Hair Pigmentation Literature Review

Drug Testing Advisory Board (DTAB) Meeting
September 3, 204

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Background: Hair Pigmentation
Literature Review Process

- 54 peer reviewed articles reviewed: 5 SMEs reviewed 6-12 articles each
- SMEs: Cone, Caplan, Walsh, Crouch and Bourland
- Literature Review Format
  - Reference
  - Keywords
  - Study Design
  - Study Findings
  - Conclusion
  - Reviewer’s Comment
Background

Resources

- Bourland slide presentation “Hair Color Bias Literature Review” - Presented to DTAB September 10, 2013

- Cone slide presentation “Hair Pigment Review” – prepared for DTAB meeting *not presented*

- Literature Review Summaries (n=54)
  - Note 43 articles presented
  - 11 articles omitted
Hair Color Bias: 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 “Hair Color Bias” for Hair Testing
Hair Pigmentation Literature Review
Article Categories:

- Animal Studies
- Human In Vitro Studies
- Human Studies General
- Human Controlled Dosing Studies
- Retrospective Statistical Studies
- Review Articles
Animal Studies:
Hair Pigmentation Literature Review
Animal Study #1 – Hair Pigmentation Review


**Description:**
- Incorporation of Methadone (MtD) in Rat Hair
- Male, Hooded, Lister Rats (n=36)
- Black pigmented and White non-pigmented hair
- RIA

**Findings:**
- Mean MtD ratio 21:1 in pigmented : non-pigmented hair
- Mean Melanin Content 3.5:1

**Conclusion(s):**
- Methadone binds at higher affinity to pigmented hair

**Comments/Limitations:**
- Animal model
- RIA
- Parent MtD, EDDP?

Description:
- Amphetamine (AMP) and N-Acetylamphetamine (N-AcAp)
- Long Evans Rats
- Black pigmented and White non-pigmented hair
- 10 mg/kg IP daily -5 days
- LC-MS/MS

Findings:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Black Hair</th>
<th>White Hair</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>6.44 ± 1.31 ng/mg</td>
<td>2.04 ± 0.58 ng/mg</td>
</tr>
<tr>
<td>N-AcAp</td>
<td>0.87 ± 0.08 ng/mg</td>
<td>0.83 ± 0.08 ng/mg</td>
</tr>
</tbody>
</table>

Conclusion(s):
- Drug Basicity plays important role in melanin binding
- Supports Hair Color Bias for Basic drugs but not Acidic drugs

Comments/Limitations:
- Animal model
- Basic v. Non-basic compounds
Animal Study #3 – Hair Pigmentation Review


Description:
- Subcutaneously administered nicotine to rats
- Also, nicotine smoke exposure and soaking in nicotine urine
- Used sodium dodecyl sulfate wash and 4X with water

Findings:
- Nicotine concentrations in hair were ~20 times higher than unpigmented hair.
- Washing removed by standard washing was up to 18% for systemic nicotine.
- After smoke exposure or soaking in nicotine urine and standard washing, nicotine remained in hair and were 2X higher in pigmented hair.

Conclusion(s):
- Hair pigmentation had a major influence on systemic uptake and a minor influence on external uptake.

Limitations /Comments:
- Animal model- No Sweat,
- Nonpigmented hair did test positive at much lower concentrations.

Description:
- Systemic administration of radiolabeled drugs to pigmented and nonpigmented mice.
- The distribution of these compounds was examined by autoradiography of skin sections containing developing hairs.
- Mice were dosed daily for 3 days and allowed to grow for an additional 21 days.

Findings:
- Skin sections at time points early (10-15 min after dosage) showed rapid association of each drug with melanin in hair bulbs.
- Parent compound was primarily deposited in hair.
- no more than 53% of the drug could be recovered.
- Drug was associated with hair below the point at which sweat and sebum have access to the hair.
- The incorporation rate for each drug into pigmented hair far exceeded the incorporation rate into nonpigmented hair.

Conclusion(s):
- Results suggested that drug was incorporated within the hair matrix and not on the surface.
- The authors concluded that interpretation of hair drug analyses is complicated by the extent of hair pigmentation.
- Drug deposition by systemic exposure was resistant to recovery suggesting that this unique pattern of deposition can be distinguished from environmental contamination.

Limitations /Comments:
- Animal model
- Melanin pigmentation has a major impact on the degree of drug incorporation into hair
- This study suggests that there are unique mechanisms of binding drug from systemic exposure that are likely to be different than from environmental contamination
Animal Study #5 – Hair Pigmentation Review


Description:
- Long Evans Rats
- Cocaine (COC) administered I.P.
  - Multiple Dose Experiments: 5, 10, 20 mg/kg; Daily -5 Days (n=8X3)
  - Single Dose Experiment: 10 mg/kg (n =4)
- 1” square white and black hair sections shaved prior to dosing
- Plasma PK study: IP. Doses 5, 10, 15 mg/kg (n=each dose per time point)
- Hair collected 14 and 28 days later: multiple dose experiment
- Single dose experiment: hair plucked 1,2,4, 6 and 24 h & 2,3,4,6,8 & 14 days.

Findings:
- Multiple Dose Experiment
  - COC, EME and NCOC preferentially incorporated
  - Dose –dependent manner
- Single Dose Experiment
  - COC analytes in pigmented hair > exceeded non-pigmented
  - COC and BE only found in non-pigmented hair
- Plasma PK Study-After normalizing for plasma concentration COC into pigmented hair was two orders of magnitude> BE

Conclusion(s):
- Cocaine preferentially incorporated into pigmented hair
- COC in hair >BE even when BE> COC in plasma

Limitations /Comments:
- Animal model
Animal Study #6 – Hair Pigmentation Review

- Description:
  - Three strains of Rats, Dark Agouti (DA), Long Evans Hooded Rat (LE) & Sprague-Dawley Albino (SD)
  - Codeine (COD) administered I.P.
    - Multiple Dose
    - Single Dose
    - Plasma PK
    - In-Vitro Binding
  - COD, MOR and MOR-Glucuronide: PICI GC ion trap MS
- Findings:
  - Hair COD, MOR, MOR-Gluc concentrations (40 mg/kg) IP COD

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>COD (ng/mg)</th>
<th>MOR (ng/mg)</th>
<th>MOR-Gluc (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0.96</td>
<td>0.34</td>
<td>0.67</td>
</tr>
<tr>
<td>DA</td>
<td>6.02</td>
<td>0.51</td>
<td>1.04</td>
</tr>
<tr>
<td>LE Nonpigmented</td>
<td>2.42</td>
<td>0.46</td>
<td>0.67</td>
</tr>
<tr>
<td>LE Pigmented</td>
<td>111.93</td>
<td>14.46</td>
<td>13.8</td>
</tr>
</tbody>
</table>
- Conclusion(s):
  - COD, MOR and MOR-Glucuronides preferentially incorporated into pigmented hair
- Limitations /Comments:
  - Animal model
Animal Study #7 – Hair Pigmentation Review

- Potsch L, Skopp G, Moeller MR. Influence of Pigmentation on the Codeine Content of Hair Fibers in Guinea Pigs, J Forensic Sci 1997; 42(6),: 1095-1098

- Description:
  - Tortoise shell guinea pigs (n=7)
  - Codeine (COD) 1 mg/mL in drinking water-3 weeks
  - Black, Reddish-Brown, and White hair collected separately before and after drug treatment
  - Hair samples washed 3X with MeOH
  - GC/MS

- Findings:

<table>
<thead>
<tr>
<th>Tortoise Shell Guinea Pig Number</th>
<th>Total Dose of Codeine-Base Ingested mg/kg</th>
<th>Codeine Content of Different Colored Hair Fibers [ng/mg Hair]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>White</td>
</tr>
<tr>
<td>1</td>
<td>254</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>305</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>328</td>
<td>2.9</td>
</tr>
<tr>
<td>4</td>
<td>468</td>
<td>9.1</td>
</tr>
<tr>
<td>5</td>
<td>678</td>
<td>18.6</td>
</tr>
<tr>
<td>6</td>
<td>1164</td>
<td>6.1</td>
</tr>
<tr>
<td>7</td>
<td>1186</td>
<td>15.9</td>
</tr>
</tbody>
</table>

- Conclusion(s):
  - Highest drug incorporation of COD was always found in Black Hair

- Limitations /Comments:
  - **Advantage:** one animal three hair color types
  - “pilot study “with small n
  - **Animal model**
Animal Study #8 – Hair Pigmentation Review


- **Description:**
  - Phenobarbital – weak acid
  - Codeine – weak base
  - Sprague Dawley and Long Evans Rats
  - 40 mg/kg COD or PB ip injection -5 days
  - Hair collected 14 days after dose start

- **Findings:**
  - Codeine incorporated 15X > PB
  - Codeine 44X higher in pigmented v. nonpigmented hair

- **Conclusion(s):**
  - Hair pigment greatly affects weak bases but not weak acids

- **Limitations /Comments:**
  - Animal model
Animal Study #9 – Hair Pigmentation Review


**Description:**
- Quantitative Method: Eumelanin and Pheomelanin in hair presented
- Permanganate oxidation used for Eumelanin
- Hydriodic analysis for Theomelanin
- Both methods produce degradation products, Eumelanin $\rightarrow$ PTCA, Pheomelanin $\rightarrow$ AHP

**Findings:**

**Conclusion(s):**
- Authors conclude quantitative analysis of eu- and pheomelanin is simple and sensitive

**Comments/Limitations:**
- *Procedure tested in animals, 1985 procedure*
Animal Study #10- Hair Pigmentation Review


Description:

- Authors develop a new method to estimate total eu- and pheomelanins in hair samples
- Completely solubilizing the melanins in hot Soluene-35- plus water
- Eumelanic hair was obtained from black, brown, dilute black, pink-eyed black and silver mice
- Pheomelanic hair obtained from lethal yellow, viable yellow, recessive yellow mice and two strains of agouti mice
- Wool samples from lambs of Tajik breeds
- Human hair collected from University of Newcastle

Findings:

- “Absorbance at 500 nm (A_{500}) pf the Soluene-350 solution correlated well with total melanin content

Conclusion(s):

- Spectrophotometric method developed for characterizing eu- and pheomelanins in hair is more convenient than HPLC
- May be useful in estimating the relative ratio of eumelanin to total melanin

Comments/Limitations:

- Method may be useful in the analysis of hair in relation to drug retention
- Could further elucidate hair color
Human *In Vitro* Studies: Hair Pigmentation Literature Review
Tsai, J.R., Tsao, L.I., and Cone, E.J. (1994) Cocaine binds in a stereospecific, saturable manner to hair: a precaution on hair testing for forensic purposes. *CPDD Abstract Form*

**Description:**
- Binding Experiments – [³H](-)Cocaine (COC)
- 2 Human Subjects (black hair and blonde hair)

**Findings:**
- Binding saturable and reached equilibrium in 60 min.
- Dissociation constant (kd) -3.5X higher in black hair

**Conclusion(s):**
- Stereospecific binding sites exist in hair for cocaine
- COC had greater affinity in black v. blonde hair

**Comments/Limitations:**
- Small n (n=2)
- Other COC metabolites not investigated
Human *In Vitro* Study #2 – Hair Pigmentation Review


**Description:**

- *In Vitro* Binding Experiments – [$^3$H] Haloperidol
- Cell lines: Pigment (Sk-Mel-1) and non-pigment producing (HaCaT)
- Cell lines separated and co-cultured
- Liquid Scintillation Spectrometry (LSS)-[$^3$H] Haloperidol
- Spectrophotometry – Melanin

**Findings/Conclusion(s):**

- After Co-culture >50% increase in [$^3$H] Haloperidol in pigmented cells

**Comments/Limitations:**

- *In vitro model demonstrated increased binding of haloperidol in melanin containing cells*
- *Demonstrated role of melanin in increased binding of certain drugs*
Human *In Vitro* Study #3 – Hair Pigmentation Review


- Description:
  - Drug-free hair was contaminated *in vitro* with cocaine from different sources and different concentrations.
  - Hair specimens were analyzed for cocaine, BE, CE, NCOC by LC/MS/MS.
  - Results were compared to drug-users hair and subjects in a controlled dosing study.

- Findings/Conclusion(s):
  - The effect of hair color was not evaluated. But the discussion stated “With these limited preliminary data, it appears that darker hair is more susceptible to drug incorporation from *in vitro* contamination than lighter hair.”

- Comments/Limitations:
  - *The limited number of hair types (3 Caucasian and 8 African-Americans) was not sufficient to evaluate the effect of hair color (pigment), but there was some suggestive evidence of effect.*
Human *In Vitro* Study #4 – Hair Pigmentation Review


**Description:**
- In vitro binding experiments were conducted with synthetic melanins.
- The melanins in the study were two black eumelanin subtypes, a reddish-brown pheomelanin, and two mixed eu-/pheomelanin copolymers.
- Cocaine (COC), BE, amphetamine (AMP) and N-acetylamphetamine (N-AcAp) were studied.
- N-acetylamphetamine was included to represent a non-basic form of amphetamine.
- Quantitation of drugs was performed with LC/MS/MS.

**Findings**
- Of the 4 drugs evaluated, only COC and AMP were found to bind with melanin.
- COC and AMP did not bind to the synthetic pheomelanin (5-CysDOPA).
- Two binding sites were involved in binding (high affinity/low capacity and low affinity/high capacity).

**Conclusion(s):**
- This study extended the principle that basic drugs like cocaine and amphetamine bind to eumelanin types, but not pheomelanin, whereas acidic drugs (BE) and neutrals (N-acetylamphetamine) do not.
- Data also suggested that drug binds with eumelanin by non-covalent attachment. These data help explain why “hair color biases exist”.

**Comments/Limitations:**
- *This is an important mechanistic study of how drugs interact with hair pigment.*
- *It highly suggests that people with high levels of eumelanin (black, brown) hair will accumulate (uptake) more basic-type drugs than blond, gray and red-headed hair.*
Human In Vitro Study #5 – Hair Pigmentation Review


**Description:**
- Hair collected from male and female subjects divided into five groups: male Africoid, female Africoid, male Caucasoid [blk/br], female Caucasoid [blk/br] and female Caucasoid [blond]- Each group n=7
- Each hair sample divided into 3 portions; one portion untreated, one bleached and one extracted to remove lipids
- Treatment with $[^{3}\text{H}](l)$ Cocaine (COC)

**Findings**
- COC binding significantly higher in male Africoid hair to all others
- Male Africoid hair 34X higher COC than blonde female Caucasoid hair
- Binding of COC to female Africoid, blk/brn male and female Caucasoid- no significant difference,

**Conclusion(s):**
- Digestion of hair and removal of insoluble melanin was not effective in removal of color bias
- Lipids play a minor role in drug binding
- Melanin is major binding site for cocaine

**Comments/Limitations:**
- Study findings support the notion of hair color bias
- Major limitation in vitro model
- Cocaine metabolites were not evaluated in this study
Human *In Vitro* Study #6 – Hair Pigmentation Review


### Description:
- *In vitro* differential binding of Benzoylcegonine (BE) to pigmented human hair samples
- *In vitro* Incorporation of BE to Black, Brown and Blonde hair types
- Hair washed 6X with EtOH, Incubated 2h with BE, Extracted, GC/MS analysis

### Findings
- Incorporation of BE Black>Brown>Blond

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incorporation of Benzoylcegonine into Human Hair</td>
</tr>
<tr>
<td>(Reconstructed Ion Current for M/z 422)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test No.</th>
<th>Black Hair</th>
<th>Brown Hair</th>
<th>Blond Hair</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,938,775</td>
<td>483,552</td>
<td>319,905</td>
</tr>
<tr>
<td>2</td>
<td>1,790,565</td>
<td>517,946</td>
<td>298,898</td>
</tr>
<tr>
<td>3</td>
<td>951,620</td>
<td>665,557</td>
<td>299,899</td>
</tr>
<tr>
<td>Mean (X)</td>
<td>1,560,319</td>
<td>555,692</td>
<td>306,234</td>
</tr>
<tr>
<td>SD</td>
<td>532,332</td>
<td>96,649</td>
<td>11,849</td>
</tr>
</tbody>
</table>

ANOVA: F = 13.543, P = 0.0006

### Conclusion(s):
- Hair color affected in vitro incorporation of BE: Black>Brown>Blond
- Melanin appeared to be a factor with BE incorporation

### Comments/Limitations:
- *Unclear why quantitation was not performed* …monitored ion current area counts m/z 422
- *In Vitro test – artificially produced BE concentrations in human hair* - not based on COC ingestion
Human *In Vitro* Study #7 – Hair Pigmentation Review


**Description:**
- $^3$H-Cocaine binding to melanin investigated
- Melanin from *Sepia officinalis* (the cuttlefish)
- Human Hair: Black, Brown, Red and Blond
- Hair soaked in $^3$H-cocaine (0.5, 1.0 and 2.5 mg) for 3 days and 1 day (Hair fibers and pulverized hair)

**Findings**
- Sepia Melanin binding maximized in 20 minutes with 0.5, 1.0 and 2.5 of $^3$H-Cocaine
- Powdered hair showed little difference in $^3$H-Cocaine uptake based on hair color
- Intact hair fibers: $^3$H-Cocaine binding – black > brown > red and blond

**Conclusion(s):**
- *In vitro* drug-soaked were not the appropriate model – melanin drug incorporation
- *In vitro* model unable to distinguish melanin v. drug bound to keratinized fibers

**Comments/Limitations:**
- *In vitro* model
- *May not reflect true drug binding in vivo*
- *Eumelanin and pheomelanin not measured*, Visual observation of hair color
Human *In Vitro* Study #8 – Hair Pigmentation Review


- Description:
  - $^3$H-Cocaine and similar analogues binding to melanin investigated

- Findings
  - 5-43 fold greater binding capacity in Dark Hair versus Light Hair
  - Male Africoid > Female Africoid
  - Africoid > Caucasoid

- Conclusion(s):
  - Melanin most likely Binding Site

- Comments/Limitations:
  - *Differences in binding appeared to be due to differences in density melanin in hair*

**Description:**

- *In vitro* model of drug incorporation
- Compared the uptake and release of amphetamine (AMP) and a non-basic analog N-acetylamphetamine (N-AcAp)
- *In vitro* culturing the drugs with keratinocytes, pigmented melanocytes (PM) and nonpigmented melanocytes (NPM)

**Findings:**

- PM – took up large amounts of AMP
- NPM & Keratinocytes – took up small amounts of AMP
- None of the cells took up N-AcAp above background levels.

**Conclusion(s):**

- Pigmented cells take up greater amounts of AMP and efflux it more slowly.
- Data support a non-diffusion mediated model for drug incorporation into hair cells

**Comments/Limitations:**

- *Consistent findings with animal in vivo model for AMP and N-AcAp*

**Description:**
- Study to investigate the mechanism of incorporation of drugs in hair
- Melanin — *sepia officinalis*
- MALDI-TOF analysis performed on products of *in vitro* synthesis of melanin in the presence of amphetamine

**Findings:**
- Several amphetamine adducts identified
- Amphetamine formed adduct with melanin intermediate L-DOPA

**Conclusion(s):**
- Author suggests the adducts are bound portions of the drug that are not accounted for during routine drug screening of hair
- Implication is likely important in understanding the quantitative values in hair testing

**Comments/Limitations:**
- Consistent with evidence that amphetamine binds to melanin
- MALDI-TOF not practical for routine analysis of drugs in hair
General Human Studies:
Hair Pigmentation Literature Review

Description:
- Graying hair collected males 33-55 yoa, Hospitalized for substance abuse
- Hairs divided into pigmented and senile white hairs; n= 29 pairs
- Cocaine (COC), Cocaethylene (CE) and Benzoylecgonine (BE)
- Extensive wash procedure; Incubated overnight in 0.05M H₂SO₄
- SPE, GC/MS (PCI)

Findings:

<table>
<thead>
<tr>
<th></th>
<th>Pigmented (ng/mg)</th>
<th>White (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC</td>
<td>31.5 ± 30.2</td>
<td>14.9 ± 19.8</td>
</tr>
<tr>
<td>CE</td>
<td>3.22 ± 5.0</td>
<td>0.52 ± 0.88</td>
</tr>
<tr>
<td>BE</td>
<td>5.1 ± 5.3</td>
<td>3.9 ± 4.8</td>
</tr>
</tbody>
</table>

Conclusion(s):
- Data supported increased binding of COC and CE in pigmented hair

Comments/Limitations:
- 5 /29 hairs concentrations not significantly different between pigment and white
- Several samples BE> in white than pigmented hair
- COC, CE and BE were detected in white senile hair

**Description:**
- Hair samples submitted for abstinence testing to obtain Germany driver’s license
- Hair tested for EtG and drugs of abuse by ELISA and MS confirmation
- Cosmetic Treatment (dyed hair) was investigated.
- 9488 positives of non-treated hair and 1026 of cosmetically treated hair analyzed

**Findings:**
- The positivity rate for drugs and EtG was equivalent in cosmetically treated hair compared to non-treated hair.

![Graph showing hair lengths analyzed](image)

**Conclusion(s):**
- Even though cosmetic treatment can reduce drug concentration, drugs remained present in dyed or bleached hair

**Comments/Limitations:**
- *Study evaluated the effect of cosmetic treatment on positivity rate*
- *Earlier studies show large reductions in drug concentrations – In Vitro*
- *Common cosmetic treatments do not have a meaningful effect on test outcome*
Description:

- 5 strands of black and white hairs obtained from human subjects treated with Ofloxacin
- Ofloxacin - an antimicrobial quinolone derivative used to treat respiratory, otolaryngeal and urinary infections in Japan
- Animal study was also conducted using Sprague-Dawley Albino and male Dark Agouti pigmented rats.
- All subjects received dosing for 5 weeks and hair samples newly-grown obtained at 6 weeks

Findings:

- Human Subjects: Ofloxacin much lower in white hair than black for the same subject.
- Human Subjects: No drug detected in 3 out of 4 white hair specimens
- Animal Study: S-D Albino rats showed significantly less drug than dark agouti rats.

Conclusion(s):

- Findings suggest Ofloxacin concentration based on “excretion” in hair closely linked to melanin content

Comments/Limitations:

- Study limited by small number of human subjects (n=4)
- 25 years old paper…older technology coupled with small sample size (5 strands) raises issues of validity of findings

**Description:**
- 2 Animal Studies and 1 Human study
- Human Study: hair collected from 10 male and 10 females taking haloperidol
- 4 subjects had “grizzled” (mixed white and dark) hair
- Samples collected by cutting
- Analysis by RIA

**Findings:**
- The concentrations of haloperidol were higher in dark hair vs. white hair in all 4 patients
- Haloperidol only detected in white hair in 1 out of 4 patients

**Conclusion(s):**
- Authors concluded that haloperidol was “excreted” into hair
- Amount “excreted” was both dose and hair color (i.e. melanin) related

**Comments/Limitations:**
- *Major limitation was number of human subjects n=4*
- *Another limitation was the use of RIA as the analysis technique*

**Description:**
- Hair collected from 15 patients (Rx therapeutic drugs or Cocaine or Heroin Users)
- Therapeutic Drugs: amitriptyline, doxepin, maprotiline, metoclopramide, carbamazepine, clorprothixene, diclofenac and indomethacin
- All subjects had “grizzled” (mixed white and dark) hair
- Both pigmented and nonpigmented hair collected from all subjects
- Extracted and analyzed by GC/MS

**Findings:**
- In general drug metabolite concentrations in white hair < than pigmented
- White:Pigmented hair ratio <1.0
- Intersubject variability: Amitriptyline 0.18-0.88; Nortriptyline 0.22-1.2

**Conclusion(s):**
- Authors concluded pigment strongly effects drug concentration in hair
- Drug can be measured in non-pigmented hair

**Comments/Limitations:**
- Majority of data presented was for therapeutic drugs only one cocaine/heroin user evaluated

**Description:**
- Effectiveness decontamination procedure tested: 67 + cocaine (COC) hair samples
- Complementary positive COC urine sample for each subject
- 7 different hair color types
- Soaking experiments: 1,000, 10,000, 50,000 ng COC/mL water -4 hair types
- Treated hair (perm) vs. untreated soaked in 10,000 ng COC/mL
- Wash procedure: IPA-15min, PO4 buffer 30 min X 3, PO4 buffer 60 min X 2
- Hair enzymatically digested; LC-MS-MS COC and metabolites (cut-off =500 pg/mg)
- Last wash analyzed by RIA, multiplied X 5, subtracted from final extract amount

**Findings:**
- Hair subjected to permanent wave treatment: significantly more COC than untreated
- Hair colors did not show significant differences in COC uptake
- Washing characteristics unrelated to hair color

**Conclusion(s):**
- Porosity not hair color determined the rate of COC uptake in solution
- Author’s criticize other hair color bias studies that exclude vigorous wash procedures

**Comments/Limitations:**
- *Porosity an important factor to consider in incorporation of drug into hair*
- *Large n however but no control of dose or history of drug use in retrospective portion of study of “real-world” hair specimens*
Human Controlled Dosing Studies: Hair Pigmentation Literature Review

**Description:**
- Codeine Controlled Dose Study
- 9 Subjects -3 males + 6 females (7 blond hair) (1 male black hair) (1 female medium brown)
- Single Oral Dose 100 mg of Codeine
- Plasma samples collected -24 hours
- Hair samples – day 7, 14, 21 and 28 post dose
- Total Melanin, Eumelanin measured by Spectrophotometry and HPLC
- Codeine analyzed by GC/MS

**Findings:**
- Higher melanin and eumelanin content in hair resulted in higher codeine concentrations
- [Codeine] correlated with total [Melanin] ($r^2 = 0.86$) and [Eumelanin] ($r^2=0.90$)

**Conclusion(s):**
- Authors concluded measurement of melanin in combo with drugs in hair important
- Recommended normalizing for melanin content

**Comments/Limitations:**
- Small number of subjects in study ($n=9$)
- Study demonstrated a correlation between melanin content and codeine supporting hair color bias – biased reduced/eliminated by normalization with melanin or eumelanin
- No codeine metabolite (morphine) levels reported although method able to detect it. Uncertain whether the morphine levels at detectable level in hair after on single oral dose of codeine.
Human Controlled Dosing Study #2
Hair Pigmentation Review


**Description:**

- **Study Group- 44 Human Subjects**
  - 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**

**Findings:**

- Codeine vs. Total Melanin ($r^2=0.73$)

**Conclusion(s):**

- Codeine results influenced by hair color
- 200 pg/mg cut-off:
  - 100% POS blk, 50% POS brn; 100% NEG blond/red
  - 50 pg/mg cut-off: All hair colors 100% POS

**Comments/Limitations:**

- Correlation of melanin to Codeine Concentration
- Lowering cut-off eliminated hair color bias for positive codeine result

Description:
- 25 “Moderate” COC users administered Cocaine-d5 (0.6-4.2 mg/kg) by IV or intranasal route
- 21 male Caucasians + 4 female non-Caucasians
- Hair color: 12 brown hair, 7 black hair, 2 blonde hair and 4 graying hair (3 dyed, 2 bleached)
- Deuterated COC and metabolites by GC/MS

Findings:
- COC-d5 major analyte identified in hair
- BE-d5 found in 10/25 subjects
- All non-Caucasians (with darker hair) had higher levels of COC-d5 than Caucasians (2-12X)

Conclusion(s):
- Authors concluded that non-Caucasians incorporated more cocaine than Caucasians
- Authors suggest coarse dark hair was the cause of greater cocaine incorporation

Comments/Limitations:
- Authors focused on ethnicity in their conclusions and not on hair color or melanin content.
- Relatively small sample size to make conclusions concerning ethnicity and hair drug incorporation
Human Controlled Dosing Study #4
Hair Pigmentation Review


Description:
- Administration of deuterated (d5)-cocaine to 9 non-Caucasians.
- Hair analyses for COC and BE by GC/MS
- Comparison of results to previous study of 6 Caucasians

Findings:
- The non-Caucasians had 2.7 times more cocaine in hair than Caucasians

Conclusion(s):
- “…there appears to be a racial bias in the incorporation of cocaine into human hair; second, cocaine in substantial amounts can be detected in hair as early as 24 h after dosing; and third, cocaine was not incorporated into the hair of one subject for reasons that remain unclear.”
- The authors opined that “individual differences in drug incorporation into hair could therefore result from differences in sweat and sebum secretion”

Comments/Limitations:
- *The study was highly limited in scope and comprised very few subjects limiting interpretation of results and extrapolation to the general population.*

**Description:**

- 7 Human subjects dosed with sustained release S-(+)-MAMP HCl 4 X 10 mg (low) -1 week
- Weekly head hair samples collected by shaving.
- 3 weeks later, 4 subjects received 4 x 20 mg (high) doses
- MAMP and AMP assayed by LC-MS-MS

**Findings:**

- Correlation MAMP and AMP Cmax / AUC and Melanin concentration

**Conclusion(s):**

- Despite large inter-individual differences, incorporation of MAMP/AMP dose related
- Observed MAMP and AMP concentrations are explained by melanin concentrations in hair
- Higher the total melanin the higher the amount of incorporated drug

**Comments/Limitations:**

- Study supports hair color bias for MAMP and AMP
- Since correlation shown may be possible to normalize to melanin to reduce bias
Retrospective Statistical Studies: Hair Pigmentation Literature Review

**Description:**
- 1852 Police Department job applicants self-classified as “black” or “white”
- Overall qualitative results for cocaine (COC) and marijuana (THC) in hair and urine
- "Racial" bias was investigated in hair compared to urine drug testing

**Findings:**
- In General: Incidence of POS drug results higher in hair than urine
  - COC ≈4X ↑ - both black and white males
  - THC ≈2X ↑ - both black and white males
  - THC same POS rate in hair and urine among “black” and “white” females
  - COC -1 urine POS → 2 Hair POS: “white” females
  - COC - 1 urine POS→ 10 Hair POS: “black” females
- Statistical treatment of data “Mantel-Haensel estimate common to odds ratio” showed no significant difference between white and black subjects, male or female:

**Conclusion(s):**
- Author concludes results of the study showed no “racial” bias for hair drug testing when compared to urine drug testing
- Use of hair increased the positive rate in both black and white applicants
- Hair Testing did not bias one race over the other based on the statistical treatment of the data

**Comments/Limitations:**
- No details given how the urine or hair testing was performed
- Provides convincing evidence in general that using hair versus urine did not introduce a bias
- However it appears that there was a significant difference for COC positives in black females, although statistically insignificant based on the statistical treatment
Retrospective Statistical Study #2
Hair Pigmentation Review


**Description:**
- 8 different sets of data examined
  - 4 Large n studies: Univ. of Glasgow (n=139), Florida “Probation Study” (n=589), APL study (n=1000), Psychemedics Study (n=998 (38))
- All 8 data sets subjected to one way ANOVA analysis or “Tukey”s Honestly Significant Difference” procedure

**Findings:**
- All data sets failed to show a significant association between hair color and analyte recovered from hair at p =0.05

**Conclusion(s):**
- Authors conclude that color plays a role in accumulation of drugs in hair but only a small part of a very complex process

**Comments/Limitations:**
- *Statistical treatment of all 8 studies showed no hair color bias*
- *Major limitation admitted by authors was the “characterization of hair color” “not done with precision but relatively casual observation.”*

**Description:**
- 3 anti-seizure medications in hair vs. hair color evaluated in 140 clinical patients
- Carbamazepine (CBZ) \(n=40\), Valproic Acid (VPA) \(n=40\), Phenytoin (PHT) \(n=60\)
- 200 mg of scalp hair from posterior vertex region
- FPIA and HPLC for CBZ and PHT; FPIA only for VPA. (2-5 aliquots- mean value)

**Findings:**
- Significant correlation between dose and drug hair concentration
- No statistically significant difference by ANOVA in CBZ and VPA in hair vs color.
- PHT significantly higher drug concentrations in darker hair vs. lighter hair
- However Brown hair (16.76 \(\mu g/g\)) vs. Black hair (16.35 \(\mu g/g\)) for PHT

**Conclusion(s):**
- CBZ no relationship between hair color and drug quantity
- VPA weak relationship between hair color and VPA concentration
- PHT significant moderate relationship: darker hair greater concentrations

**Comments/Limitations:**
- Results for PHT fit the hair color bias model
- Results for CBZ and VPA not supportive of the hair color bias model
Retrospective Statistical Study #4
Hair Pigmentation Review


**Description:**
- Hair test results for THC-COOH examined for any correlation with hair color
- ~80,000 specimens, 3,886 POS RIA, 3,678 POS GC/MS/MS for THC-COOH
- Hair color assigned by lab personnel with color charts
- Hair washed IPA, 3 phosphate buffer washes, enzyme digestion RIA→GC/MS/MS

**Findings:**
- Not significant by ANOVA

**Conclusion(s):**
- No significant relationship [THC-COOH] vs. hair color

**Comments/Limitations:**
- *Most studies higher conc. for weak bases, acidic metabolites not expected*
- *Small n for red and blond hair*

**Description:**
- Study describes 3 separate studies; 3 distinct subject pools
- **SP1:** 2000 Hair samples, 500 NEG, 500 COC +, 500 AMP*+ and 500 THC, Hair color determined for each sample (7 categories)
- **SP2:** 2000 Urine results, donor photos and surnames examined and ethnic/racial category assigned: Caucasian, African-American, Hispanic, Asian-Pacific, and Other (originally collected to determine hair color – unable)
- **SP3:** 2017 urine results and record at collection of hair color (7 categories): Hair color matched to urine test results using donor’s name and date of collection

**Findings:**
- Highest percentage of COC positives in darker Hair
- No discernable pattern in THC positives
- Amphetamines* higher in brown hair than black
- Highest percentage of COC urine positives in donors with recorded black hair color

**Conclusion(s):**
- Author’s conclude that statistical treatment failed to show hair color bias
- Author’s offer the possibility of ethnic preference for drug type as possible reason for differences in positive rates since also demonstrated in the urine matrix

**Comments/Limitations:**
- *Hair color determination subjective, not precise and didn’t account for hair treatment etc.*
- *Ethnicity determination not precise – done by one individual*
- *Cocaine appeared to follow the hair color bias model strongly yet the statistical treatment contradicted this*

**Description:**
- The purpose was to evaluate the hypothesis that hair assays for cocaine (COC) will show racial bias.
- Hair, urine and survey data from 315 African Americans (AA) and 846 Caucasians (C) collected by staff at the Pinellas County [FL] jail – 4- 6 month periods -2 yrs

**Findings:**
- **Positive Assays:**

<table>
<thead>
<tr>
<th>Positivity</th>
<th>Caucasian</th>
<th>Afr American</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Urine +</td>
<td>16.5%</td>
<td>35.9%</td>
<td>2.18</td>
</tr>
<tr>
<td>% Hair +</td>
<td>36.15%</td>
<td>62.5%</td>
<td>1.73</td>
</tr>
</tbody>
</table>

- Self Reports: Ratio AA/C 48h-2.05, 30day-1.71, 60day-1.62, Ever used 1.01

**Conclusion(s):**
- Authors conclude that data does not suggest a bias based on racial group

**Comments/Limitations:**
- 4 waves or periods treated as a single cohort but wave 3 showed more COC + in C
- Comparisons are not matched
Retrospective Statistical Study #7
Hair Pigmentation Review


**Description:**
- ~1500 arrestees hair and urine specimens, 1463 males, 149 females
- Cohort 27.6% Black, 70.9% white, 1.1% Hispanic, and 0.5% Other
- 1,117 hair and 1498 urine specimens collected (70% and 90% of subjects)
- Urine samples Immunoassay, Hair samples RIA and GC/MS
- Subjects self-reported race and cocaine use in last 48 h, 60 days or during lifetime

**Findings:**

<table>
<thead>
<tr>
<th>Table 9. Ratios of Cocaine (+) Urinalyses, Hair Assays, &amp; Self-Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Urine (+)</td>
</tr>
<tr>
<td>36.7</td>
</tr>
<tr>
<td>% (+) S/R 48 Hrs.</td>
</tr>
<tr>
<td>13.3</td>
</tr>
<tr>
<td>% Hair (+)</td>
</tr>
<tr>
<td>63.4</td>
</tr>
<tr>
<td>% (+) S/R 60 Days</td>
</tr>
<tr>
<td>17</td>
</tr>
</tbody>
</table>

**Conclusion(s):**
- Black subjects more likely to test positive than white subjects in hair and urine and have higher hair drug concentrations

**Comments/Limitations:**
- *Dose of drug unknown, illicit source.*
- Self-reported race
- Hair morphology not reported
Review Articles:
Hair Pigmentation Literature Review

Description:
- 1996 review article of addressing issue of pigmentation and effects on hair tests
- Article reviews drug binding in animals and humans

Findings
- Melanin primary pigment in hair, 3 types eumelanin and pheomelanin
- Hair morphology refers to cross-sectional shape of hair and gross appearance
- Potential binding sites include keratin (protein) and melanin (pigment)
- Animal study results similar to human studies

Conclusion(s):
- Hair color appears to affect accumulation and retention of drugs such as cocaine and PCP in hair

Comments/Limitations:
- Early review provides detailed description of chemistry and morphology of hair
- Evaluates possible mechanisms of drug binding (ionic binding of melanin to basic drugs)
- Data available described observed drug concentrations in different types of hair rather than detailed studies of binding mechanism.
Review Article #2 – Hair Pigmentation Review


Description:
- 1997 review of morphology of hair
- Reviews the biochemical process of synthesis and genetic factors that influence hair color

Findings
- Melanins combinations produce various shades of hair from yellow and red to black.
- Melanins produced by melanosomes (specialized dendritic cells)
- ~100,000 hair follicle in the adult male with significant loss with age
- Melanin granules are mainly in the cortex
- Whatever the color human hair contains various portions of eumelanin and pheomelanin
- Eumelanins-black to brown, insoluble in solvents
- Pheomelanins-reddish brown and alkalai-soluble

Conclusion(s):
- Coat color of mice varies from black, brown, yellow, gray and white.
- 60 loci and more than 150 mutations involved in mice coat color

Comments/Limitations:
- Review provides good understanding of the complex dependence of hair color to biochemical factors involved in melanin synthesis and genetic factors linked to determining hair color.

Description:
- Explores using hair testing as an adjunct to U.S. Navy’s drug testing program
- Review several topics concerning hair testing including mechanism’s of drug incorporation, passive exposure, mechanisms of drug retention, metabolite screening and hair color

Findings:
- Several studies cited showing significant differences between hair of different colors
- Several studies [Cocaine] more likely to be detected in black vs. brown hair
- In Vitro data presented for PCP soaking where wash procedures removed PCP from brown hair more readily than black hair.

Conclusion(s):
- Author concludes that the use of hair “will be severely limited” due to inter-individual differences such as hair thickness or hair color

Comments/Limitations:
- Review 22 years old and does not include the majority of literature covered in presentation
- Many issues raised are still being raised today in regards to hair testing for drugs of abuse

**Description:**
- Chapter review on potential mechanisms of incorporation and removal of drugs in/from hair
- Substantial portion focused on contamination

**Findings**
- “Ambiguous” *in vitro* studies reported: black hair (Asian or Afr Am.) might incorporate more COC than brown or blond (Caucasian), however brown or blond (Caucasian) hair contained more drug than black hair (Hispanic or Italian)

**Conclusion(s):**
- Authors concluded from *in vitro* experiments, regardless of hair type and digestion that “little cocaine was associated with melanin” (association based on true chemical association)

**Comments/Limitations:**
- 19 year old review that summarized the literature and state of the science
- Discussion of hair color bias by the authors is minimal in this review and is primarily focused on whether actual chemical binding of drug(s) to melanin

**Description:**
- The paper describes several early *in vitro* studies and contains an extensive literature review.

**Findings**
- Cocaine and Morphine incorporates at higher rate in hair of African American females Vs. Caucasian males or females.

**Conclusion(s):**
- Four reasons proposed to account for differences in drug uptake in hair: 1) Permeability 2) Cosmetic hair treatments 3) Personal hygiene and 4) Route of drug administration or exposure.

**Comments/Limitations:**
- *The study supports hair color bias based on numerous in vitro studies*
End of Presentation