FORENSIC TOXICOLOGY 2020

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Lecture Overview

What is Forensic Toxicology?

How are drugs and toxins handled by the body?

How are drugs detected, identified and quantified by Forensic Toxicologists?

Some Case Studies

- Ethanol
- THC
- Opioids

What is Forensic Toxicology?

According to the Canadian Society of Forensic Science:

"Forensic toxicology deals with the study of the adverse effects of drugs and chemicals on biological systems, and the interpretation of those results for legal purposes."

https://www.csfs.ca/what-we-do/disciplines-sections/toxicology/

According to Wikipedia: "**Forensic toxicology** is the use of toxicology and disciplines such as analytical chemistry, pharmacology and clinical chemistry to aid medical or legal investigation of death, poisoning, and drug use. The primary concern for forensic toxicology is not the legal outcome of the toxicological investigation or the technology utilized, but rather the obtainment and interpretation of results."

What do Forensic Toxicologists Do?

Forensic Toxicologists:

- Detect and identify drugs and toxins in body fluids, tissues and organs
- Interpret the potential biological consequences of the detected drugs or toxins

Close ties to Analytical Toxicology and Analytical Chemistry

Drugs and Toxins

A drug is any substance, other than a normal constituent of the body, that when applied to or introduced into a living organism has the effect of altering body functions

Drugs and Toxins

Paracelsus (1493-1541) - paraphrased

- Physician-alchemist

‘All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy.’

How are drugs and toxins handled by the body?

To understand what tissues to analyze and how to interpret the results, the Forensic Toxicologist needs to understand how drugs are handled by the body

The description of how drugs are handled by the body is often described as occurring in four phases:

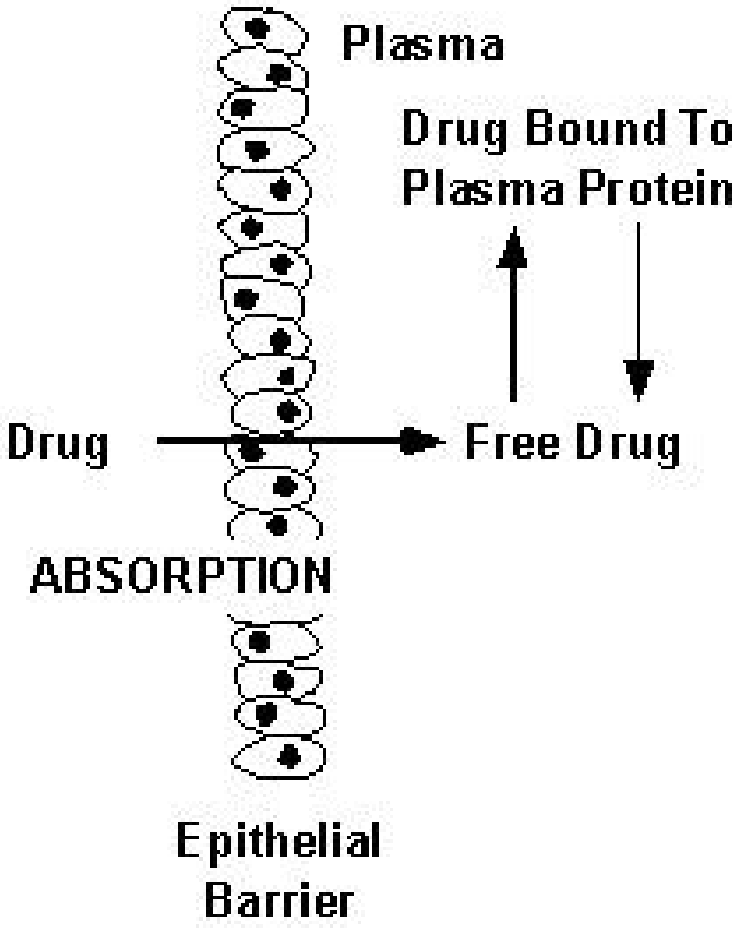
- Absorption, Distribution, Metabolism and Excretion
- "ADME"

ADME

Absorption:

- Drugs must pass through an epithelial cell layer to enter the body

Drug Absorption



ADME

Absorption:

- Common routes of drug administration and absorption:
  - Gastrointestinal tract (stomach, small intestine, large intestine)
  - Sub-lingual
  - Lungs (inhalation)
  - Skin (dermal)
  - Injection – subcutaneous, intramuscular, intravenous

ADME

Absorption - Implications:

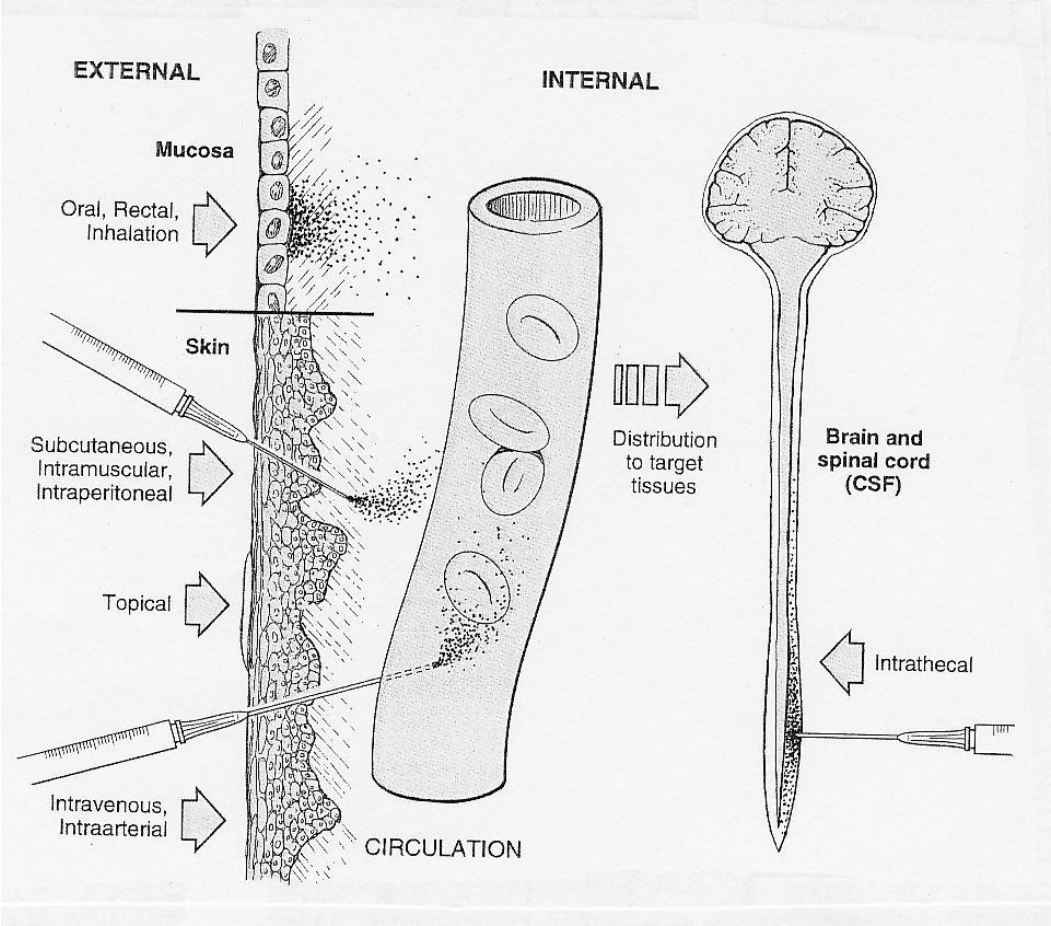
- The optimal site for maximum drug absorption is dependent on the physicochemical properties of the drug
- Route of administration can influence how quickly the drug reaches the blood stream and the brain
- After death, high local concentrations of drugs in the GI tract or lungs can be a clue as to the site of administration

ADME

Distribution:

- Once drugs pass through the cellular membranes at the site of administration, most end up circulating in the blood
- The blood is responsible for distributing the drug to different tissues including the ultimate site of action (e.g. the brain)
- Blood (plasma or serum) concentrations are used as a surrogate measure of the levels of drug at the site of action
- Blood concentration versus effect relationships

Distribution with different administration methods



ADME

Metabolism (Biotransformation):

- Many drugs undergo biotransformation to metabolites before they are excreted from the body
- Enzymes located in body tissues catalyze this biotransformation
  - Oxidation, reduction, conjugation reactions
- The liver is the most common site for metabolic conversion
  - Other tissues do play a role, dependent on the drug
- Each drug has characteristic metabolic pathways

ADME

Metabolism – Implications

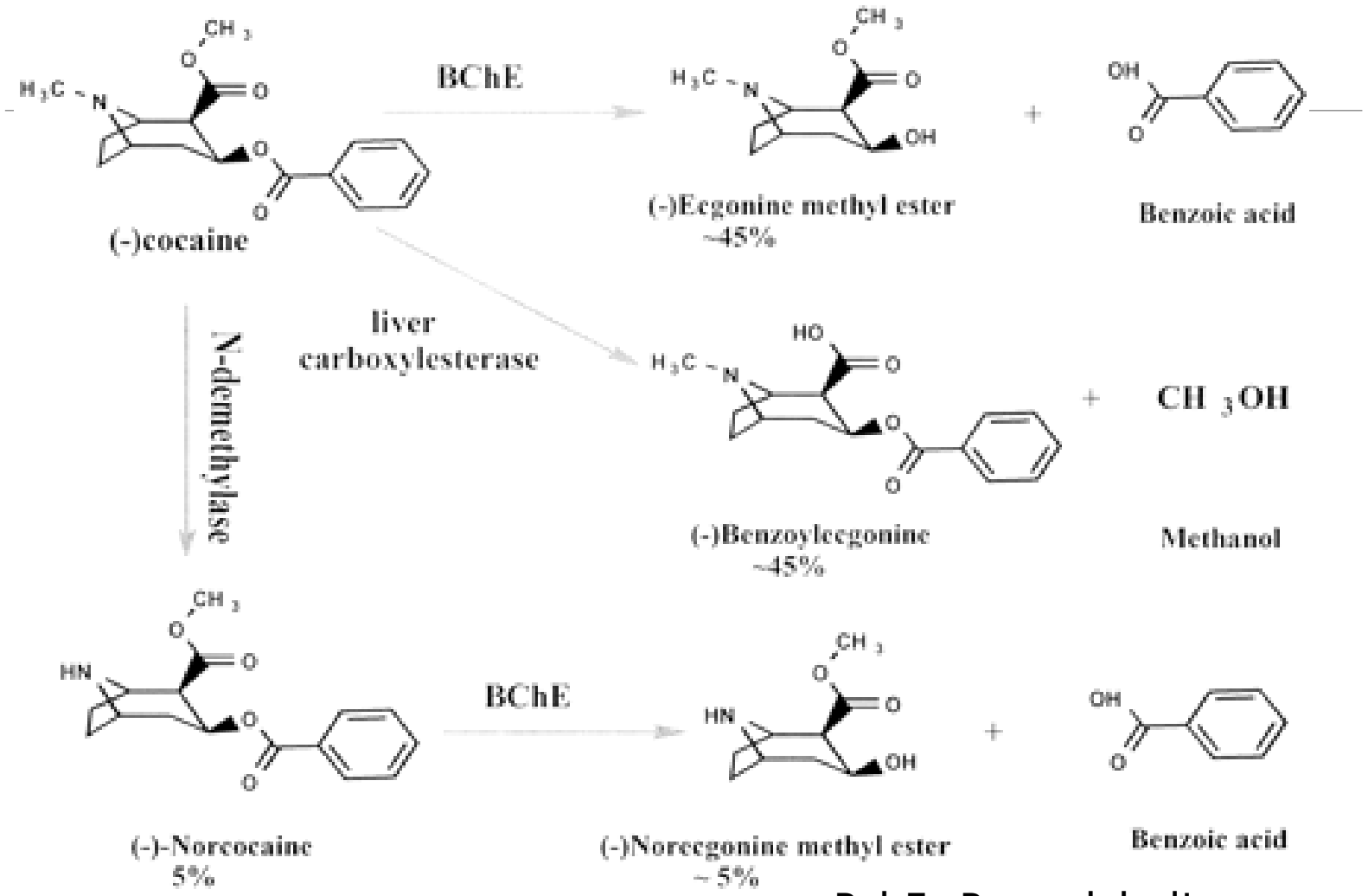
- The Forensic toxicologist needs to know the identity of metabolites
  - Some drugs are metabolized so quickly that the only way to detect their presence is through the measurement of metabolites
  - Some metabolites are pharmacologically active and can increase the length of time a subject is under the drug's influence
  - Metabolites can remain in the body for long periods of time, influencing the time frame for detection

ADME

Metabolism – Implications

- Example – Cocaine
  - Extensively metabolized
  - Only 1% excreted unchanged in the urine
  - Major metabolites – benzoylecgonine, ecgonine methyl ester
  - Other metabolites - ecgonine
  - Cocaine combines with ethanol in the liver to form norcocaine, cocaethylene, and norcocaethylene, pharmacologically active metabolites

Cocaine Metabolism



BchE=Butyrylcholinesterase

http://jpet.aspetjournals.org/content/jpet/302/2/710/F1.large.jpg

ADME

Excretion:

- Most drugs are eliminated either through the urine or through the feces
- Some limited number of small volatile drugs can be eliminated via the breath
- To be excreted into the urine, drugs must be water soluble
  - Drugs that are fat soluble are metabolized to water-soluble forms by the body prior to excretion in the urine
- The relative importance of the routes of elimination vary depending on the drug

ADME

Excretion – Implications:

- The rate at which a drug is metabolized and excreted will influence the half-life of the drug in the body
- Elimination rate is important in determining the length of time a person can be influenced by the drug:
  - Cocaine has a half-life of 60 to 90 minutes
  - THC has a half-life of 1.3 – 13 days*

*Sharma et al 2012 Iran J Psychiatry 2012; 7:4: 149-156

Absorption, Distribution, Metabolism and Excretion - Conclusions

- Understanding the ADME profile for a drug is important for understanding the results of blood and tissue analysis
  - Understanding the time frame between administration and effect
  - Interpreting what tissue concentrations mean
  - Knowing what metabolites to look for
  - Knowing what tissues samples to look at

Detecting and Measuring Drugs

- Collecting samples for drug analysis
- Sampling living individuals
  - Blood
  - Urine
  - Breath
    - Ethanol
  - Hair
    - Cocaine
- Sampling deceased individuals
  - All of the above, except breath, plus tissue sampling

Detecting and Measuring Drugs

Extraction techniques:

- Most drugs must be extracted from biological tissues (aqueous solutions) into organic solvents before they can be analyzed
- A common technique is to use pH to selectively extract acidic and basic drugs from the biological media
  - Adjusting the pH of an aqueous solution to less than 7 will allow acidic drugs to be extracted into an organic solvents
  - Basic drugs can be extracted from aqueous solutions that have a pH above 7
  - The separate fractions are then concentrated and analyzed

Detecting and Measuring Drugs

Measurement techniques:

- In most cases the identity of the drug is unknown
- Screening assays are used first to get an initial assessment of what might be present
  - Thin layer chromatography
  - Gas chromatography
  - Immunoassay
- The screening assays are followed with confirmatory tests that are more accurate and specific to given dugs
  - Gas chromatography/mass spectrometry (GS-MS) is the “Gold Standard”

Detecting and Measuring Drugs

Interpretation – what was the biological effect of the detected drug or toxin?

- Direct cause of death
- Indirect cause of death
- Influence on subject behavior
- Placement at the scene of a crime
  - Example, carbon monoxide exposure in fire deaths
- Absence of a therapeutic drug that may have influenced behavior
  - Example, seizure or heart attack due to a missed pharmaceutical drug treatment

Detecting and Measuring Drugs

Challenges

- Even the most experienced Forensic toxicologist needs help from other crime scene information to optimize analytical testing and to interpret toxicological findings
  - Victim's symptoms
  - Postmortem pathological findings
  - Examination of the victim's personal effects including presence of drug containers or household chemicals

Ethanol

Ethanol is one of the most widely used and abused drugs in Western countries

Most jurisdictions have specific laws regulating ethanol

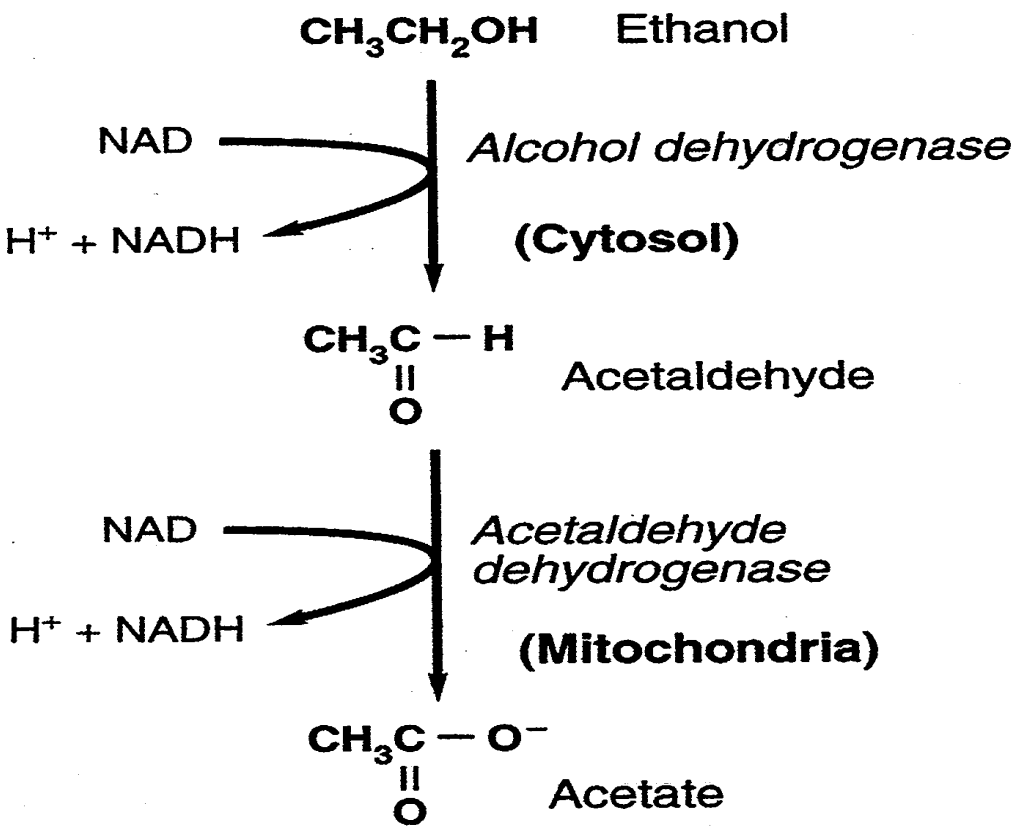
For this reason, legal cases involving ethanol typically represent a significant percent of the Forensic Toxicologist's work load

Ethanol

Ethanol ADME

- Simple molecule, very water soluble, volatile
- Readily absorbed in the gut with oral ingestion
    - Appears in the blood within minutes of ingestion
- Once in the blood stream, distributes evenly in the total body water
- Metabolized largely in the liver (>90%)
- Ultimately most – 95-98% - of the ingested ethanol is metabolized to carbon dioxide and water (generating energy in the process); a small percent is excreted intact in the breath

Ethanol


CH3CH2OH
Ethanol
NAD
H+ + NADH
Alcohol dehydrogenase
(Cytosol)
CH3C — H
O
Acetaldehyde
NAD
H+ + NADH
Acetaldehyde dehydrogenase
(Mitochondria)
CH3C — O−
O
Acetate

Ethanol

Measurement of Ethanol Exposure

- Intact ethanol can be measured in the blood
- Excretion of intact ethanol in urine is small
- Excretion of intact ethanol in breath is small but measurable
- Metabolites are not helpful in detection or quantification

Ethanol

Blood Alcohol Concentration (BAC)

- Blood alcohol concentration (BAC) is most commonly used as a metric of alcohol intoxication for legal or medical purposes
- Blood alcohol content is usually expressed as a percentage of alcohol per volume in the blood
- For instance, a BAC of 0.10 means that 0.10% (one tenth of one percent) of a person's blood, by volume, is alcohol.
    - 1 percent (%) is equivalent to 1 g/100 mL = 1 g/dL
    - 0.01% is equivalent to 10 mg/100 mL

Ethanol

There is a very good relationship between concentration of alcohol in the blood and pharmacological effects

- Supported by years of experimental data

There is also a good relationship between ethanol concentrations in the blood and concentrations in the breath

- The ratio of ethanol in the blood to ethanol in alveolar air is approximately 2,100 to 1 at 34^{o}C

Ethanol

BAC (% by vol.)	Progressive effects of ethanol	
	Behavior	Impairment
0.010–0.029	• Average individual appears normal	• Subtle effects that can be detected with special tests
0.030–0.059	• Mild euphoria, relaxation, decreased inhibition	• Concentration
0.06–0.09	• Blunted feelings, disinhibition, extraversion	• Reasoning, depth perception, peripheral vision, glare recovery
0.10–0.19	• Over-expression, emotional swings	• Reflexes, reaction time, gross motor control, staggering, slurred speech
0.20–0.29	• Stupor, loss of understanding, impaired sensations	• Severe motor impairment, memory blackout, loss of consciousness
0.30–0.39	• Severe CNS depression, unconsciousness • Death is possible	• Bladder function, breathing, heart rate

Ethanol

Blood Alcohol Concentration

- BAC is tied to the number of drinks and the time frame over which they were ingested
- Also Influenced by:
  - Body weight; the higher the body weight, the lower the BAC per drink
  - Body fat, the higher the percent body fat, the higher the BAC per drink
  - Gender, Females have higher BAC for the same number of drinks as males of the same body weight
  - Food, the presence of food will slow the rate of absorption

Ethanol

Rate of Elimination:

Ethanol is cleared from the system at an average rate of 0.015% (blood volume percent) per hour

Ethanol

Ethanol and the law

- For the purposes of law enforcement, BAC is used to define intoxication and provides a measure of impairment
- In Ontario and the rest of Canada, the maximum legal BAC for fully licensed drivers is 80 milligrams of alcohol in 100 millilitres of blood (0.08%)
- Driving with BAC in excess of 0.08 is a criminal offence

Ethanol and the law

- As of May 1, 2009, Ontario revised the law such that drivers who register a BAC from 0.05 to 0.08 (known as the "warn range") lose their license at roadside for 3, 7 or 30 days. Consequences get tougher for repeat occurrences.
- Federal Law reformed June 21, 2018. In relation to alcohol-impaired driving, these reforms are an improvement that:
  - authorize mandatory alcohol screening at the roadside
  - repeal and replace all transportation offences with a modern, simplified and coherent structure
  - increase some minimum fines and maximum penalties
  - facilitate investigation and proof of blood alcohol concentration
  - eliminate and restrict defences that encouraged risk-taking behaviour
  - clarify what is required to be disclosed by the Crown to the defence with respect to proving blood alcohol concentration
  - permit an earlier enrolment in provincial ignition interlock programs, where they are available

https://www.justice.gc.ca/eng/cj-jp/sidl-rlcfa/qa_c46-qr_c46.html

Infographic: New alcohol-impaired driving laws

https://www.justice.gc.ca/eng/cj-jp/sidl-rlcfa/longdesc.html

Ethanol

Breath testing for Ethanol

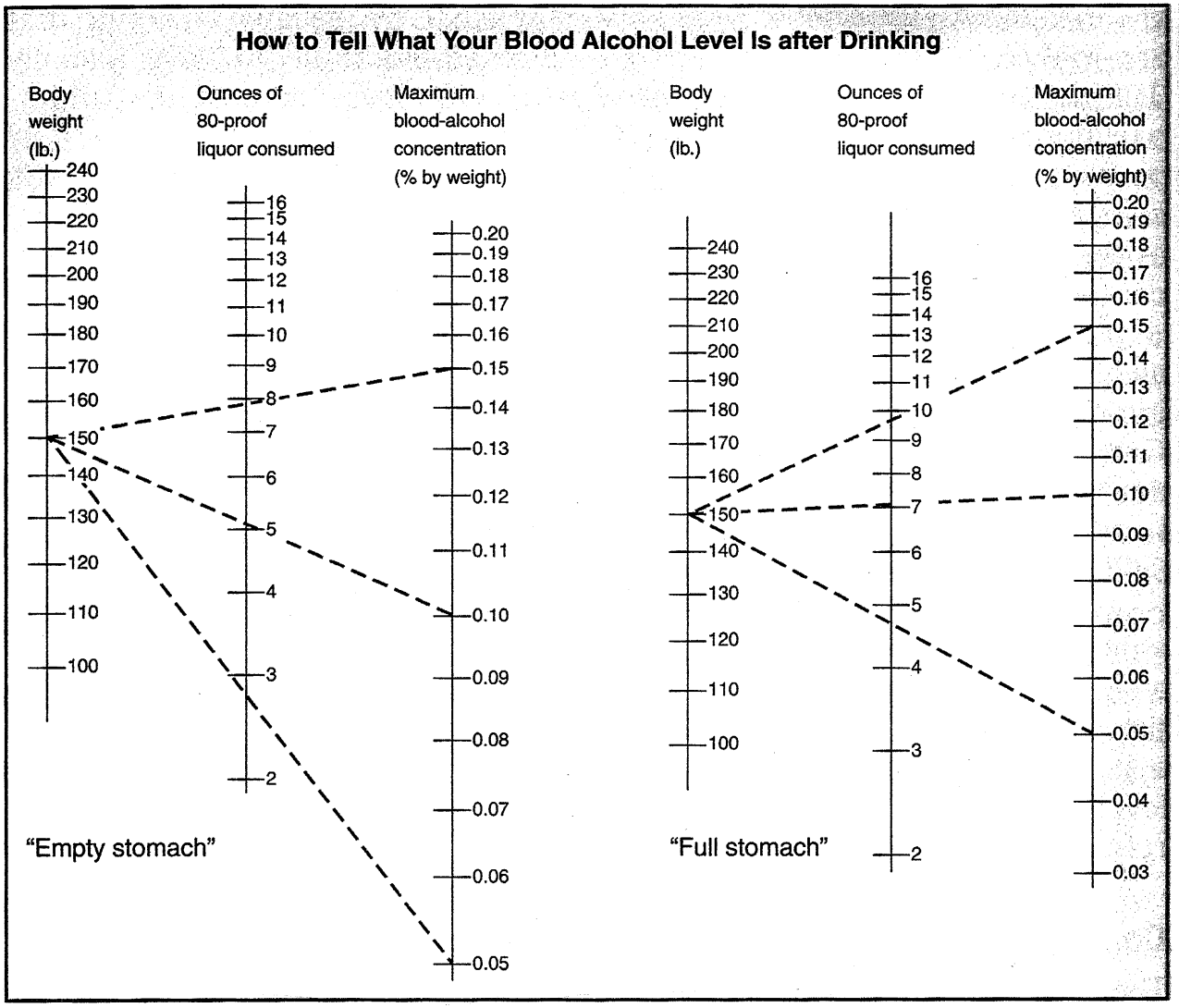
- The amount of ethanol exhaled in the breath is in direct proportion to the concentration in the blood
  - A unique property of ethanol due to its volatility and distribution in total body water
- Breath testers operate on the fact that at 34 C, the ratio of alcohol in the blood to alcohol in alveolar breath is approximately 2,100 to 1
- Many types of breath testers are designed to analyze a set volume of breath
- The captured breath is exposed to infrared light; the degree of interaction of the light with alcohol in the captured breath sample allows the instrument to measure ethanol

Ethanol

Measurement of Blood Alcohol Concentration

- Gas chromatography offers the toxicologist the most widely used approach for determining alcohol levels in blood
- Blood must always be drawn under medically accepted conditions by a qualified individual using a nonalcoholic disinfectant
- Once blood is removed from an individual, its preservation is best ensured when it is sealed in an airtight container after an anticoagulant and a preservative have been added and stored in a refrigerator
    - Avoidance of loss of ethanol from samples due to volatility

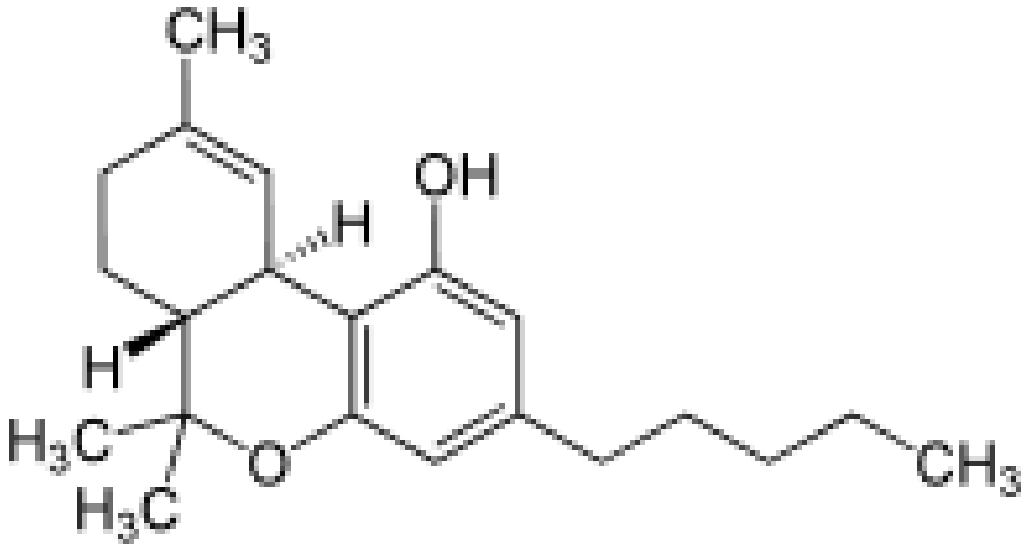
Ethanol


How to Tell What Your Blood Alcohol Level Is after Drinking
Body weight (lb.)
Ounces of 80-proof liquor consumed
Maximum blood-alcohol concentration (% by weight)
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
"Empty stomach"
Body weight (lb.)
Ounces of 80-proof liquor consumed
Maximum blood-alcohol concentration (% by weight)
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
"Full stomach"

THC

Tetrahydrocannabinol (THC)

- The principal psychoactive substance from the Cannabis plant



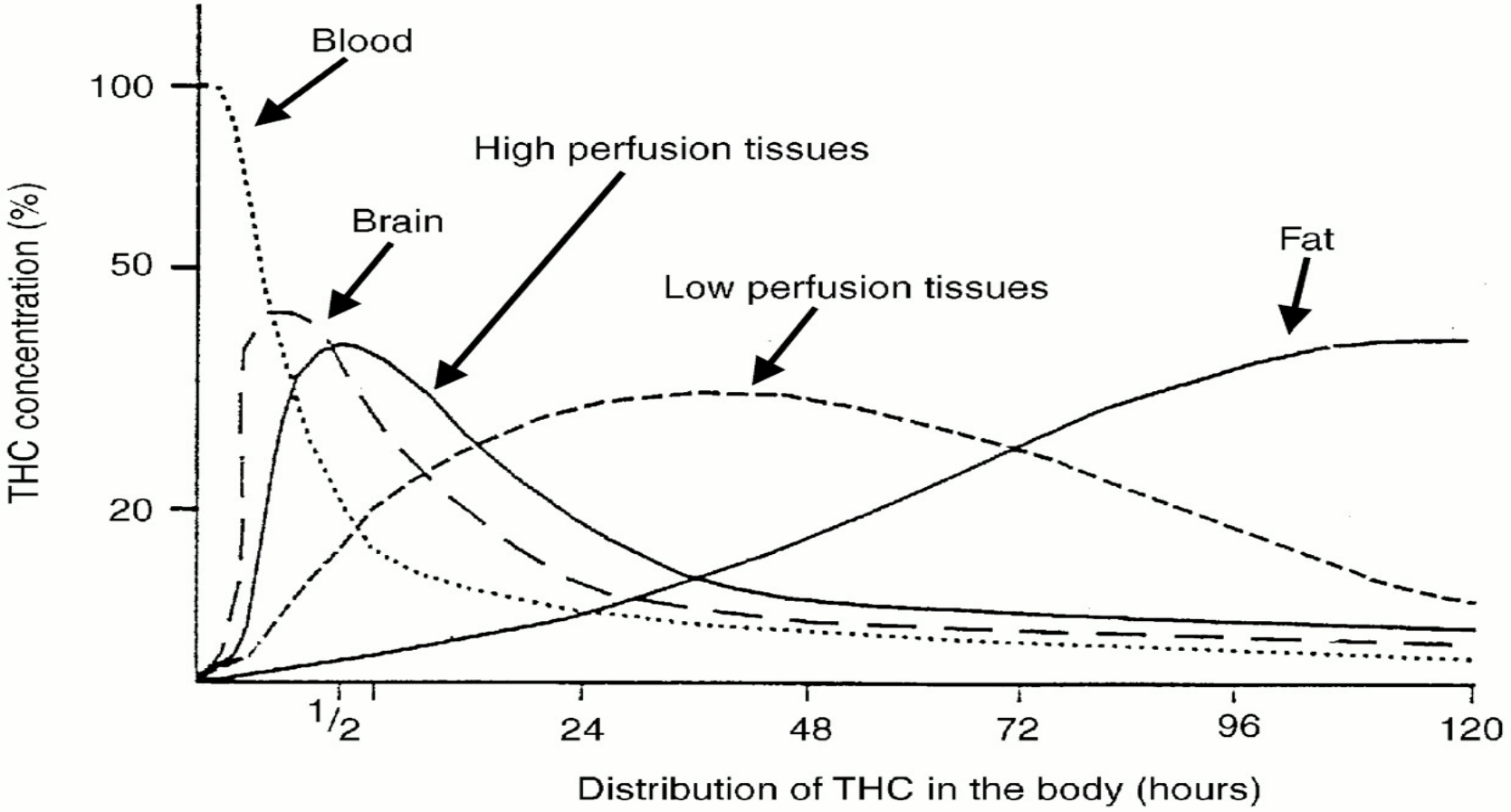
THC

THC is very different from ethanol in its ADME characteristics

- THC is very lipid soluble
- It is not absorbed well from the GI tract - oral bioavailability 5-10%, and after oral ingestion there is a lag before pharmacological effects are seen
- Absorption from the lungs is very rapid, effects seen within minutes (serum levels peak within 10 minutes)

THC

- Highly concentrated in tissues over the blood, concentrates in fat
- Drug can then be re-released from fat tissues over time
- THC is metabolized to 11-hydroxy-THC which is also pharmacologically active
- These combined characteristics result in a very long biological half-life
  - Tissue elimination is about 7 days, and complete elimination of a single dose may take up to 30 days


Blood
100
High perfusion tissues
Brain
50
Low perfusion tissues
Fat
20
THC concentration (%)
1/2
24
48
72
96
120
Distribution of THC in the body (hours)

THC

Detection of THC or 11-hydroxy-THC in urine is indicative of exposure but not necessarily of impairment

- Actual psychological effects may dissipate within 3-5 hours despite the long half life in the system
- The relationship between blood levels of THC and pharmacological effect is not well established
- Debate within the scientific and legal community on relevance

Impact of THC levels on driving ability also debated

THC

Interpretation of forensic toxicology results for THC are not as straightforward as those for ethanol

Opioid Metabolism

Has a genetic component, ie allelic variation in cytochrome P450s that metabolize opioids

For some background:

A Z DePriest, B L Puet, A C Holt, A Roberts, E J Cone. Metabolism and Disposition of Prescription Opioids: A Review

Forensic Sci Rev 27 (2), 115-45 Jul 2015

Fentanyl

Synthetic opiate

Variable excretion rates

Data suggests this may be the result of differential distribution about the body

10% is cleared from the body through renal excretion after being acted on by a P450 the rest is liver-based

Mather Clin. Pharmacokinet. 1993 5:422

Updated review:

Kuip et al (2017) A Review of Factors Explaining Variability in Fentanyl Pharmacokinetics; Focus on Implications for Cancer Patients. Br J Clin Pharmacol, 83 (2), 294-313

Recap

Forensic Toxicologists:

- Detect and identify drugs and toxins in biological samples
- Interpret the results in the context of forensic applications
    - Can be complex and challenging
- Ethanol cases form a large percentage of case load for forensic toxicologists
- The ADME characteristics of ethanol are well understood
    - Makes interpretation of results more straightforward than other drugs

Recap

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

“It is the dose that makes the poison.”