NMS LABS

Toxicology for Death Investigations

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Disclaimer

- I am a paid employee of NMS Labs, a commercial provider of Toxicology and other forensic testing services.

Toxicology

- Paracelsus (1493-1541)

“All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.”

WHY Forensic Toxicology?

Forensic toxicology exists because:

- People MISUSE drugs
- People take TOO MUCH drug
- People take TOO LITTLE drug
- People take the WRONG drugs

Forensic Toxicologist in Death Investigation

- Interact with investigators and pathologists
- Recommend samples
- Select methods of analyses
- Perform toxicological analyses
- Gather data, interpret the results, and generate a report

Toxicology in the Perfect World

- Autopsy is performed immediately following death
- Toxicology testing performed immediately following autopsy
- Concentration of drugs measured in the blood will accurately reflect concentration that was circulating at the time of death.

This will never happen…..

So…

- What happens to drugs in the specimen between death and autopsy?
- What happens to drugs in the specimen between collection and testing?
- Can post-mortem toxicology results be interpreted in a useful manner?

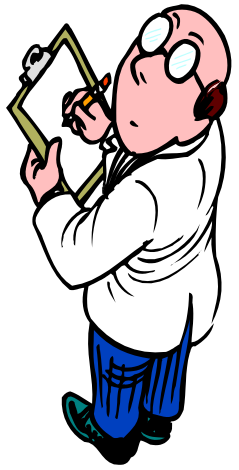
What can’t we control?

- What happens to drugs in the specimen between death and autopsy?

- Post-mortem interval
- Environmental conditions

The *False* Assumptions

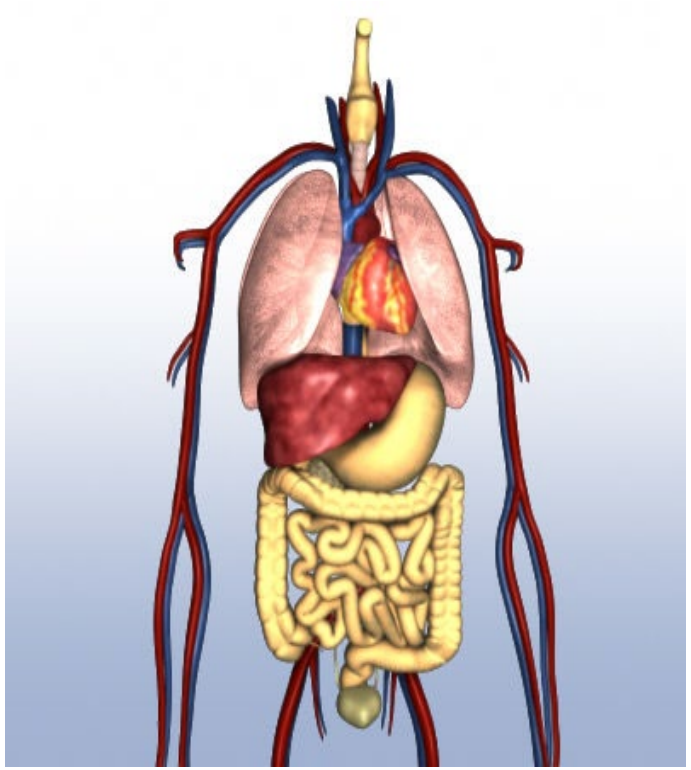
- Postmortem blood drug concentrations reflect those at the moment of death
- Blood drug concentrations are reasonably predictable
- Pharmacokinetics is useful in postmortem cases
- Drug dose can be estimated from postmortem blood concentrations



From CAT 2003 Quarterly Meeting; Dr. Graham Jones; Presentation on Interpretation of PM Findings

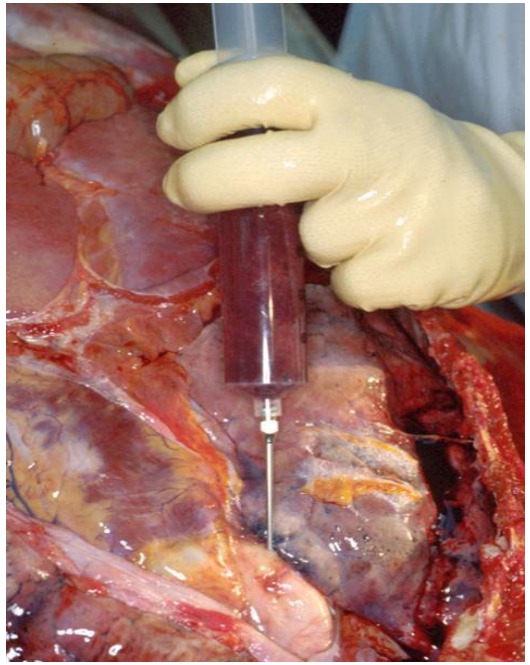
Post-mortem interval

- Post-mortem redistribution
  - Redistribution and diffusion of drugs after death
  - Time and concentration dependent
  - Basic, lipophilic drugs with higher volumes of distribution
- Decomposition
  - Alcohol formation
  - Specimen issues



Sample Integrity

- Where is blood from?
  - Is it cardiac blood or “chest” blood?
  - How good is a large volume of femoral blood?
- Is the blood/tissue contaminated?
  - Diffusion from stomach?
  - Ruptured stomach/diaphragm?
  - Aspiration of gastric contents?
- Is the “blood” really “blood”?
  - Or is it pleural fluid, bloody chest fluid?



From CAT 2003 Quarterly Meeting; Dr. Graham Jones; Presentation on Interpretation of PM Findings

Mechanisms of PMR

- Diffusion of drug from a reservoir
  - Gastrointestinal tract, liver, lung, myocardium, fat
  - Diffusion through blood vessels or across tissue
- Cell and Tissue Changes
  - Cell death, blood movement, blood stasis and lysis, putrefactive processes
- Determinative Factors
  - Drug kinetics and properties
  - Acid/base properties, lipophilicity, protein binding, potential for postmortem metabolism

WHAT CAN WE CONTROL?

SAMPLE COLLECTION, STORAGE & ANALYSIS

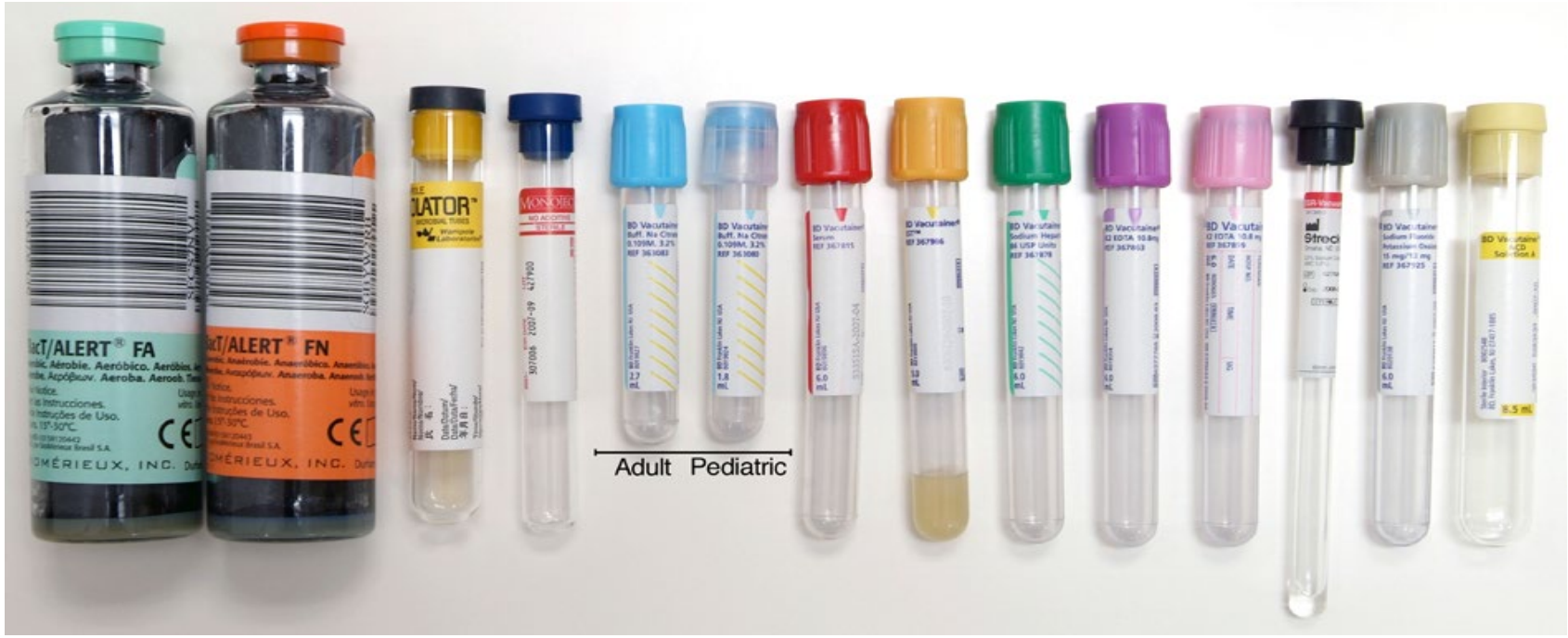
Analytical Considerations

- What specimen(s)?
  - Blood – peripheral v. cardiac
  - Tissue – liver, kidney, brain, others?
  - Urine
  - Vitreous fluid
- Testing
  - Immunoassays
  - HRMS Screens
  - Confirmation/Quantification

Collection Containers

- What happens to drugs in the specimen between collection and testing?
  - Collection container
  - Storage temperature
  - Time between collection and testing

Collection Tube Types



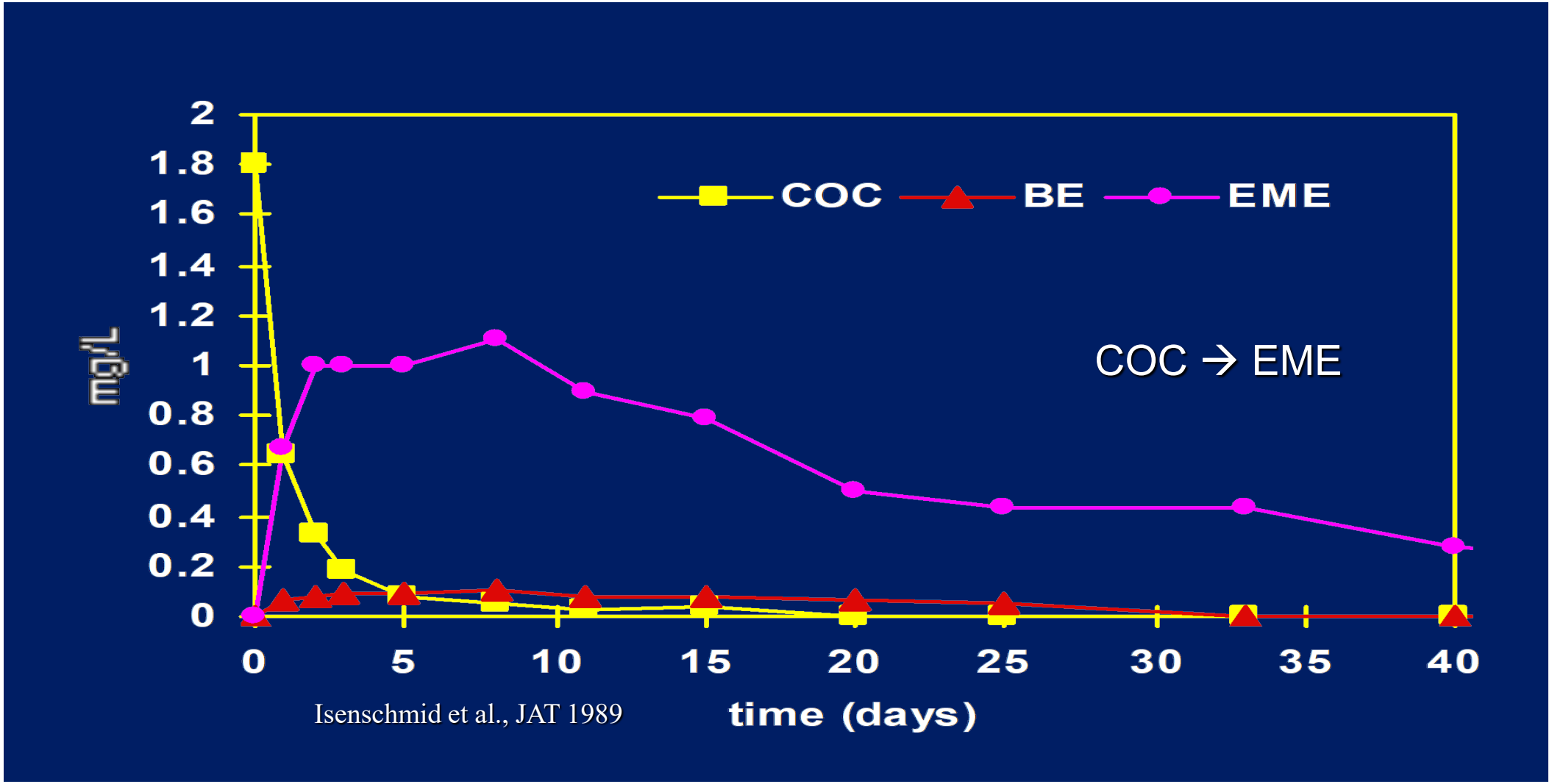
Gray Top Tube

- 1% NaF to inhibit enzymatic and microbial activity
  - fill 10 mL if possible
- Most important for alcohol
- Oxalate anticoagulant
- Lavender top (EDTA) is next best for most other tests

Why Gray Top Tubes?

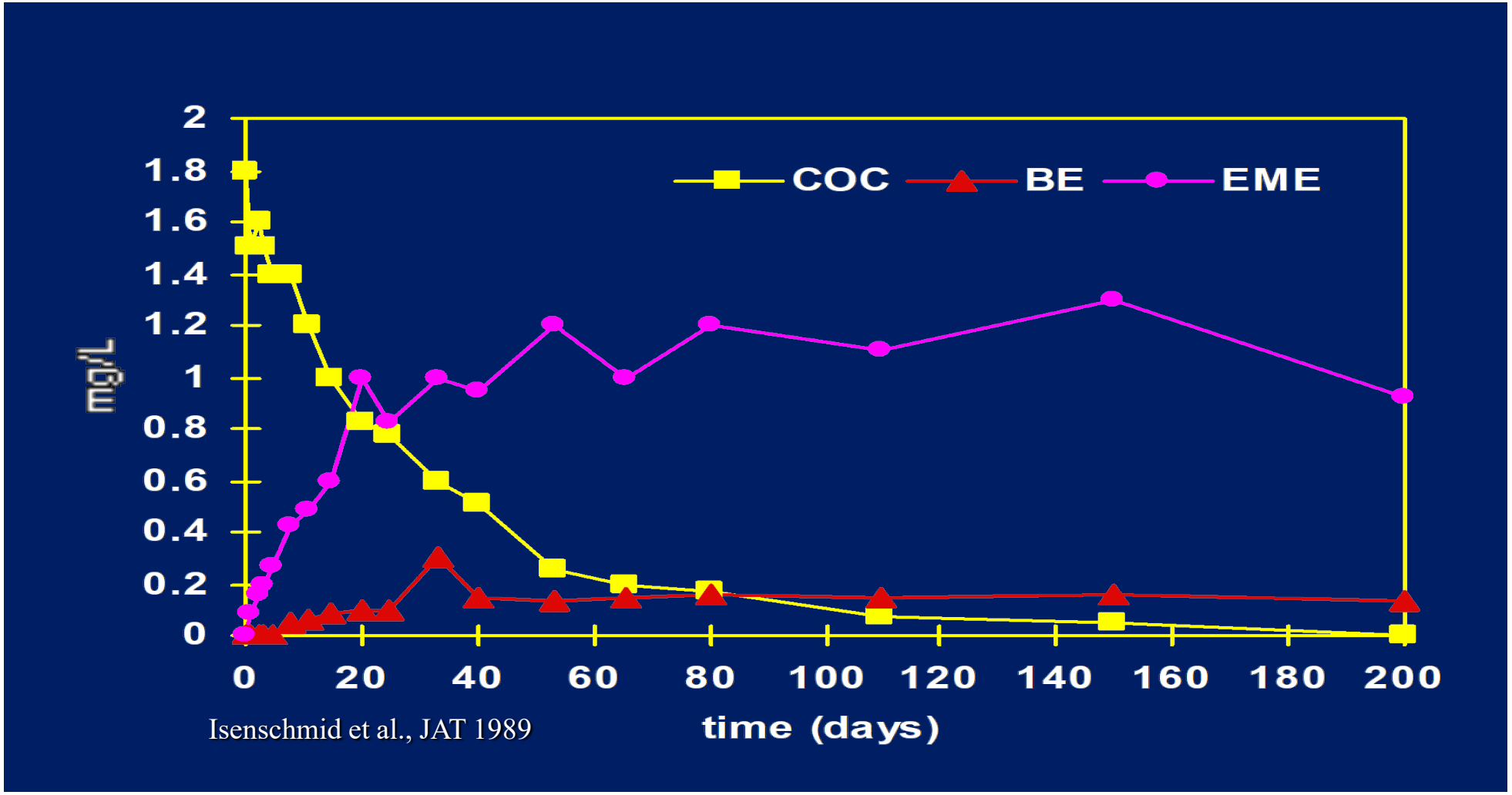
- Fluoride is added to inhibit:
  - microorganism-mediated conversion of glucose to ethanol
  - microorganism-mediated oxidation of ethanol to acetaldehyde
  - post-mortem conversion of cocaine to inactive cocaine metabolites by pseudocholinesterase
  - enzymatic loss of other esters such as 6-MAM

Stability in Blood at 25 °C
Unpreserved, Physiological pH

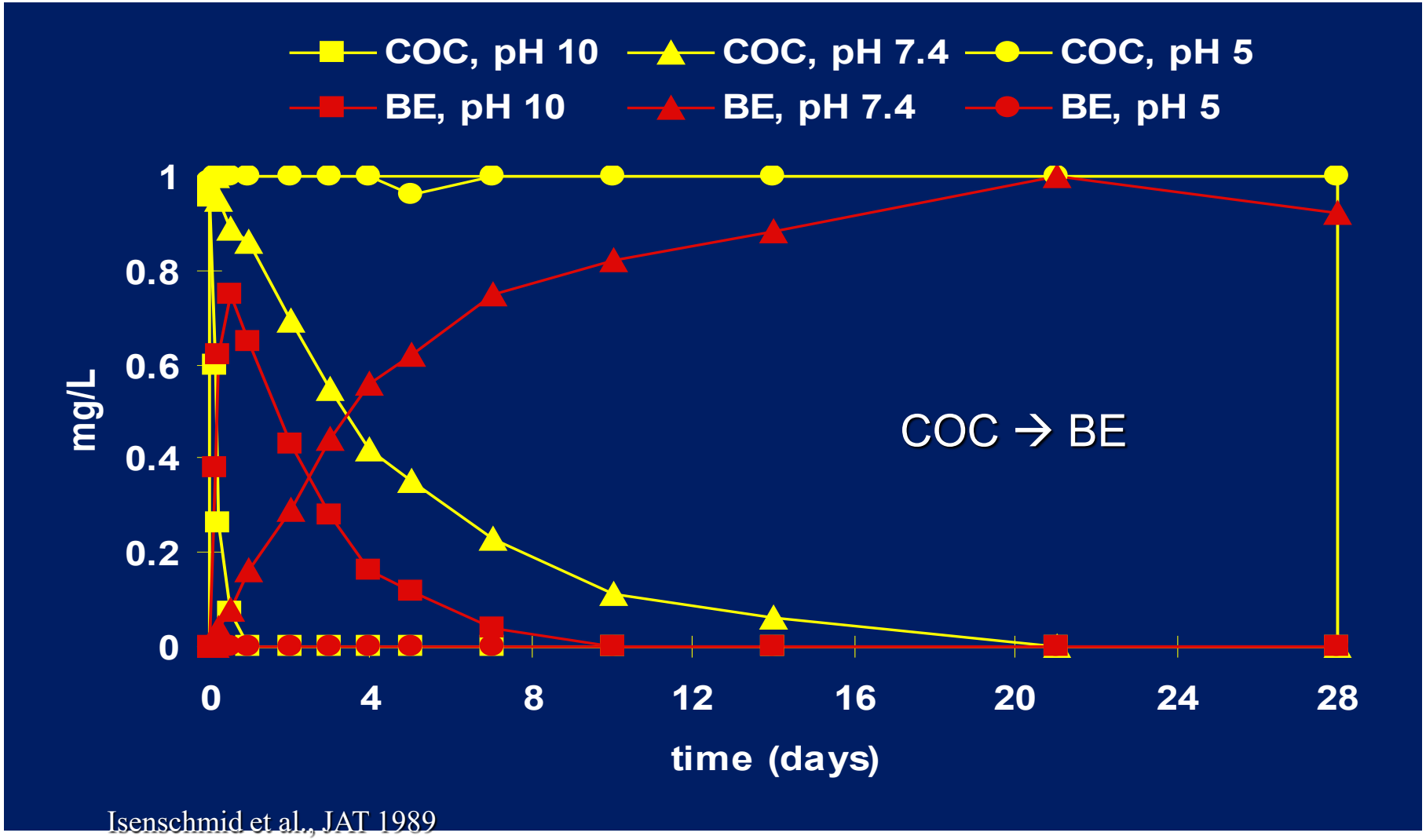


Isenschmid et al., JAT 1989

Stability in Blood at 4 °C
Unpreserved, Physiological pH

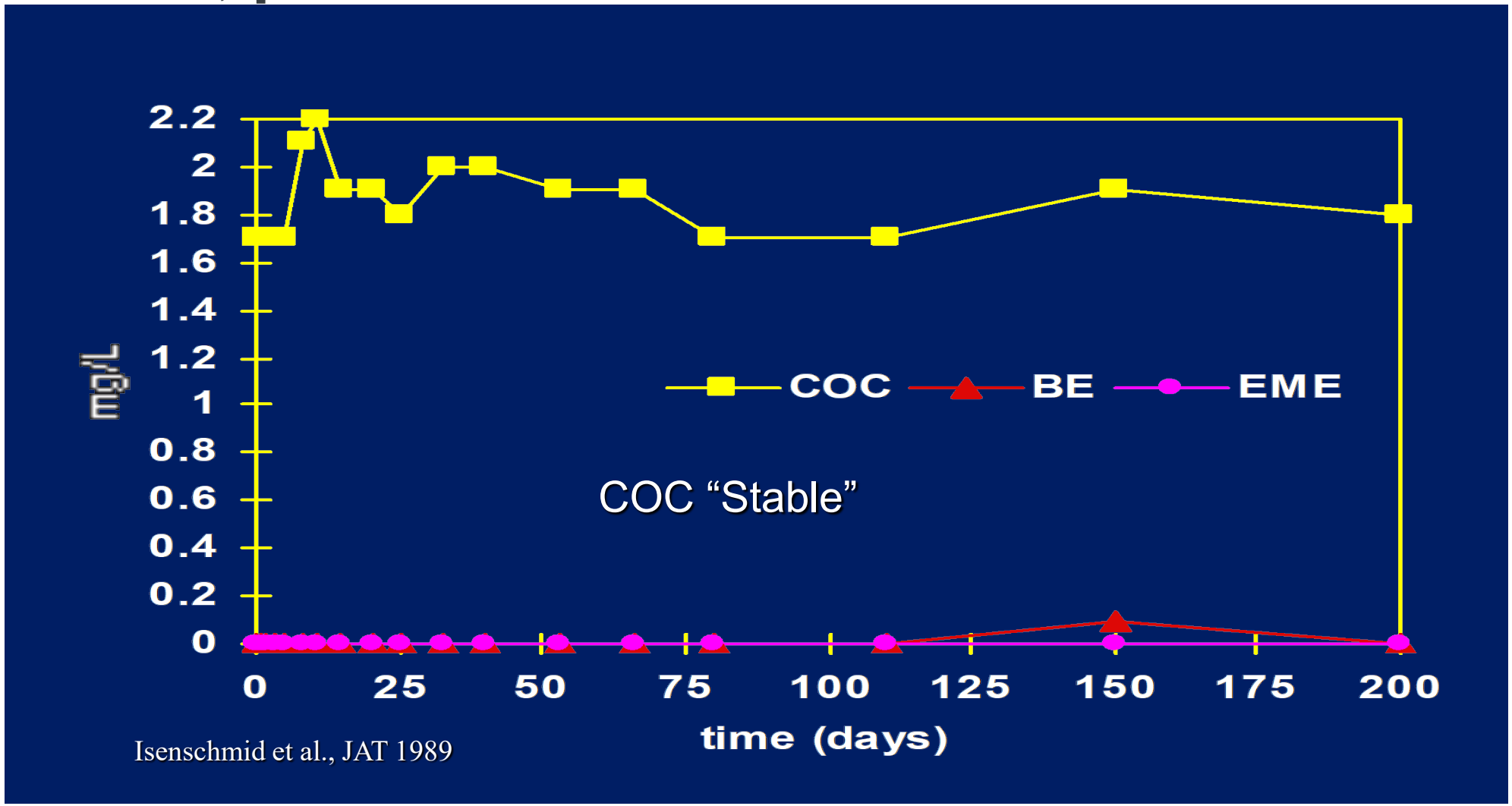


Stability in Buffers, 25 °C



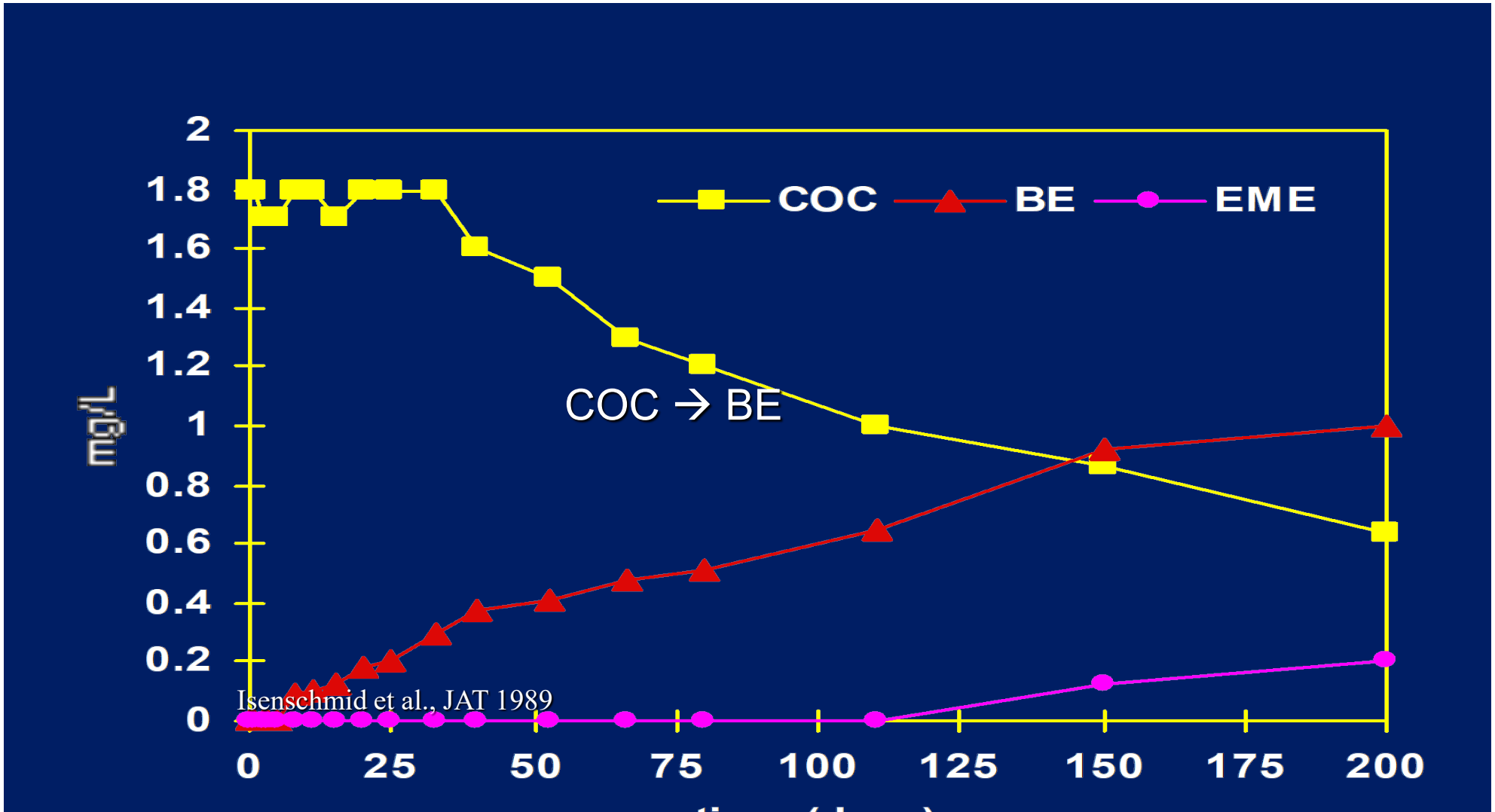
Isenschmid et al., JAT 1989

Stability in Blood at 4 °C
2% NaF, pH 5



Isenschmid et al., JAT 1989

Stability in Blood at 4 °C
2% NaF, Physiological pH



Isenschmid et al., JAT 1989

Royal Blue Top Tubes

- Lilac Label
  - Contains disodium EDTA
  - Free of trace metals
  - Used for trace metal analyses in blood
- Red Label
  - Contains no additive
  - Free of trace metals
  - Used for trace metal analyses in serum

Serum Separator Tubes (tiger top)

- Drugs or poisons most often have lipid soluble properties.
- These compounds may diffuse into the wax-like polymer from the liquid, reducing the measureable concentration in the serum
- Therefore, the analysis may provide a value lower than was actually present in the blood.

What should be collected?

Best Practice Recommendation

ANSI/ASB BEST PRACTICE RECOMMENDATION 156

Best Practices for Specimen Collection and Preservation for Forensic Toxicology

First Edition, 2023

Best Practice Recommendation Consensus Body: Toxicology

Published

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What should be collected?

Best Practice Recommendation

ANSI/ASB BEST PRACTICE RECOMMENDATION 156

Best Practices for Specimen Collection and Preservation for Forensic Toxicology

First Edition, 2023

Best Practice Recommendation Consensus Body: Toxicology

Published

This document delineates guidelines for the collection of forensic toxicology specimens, their amounts, preservatives, and storage conditions. This guideline applies to specimens collected for laboratories performing forensic toxicological analysis in the following sub-disciplines: postmortem toxicology, human performance toxicology (e.g., drug-facilitated crimes and driving-under-the-influence of alcohol or drugs) and other forensic testing (e.g., court-ordered toxicology, general forensic toxicology). It is not intended for the area of breath alcohol toxicology.

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https://www.aafs.org/asb-standard/best-practices-specimen-collection-and-preservation-forensic-toxicology

More is Better!

- A single postmortem blood specimen is often insufficient (regardless of the source) without additional, reliable case history or supportive data
- Two is better than one!
  - Cardiac and peripheral – Postmortem Redistribution
- More is best – when needed
  - Add Tissue (Liver, Spleen, Brain, Psoas muscle), VH, urine, bile, gastric contents (caution), AM samples

WHAT DO THE RESULTS MEAN?

Pharmacokinetics & Pharmacodynamics

Pharmacokinetics

- Absorption
  - Oral, injection, smoking, transdermal
- Distribution
  - Lipophilicity enhances access to CNS (Brain = Butter)
- Metabolism
- Excretion
  - Conjugation with glucuronide

Pharmacodynamics

- Therapeutic, adverse and toxic effects
- Comparison to reference ranges
  - Therapeutic: dose v. concentration
  - Toxic
  - Fatal
- Basic understanding of receptors, potency and efficacy can help with interpretation

What is a receptor?

What is an agonist?

What is an antagonist?

What is a partial agonist?

Affinity and Potency

- Affinity – how tightly the drug is bound to the receptor
  - Drugs with higher affinity can knock lower affinity drugs off the receptor
- Potency – how much is required to reach a certain effect
  - More potent drugs require lower doses to achieve same effect

Tolerance and Drug Interaction

- Tolerance – more drug required to obtain same effect
  - Not completely understood
  - Alterations to metabolic enzymes?
  - Change in # or affinity of receptors?
- Drug Interactions
  - Cross-tolerance
  - Metabolic interactions – inhibition or induction of CYP2D6
  - Drug-drug interactions

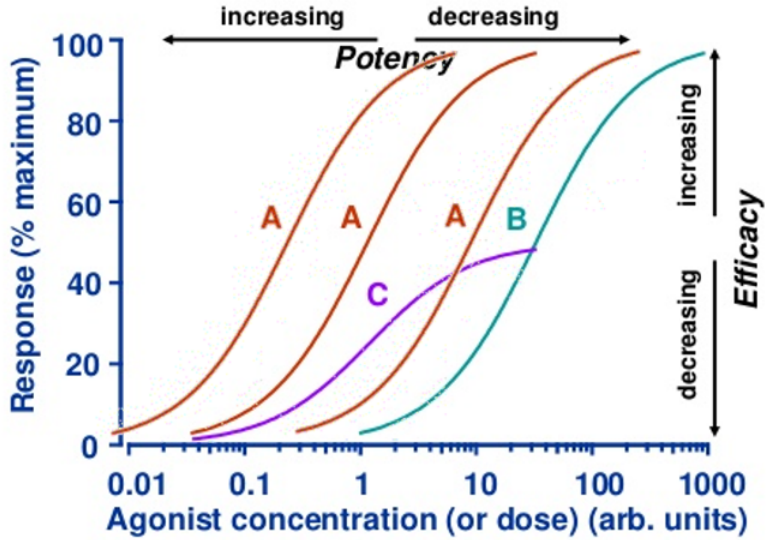
Drug Interactions

- Additive: 1+1 =2
- Synergistic: 1+1 = 3
- Antagonistic: 1+1 = 0

- Typically for opioid agonists the effects will be “at least additive”
- Opioids + Benzodiazepines = increased respiratory depression

Is the concentration of <insert drug name> lethal?
Short answer: It depends.

- Potency & Efficacy
- Tolerance
- Drug interactions



- A & B are *full* agonists
- A is a more *potent* agonist than B but has equal *efficacy*
- A & C are equipotent
- C is a *partial* agonist with a lower *efficacy* than either A or B

https://www.slideshare.net/PharmacologyEducationProject/introductory-receptor-pharmacology201415jap

Interpretation

- Toxicology results cannot be interpreted in a vacuum
- Information that can aid interpretation:
  - Was the decedent a regular drug user?
  - Was the medication prescribed? If so, what was the prescribed dose?
  - Were there signs of drug effects prior to death?
  - Were there signs of drug overdose at autopsy?

How much did they take?

- Dose cannot be calculated from concentration
- Use published therapeutic/lethal range information carefully
- Concentrations that may be lethal to a naïve user could be well tolerated in a pain management patient

When was it taken?

How much did they take?

Kinetic Parameters (Chart)	oral bio-availability (avg)	onset of effect	average half life (hr.)	plasma protein binding	typical duration
codeine	70-90%	45-60m	prodrug	7-25%	4-6h
pethidine	40-60%	20-40m	3-5h	60-80%	2-4h
morphine	30-40%	30-45m	2-4h	35%	3-4h
oxycodone	60-80%	45-60m	3.5h	45%	4-6h
hydrocodone	60-80%	45-60m	3.5h	unknown	4-6h
hydromorphone	24%	30m	2.6h	8-19%	2-3h
oxymorphone	10%	20-40m	1.3h	10-12%	3-4h
levorphanol	~50%	20-40m	11-16h	40%	4-8h
methadone	80%	60-90m	22h	80-90%	6-12h
fentanyl	~10-15%	10-20m	3.5h	85%	1-2h
buprenorphine	~10-15%	60m	36h	96%	4-12h
tramadol	70%	60-90m	6-7h	20%	4-6h
tapentadol	30-40%	30-45m	4.5h	20%	2-4h

When was it taken?

Novel Opioid/Fentalog?

How does naloxone effect the concentrations of opioids in the blood?

It doesn’t.

If you take away all the other findings would drug X have killed this individual?

Analysis and Comments	Result	Units	Rpt. Limit	Specimen Source	Analysis By
Amphetamine	81	ng/mL	5.0	001 - Blood	LC-MS/MS
Methamphetamine	210	ng/mL	5.0	001 - Blood	LC-MS/MS
Clonazepam	6.3	ng/mL	2.0	001 - Blood	LC-MS/MS
7-Amino Clonazepam	6.5	ng/mL	5.0	001 - Blood	LC-MS/MS
Alprazolam	7.0	ng/mL	5.0	001 - Blood	LC-MS/MS
Fentanyl	210	ng/mL	1.0	001 - Blood	LC-MS/MS
Norfentanyl	95	ng/mL	2.0	001 - Blood	LC-MS/MS

I don’t know.

If you take away all the other findings would drug X have killed this individual?

CHARGES

#	Charge	Grade	Description	Offense Dt.	Disposition
1	75 § 3802 §§ D1i*	M	DUI: Controlled Substance - Schedule 1 - 1st Offense	12/30/2019	Held for Court

I don't know.

If you take away all the other findings would drug X have killed this individual?

Compound	Result	Units
Caffeine	Positive	mcg/mL
Alprazolam	21	ng/mL
Morphine - Free	30	ng/mL
6-Monoacetylmorphine - Free	2.0	ng/mL
Oxycodone - Free	90	ng/mL
Oxymorphone - Free	2.4	ng/mL
Phencyclidine	150	ng/mL
Delta-9 THC	0.86	ng/mL
Fentanyl	5.8	ng/mL
Acetyl Fentanyl	0.26	ng/mL

I don’t know.

If you take away all the other findings
would drug X have killed this
individual?

- Law enforcement traced heroin back to dealer
- Believed heroin was contaminated with fentanyl
- Cause of death – mixed drug toxicity

I don’t know.

Are postmortem cannabinoid concentrations forensically reliable?

The American Journal of Forensic Medicine and Pathology

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LETTERS TO THE EDITOR

Are Postmortem Cannabinoid Concentrations Forensically Reliable?

Kacinko, Sherri L. PhD; Isenschmid, Daniel S. PhD; Logan, Barry K. PhD

Author Information

The American Journal of Forensic Medicine and Pathology 45(1):p 92-93, March 2024. | **DOI:** 10.1097/PAF.0000000000000887

with respect to impairment, is likely not warranted. At best, the detection of delta-9-THC in PM blood is evidence of ingestion of the drug at some indeterminate time before death.

Given these considerations, PM blood concentrations should not be considered forensically reliable.

No.

What is the risk to first responders?

SCIENCE Drugs circa

Fentanyl looks like heroin. But just touching it could kill cops and first responders.

Lifeline

Baltimore Co. Police Evacuate Entire Precinct After 3 Officers Treated for Fentanyl Exposure

He became dizzy and his heart began to beat rapidly, symptoms typical of opioid exposure.

> *"She became nauseous, suddenly had an increased heart rate and started to perspire,"* Miller said. *"She was exhibiting symptoms of suffering some kind of second-hand exposure to a synthetic drug. She was at the hospital for a few hours and released,"* he added.

ACMT and AACT Position Statement: Preventing Occupational Fentanyl and Fentanyl Analog Exposure to Emergency Responders

The position of the American College of Medical Toxicology (ACMT) and American Academy of Clinical Toxicology (AACT), is as follows:

Fentanyl and its analogs are potent opioid receptor agonists, but the risk of clinically significant exposure to emergency responders is extremely low. To date, we have not seen reports of emergency responders developing signs or symptoms consistent with opioid toxicity from incidental contact with opioids. Incidental dermal absorption is unlikely to cause opioid toxicity. For routine handling of drug, nitrile gloves provide sufficient dermal protection. In exceptional circumstances where there are drug particles or droplets suspended in the air, an N95 respirator provides sufficient protection. Workers who may encounter fentanyl or fentanyl analogs should be trained to recognize the signs and symptoms of opioid intoxication, have naloxone readily available, and be trained to administer naloxone and provide active medical assistance. In the unlikely event of poisoning, naloxone should be administered to those with objective signs of hypoventilation or a depressed level of consciousness, and not for vague concerns such as dizziness or anxiety. In the absence of prolonged hypoxia, no persistent effects are expected following fentanyl or fentanyl analog exposures. Those with small subclinical exposures and those who awaken normally following naloxone administration will not experience long-term effects. While individual practitioners may differ, these are the positions of American College of Medical Toxicology and American Academy of Clinical Toxicology at the time written, after a review of the issue and scientific literature.

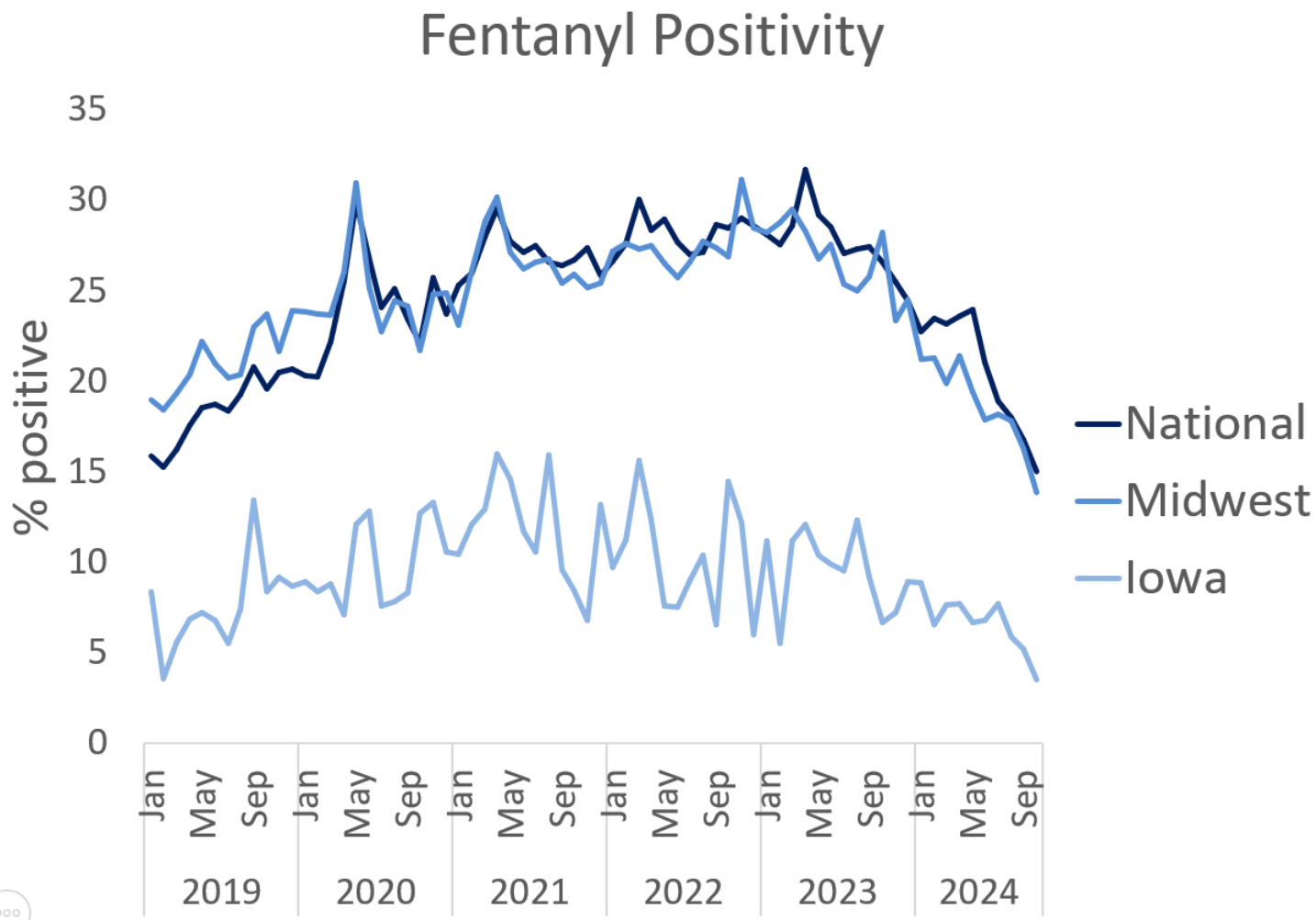
What drugs are commonly found in post-mortem cases?

It varies!

What drugs are commonly found in post-mortem cases?

National	Midwest	Iowa
Fentanyl	Fentanyl	Ethanol
Ethanol	Ethanol	Cannabinoids
Cannabinoids	Cannabinoids	Methamphetamine
Methamphetamine	Cocaine	7-Amino Clonazepam
Cocaine	Methamphetamine	Alprazolam
Gabapentin	Diphenhydramine	Citalopram / Escitalopram
Diphenhydramine	Gabapentin	Diphenhydramine
Xylazine	Acetaminophen	Hydrocodone
Acetaminophen	Trazodone	Oxycodone
Morphine	Hydrocodone	Tramadol
Desmethylsertraline	Alprazolam	Clonazepam

What drugs are commonly found in post-mortem cases?



Where can I learn more about pharmacology and post-mortem toxicology interpretation?

Center for Forensic Science Research and Education

The Role of Comprehensive Medicolegal Death Investigation as Part of a Public Health Improvement Strategy

Archived Twelve-Part Webinar Series

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Acknowledgement

- The “old school” NMS toxicology team: Wendy Adams, Bill Anderson, Ed Barbieri, Jolene Bierly, Lee Blum, Aya Chan-Hosakawa, Dan Isenchmid Laura Labay, Mike Lamb, Barry Logan, Matt McMullin, Rob Middleburg, Donna Papsun


Questions?
Answers.