Expanded Panel Drug Testing

Opiates/Opioids, Hallucinogens,
Benzodiazepines,
Barbiturates, Designer Drugs

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Disclosure

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Employer Solutions

Quest Diagnostics

NSDUH

- The National Survey on Drug Use and Health
- Annual survey of the civilian, noninstitutionalized population of the United States aged 12 years old or older.
- Self reported illicit (illegal and non-Rx) drug, alcohol and tobacco use
- Data from NSDUH provide information on illicit drug use, alcohol use, substance use disorder (SUD), substance use treatment, reasons for not receiving substance use treatment, mental health issues (including mental health service use), and co-occurring SUD and mental health issues.
- The NSDUH interviews approximately 67,500 persons annually.

2019 NSDUH (past month)

- 35.8 million people, 13% (mainly driven by marijuana and opioids) of the US population admitted current (past month) illicit drug use, including:
  - Marijuana – 31.6 million (11.5%)
  - ***Prescription*** Pain Relievers – 2.8 million (1.0%)
  - Cocaine – 2.0 million (0.7%)
  - ***Prescription*** Pain Tranquilizers or Sedatives – 2.0 million (0.7%)
  - Hallucinogens (e.g. LSD, MDMA, PCP) – 1.9 million (0.7%)
  - ***Prescription*** Stimulants – 1.6 million (0.6%)
  - Methamphetamine – 1.2 million (0.4%)
  - Inhalants - 0.8 million (0.3%)
  - Heroin - 0.43 million (0.1%)

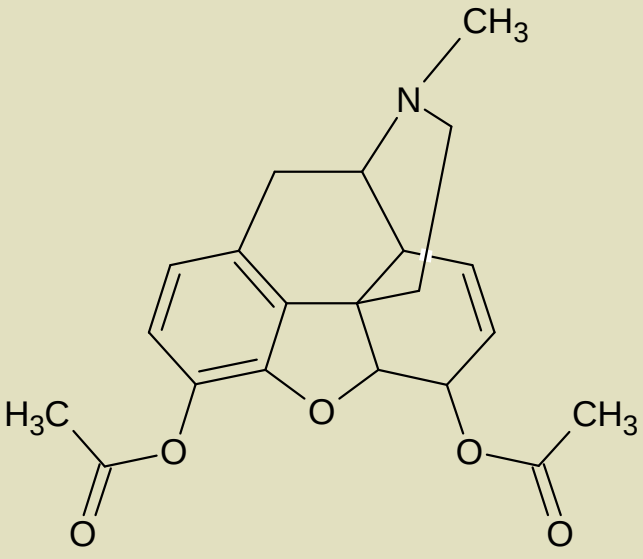
2019 NSDUH (past year)

- 57.2 million people, 20.8% of the US population admitted past year illicit drug use, including:
  - 4.3 million adolescents aged 12-17
  - 65.8 million aged 12 or older were ***past month*** binge drinkers, including 1.2 million adolescents
- 20.4 million (7.4%) aged 12 or older had an SUD (alcohol, illicit drug use, or both)
- 1.1 million (4.5%) aged 12-17 had an SUD
- 4.8 million (14.1%) aged 18-25 had an SUD
- 14.5 million (6.7%) aged 26 or older had an SUD

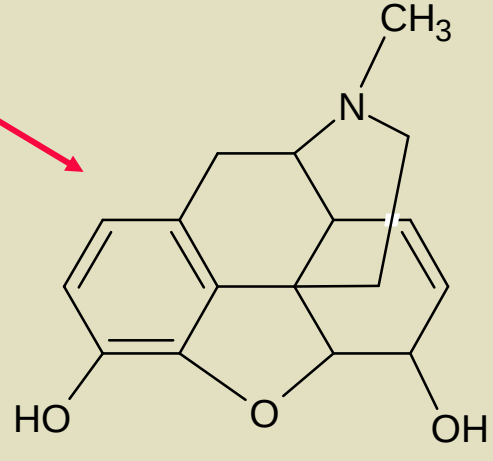
Opiates

- Opiates – refers to any opiate drug and those that share many of the properties of the opiates.
- Derived from the phenanthrene group of alkaloids found in the opium poppy
  - Codeine
  - Morphine
  - Heroin
- Semi-Synthetic – refers to those opiates that are synthesized from naturally occurring opiates (i.e. thebaine)
  - Hydrocodone/Hydromorphone
  - Oxycodone/Oxymorphone
- Opiates vs. Opioids
  - Opiates have a similar structure and are derived from the opium poppy
  - ***Opioids*** are all compounds that act at the opiate receptor, but may not have classical opiate structure (e.g. fentanyl)

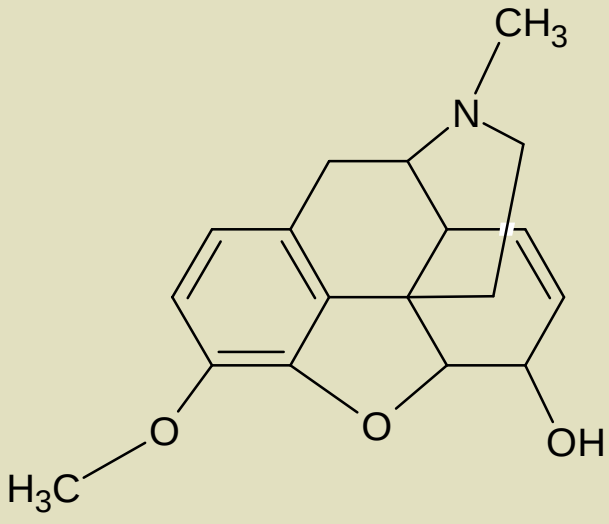
Heroin-Codeine-Morphine Relationship



Heroin



Morphine



Codeine

Opioids

Methadone
Dihydromorphine
Buprenorphine (Buprenex)

Hydromorphone (Dilaudid)

Hydrocodone (Vicodin)

Oxymorphone (Numorphan)

Oxycodone (Percodan, Percocet, OxyContin)
Tramadol (Ultram)
Fentanyl (Sublimaze, Actiq)

Nalbuphine (Nubain)

Remifentanil (Ultiva)

Pentazocine (Talwin)
Dextramoramide
Levorphanol (Levo-Dromoran)
Alfentanil (Alfenta)

- Dezocine (Dalgan)
- Butorphanol (Stadol)
- Meperidine (Demerol)

Opioids and Drug Testing 1

Why do we test for opioids in the workforce?

- Many cognitive and psychomotor functions are impaired
- Opioids are potent CNS depressants
- Their use poses a potential risk associated with safety sensitive jobs
- The use of illicit opioid drugs is associated with theft and distribution

Opioids and Drug Testing 2

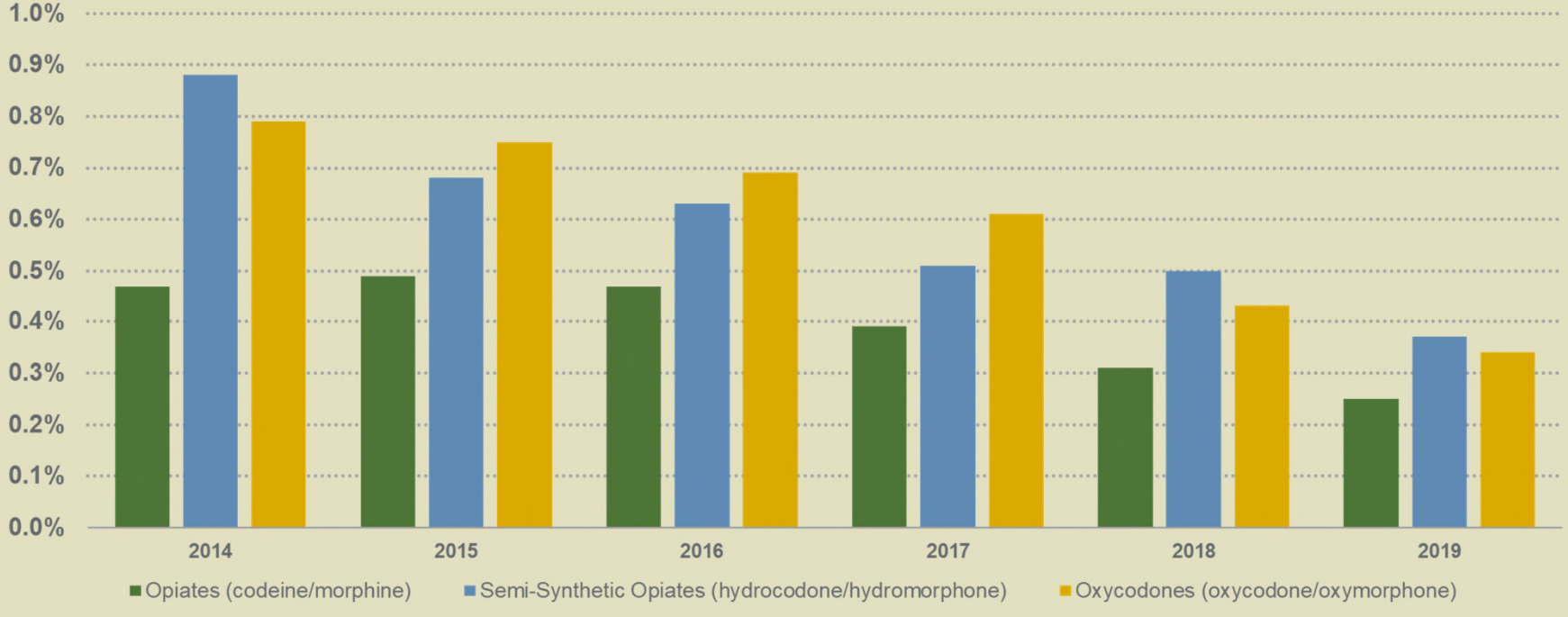
- How do we test for opioids in the workforce?
  - The HHS Mandatory Guidelines have traditionally required an immunoassay for initial testing and GC-MS for confirmatory testing and since 10/2008, other chromatographic-mass spectrometric (e.g. LC-MS/MS) technology for confirmation.
  - The current revised (10/2017) HHS Guidelines continue to permit immunoassay for initial testing, and now also allow confirmatory technologies that use chromatography coupled with mass spectrometry (LC-MS, GC-MS/MS, LC-MS/MS) for both the initial and confirmatory test.

Opioids and Drug Testing 3

Which opiates do we test for DOT Testing?

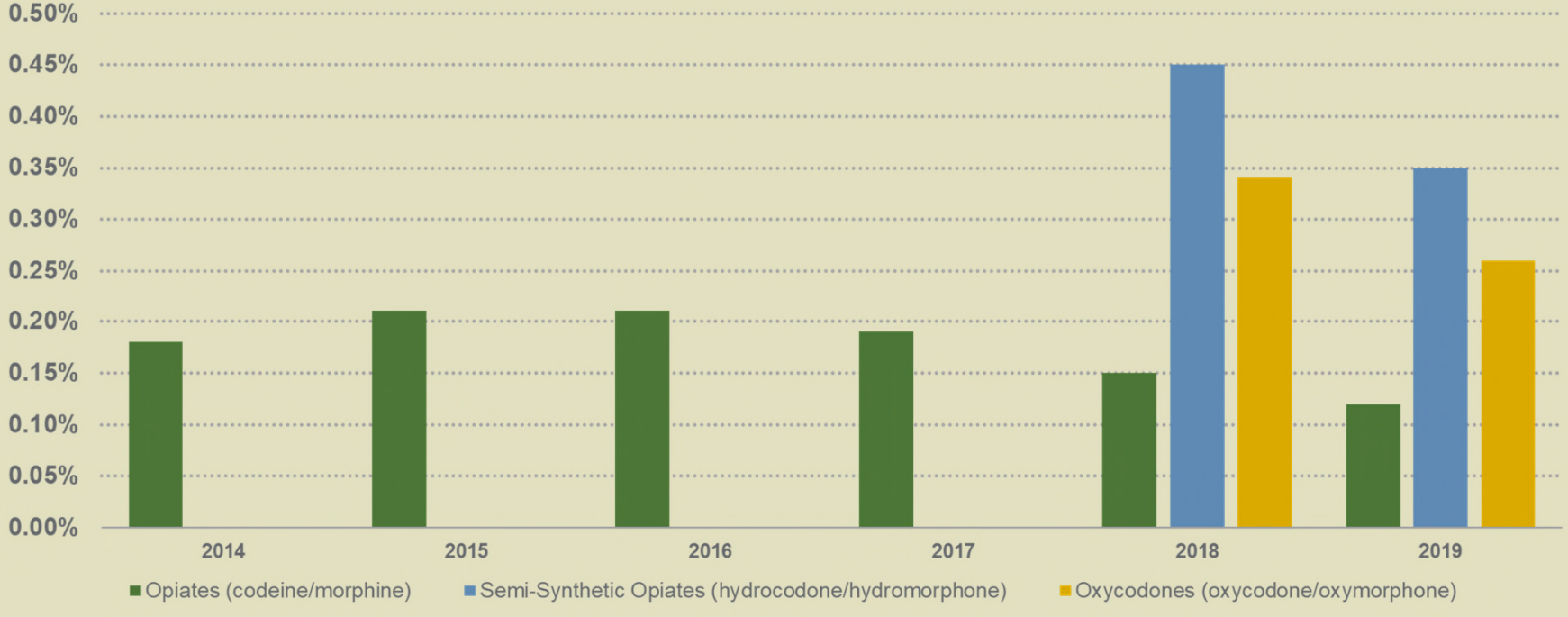
- Codeine, Morphine, 6-acetylmorphine
- Eff. 1/2018, also hydrocodone, hydromorphone, oxycodone, oxymorphone

Opiates in urine general U.S. workforce tests



- Source: Quest Diagnostics Drug Testing Index Full Year 2019

Opiates in urine federally mandated, safety-sensitive workforce tests



- Source: Quest Diagnostics Drug Testing Index Full Year 2019

Drug Testing Technology OVERVIEW

EIA

Enzyme immunoassay (EIA)

GC/MS

Gas chromatography/mass spectrometry (GC/MS) is the "gold standard" of the industry and provides highly accurate test results.

LC-MS/MS

Liquid chromatography/tandem mass spectrometry is the ”platinum standard" of the industry and provides highly accurate test results.

Immunoassays: Presumptive results

- **Immunoassay Technology Products**
  - POCT Test Cups and Strips
  - Enzyme Immunoassay (EIA Lab Based using instrumentation)
  - ELISA (Enzyme-Linked Immunosorbent Assay)
  - RIA (Radio Immunoassay)
  - CEDIA (Cloned Enzyme Donor Immunoassay)
  - FPIA (Fluorescent Polarization Immunoassay)

Testing Overview

- Clinical Toxicology Lab Protocols

1. Screen by Immunoassay and Confirm the EIA positive by GC-MS or LC-MS/MS
2. Analyze on LC-MS/MS only
3. Simultaneously analyze by EIA and LC-MS/MS

Testing Overview II

- **Lab Reflexes Immunoassay to LC-MS/MS:**

What this protocol may miss:

- Longer Detection window
- Hydromorphone
- Lorazepam
- Clonazepam
- Buprenorphine
- Pain Management drugs

Limitations of Immunoassay

- Limited Test Menu
- Limited specificity (cannot identify specific drugs within drug class; examples, opiates, benzodiazepines)
- Limited or no sensitivity for specific drugs within certain drug classes (examples, clonazepam and lorazepam does not detect; hyromorphone only gives positive at very high concentrations)
- No Quantitative Value
- Does not provide the detection of drug and its metabolites to ‘detect pill scraping’
- False Negatives and False Positives
- Cutoff Level much higher for zero-tolerance detection
- **The Lower the cutoff, the longer the detection Window**

Limitations of Immunoassay: Cutoff

- Cutoffs may not provide adequate sensitivity
- Example:
    1. Positivity for cocaine increased 33% when SAMHSA lowered the cutoff from 300 ng/mL to 150 ng/mL
    2. Amphetamine positivity increased 26% when SAMHSA lowered the cutoff from 1000 ng/mL to 500 ng/mL

The Lower the cutoff concentration, the longer the detection window

Alere Toxicology data: 90,000 specimens:

- Thirty five (35%) percent of the cocaine metabolite positives were below POCT and EIA cutoff and we would have missed **1104** specimens.
- Thirty four(**34%) percent of methamphetamine** positive were below POCT and EIA cutoff and we would have missed **254** specimens.
- One thousand two hundred twenty five (**1225) heroin** (6-AM) where identified by LC-MS/MS and no POCT for 6-AM
- *Total **2583** positive specimens would have been missed if only POCT were performed and this is **2.58% of the total specimens***

Opioids and Drug Testing 4

Which opioids can we test ?

- Methadone
- Fentanyl
- Tramadol
- Hyrocodone
- Hydromorphone
- Oxycodone
- Oxymorphone
- Meperidine

Opioids and Drug Testing 5

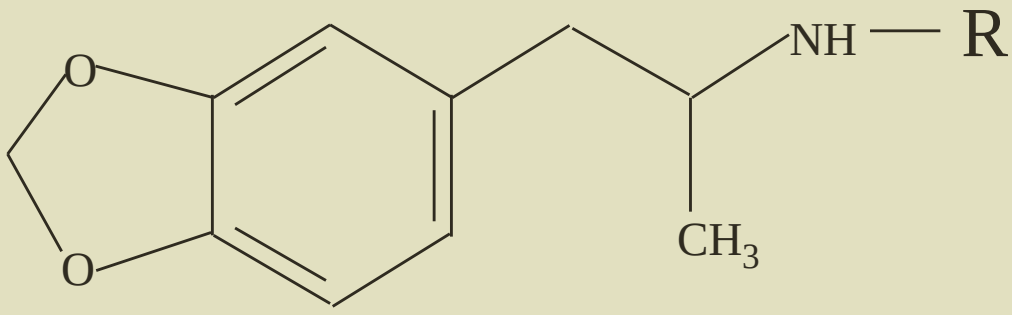
How are opioid testing results interpreted?

- Test results for methadone, fentanyl, tramadol, dihydromorphine, meperidine, hydrocodone, hydromorphone, oxycodone and oxymorphone should follow MRO guidelines
- Verification of prescription and prescription history, (e.g., what other drugs are being taken?)
- Consider fitness-for-duty evaluation, even though tolerance develops with chronic use of opioids

Drug Testing
Expanded Panel

Opiates, **Hallucinogens**,
Benzodiazepines,
Barbiturates

MDMA, MDA, MDEA



R	Drug
- CH_3	MDMA, Methylenedioxymethamphetamine
- H	MDA, Methylenedioxyamphetamine
- CH_2CH_3	MDEA, Methylenedioxyethylamphetamine

MDMA, MDA, MDEA

- **MDMA**: hallucinogen; derivative of methamphetamine first synthesized in 1914
- **MDA**: derivative of amphetamine first synthesized in 1910; central stimulant, may be hallucinogenic in large doses; minor metabolite of MDMA
- **MDEA**: hallucinogen; close chemical analogue of MDMA
- DEA (2005): About 10% of seized MDMA-positive samples also contain MDA and MDEA, while another 10% contain amphetamine, methamphetamine, or both.

MDMA Background 1

- Patented by Merck in 1914; initially intended as an appetite suppressant
- Also induces psychomotor agitation that can be pleasurably relieved by dancing - the ideal party drug.
  - "Empathy drug"
  - Most profound effect appears to be the experience of intense emotions and perception of experiencing the emotions of others
  - Use of MDMA and LSD flourished amongst "Dead Head" followers of Grateful Dead concerts who attempted to achieve a spiritual state valuing beauty, generosity and love

From: R. Millman and A.B. Beeder, *The New Psychedelic culture: LSD, Ecstasy, "Rave" Parties and The Grateful Dead,"* in *Psychiatric Annals*, 24:148-150 (1994).

MDMA Background 2

- Widespread use in early ‘80s by partygoers, “new age seekers,” and psychotherapists
- Regulated by DEA on Schedule I in 1985 along with other psychedelic drugs
- In the ’90s, popular “club drug” used in nightclubs and dance parties (raves)
- Recent resurgence in use among U.S. teens (self-reported past year use up 67% from 2008 to 2009)*

MDMA Tablets with “man-in-the-moon” design.

*2009 Partnership for a DrugFree America/MetLife Foundation Attitude Tracking Study (PATS)

MDMA Pharmacology – Human 1

- Usually taken as oral dosage of 100-150 mg
- Initial “amphetamine-like rush” 20 - 40 min
- Causes serotonin release, blocks reuptake

From: D.M. McDowell and H.D. Kleber, *MDMA: Its History and Pharmacology*, in *Psychiatric Annals,* 24(3):127-130, 1994.

MDMA Pharmacology – Human 2

- Peak effect of "world relatedness" at 3-4 hours - other subjective effects during the plateau phase
  - altered time perception
  - increased ability to interact with others
  - decreased defensiveness and aggression
  - changes in visual perception and speech
  - increased awareness of emotions
  - decreased obsessiveness, restlessness, impassivity

From: D.M. McDowell and H.D. Kleber, *MDMA: Its History and Pharmacology*, in *Psychiatric Annals,* 24(3):127-130, 1994.

MDMA Pharmacology – Human 3

- Duration of general effects
  - MDA 10-12 hours with pronounced day-after sluggishness
  - MDMA 4-6 hours with gentler effects and less day-after fatigue

From: D.M. McDowell and H.D. Kleber, *MDMA: Its History and Pharmacology*, in *Psychiatric Annals,* 24(3):127-130, 1994.

MDMA Pharmacology – Human 4

- After effects last 24 hr or more
  - profound anhedonia (loss of pleasure feeling)
  - side effects include bruxism, trismus (jaw clenching), disphoresis, anorexia
  - at high doses adverse effects include suspicion and paranoia, tachycardia, psychosis and violence
  - long term effects include memory loss, confusion, fatigue, sleep dysfunction, aggravation of pre-existing medical conditions

From: D.M. McDowell and H.D. Kleber, *MDMA: Its History and Pharmacology*, in *Psychiatric Annals,* 24(3):127-130, 1994.

MDMA Analytical Methods 1

- MDMA cross-reacts with many amphetamine class immunoassays at the 1000 ng/mL cutoff:
  - Roche Online 197%
  - ThermoFisher DRI 76.9%
  - ThermoFisher CEDIA 69%
  - Siemens EMIT II Plus 2.9%

MDMA Analytical Methods 2

- Specific MDMA immunoassays are available:
  - Siemens EMIT II Plus Ecstasy
  - ThermoFisher DRI Ecstasy
  - ThermoFisher CEDIA Amphetamine/Ecstasy

Workforce Drug Testing

- MDMA and MDA are routinely tested in current workforce testing
- Lower cutoff amphetamines assays and specific MDMA immunoassay reagents are now available, greatly improving detection
- HHS Mandatory Guidelines require MDMA and MDA as initial test analytes, and MDMA and MDA as confirmatory test analytes.

Drug Testing
Expanded Panel

Opiates, Hallucinogens,
Benzodiazepines,
Barbiturates

Regulation of Benzodiazepines

- DEA Schedule IV (limited abuse potential)
- Excluded from HHS Guidelines and DOT Regulations - not considered “illegal” drugs

Benzodiazepines

Four Therapeutic Actions

- Anxiolytics (tranquilizers)
  {e.g., Chlordiazepoxide, Alprazolam}
- Sedative-hypnotics (sleep inducers)
  {e.g., Triazolam}
- Anticonvulsants (anti-epileptics)
  {e.g., Clonazepam}
- Muscle relaxants {e.g., Diazepam}

Pharmacological Parameters

Generic Name (Brand)	Half-Life (hrs.)	Therapeutic Dose (mg/day)	Plasma Conc. (ng/mL)	Detectable Urinary Drug/Metabolites
Diazepam (Valium)	21-37	< 40	< 1500	Nordiazepam Oxazepam Temazepam
Chlordiazepoxide (Librium)	16-27	< 100	< 1600	Norchlorodiazepoxide Demoxepam Nordiazepam Oxazepam
Alprazolam (Xanax)	13-16	≤ 6	< 55	a-Hydroxyalprazolam 4-Hydroxyalprazolam a,4-Dihydroxyalprazolam
Triazolam (Halcion)	1.8-3.9	< 0.5	< 10	1-Hydroxymethyltriazolam 4-Hydroxytriazolam

Newer Benzodiazepines

- Shorter half-lives
- Lower therapeutic dosages
- Lower plasma concentrations
- Significant chemical differences
- Significantly different profile of metabolites

Benzodiazepines

Type	Drug	Detection Time in Urine
Long-Acting	Diazepam	Weeks or months after chronic use
	Nordiazepam	
	Chlordiazepoxide	
	Oxazepam	
Short-Acting	Alprazolam	A few days, if at all
	Triazolam	

Benzodiazepines and their Major Metabolites

After Hydrolysis using ß-Glucuronidase

Generic Name	Brand Name	Urinary Metabolite
Oxazepam	Serax	Oxazepam
Temazepam	Restoril	Temazepam Oxazepam
Lorazepam	Ativan	Lorazepam
Flurazepam	Dalmane	Hydroxyethylflurazepam Desalkylflurazepam
Chlordiazepoxide	Librium	Oxazepam Nordiazepam

Benzodiazepines and their Major Metabolites

After Hydrolysis using ß-Glucuronidase

Generic Name	Brand Name	Urinary Metabolite
Diazepam	Valium	Temazepam Nordiazepam Oxazepam
Alprazolam	Xanax	a-Hydroxyalprazolam
Triazolam	Halcion	a-Hydroxytriazolam
Midazolam	Versed	a-Hydroxymidazolam
Prazepam	Centrax Verstran	Oxazepam 3-Hydroxyprazepam

Benzodiazepines and their Major Metabolites

After Hydrolysis using ß-Glucuronidase

Generic Name	Brand Name	Urinary Metabolite
Clonazepam	Klonopin Rivotril	7-Aminoclonazepam
Clorazepate	Tranxene	Oxazepam Nordiazepam
Quazepam	Doral	N-Desalkyl-2-Oxoquazepam 3-Hydroxy-2-Oxoquazepam 3-Hydroxy-N-Desalkyl-2-Oxoquazepam
Estazolam	ProSom	4-Hydroxyestazolam 1-Oxoestazolam

Analytical Issues

- Most immunoassays calibrated with Oxazepam or Nordiazepam; excellent cross-reactivity with other benzodiazepines
- Most confirmatory tests minimally detect
  - Oxazepam
  - Nordiazepam
  - Temazepam
  - Alpha-hydroxyalprazolam
  - Diazepam

POCT misses 50% of Benzodiazepines

- Limitations of Benzodiazepine Immunoassay:
  - Clonazepam (metabolite) does not trigger positive
  - Lorazepam does not trigger positive
  - Oxazepam glucuronide does not trigger positive

Analytical Issues

- Other benzodiazepines (or those not metabolized to these compounds) are often not reported unless tested by LC-MS/MS technology, such as:
  - Flurazepam
  - Lorazepam
  - Clonazepam
  - Triazolam

Analytical Issues

- Wide range of benzodiazepine chemical differences may miss some of the benzodiazepines by immunoassay.
- The best method to detect all the bezodiazepines is LC-MS/MS
- Assignment of cut-off concentrations difficult, but most use 200 or 300 ng/mL
- Potential for misidentification of which drug was actually taken due to overlapping metabolic pathways

Explanation for Some Laboratory Positives for Urinary Benzodiazepines

- Unexplained incidences of some GC-MS confirmed positive results for diazepam
- MRO inquiries revealed no prescription benzodiazepine intake, but rather self-administration of Chinese herb pills
  - *Cows Head Pills*
  - *Miracle Herb Pills*
  - *PotentSex Pills*
- Results of GC-MS analysis of pills obtained by mail-order from Chinese health food stores confirmed diazepam and the NSAID diclofenac in these pills

From: E.D. Lykissa and S.E. Harris, abstract presented at TIAFT/SOFT Annual Meeting, Oct. 1994, Tampa, FL.

Black Pearls

- Pearl-sized black or brown pills; may have a spicy pungent aroma
  - *Tung Sheuh Pills*
  - *Chuifong Toukuwan ("chase-the-wind-from-the-bones")*
- Sold in packages of 50-100 pills; writing in Chinese characters; may have a brief explanation in English
- Ingredients list contains 20 to 23 herbs
- Recommended for strengthening bones and muscles, stimulating circulation, causing the muscles and joints to relax
- Dose: 6 to 8 pills, twice daily; available in oriental food market, mail order or door-to-door sales.

International Incidents of Black Pearls

Use Reported in:
England, Holland, Japan, Canada, Australia

Undeclared Drugs Present in Black Pearls	
Acetaminophen	Hydrochlorothiazide
Aminopyrine	Indomethacin
Chlordiazepoxide	Mefenamic Acid
Clorzoxazone	Methyltestosterone
Dexamethasone	Phenylbutazone
Diazepam	Prednisone
Diclofenac Sodium	

Drug Testing
Expanded Panel

Opiates, Hallucinogens,
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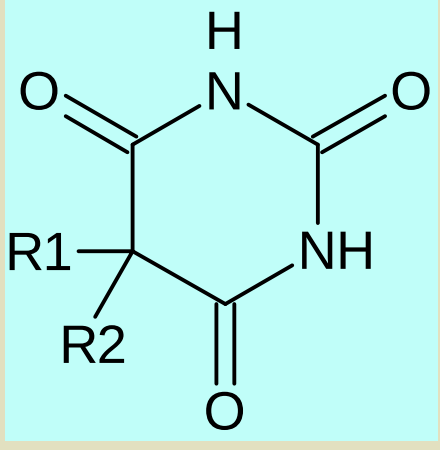
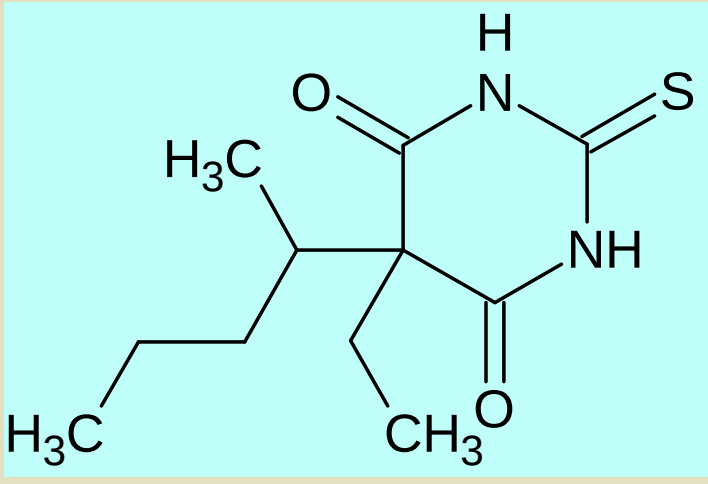
Barbiturates

Barbiturates: Structures, Names and DEA Scheduling

Representative Barbiturate



Thiopental =



Name	R_1	R_2	DEA Schedule
Amobarbital	- C_2H_5	- $CH_2CH_2CH(CH_3)_2$	II
Butalbital	- $CH_2CH{=}CH_2$	- $CH_2CH(CH_3)_2$	III
Pentobarbital	- C_2H_5	-$CH(CH_3)C_3H_7$	III
Phenobarbital	- C_2H_5	- C_6H_5	IV
Secobarbital	-$CH_2CH{=}CH_2$	-$CH(CH_3)C_3H_7$	II
Thiopental (see above)	-$CH(CH_3)C_3H_7$	-C_2H_5	III

Pharmacologic Parameters

Generic Name	Class	Plasma Half- Life
Amobarbital	Intermediate acting	24 hours
Bultalbital	Short acting	6.5 hours
Pentobarbital	Short acting	20 – 30 hours
Phenobarbital	Long acting	4 days
Secobarbital	Short acting	22 – 29 hours
Thiopental	Ultrashort acting	6 – 26 hours

Barbiturates Today

- Anxiolytic and Muscle Relaxant/Analgesic:
  - Butalbital + Salicylates + Caffeine (Fiorinal®)
  - Butalbital + Acetaminophen + Caffeine (Fioricet®)
- Anti-Convulsants:
  - Mephobarbital
  - Phenobarbital

Barbiturates

Type	Detection Time in Urine, Primarily Parent Drug
Long acting -Phenobarbital	Several weeks after chronic use
Intermediate acting	2 – 4 days
Short and Ultrashort acting	1 day or less, if at all

Barbiturate Analysis

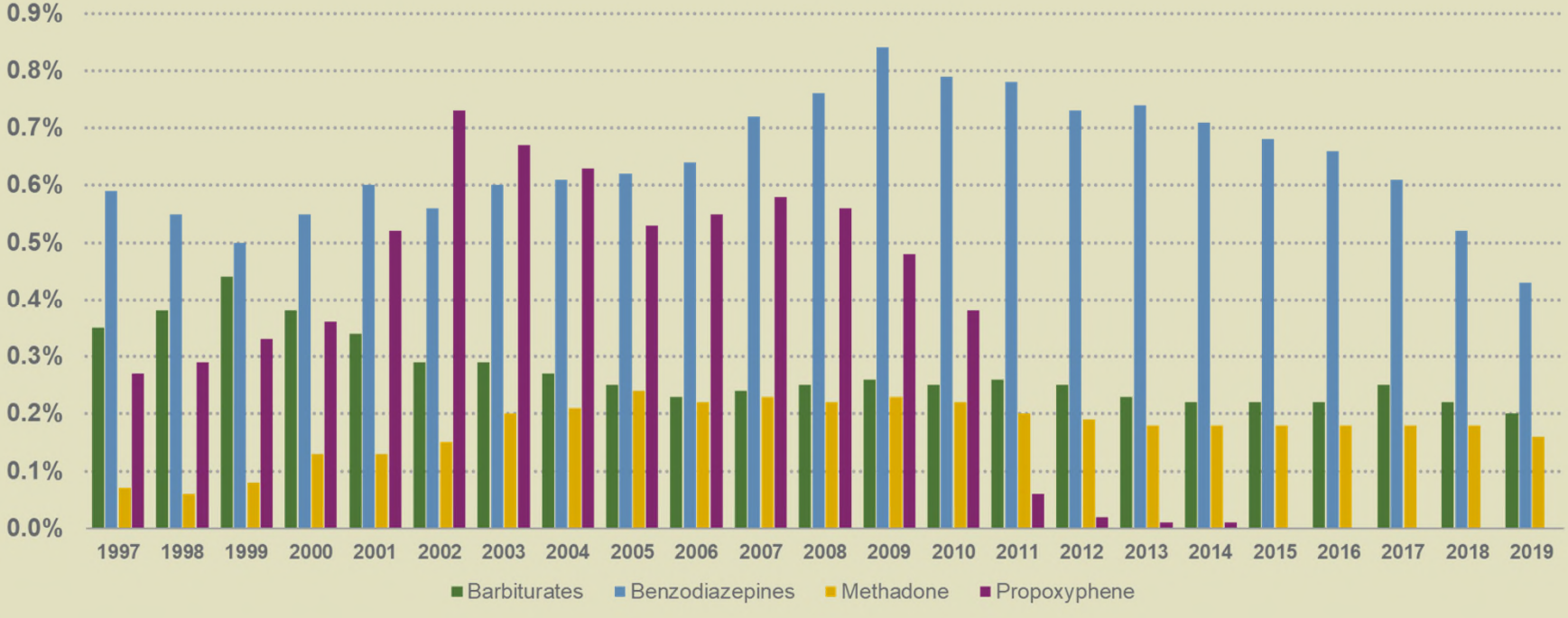
- Immunoassay usually designed for Secobarbital at either 200 or 300 ng/mL cutoff with wide cross-reactivity to other barbiturates
- Confirmatory: Small molecule, so small number of diagnostic ions
- Most confirmatory test panels include:

Amobarbital	Phenobarbital
Butalbital	Secobarbital
Pentobarbital	Butabarbital (less common)

Workforce Drug Testing

- Positive rate in workforce extremely low
- Confirmation rare for other than Butalbital and Phenobarbital
- Result interpretation - vast majority of laboratory positives due to prescription use.
- Diminished return of positive test results does not justify cost of test, especially in a deterrence program; not tested under HHS Guidelines or DOT Regulations.
- A number of companies test for barbiturates in safety-sensitive positions.

Drug Summary – General Workforce



Source: Quest Diagnostics Drug Testing Index™

- Source: Quest Diagnostics Drug Testing Index Full Year 2019

Conclusion

- Drug Abuse is changing:
- Marijuana to “K2”-Species
- Methamphetamine to “Bath Salts”
- Prescription Opioids abuse is higher than heroin
- Challenging:
  - Law enforcement officials
  - Toxicologists
  - MROs
  - Law makers