



Alere™

# Practical Aspects of Drug Testing in Human Hair

## Laboratory Perspective

James A. Bourland, Ph.D., DABFT



# Overview of Collection Process



# Hair Collection

- Site of Collection - Occipital Crown / Posterior Vertex
- Cut with Scissors in 2-3 locations close to the scalp
- Hair aligned in collection container root and distal ends





# Hair Collection Challenges

- No head hair
- Body Hair
- Weaves
- Short Head Hair
- Contamination Claims
- Variability cutting from the scalp





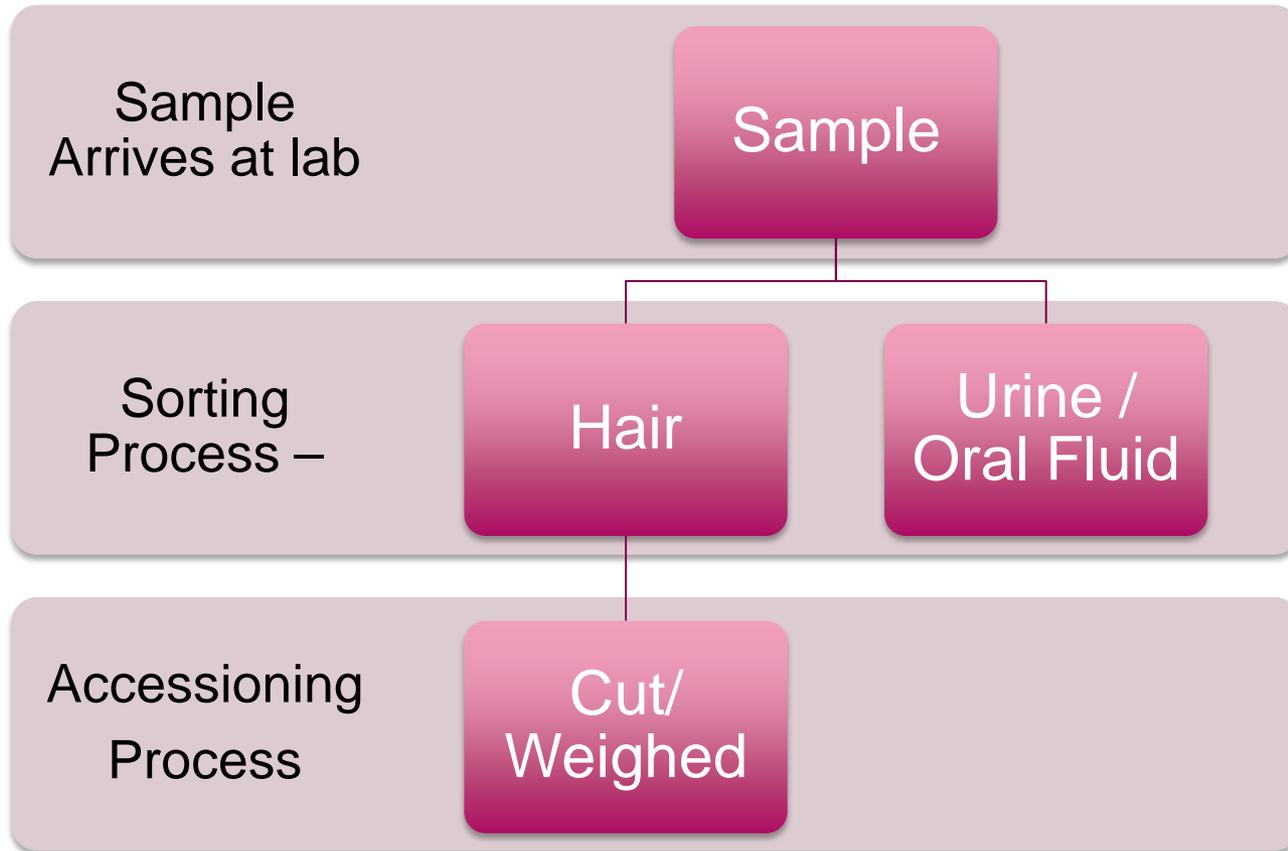


# Overview of Accessioning Process



# Overview

## Hair Accessioning





# Hair Accessioning Requirements

- Ideal to have Separate Area Isolated
- Each Station
  - Scissors
  - Cleaning Solution for Scissors
  - Scale
  - Sharpie
  - Disposable Pads
- Accurate Scales Needed to Weigh hair
- Humidity Control (Reduce static electricity)



# Aliquoting of Hair

- Hair cut at 3.9 cm from root end
- 3.9 cm segment of hairs cut into 2-5 mm segments
- Cut hair mixed for homogeneity
- 20 mg weighed
  - 1<sup>st</sup> aliquot: ELISA screen
  - 2<sup>nd</sup> aliquot: GC/MS and/or GC/MS/MS confirm



# Pre-Analytical Process



# Pre-Analytical Process

- Not a liquid Matrix
- Pre-Analytical Process Needed Prior to :
  - Screening by Immunoassay
  - Extraction for GC/MS, GC/MS/MS or LC/MS/MS
- Pre-Analytical Process should include Wash Procedure(s) to remove sources of external contamination
- Two main approaches
  - Digestion of the hair matrix to a liquid
  - Pre-Extraction of the Drug dissolved in a liquid
- Challenges
  - Drugs and metabolites must remain intact
  - Extraction or Digestion Efficiency
  - Time – Balance between Efficiency and TAT



# Hair Confirmation: Extractions

- Cocaine and Amphetamines
  - 4 washes
  - Dilute Acid + Heat Extraction
- Opiates
  - 4 washes
  - Methanol/Buffer/KOH Digestion
- PCP
  - 4 washes
  - Methanol + Heat Extraction
- THC/THCA
  - 4 washes
  - NaOH + Heat Digestion
- All Drugs: SPE



# Extraction



# Extraction

- After Pre-analytical Process Extraction is similar to other matrices
- Solid Phase Extraction
- Liquid-Liquid
- Derivatization
- Deuterated Internal Standards
- Calibration Curve
- Quality Controls



# Confirmation Technology

- GC/MS
- GC/GC/MS
- GC/MS/MS
- LC/MS
- LC/MS/MS



# Drugs/ Metabolites Tested & Cut-Off Values in Hair



# Quest Diagnostics

DRUG CLASS	INITIAL TEST LEVEL	CONFIRMATORY LEVEL
<b>AMPHETAMINES</b>	<b>300 pg/mg</b>	
Amphetamine		300 pg/mg
Methamphetamine		300 pg/mg
MDA		300 pg/mg
MDMA		300 pg/mg
<b>COCAINE/METABOLITES</b>	<b>300 pg/mg</b>	
Benzoylcegonine		300 pg/mg
Cocaine		300 pg/mg
Cocaethylene		300 pg/mg
Norcocaine		300 pg/mg
<b>MARIJUANA</b>	<b>1.0 pg/mg</b>	
THC Metabolite		0.1 pg/mg
<b>OPIATES</b>	<b>500 pg/mg</b>	
Morphine		500 pg/mg
Codeine		500 pg/mg
6-Acetylmorphine (6-AM)		500 pg/mg
Hydrocodone*		500 pg/mg
Hydromorphone*		500 pg/mg
Oxycodone*		500 pg/mg
Oxymorphone*		500 pg/mg



# Omega Laboratories

DRUG CLASS	INITIAL TEST LEVEL	CONFIRMATORY LEVEL
AMPHETAMINES	<b>500 pg/mg</b>	
<i>Amphetamine</i>		500 pg/mg
Methamphetamine		500 pg/mg
MDA		500 pg/mg
MDMA		500 pg/mg
COCAINE/METABOLITES	<b>500 pg/mg</b>	
Benzoylcegonine		500 pg/mg
Cocaine		500 pg/mg
Cocaethylene		500 pg/mg
Norcocaine		500 pg/mg
MARIJUANA	<b>1.0 pg/mg</b>	
THC Metabolite		0.3 pg/mg
OPIATES	<b>300 pg/mg</b>	
Morphine		300 pg/mg
Codeine		300 pg/mg
6-Acetylmorphine (6-AM)		300 pg/mg
Hydrocodone*		300 pg/mg
Hydromorphone*		300 pg/mg
Oxycodone*		300 pg/mg
Oxymorphone*		300 pg/mg



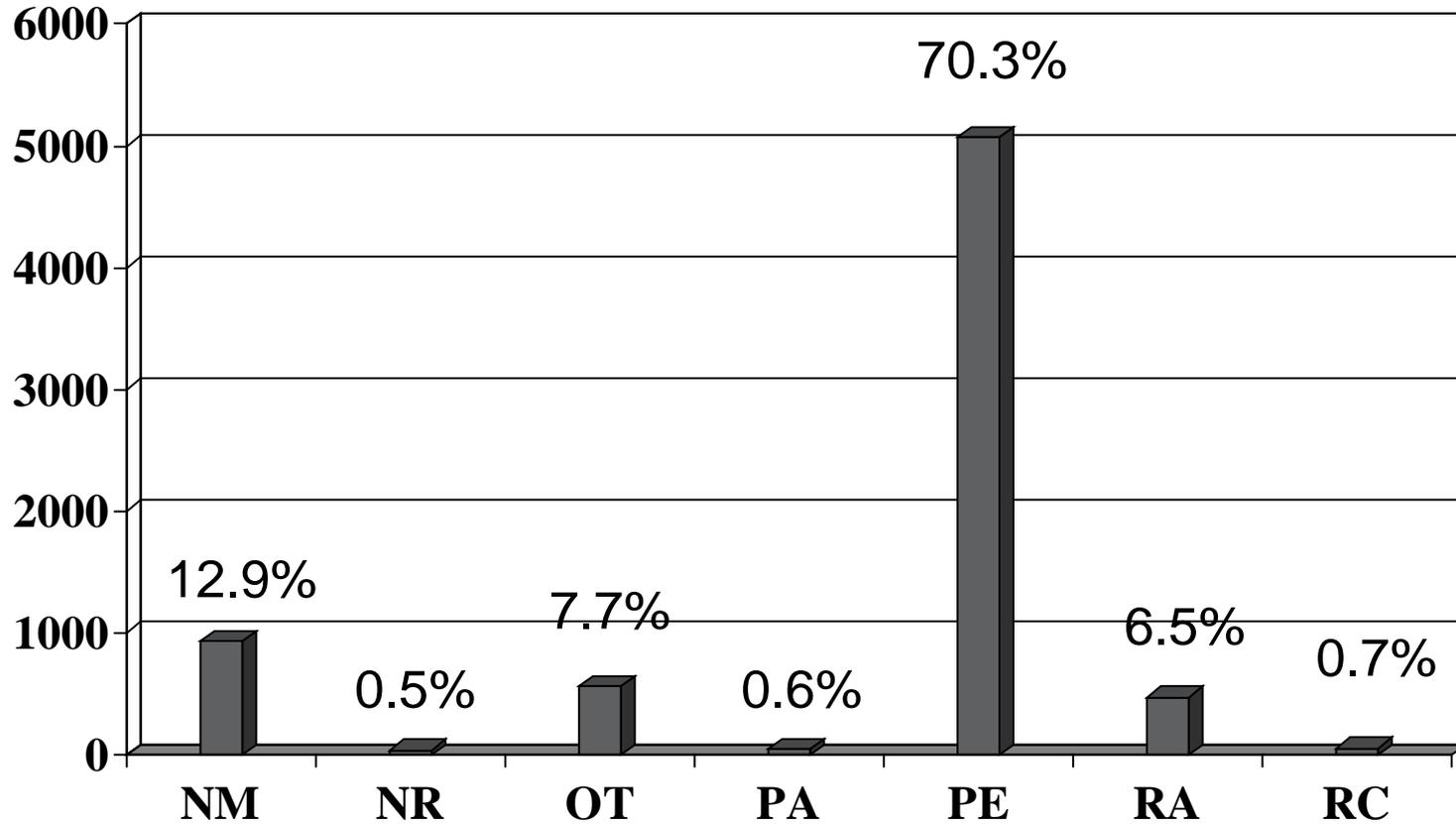
# Drug Test Results in Hair v. Urine



# Combined Hair + Urine Profile

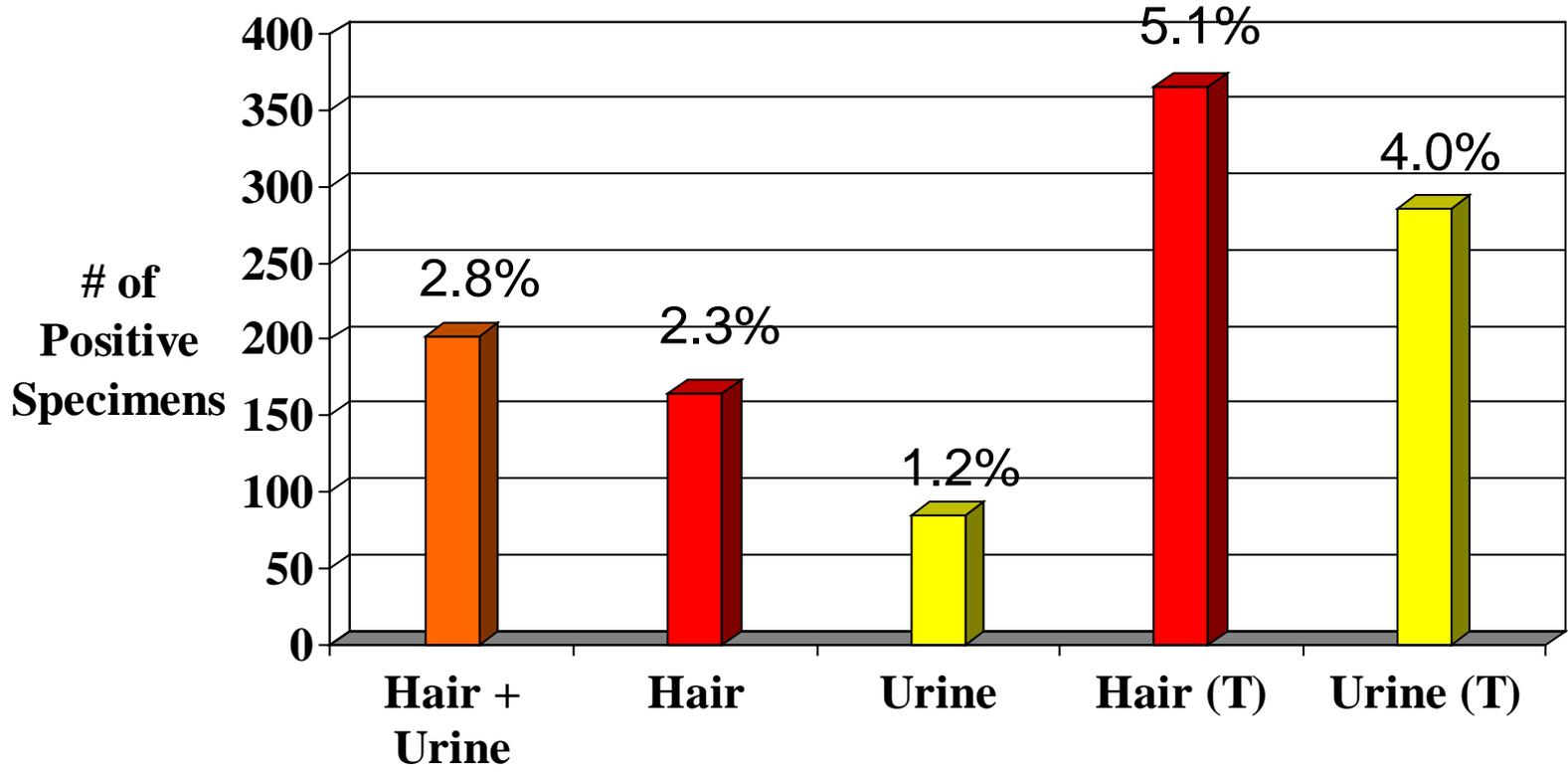
- Hair and Urine collected
- Urine 7-panel
  - THC, Cocaine, Amps, Opiates and PCP
  - Barbs and Ethanol
- Hair 5-panel
  - THC, Cocaine, Amps\*, Opiates and PCP
  - \*MDMA and MDA
  
- N = ~ 7,000 samples

# Reason For Test



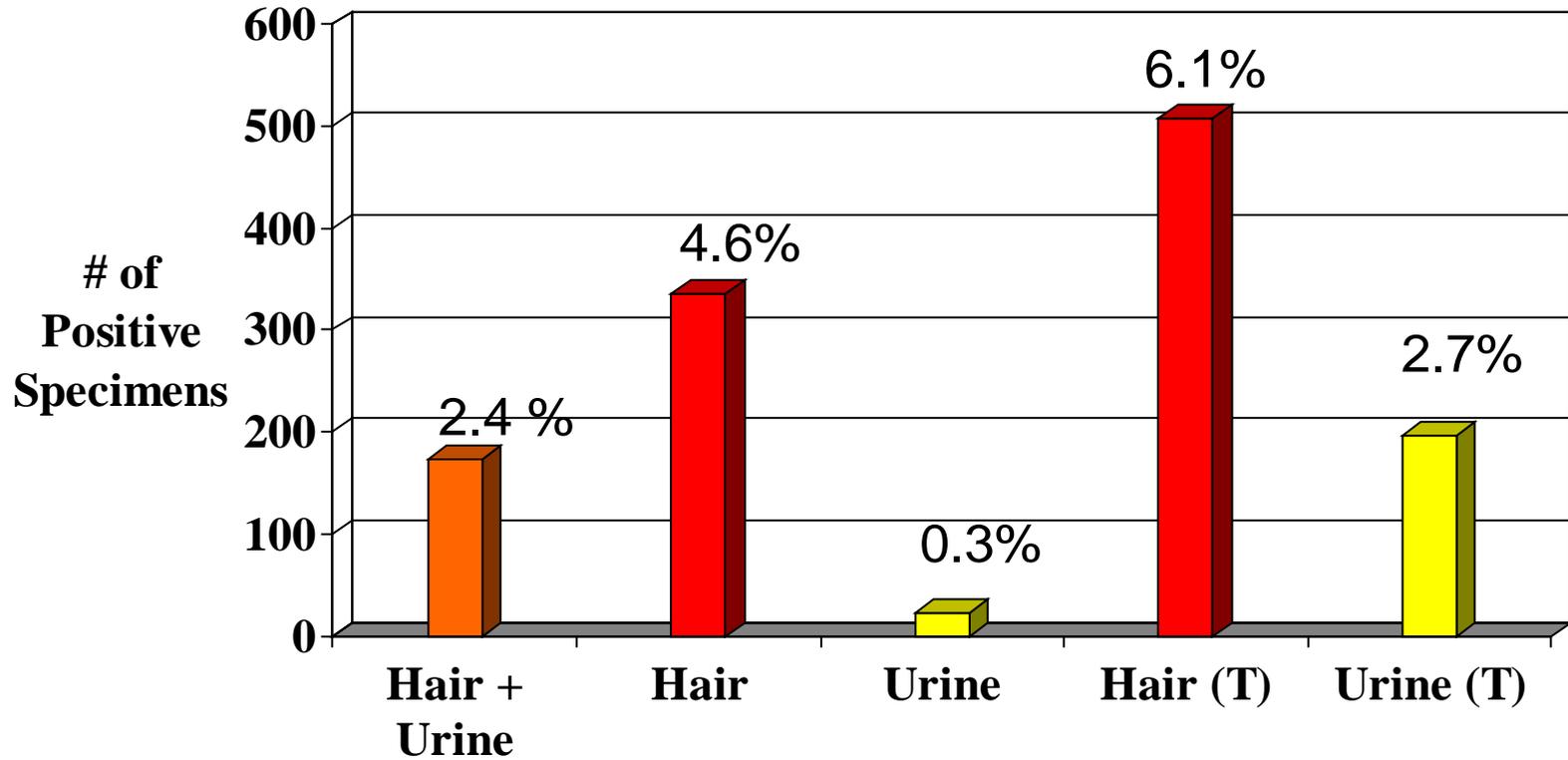


# Marijuana

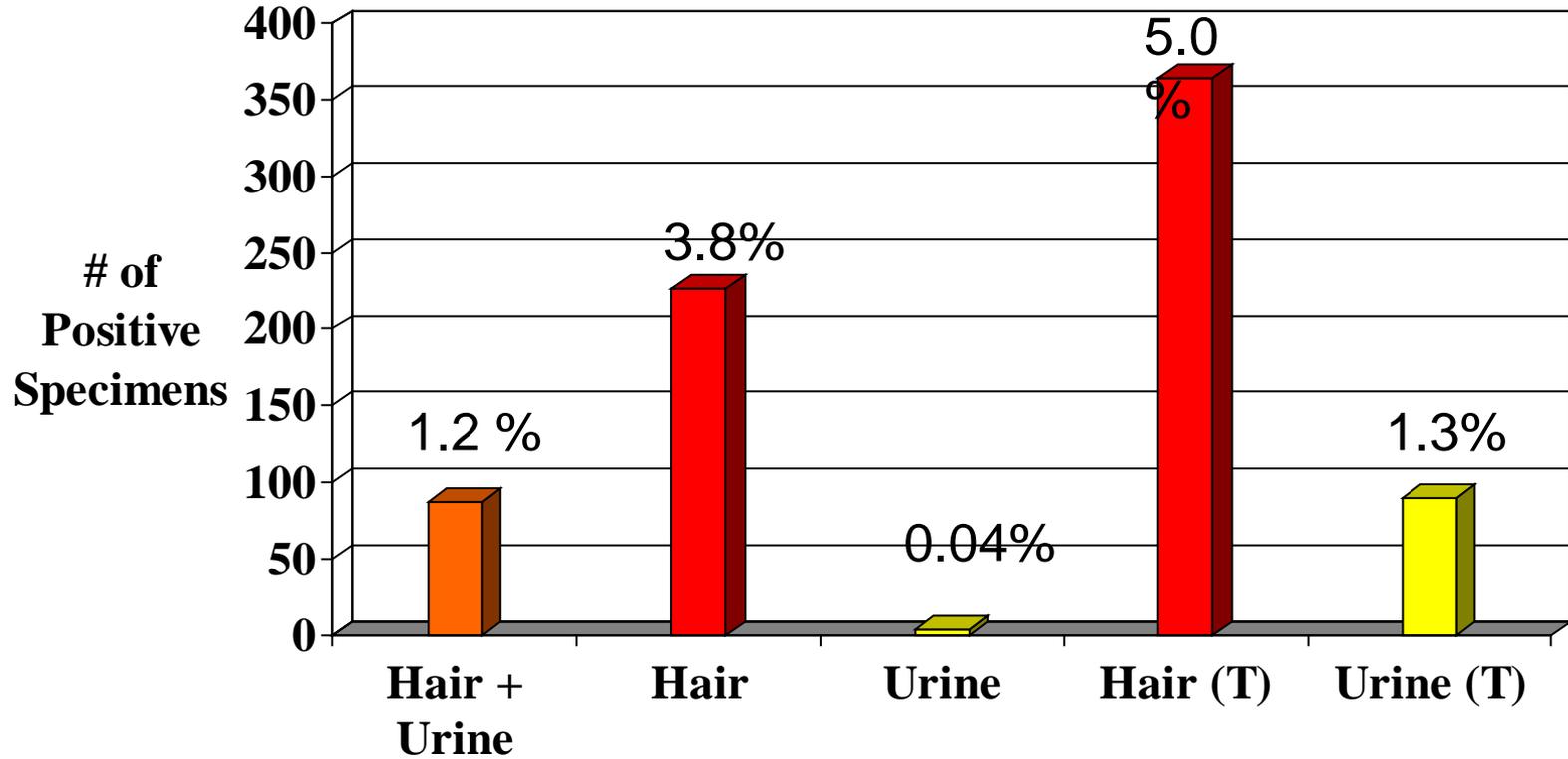




# Amphetamines

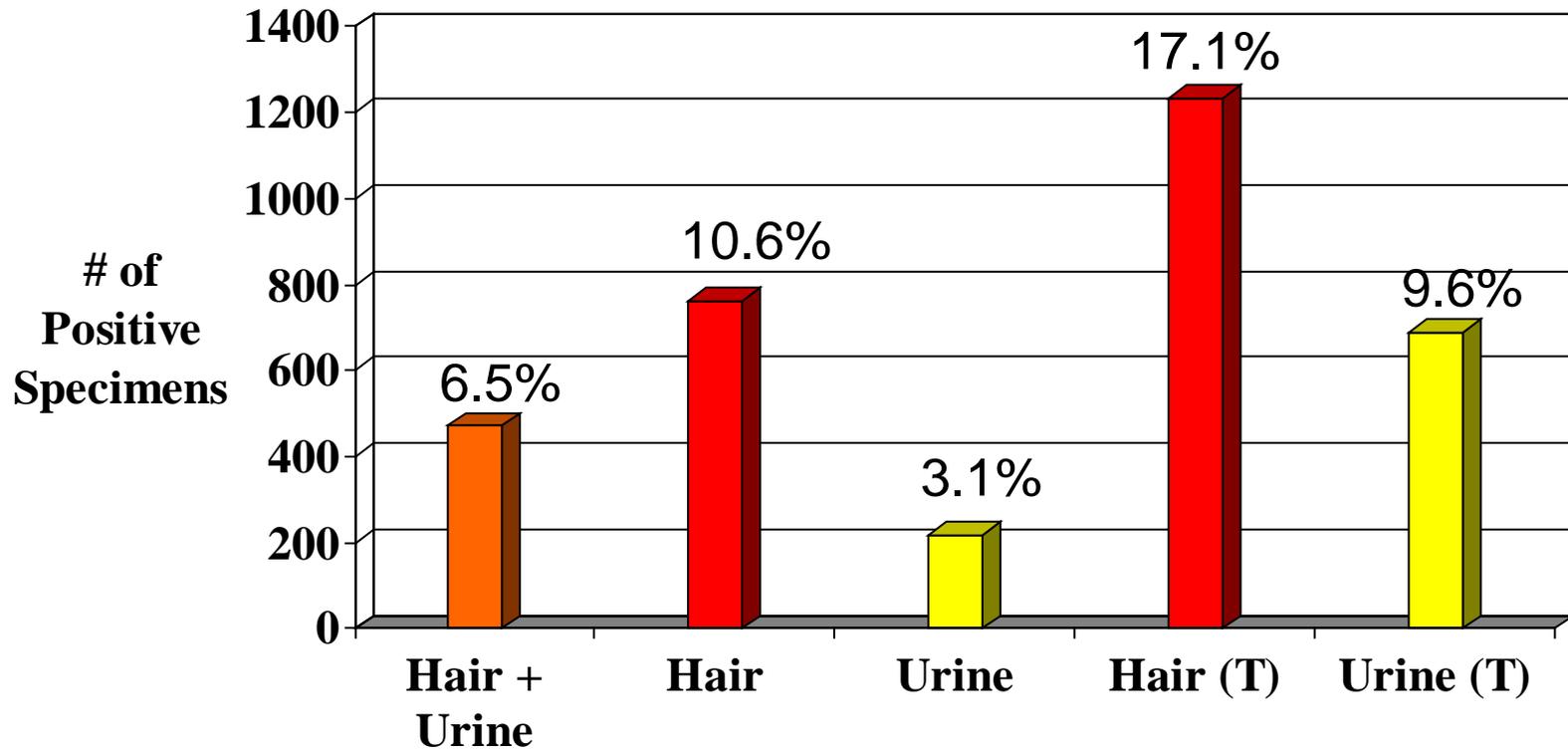


# Cocaine





# Total : All Drug Categories (Combined Hair + Urine Profile)





# Conclusions

- Drug Positive Rates: Higher in Hair v. Urine
- Most Dramatic Differences : Cocaine and Methamphetamine
  - Pharmacokinetics
  - Drug Use Patterns
- Combination of Both Matrices: Highest Detection Rate



# INTERPRETATION ISSUES



# Two Major Roadblocks: Interpretation ??????

- EXTERNAL CONTAMINATION
  - Use or Exposure?
  - Many claims of exposure
    - Marijuana
    - Cocaine
  - Hair Positives in Children
  - Published studies showing Contamination
  - Approaches to Combat
    - Wash Procedures
    - Metabolite Ratios
- HAIR COLOR BIAS
  - Melanin Content
  - Positives in Gray Hair
  - Published studies showing Bias
  - Approaches to combat claims
    - Statistics
    - Procedural



# Methamphetamine Exposure Results

- 80 specimens: Methamphetamine only (no metabolite detected)
- 3 specimens: Amphetamine only
- 14 specimens :
  - Methamphetamine Detected
  - Amp < 50 pg/mg
- 20 specimens :
  - Methamphetamine > 300 pg/mg and
  - Amphetamine >50 pg/mg



## 20 “Exposure” Specimens: Methamphetamine “Reportable”

- 2 Specimens
  - 5 month and 7 month old
  - Prenatal Exposure?
- 15 specimens
  - Ages 1-5 (8- 2 year olds)
  - Passive Ingestion?
- 1 specimen
  - 7 year old
- 2 specimens
  - 11 and 12 year old
  - Drug Use?



# Methamphetamine Adult Use Results

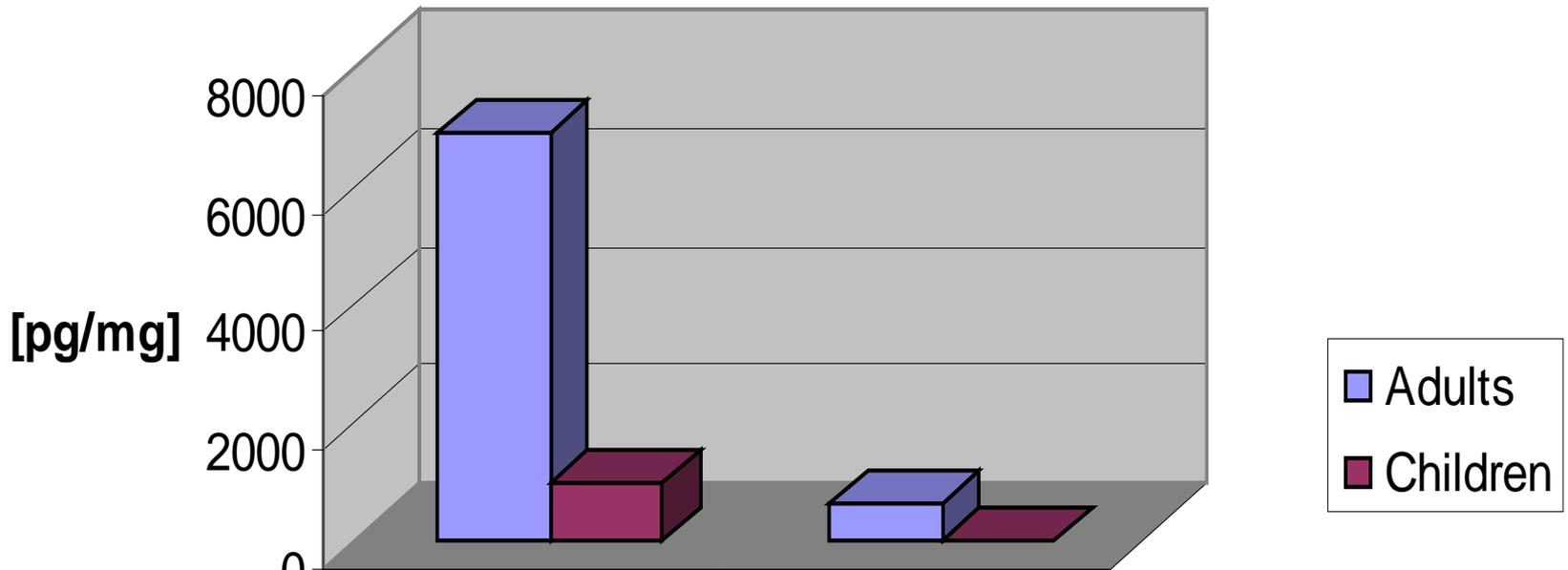
- 110 specimens met reporting criteria
- 6 specimens: amphetamine < 50 pg/mg
- 1 specimen: no amphetamine detected



# Methamphetamine Parent / Metabolite Ratios: Adult v. Children (Exposure)

	<u><b>Methamphetamine</b></u>	<u><b>Amphetamine</b></u>
<b>CHILDREN:</b>		
Mean (pg/mg)	979	27
range	19-10,598	0-398
n =	114	114
ratio	36:1	
<b>ADULTS:</b>		
Mean (pg/mg)	6944	663
range	204-62,170	0-8,083
n =	117	117
ratio	10:1	

## Methamphetamine in Hair: Adults v. Children



Adults	6944	663
Children	979	27



# Marijuana (THC) Exposure

- 126 requests for directed analysis for THC in children's hair (01/03-06/03)
- 104 samples positive for THC
- Mean Age = 4.15 years
- Age range (3 months to 16 years)



## Marijuana (THC) Exposure Results

- 94 specimens: THC only (no metabolite detected)
- 1 specimen:
  - THC < 5.0 pg/mg
  - THCA > 0.1pg/mg
- 4 specimens
  - THC Detected
  - THCA < 0.10 pg/mg
- 5 specimens :
  - THC > 5.0 pg/mg and
  - THCA > 0.1 pg/mg



## 5 “Exposure” Specimens: Marijuana “Reportable”

- 2 Specimens
  - 14 year old male and 16 year old female
  - Drug Use?
- 3 specimens
  - Ages 1-3
  - High Parent THC concentrations
  - Passive Ingestion?
  - 424.9/0.36; 188.5/0.10; 143.6/0.26



# Marijuana (THC) Adult Use Results

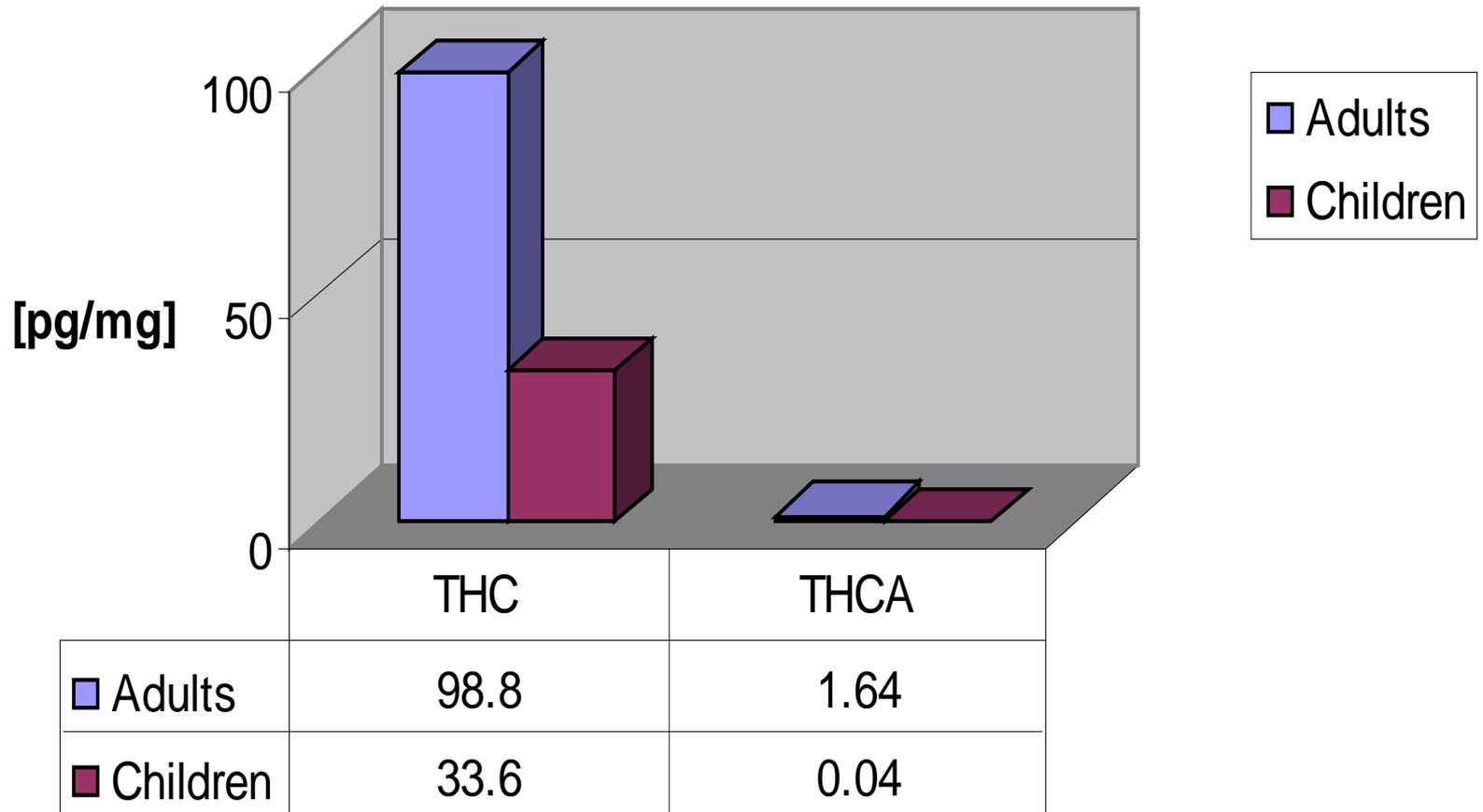
- 88 specimens met reporting criteria
- 15 specimens: no THC metabolite detected
- 1 specimen: THC metabolite below reporting cut-off



# Marijuana Parent / Metabolite Ratios: Adult v. Children (Exposure)

	<u>THC</u>	<u>THCA</u>
<b>CHILDREN:</b>		
Mean (pg/mg)	33.6	0.04
range	0.5-464.9	0-2.5
n =	104	104
ratio		840:1
<b>ADULTS:</b>		
Mean (pg/mg)	98.8	1.6
range	0-643.2	0-17.0
n =	104	104
ratio		62:1

## Marijuana in Hair Adults v. Children





# Interpretation ??????

- How many times did the person use in the last 90 days?
- Is the use decreasing, increasing or remaining the same?
- The hair test was negative but the person admits to taking X?
- The hair test was positive but the person says “no way...I don’t do drugs”?
- Could “Y” cause a positive result for “X”?
- The person collecting my hair contaminated it with drug...?
- How long does “X” stay in body hair? What’s the timeline?



Thank you. Questions?

[james.bourland@alere.com](mailto:james.bourland@alere.com)



[aleretoxicology.com](http://aleretoxicology.com)