	184	n/a	Negative
				84	14	70	ND	n/a	186	n/a	Negative
22	14	~1 week: multiple cases daily	Dup	66	11	55	389	<500	170	n/a	Negative
				117	20	98	ND	n/a	130	n/a	Negative
23[b]	12.5	Tested 9 samples this morning	Dup	71	12	59	ND	n/a	ND	n/a	Negative
				70	12	58	ND	n/a	ND	n/a	Negative
24[b]	10.5	Tested 4 samples this morning	Dup	263	44	219	699	<500	359	0.51	EC
				254	42	212	602	<500	325	0.54	EC
25	27	Most days of the week	Dup	ND	n/a	n/a	ND	n/a	ND	n/a	Negative
				ND	n/a	n/a	ND	n/a	ND	n/a	Negative
26[b]	3	1/2 h few days ago	Dup	363	61	303	361	59	ND	n/a	Negative
				148	25	123	235	112	ND	n/a	Negative
27[b]	28	Daily	Dup	282	47	235	ND	n/a	ND	n/a	Negative
				825	138	688	ND	n/a	ND	n/a	Negative

No NC, CE or hydroxycocaine metabolites detected.
EC, externally contaminated; ND, not detected; n/a, not applicable.
[a]Chemist sharing environmental workspace not processing COC samples.
[b]Trace BE in wash.
[c]Value corrected to not more than three significant figures.

the sample is reported as *externally contaminated*, regardless of the amount of COC identified in the hair.

The 500 pg/mg cutoff for COC positive hair samples has been recommended by the Society of Hair Testing (3) and has been proposed in the US Federal Register (20). Although this research supports this cutoff, the implementation of extensively washing hair is imperative. The main difference between the various reporting scenarios presented here are the differentiation between COC exposure and COC use. Seized COC samples have never been found to contain the aryl hydroxycocaines (*para* and *ortho*) and only very trace levels of *meta*-hydroxycocaine. The detection of two aryl hydroxycocaines can therefore be reported as positive for COC use. On the other hand, seized COC samples have been found to contain CE and NC. Although their detection may be due to COC use from drug metabolism, it may also be due to inherent contamination. The current prevalence of CE and NC in seized drug samples is not known. Therefore, to avoid using ratios of CE to COC and NC to COC, the FBI Laboratory will report such hair samples as positive for COC exposure.

The use of decontamination procedures is currently not a mandatory part of COC hair testing guidelines. Some researchers omit washes out of concern that COC may be excessively removed from the sample (3). Although many researchers have discussed the utility of washes, few have adopted extensive washing practices and no consensus standards exist. The thorough washing of hair samples not only removes potential environmental contamination, but also drug that is incorporated via sweat. Without the utilization of a cocaine extended wash kinetics calculation, the extent of COC contamination is not apparent. When hair is thoroughly washed and a cocaine extended wash kinetics calculation is applied, the results of this study show that one can reliably detect hair that has been externally contaminated with COC. However, some instances of acute COC use may not be identified using this approach as it leads to conservative conclusions. Likewise, a chronic COC user's hair may be so grossly contaminated that the COC_{final} level is below the cutoff, yet the COC metabolites are identified at significant levels. These samples are reported as contaminated hair that is positive for chronic COC use because of the presence of metabolites. Our previously stated concern (19) was that those who have legitimate contact with COC in the course of their profession might be identified as COC positive. When hair is extensively washed and a cocaine extended wash kinetics calculation is applied, these individuals (e.g., attorneys involved in prosecuting or defending drug charges, law enforcement officers handling drug evidence and crime laboratory employees) will no longer be at risk of being identified as a COC user.

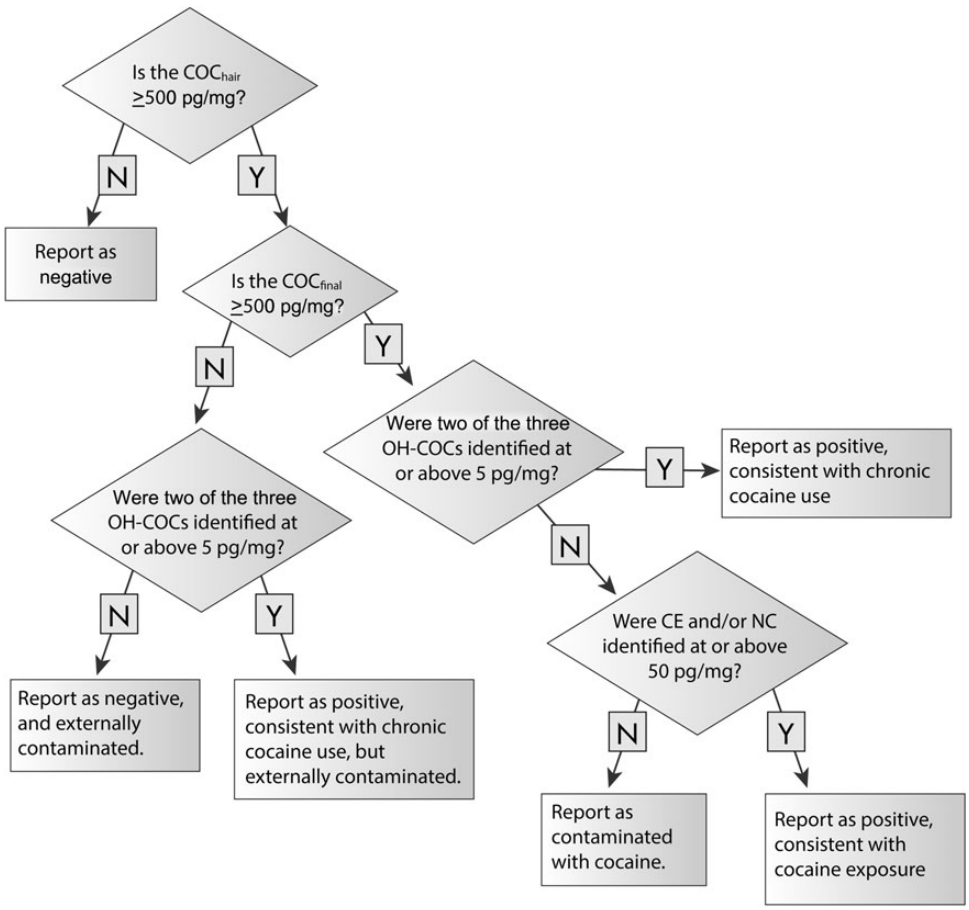

Figure 1. Reporting criteria. *Note*. If the amount of COC in the last wash is either above the curve (0.1 mL aliquot) or 10 times above the curve (1 mL aliquot), the sample is reported as *externally contaminated*, regardless of the amount of COC identified in the hair.

Conclusion

The FBI Laboratory suspended COC analyses in hair for most cases in 2009 (19). Prior to this suspension, the FBI Laboratory utilized the proposed 2004 federal guidelines (20) for interpretation of analytical results. Brief methanol and water washes were used, and when the ratio of the amount of COC in the final methanol wash to the amount of COC in the hair was 10% or more, the sample was considered externally contaminated. Since then, we have evaluated the utility of an extensive wash procedure, followed by the application of a cocaine extended wash kinetics calculation, as well as the identification of additional COC metabolites (NC, CE, aryl hydroxycocaine metabolites) to strengthen our interpretation of results.

Despite the potential presence of BE, CE and NC in street samples, hair samples collected from drug chemists only contained BE with no other COC metabolites detected. Only hair from chronic COC users contained NC, CE and aryl hydroxycocaine metabolites. The implementation of extensively washing hair and applying a cocaine extended wash kinetics calculation along with the detection of pertinent metabolites can be used to differentiate passive exposure to COC from active use of COC. Although these guidelines are conservative and may lead to the occasional underreporting of COC users', the use of these guidelines may prevent an innocent individual from being accused of COC use.

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