

# Unique Components of Hair

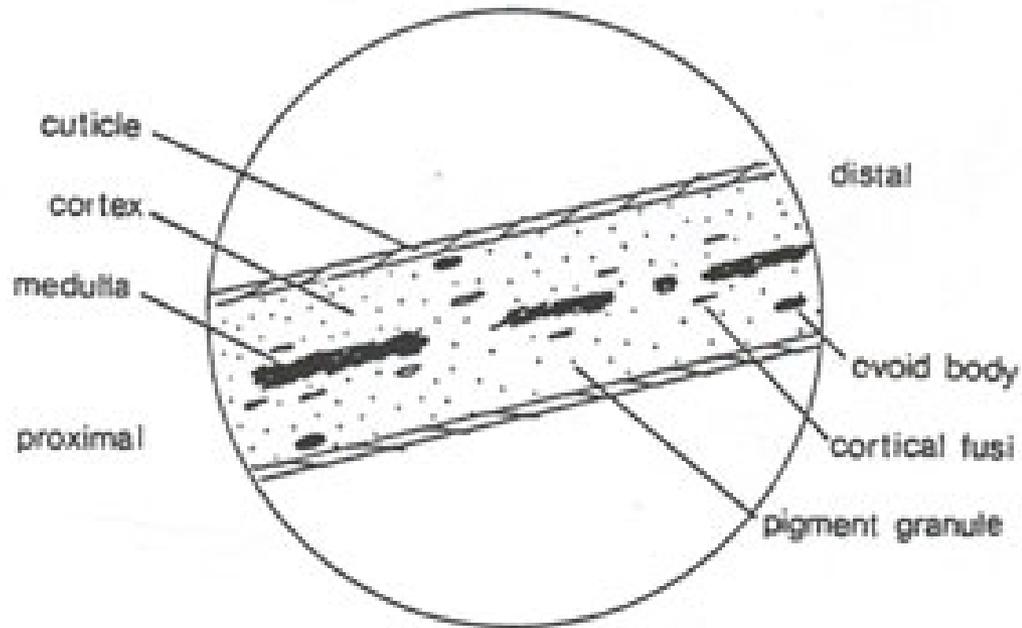
Peter R. Stout  
RTI International, Research  
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# Hair

- Morphology highly variable
- Fiber identification is included with “. . .the most vulnerable forensic sciences– hair microscopy, bite marks and handwriting. . . ” (NAS, p 107)
  - *“As an example, in a FBI publication on the correlation of microscopic and mitochondrial DNA hair comparisons, the authors found that even competent hair examiners can make significant errors”*
- M. Houck and B. Budowle. 2002. Correlation of microscopic and mitochondrial DNA hair comparisons. *Journal of Forensic Sciences* 47(5):964-967

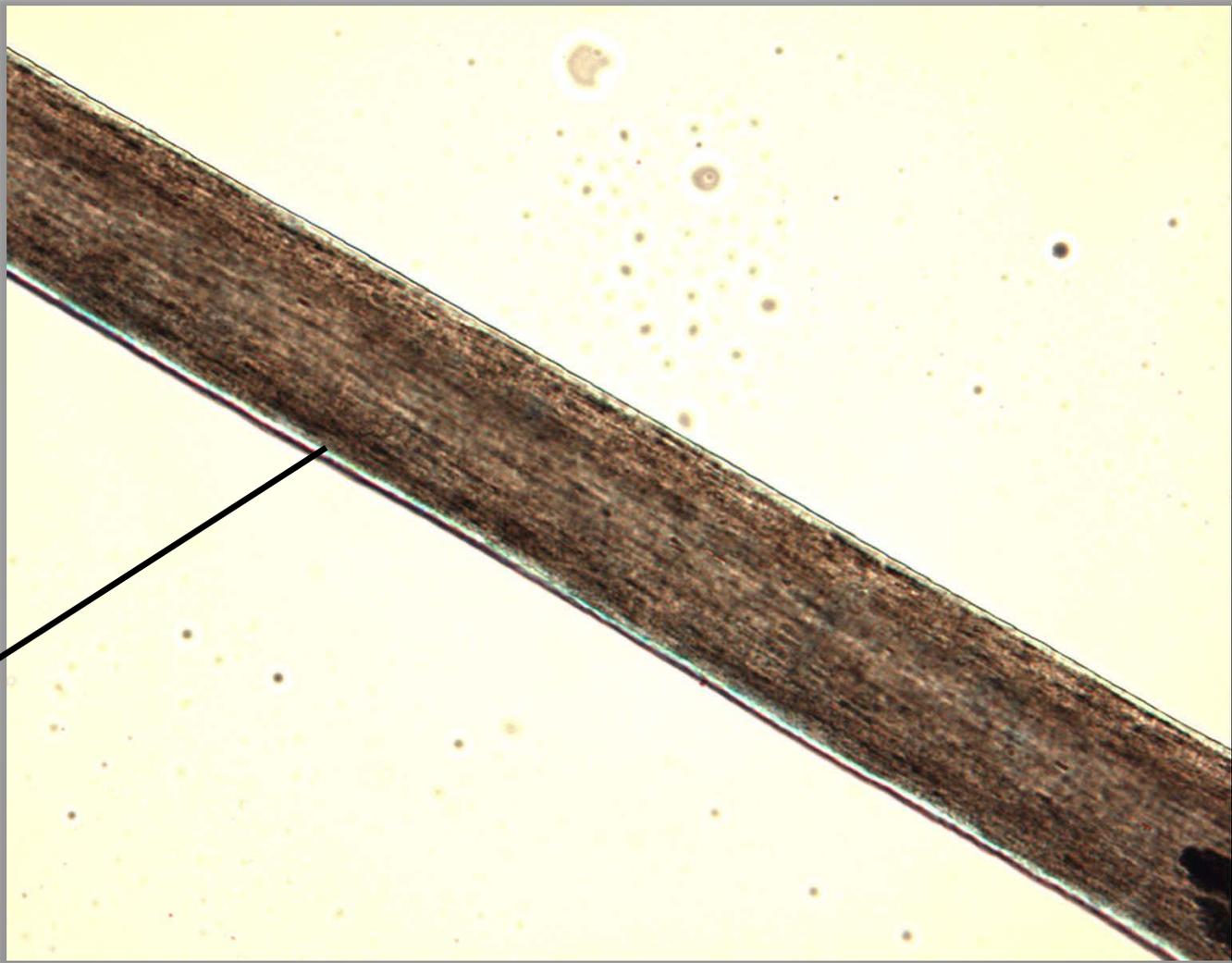
# Structure of Hair

[http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/research/2004\\_03\\_research02.htm](http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/research/2004_03_research02.htm)

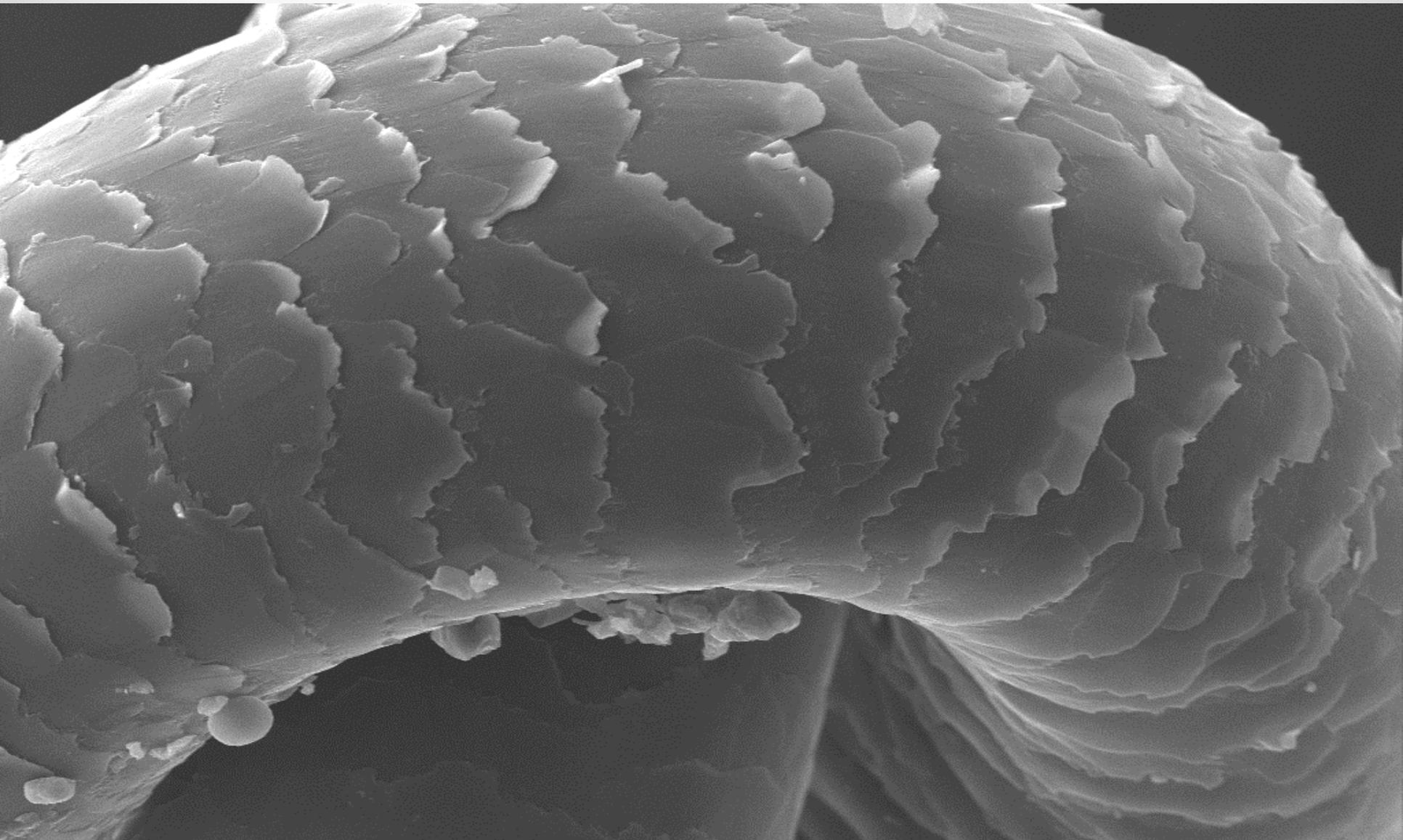


# Hair\*

Cuticle



# Morphology of Human Hair

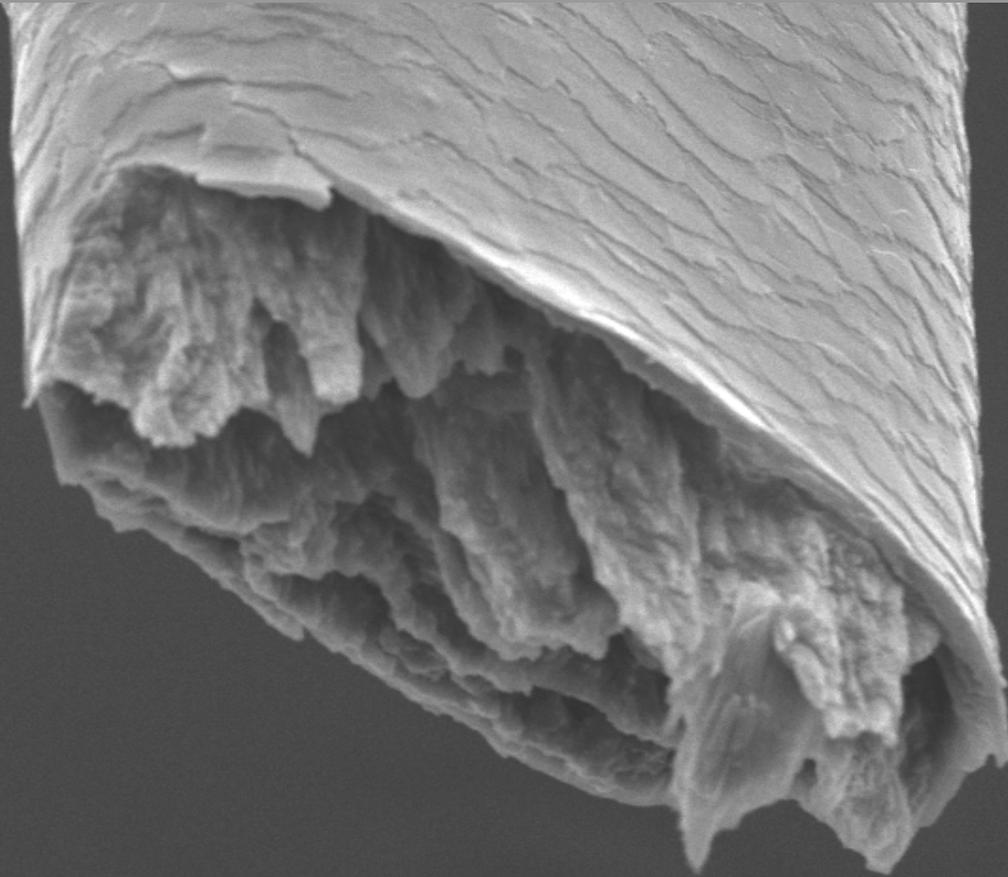


HV	Spot	WD	Det
15.0 kV	3.0	9.7 mm	ETD

50.0µm

11367-150-28 Pre Strand A

# Morphology of Human Hair\*

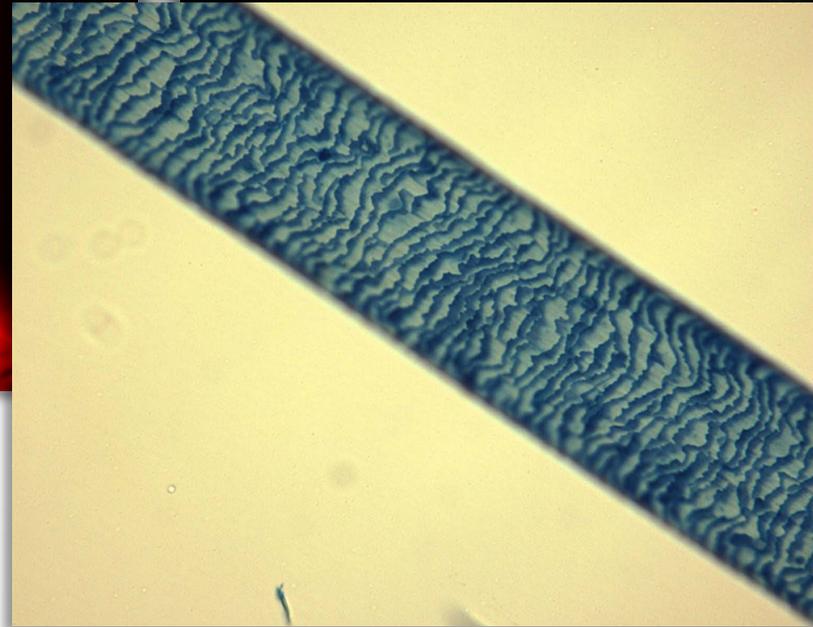
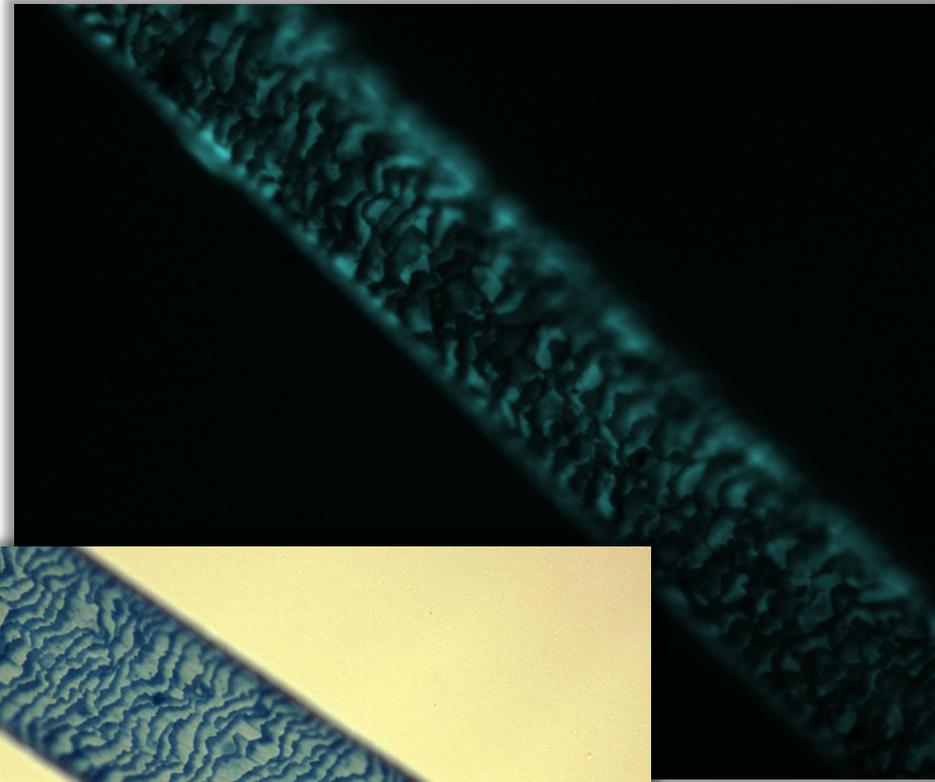
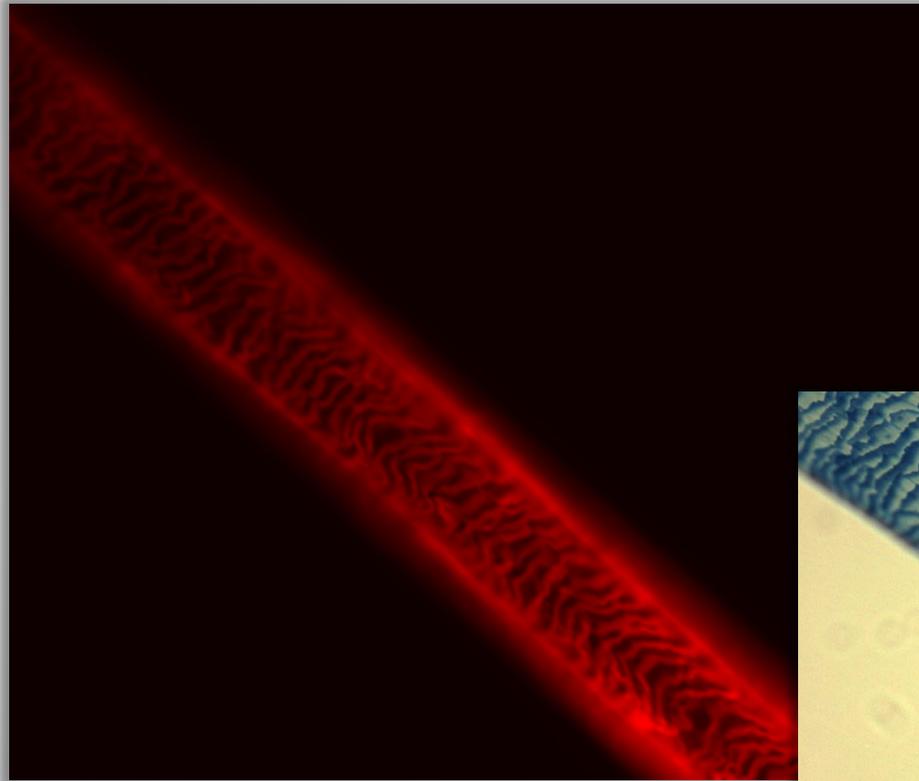


HV	Spot	WD	Det
15.0 kV	3.0	19.8 mm	ETD

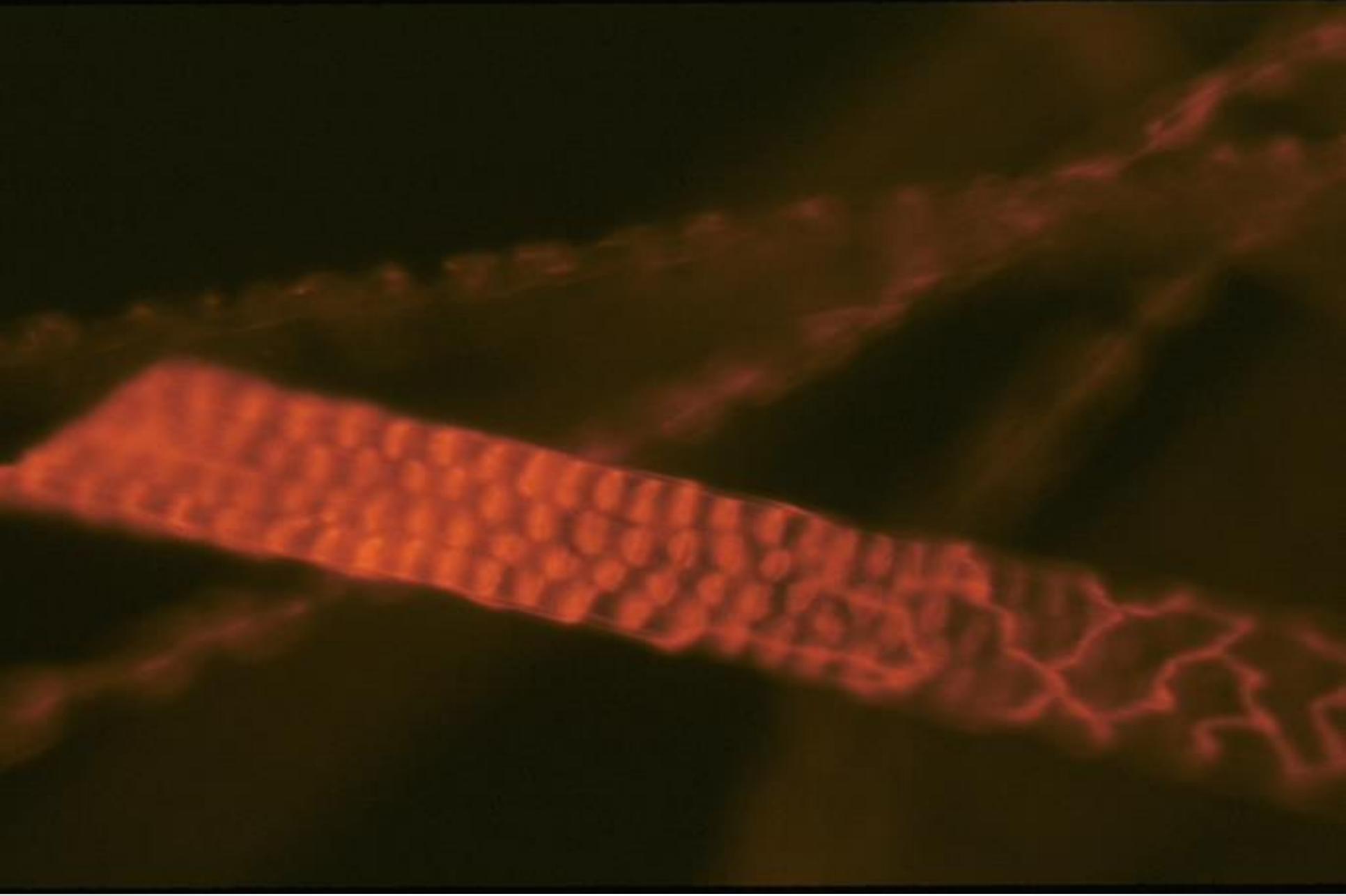
100.0µm

FF 11367-150-01 Pre 2a

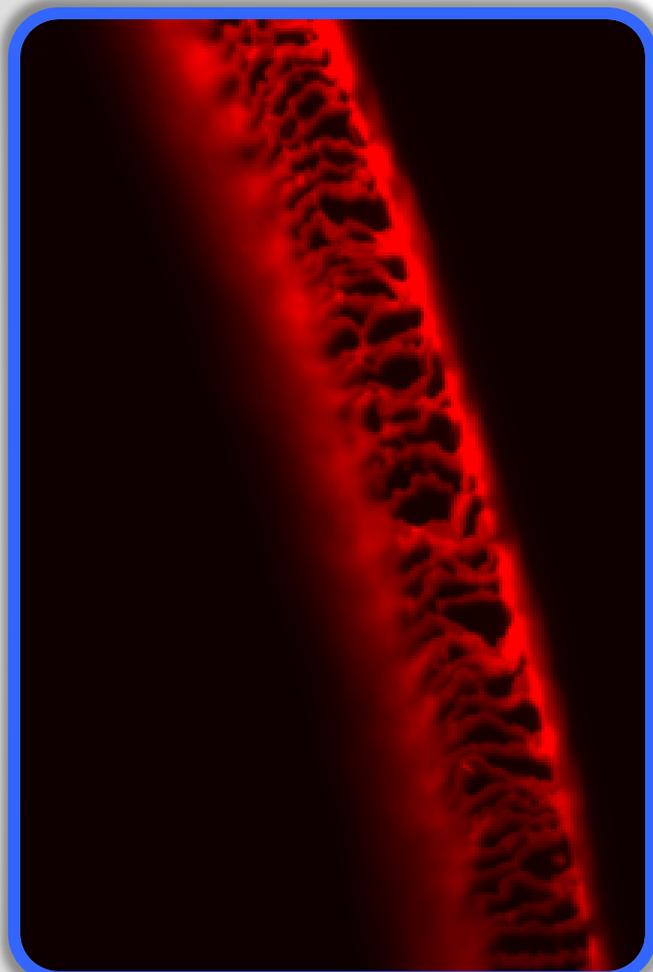
# External dye (human)



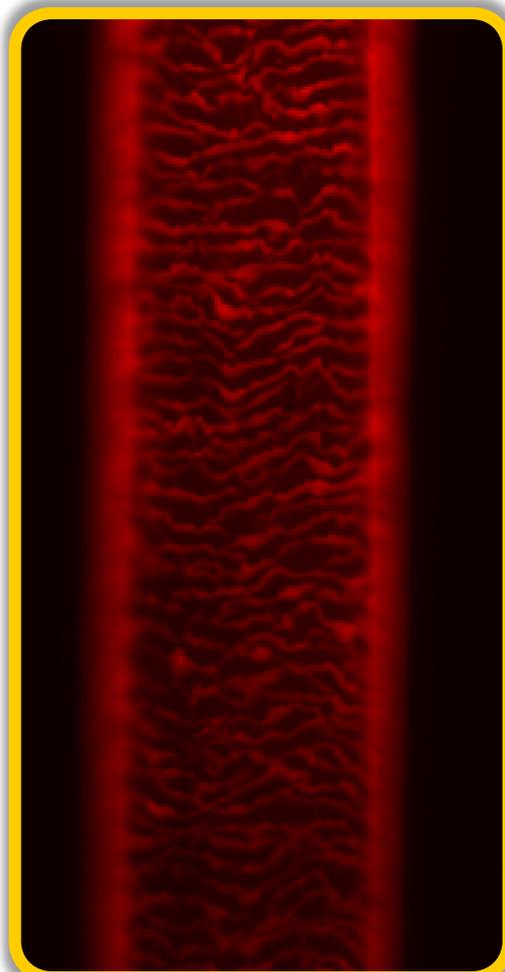
# External rhodamine (mouse)



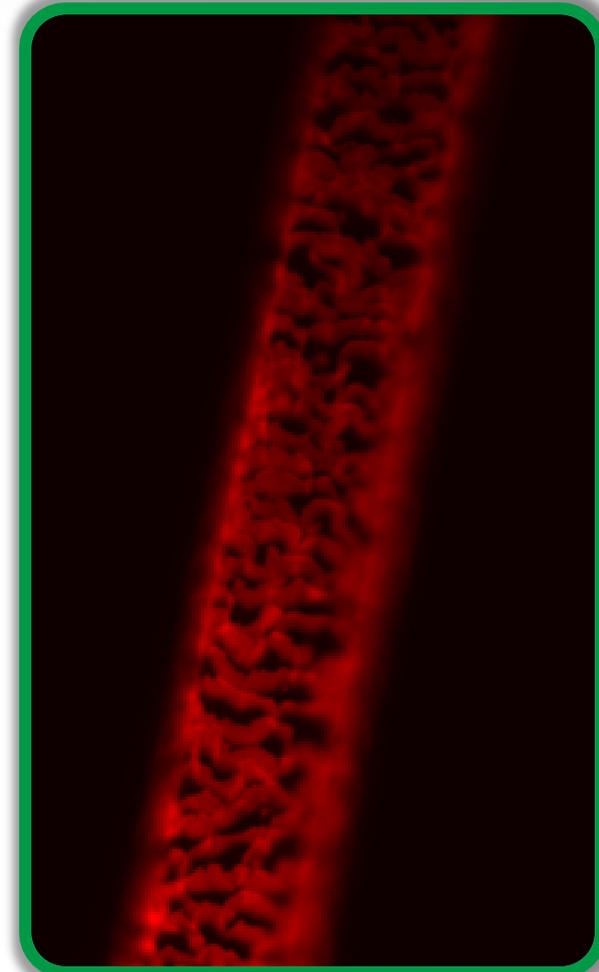
# Staining Penetration Images



Sample #37, African American, pre-treatment, stained with rhodamine B for 5 min (200x)



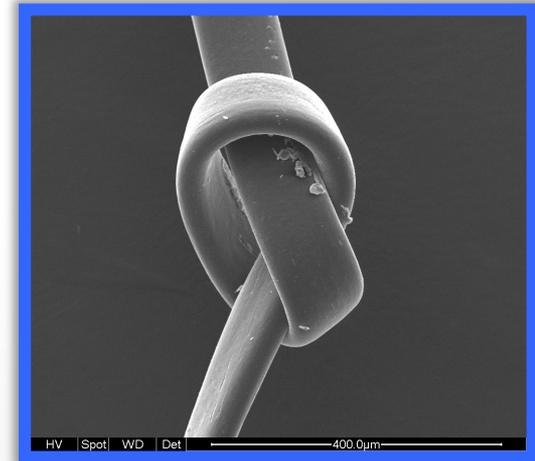
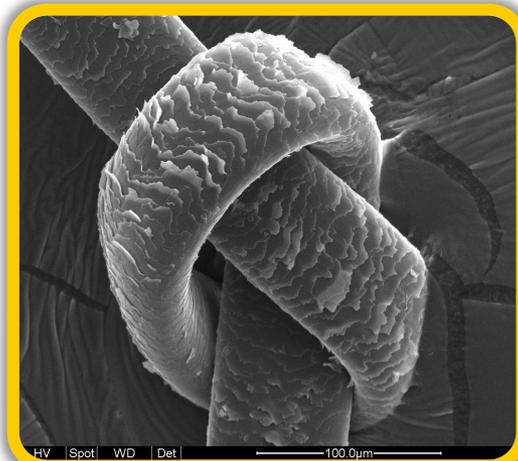
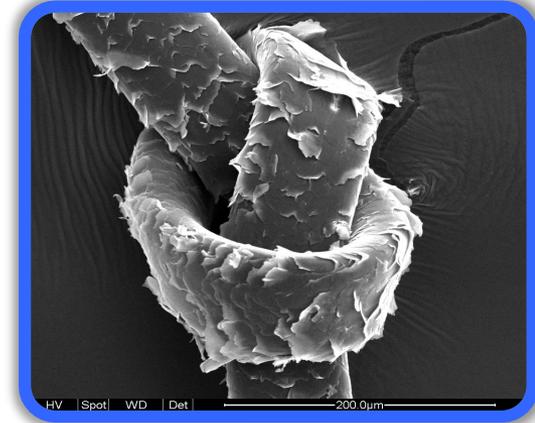
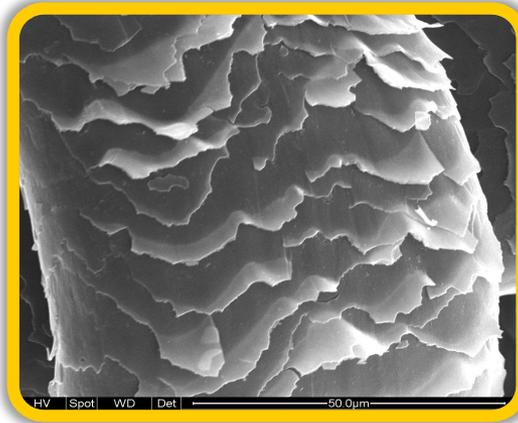
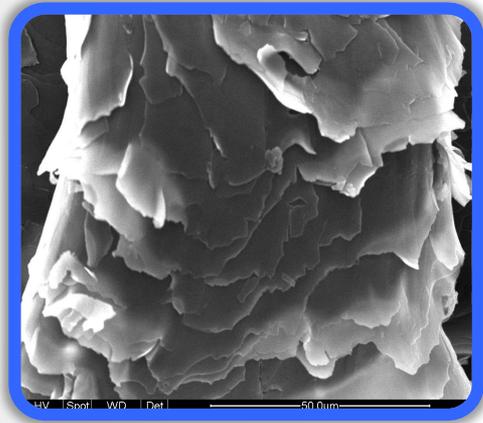
Sample #20, Caucasian Blond, pre-treatment, stained with rhodamine B for 5 min (200x)



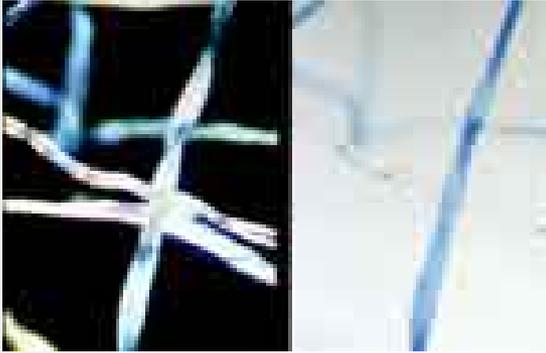
Sample #31, Caucasian Black, pre-treatment, stained with rhodamine B for 5 min (200x)

# Scanning Electron Microscopy

- Looked at ultrastructural cuticle appearance
- Wide variability in cuticle appearance both within and between individual



# Fibers



Cotton Fibers



Wool Fibers

Nylon Fiber Cross-Section

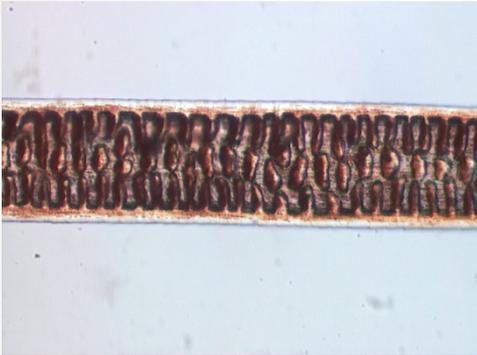


Acrylic Fiber

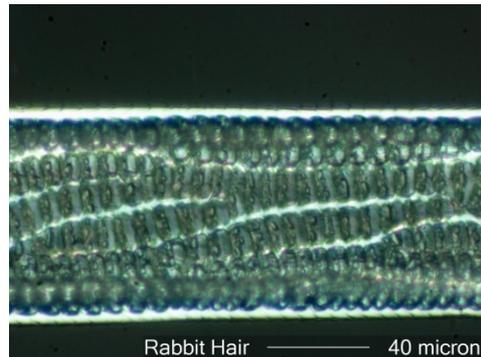


Spandex Fiber

# Mammalian Hair



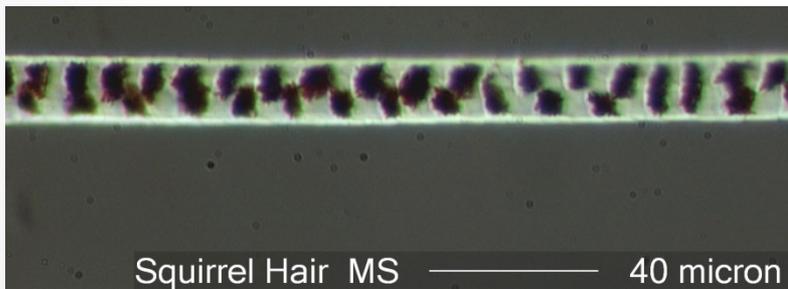
Mouse



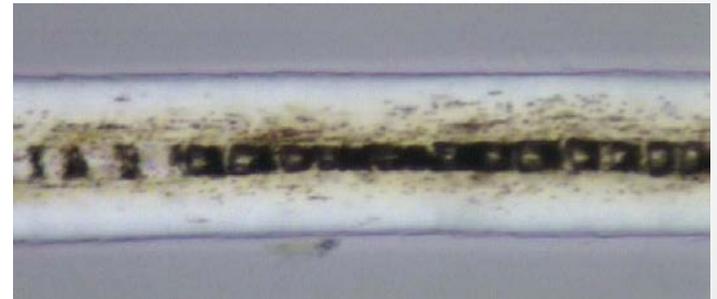
Rabbit



Cat



Squirrel



Dog

[http://www.fbi.gov/hq/lab/fsc/backissu/july2004/research/2004\\_03\\_research02.htm](http://www.fbi.gov/hq/lab/fsc/backissu/july2004/research/2004_03_research02.htm)

<http://www.microlabgallery.com/hair.aspx>

# Unique Drug Metabolites in Hair

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# Metabolites as Markers for Drug Use

- Drugs and their metabolites can be measured in hair
- Parent drug is mostly present, while metabolites are present in very small concentrations, if at all

# Ethanol, THC, and Heroin

- Fatty acid ethyl esters biomarkers for excessive alcohol consumption (Auwarter et al, 2001)
  - Ethyl glucuronide considered specific biomarker
- External contamination from herbal hair tonics
  - Ferreira et al., 2012– EtG a specific marker
  - Arndt et al., 2013. – EtG a contaminant in hair treatments
- THCA undisputed as only forming in vivo and not confused with external contamination of THC
- 6-AM was thought to be biomarker for heroin use but a hair study showed that presence of 6-AM in hair could form from in situ formation from heroin contamination (Romano et al., 2003)

# Opioid

- Moore et al. (2006) self reported opioid use. Detected hydrocodone, hydromorphone, morphine, codeine, 6-AM. No apparent correlation between reported dose and concentration
- Gryczynski et al (2014) Low sensitivity (2.9%) of hair testing to self reported opioid use.

Moore C, Feldman M, Harrison E, Rana S, Coulter C, Kuntz D, Agrawal A, Vincent M, Soares J. Disposition of hydrocodone in hair. [J Anal Toxicol](#). 2006 Jul-Aug;30(6):353-9

Gryczynski J, Schwartz RP, Mitchell SG, O'Grady KE, Ondersma SJ. Hair drug testing results and self-reported drug use among primary care patients with moderate-risk illicit drug use. [Drug Alcohol Depend](#). 2014 Aug 1;141:44-50. doi: 10.1016/j.drugalcdep.2014.05.001. Epub 2014 May 17.

# Meth and PCP

- Some work on methamphetamine, PCP – not extensive

# Adducts of Melanin Intermediates

## Potential biomarkers

- Formed during melanin synthesis and is less likely formed by drug exposure
- Adduct biomarker harder to remove by chemical treatments and hair washing
- Amphetamine, nicotine and cotinine adduct with melanin intermediate detected using MALDI-TOF
  - different adduct formation pathways for amphetamine and nicotine
- Need for in vivo studies

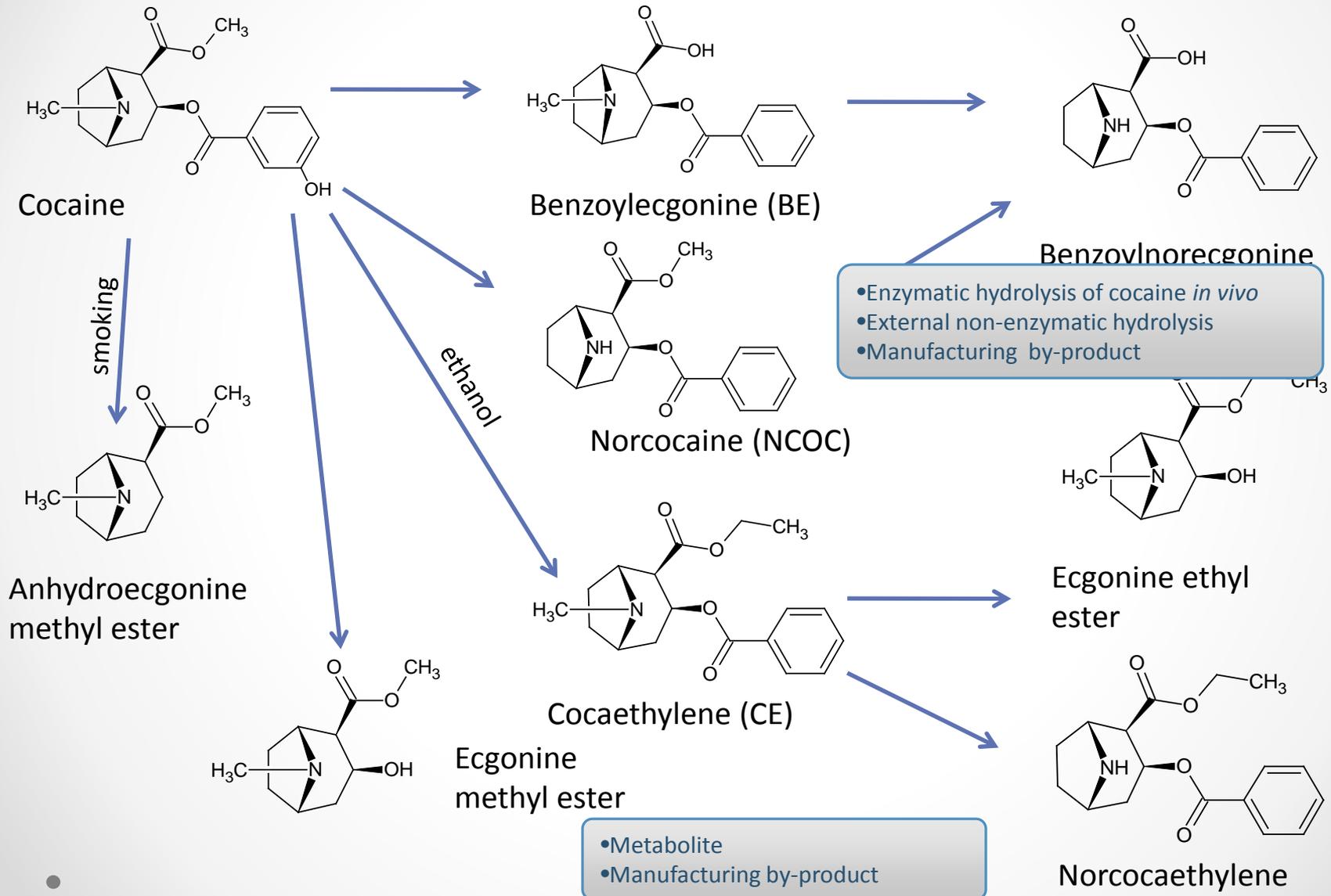
# Thiol Adducts

## Potential biomarkers

- Sulfur containing adducts only form in vivo therefore thiol adducts could be used to identify consumption vs. external contamination
- Studies have found thiol adduct formation with amphetamines, cocaine, opiates, and PCP
  - Schneider and DeCaprio (2013) demonstrating thiol adducts

# Cocaine Metabolism

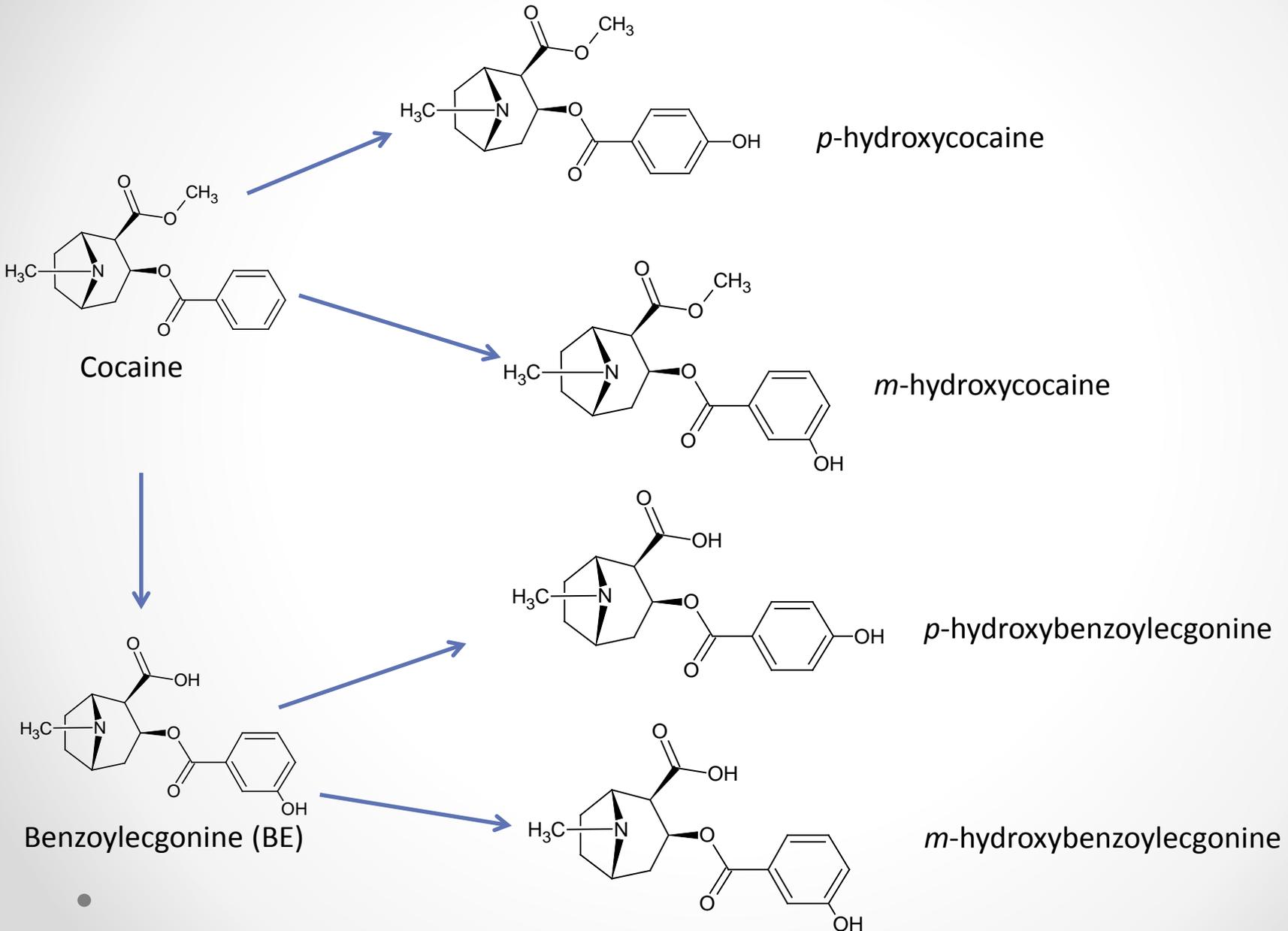
- Cocaine and ethanol ingested
- Manufacturing by-product



# Cocaine Metabolism\*

- Norcocaine is a minor constituent in Coca leaves
- Norcocaine is formed using potassium permanganate in illicit cocaine production
- Cocaethylene can form using ethanol in the production of cocaine base

# Cocaine Metabolism\*\*



# Recent FBI Study

- In 2014, FBI published study evaluating extensively washed hair
- Examined hair specimens from 27 drug chemists and 20 drug users for COC and 6 COC metabolites
- Results used to develop laboratory's criteria for hair analysis of COC
- *Morris-Kukoski, C.L., Montgomery, M.A., and Hammer, R.L. (2014) Analysis of Extensively Washed Hair from Cocaine Users and Drug Chemists to Establish New Reporting Criteria. Journal of Analytical Toxicology X, 1-9.*

# Recent FBI Study\*

- Results of COC user hair (antemortem)
  - All washes contained COC and BE but **no other metabolites detected**
  - Segments #1-#3 hair digests
    - COC from 1,300- 4,270 pg/mg
  - Segments #4-#6 hair digests
    - Negative for COC when applying the  $\geq 500$  pg/mL cutoff
  - After applying COC extended wash kinetics calculation segments #1-#3 remained positive
    - Segments #1 - #3 contained BE  $>1,000$  pg/mg, CE  $>100$  pg/mg and NC $>100$  pg/mg
    - ***p*-and *m*-OH-COC were positive in segments #1-#3**
- Results of 19 drug users (postmortem)
  - 18 washes contained COC, 6 washes contained CE, 3 washes contained NC and 10 washes contained BE
  - **No hydroxycocaine metabolites detected** in wash
  - 10 hair digests contained COC<sub>final</sub>  $> 2700$  pg/mg, NC  $>100$  pg/mg, and CE $>68$  pg/mL
  - **13 positive for *p*-OH-COC,**
    - **14 positive for *m*-OH-COC and 6 positive for *o*-OH-COC**

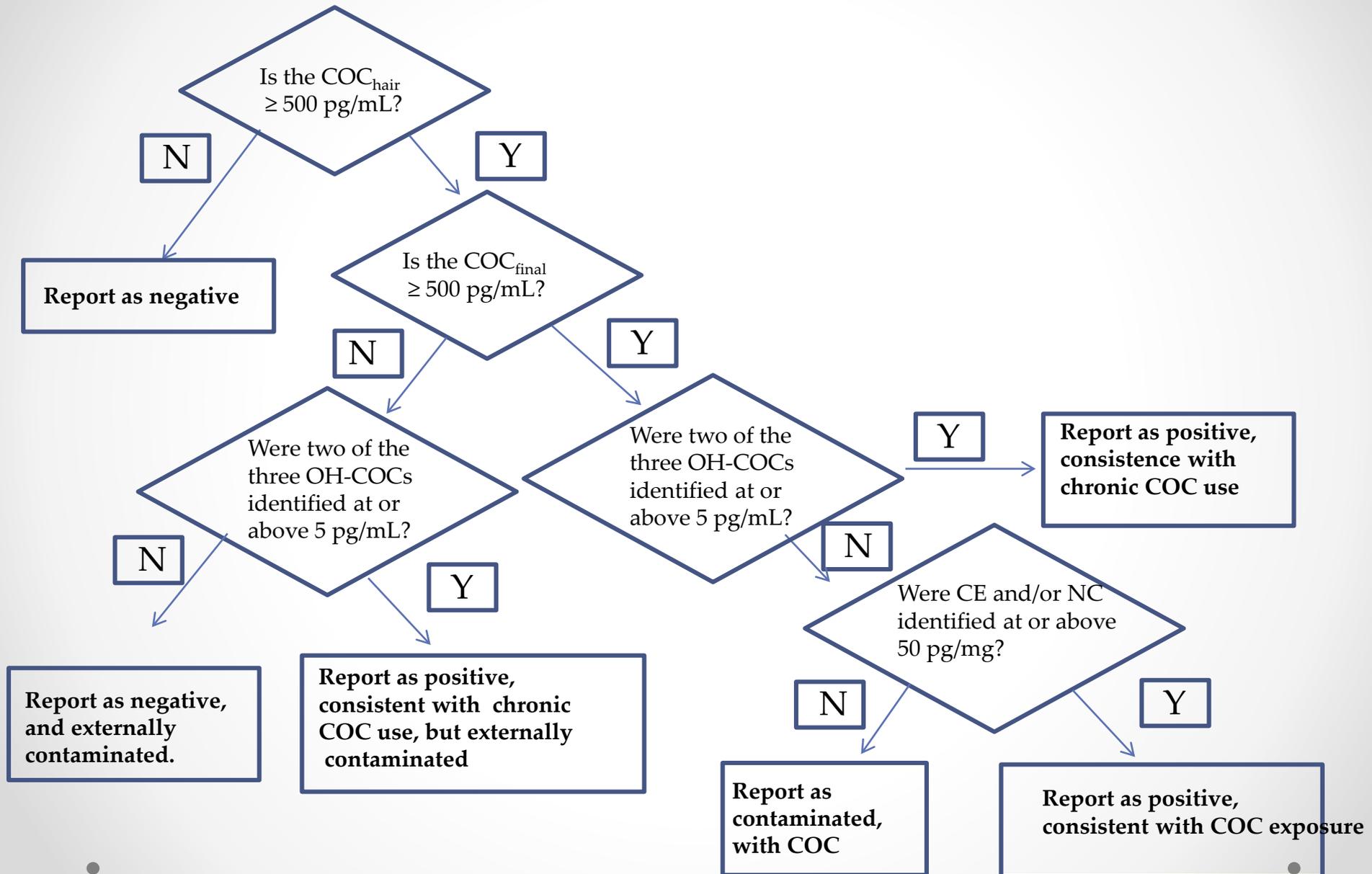
# Recent FBI Study\*\*

- Results of hair from 27 drug chemists
  - 11 washes contained COC and 4 contained BE but no **other metabolites detected**
  - 8 hair digests contained COC
  - 8 hair digests  $BE \geq 100$  and BE/COC ranged from 0.24-0.73
  - **NC, CE or hydroxycocaine metabolites were not detected**
  - Once extended wash kinetics calculation was applied, the 4 samples with  $COC \geq 500$  pg/mL had  $COC_{final} > 500$  pg/mL
  - If calculation was not applied and the cutoff of  $BE \geq 100$  pg/L or the  $BE/COC \geq 0.05$  was applied per the 2004 proposed guidelines, 4 of the drug chemists may have been reported as either a COC user or chronically exposed to COC.

# Recent FBI Study\*\*\*

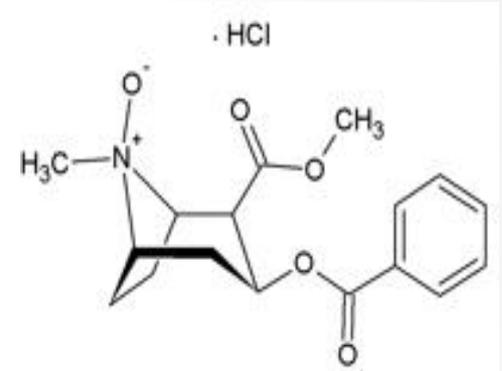
- Determined little use in measuring BE or BE/COC ratio in hair
- Suggests the presence of NC, CE and aryl hydroxycocaine metabolites in hair samples provide positive interpretative value
- m-OH-COC has been identified as a COC impurity while p-OH-COC and o-OH-COC have not

# Recent FBI Study\*\*\*\*



# Cocaine N-Oxide

- Labile
  - Not amenable to GC/MS analysis
- Decomposes in solution
- Standards available
  - <http://www.cerilliant.com/Shoponline/OpenDocument.aspx?DocumentId=228>



# Previous RTI Studies

**Cocaine analytes in human hair: evaluation of concentration ratios in different cocaine sources, drug-user populations and surface-contaminated specimens.**

Ropero-Miller JD, Huestis MA, Stout PR.

J Anal Toxicol. 2012 Jul;36(6):390-8

- Drug-free hair contaminated *in vitro* with COC from different sources at varied concentrations
- Analyzed for COC, BE, CE and NCOC by LC-MS/MS
- Results compared to COC analyte concentrations in drug users' hair following self-reported use (Street) and in hair from participants in controlled administration (Clinical)

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- Mean BE/cocaine ratios can exceed 0.05
- Incorporation of cocaethylene and norcocaine did not improve distinguishing cocaine use from contamination