Hair External Contamination Literature Review

James A. Bourland, Ph.D., D-ABFT

Postulated Mechanisms of Drug Incorporation into Hair

- Drug
  - Blood
    - Drug and metabolites incorporated into hair
    - Transferred to sweat/sebum
      - Drug and metabolites incorporated into hair
- Drug
  - External exposure
    - Drug deposited onto hair
    - Drug transferred to sweat/sebum
      - Drug deposited onto hair

How do drugs get into hair?

- Blood
- Sebum/sweat
- External exposure

External Contamination: Definition

- An evidentiary false positive that is the result of exogenous exposure to drug(s) in the environment.
- The drug positive result is not due to the ingestion or use of drug by any route of administration.
- Drug(s) in sweat or sebum from a source other than the user contacting hair to cause a drug positive result.

Henderson *et al.* 1993

- In summary, our studies show that hair analysis with a sensitive and specific method like GC/MS can be used to detect cocaine use or exposure.
- However, it is our opinion that the mechanism(s) for cocaine incorporation into hair appear to be more complex than previously thought.
- Thus, there is not, at present, the necessary scientific foundation for hair analysis to be used to determine either the time or amount of cocaine use.
- Further, because external contamination may be a possible source for evidentiary "false" positives for cocaine (i.e., drug is present, but not due to ingestion), all hair testing procedures for cocaine must be designed to rigorously guard against any inadvertent contamination of the sample during collection or analysis and external contamination must be ruled out when interpreting hair analysis results.

External Contamination in Hair Literature Review

- Child Exposure Studies
- Narcotic Officer Exposure Studies
- THC Exposure
- Lab Procedures / Approaches to External Contamination Issues
- *In Vitro* Contamination Studies

Child Exposure Studies

External Contamination in Hair

Knight *et al.* (1996) - *CHEST* Passive Smoking in Children

- Passive Nicotine Exposure Adversely effects Health of children
- Correlation: Number of Cigarettes per day vs. Cotinine Concentrations detected in Urine and Hair
- African American Children higher concentrations in both Hair and Urine than Caucasian Children with less # of cigarettes

Lewis *et al.* (1997) – *Forensic Sci Int*

Determination of drug exposure using hair: application to child protective cases

- Children exposed: Majority positive for Cocaine and Methamphetamine
- N= 23, Age 6 mo- 13 yrs
- N= 3 -Adults aged 19, 24, 30 yrs
- Benzoylecgonine Detected in 6/12 Cocaine Positive Exposed Children, 2/3 Adults.
- Stated “Some” Positive for Cocaethylene – No data presented

Smith *et al.* (1996) - *Forensic Sci Int*

Cocaine in hair, saliva, skin swabs, and urine of cocaine user's children

- Compared COC and BE levels in children of Cocaine-using mothers
- Adults 15/16 COC Positive in Hair; Children 22/24 COC Positive in Hair
- COC/BE Concentrations
  - Adult mean 2.4/0.39 ng/mg
  - Children Mean 2.4/0.74 ng/mg
- 0/22 POS COC Urine <300ng/mL
- 1/17 POS in Saliva
- Skin 19/26 COC POS, 7/26 BE POS

Bassindale (2012) – *Forensic Sci Int*

Quantitative analysis of methamphetamine in hair of children removed from clandestine laboratories - Evidence of passive exposure

- New Zealand Study (52 cases)
- Children removed form Clandestine Labs
- 3X MeOH wash employed prior to SPE
- Hair samples analyzed by LC-MS/MS for MAMP & AMP
- 38 (73%) Positive METH (>0.1 ng/mg)
- AMP detected in 34/38 POS METH samples
- Levels Similar to Adult Users
  - 7.03 ng/mg Mean MAMP: Children
  - 6.28 ng/mg Mean MAMP: Adults

Papaseit *et al.* (2011) - J Med Case Reports

- Case Report on female toddler (2 yrs)
- Chronic Crack Cocaine Exposure
- Both Parents Admitted Crack Users
- 2 Segmental Sections: 0-3 cm & >3 cm

Results

Hair Segment	Toddler Hair (ng/mg)	Maternal Hair (ng/mg)	Paternal Hair (ng/mg)
0-3 cm	1.9	7.88	13.06
> 3 cm	7.04	6.39	12.97

Narcotic Officer Exposure Studies

External Contamination in Hair

Mieckowski (1995) – *Microgram*
Passive Contamination of Undercover Narcotics Officers by Cocaine

- 9 Officers; 7-male, 2-female
- Mean Age 33 yrs
- Majority of “buy and bust” Cocaine (60%)
- Majority Cocaine “Crack” Cases (79.2%)
- Activity:
  - 5-Several times per week
  - 4-Several times per month

Mieckowski (1995) – *Microgram*

Passive Contamination of Undercover Narcotics Officers by Cocaine

Case #	Alcohol wash (ng/10 mg)	PO_4 buffer wash #1 (ng/10 mg)	PO_4 buffer wash #2 (ng/10 mg)	PO_4 buffer wash #3 (ng/10 mg)	Hair digest (ng/10 mg)
1	0.9	1.30	0.14	0.0	0.0
2	0.0	1.00	0.10	0.0	0.0
3	0.0	1.50	0.14	0.0	0.0
4	0.0	3.40	0.30	0.2	0.0
5	0.0	0.80	0.08	0.0	0.0
6	1.1	1.20	0.00	0.0	0.0
7	0.0	3.40	0.10	0.10	0.0
8	0.0	1.10	0.07	0.0	0.0
9	0.0	1.50	0.18	0.0	0.0

Villain *et al.* (2010) – *Forensic Sci Int*

Heroin markers in hair of narcotic police officer: Active or passive exposure?

- Police Officer and Clerk arrested drug trafficking
- Resale of seized drugs
- 2 Subjects: POS 6-AM +MOR in Hair
- Claimed External Contamination
- 11 other Police Officers: NEG

- ND – not detected below LOQ

Subject	Hair	Heroin, ng/mg	Other drugs of abuse
Police officer	chest	6-AM: 0.5 Morphine: 0. 2	ND
Clerk	head	6-AM: 0.8 Morphine: 0.4	ND
Informer	chest	ND	ND
Police officer 1	chest	ND	ND
Police officer 2	head	ND	ND
Police officer 3	head	ND	ND
Police officer 4	chest	ND	ND
Police officer 5	chest	ND	ND
Police officer 6	head	ND	ND
Police officer 7	chest	ND	ND
Police officer 8	chest	ND	ND
Police officer 9	head	ND	ND
Police officer 10	chest	ND	ND
Police officer 11	chest	ND	ND

THC External Contamination?

External Contamination in Hair

Kintz *et al*. (1995) – *J Forensic Sci*

Testing Human Hair for Cannabis II. Identification of THC-COOH by GC-MS-NCI As a Unique Proof

- Identification of THC metabolite unique marker of marijuana use
- Distinguishes External Exposure to Smoke
- THC parent possibly present due to exposure to marijuana smoke

Sachs *et al.* (1999) – *Forensic Sci Int*

- Method for THC-COOH in Hair by MSD-NCI
- “Detection of THCCOOH should be offered to prove consumption and *metabolism* of THC”

Auwarter *et al.* (2010) – *Forensic Sci Int*

- Widely Accepted Strategy – THC-COOH detection to prove active Cannabis Consumption
- THCA A : preliminary end product of THC biosynthesis in Cannabis
- Oral intake of THCA , regular basis, No incorporation into Hair
- THCA A potentially a marker for exposure to Cannabis side stream smoke

Lab Procedures and Approaches to External Contamination

External Contamination in Hair

Kippenberger (1995) – *A Wash Kinetic –Digestion Method of Hair Analysis for Distinguishing between Drug Use and Contamination.*

- Lab Procedure Approach to External Contamination
- Procedure claims distinguishing external contamination from drug use by:
  - Measure Wash Kinetic/Digest Profiles
  - Different for Use v. Contamination
- Divides Hair Regions into:
  - Accessible Domain
  - Semi-Accessible Domain
  - Inaccessible Domain

Cairns *et al.* (2004) – *Forensic Sci Int*

Removing and identifying drug contamination in the analysis of human hair

- 2 Models , Soaking and Sweat to contaminate Drug free Hair
- Multi-Part Washed Procedure Described & Used
- Wash Criterion Used
- Contaminated Samples Identified
- Hair from Drug Users (Urine COC +)
- Application of Wash Procedure and Criterion ID’d COC Users

Bourland *et al.* (2000) – *J Anal Toxicol*

- Analytical Paper: GC/MS/MS procedure Detection of COC, BE, EME, CE & NCOC in Hair
- No Decontamination Procedure Employed
- N=30 retrospective hair cocaine positives
- CE detected in 19/30 samples, NCOC 29/30
- % to COC Hair : BE=12.8%, EME=1.8%, CE=15.4% & NCOC= 2.5%
- % to COC in confiscated “Street Samples”: BE=0.7 %, EME=2.6%, CE*=n.d., NCOC= 0.2%
- Proposed CE and NCOC as potential biomarkers to distinguish Use v. Exposure

Ropero-Miller *et al. (2012) – J Anal Toxicol*

Cocaine Analytes in Human Hair

- Drug-free Hair contaminated *in vitro* compared to "Street" User and "Clinical" Cocaine Positives
- LC-MS/MS : COC, BE, CE and NCOC
- Hair Decontamination Procedure used
- Street Cocaine User Hair % to COC
  - BE-29%
  - CE-3%
  - NCOC-1%
- Criteria –Use v. Contamination did not improve with addition of CE and NCOC

In Vitro Contamination Studies

External Contamination in Hair

Romano *et al.* (2001) - *Forensic Sci Int*

Hair Testing for Drugs of abuse: evaluation of external cocaine contamination and risk of false positives

- N = 4 “drug free volunteers”
- Applied 10 mg of COC HCL to hands then rubbed in Hair , roots to ends
- Decontamination Procedures proposed by Baumgartner and Hill
- Results : After 10 washes Positive COC and BE results remained in final extraction
- COC > 1ng/mg ; BE > 0.5 ng/mg
- BE/COC Ratio > 0.05

Stout *et al.* (2006) - *J Anal Toxicol*

- Evaluation of Hair Testing Industry's decontamination procedures
- Hair Locks (5 subjects)
- Contaminated with COC HCL (15 mg) to 5 (12g) hair locks
  - Treated with Synthetic Sweat Solution & "Hygienic" Treatments
  - Shampooed Daily (M-F) 10 weeks
- COC HCL purity examined; 0.6% CE, 0.1% NCOC
- 3 Commercial Analytical Laboratories
- 3 Protocols
  - No Decontamination
  - Lab Decontamination Procedure
  - RTI Decontamination Procedure

Stout *et al.* (2006) – *JAT RESULTS of STUDY*

- Labs Reported Quant Results :
  - Cocaine (COC)
  - Benzoylecgonine (BE)
  - Cocaethylene (CE)
  - Norcocaine (NCOC)
- Hair not contaminated COC:BE ratio more significant
- Only hair samples with all 4 drugs/metabolites below detection limits – Decontaminated 1 hour after contamination
- BE/COC ratios increased over 10 week period
- From 21 days to end of Study: BE/COC ratio >0.05 Labs Reported Quant Results :
  - Cocaine (COC)
  - Benzoylecgonine (BE)
  - Cocaethylene (CE)
  - Norcocaine (NCOC)
- Hair not contaminated COC:BE ratio more significant
- Only hair samples with all 4 drugs/metabolites below detection limits – Decontaminated 1 hour after contamination
- BE/COC ratios increased over 10 week period
- From 21 days to end of Study: BE/COC ratio >0.05

Hill *et al.* (2008) – *Forensic Sci Int*

- Hair samples contaminated with Cocaine:
  - rubbing 15 mg COC HCL into 5 (12g) hair samples
  - Sweat application / Shampoo treatments
  - Repeated Stout's Protocol
- Amt of Drug in last wash used as wash criterion
- LC-MS/MS Analysis of Cocaine and metabolites
- Procedure applied to 2 sets of samples:
  - Previous "Govt-Sponsored Cooperative Study"
  - Parallel in-House Study
- All contaminated samples identified as contaminated

Hill *et al.* (2008) – *RESULTS*

- Analytical results after washing (ng/10 mg hair)

Sample 1

Hair treatment step	Total cocaine on hair (ng/10 mg hair	Cocaine	BE	LW[b]	Cocaine - 5 (LW)	Interpretative result[c]
Pre-Sweat	555	7.5	0	1.48	0	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	669	5.8	0	2.05	−4.4	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	443	11	0.1	5.64	−17.1	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
Plus Sweat	571	7.9	0	5.1	−17.4	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	510	5.3	0.1	2.7	−8.1	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	Lab accident	5.8	0	2.7	−7.7	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
Plus Sweat, 1 week[d]	242	7.2	0.2	11.7	−51.1	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	225	6.5	0.2	10.9	−48.2	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	225	5.8	0.2	10.9	−48.5	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
Plus Sweat, 2 weeks	35	5.3	0.27	1.13	−0.33	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	29	5.1	0.21	0.76	1.33	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
	32	3.8	0.17	1.35	NA	Negative: BE < 0.5, %BE < 5; Wash Criterion fails
Plus Sweat, 3 weeks	30.2	5.2	0.3	1.45	−2.06	Negative: BE < 0.5, Wash Criterion fails
	78.7	4.6	0.27	1.39	NA	Negative: Cocaine < 5
	31.3	5.6	0.31	1.5	−1.86	Negative: BE < 0.5, Wash Criterion fails
Plus Sweat, 4 weeks	29.1	2.7	0.18	1.19	NA	Negative: Cocaine < 5
	26.2	2.6	0.17	1.03	NA	Negative: Cocaine < 5
	25.6	2.4	0.12	1.1	NA	Negative: Cocaine < 5

Sample 2

Hair treatment step	Total cocaine on hair (ng/10 mg hair	Cocaine	BE	LW[b]	Cocaine - 5 (LW)	Interpretative result[c]
Pre-Sweat	625	2.2	0	1.0	NA	Negative: Cocaine < 5
	619	2.1	0	0.6	NA	Negative: Cocaine < 5
	589	2.0	0.1	1.0	NA	Negative: Cocaine < 5
Plus Sweat	187	6.7	0	1.1	1.1	Negative: no BE, Wash Criterion fails
	191	5.2	0	1.2	−0.7	Negative: no BE, Wash Criterion fails
	Lab accident	5.7	0	1.26	−0.6	
Plus Sweat, 1 week	134	3.0	0	1.3	NA	Negative: Cocaine < 5
	141	2.3	0	5.2	NA	Negative: Cocaine < 5
	147	2.8	0	6.0	NA	Negative: Cocaine < 5
Plus Sweat, 2 weeks	39.1	2.8	0	0.42	NA	Negative: Cocaine < 5
	37	2.8	0	1.7	NA	Negative: Cocaine < 5
	38.3	2.9	0	2.1	NA	Negative: Cocaine < 5
Plus Sweat, 3 weeks	4.7	2.1	0	0.21	NA	Negative: Cocaine < 5
	4.3	1.8	0	.16	NA	Negative: Cocaine < 5
	5.6	2.2	0	.19	NA	Negative: Cocaine < 5
Plus Sweat, 4 weeks	8.8	2.4	0	0	NA	Negative: Cocaine < 5
	9.3	2.6	0	1.27	NA	Negative: Cocaine < 5
	5.8	2.4	0	1.33	NA	Negative: Cocaine < 5

External Contamination Issue in Hair: Observations

- Hair may be an ideal matrix to test for exposure of drugs in children; Distinguishing exposure and ingestion proves difficult in studies reviewed
- Exposure to Drugs by Narcotic Officers: Real concern: Able to distinguish Use v. Exposure
- THC Exposure in Hair: Little Argument: THCA detection appears to be evidence of Use

External Contamination Issue in Hair: Observations

- Wash Procedures and Wash Criterion appear to be effective for distinguishing Contamination vs. Use based on Studies
- Past Proposed Unique Biomarkers for Cocaine Use not unique – Search continues
- *In Vitro* Studies appear contradictory to Lab Procedure *In Vitro* Studies to distinguish Cocaine Exposure vs. Use

Questions?