Hair Color Bias Literature Review

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

Hair Color Bias Model: Defined

- Drugs bind to melanin
- Melanin Content greater in Darker Hair
- Incorporation and binding of drugs into hair greater in Pigmented versus Non-pigmented Hair
- Higher drug concentrations demonstrated with same dose in dark hair versus light hair
- Bias introduced could mean the difference between a positive or negative drug test outcome
- Implies potential “Racial” Bias to Hair Testing

Hair Color Bias Literature Review:

- Description of Hair Morphology, Chemical make up, Racial/ Ethnic Differences
- Animal Studies
- Human *In Vitro* Studies
- Human Studies General
- Human Controlled Dosing Studies
- Retrospective Statistical Studies

Hair Morphology

Hair Morphology

- Spearman *et al.* (1960) - *AmJPhysicalAnthro*
  - African American Hair
  - Marked Asymmetry of pigmentation
  - Concentration of Melanin Higher African A v. Asian
  - No Asymmetry in Asian Hair
- Lindelof *et al.* (1988) – *ArchDermatol*
  - African American Hair – Helical Factor
  - Asian Hair – Completely Straight
  - Caucasian Hair - Variation

Hair Morphology

- Hardy *et al.* (1973) - *AmJPhysicalAnthro*
  - Studied Hair from 7 populations
  - Used 8 Factors
    - Ave Diameter
    - Medullation
    - Scale
    - Kinky
    - Ave Curvature
    - Ratio of Max to Min Curvature
    - Crimp
    - Ratio of “natural” to “straight” length
  - Racial Differences
  - African to Malaysian

Hair Morphology

- Hopps *et al.* (1977) - *SciTotalEnviron*
  - Diameter of Hairs range from 0.05-0.125 mm, Mean = 0.09 mm
  - Sebaceous and Sweat Glands assoc with hair
  - Growth Rest Cycle of Scalp Hair -1000 days
    - Anagen=Growth Phase: ~900 days,
    - Catagen=Degeneration transitional: several days
    - Telogen=Resting Phase ~100 days

Hair Color Bias

Animal Studies

Animal Studies – Hair Color Bias

- Green *et al.* (1996) – *JAT*
  - Incorporation of Methadone (MtD) in Rat Hair
  - Male, Hooded, Lister Rats (n=36)
  - Black pigmented and White non-pigmented hair
  - Mean MtD ratio 21:1 in pigmented : non-pigmented hair
  - Mean Melanin Content 3.5:1
- Gygi *et al.* (1996) - *DrugMetabDispos*
  - Male Sprague Dawley Rats
  - Distribution of Codeine and Metabolites
  - Codeine IP injection daily for 5 days different doses
  - Conclusion Dose dependent concentrations in hair

Animal Studies – Hair Color Bias

- Gygi *et al.* (1996) - *DrugMetabDispos*
  - Incorporation of Codeine: Role of Pigmentation
  - Sprague-Dawley (SD) : White non-pigmented
  - Dark Agouti (DA): Brown pigmented
  - Hooded Long Evans (LE): Black Pigmented and White Non-Pigmented
  - Measured Codeine, Morphine and Mor-GLuc
  - Codeine 40 mg/kg IP injection daily for 5 days

Gygi *et al.* (1996) - *DrugMetabDispos*

- **Hair Drug Mean Conc (ng/mg)**

Drug	SD	DA	P-LE
Codeine	0.98	5.99	111.93
Morphine	0.34	0.51	14.46
MOR-gluc	0.67	1.04	13.80

Animal Studies – Hair Color Bias

- Wilkins *et al.* (1998) - *JPharmSci*
  - Incorporation of Drug in Pigmented v. Non-Pigmented Hair
  - L-α-Acetylmethadol, Buprenorphine or Methadone
  - Male Long Evans (LE): Black Pigmented and White Non-Pigmented Hair
  - Conclusion: Concentration of each drug at either high or low dose was considerably less in non-pigmented v. pigmented hair

Animal Studies – Hair Color Bias

- Borges *et al.* (2001) - *JAnalToxicol*
  - Incorporation of Amphetamine and N-Acetylamphetamine in Hair
  - Role of Basicity in Hair Color Bias
  - Male Long Evans (LE): Black Pigmented and White Non-Pigmented Hair
  - Rats dosed with Amphetamine >3X more amphetamine in black versus white hair pigmented hair
  - No significant difference in concentrations of N-Acetylamphetamine among black or white hair

Borges *et al.* (2001) - *JAnalToxicol* *Incorporation of Amphetamine and N-Acetylamphetamine in Black and White LE Rat Hair*

- Figure 4. Concentrations of amphetamine found in white and black rat hair and N-AcAp found in white and black hair. N = 8 for each drug. *p < 0.001

Drug	**White hair**	**Black hair**
Amphetamine, ng/mg	2.04 (SD = 0.58)	6.44 (SD = 1.31)*
N-Acetylamphetamine, ng/mg	0.83 (SD = 0.15)	0.87 (S.D. = 0.08)

Hair Color Bias

Human *In Vitro* Studies

Reid *et al.* (1994) - *JToxicolClinToxicol*

- *In vitro* differential binding of BE to pigmented human hair samples
- In vitro Incorporation of BE to Black, Brown and Blonde hair types
- Incorporation of BE Black>Brown>Blond
  - Table: incorporation of BE into human hair (reconstructed ion current m/z 422)
    - ANOVA: F = 13.543; p = 0.0006

Test No.	Black Hair	Brown Hair	Blond hair
1	1,938,775	483,552	319,905
2	1,790,565	517,746	298,899
3	951,620	665,557	299,899
Mean	1,560,319	555,692	306,234
SD	532,332	96,649	11,849

Joseph *et al.* (1994) - TIAFT Meeting

- *In vitro* binding of [^{3}H](-) Cocaine to human hair samples
- Subjects
  - “Light Colored” Female Caucasian Hair (n=8)
  - Female African American Hair (n=9)
- Hair homogenized - 100uM [^{3}H](-) Cocaine
- Amt binding defined amount of [^{3}H](-) Cocaine displaced by 10 uM of (-) Cocaine
- Radioactivity measured
  - 592 488 Female Caucasian Hair
  - 11,080 8,225 Female African American Hair

Joseph *et al. –JPET :1997*

In Vitro Characteristics of Cocaine Binding Sites in Human Hair

- ^{3}H-Cocaine
- Radio Ligand Binding:
  - Male Africoid >Female Africoid
  - Africoid > Caucasoid
- Melanin most likely Binding Site
- 5-43 fold greater binding capacity in Dark Hair versus Light Hair

Hair Color Bias

Human Studies General

Rothe *et al.* (1997) – *ForensicSciInt*

- 15 patients
- Medical treatment with:
  - Carbamazepine, Amitriptyline,Chlorprothixene, Diclofenac, Doxepine, Indomethacin, Maprotiline,Metaclopramide
- Chronic Heroin and Cocaine Abuse
- Hair separated into pigmented and white non-pigmented fractions

Rothe *et al.* (1997) – *ForensicSciInt Conclusions:*

1. Pigmentation strongly affects the drug concentration in hair. Therefore the natural colour or the melanin content of the sample should be registered and taken into account, if forensic or clinical conclusions are drawn from the data.
2. Drugs can also be determined in weakly or non-pigmented hair. White- haired and very fair-haired people are not excluded from hair analysis.

Hair Color Bias

Human Controlled Dosing Studies

Henderson *et al.* (1998) – JAnalToxicol
Incorporation of Isotopically Labeled Cocaine into Human Hair: Race as a Factor

- Cocaine-D5
- *In Vivo* Study
  - 2 mg/kg COC-d5
  - Intranasal Administration
- Subjects
  - 8 Non Caucasians
  - 6 Caucasians from previous study
- 2.7 X more COC-d5 in Non-Caucasian Group
- Not Conclusive - Small Sample Size

Kronstrand *et al.* (1999) - *ClinChem*

- Codeine Controlled Dose Study
- 9 Subjects – Single Oral Dose
- Plasma samples collected -24hours
- Hair samples – Weekly –one month
- Total Melanin, Eumelanin and Codeine Assayed
- Codeine vs. Total Melanin (r^2=0.86)
- Codeine vs. Eumelanin (r^2=0.90)
- Suggests Normalizing for Melanin Content

Mieczkowski-1995

Ratios of Cocaine(+) Urinalyses, Hair Assays, & Self-Reports

Percentages	Black Arrestees	White Arrestees	Ratio: Black/White
%Urine(+)	36.7	16.04	2.29
%(+)S/R 48 Hrs.	13.3	6.3	2.11
%Hair(+)	63.4	32.5	1.95
% (+)S/R 60 Days	17	10.98	1.56

Rollins *et al.* (2003) – *JAnalToxicol*
The Effect of Hair Color on the Incorporation of Codeine in Human Hair

- Study Group
  - Caucasian: black hair (n=6), brown (n=12), blond (n=8),red (n=6)
  - Non-Caucasian: black hair (n=12)
- Oral Codeine Syrup – 30 mg, 3X/day -5 days
- Plasma samples collected -24hours
- Hair samples –Week 4,5,6 & 7
- Codeine vs. Total Melanin (r^2=0.73)

Rollins *et al.* (2003) – *JAnalToxicol*

RESULTS

Hair Type	**[Codeine] (pg/mg)**
Black (n=18)	1429 249
Brown (n=12)	208 17
Blond (n=8)	99 10
Red (n=6)	69 11
Asian Black (n=12)	2564 170
Caucasian Black (n=6)	865 162

Hair Color Bias

Retrospective Statistical Studies

Mieczkowski - 1995

Cocaine Hair Assay: Overall Mean Concentration [ng/mg]

Arrestees	Cocaine Mean Concentration	Standard Deviation	#of Cases
All Arrestees	15.2	35.1	382
Black Arrestees	24.9	52.7	116
White Arrestees	11.1	22.9	258

Kidwell et al. (2000) – *ForensicSciInt* Evidence for Bias in Hair Testing…

- “Cultural Bias” v. “Hair Color Bias”
- << Cocaine in black hair of Asian-Caucasian males than African American females
- Cosmetic Treatments, Hygiene
- Cut-off could determine whether bias is observed
- Heavy Use population - little no bias
- Light Use population –bias more likely

Hoffman - 1999:
Analysis of Race Effect on Drug Test Results

- Urine

Sex/Result	Black	White	OR
Males			
Positive	11	5	
Negative	473	857	3.99
Females			
Positive	1	1	
Negative	278	226	0.81

- Hair

Sex/Result	Black	White	OR
Males			
Positive	41	20	
Negative	443	842	3.90
Females			
Positive	10	2	
Negative	269	225	4.18

Kelly *et. al.* (2000) - *ForensicSciInt*

Hair Analysis for drugs of abuse. Hair color and race differentials or systematic differences in drug preferences?

- Large Retrospective Population
- 3 Groups (2000 each) (n=6,000)
- Hair color : 7 Categories
- Only COC, MAMP/AMP, THCA Positives and No Drug Detected
- SP1: Urine Drug Testing (UDT) Results v. Hair Color (Recorded at Collection Site)
- SP2: UDT Results v. Ethnicity Determination via Photographs and Surnames
- SP3: Hair Drug Testing Results v. Hair Color Determination

Cocaine Positives: Pattern in Urine and Hair
Kelly *et. al. (2000)- ForensicSciInt*

Race	% of Positives
Caucasian	1.95
African American	13.07
Hispanic	4.28
Asian Pacific	0

Hair color	Negative, %	Positive, %
Red	1.4	1
Grey	5.6	1
Blond	6.8	4
Light Brown	10.2	6.2
Medium Brown	25.6	18.6
Dark Brown	25	28.8
Black	25.4	40.4

Amphetamine Positives: Pattern in Urine and Hair - Kelly *et. al. (2000) ForensicSciInt*

Race	% of Positives
Caucasian	12.2
African American	0.57
Hispanic	5.88
Asian Pacific	0

Hair color	Negative, %	Positive, %
Red	1.4	1.2
Grey	5.6	0.8
Blond	6.8	7.6
Light Brown	10.2	14.4
Medium Brown	25.6	36
Dark Brown	25	28
Black	25.4	12

Kelly *et. al.* (2000) *ForensicSciInt*

- Our summary impression is that the observed outcome patterns were largely consistent with differences in drug preferences among the various societal groups. There was little evidence of a pattern attributable to hair color bias alone or selective binding of drugs to hair of a particular color. Likewise, there was no discernible pattern associated with race or ethnicity that would lend support to a "race effect" in drug analysis.

HAIR COLOR BIAS OBSERVATIONS

- Drugs /metabolites: bind to melanin
  - Authors suggest normalizing
  - Others suggest Lab Procedures to compensate
- Equal doses of many basic drugs are detected in significantly greater concentrations in pigmented than non-pigmented hair in animals and humans.
- Differences in binding and ultimate concentrations based on:
  - Hair Color
  - Hair Type

HAIR COLOR BIAS OBSERVATIONS - Continued

- Hair Color Bias Results may be dependent on
  - Cut-off Concentrations
  - Type of User: Recreational –Heavy
- Statistical Findings : Bias in Urine similar to Bias in Hair among different ethnic groups.
- Retrospective Studies Hair Color Bias Model:
  - Cocaine –Fits Model
  - Amphetamine- Contrary to Model
  - THC-COOH-Not Supportive of Model

Questions?