Learning Objectives

• Become familiar with the OSAC/NAME recommendations and guidelines
• Know when to order toxicology labs
• Know the best locations for samples
• Know what to include in a toxicology analysis
• Know how to report the toxicology results
Medicolegal Death Investigation Subcommittee Recommendations

• Complete autopsy is necessary for optimal interpretation of toxicology results, which must also be considered in the context of the circumstances surrounding death, medical history, and scene findings

• A complete scene investigation extends to reconciliation of prescription information and pill counts

• Blood, urine, and vitreous humor when available, should be retained in all cases

• A toxicological panel should be comprehensive and include opioid and benzodiazepines, as well as other potent depressant, stimulant, and anti-depressant medications

• Interpretation of postmortem opioid concentrations requires correlation with medical history, scene investigation, and autopsy findings

• If death is attributed to any drug or combination of drugs, the certifier should list all the responsible substances by generic name in the autopsy report and on the death certificate
Need for Autopsy

• Even the fastest forensic toxicology laboratory will not allow for results in advance of making a decision to autopsy.

• There have been attempts to use screening point of service kits, but many of these are too specific, not made for available specimens and will miss some of the new emerging substances.
External Examination

- The external examination documents identifying features, signs of or absence of disease and trauma, and signs of death.
- Recording identifying features provides evidence for or against a putative identification.
- Recording signs of disease and trauma is a primary purpose of the forensic autopsy.
- Recording rigor mortis documents a sign of death that cannot be captured by photography.
- Recording livor mortis helps to answer later questions about bruises and body position.
- Notation of postmortem artifacts is useful for interpretation of subsequent forensic autopsy findings.
X-Ray and Photography

- It is highly desirable to have facilities available to photograph and x-ray any findings of particular interest.
- Photographic documentation complements written documentation of wounds and creates a permanent record of forensic autopsy details.
- Photographic documentation of major wounds and injury shall include a reference scale in at least one photograph of the wound or injury to allow for 1:1 reproduction.
- Radiographs detect and locate foreign bodies and projectiles. Charred remains have lost external evidence of penetrating injury and identifying features.
Injection Sites

• Needle track marks
• Recent needle punctures
• Skin popping
• Upper and lower extremities
Direct toxic effects of Drugs

- Transient hypertension (cocaine)
- Intracerebral hemorrhage
- Aortic dissection
- Acute cardiac necrosis
- Arrhythmia
- Cardiorespiratory depression (opiates, ethanol, antidepressants)
- Biochemical imbalance (including ‘water overdose’ in ecstasy).
Chronic Indirect Effects of Drugs

Due to previous or current continuous drug use include:

- Natural disease arising due to drug use
- Cirrhosis (alcohol)
- Cardiac fibrosis (amphetamine and cocaine abuse)
- Infective endocarditis and mycotic aneurysm
- HCV, HBV and HIV infection
- Pulmonary hypertension
- Injection abscess
- Secondary amyloidosis
Subacute Direct Toxic Effects of Drugs

The inciting drug itself may not be detectable.

• These effects include:
  • Pulmonary edema
  • Hypoxic encephalopathy
  • Aspiration of gastric contents and inhalational pneumonia.
Central Nervous System

The major pathological finding was cerebral edema.

• As shown with fentanyl, acetylfentanyl, butyrylfentanyl, furanylfentanyl, ocfentanil, AH-7921, U-47700, MT-45.

• A case of fatal cerebral hemorrhage induced by acetylfentanyl was reported.

• Another case of a 19-month-old girl poisoned by a transdermal administration of fentanyl who developed leukoencephalopathy was described.
Cardiovascular System

• Uncommon intoxication symptom is chest pain mimicking acute coronary syndrome with non-specific T-wave changes on the electrocardiogram.

• Necessary to distinguish the alterations induced acutely by the drug from those due to pre-existing pathologies.

• Most of the observed cardiovascular pathological findings, such as hypertrophy, cardiomegaly, cardiac fibrosis, atherosclerosis, are not attributable to an acute intoxication but may be compatible with chronic drug intake.

• Pericardial petechiae can be interpreted as a generic sign of asphyxia, due to opioid-related respiratory failure.
Pulmonary

• The major pathological findings are pulmonary congestion and pulmonary edema, which are common to all the opioids.
• Signs found occasionally are petechiae on the pleura and aspiration of gastric contents inside the trachea and bronchi.
• A few cases of fentanyl patch aspiration have been reported, where the patch was found in the airways.
• Microscopically, small amounts of foreign material have been reported in the lungs, consistent with prior intravenous drug abuse.
Pulmonary

- The main effect of fentanyl and its analogues on the respiratory system is respiratory depression.
- Fentanyl can cause chest wall rigidity and apnea, particularly with rapid intravenous administration, a factor that can contribute to respiratory failure.
- Rare adverse effects after fentanyl usage include diffuse alveolar hemorrhage immediately after insufflating fentanyl powder.
Pulmonary edema

- Heavy, congested lungs
- Lung weights exceed 500 g, even more than 1000 g
- Protein content of opioid pulmonary edema higher than cardiac related pulmonary edema
- Can also be caused by drowning, congestive heart failure, epileptic seizure, and traumatic head injury
Other Pathological Findings

• Another common sign is generalized visceral congestion
• Hepatic parenchyma alterations, such as liver cirrhosis, chronic active hepatitis, fatty degeneration, hepatomegaly, are common but due to pre-existing conditions or chronic abuse of narcotics.
Negative Autopsy

• Many deaths caused by suspected illicit drugs do not show significant pathology at the time of autopsy.

• Partially due to the younger age of these patients

• Elderly are more likely to have existing natural disease that is significant to count as a cause of death ‘on the balance of probabilities’

• Toxicology may come back negative
  • Advisable to take full histology and toxicology samples in cases where a likely cause of death is not identified at autopsy

• Negative autopsies are not a sign of failure on the part of the pathologist but rather confidence that any reasonable natural death has been routinely excluded

• The documentation of absence of significant other findings is in itself an important negative finding
Specimen Collection

• Selection
  • Multiple tissues, varied sites of collection

• Collection
  • Method of collection, autopsy or external exam
  • Adequate volumes for toxicology analysis

• Proper storage and handling

• Important to ensure accurate analytical results and solid interpretation
Best sources for samples

• Collect blood, urine, and vitreous humor when available
• Best source of blood sample for toxicology sample is ilio-femoral vein
• If femoral vein blood is not available then blood from the subclavian vein, the right atrium of the heart, or any other intact blood vessel is the next choice, listed in decreasing order of desirability
• Gastric contents are also relevant to deaths related to opioids
Determining Analysis

- Case history
  - Scene findings
  - Medical history, treatment before death
  - Autopsy findings, pathological findings
  - Symptoms
- Available specimen amount
- Specimen nature, excessive trauma, decomposed
- Medico-legal policies, office policies
  - Death in custody, homicides, traffic
When to order toxicology

• History of prescription opioid or illicit drug use, misuse, or abuse
• Evidence of prescription opioid or illicit drug abuse revealed by scene investigation
• Autopsy findings suggesting a history of illicit drug abuse
• Massive lung edema and froth in airways with no grossly visible explanation (e.g. heart disease) or nontoxicological explanation (e.g. epileptic seizures)
• Potential or suspected smugglers of illicit drugs
• No unequivocal cause of death identified at autopsy
• Decedents with a potential natural cause of death visible at autopsy whenever a drug may have been precipitated or contributed to death
Typical autopsy specimens

- Blood
- Urine
- Bile
- Vitreous humor
- Stomach contents
- Liver
- Spleen
- Brain
- Kidney
Toxicology testing

- Opiates
- Benzodiazepines
- Antidepressants
- Muscle relaxants
- Sleep aids
- Ethanol
- Stimulants (both pharmaceutical and illicit)
Opioids

- Hydrocodone (e.g. Vicodin)
- Oxycodone (e.g. Oxycontin, Percocet)
- Oxymorphone (e.g. Opana)
- Meperidine (e.g. Demerol)
- Methadone (e.g. Dolophine)
- Fentanyl (e.g. Ultiva, Sublimaze, Duragesic patch)
- Buprenorphine (Suboxone)
- Hydromorphone
- Propoxyphene
- Tapentadol
- Tramadol
- U-47700, Synthetic opioids
Common Benzodiazepines

- Alprazolam (e.g. Xanax)
- Clonazepam (e.g. Klonopin)
- Diazepam (e.g. Valium)
- Lorazepam (e.g. Ativan)
- Triazolam (e.g. Halcion)
- Temazepam (e.g. Restoril)
Common Antidepressants

- Fluoxetine (e.g. Prozac)
- Citalopram (e.g. Celexa)
- Sertraline (e.g. Zoloft)
- Paroxetine (e.g. Paxil)
- Escitalopram (e.g. Lexapro)
- Duloxetine (e.g. Cymbalta)
Common Muscle Relaxants

• Diazepam (e.g. Valium)
• Methocarbamol (e.g. Robaxin)
• Tizanidine (e.g. Zanaflex)
• Metaxalone (e.g. Skelaxin)
• Baclofen
• Chlorzoxazone
• Carisoprodol (Soma)
• Cyclobenzaprine (Flexeril)
Common Sleep Aids

- Zolpidem (e.g. Ambien, Edluar, Intermezzo)
- Doxepin (e.g. Silenor)
- Eszopicolone (e.g. Lunesta)
- Ramelteon (e.g. Rozerem)
- Suvorexant (e.g. Belsomra)
- Zaleplon (e.g. Sonata)
Scenario 8
The Scene

• A 25 year old man, named X, is found on his side on the ground at a friend’s house.
• His friend, Y, says he found X on the ground and unconscious after waking up from a nap.
• Near the decedent, you see a grey powder.
• What do you want to know?
Scenario 8

What do you want to know?

• What is the grey powder?
• How did this person consume the drugs?
• Any medical diagnoses? Any medications? Recent hospitalizations? Recent procedures (i.e. surgeries or dental procedures)?
• Any previous overdoses?
• Any stints in rehab or prison?
• Any other drug paraphernalia or drugs in the vicinity or in the decedent’s system?
• Did the decedent have any allergies?
Scenario 8
Further Investigation

- The gray powder is called Gray Death, a combination of heroin, fentanyl, carfentanil (large animal tranquilizer), U-47700, and possibly other opioids or unidentified drugs
- **DO NOT TOUCH** as it is very lethal in small doses and by skin contact and inhalation
- Use personal protective equipment when handling
- Rolled up bills are found near the decedent, however some is also found on his fingers
- No previous overdoses, stints in the hospital, prison, or rehab, no significant medical history, and no other medications
Scenario 9
The Scene

• A 55 year old man was found unconscious and hunched over while sitting on the toilet.
• Upon examination the decedent had fresh injection sites in the abdomen and old injection sites in the lateral thigh and posterior arm.
• His girlfriend said they had just returned from lunch where they celebrated him returning from a trip for the past 3 weeks.
• When investigating his bathroom you find used and unused needles, insulin, a cooker spoon (recently used), a tourniquet, a half full alprazolam bottle, and 1 empty acetaminophen/hydrocodone bottle.
• What else do we want to know?
Scenario 9
What else do we want to know?

• Did he have any past medical conditions? (diabetes, depression, anxiety, pain)
• What medications is he taking?
• Were the alprazolam and hydrocodone prescribed to him? Dosage? Date filled? Quantity? Remaining? Refills? Instructions? Prescriber(s)?
• Where did he go for the past 3 weeks? (rehab, prison, hospital)
• Did he take the alprazolam and hydrocodone? (need toxicology report)
• Who used the needles, cooker spoon, and tourniquet? (Him and/or his girlfriend)
• Any other drug paraphernalia in the apartment?
Scenario 9
Further Investigation

• Diagnosed with T2DM 5 years ago and has been on insulin for 1 year
• Taking pain medication for osteoarthritis for the past 3 years
• Very anxious recently because of an upcoming job interview, but he has never been diagnosed with chronic anxiety or depression.
• The acetaminophen/hydrocodone was prescribed to him by Dr. X for his osteoarthritis 4 weeks prior. He was meant to take the pill every 4-6 hours as needed. The prescription originally had 40 pills but was now empty.
• The alprazolam prescribed to his girlfriend by Dr. Y for her chronic anxiety 4 weeks prior. She was meant to take the pill every 5 hours as needed. The prescription originally had 40 pills but was now empty.
Scenario 9
Further Investigation

• In jail for the past 3 weeks for a minor offense, but he did not have the funds to pay for bail until today
• Able to take his insulin, but not the acetaminophen/hydrocodone. The ordeal and loss of bail money has caused extra anxiety, according to his girlfriend.
• Heroin is found at the scene, hidden in a drawer in the kitchen.
• The toxicology report comes back positive for alprazolam and hydrocodone, negative for heroin and heroin metabolites.
Scenario 9
What happened?

• The decedent, who has T2DM and takes insulin for it, was in jail for 3 weeks where he experienced withdrawal symptoms and a decreased tolerance

• The injection sites are likely from his insulin injections and not heroin injections

• The bail payment, jail time, and upcoming job interview have stressed him out which led him to take some of his girlfriend’s alprazolam to relax him

• For osteoarthritic pain he continued taking his acetaminophen/hydrocodone, but took extra because of the increased pain

• The heroin belongs to his girlfriend and she used it after lunch with one of his insulin needles
Current issues in toxicology testing

• Scope of testing (list of analytes) not standardized
• Different methodologies
• No clear reference laboratory system
• Inadequate capacity (specially in the forensic area)
• Inadequate capability to test for novel analogs-”designer opioids”
Barriers to a standardized approach

• Expensive instrumentation
• Expensive calibration/IS/QC standards for isotope dilution LC/MS/MS
• Differing accreditation requirements for clinical vs. forensic laboratories
• Regulatory oversight
• Lack of standardization of methodology or defined list of target analytes
Postmortem Forensic Toxicology

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Postmortem Forensic Toxicology

• Perform the qualitative and quantitative analysis of drugs, their metabolites or poisons in biological specimens collected at autopsy

• Interpretation of findings
  • Determining or contributing factor to death
  • Physiological effect at time of death
  • Behavioral effect at time of death
Quantitative vs. Qualitative

- Qualitative analysis – determines the presence or absence of a drug or poison in a sample
  - Was this person exposed to the drug?
- Quantitative analysis – determines the amount of drug or poison that is present in the sample
  - How much is present and at what level?
Route of Drugs

- Drugs will leave from the stomach
- Pass through the Liver
- Enter into Blood stream
- Leave through Liver/Kidneys
- Pass into Urine
Blood

- Antemortem blood is the ideal blood sample
  - Hospital Blood, admission blood, or taken right before death
- Postmortem blood is not truly “blood”
  - Mixture of blood and cell components, bacteria
- Anatomical site of collection at autopsy should be noted
- Different sites can give different drug concentrations
Blood Types

- Central sites
  - Heart
- Peripheral sites
  - Femoral
  - Iliac
  - Subclavian
- Other sites
  - Hematoma blood
Postmortem Blood Description

- Postmortem, the composition and distribution of body fluids change
- Postmortem blood sediments and clots
- Hemolysis occurs
- Samples could be clotted, completely fluid or in between
- pH changes
- Variation in water content
- All these can effect drug levels
Urine

- Produced by the kidneys
- Blood filtered by the kidneys
- Contains drugs, metabolites
- Urine is qualitative, it shows the presence of a drug. It indicates that some time prior to death that a drug was present in the blood
Gastric Contents

- Best practice to take all content at autopsy
- May contain pills or tablets
- Ingested drugs may be detected
- Drugs administered by other routes may also diffuse into gastric contents from the blood
- Mainly qualitative:
  - Gastric contents are not homogeneous
- Useful for directing further analysis
- What drugs are contained in contents?
Case Example

- A 26 year old woman is found dead in bed
- Numerous medications at scene:
  - Amitriptyline, Oxycodone, Morphine, Paroxetine, Diphenhydramine, Pseudoephedrine, Phenobarbital, Codeine, Temazepam, Diazepam
- Only 3 mL of blood collected at autopsy
- Qualitative analysis of gastric contents:
  - Amitriptyline and Nortriptyline: detected
- Quantitation can now be performed in blood
Liver

- Drug metabolism occurs
- Both parent compounds and metabolites may be present in higher concentrations in the liver than in the blood, making detection easier
- Drugs are not evenly distributed in the liver
- Different samples of liver can give different concentrations
Bile

- Digestive secretion
- Continuously produced by the liver
- Stored in the gallbladder
- Drug concentrations usually higher
- **Qualitative**
  - Presence of a drug in the bile of an individual indicates that sometime prior to death that the individual was exposed to the drug
Vitreous humor

- Fluid that occupies the space between the lens and the retina of the eye.
- Isolated from putrefaction, charring and trauma, bacteria.
- Useful with advanced decomposition, exhumation or fire deaths
Hair

- Recent specimen of interest
- Metabolism does not occur in hair
- Can provide a historical record of drug or poison exposure because it grows at a predictable rate
- Not for recent drug use detection
Non-biological submissions

• Physical evidence from the scene
  • Can target testing done based on the results
• May indicate the nature of substances that may have been ingested, inhaled or injected
• Examples:
  • Containers found at the scene
  • Syringes
  • Both identified and unidentified pills or liquids
• Drugs at scene could not be in tox, drugs in tox not at the scene
Autopsy Specimens of Limited Value

- Pleural fluid, from around the lungs
- Chest cavity blood
- Samples taken after embalming
- Samples taken after transfusion in hospital
Chest Cavity Fluid

• Not readily definable
• Most likely to be collected:
  • Traumatic injury to the chest, car/train accident, fall from height
  • Advanced decomposition
• A “contaminated” blood sample, chest cavity fluid may contain fluids from stomach, heart, lungs etc.
Embalmed Samples

- Methanol is a main component of embalming fluid
- Most drugs are soluble in methanol
- Embalming fluid essentially “wash” the vasculature and tissues
- Drugs can be completely removed or just diluted
- Qualitative analysis can be performed on body tissues
Storage and Handling

• Not always possible to analyze specimens immediately
• Sample should be in sealed sterile containers
• Use preservatives and anti-coagulants
• Refrigeration or freezing
  • Both inhibit bacterial action; esp. freezing
  • Freezing results in prep time before analysis
  • Freeze-thaw cycle may promote breakdown
Storage of Samples

• Preservative
  • Sodium fluoride

• Anti-coagulants, not necessary because blood is hemolyzed
  • Sodium citrate
  • Potassium oxalate
  • EDTA
  • Heparin

• Changes in blood cannot be reversed
The Analytical Process

• Sample receipt
  • Chain-of-custody
• Review request and information
• Decide on testing to be performed
• Analytical testing
• Review, evaluate and interpret results
Analysis of Forensic Specimens

Analytical Process
  • Separation: Extraction of drugs
  • Identification: Screening
  • Confirmation
  • Quantitation
Drug Analysis

Screening
• Immunoassay
• ELISA/EMIT

Confirmation/Quantitation
• Gas Chromatography (FID, ECD, NPD, MS)
• Liquid Chromatography (MS/MS)
Forensic Drug Testing

One Comprehensive Approach

[Diagram: Comprehensive Drug Testing - One Approach]

- Biological Specimen
  - DA Immunoassay
    - Neg
    - Pos
      - GC/MS Confirmation
  - Acid/Neutral Screen
    - Neg
    - Pos
      - GC/MS Confirmation
  - Base Screen
    - Neg
    - Pos
      - GC/MS Confirmation
      - Quantitation by GC
  - Color Tests
Case Example

- 30’s male
- Heart condition that would lead to an early death
- At first look, case looked like a natural death
- Toxicology analysis performed found 4 drugs at high toxic and lethal levels
Case Example

- 30’s overweight female
- Asthma, oversized heart
- Toxicology analyses found high lethal levels of codeine and other painkillers
- Cause of death was a stab wound to the abdomen
Case Example

- 30’s female
- Found with laceration to the head due to fall in the shower
- Carbon monoxide analysis showed high level, possible lethal level, high enough to cause unconsciousness
Case Example

- 40’s Female
- Found with 10 prescription drugs
- Empty bottles few days after prescription filled
- These drugs were not found in system
Case Example

- 50's female
- History of depression
- Suicide, jump from height
- Toxicology analysis came up positive acetone 650 mg/dL