FORENSIC TOXICOLOGY:

FROM CRIME SCENE TO VIRTUAL LAB

MODULE 1

Chapter 2: Opiates

CURRENT OPIATE DETECTION TOOLS

DRUG DETECTION DEVICE

Indiana Police Now Using Drug-Detection Device on Drivers

The device called the SoToxa Mobile Test System is a handheld analyzer that uses an oral fluid swab to detect the presence of drugs

Published December 20, 2020 • Updated on December 20, 2020 at 4:38 pm

Sotoxa™ Mobile Test System

Sotoxa is a handheld analyzer that allows for **drug testing at roadside.** It can detect up to 6 drug classes within 5 minutes from a **single oral fluid** collection sample, including **opiates**!

DRÄGER DRUGCHECK® 3000

Dräger

D-16444-2017

The Dräger DrugCheck® 3000 immediately detects whether someone has recently consumed drugs or is possibly under the influence. This compact, saliva-based rapid drug test is simple to use, cost-effective and hygienic. With the DrugCheck 3000, you can test for up to six classes of substances at once: cocaine, opiates, amphetamines, methamphetamine/designer drugs such as ecstasy, benzodiazepines and cannabis (THC). The pocket-sized DrugCheck 3000 requires no electricity, so it can be used anywhere.

DrugCheck 3000 can accurately measure six classes of drugs at once as well (including opiates).
Using saliva, these are ideal for police and industrial use.

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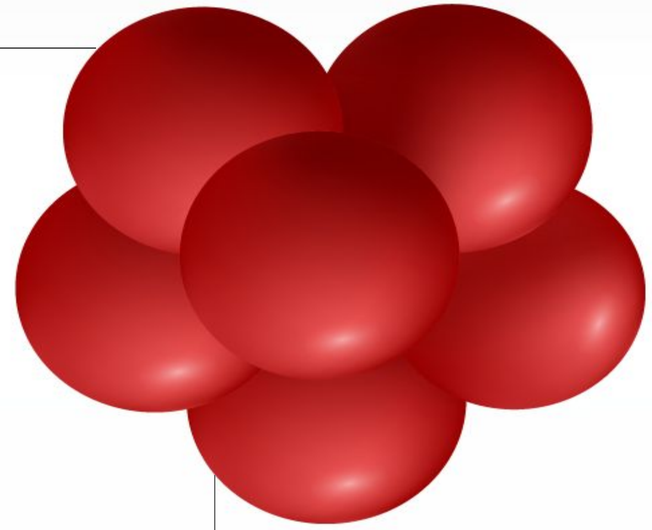
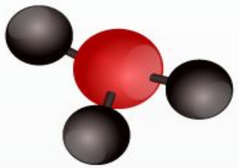
01

INTRODUCTION TO OPIATES

*What are **opiates**?*
*Where do opiates **come from**?*
*What are the **common types** of opiates?*
*Differences between **opiate** and **opioid**?*





01

WHAT ARE OPIATES & WHERE DO THEY COME FROM?

Opiates - chemical substances derived from opium, which is present naturally in poppy plants and seeds. Opium is a mixture of natural alkaloids some of which are effective drugs that treat mild to severe pain ("painkillers").

Poppy Plant

Inside of poppy seed

Processed opium

Two main classifications include:

1) **Antagonists:** less addictive, assist with detoxification
2) **Agonists:** mimic the effects of naturally-occurring endorphins in the body and produce opiate effect in the brain

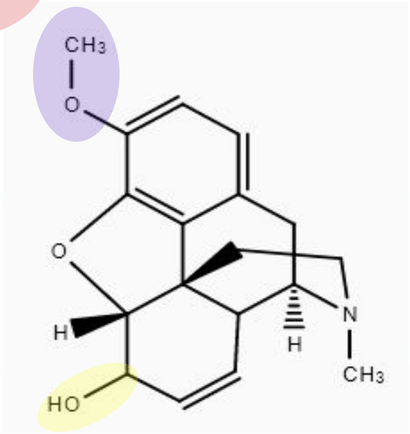
Antagonists	Agonists	
Naltrexone Naloxone	Morphine Fentanyl Hydrocodone	Oxycodone Heroin Buprenorphine

01

WHAT ARE THE COMMON TYPES OF OPIATES?

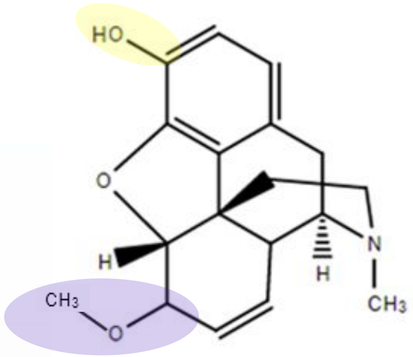
Opiate agonists interact with **specific receptor sites** in the brain, causing the feelings of '**high**' one feels when intoxicated. **Abuse of opiates shown below = <u>opiate crisis</u>**

1



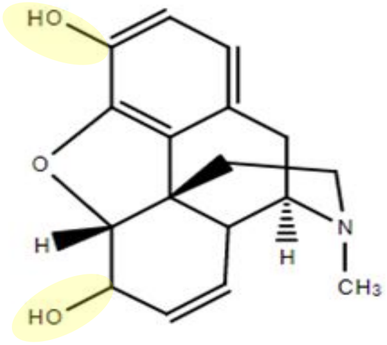
Codeine

2



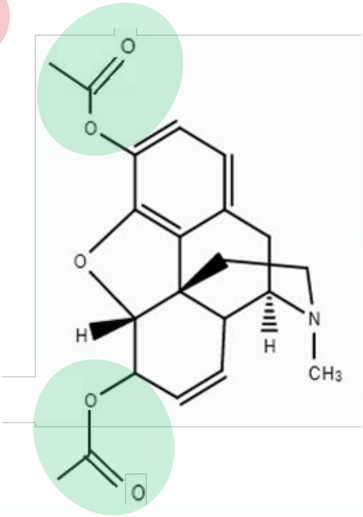
Thebaine

3



Morphine

4



Heroin

01

DIFFERENCES BETWEEN OPIATES & OPIOIDS

OPIATES

Naturally-derived substances that contain active ingredients from opium found in poppy plants

Example:

- **Morphine**

OPIOIDS

Synthetically-manufactured ('manmade') substances that mimic the natural effects of opium. Some are fully synthetic, while others contain natural opium in part (**Synthetic** vs. **Semi-Synthetic**).

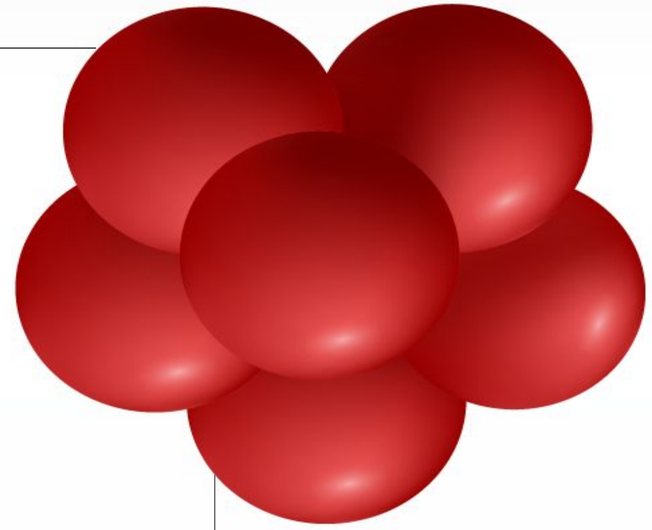
Examples:

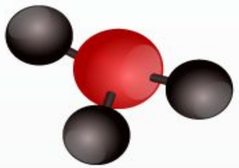
- **Fentanyl** (synthetic)
- **Hydrocodone** (semi-synthetic)

Both **opiates** and **opioids** activate Mu receptors in the brain through the **depression** of the brain (central nervous system, CNS). When these receptors are activated, they release chemicals called "***endorphins***" that induce a feeling of pleasure, relaxation, and calmness, feelings that can be highly addictive.

HISTORICAL & CURRENT USES

***Ancient** Uses*
Current** Uses in **Canada





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Postcard with opium smoker *(c. 1905)*

ANCIENT USES OF OPIATES:

3400 BC – 1996

3400 B.C. — Opium poppy was cultivated in lower **Mesopotamia**, known as "*the joy plant.*" Sumerians pass along euphoric effect knowledge to Assyrians, then to Babylonians and Egyptians.

1300 B.C. — **Thebes, Egypt:** Began cultivating opium thebaicum in their poppy fields. Opium trade exploded during King Tutankhamen's reign, making its way into the Mediterranean Sea, to Greece and Europe.

460 B.C. — **Hippocrates** (*Father of Medicine*) dismissed magical features of opium but states its usefulness as a narcotic in treating internal diseases, diseases in women, and epidemics.

330 B.C. — **Alexander the Great** introduces opium to **Persia** and **India**.

1500 — **The Portuguese**: initiated smoking opium during the East China Sea trade. Effects of opium were instantaneous, but it was a method the Chinese found barbaric.

1527 — **Reformation**: Opium is reintroduced into European medical literature by Paracelsus (*laudanum*). The medical pills were made of opium thebaicum, citrus juice, & gold – prescribed as painkillers.

1600s — **Persians & Indians:** initiated ingestion of opium mixtures for recreational uses.

1700 — **Dutch:** Introduces smoking opium in a tobacco pipe to the Chinese.

1750 — British shipping dominates the opium trade out of **Calcutta to China.**

1803 — Friedrich Sertuerner (Germany): Discover the **active ingredient** of opium by dissolving it in acid, then neutralizing it with ammonia.
RESULT:
Alkaloids (*Principium somniferum* or *MORPHINE*)

1827 — **E. Merck & Company** (Germany): Begins commercially producing **morphine**.

1895 — **The Bayer Company** (Germany): Diluting **morphine** with **acetyls** produces drug without the side effects; coins term ***heroin*** – introduced officially as "**Heroin**" three years later **(1897)**

1874 — **C.R. Wright:** 1st synthesizes **heroin** (diacetylmorphine) by boiling morphine over stove.

1903 — **Heroin addiction** rises to alarming rates.

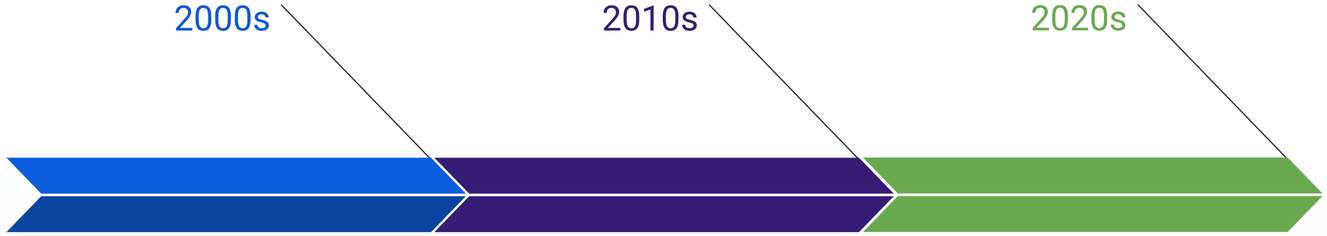
1923 — The US Treasury Department's Narcotics Division **bans all legal narcotics sales.**

1973 — US President Nixon creates **the DEA (Drug Enforcement Administration)**

1996 — **International drug trafficking** organizations began aggressively marketing heroin in the US and Europe.

CURRENT USES OF OPIATES:

Today, Canada is currently the second highest consumer of opiates and opioids in the world. The use of medicinal and recreational opiates has increased steadily over the last two decades, with the highest numbers recorded in misuse, disorders, and death cases. In 2015, an estimated 2,000 Canadians died from opioid overdoses, and while these statistics include synthetic and semi-synthetic opiates, a large portion is attributable to heroin, codeine and morphine opiates. A timeline of the **Opioid Crisis in Canada** is listed below.



Morphine & Codeine

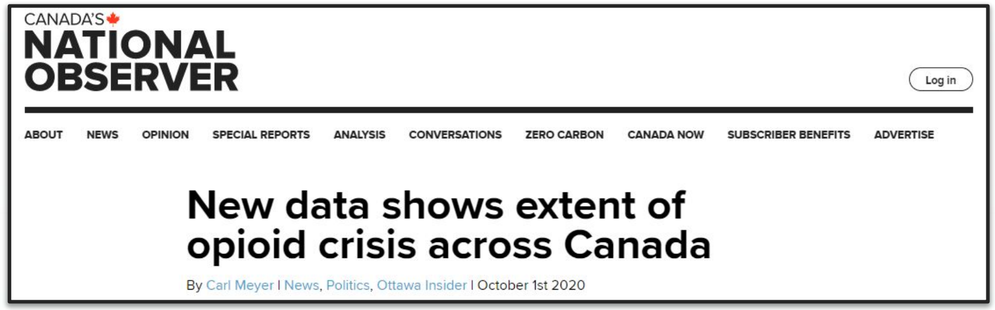
The overprescribing of opiate painkillers increased the prevalence of misuse cases globally, increasing the global populations addiction to opiates & fueling the opioid crisis epidemic

Pinnacle of Opioid Crisis in Canada

In 2016, Canada declared a national opioid crisis. Between January 2016 and December 2018, more than 11,500 opioid-related deaths were reported in Canada alone, suggesting the opioid crisis is a leading public health and safety concern.

COVID-19 Pandemic + Opioid Crisis

77% of deaths across Canada involved some form of opiate/opioid (including fentanyl & analogues), as seen in data collected up until March 2020. The COVID-19 pandemic had paused most data collection of opioid-related deaths, as the virus was a more gruesome and deadly killer at the time. However, more than 4560 suspected overdoses were reported in early 2020.


CANADA'S NATIONAL OBSERVER

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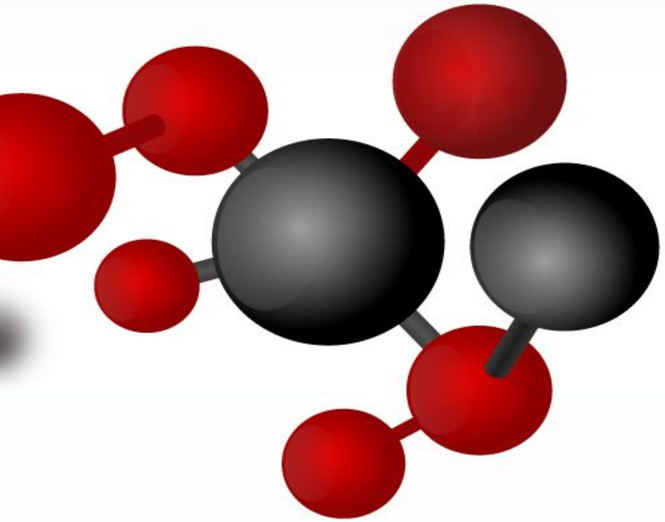
ABOUT NEWS OPINION SPECIAL REPORTS ANALYSIS CONVERSATIONS ZERO CARBON CANADA NOW SUBSCRIBER BENEFITS ADVERTISE

New data shows extent of opioid crisis across Canada

By Carl Meyer | News, Politics, Ottawa Insider | October 1st 2020

03



EXTRACTION OF OPIUM ALKALOIDS

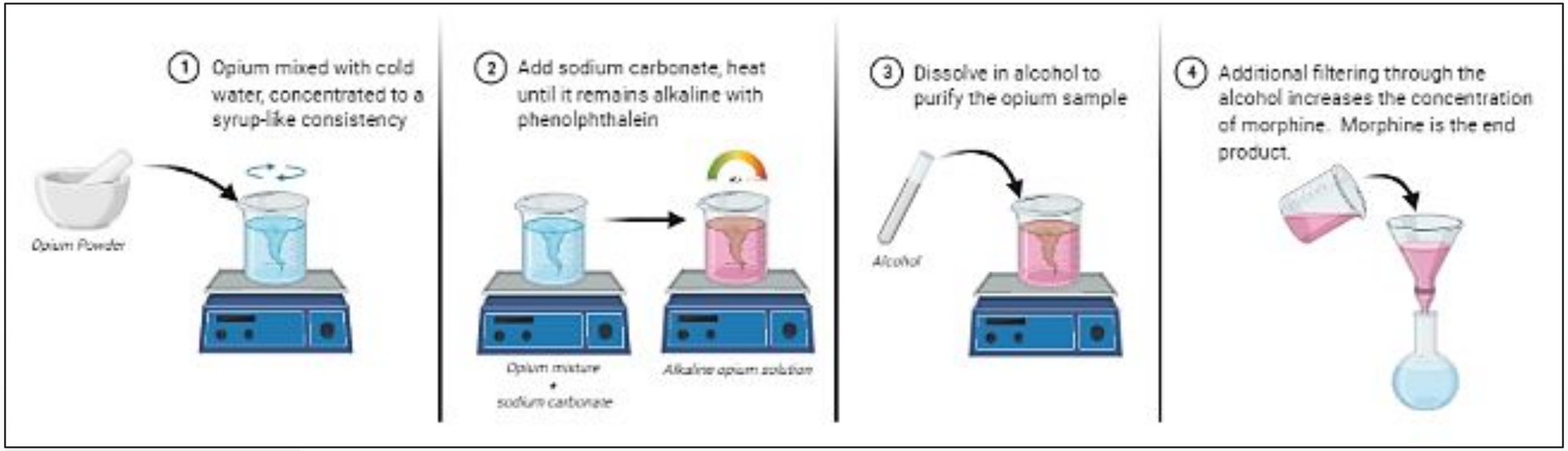
From Poppy Plant to Drug

EXTRACTION OF OPIUM ALKALOIDS

To chemically process the opium seeds for its alkaloid properties, three classical and ancient extraction methods exist for the extraction of morphine, including the **Merck Process**, the **Thiboumery and Mohr Process**, and the **Robertson-Gregory Process**.

Merck Process

1. Opium exhausted with cold water, product is concentrated to a syrup-like consistency
2. Precipitated with powdered sodium carbonate and heated until it remains alkaline with phenolphthalein indicator
3. Precipitate filtered then dissolved in alcohol to purify the sample
4. Acidification was done to neutralize solution and isolate morphine

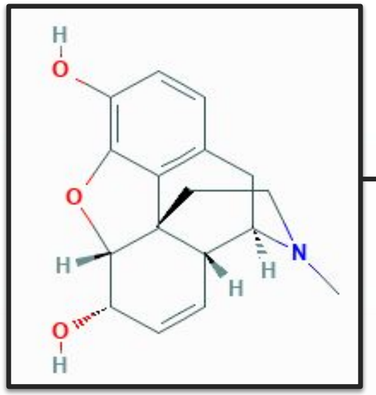


Created in BioRender.com

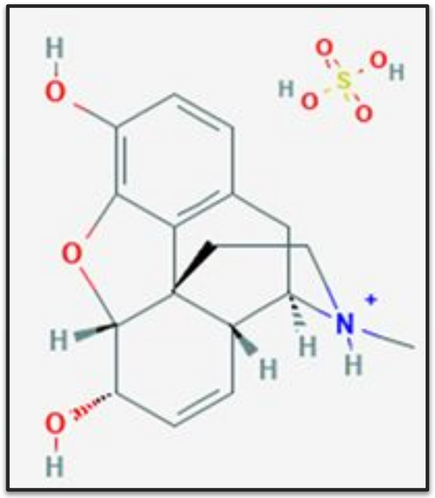
EXTRACTION OF OPIUM ALKALOIDS

The current industrial extraction process of opium alkaloids used today are listed below.

A. Extraction of Opium
B. Concentration of Liquids
C. Precipitation of Total Alkaloids
D. Extraction of Secondary Alkaloids
E. Preparation of Morphine Acid Tartrate
F. Precipitation of Morphine Base
G. Treatment of Secondary Alkaloids
H. Extraction of Alkaline Liquids
I. **Natural Codeine**



Morphine Base

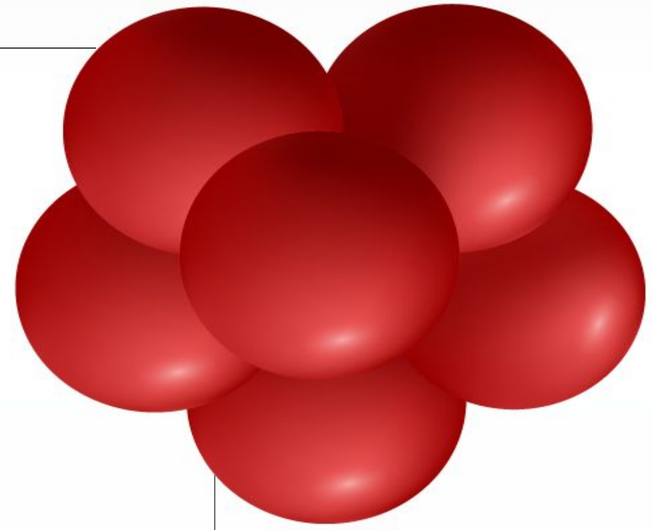


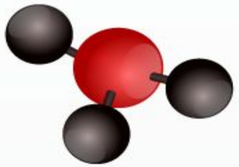
Morphine Sulfate (salt)

Morphine sulfate is the sulfate salt of morphine (opiate alkaloid). Other common names are ***Kadian***, which is a DEA controlled drug (narcotic).

STRUCTURES OF OPIATES

Naturally-Occurring Products:
Codeine, Morphine, Heroin, Thebaine

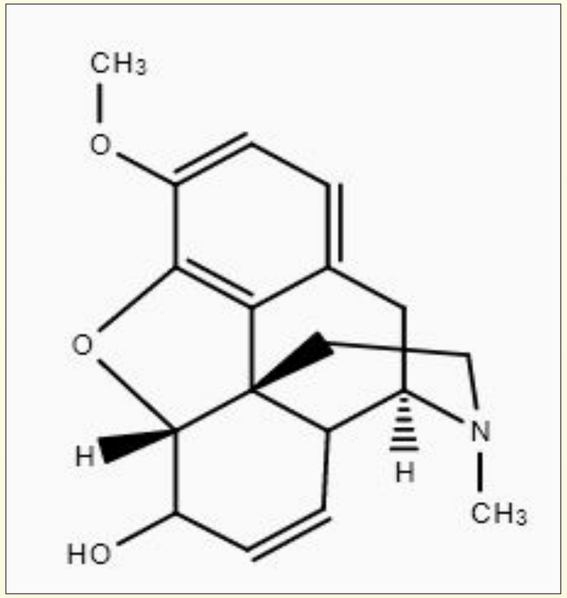




CODEINE: STRUCTURES

Codeine is an **opiate analgesic** used to treat moderate to high pain. This drug was first approved by the US in 1950 as a treatment method for increasing pain threshold without altering the conscious state and sensory functions. Codeine is an opiate **derived from the poppy plant**, with the codeine content in opium being highly dependent on the type of opium produced among various countries.

- **Naturally-occurring phenanthrene** alkaloid and opioid agonist
- Mimics actions of endogenous opioids by binding to the opioid receptor in the brain and in the CNS
  - Stimulates ***mu* opioid receptors**, resulting in a decrease in nociceptive neurotransmitters (GABA, dopamine, noradrenaline)
  - *Metabolite*: **morphine** induces opening of G-protein-coupled inwardly rectifying potassium (GIRK) channels, blocks calcium channels, leads to hyperpolarization and excitability

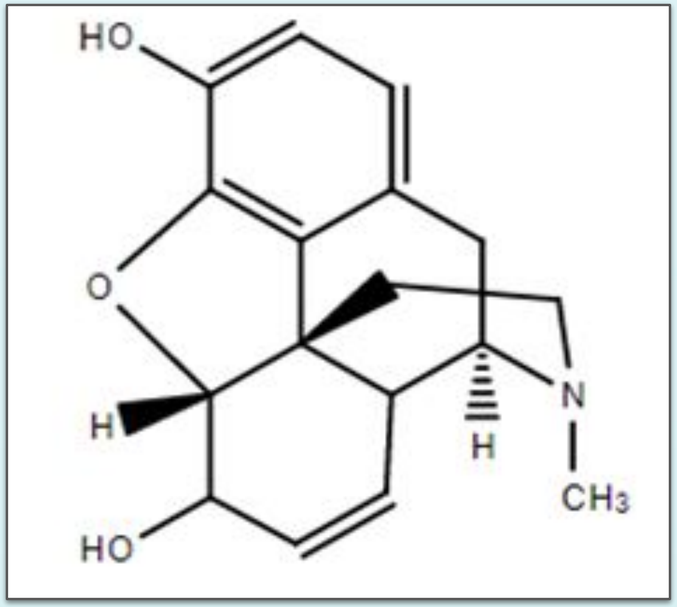


Codeine

MORPHINE: STRUCTURES

Morphine is a potent **opioid agonist** used to treat moderate to severe and chronic pain with analgesic effects on the body. First isolated from poppy seeds in 1805, this drug is the main chemical **alkaloid** in opium and is routinely used for medicinal purposes today.

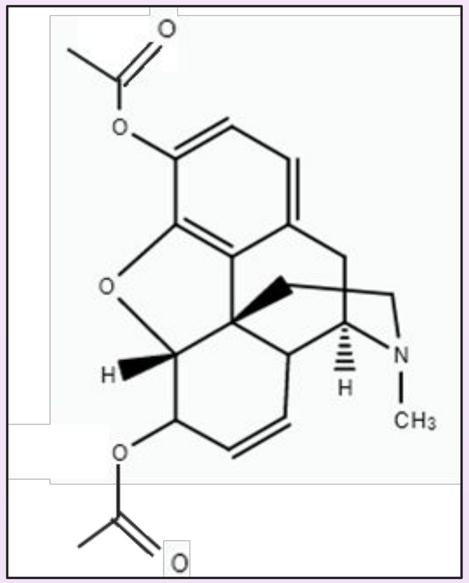
- Morphine belongs to the organic compound class ***morphinans***
- **Polycyclic** compounds with a 4-ring basic skeleton and 3-condensed 6-member rings that form a partially hydrogenated phenanthrene group
- Free hydroxyl groups
- Known transformation product of **codeine** (**metabolite**)
- Induces opening of G-protein-coupled inwardly rectifying potassium (GIRK) channels, blocks calcium channels, leads to hyperpolarization and excitability



Morphine

HEROIN: STRUCTURES

Diamorphine (**heroin**) is a morphinane alkaloid that is structurally similar to morphine with two acetyl groups on the O-3 and O-6 positions. Heroin is used as an **analgesic** for the relief of severe pain and as a recreational drug, where when ingested, binds to *mu*-receptors and acts as a prodrug.



Heroin

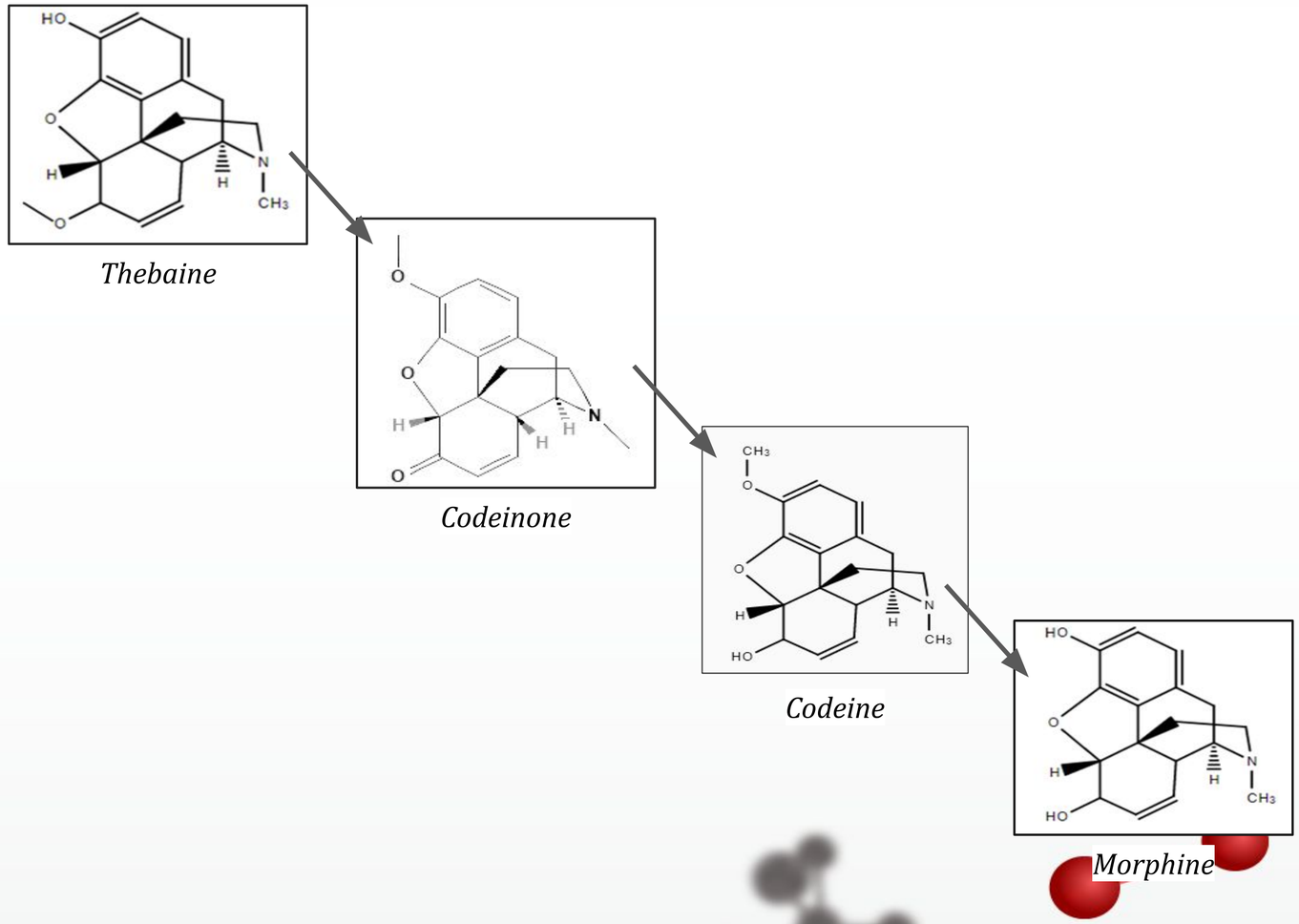
STRUCTURE:

- **Heroin** is comprised of 2-acetyl groups that give rise to its chemical name
- It derives from a **morphine** drug
- The drug is one of the **most potent opiates** currently banned in most countries, as people using the drug often develop liver disease due to substance abuse
- Heroin is **deacetylated** via esterase enzymes to form active metabolites

THEBAINE: STRUCTURES

Thebaine is an alkaloid opiate used as an **intermediate** for the synthesis of other opioid drugs. It's classification is a **morphinane alkaloid** and an organic **heteropentacyclic** chemical.

- Thebaine belongs to the organic compound class ***morphinans***
- Once metabolized, structures formed are neopinone, codeinone, codeine, and eventually morphine (active chemical compound)



Canada
Justice Laws Website

CONTROLLED DRUGS AND SUBSTANCES ACT

The **Controlled Drugs and Substances Act (CDSA)** establishes a legislative framework for the regulation of import, export, possession, production, assembly, sale, transport, sending, and delivery of controlled substances used for the manufacturing of **illegal drugs**. The CDSA is regulated by **Health Canada**, where all activities are prohibited unless authorized by the Act. The term **'controlled substance'** refers to the substances listed in **Schedules I-IX** including narcotics, restricted substances/drugs, benzodiazepines, and targeted substances. The Schedules are listed in order of decreasing potential for abuse (*i.e., Schedule 1 has a higher potential for abuse than Schedule 2*).

Schedule I

1. Opium Poppy
   a. **Opium, codeine, morphine, thebaine & their salts, derivatives, & alkaloids**
   b. **Naloxone, papaverine, poppy seeds & their salts, derivatives, & alkaloids**
2. Coca Plant
   a. Coca leaves, cocaine, ecgonine and their salts, derivatives, & alkaloids
3. Phenylpiperidines
   a. Intermediates, salts, derivatives, & analogues
4. Morphinans
   a. Buprenorphine and their derivatives & salts
5. Fentanyls
   a. Salts, derivatives, & analogues of salts
6. Methamphetamine
   a. Salts, derivatives, isomers, & analogues
7. Amphetamines
   a. Salts, derivatives, isomers, & analogues
8. 4-hydroxybutanoic acid (GHB)
   a. Any of its salts

Schedule II

1. **Synthetic cannabinoid receptor type 1 agonists**
   a. Their salts, derivatives, isomers, & salts of derivatives/isomers
2. **Any substance that has a 3-(1-naphthoyl)indole structure with substitution at the N atom of the ring**
   a. (ex.) 1-pentyl-3-(1-naphthoyl)-indole

Schedule III

1. **Methylphenidate** & its salts, derivatives, isomers, & analogues
2. **Psilocin**
3. **Psilocybin**
4. **Cathinone**

Schedule IV

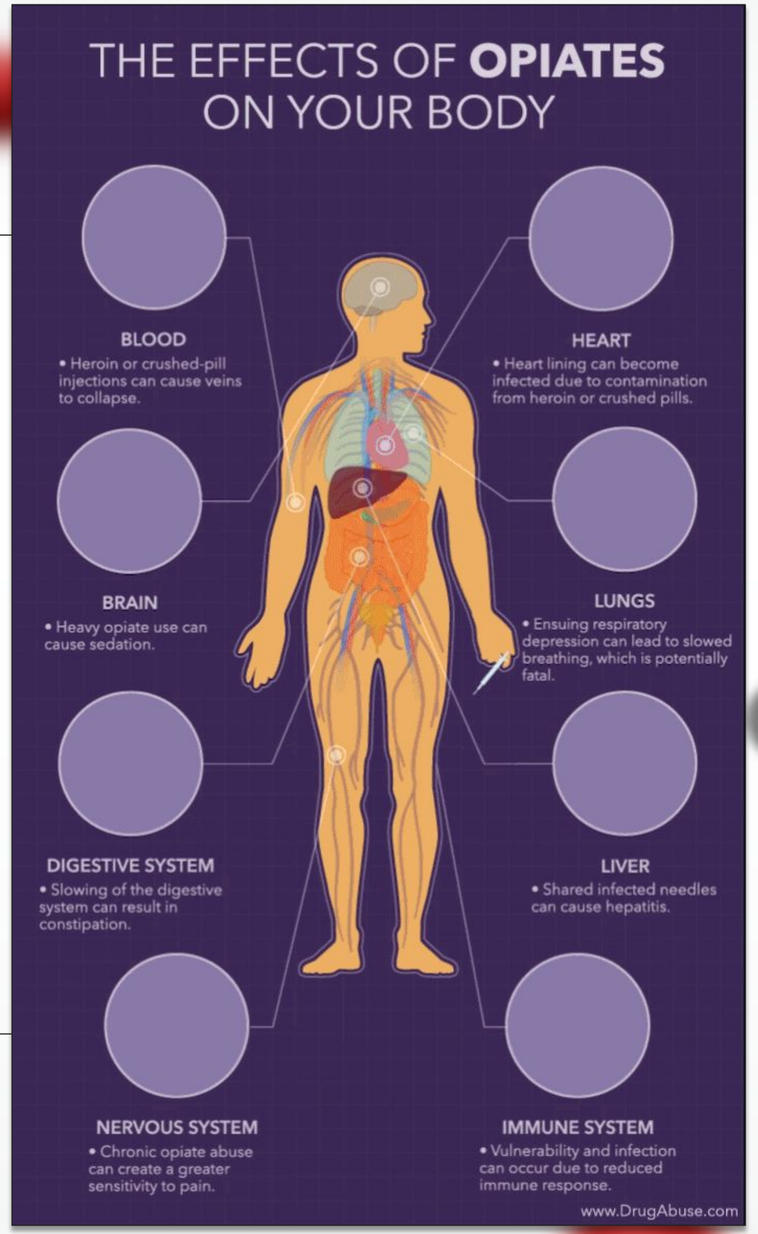
1. **Barbiturates** (Salts & derivatives)
2. **Thiobarbiturates** (Salts & derivatives)
3. **Benzodiazepines** (Salts & derivatives)
4. **Anabolic steroids** (Salts & derivatives)

Government of Canada / Gouvernement du Canada

OPIATE EFFECTS ON THE BODY

***Toxic Effects** on:*

- *Heart, Brain, Liver*
- *Digestive, Nervous, Respiratory and Immune Systems*



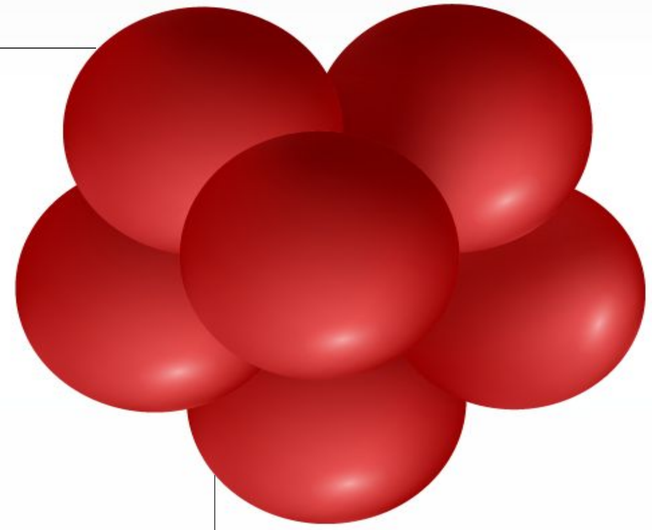
OPIATE EFFECTS ON THE BODY

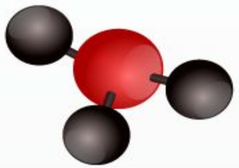
Liver	➔ Since many painkillers are combined with acetaminophen, excessive uses can cause **acetaminophen toxicity** ➔ Adding alcohol to the drug use also decreases the liver's ability to process the combination of alcohol and acetaminophen, thus inducing **severe liver injuries**
Brain	➔ Opiate painkillers increase the likelihood of **daytime sleepiness**, which could require additional stimulant medications to counteract the tiredness ➔ (ex.) Heroin can elicit severe drowsiness, with abusers experiencing rounds of 'nodding off' and increased risk of developing **depression**
Nervous System	➔ Chronic use of opiates can lead to **hyperalgesic** states: **increased sensitivity** to **pain** ➔ Opiate use is linked to **psychomotor impairment** -- slowing of a person's physical abilities and loss of coordination ➔ Stimulation of dopamine reward system that increases pleasure and greater sensitivity to pain (leading to dependence)
Respiratory System	➔ Overdosing on painkillers (codeine, morphine) or heroin can lead to **respiratory depression** (sharp decrease in breathing) ➔ At sufficient doses, respiratory breathing stops and damages the brain and body by decreasing the amount of oxygen present (debilitating and/or fatal)
Digestive System	➔ Opiates cause **constipation** in the digestive system, leading to slower metabolism and breaking down of nutrients ➔ This lack of motility can lead to chronic constipation in abusers and can increase the risk of small bowel obstruction, perforation, and peritonitis, as well as **nausea** and **vomiting**
Immune System	➔ Excessive opiate use can suppress the immune system by increasing the **vulnerability** and **risk** of **infection** in the body, inducing by a severely **reduced** immune response

OPIATE EFFECTS ON THE BODY

PHARMACODYNAMICS OF OPIATES

Opiate Receptor Binding

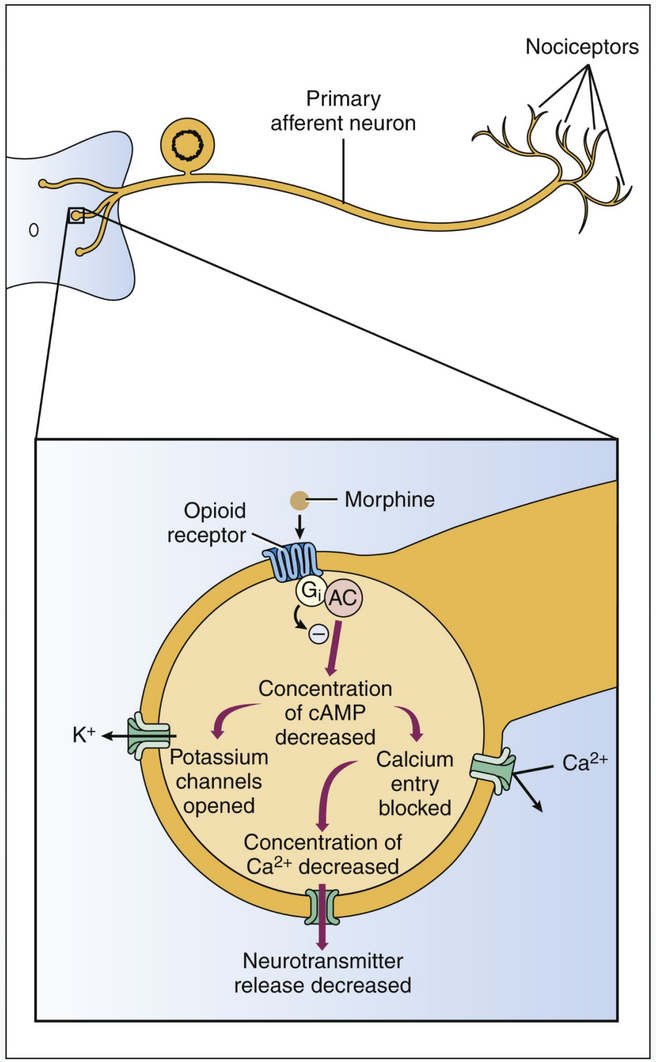


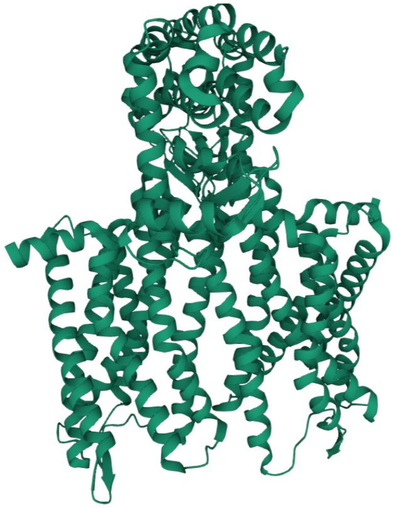


PHARMACODYNAMICS OF OPIATES:

Receptor Binding

- Each opiate has a different affinity for each receptor
- Opiates commonly bind to, the ***mu*** receptor but also to **kappa** (**κ**) and **delta** (**δ**) receptors
- Opiate metabolites can exhibit high affinities for the receptors, contributing to the overall analgesic effects of the drugs
- Morphine binds to mu receptor with Ki ~ 5 nM





Crystal structure of the mu-opioid receptor

PHARMACODYNAMICS OF OPIATES:

Receptor Binding

The **3 main receptors** in the CNS and peripheral tissues for opioid binding differ based on their prototype agonists and are typically stimulated by endogenous peptides (endorphins, enkephalins, and dynorphins).

Mu (μ)

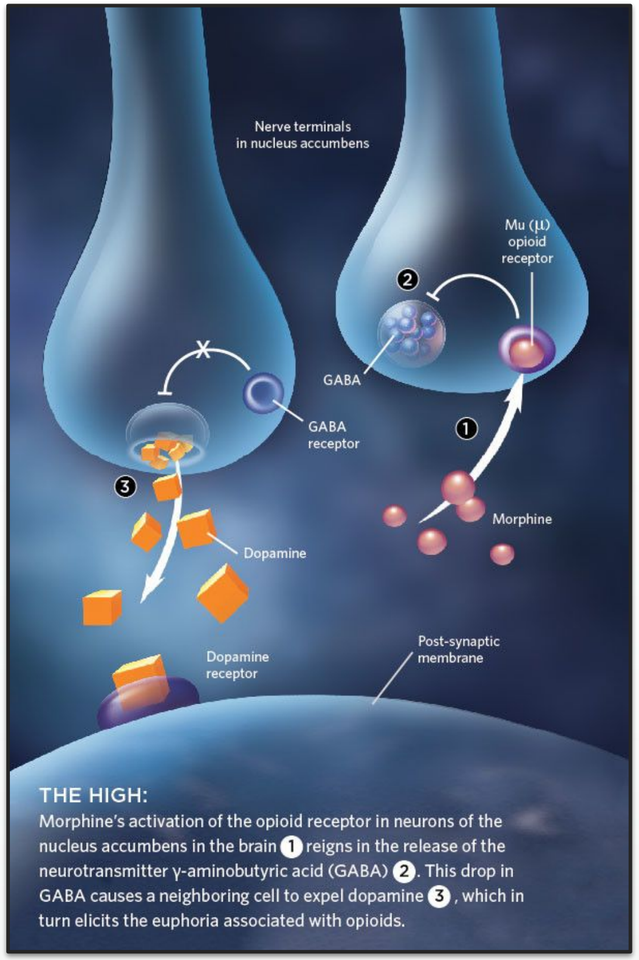
- Found primarily in the brainstem and medial thalamus
- Responsible for supraspinal analgesia, euphoria, sedation, & physical dependence
- Also known as **OP3** or **MOR** (morphine opioid receptors)
- ***Agonist morphine***

Delta (δ)

- Located in the brain, effects not well-studied
- May be responsible for psychomimetic and dysphoric effects
- Also known as OP1 and DOR (delta opioid receptors)
- ***Agonist delta-alanine-delta-leucine enkephalin)***

Kappa (κ)

- Found in the limbic and diencephalic areas, brainstem, and spinal cord
- Responsible for spinal analgesia, sedation, dyspnea, dependence, and respiratory depression
- Also known as **OP2** or **KOR** (kappa opioid receptors)
- ***Agonist ketocyclazocine***



Ka (binding constant); Kd =1/Kb (dissociation constant):

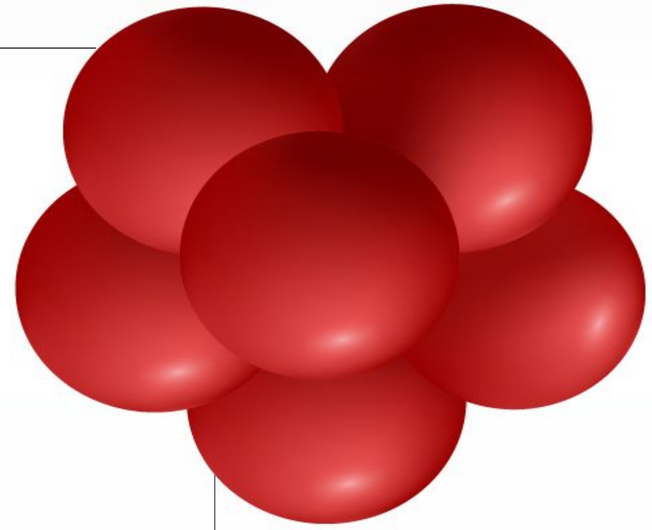
- EXpression used to describe the reversible reaction of a **receptor (R) and ligand (L; *opiate drug*)**:

$$R + L \leftrightarrows RL$$

- K value determines the strength of the affinity the ligand (drug) has for the receptor
- K values determines the stability of the complex

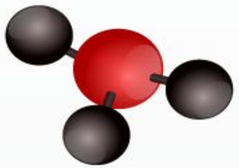
Morphine's Kd is ~ 5 nM at the *mu* receptor. **Fentanyl's** Kd is ~ 1 nM at the mu receptor.

Dissociation constants below 10 nM indicate high binding affinity of drug to receptor



PHARMACOKINETICS OF OPIATES

***ADME**:*
Absorption, Distribution, Metabolism, Excretion



PHARMACOKINETICS OF OPIATES

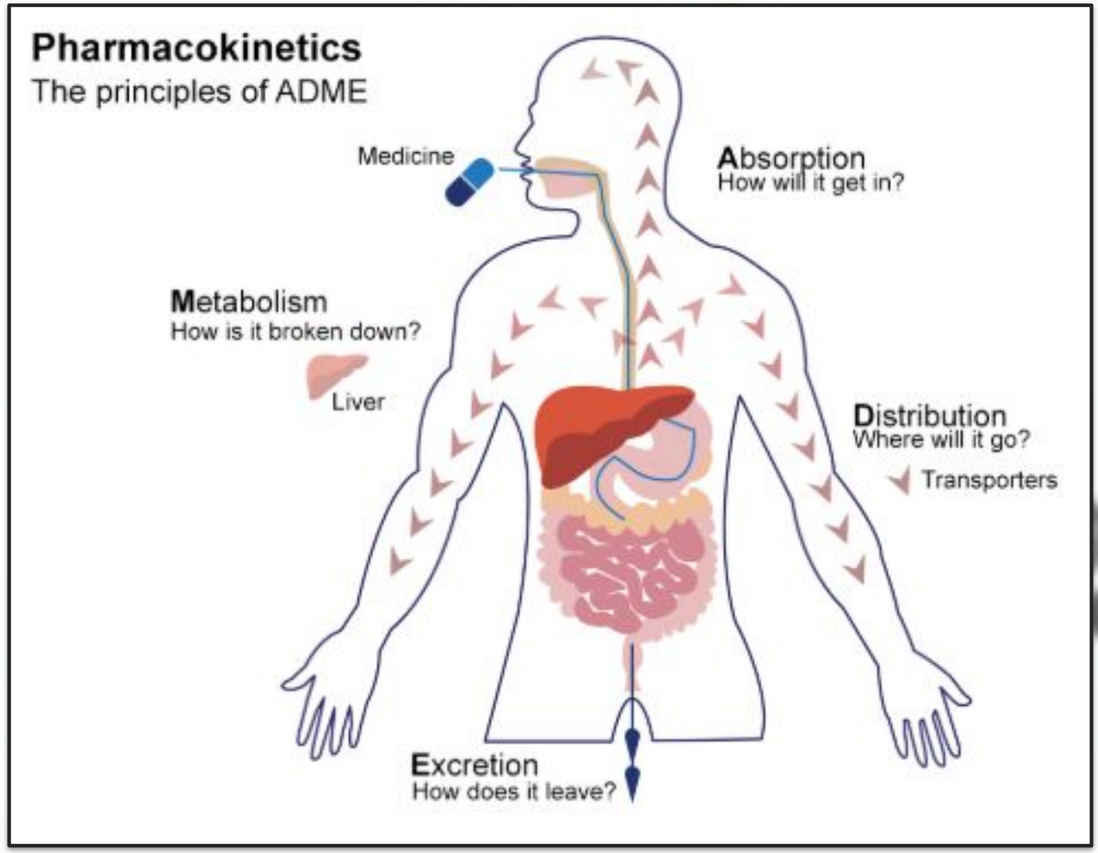
Pharmacokinetics is the processing of compounds in the body that undergo 4 fundamental stages: **Absorption**, **Distribution**, **Metabolism**, and **Elimination**. These processes describe the interaction of drugs with target tissues throughout the body and can be broken down into *3 Chemical Structure Classes*:

CHEMICAL STRUCTURE CLASSES

Class 1:
4,5-epoxymorphinan ring → Opium, codeine, morphine, thebaine

Class 2:
Phenylpiperidines → Various opioids

Class 3:
Diphenylheptylamines → Various designer drugs

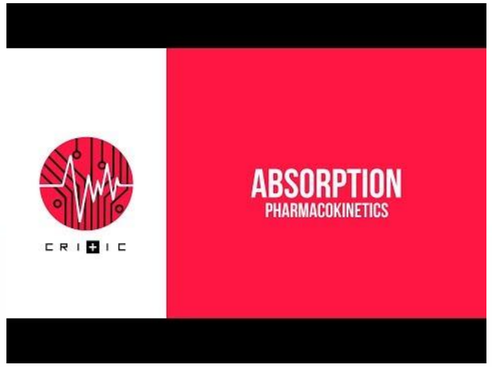


Pharmacokinetics of the Human Body:
ADME

PHARMACOKINETICS OF OPIATES

Opiates share many similar structural features, but what separates them are their varying functional groups. Because of these differences, each compound elicits a slightly different **pharmacological effect** and can bind to different neuronal receptors. To understand individual responses in pharmacokinetics, general aspects of opiate pharmacology are below (**absorption**, **distribution**, **metabolism**, and **excretion**).

ABSORPTION



- The majority of opiates present high gastrointestinal (GI) permeability and are readily absorbed from the GI tract into the bloodstream after oral ingestion.
- These drugs include morphine and codeine, with heroin and other metabolites exhibiting poor absorption and must undergo extensive metabolism to be processed.

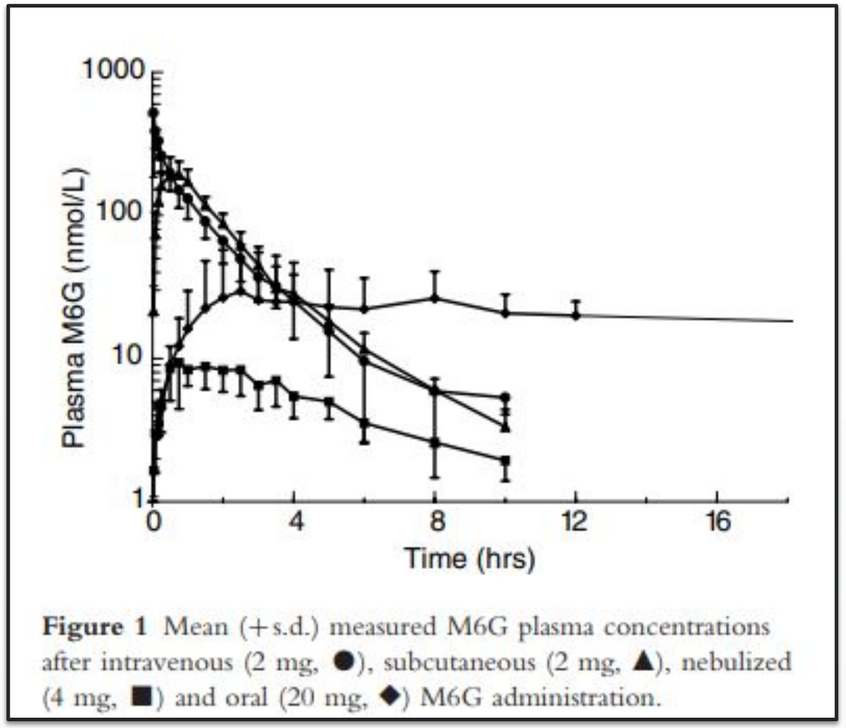


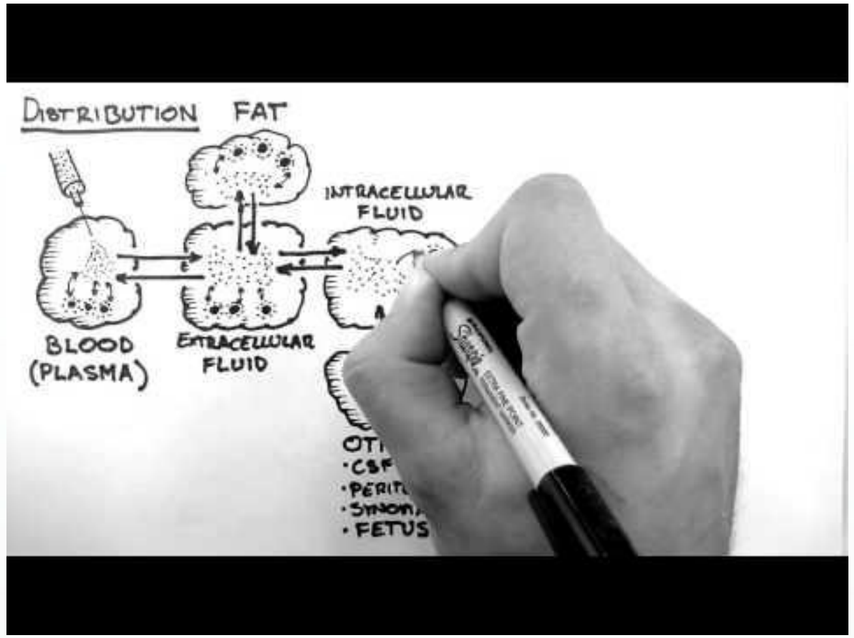
Figure 1 Mean (+s.d.) measured M6G plasma concentrations after intravenous (2 mg, ●), subcutaneous (2 mg, ▲), nebulized (4 mg, ■) and oral (20 mg, ◆) M6G administration.

J. Clin. Pharmacol. 2002, 53, 347-354

- The mean plasma concentrations of M6G after each route of administration of morphine: **intravenous, subcutaneous, nebulized, or oral**
- Absorption from the intravenous and subcutaneous was fairly **rapid** (<10 mins).
- Absorption from the oral was **slowest** (~2.5h)
- Fast peaking = fast elimination
- At 10 h, the highest M6G was after oral administration and the lowest was from nebulized

PHARMACOKINETICS OF OPIATES

How drug travels through the body?
Pay attention to the Vd, pH, free drug and other concepts in this video.



DISTRIBUTION

- After being absorbed into the body, opiates target the main action site within the central nervous system (CNS).
- In order to effectively enter the CNS, the drug compounds must pass through the blood-brain-barrier (BBB) in order to affect the CNS through various absorption pathways, followed by the distributing of the drug in the blood plasma and circulatory system.
- The body is made of 4 different compartments: **Blood** (**plasma**), **Fat**, **Extracellular Fluid**, and **Intracellular Fluid**. Other compartments include CSF, peritoneum, synovial fluid, and fetal fluid.

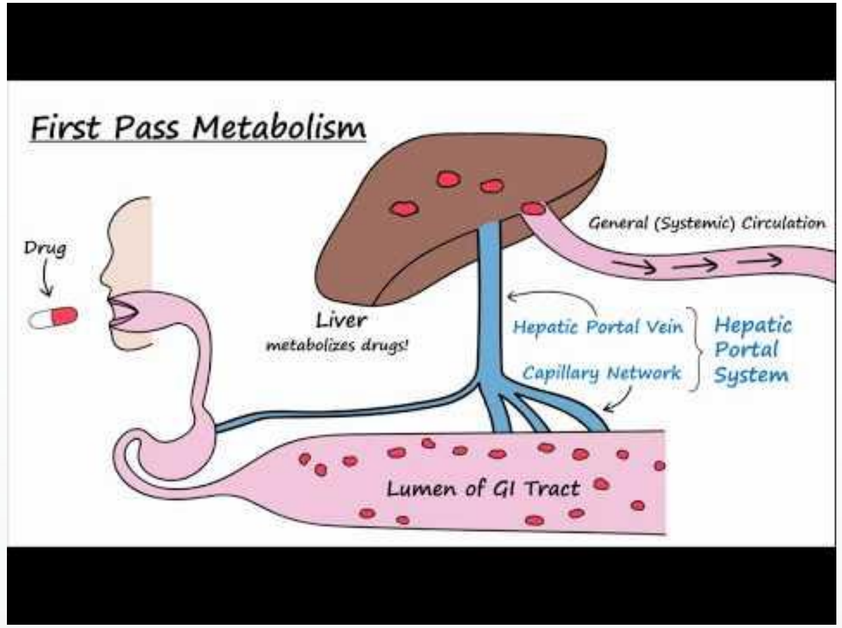
PHARMACOKINETICS OF OPIATES

METABOLISM

- If taken orally, most opiates undergo **first pass metabolism** in the liver, which excretes most of the drug almost immediately out of the body once ingested.
- However 100% of opiate is bioavailable if taken intravenously.
- **Bioavailability** is the percentage of a drug that makes it to systemic circulation, which defines how much of an ingested drug will affect the the body from the amount taken in (SEE THE VIDEO ON "METABOLISM AND BIOAVAILABILITY").

- Heroin is subject to hydrolysis
- Codeine is subject to *O*-dealkylation by **CYP2D6** enzymes to produce morphine
- Opiate are also prone to *N*-alkylation by **CYP3A4** enzymes
- Lastly, these drugs undergo glucuronidation, forming glucuronide-based metabolites at the hydroxyl group by **UGT2B7** enzyme

METABOLISM AND BIOAVAILABILITY

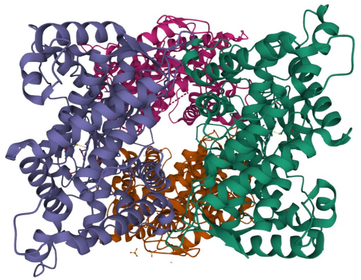


PHARMACOKINETICS OF OPIATES

Phase I

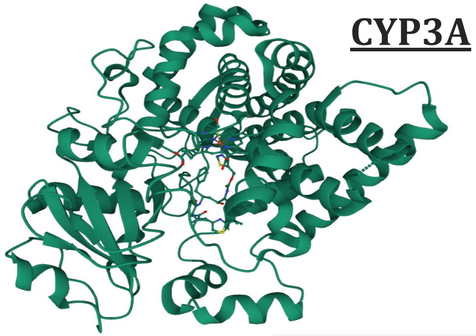
Phase I refers to the modulation of drug compounds through chemical reactions like dealkylation, oxidation, reduction or hydrolysis. The CYP450 enzymes are responsible for these reactions and work on drugs including; heroine, codeine and morphine.

CYP2D6



- Metabolizes several opiate analgesics
- Metabolizing phenotypes are described as ultrarapid, extensive, intermediate, and poor metabolizers (UM, EM, IM, PM)

CYP3A

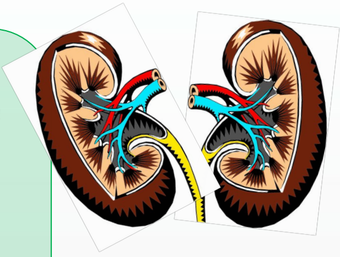


- Metabolizes ~50% of all drugs

Phase II

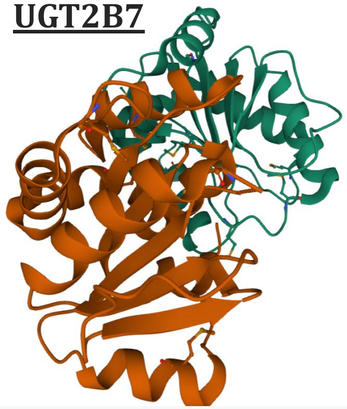
Phase II refers to chemical reactions in the drugs that occur by conjugation, such as a glucuronide, which promotes drug excretion through the kidneys. For nearly all drugs, the conjugated drug is inactive and loses biological activity. Morphine represents an exception, as its metabolite, morphine-6-glucuronide (M6G) retains its analgesic properties. These metabolites are commonly catalyzed by **UGT2B7**.

- Most abundant enzyme in Phase II metabolism
- Primary route of elimination for morphine and similar opiates



KIDNEYS

UGT2B7



PHARMACOKINETICS OF OPIATES

Opiates share many similar structural features, but what separates them are their varying functional groups. Because of these differences, each compound elicits a slightly different **pharmacological effect** and can bind to different neuronal receptors. To understand individual responses in pharmacokinetics, general aspects of opiate pharmacology are below (**absorption**, **distribution**, **metabolism**, and **excretion**).

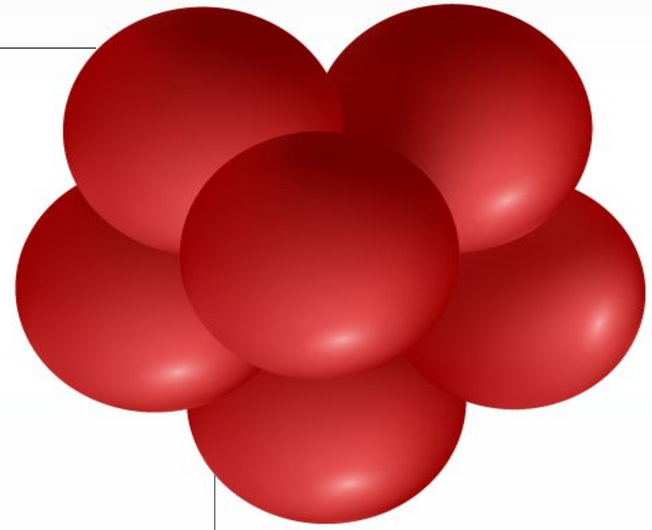
EXCRETION

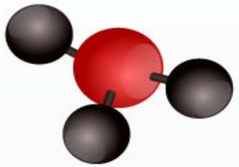
- **Drug excretion** is the removal of drugs from the body, either as a metabolite or as an unchanged drug.
- After absorption, distribution and metabolism, most of the opiates are transformed into pharmacologically active metabolites and further excreted through the **kidneys**.
- Routes of excretion include **urine, bile, saliva, sweat, milk, tears,** and **feces**.
- These metabolites are typically inactive, with the exception of morphine, where the active metabolites (M6G, M3G) may decrease the function of the kidneys with the accumulation of the metabolites, as their elicited effects may damage the overall activity of the organs.

Excretion of Drugs Through the Body

METABOLISM OF OPIATES

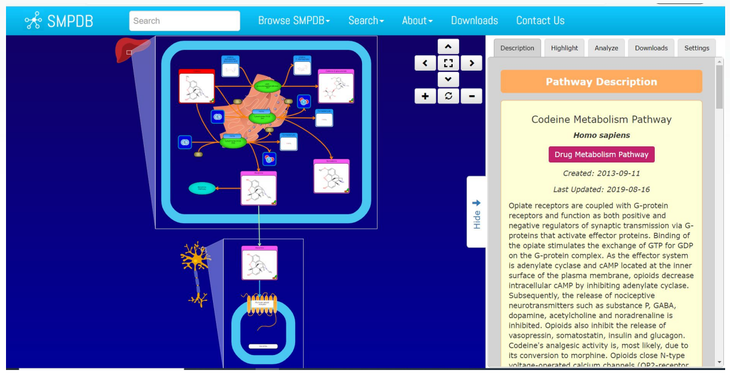
Naturally-Occurring Products:
Codeine, Morphine, Heroin, Thebaine



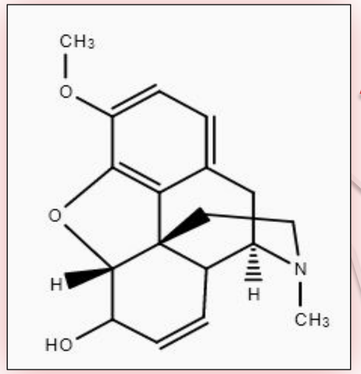


08

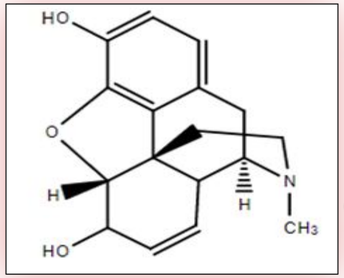
CODEINE METABOLISM



Codeine

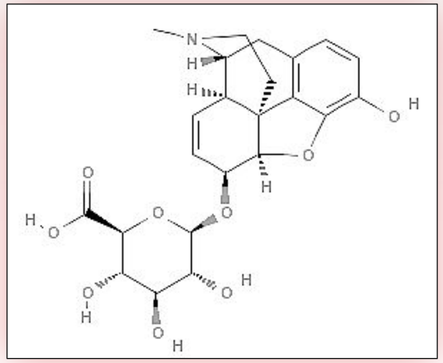


***O*-Demethylation**
Enzyme:
CYP2D6



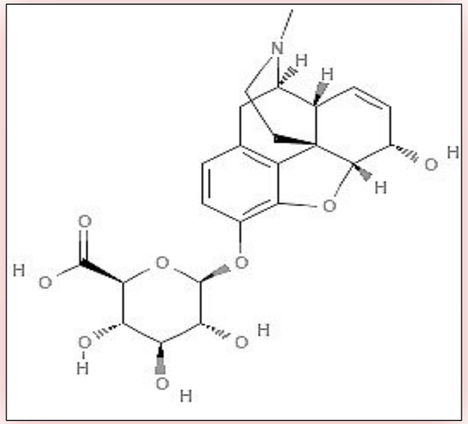
Morphine

Glucuronidation
Enzyme:
UGT2B7
UGT1A1



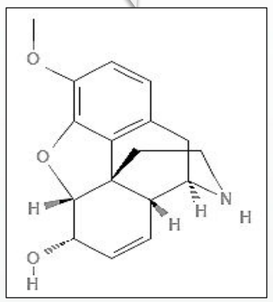
Morphine-6-glucuronide

Enzyme:
UGT2B7
UGT1A1



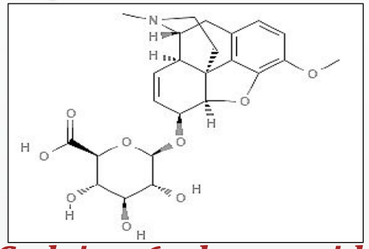
Morphine-3-glucuronide

Enzyme:
UGT2B7



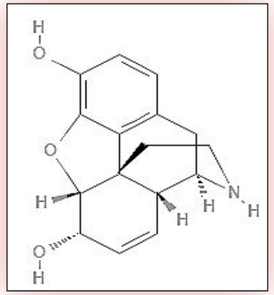
Norcodeine

Enzyme:
CYP3A4



Codeine-6-glucuronide

***N*-Demethylation**

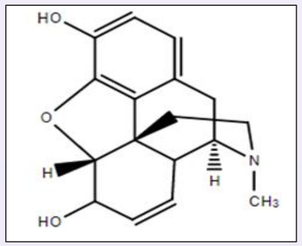


Normorphine

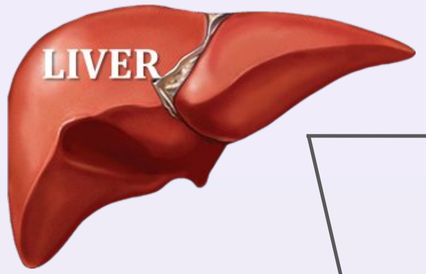
MORPHINE METABOLISM

When morphine enters the body, it enters **PHASE 2 METABOLISM**, which is the process of glucuronidation in the liver using UGT2B7 enzyme. This process breaks 90% of the morphine into M3G metabolite. During **PHASE 1 METABOLISM**, morphine is converted to normorphine, and other metabolites.

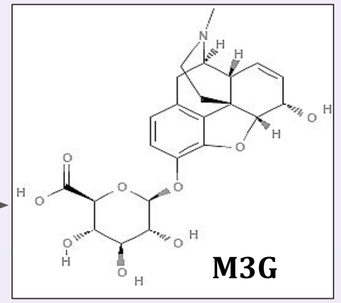
PHASE 2 METABOLISM



Morphine

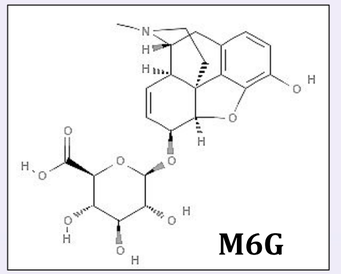


UGT2B7



M3G

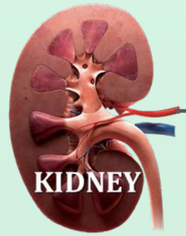
90% of the morphine dose is metabolized by the enzyme **UGT2B7** in the liver to produce the metabolite **M3G**. Other Gluc-metabolite is **M6G**.



M6G

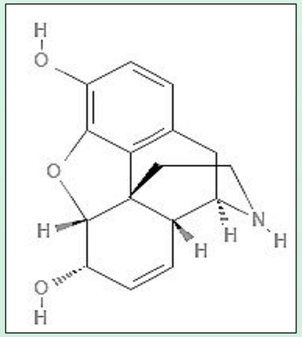
PHASE 2 METABOLISM

The remaining **10% of morphine** is metabolized by **UGT2B7** and **UGT1A1** in the kidneys and excreted in the urine/feces.

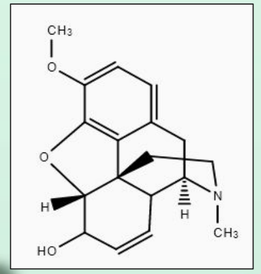


UGT2B7
UGT1A1

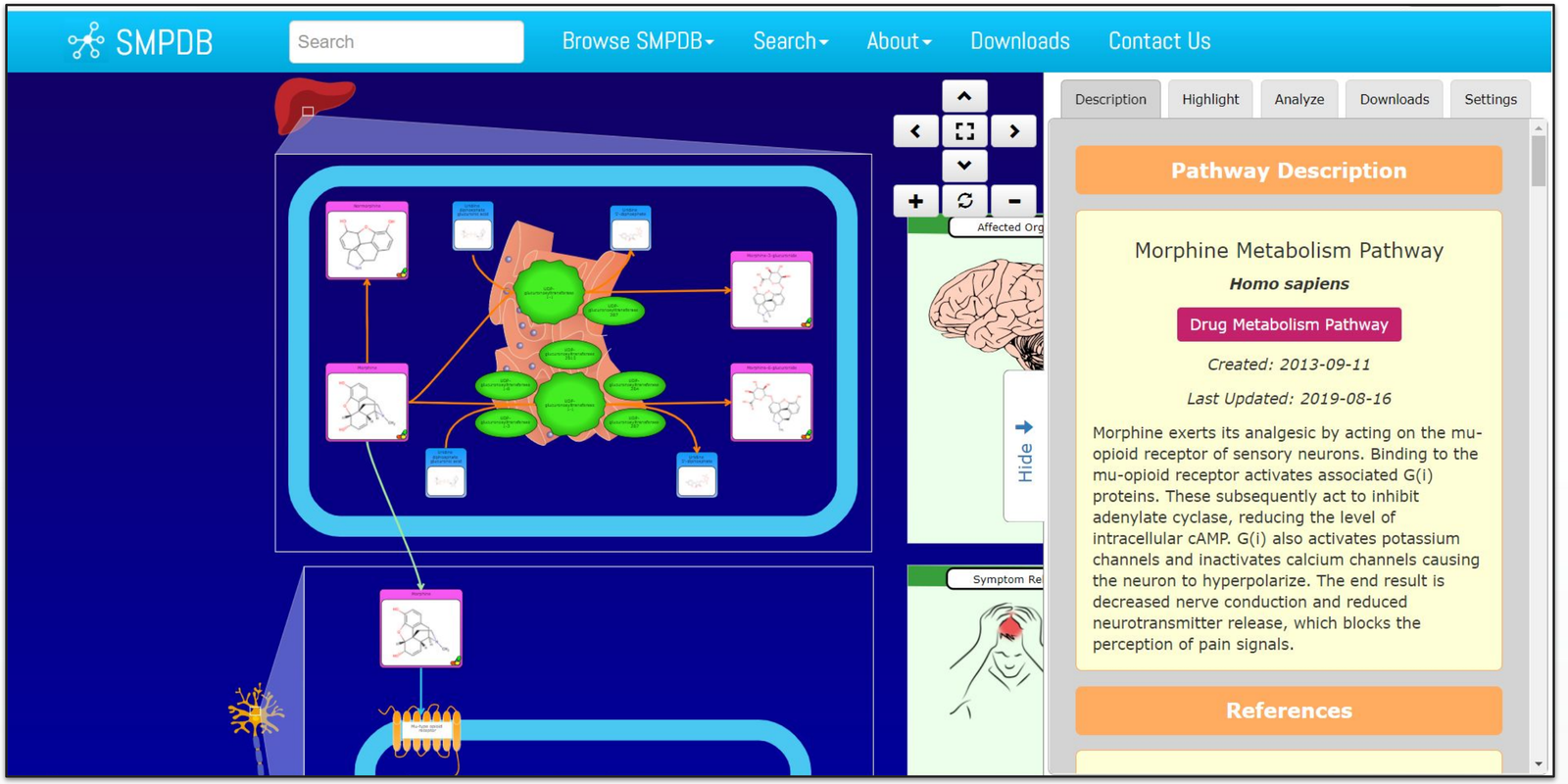
Normorphine



Codeine



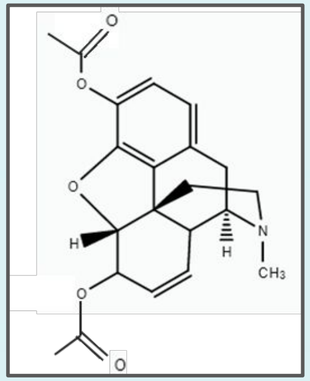
Explore the Interactive Morphine Metabolism Pathway!



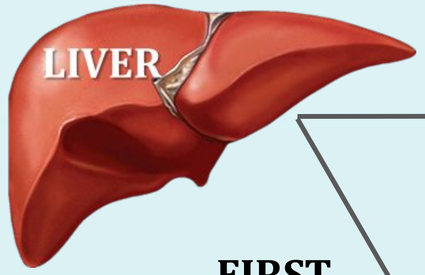
HEROIN METABOLISM

The majority of heroin is excreted through the kidneys as **glucuronides** and **morphine**, to a lesser degree. **7-10%** of the dose is eliminated through the feces. If taken by mouth, heroin is completely metabolized by extensive **first pass metabolism** into morphine before entering the BBB. When injected, heroin's **acetyl** groups facilitate quicker crossing of the BBB, where it is rapidly **metabolized** into **morphine** through the removal of acetyl groups.

PHASE 1 METABOLISM

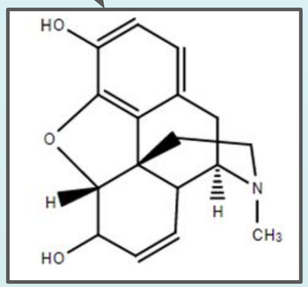


Heroin

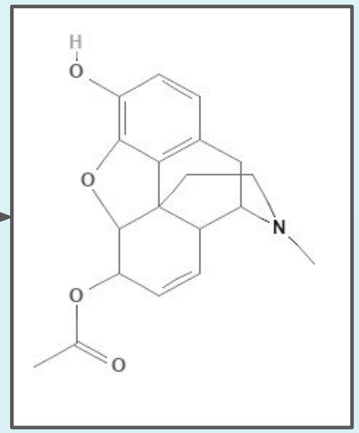


ESTERASES: hCE-1, hCE-2

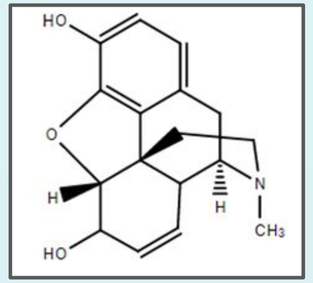
FIRST PASS



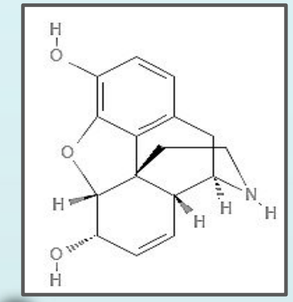
Morphine



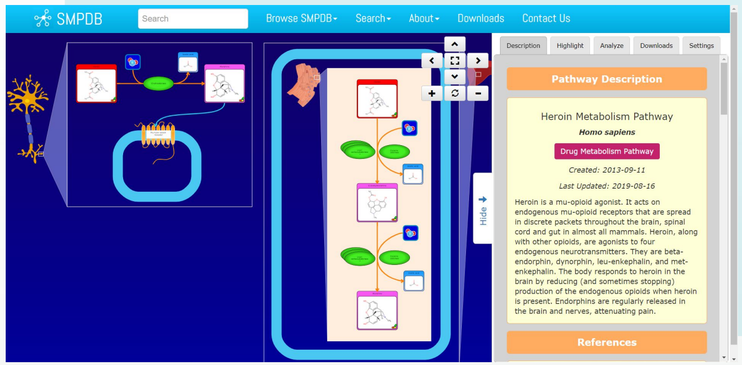
6-monoacetylmorphine



Morphine



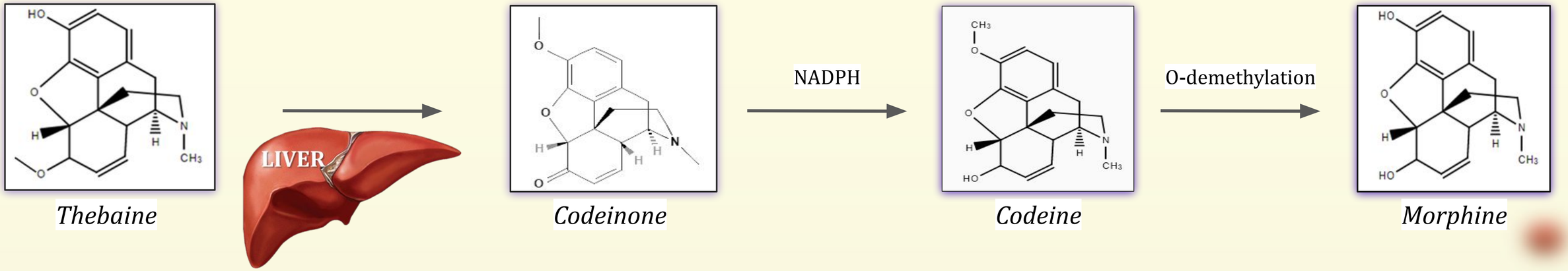
Normorphine



THEBAINE METABOLISM

Thebaine transforms into **codeinone**, where it reacts with **NADPH** to form **codeine** and subsequently **morphine** (**demethylated**).

PHASE 1 METABOLISM

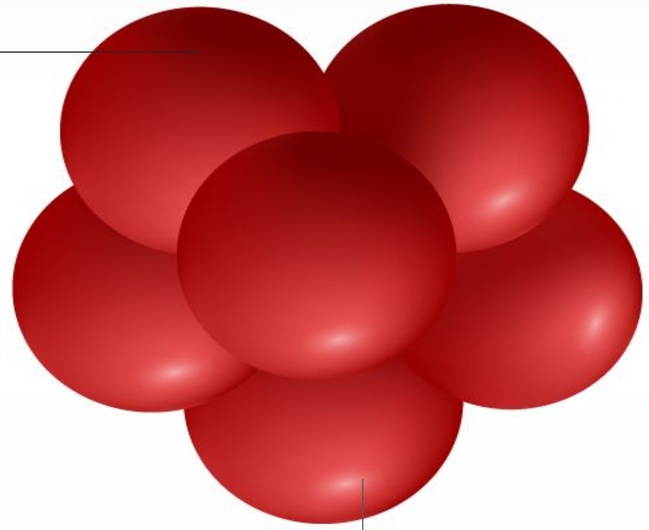


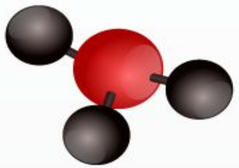
Thebaine *Codeinone* *Codeine* *Morphine*

09

OPIATES IN BIOLOGICAL FLUIDS

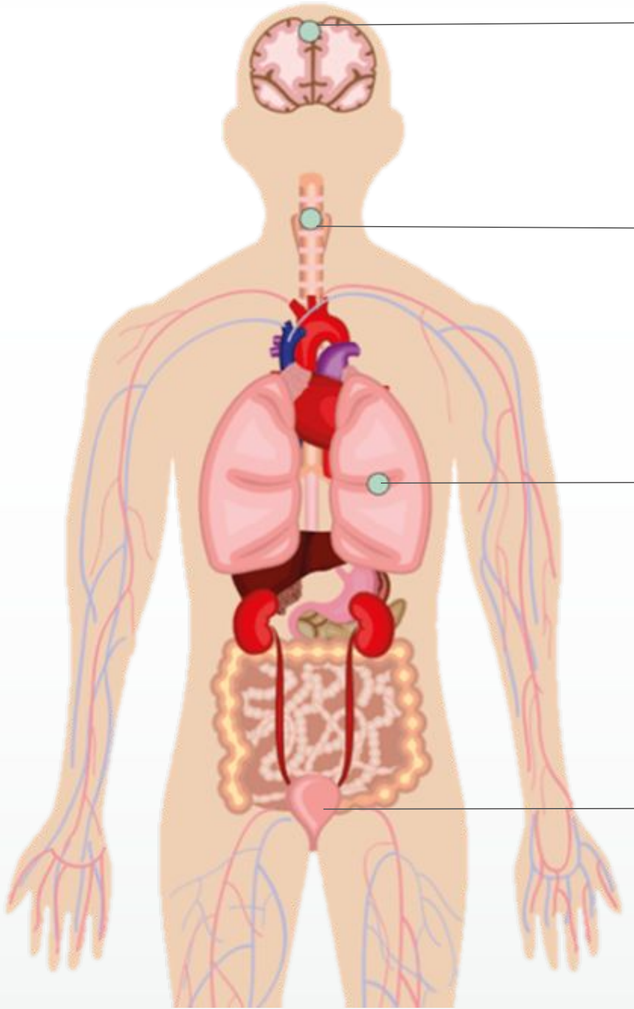
Morphine, Heroin, 6-MAM, Codeine





OPIATES IN BIOLOGICAL FLUIDS

All opiates are different, but there is a general rule of thumb for detectable drugs in bodily fluids. People who have taken opiates can have the drugs detected in hair, saliva, blood, and urine even up to 90 days after use.



90 DAYS:
Opiate residue can be found in **hair follicles**

2-3 DAYS (24-36 h):
Opiate residue can be found in **saliva**

1 DAY (24 h):
Opiate residue can be found in **blood**

3 DAYS:
Opiate residue can be found in **urine**

Opium Alkaloids

- Papaverine and its metabolites are often detected in heroin seizures, but are also detected in individuals that have consumed poppy seeds
- Thebaine has also been observed in heroin users, but also detected among poppy seed eaters
- These crossovers lead to false positive and non-specific results

6-MAM

- The presence of morphine does not indicate which opiate was consumed, so heroin and its metabolite (6-MAM) need to be monitored using the short half-life and instability of heroin in the blood
- Detection of 6-MAM in blood (or urine) is a good indicator of heroin use (quantified using GC-MS)
- 6-MAM half-life = 10-20 mins, window of detection = 1-2 h after intake (undergoes rapid deacetylation at room temperature, but it stable in frozen urine at -20 °C for at least 2 years)

Morphine/Codeine Ratio

- Criteria have been collected for urine analysis, especially when no 6-MAM is detected:
  - ***A detectable amount of free morphine exists and [total morphine] = >10 µg/mL***
  - ***A detectable amount of codeine exists***
  - ***Morphine: codeine ratio is higher than 2 for both free forms and total amounts of opiates***
- When 6-MAM observed in urine, morphine/codeine ratio in blood was always greater. When urine was not available, higher morphine/codeine ratio in blood suggests heroin use, not medicinal codeine

Poppy Seed Alkaloids

- 6-MAM in urine is indisputably heroin use, so unconjugated morphine/codeine found in blood samples after eating poppy seeds can have urine levels of opiates up to or more than 300 ng/mL
- Presence of opiates higher than 2000 ng/mL excludes poppy seed consumption and suggests drug use

OPIATES IN BIOLOGICAL FLUIDS: Blood

Biomarkers in Biological Fluids	
DRUG USE	**BLOOD**
Heroin	• 6-MAM (not always detected) • Morphine to codeine ratio = (>>1)
Morphine	• Morphine to codeine ratio = (>1)
Codeine	• Morphine to codeine ratio = (<1)
Poppy Seeds	• Morphine detected after hydrolysis

- The ratio of **morphine/codeine** is the difference between morphine origin and its metabolites.
- Since both heroin (illicit) and codeine (prescription) are metabolised to morphine, interpretations of the biological samples is complicated due to opiate-positive samples containing the same metabolite.
- Thus, the **high** codeine/morphine ratio likely represents the ingestion of the prescription drug, codeine, rather than heroin.

OPIATES IN BIOLOGICAL FLUIDS: Blood Plasma

- The **plasma** elimination rates of codeine and its metabolite (C6G) and morphine and its metabolites (M3G, M6G)
- The plasma **morphine is cleared from the body at a rate much slower** than codeine
- The concentration of morphine was lower than the concentration of its metabolites M3G or M6G.
- The concentration of codeine was higher than that of morphine.
- The codeine metabolite, C6G, concentration was higher than the concentration of parent drug codeine.

J. Anal. Toxicol. 1996, 20, 541-546

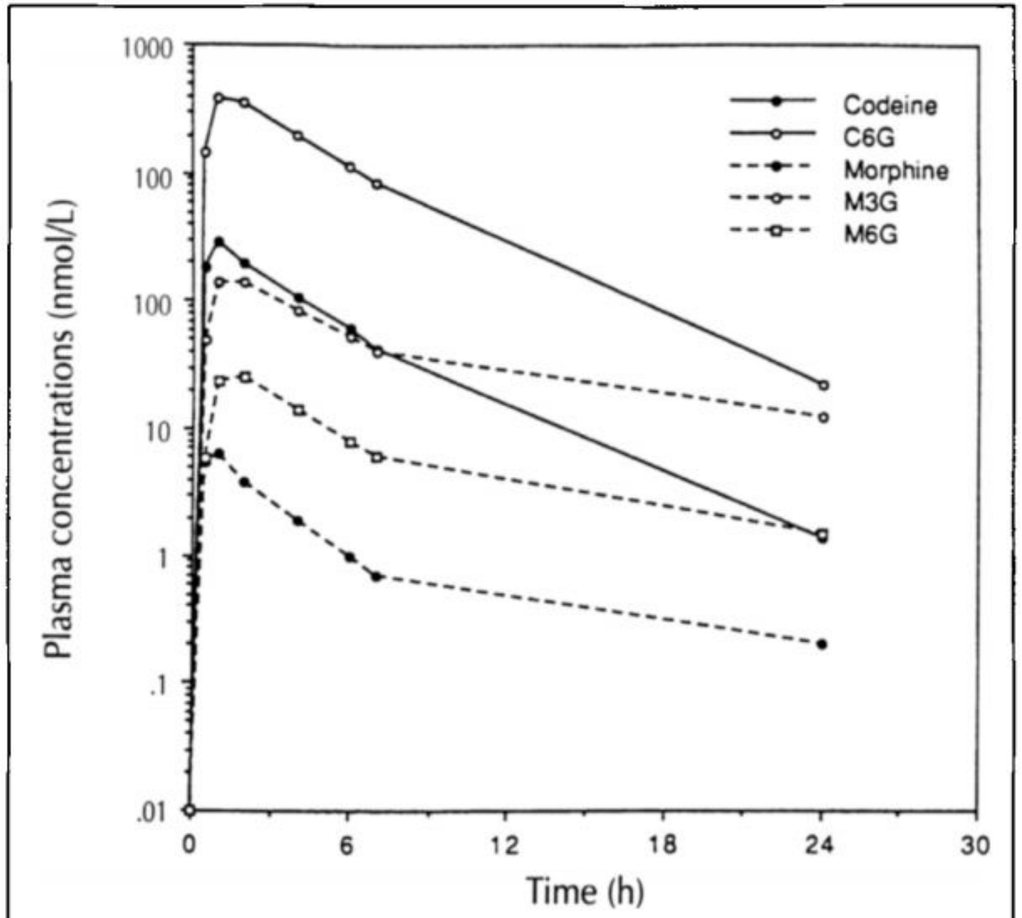


Figure 3. Mean plasma concentrations of codeine, C6G, morphine, M3G, and M6G in 13 healthy volunteers receiving 50 mg codeine orally at time zero.

OPIATES IN BIOLOGICAL FLUIDS: Urine

Biomarkers in Biological Fluids	
DRUG USE	**URINE**
Heroin	• 6-MAM (not always detected) • Codeine to morphine ratio = (<0.5), total morphine = >200 ng/mL • Acetylcodeine
Morphine	• Codeine to morphine ratio = (<0.5), total morphine = >200 ng/mL
Codeine	• Codeine to morphine ratio = (<0.5), total morphine = <200 ng/mL
Poppy Seeds	• Thebaine, papaverine, or noscapine and codeine to morphine ratio = (<0.02) from poppy seed ingestion • Total morphine = >2000 ng/mL (in absence of poppy seeds consumption)

- In immunoassay screening tests, a positive result indicates that an opiate is present in the urine sample at or above the **LOD** (limit of detection), and should be confirmed with a **confirmatory test**, such as gas-chromatography- mass spectrometry (GC-MS).
- Approximately 2-10% of unchanged morphine is excreted in urine, with 7-10% is excreted in the feces. Overall, 70-80% of the dose is excreted within 48 hours.

OPIATES IN BIOLOGICAL FLUIDS

The **half-life** of opiates can range from 1-5 hours:

Morphine: *2-4 hrs*
Codeine: *3 hrs*
Heroin: *a few minutes*

Approximate Detection Times

Opiates	6-MAM
LOQ (ng/mL)	5
Detection Time* up to	<1 day
Opiates	Codeine
LOQ (ng/mL)	25
Detection Time* up to	3 days
Opiates	Morphine
LOQ (ng/mL)	25
Detection Time* up to	3 days

These detection times are approximated based on metabolites in urine samples.

The actual value is dependent on the dose of the drug, the frequency used, and metabolism of the individual.

OPIATES IN BIOLOGICAL FLUIDS: Urine

- **Urinary** elimination rates of codeine and morphine
- Similar to blood plasma, the morphine concentration in urine had a **slower excretion rate** (longer half-life) than codeine in all volunteers
- **Morphine** may be present in concentrations **above** those of codeine (after 30 h), even after intake of only codeine in small doses, suggesting that confirmed-positive opiate finding must be analyzed critically for the truest results.

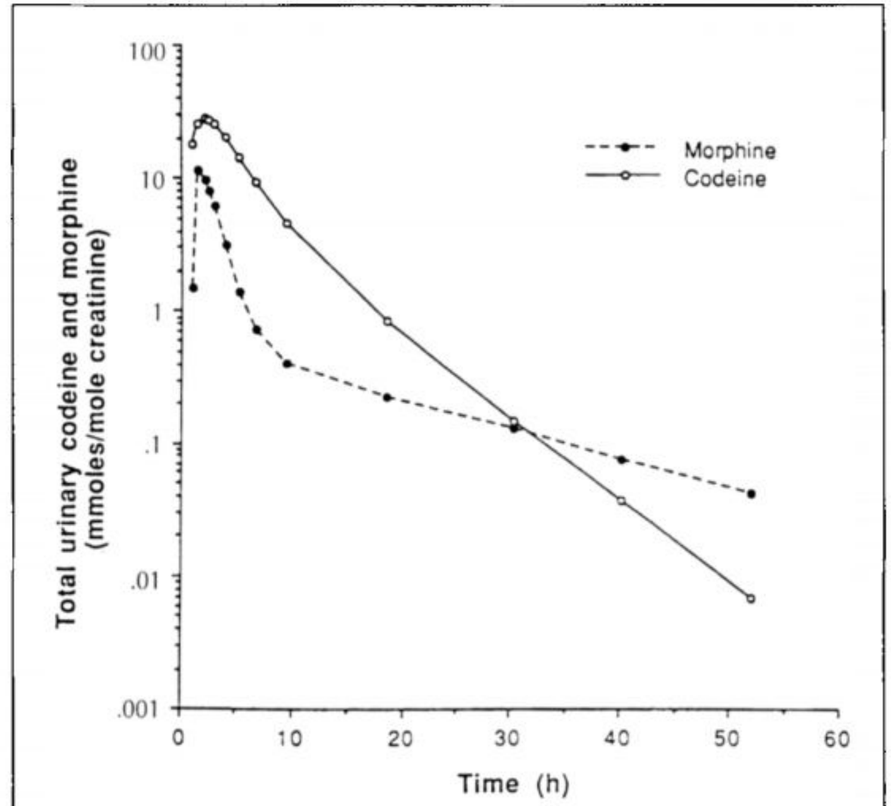
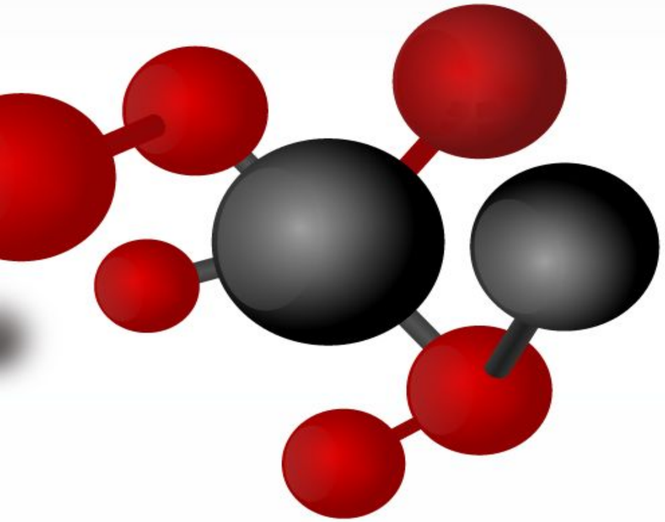


Figure 1. The urinary excretion of total codeine and morphine after single dose intake of 50 mg codeine orally by 13 healthy volunteers. The graph was constructed by fitting individual data to a two-compartment model (three exponentials, weighting factor $1/y^2$).

J. Anal. Toxicol. 1996, 20, 541-546

Now, let's consider a case study on the toxicological and legal implications of opiate use in Canada!



FROM THE CRIME SCENE TO THE LAB:

Opiate Case Files

2014 ONCJ 153 | R. v. Biagi (CanLII)

Ontario Court of Justice

CAN-LII CASE TIMELINE FROM ARREST TO DRE:

R. v. Biagi

May 22, 2011

3:00 pm

Sunday, May 22, 2011, police were notified of a possible impaired driver on the Queen Elizabeth Way (QEW).

3:11 pm

Officer P.C. Bryan pulled over Daniele Biagi and noted Mr. Biagi's eyes were red, glossy, and smelled of alcohol, as well as droopy eyelids and fumbling. The officer arrested the suspect at 3:11pm.

3:33 pm

The officer questioned Mr. Biagi who admitted to having a beer earlier that day. She did not conduct a Standard Field Sobriety test, but read the breath demand to him at 3:33 pm and transported him to the nearest police station for breath sample collection.

4:00 pm

Three unidentified bottles containing a number of pills was found in the suspect's car, later identified as morphine, oxycodone, and lorazepam. At 4:00 pm, a breathalyzer was taken where the suspect gave two breaths.

4:37 pm

The suspect passed the breath test, and the officer concluded his ability to operate a motor vehicle was impaired by a drug.

5:07 pm

Officer P.C. Bryan conducted a DRE from 5:07 pm until 6:15 pm, where she concluded Mr. Biagi's ability to operate a motor vehicle was indeed impaired by a drug, namely a CNS depressant and narcotic analgesic.

6:15 pm

DRE completed, urine sample collected from the sample. The urine sample was given to the Centre of Forensic Sciences (CFS), which was later identified as morphine, oxycodone, and lorazepam in the urine (as well as their metabolites).

FROM CRIME SCENE TO SCREENING LAB

CRIME SCENE

1. Police officer detects possible **impairment**
2. Roadside oral & physical (movement) drug test
3. **Drug Recognition Expert** (DRE) evaluates individual

POLICE STATION

1. Additional **screening tests** performed to determine level of impairment
2. Biological **sample collection** for drug testing

LAB

1. Screening test:

   Immunoassay □ based on drug class

   Broad spectrum □ unknown sample screening *via* mass spectrometry

2. Definitive testing of positive drug samples

FROM CRIME SCENE TO SCREENING LAB: DRE ROLLING LOGS

CRIME SCENE

1. Police officer detects possible **impairment**
2. Roadside oral & physical (movement) drug test
3. **Drug Recognition Expert** (DRE) evaluates individual

- A **Drug Recognition Expert (DRE)** is a police officer who is trained and certified by the International Association of Chiefs of Police (IACP) to correctly identify drug-impaired persons.
- In Canada, all DRE officers are trained by the Royal Canadian Mounted Police (RCMP).
- DREs are trained on a **12-step protocol** called the Drug Influence Evaluation to determine:

[1] If the driver is actually impaired and,

[2] The classification of the drug causing the incident.

09

FROM CRIME SCENE TO SCREENING LAB: DRE ROLLING LOGS

CRIME SCENE

1. Police officer detects possible **impairment**
2. Roadside oral & physical (movement) drug test
3. **Drug Recognition Expert** (DRE) evaluates individual

CLICK LINK FOR DRE EXAMPLES HERE

12-Step DRE Protocol:

DRE EXAMINATION EVIDENCE:

1. Breath Alcohol Test → **Polite attitude, relaxed coordination, alcohol on breath, slurred speech**
2. Interview of the Arresting Officer
3. Preliminary Examination and First Pulse
4. Eye Examinations

→ **Eye tracking was smooth and consistent, pupils were equal, eyes bloodshot, eyelids droopy**

BP: below normal range, temperature was normal

5. Divided Attention Psychophysical Tests
6. Vital Signs and Second Pulse
7. Dark Room Examinations
8. Examination for Muscle Tone

→ ***Balance Test:*** **Rigid, eyelids fluttering** *(indication of a drug)*

Trembling while holding a position, could not keep balance

***Finger-to-Nose Test*: Touched the tip of his nose with his finger very slowly and carefully, missed the tip of his nose on the 3rd and 6th attempts (touched side of nose)** *(indication of a drug)*

9. Check for Injection Sites and Third Pulse
10. Subject's Statements and Other Observations
11. Analysis and Opinions of the Evaluator

→ **No constriction of eyes when lights were turned on** *(indication of drugs)*

Overall, DRE officer determined Mr. Biagi's results were **positively affected** by the **influence of drugs**

12. **After completing the evaluation, the DRE normally requests a urine, blood marker, and/or OF sample from the subject for a toxicology lab analysis**

09

FROM CRIME SCENE TO SCREENING LAB: DRE ROLLING LOGS

CRIME SCENE

1. Police officer detects possible **impairment**
2. Roadside oral & physical (movement) drug test
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CLICK LINK FOR DRE EXAMPLES HERE

R. v. Biagi

May 22, 2011

Officer P.C. Bryan conducted a DRE from 5:07 pm until 6:15 pm, where she concluded Mr. Biagi's ability to operate a motor vehicle was indeed impaired by a drug, namely a CNS depressant and narcotic analgesic.

Drug Symptom Matrix

	CNS Depressant	Inhalants	PCP	Cannabis	CNS Stimulants	Hallucinogens	Narcotic Analgesics
HGN	Present	Present	Present	None	None	None	None
VERTICAL NYSTAGMUS	Present* (High Dose)	Present* (High Dose)	Present	None	None	None	None
LACK of CONVERGENCE	Present	Present	Present	Present	None	None	None
PUPIL SIZE	Normal (1)	Normal (4)	Normal	Dilated (6)	Dilated	Dilated	Constricted
REACTION to LIGHT	Slow	Slow	Normal	Normal	Slow	Normal (3)	Little to none visible
PULSE RATE	Down (2)	Up	Up	Up	Up	Up	Down
BLOOD PRESSURE	Down	Up/Down (5)	Up	Up	Up	Up	Down
BODY TEMPERATURE	Normal	Up/Down/ Normal	Up	Normal	Up	Up	Down

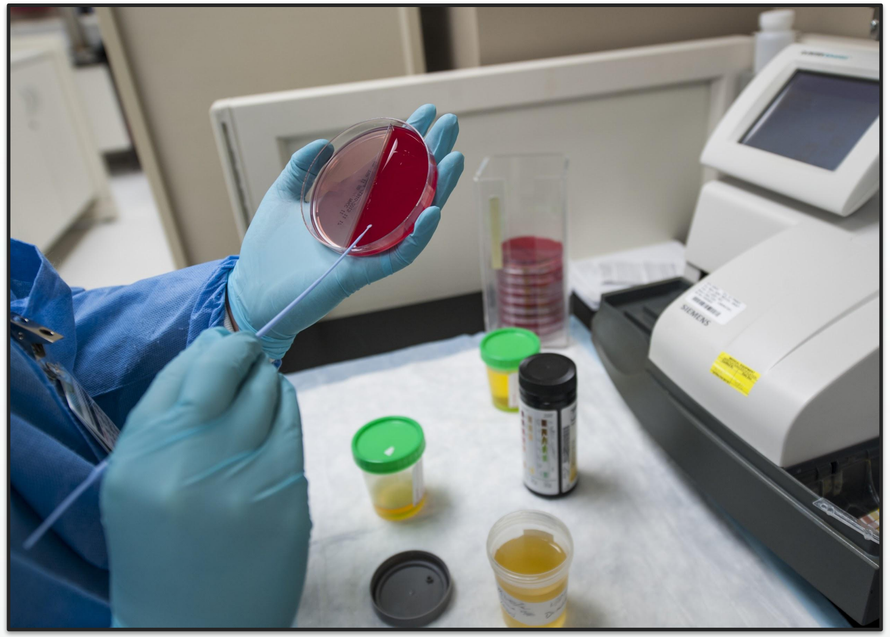
FROM CRIME SCENE TO SCREENING LAB

POLICE STATION

4. Additional **screening tests** performed to determine level of impairment
5. Biological **sample collection** for drug testing

After the DRE is administered, the officer decides based on the results if the suspect should give a biological sample for further analysis (i.e., blood or urine). Based on the DRE evaluation and the discretion of the experienced officer, a **urine sample** was collected from the suspect and sent for analysis to the Center of Forensic Science (CFS).

LAB



FROM CRIME SCENE TO SCREENING LAB

LAB

6. Screening test:

   (ex.) **Immunoassay** → based on drug class

   Broad spectrum → unknown sample screening using mass spectrometry

7. Definitive testing of positive drug samples

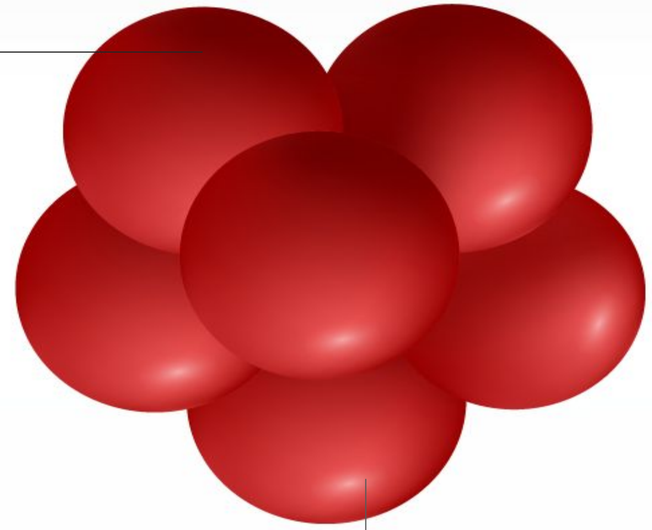
After the sample is collected and sent to the CFS, it is **analyzed and screened** through many different tests to determine the composition of the drugs in the sample.
Based on the current case study, answer the following questions from the CanLii case:

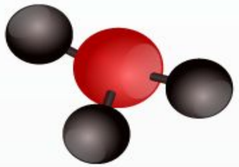
1. **Ms. Patricia Solbeck is a forensic toxicologist for the CFS and was the dominant personnel responsible for analyzing Mr. Biagi's urine sample. Which drugs did she accurately find in the sample? Additionally, which other drug classes did she test for but did not find?**

1. **What limitations made it difficult to analyze the urine sample?**

1. **Based on Ms. Solbeck's testimony, what side effects did she mention could be present in suspects with the above drugs in their systems? What is the metabolism of each drug based on her expert advice?**

LIST OF REFERENCES





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