



***Hair Analysis for Drugs:
Cut-off Concentrations
Analytes
Stability***

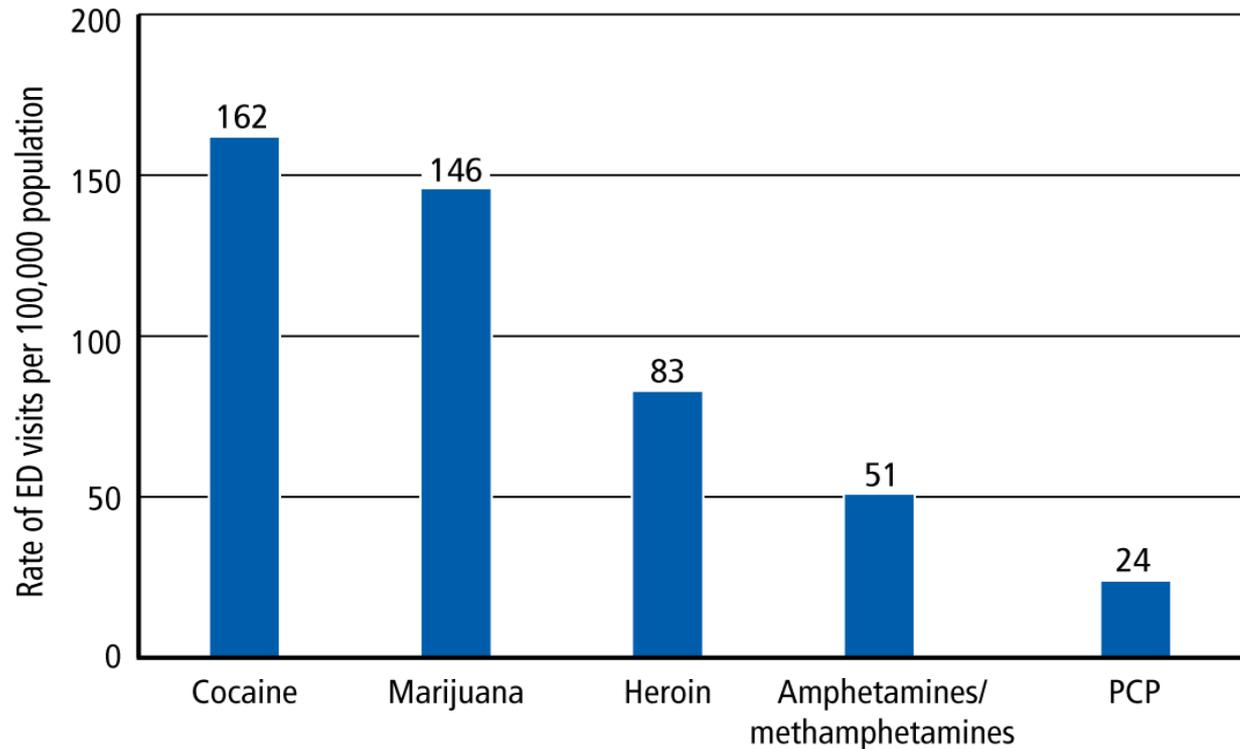
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Immunoanalysis Corporation*

***Drug Testing Advisory Board
July 15th 2013***

Overview

- ***Proposed guidelines***
 - *Cut-off concentrations*
 - *Analytes*
 - *Drug stability*
- ***Further considerations for program***

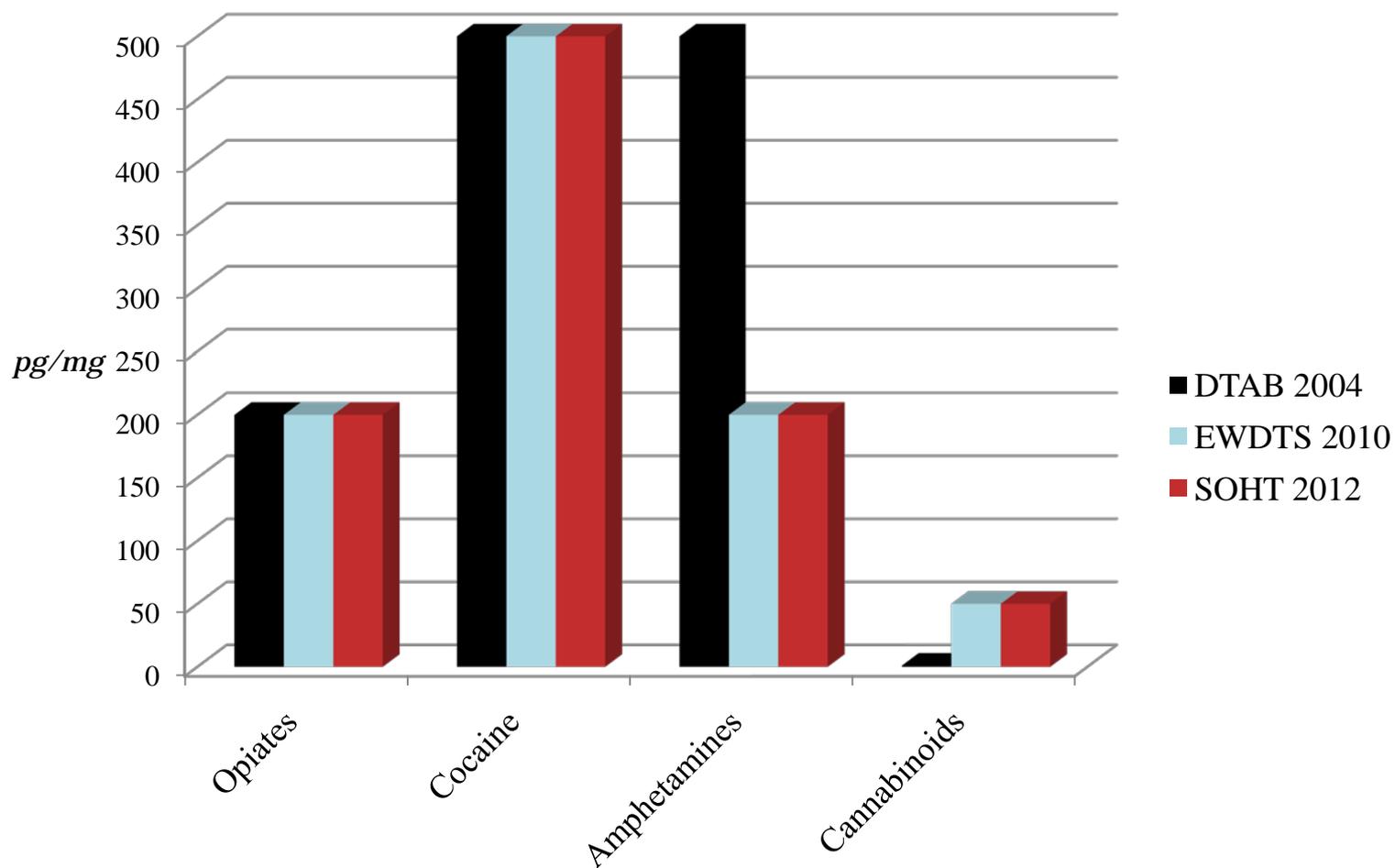
Rates of ED visits per 100,000 population involving illicit drugs, 2011



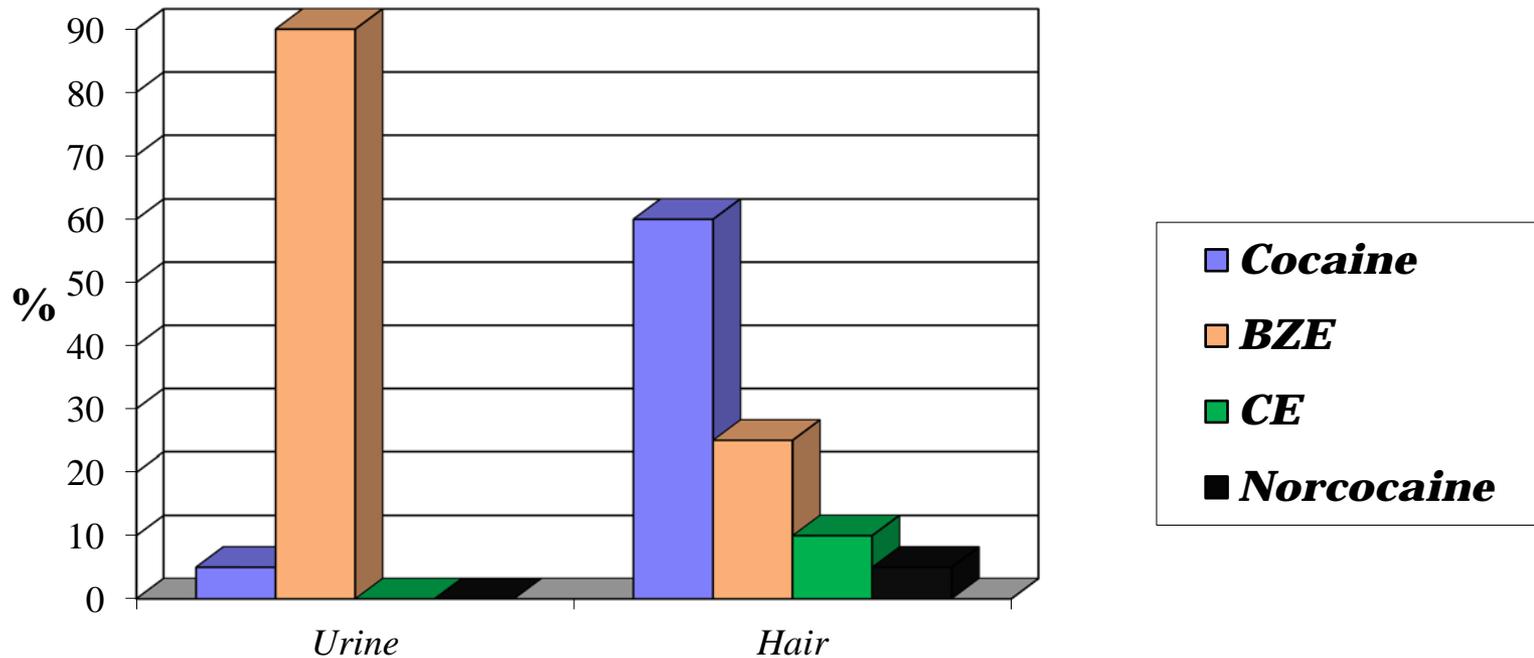
Proposed guidelines: Immunoassay / Screening

| <i>Drug</i> | <i>Recommended cut-off (pg/mg)</i> | | |
|------------------------|------------------------------------|-------------------|------------------|
| | <i>DTAB 2004</i> | <i>EWDTs 2010</i> | <i>SOHT 2012</i> |
| Phencyclidine | 300 | | |
| Opiates | 200 | 200 | 200 |
| Cocaine | 500 | 500 | 500 |
| Amphetamines | 500 | 200 | 200 |
| Cannabinoids | 1 | 50 | 50 |
| Methadone | | | 200 |
| Buprenorphine | | | 10 |
| Benzodiazepines | | 50 | |

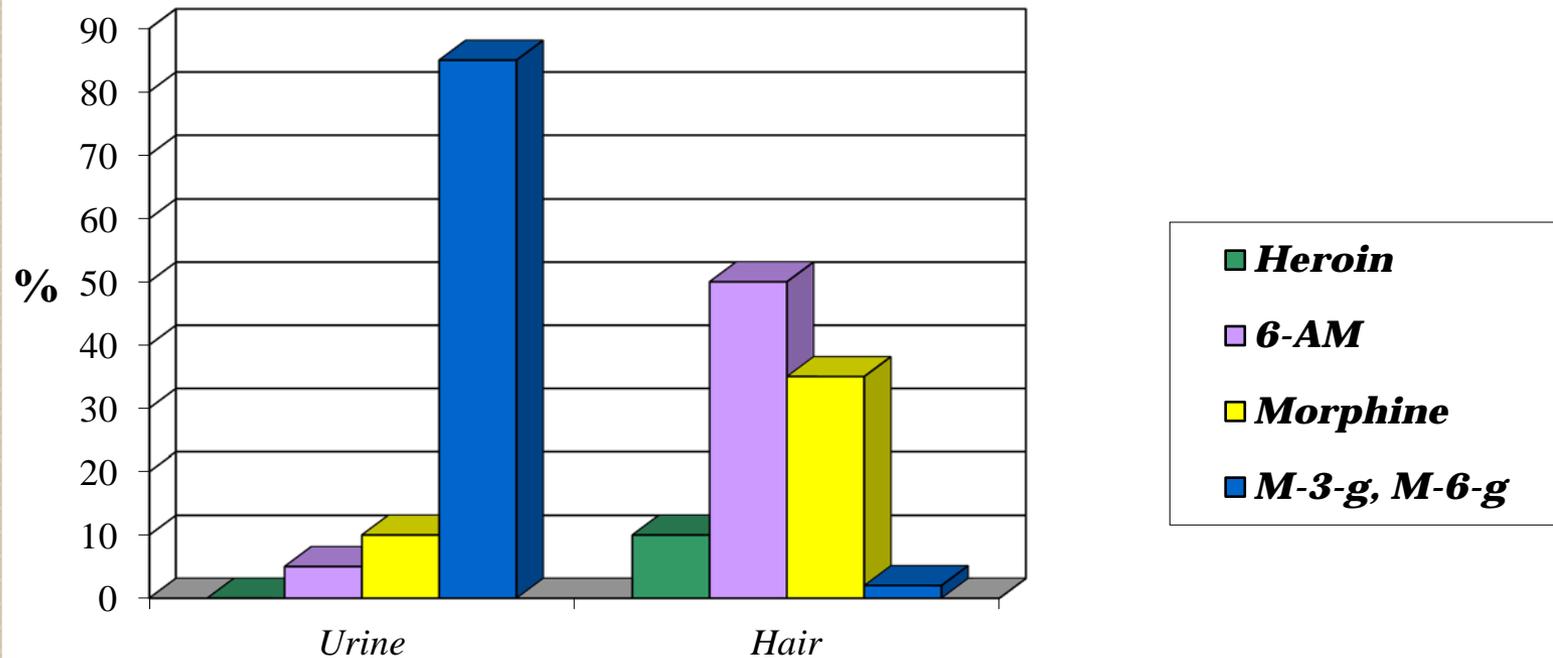
Proposed screening cut-off concentrations



Considerations for immunoassay: Cocaine



Considerations for immunoassay: Heroin



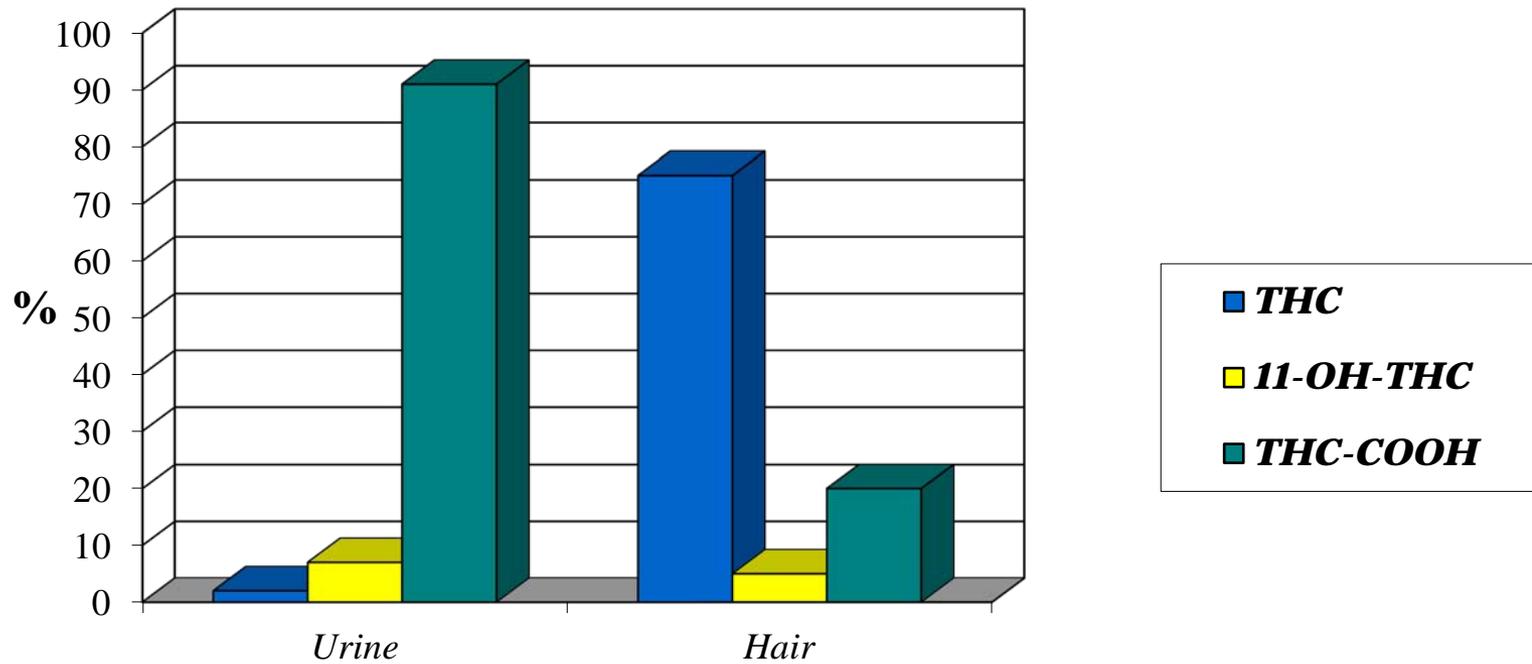
Targeted immunoassay screens

- ***Basic drugs:***

- *Incorporate well into hair*
- *Parent compound (e.g. cocaine) incorporated to greater extent than metabolites (e.g. BZE)*
- *So immunoassay must target cocaine, OR, if urine immunoassay used, degree of conversion of cocaine to BZE in method must be measured*

- *6-AM in higher concentration than morphine*
- *Immunoassay should target 6-AM, OR, degree of conversion to morphine must be measured*

Considerations for immunoassay: Cannabinoids



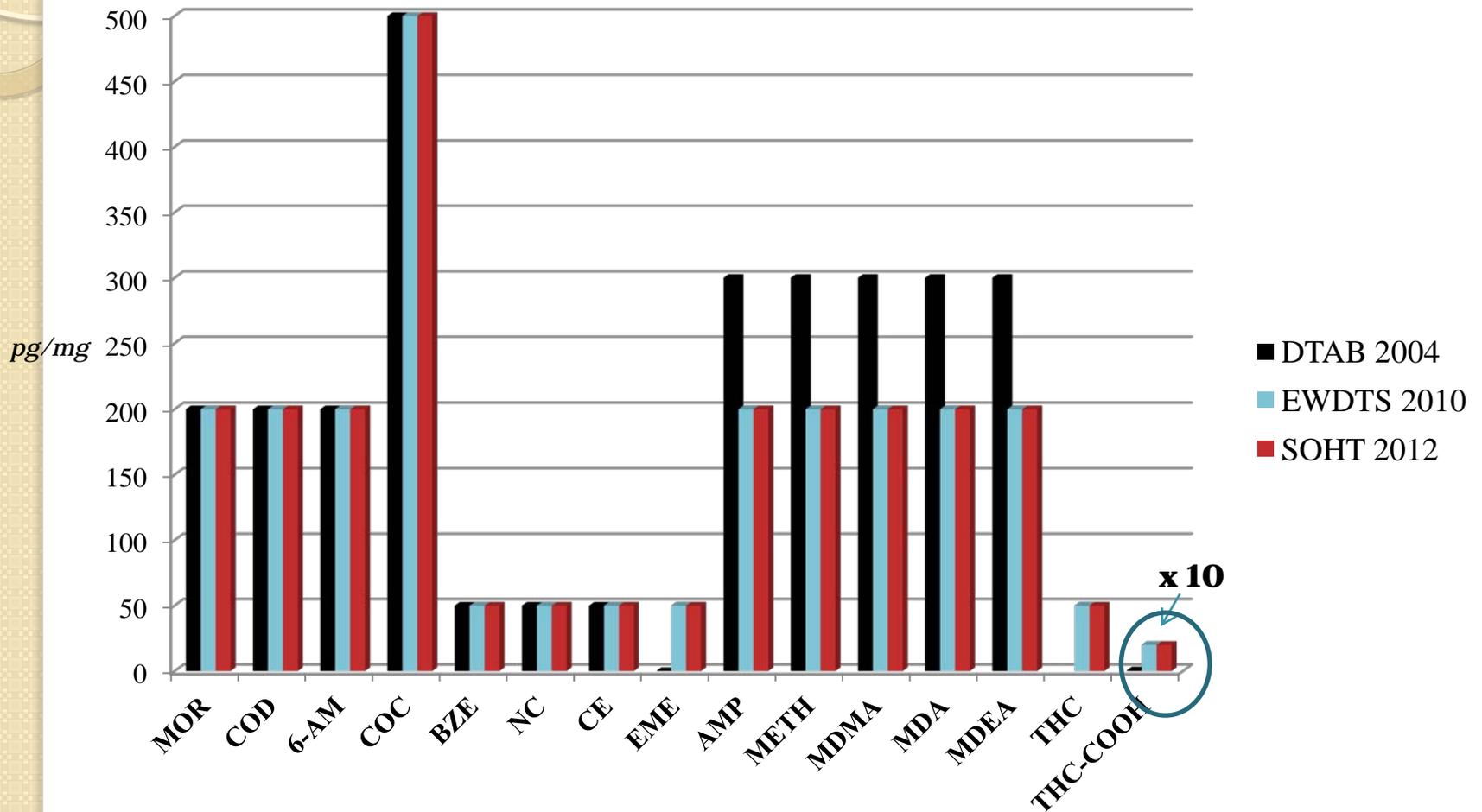
Targeted immunoassay screens

- *Acidic drugs:*
 - *Do not incorporate well (e.g., marijuana)*
 - *THC in higher concentration in hair than metabolite*
 - *Why not use an immunoassay targeted to THC ?*
 - *Confirmatory procedure would identify metabolite THC-COOH*

Mass Spectrometry / Confirmation

| Drug | Recommended cut-off (pg/mg) | | |
|------------------------|---|--|--|
| | DTAB 2004 Federal Register | EWDTS 2010 DTA 2(8): 367-376 | SOHT 2012 FSI 218: 20 - 24 |
| Phencyclidine | PCP: 300 | | |
| Opiates | MOR, COD, 6-AM: 200 | MOR, COD, 6-AM: 200 | MOR, COD, 6-AM: 200 |
| Cocaine | Cocaine: 500 BZE, Norcocaine, CE: 50 | Cocaine: 500 BZE, Norcocaine, CE, EME: 50 | Cocaine: 500 BZE, Norcocaine, CE, EME: 50 |
| Amphetamines | AMP, METH, MDMA, MDA, MDEA: 300 | AMP, METH, MDMA, MDA, MDEA: 200 | AMP, METH, MDMA, MDA: 200 |
| Cannabinoids | THC-COOH: 0.05 | THC: 50 THC-COOH: 0.2 | THC : 50 THC-COOH: 0.2 |
| Methadone | | | Methadone: 200 EDDP: 50 |
| Buprenorphine | | | Buprenorphine: 10 Nor-BUP: 10 |
| Benzodiazepines | | Bromazepam, Nordiazepam, Oxazepam, Lorazepam, Alprazolam, Diazepam, Flunitrazepam: 50 | |

Proposed confirmatory cut-off concentrations





***Are proposed cut-offs
appropriate ?***

Phencyclidine

- *Only North America suggests inclusion*
- *Nakahara et al. 1997 J Anal Toxicol. 21(5):356-62.*
- *Hair analysis for drugs of abuse. XVII. Simultaneous detection of PCP, PCHP, and PCP-diol in human hair for confirmation of PCP use.*

- *8 PCP users*
- *Positive: 330 – 14,000 pg/mg*
- *Minor metabolites also detected in lower concentration*

- *Suggested cut-off appears appropriate*

Cocaine

- *Good agreement between professional societies*
- *Detection of metabolites mandated*
- *However, benzoylecgonine (& EME) not indicative of ingestion due to “in vitro” cocaine degradation*
- *Cocaethylene and norcocaine were initially thought to be indicative of use, but are apparently present in street cocaine*
- *Some discussion of m-OH and p-OH BZE metabolites*

Cocaine users

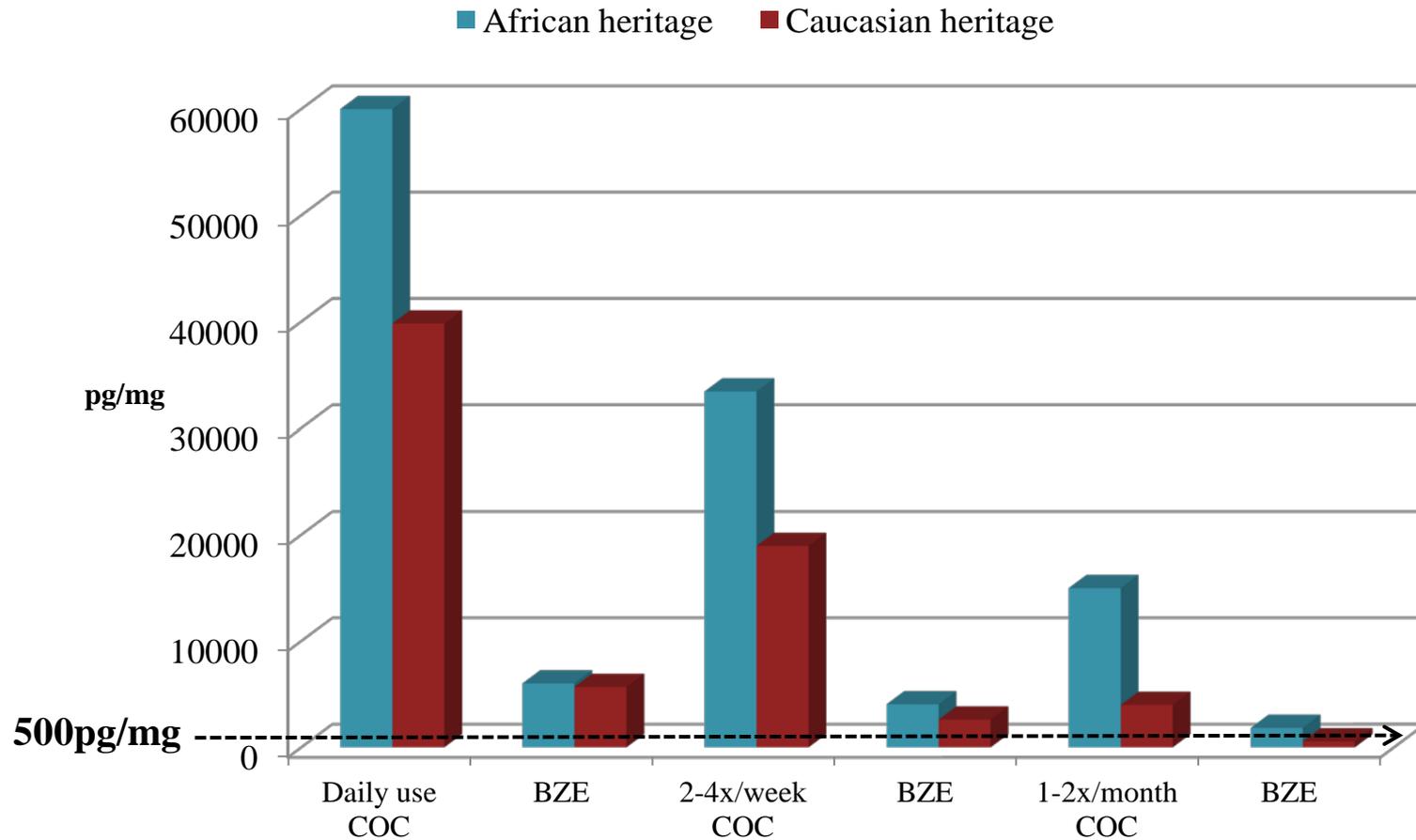
- *Lachenmeier et al. 2006 Forensic Sci. Int. 159(2-3):189-99*
- *Determination of opiates and cocaine in hair using automated enzyme immunoassay screening methodologies followed by GC-MS confirmation*

- *Authentic samples from cocaine users (n=103)*
- *GC/MS confirmation results:*
- *Cocaine: 100 – 21,370 pg/mg* ***Mean: 2,610***
- *BZE: 30 – 10,510 pg/mg* ***Mean: 1,110***
- *CE: 50 – 1260 pg/mg* ***Mean: 270***

- *Users have high cocaine concentrations in hair*

Self report of cocaine use

- *Vignali et al. 2012 Forensic Sci. Int. 215: 77-80.*
- *Hair testing and self-report of cocaine use*



Proposed cut-offs will identify cocaine users

Amphetamines

- *Some agreement between professional societies*
- *Polettini et al. 2012 Anal Chim Acta. 726:35-43*
- *Incorporation of methamphetamine and amphetamine in human hair following controlled oral methamphetamine administration*

- *7 volunteers: METH administration at low and high doses*
- ***Maximum*** detected concentrations:
 - *METH: 600 – 3500 pg/mg; AMP 100 – 300 pg/mg (low dose)*
 - *METH 1200 – 5300 pg mg; AMP 200 – 500 pg mg (high dose)*
 - *AMP/MAMP ratio: Mean: 0.15; Median: 0.13*

 - ***Proposed cut-off: 300pg/mg; At least 50pg/mg of AMP if 300pg/mg METH (0.16)***

 - *Suggested cut-off appears reasonable for METH with AMP metabolite*

MDMA in Hair

- *Cheze et al. 2007 Forensic Sci Int. 170(2-3):100-4.*
- *Simultaneous analysis of six amphetamines and analogues in hair, blood and urine by LC-ESI-MS/MS. Application to the determination of MDMA after low ecstasy intake.*

- *Detection of MDMA in a forensic case*
- *Single administration of ecstasy to a 16-year-old female without her knowledge during a party*
- *Hair collected 60 days after incident*
- *Hair positive for MDMA (22 pg/mg); no MDA*

Amphetamines

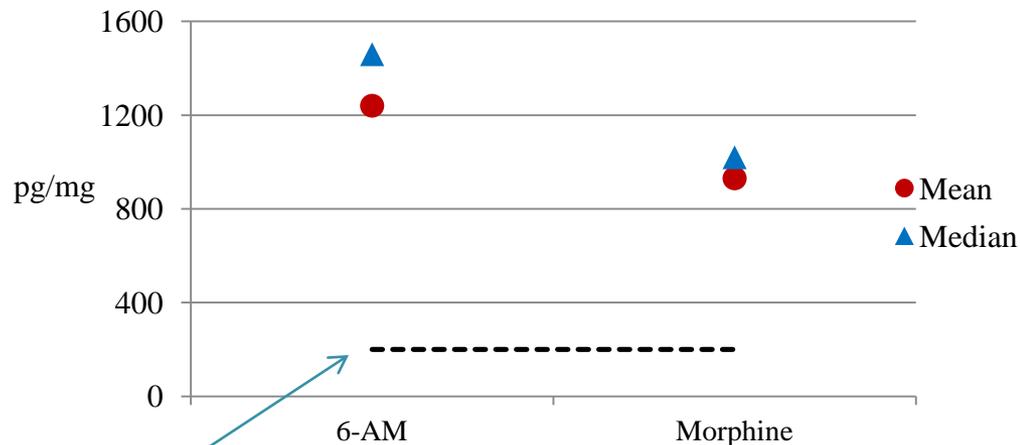
- *DTAB proposals higher than other societies*
 - *Discussion of lower threshold values*
- *METH with AMP as a metabolite appears appropriate*
- *If MDMA/MDA and MDEA are included in test profile, consider a lower cut-off*
- *Consider including ratio requirement for MDMA/MDA*

THC

- *Limited agreement between professional societies*
- *Parent THC in higher concentration than metabolite (similar to oral fluid analysis)*
- *Screening for parent THC proposed in other organizations*
- *Consensus that THC-COOH minimizes claim of passive exposure*
- *Consider allowing THC screen with THC-COOH confirmation*

Opiates

- *Good agreement between professional societies*
- *Detection of 6-AM identifies heroin usage*
- *Distinct advantage over urine testing*
 - *Musshoff et al. 2005 J Anal Toxicol 29(5): 345-52*
 - *Opiate concentrations in hair from subjects in a controlled heroin-maintenance program and from opiate-associated fatalities*
 - *46 individuals tested – all different hair colors*
 - *100% positive for 6-AM; 89% positive for morphine*

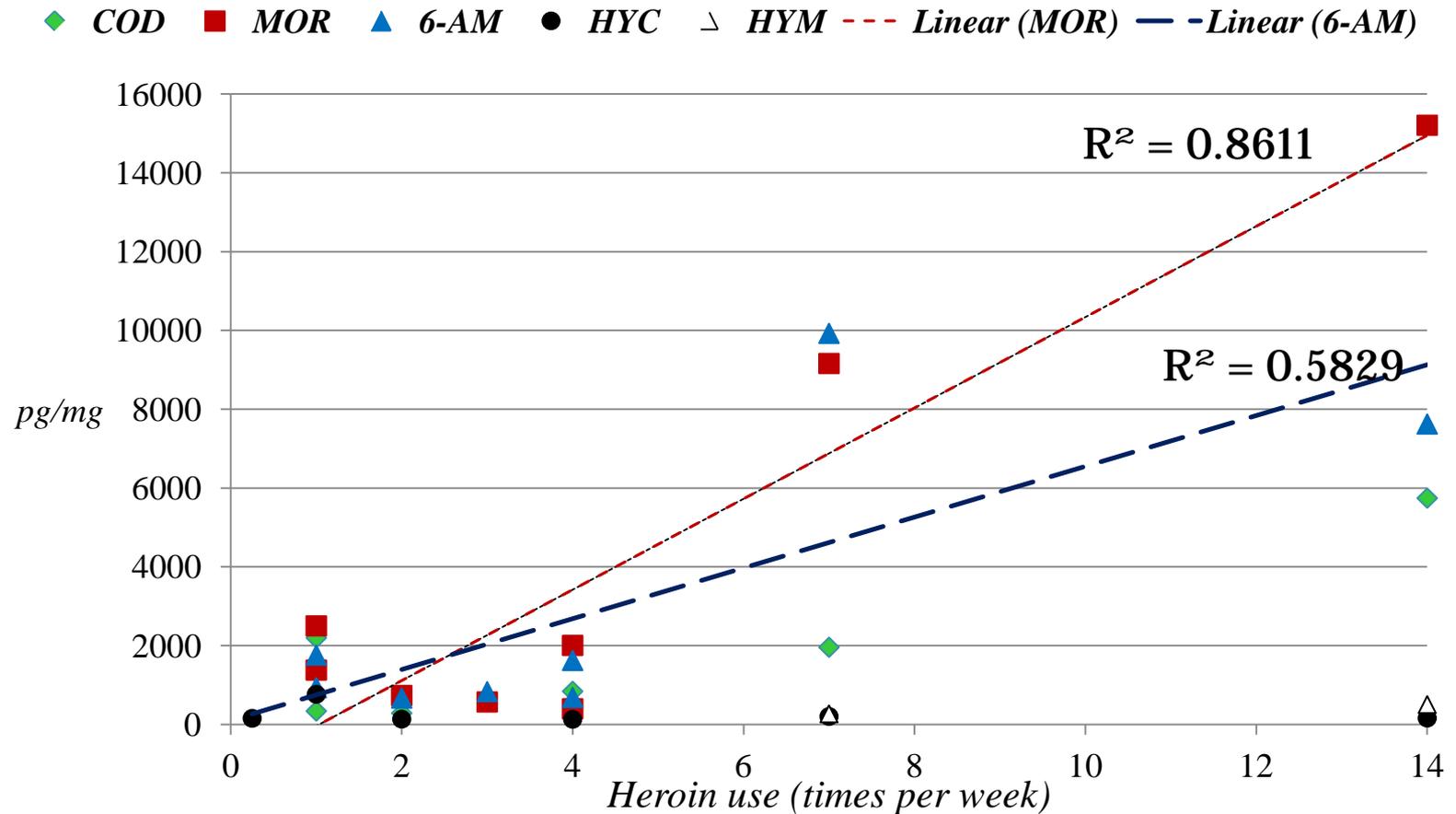


Suggested cut-off appears appropriate

What about other analytes ?

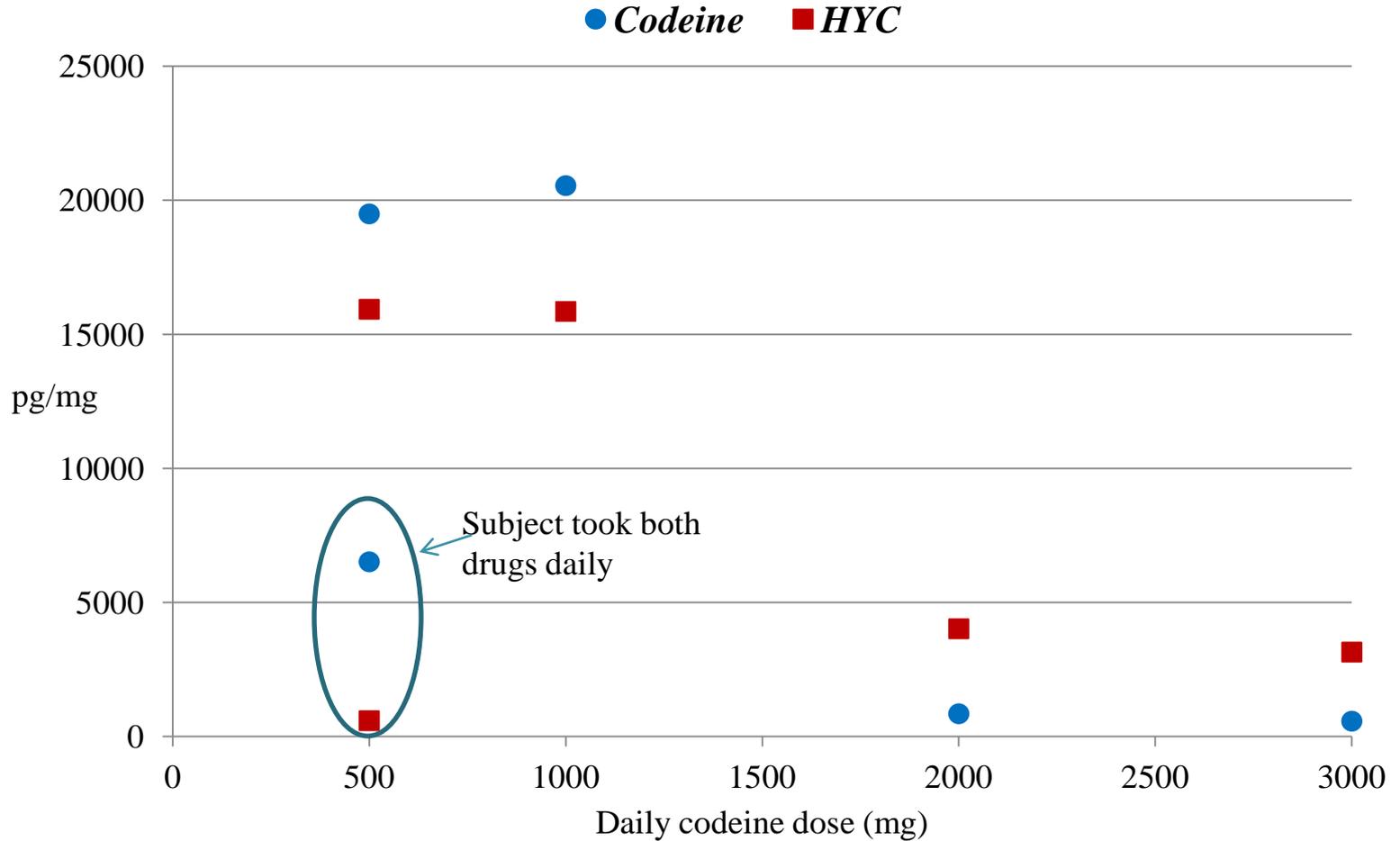
- *Currently, additional drugs being considered for other matrices, so potential addition of:*
 - *Hydrocodone*
 - *Hydromorphone*
 - *Oxycodone*
 - *Oxymorphone*
- *What data on these drugs in hair is available ?*

Drug concentrations in hair following self reported heroin use



Moore et al. JAT 2006 30; 353-359

Drug concentrations in hair following self reported codeine use



Heroin / Codeine

Heroin:

- *9 self-reported heroin users: 8 provided hair specimens positive for MOR and 6-AM (89%) (9th subject once a month use)*
- *6 samples also contained COD, 5 of those had HYC and 2 HYM*

Codeine:

- *5 subjects reported daily codeine intake*
- *MOR not detected in any hair specimens, but both COD and HYC were present*

◦ ***Summary:***

- *Presence of MOR suggests heroin or morphine intake, not codeine*
- *Apparent linear relationship between reported frequency of heroin use and morphine and 6-AM concentrations in hair*

Oxycodone /Oxymorphone

- *Notable paucity of information and literature*
- *Oxycodone in hair PubMed: 8 citations*
 - *None discuss concentrations from users*
- *Oxymorphone in hair PubMed: 4 citations*
 - *2 to do with cats/dogs (surgery)*
 - *1 was general analytical screen*
 - *One discussed real patients, but focused on other pain medications (tramadol, fentanyl)*

2007 Wayne County, MI: Post-mortem hair specimens

- *All cases had narcotic paraphernalia at the scene, **or***
- *History of previous drug overdose, **or***
- *Witnessed drug use*

- *Target cases:*
 - ***Acute drug intoxication suspected***
- *Hair samples were collected from root end*

Case 1

| Drug | Heart Blood (mg/L) | Hair (pg/mg) |
|---------------------------|---------------------------|---------------------|
| <i>Cocaine</i> | <i><0.025</i> | <i>>10,000</i> |
| <i>BZE</i> | <i>2.9</i> | <i>>10,000</i> |
| <i>CE</i> | <i>ND</i> | <i>1041</i> |
| <i>Norcocaine</i> | <i>NA</i> | <i>532</i> |
| <i>Oxycodone</i> | <i>0.066</i> | <i>2079</i> |
| <i>Hydrocodone</i> | <i>ND</i> | <i>2231</i> |
| <i>Alprazolam</i> | <i>ND</i> | <i>261</i> |

Other PM Findings:

Urine: EME, COC, Diltiazem, Levamisole, Clonidine, Oxycodone

Cause of Death: Cocaine use

Manner of Death: Accident

Case 3

| Drug | Liver (mg/kg) | Hair (pg/mg) |
|---------------------|----------------------|---------------------|
| <i>Morphine</i> | <i>6.4</i> | <i>541</i> |
| Hydrocodone | 0.67 | ND |
| Oxycodone | 279 | >10,000 |
| Oxymorphone | NA | 1236 |
| <i>Propoxyphene</i> | <i>ND</i> | <i>375</i> |

Other PM Findings: None

Cause of Death: Oxycodone Intoxication

Manner of Death: Accident

Case 12

| Drug | Spleen (mg/kg) | Hair (pg/mg) |
|---------------------------|-----------------------|---------------------|
| <i>Cocaine</i> | <i>ND</i> | <i>977</i> |
| <i>BZE</i> | | <i>229</i> |
| <i>Morphine</i> | <i>0.78</i> | <i>288</i> |
| <i>Codeine</i> | <i>0.084</i> | <i>86</i> |
| <i>Oxycodone</i> | <i>ND</i> | <i>82</i> |
| <i>Hydrocodone</i> | <i>ND</i> | <i>704</i> |
| <i>6-AM / 6-AC</i> | <i>ND</i> | <i>865 / 76</i> |

Other PM Findings: None

Cause of Death: Opiate Use

Manner of Death: Accident

Summary of Results – 14 Cases

| Drug Class | Traditional PM Samples | Hair |
|--|-------------------------------|----------------------|
| <i>Cocaine / BE / CE</i> | <i>3 / 4 / 0</i> | <i>12 / 12 / 4</i> |
| <i>Morphine / 6-AM</i> | <i>8 / 5</i> | <i>10 / 11</i> |
| <i>Codeine / HYC</i> | <i>7 / 4</i> | <i>10 / 8</i> |
| <i>Oxycodone</i> | <i>2</i> | <i>6</i> |
| <i>Benzodiazepines</i> | <i>5</i> | <i>6</i> |
| <i>Methadone</i> | <i>1</i> | <i>3</i> |
| <i>Fentanyl</i> | <i>4</i> | <i>4</i> |
| <i>Tramadol / PPX / Carisoprodol</i> | <i>1 / 0 / 0</i> | <i>1 / 1 / 1</i> |

Additional drugs

- *Summary of post-mortem cases shows OXYC, OXYM, HYC, HYM detected in hair, often when more traditional specimens were negative*
- *Should be relatively straight-forward to include in workplace drug testing program*

Drug stability in hair

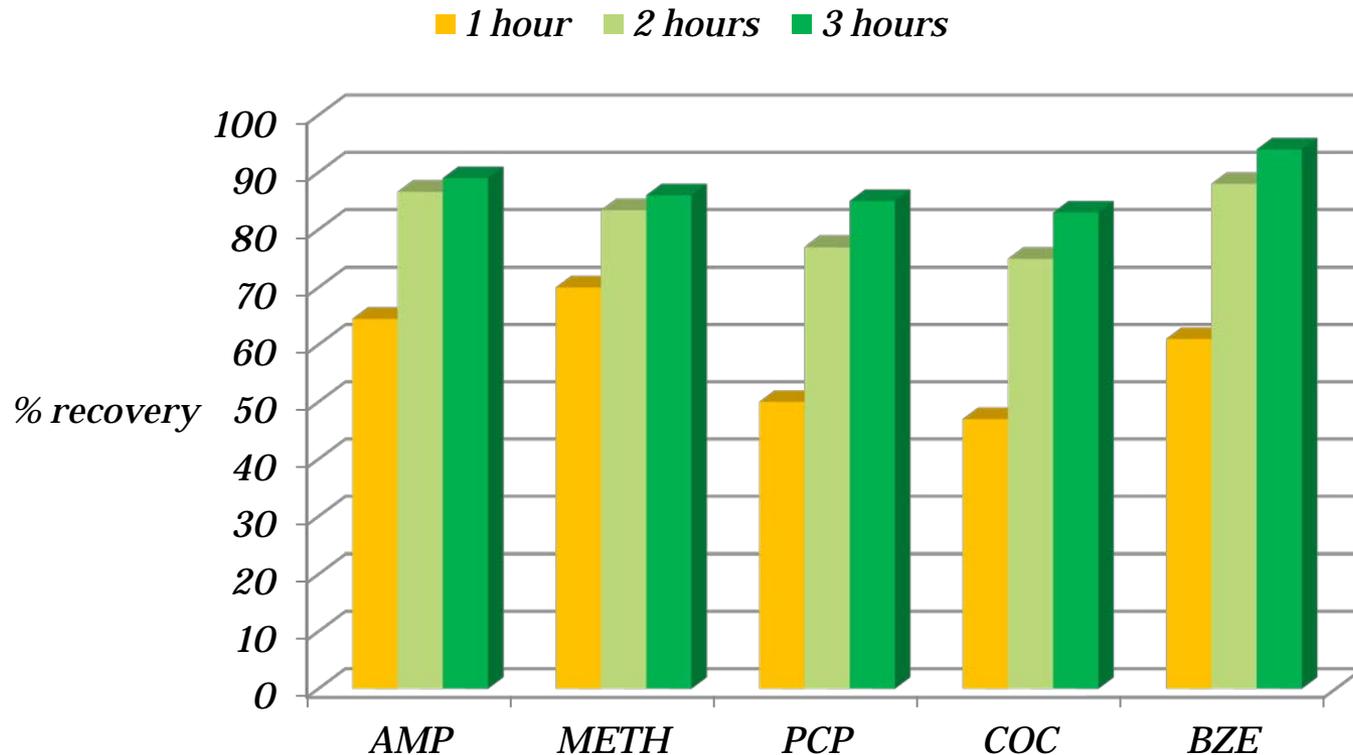
- *Several papers state that hair can be “stored for years”*
- *No supportive data*
- *Some conditions listed:*
 - *Cool*
 - *Dark*
 - *No plastic bags*
 - *No refrigeration*
- *Surprising lack of literature*

Other areas for DTAB discussion

- *1. Extraction efficiency from authentic hair specimens (solid matrix, different issues)*
- *2. Extent of drug conversion during extraction procedure (BZE, 6-AM)*
- *3. Drug stability in storage and during routine transportation*
- *4. Addition of other analytes*

1. Extraction efficiency

- *Many different published procedures*
- *Drug recovery from authentic hair*
- *Point of diminishing returns*



2. Drug conversion

- *Cocaine is converted to BZE in many methods (sometimes deliberately to use a BZE immunoassay targeted screen)*
- *6-AM may convert to morphine*
- *Degree of conversion obviously critical if interpretative results are based on drug - metabolite ratio / concentration*

3. Drug stability

- *Demonstrate drug stability in various storage conditions*
- *Demonstrate drug stability during transportation*

- *Re-analysis of collected specimens*
 - *Brings segmental analysis into the discussion*
 - *Is re-analysis of “same hair” or hair extract ?*
 - *Is a different “segment” analyzed ?*

4. Addition of other analytes

- *As discussed earlier, to harmonize with other matrices:*
 - *MDMA*
 - *MDA*
 - *MDEA*
 - *Oxycodone*
 - *Oxymorphone*
 - *Hydrocodone*
 - *Hydromorphone*

Summary

- *Proposed cut-offs seem appropriate for PCP, opiates and cocaine*
- *Amphetamines and cannabinoids may require further discussion*
- *Expand analyte list to be in harmony with other matrices*
- *Drug stability in hair needs far more research*
- *Good alternative or addition to urine/oral fluid*
- *Provides different information and should be used depending on circumstances for testing*
- *Method validation may have different requirements to urine and oral fluid*



Thank you.....