Hair Pigmentation Literature Review

Drug Testing Advisory Board (DTAB) Meeting

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DISCLAIMER

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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

- Articles published 1992-2014 (*exception 1-1985, 1- 1974*)

- Literature Review Format
  - Reference
  - Keywords
  - Study Design
  - Study Findings
  - Conclusion
  - Reviewer's Comment

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

- Green, S. J. and Wilson, J.F. (1996) The effect of hair color on the incorporation of methadone into hair in the rat. *Journal of analytical Toxicology* **20**, 121-123

- 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?*

Animal Study #2 – Hair Pigmentation Review

- Borges, C.R., Wilkins, D.G. and Rollins, D.E. (2001) Amphetamine and N-acetylamphetamine incorporation into hair: An investigation of the potential role of drug basicity in hair color bias. *Journal of Analytical Toxicology* **25,** 221-227

- 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:

- 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

- Gerstenberg, B., Schepers, G., Voncken, P., Volkel, H. (1995) Nicotine and cotinine accumulation in pigmented and unpigmented rat hair. *Drug Metabolism and Disposition*, **23**, 143-148.**25,** 221-227

- 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.*

Animal Study #4 – Hair Pigmentation Review

- Stout, P.R. and Ruth, J.A. (1999) Deposition of [3H]cocaine, [3H]nicotine, and [3H]flunitrazepam in mouse hair melanosomes after systemic administration. *Drug Metab Dispos.*, **27**, 731-735.

- 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

- Hubbard DL, Wilkins DG, and Rollins DE, (2000)The Incorporation of Cocaine and Metabolites into Hair: Effects of Dose and Hair Pigmentation, Drug Metabolism and Deposition, Vol. 28, No. 12

- 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

- Gygi SP, Joseph RE, Cone EJ, Wilkins DG, Rollins DE (1996) Drug Metabolism and Disposition, Vol.24 (4) 495-501

- 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

- 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 1—*Codeine content of regrown hair fibers collected from different colored regions of tortoise shell guinea pigs.*

| Tortoise Shell Guinea Pig Number	Total Dose of Codeine-Base Ingested mg/kg	Codeine Content of Different Colored Hair Fibers [ng/mg Hair]		
		white	reddish-brown	black
1	254	2,7	2,6	5,0
2	305	3,3	4,6	13,3
3	328	2,9	4,4	5,1
4	468	9,1	16,4	28,1
5	678	18,6	22,6	50,2
6	1164	6,1	38,4	76,2
7	1186	15,9	32,4	72,4

- 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*

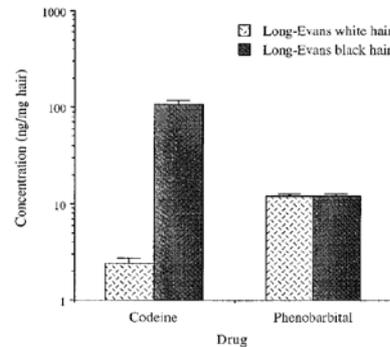# Animal Study #8 – Hair Pigmentation Review

- Gygi, S. p., Wilkins, D.G., and Rollins D. E. (1996) A comparison of phenobarbital and codeine incorporation into pigmented and nonpigmented rat hair. *Journal of Pharmaceutical* Sciences 86 (2): 209-214

- 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
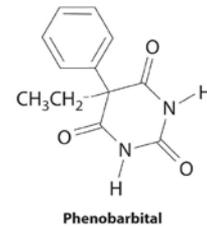
- Findings:

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*

- Ito, S. and Fujita, K. (1985) Microanalysis of eumelanin and pheomelanin in hair and melanomas by chemical digestion and liquid chromatography. *Analytical Biochemistry* **144,** 527-536

- Description:

    - Quantitative Method : Eumelanin and Pheomelanin in hair presented
    - Permanganate oxidation used for Eumelanin
    - Hydriodic anlaysis for Theomelanin
    - Both methods produce degradation products,

        Eumelanin ⟶ PTCA, Pheomelanin ⟶ AHP

- Findings:
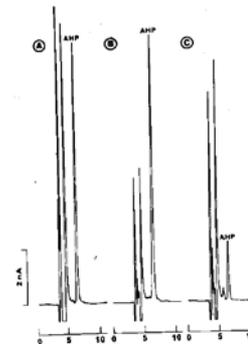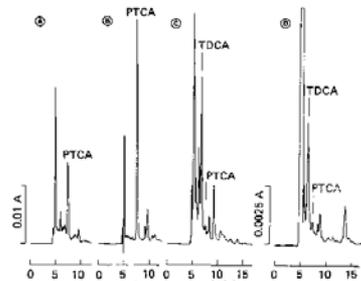
- Conclusion(s):

    - 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

- Ozeki H, Ito I, Wakamatsu K, and Thody AJ (1996) Spectrophotometric Characterization of Eumelanin and Pheomelanin in Hair. *Pigment Cell Research*, **9,** 265-276

- 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):

    - Spectrophotmetric 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 – [^{3}H](-)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):

    - Stereospecifc 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*

- Potsch, L. Emmerich, P. and Skopp, G. (2002) Preliminary approach to elucidate the role of pigment as a binding site for drugs and chemicals in anagen hairs: pigments as carriers for ^{3}H-haloperiodol in HaCaT/Sk-Mel-1 co-cultures. *International Journal of Legal Medicine* **116,**12-16

- Description:

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

- Findings/Conclusion(s):

    - After Co-culture >50% increase in [3H] 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*

- Ropero-Miller, J.D., Huestis, M.A., Stout, P.R. (2012) Cocaine analytes in human hair: evaluation of concentration ratios in different cocaine sources, drug-user populations and surface-contaminated specimens. *Journal of Analytical Toxicology*, **36**, 390-398.

- 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.*

- Borges, C.R., Roberts, J.C., Wilkins, D.G., Rollins, D.E. (2003) Cocaine, benzoylecgonine, amphetamine, and N-acetylamphetamine binding to melanin subtypes. *Journal of Analytical Toxicology*, **27**, 125-134.

- 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.*

- Joseph RE, Su T-P, Cone EJ (1996) In Vitro Binding Studies of Drugs to Hair: Influence of Melanin and Lipids on Cocaine Binding to Caucasoid and Africoid Hair. *Journal of Analytical Toxicology 20, 338-344*

- Description:

    - Hair collected from male and female subjects divided into five groups: male Africoid, female Africoid, male Caucasoid [blk/br], female Caucsoid [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}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*

- Reid R.W., O'Connor, L. and Crayton, J.W. (1994) The in vitro binding of benzoylecgonine to pigmented hair samples. *Clin Tox.* **32 (4),** 405-410.

- Description:

    - *In vitro* differential binding of Benzoylecgonine (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 1

Incorporation of Benzoylecgonine into Human Hair
(Reconstructed Ion Current for M/z 422)

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 (X)	1,560,319	555,692	306,234
SD	532,332	96,649	11,849

- Conclusion(s): ANOVA: $F = 13.543$; $P = 0.0006$

    - 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*

- Potsch, L., Skopp, G. and Rippin, G. (1997) A comparison of ^{3}H-cocaine binding on melanin granules and human hair *in vitro*. *J Legal Medicine.* **110**, 55-62

- Description:

  - ^{3}H-Cocaine binding to melanin investigated
  - Melanin from *Sepia officinalis* (the cuttle fish)
  - 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*

- Joseph, R.E., Tsai, W-J, Tsao, L-J, Su, T-P and Cone, E.J. (1997) In vitro characterization of cocaine binding sites in human hair. *The Journal of Pharmacology and Experimental Therapeutics* **282**, 1228-1241

- 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*
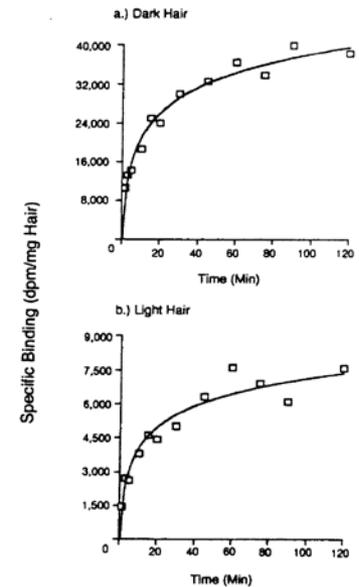
- Borges, C. R., Martin, S.D., Meyer, L.J., Wilkins, D. G. and Rollins, D. E. (2002) Influx and efflux of amphetamine and N-acetylamphetamine in keratinocytes, pigmented melanocytes, and nonpigmented melanocytes. *Journal of Pharmaceutical Sciences* **91**, 1523-1535

- 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*
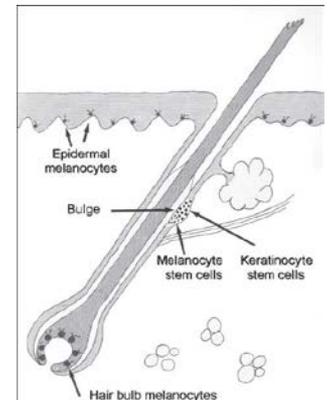
- Claffey, D. J. and Ruth, J. A. (2001) Amphetamine adducts of melanin intermediates demonstrated by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Chemical Research in Toxicology* **14,** 1339-1344

- 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

- Reid, R.W., O'Conner, F. L., Deakin A. G., Ivery, D.M. and Crayton, J.W. (1996) Cocaine and metabolites in human graying hair: Pigmentary relationship *Clinical Toxicology* **34**, 685-690

- 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 H_2SO_4

    - SPE, GC/MS (PCI)

- Findings:

- 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*

Alere™

- Agius, R. (2014) Utility of coloured hair for the detection of drugs and alcohol. *Drug Test.Anal.*, **6 Suppl 1**, 110-119.

- 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 postives 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.
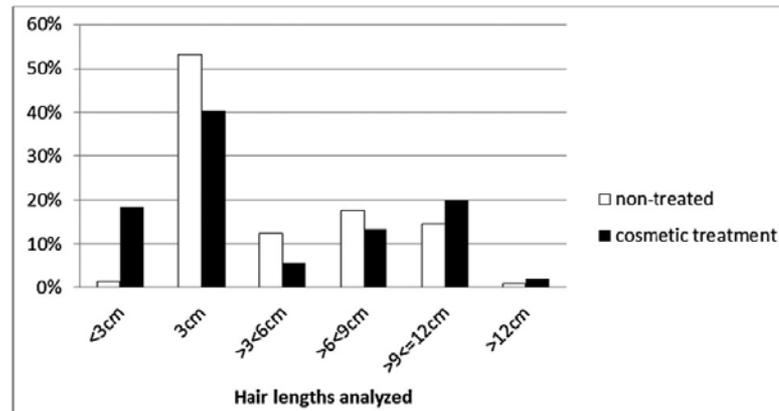
- Conclusion(s):

    - 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*

- T. Uematsu, N. Miyazawa, O. Okazaki, and M. Nakashima, Possible Effect of Pigment on the Pharmacokinetics of Ofloxacin and its Excretion in Hair, *J. of Pharmaceutical Sciences,* 45, vol. 81, No. 1, January 1992, pp 45-48

- 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*

- Uematsu T., Sato R., Fujimori O. and Nakashima. (1990) Human scalp hair as evidence of individual dosage history of haloperidol: a possible linkage of haloperidol excretion into hair with hair pigment. *Archives of Dermatologic Research*. **282,** 120-125.

- 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*

- Rothe, M., Pragst, F., Thor, S. and Hunger, J. (1997) Effect of pigmentation on the drug deposition in hair of grey-haired subjects. *For Sci Inter*. **84,** 53-60.

- Description:

  - Hair collected from 15 patients (Rx therapeutic drugs or Cocaine or Heroin Users)
  - Therapeutic Drugs: amitriptyline, doxepin, maprotiline, metoclopromide, carbamazeoine, 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 variablility: 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*

- Schaffer, M., Hill, V. and Cairns, T. (2005) Hair analysis for Cocaine: The requirement for effective wash procedures and effects of drug concentration and hair porosity in contamination and decontamination. *Journal of Analytical Toxicology* **29,** 319-326

- 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 (permed) 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

- Kronstrand, R., Forstberg-Peterson, S., Kagedal, B. Ahlner, J. and Larson, G. (1996) Codeine concentration in hair after oral administration is dependent on melanin content. *Clinical Chemistry* **45,**1485-1494

- 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 -24hours
  - 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

- Rollins, D. E., Wilkins, D. G., Krueger, G.G., Augsburger, M.P, Mizuno, A., O'Neal, C., Borges, C.R. and Slawson, M. H.(2003) The effect of hair color on the incorporation of codeine in human hair. *Journal of Analytical Toxicology* **27,**545-551

- 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*

- Henderson, G.L., Harkey, M.R., Zhou, C., Jones, R.T., Jacob III, P. (1996) Incorporation of isotopically labeled cocaine and metabolites into human hair: 1. Dose-response relationships. *Journal of Analytical Toxicology*, **20**, 1-12.

- 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

- Henderson, G.L., Harkey, M.R., Zhou, C., Jones, R.T., Jacob, P., III (1998) Incorporation of isotopically labeled cocaine into human hair: race as a factor. *Journal of Analytical Toxicology*, **22**, 156-165.

- 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.*

- Polettini,A., Cone,E.J., Gorelick, D.A. and Huestes, M.A. (2012) Incorporation of methamphetamine and amphetamine in human hair following controlled oral methamphetamine administration. *Analyttca Chimica Acta* **726**, 35-43

- 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
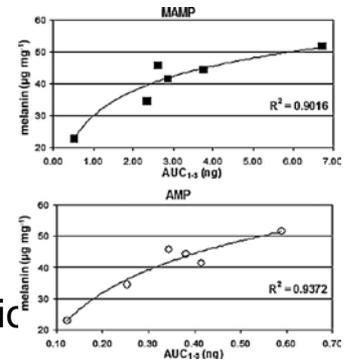
- Findings:

    - Correlation MAMP and AMP Cmax / AUC and Melanin concentratic

- 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

- Hoffman, B.H. (1999) Analysis of Race effects on drug-test results. *Journal of Occupational and Environmental Medicine* **41,** 612-614

- 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*

- Miecczkowski, T. and Newel R. (2000) Statitstical examination of hair color as a potential biasing factor in hair analysis. *Forensic Science International* **107,** 13-38

- Description:

    - 8 different sets of data examined

    - 4 Small n studies : Goldberger *et al.* (1991) n= 20, Kintz *et al.* (1998) *n*=20, Cone *et* al. (1991) *n*= 10 and Henderson *et al.* (1998) *n*= 15

    - 4 Large n studies: Univ. of Glascow (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."*

Retrospective Statistical Study #3 Hair Pigmentation Review

- Miecczkowski, T. Tsatsakis, A..M., Kruger,M. and Psillakis, T. (2001) The concentration of three anti-seizure medications in hair: the effects of hair color, controlling for dose and age. *BMC Clinical Pharmacology* **1:2**

- 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 μg/g) vs. Black hair (16.35 μg/g) fro 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 cocnetrations

- *Comments/Limitations:*
    - *Results for PHT fit the hair color bias model*
    - *Results for CBZ and VPA not supportive of the hair color bias model*

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- Mieczkowski, T. (2003) Assessing the potential of a "color effect" for hair analysis of 11-nor-9-carboxy-delta(9)-tetrahydrocannabinol: analysis of a large sample of hair specimens. *Life Sciences*, **74**, 463-469.

- 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*

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- Kelly RC, Mieczkowski T, Sweeney SA, and Bourland JA (2000) Hair analysis for drugs of abuse: Hair color and race differentials or systematic differences in drug preferences? *Forensic Science International* **107**, 63-86

- 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*

- Mieczkowski, T and Newel, R. (1993) An evaluation of patterns of racial bias in hair assays for cocaine: black and white arrestees compared. *Forensic Science International* **63,** 85-98

- 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:

Positivity	Caucasian	Afr American	Ratio
% Urine +	16.5%	35.9%	2.18
% Hair +	36.15%	62.5%	1.73

    - 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*

- Mieczkowski T. and Madea B. (1995) An evaluation of patterns of race and sex bias in cocaine assays of human hair. *Proceedings of the 1995 International Conference and Workshop on Hair Analysis Forensic Toxicology.* 90-106

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

	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

- 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

Review Article #1 – Hair Pigmentation Review

- Cone,E.J. and Joseph,R.E., Jr. (1996): The potential for bias in hair testing for drugs of abuse. In: *Drug Testing in Hair*, edited by P.Kintz, pp. 69-93. CRC Press, Boca Raton, FL.

- 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 sties 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.*

- Castanet,J. and Ortonne,J.-P. (1997): Hair melanin and hair color. In: *Formation and Structure of Human Hair*, edited by P.Jolles, et al, pp. 209-225. Birkhauser Verlag Basel,Switzerland.

- 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.*

Review Article #3 – Hair Pigmentation Review

- Kidwell DA, Blank DL. Hair analysis: techniques and potential problems. In: Sunshine I, ed. *Recent Developments in Therapeutic Drug Monitoring and Clinical Toxicology*. New York, New York: Marcel Dekker, Inc.; 1992;555-563. Ref ID: 256

- 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*

- Kidwell, D.K. and Blank, D.L. (1995) Mechanisms of Incorporation of Drugs into hair and the interpretation of hair analysis data. In: *Hair Testing of Drugs of Abuse: International Research in Standard and Technology*. NIH Pub. No. 95-3727, National Institute on Drug Abuse, Rockville, MD. 19-90.

- 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*

Review Article #5 – Hair Pigmentation Review

- Kidwell, D.A., Lee, E. H. and DeLauder, S.F. (2000) Evidence for bias in hair testing and procedures to correct bias. *Forensic Science International* **107,** 39-61

- 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.
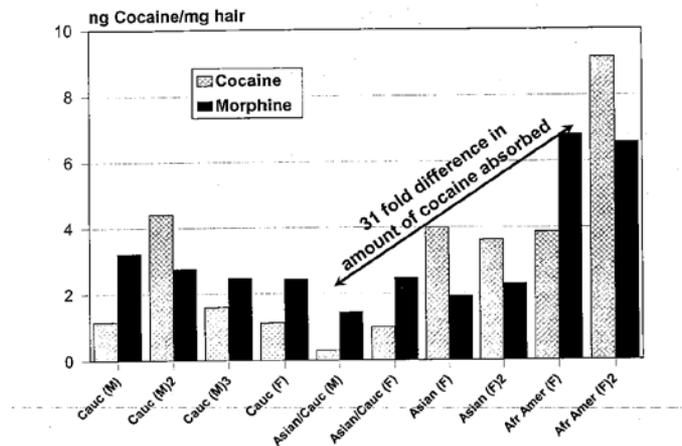
- Conclusion(s):

    - Four reasons proposed to account for differences in drug uptake in hair: 1) Permeability 20 Cosmetic hair treatments 3) Personal hygiene and 4) Route of drug adminsitration or exposure

- *Comments/Limitations:*

    - *The study supports hair color bias based on numerous in vitro studies*

End of Presentation