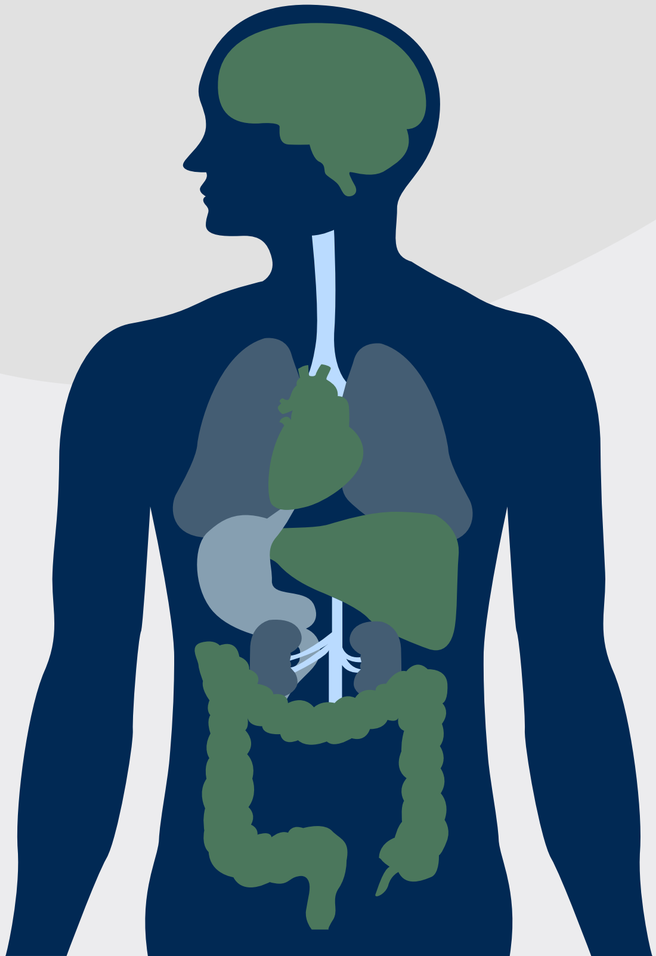
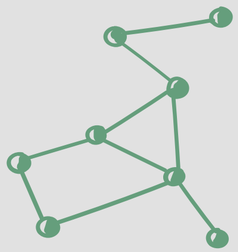
FORENSIC TOXICOLOGY:

FROM CRIME SCENE TO VIRTUAL LAB



MODULE 1

Chapter 3: **OPIOIDS**



DISPENSION'S BIOMETRIC OPIOID VENDING MACHINES:
Canada-Wide Rollout

Broward Sheriff's Office Unveils Newest Drug Detection Devices

Called MX 908, the devices can be used in multiple places that include the field, while processing inmates and during traffic stops

Opioid overdoses have claimed over 15,000 Canadian lives since January 2016.

Dispension has created the Verified Identity Dispenser (VID) – a platform that uses access control software and tamper-resistant hardware to provide contactless hydromorphone pills to replace heroin.

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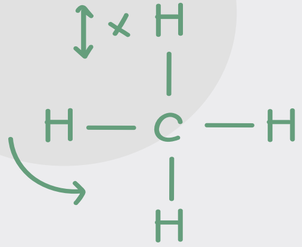
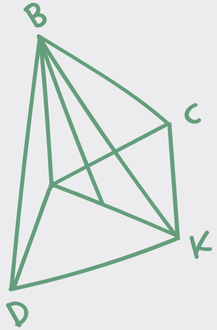
R. v. Macdonald

01





INTRODUCTION TO OPIOIDS

What are opioids?
Overview of Common Opioids

01

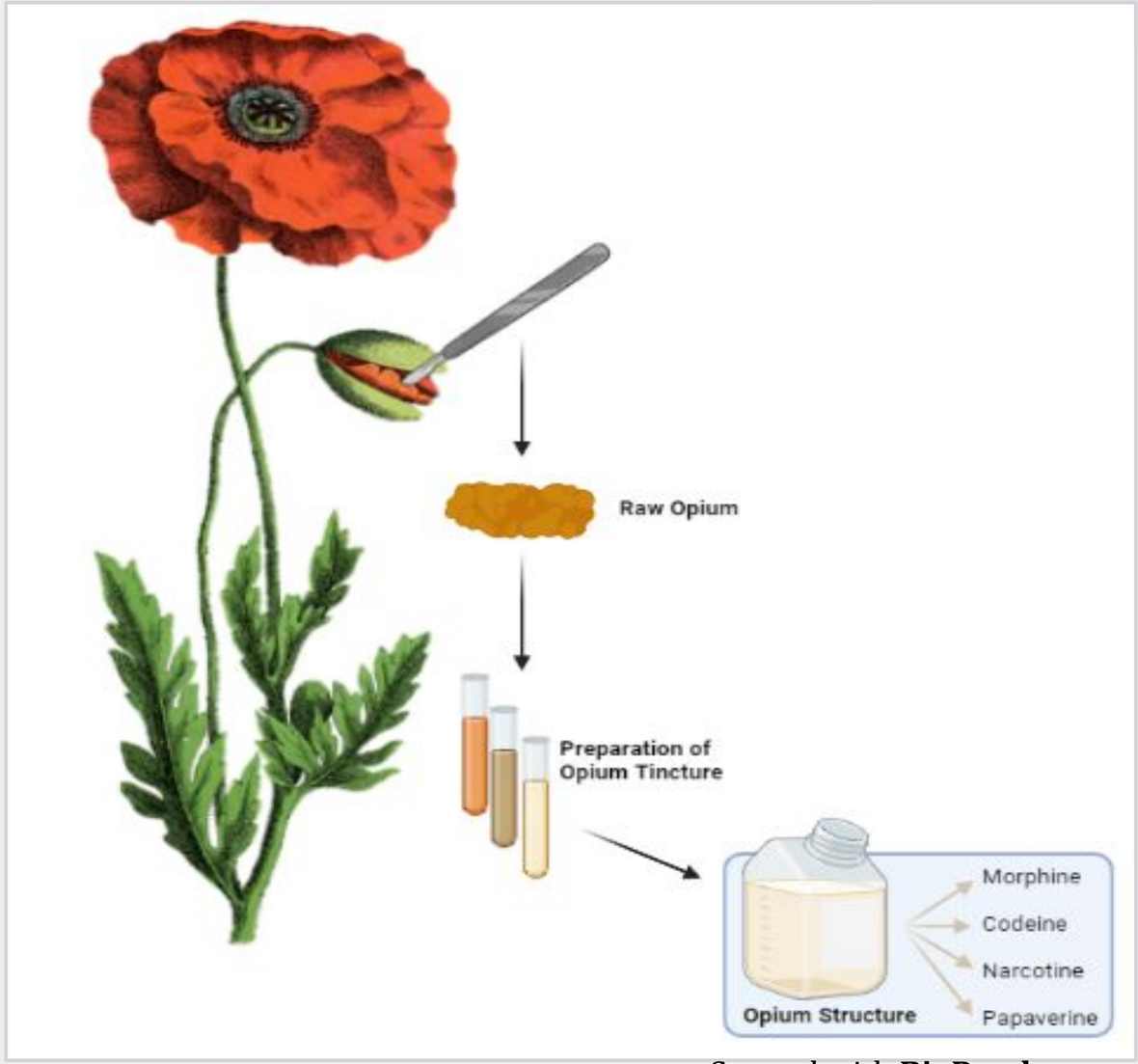
INTRODUCTION TO OPIOIDS

WHAT ARE OPIOIDS?

"**Opioid**" is a term used to characterize the entire family of opiate drugs, including **natural**, **semi-synthetic,** and **synthetic** drugs.

Similar to opiates, **opioids** are produced and used most commonly to treat pain.

Opioids exist in both *licit* and *illicit* forms, either consuming them for medical or non-medical purposes. Since these drugs cause feelings of pleasure and a 'high,' they are highly addictive and can cause long-term drug addictions.



Created with **BioRender.com**

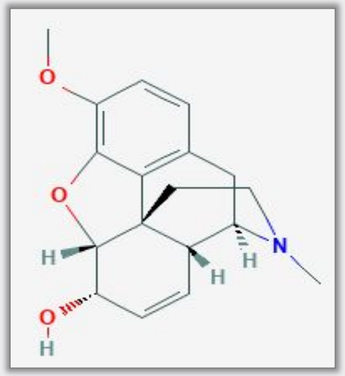
01

OVERVIEW OF COMMON OPIOIDS

Currently, hundreds of **opioids** exist in the medicinal and illegal markets. Of these, the most common opioids used and misused are prescription opioids mixed with synthetic chemicals to form the most potent and addictive compounds. Eight of the most used & misused opioids are listed below with their chemical structures:

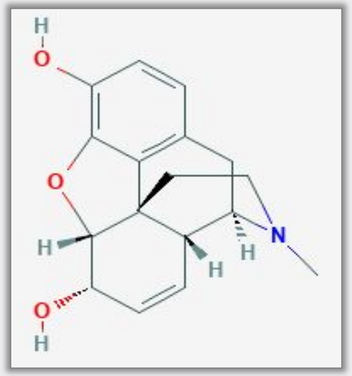
CODEINE

Opiate and prodrug of morphine used for treating pain



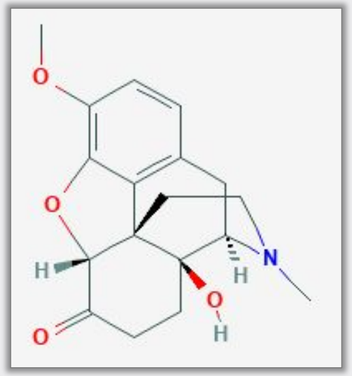
MORPHINE

Pain medication of the opiate family found naturally in the poppy plant



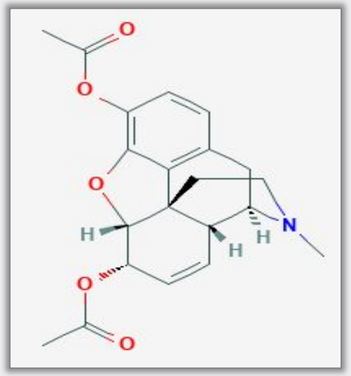
OXYCODONE

Highly addictive opioid medication used for the treatment of moderate to severe pain



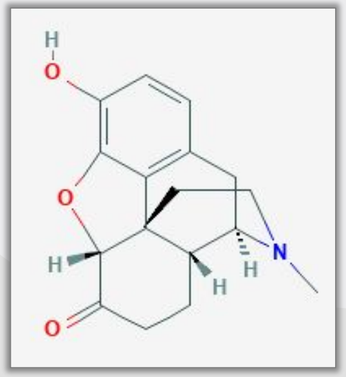
HEROIN

Highly addictive illicit drug made from morphine that is part of of the opioid drug class



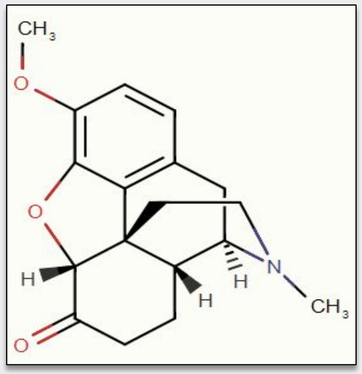
HYDROMORPHONE

Opioid analgesic used to treat moderate to severe pain



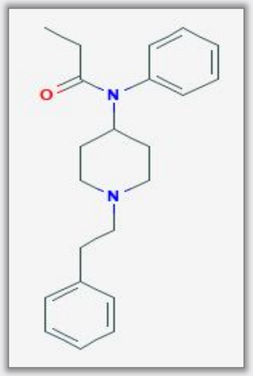
HYDROCODONE

Opioid agonist used as an analgesic and antitussive agent



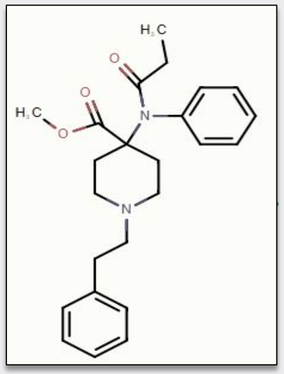
FENTANYL

Powerful opioid used as a pain medication and for anesthesia, illicitly used as a recreational drug



CARFENTANIL

Popular synthetic opioid analgesic (fentanyl analogue), one of the most potent opioids in the world



Canadian Drug Schedule Classification: Opioids

In Canada, opioids including hydrocodone, hydromorphone, oxycodone, oxymorphone, and fentanyls (& their salts / derivatives) are classified under **Schedule I** in the Controlled Drugs and Substances Act.

Saskatoon

Codeine to carfentanyl: A guide to Saskatchewan's deadliest drugs

Addiction expert digs into Coroners Service report on drug toxicity deaths

Dan Zakreski · CBC News · Posted: Aug 11, 2021 4:00 AM CT | Last Updated: August 11

Drug overdoses are killing hundreds in Saskatchewan. (Dan Zakreski/CBC)

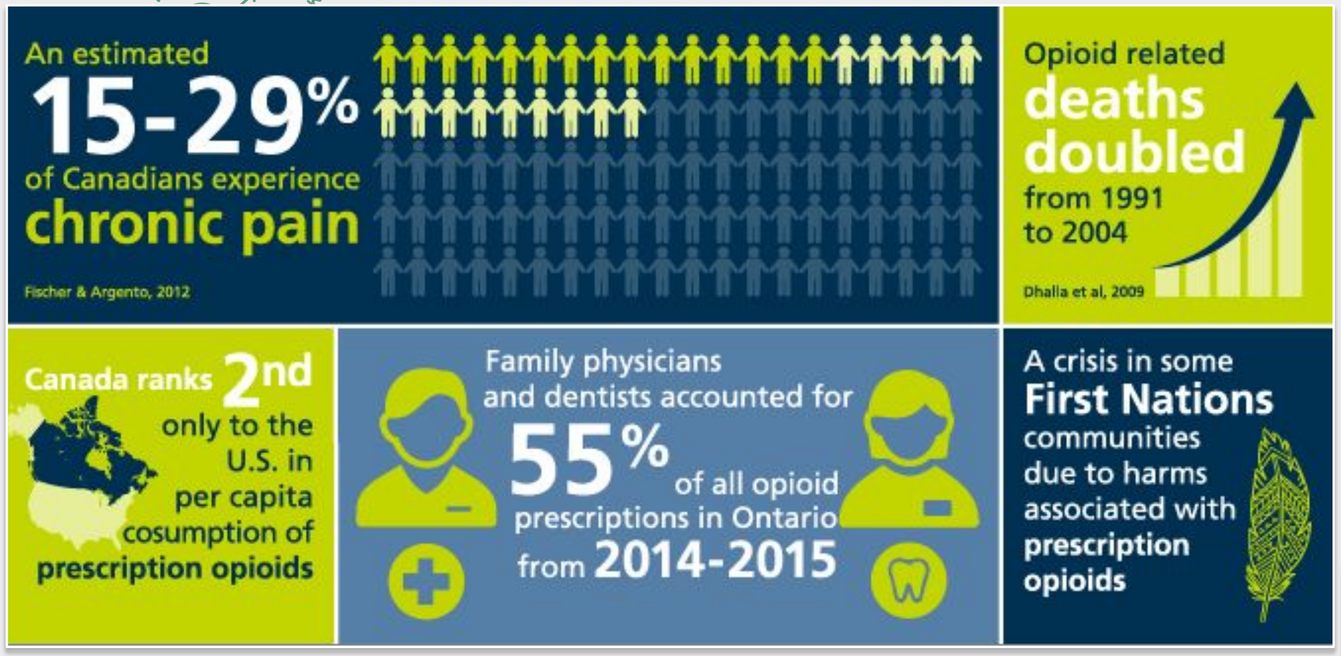
By definition, Schedule I drugs:

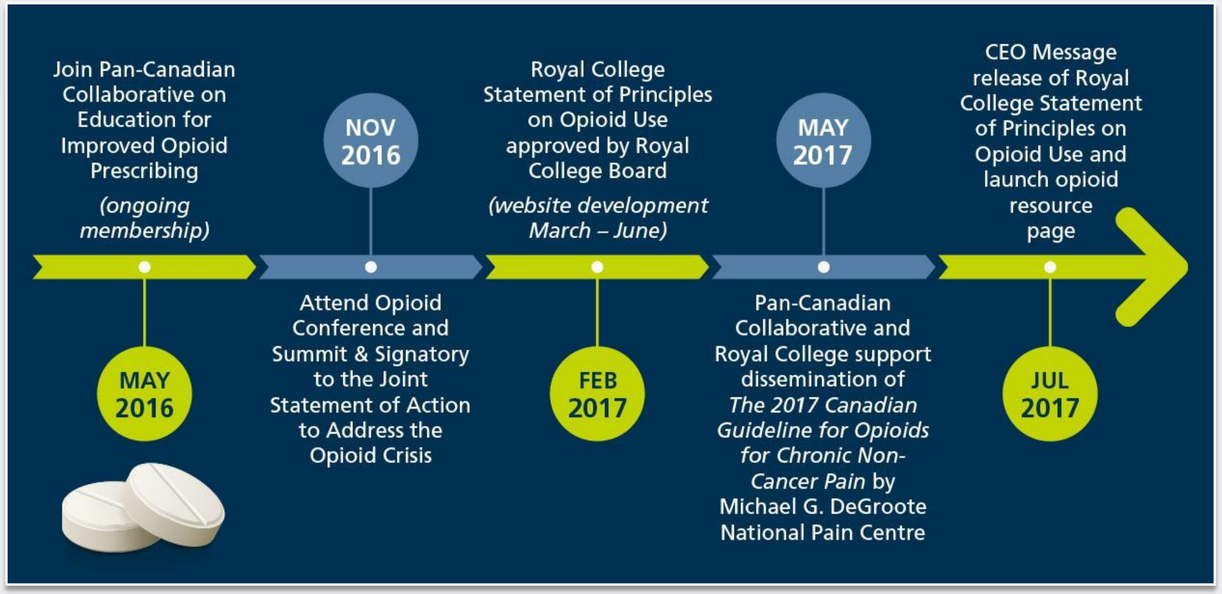
- require a prescription for sale;
- are provided to the public by a pharmacist following diagnosis and professional intervention; and
- are controlled in a regulated environment as defined by provincial pharmacy legislation

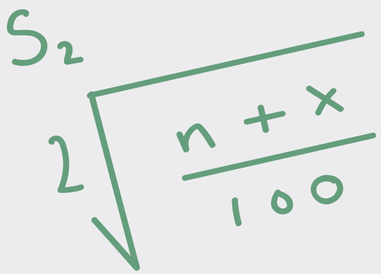
This article describes the differences between opioids and the effects of opioid-based painkillers on the human brain.

Check out out the video embedded in the article linked here!

INTRODUCTION TO OPIOIDS







02

HISTORICAL & CURRENT USES

HISTORICAL TIMELINE OF OPIOIDS

3400 BC

Poppy plants began growing and being cultivated in lower Mesopotamia

1500s

Smoking opium was increasing in Portugal, laudenaum was created by Paracelsus (mixed with crushed pearls, musk, opium, and alcohol)

1839-1842

OPIUM WAR:
Huge shipments of opium were destroyed, lasted until 1842. Alexander Wood discovered morphine can be injected

1860

The Treaty of Beijing was instrumental in Britain's control of Hong Kong

1973

US President Nixon created the Drug Enforcement Administration (DEA), war on opium drugs increased

1980-1990s

Use of opioids was gaining traction for medicinally treating pain

CURRENT USES OF OPIOIDS

- Today (2021) Canada is currently the second highest consumer of opiates and opiates in the world.
- In 2015, an estimated 2,000 Canadians died from opioid overdoses.

1950s

Synthetic Medicinal Opioids

Synthetic opioids introduced into the prescription market to mimic the uses of natural opiates, including oxycodone, synthetic heroin, and synthetic morphine.

1990s

Slow-Release Formulations

Slow-release opioids for medicinal uses were introduced and commonly prescribed by doctors for pain relief (chronic and acute).

2000s

More Opioids Involved in Opioid Dependence

Among slow-release opioid pills, fentanyl patches, hydromorphone, and OxyContin were made by US-based Purdue Pharma. These drugs were marketed to new medical students and pharmacies as a highly effective painkiller "without side effects.'

2007

Opioid Dependence Increases

Prescription numbers increased significantly in Canada and the US, including addiction reports and overdose deaths. Later, the truth about *OxyContin* was revealed (manufacturers knew people taking the drug would become addicted).

2010s

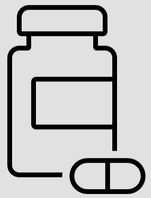
Pharmacies Increase Opioid Sales

In 2012, Canadian retail pharmacies dispensed almost 19 million opioid prescriptions; In 2013, that number was doubled to more than 30 million, bringing in nearly $881 million in sales.

2020

Opioid Toxicity Rises

From July 2019 to September 2020, a 120% increase in opioid toxicity deaths has been reported in Canada. Most of these death were among males aged 20-49 years old. Fentanyl and its analogues continue to be the main contributors to the crisis.



02

CURRENT GLOBAL OPIOID CRISIS

THE OPIOID EPIDEMIC BY THE NUMBERS

70,630
people died from drug overdose in 2019[2]

10.1 million
people misused prescription opioids in the past year[1]

1.6 million
people had an opioid use disorder in the past year[1]

2 million
people used methamphetamine in the past year[1]

745,000
people used heroin in the past year[1]

50,000
people used heroin for the first time[1]

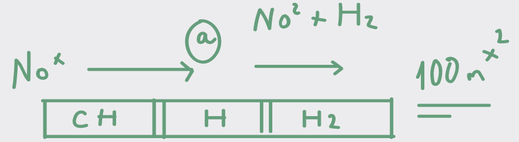
1.6 million
people misused prescription pain relievers for the first time[1]

14,480
deaths attributed to overdosing on heroin (in 12-month period ending June 2020)[3]

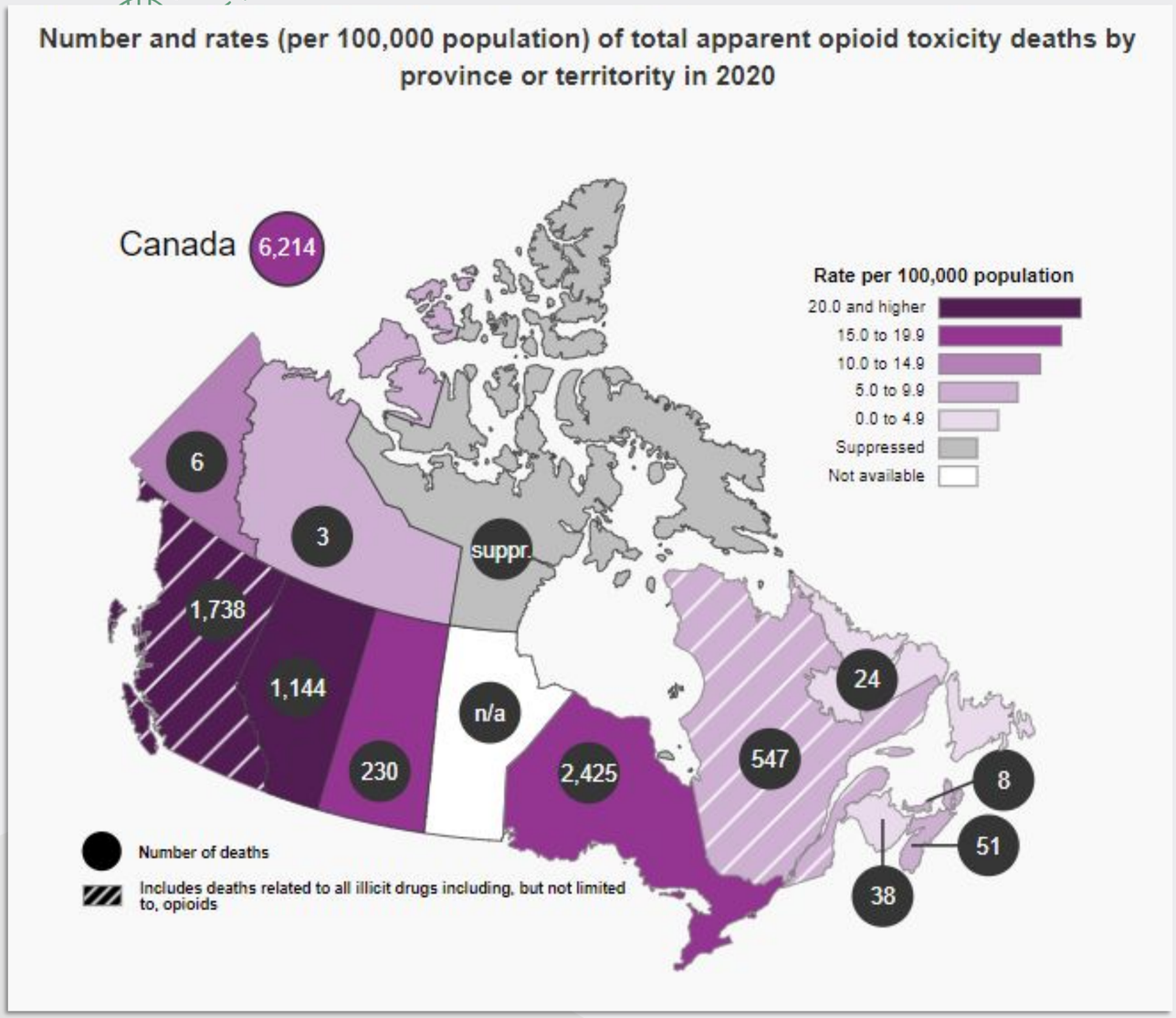
48,006
deaths attributed to overdosing on synthetic opioids other than methadone (in 12-month period ending June 2020)[3]

SOURCES

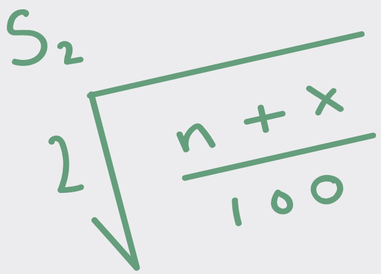
1. 2019 National Survey on Drug Use and Health, 2020.
2. NCHS Data Brief No. 394, December 2020.
3. NCHS, National Vital Statistics System. Provisional drug overdose death counts.



CURRENT GLOBAL OPIOID CRISIS



- By 2016, the apparent opioid-related death rates revealed a national public health crisis, where this epidemic affected communities across the country with a opioid-related death rate of **7.9 per 100,000 people**.
- To date, there exists pronounced regional differences.
- Follow the link attached to the image to see the **Interactive Map of the Opioid Crisis** from June 2021.



03

CLASSIFICATION OF OPIOIDS

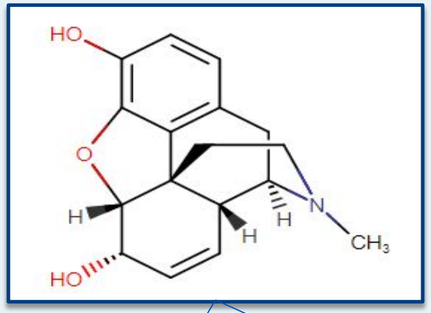
Natural, Semi-Synthetic, & Synthetic

SEMI-SYNTHETIC OPIOIDS: PRODUCTION

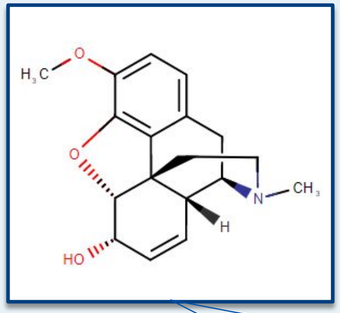
Semi-Synthetic opioids are created in labs from the use of natural opiates. Most semi-synthetic opioids emerged from their potent uses as pain analgesics but have been of the most widely misused opioids globally.

NATURAL OPIATES

Morphine



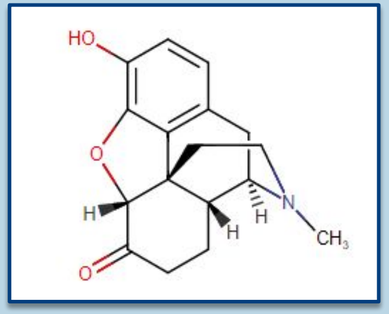
Codeine



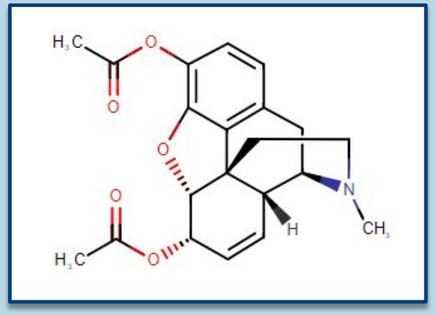
Synthesis from morphine and codeine through chemical reactions to generate semi-synthetic opioids
SEE BELOW!

SEMI-SYNTHETIC OPIOIDS

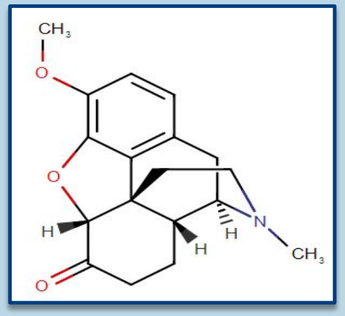
Hydromorphone



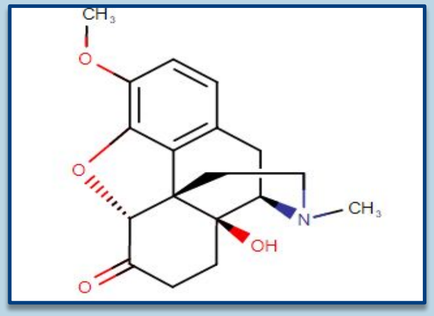
Heroin (*Diamorphine*)



Hydrocodone



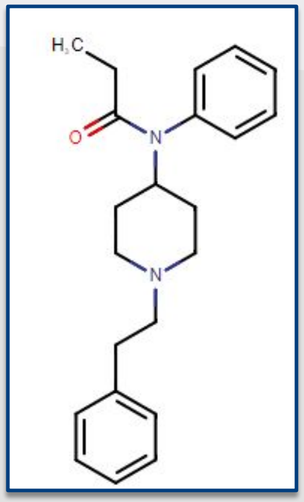
Oxycodone



SYNTHETIC OPIOIDS: PRODUCTION

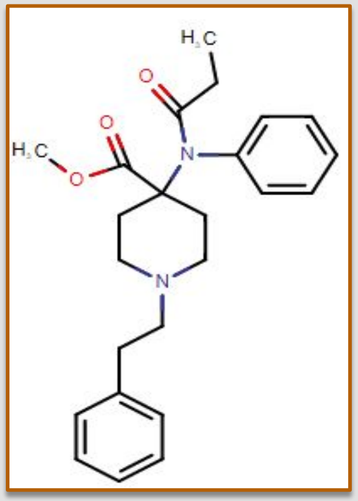
Synthetic opioids are substances synthesized in a **laboratory** that target the brain ANALOGOUS TO natural opioids.

Fentanyl



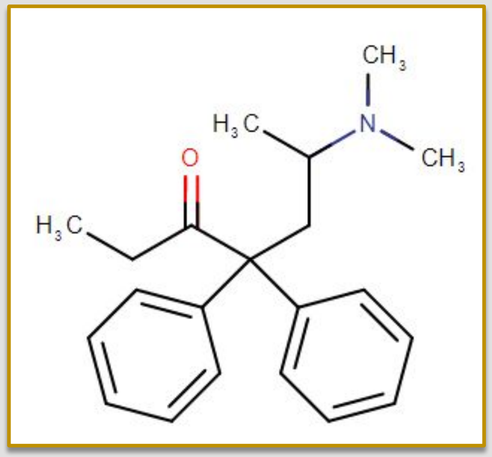
Fentanyl is the most widely misused and addictive drug on the market today, with a potency of **50-100 times** stronger than morphine. It is both used in pain relief and illegal street distribution.

Carfentanil

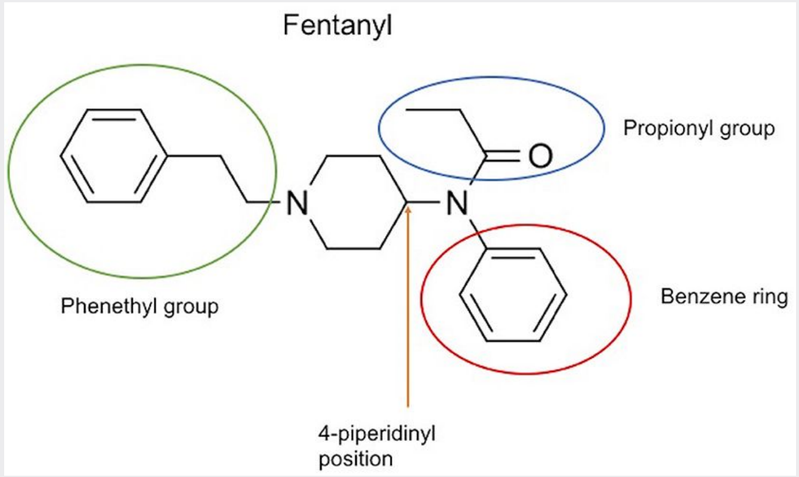


Carfentanil is another extremely powerful opioid and is **10,000 times** stronger than morphine, originally created as an elephant tranquilizer. The powder solid form is commonly used in combination with heroin.

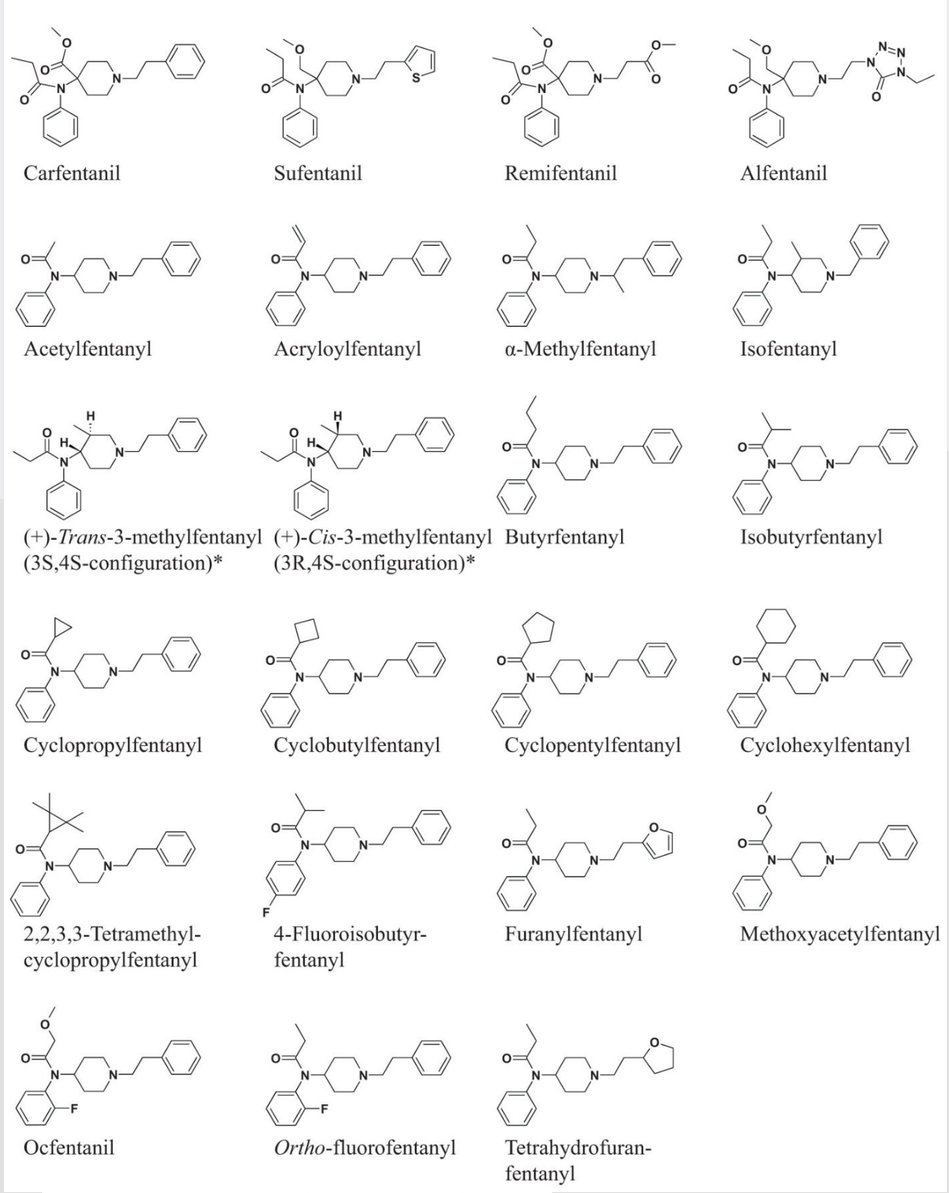
Methadone



Methadone is a synthetic opioid commonly used in **medication-assisted treatment** for patients addicted to opioids (therapeutic weaning).



- The chemical parameter log P, the decimal logarithm of the partition coefficient Kp, are useful indications of the lipophilicity of a compound
- Fentanyl and analogs show a log P between 1.5 and 4.3.
- **The high lipophilicity enables rapid diffusion through membranes, including the blood-brain barrier.**
- The high lipophilicity along with their basic characteristics make these group of drugs candidates to undergo postmortem redistribution.
- Cyclopropyl fentanyl and crotonyl fentanyl, have exactly the same molecular formula, and therefore, the same molecular weight.

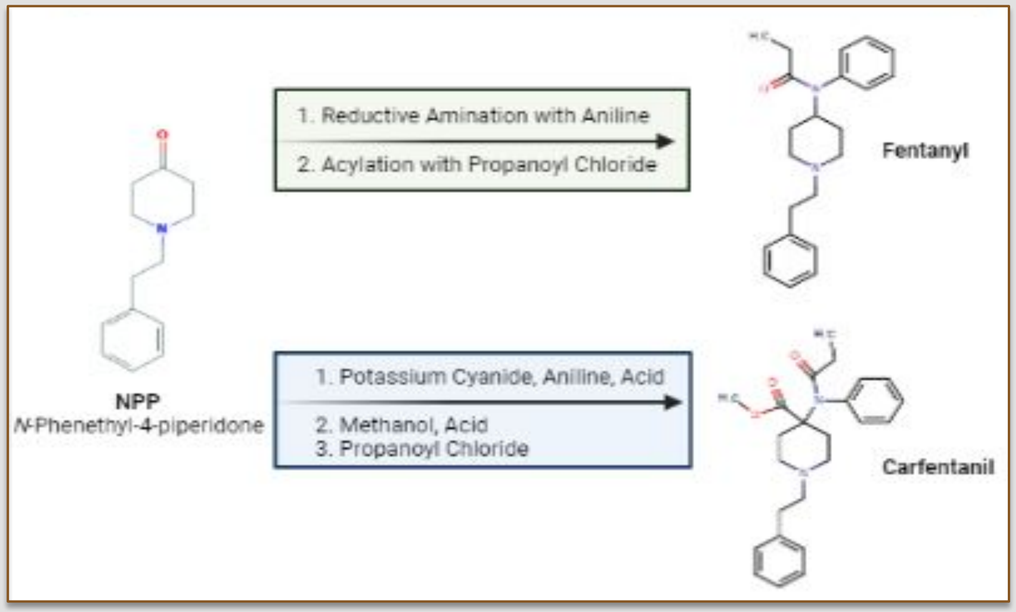


Front. Pharmacol., 05 April 2019
Front. Pharmacol., 26 October 2018

A+B=C

STRUCTURAL ANALOGUES OF FENTANYL

SYNTHETIC OPIOIDS: PRODUCTION



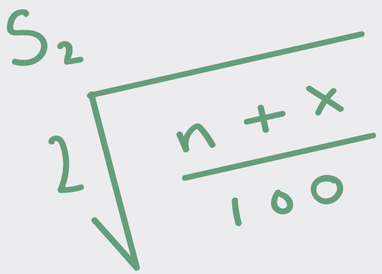
A+D

NPP (*N*-Phenethyl-4-piperidone) is an intermediate used in the production of **fentanyl** and similar illicit opioids. Once combined with other important chemical compounds, pure fentanyl is produced and dried into a powder, where it is sold as a **pain relief medication** or used by **illicit means.**

When NPP is added to the solution, it can form two drugs:

1. **Fentanyl** – formed through reductive amination with aniline, followed by acylation with propanoyl chloride.

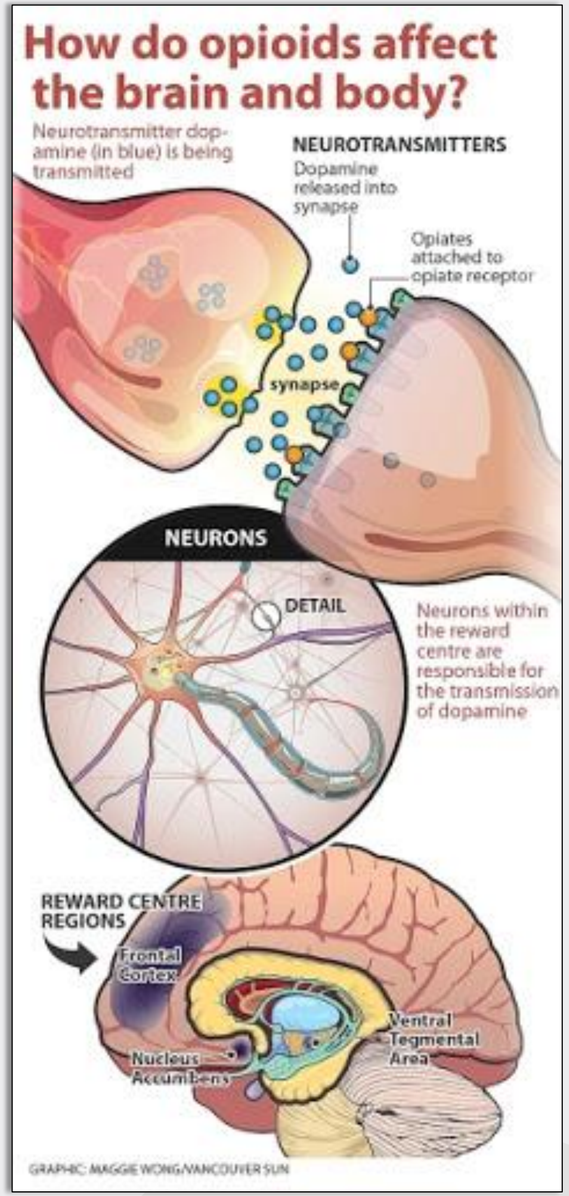
1. **Carfentanil** – formed through the additions of potassium cyanide/ aniline/ and acid; followed by methanol/ acid; and the addition of propanoyl chloride



04

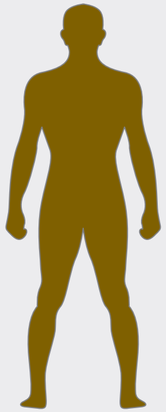
OPIOID EFFECTS ON THE BODY

OPIOID EFFECTS ON THE BODY



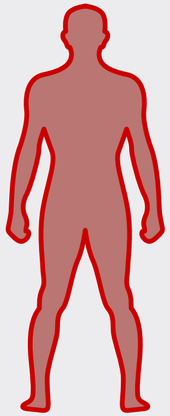
Opioids attach to receptors in the body (brain, spinal cord) to reduce the sending of pain messages to the brain in order to reduce the sensation of pain.
The drugs bind to the opioid receptors on neurons in the brain and block the binding of natural dopamine neurotransmitters to induce a much stronger feeling of pleasure (see figure on the left).

Neurons are responsible for the **reward centre** and are thus responsible for the transmission of positive feelings in the body.



Short-Term Effects:

- Fatigue
- Numbness *(lack of feeling)*
- Euphoria
- Drowsiness
- Nausea



Long-Term Effects:

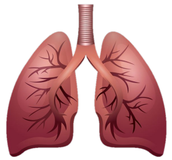
- Hallucinations
- Anxiety
- Depression
- Hypoxia *(lack of oxygen)*
- Hyperalgesia *(sensitivity to pain)*

A+B=C

OPIOID EFFECTS ON THE BODY

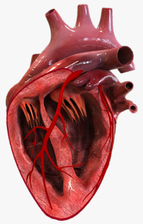
Although opioids are widely used for the analgesic properties for their pain relief, misuse or overuse has serious and severe side effects on the bodily systems, including the **Respiratory**, **Circulatory**, and **Nervous Systems**.

Respiratory System



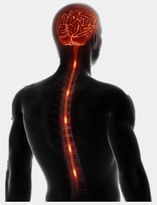
- Opioids cause respiration to slow and become irregular, leading to hypercapnia & hypoxia
- Role of carotid bodies remains unclear, even though opioid receptors are present there –mediate hypoxic & hypercapnic responses
- Only few studies have analyzed opioids effects on respiration, most concluding opioids affect the effectiveness of the brainstem

Circulatory System



- Opioid users have a 34% increased risk in developing **Atrial Fibrillation (AFib):** heartbeat quivers & does not move blood correctly from the heart's upper chamber (atria) to the ventricles (increased risk of blood clots in the heart and brain, leading to stroke)
- Chronic heroin injection can cause scars in the blood veins, as well as bacterial infections in the veins & heart valves

Nervous System



- Opioids act on the central nervous system (CNS) to provide pain relief and changes the brain chemistry to allow for a higher pain tolerance, regardless of addiction
- Once administered, opioids bind to receptors (mu, μ) located in both the 'reward pathway' or the 'pain pathway': **Pain pathway**: pain relief; **Reward pathway**: cause euphoria & release dopamine to create a pleasurable feeling ("*high*"), thus repeated use causes increased concentration of the drug to achieve the same level of pleasure as the first time

05

PHARMACODYNAMICS OF OPIOIDS

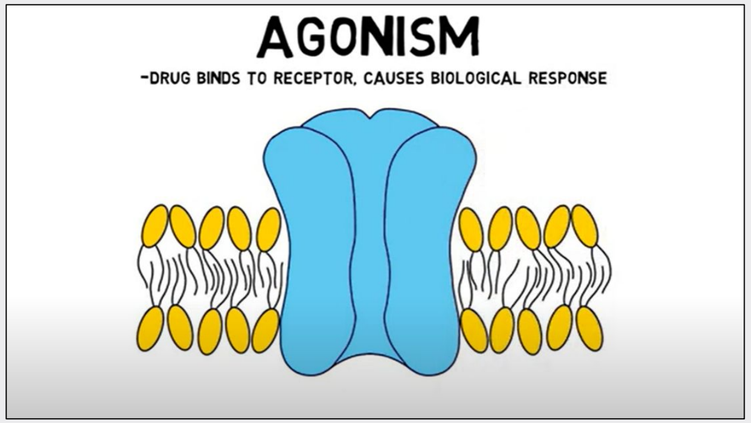
Receptor Binding Classifications
Intrinsic Activity

PHARMACODYNAMICS OF OPIOIDS

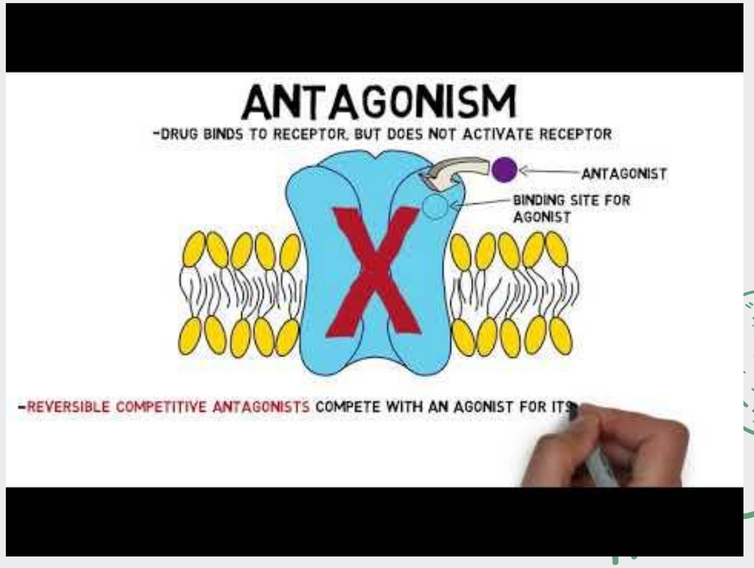
Intrinsic Activity

The relationship between opioid receptor binding and corresponding responses typically fall into 3 categories:

1. **AGONIST**: when bound, initiates a physiological response when combined with receptor
1. **ANTAGONIST**: when bound, interferes with or inhibits the physiological action of another (agonist)
1. **PARTIAL AGONIST**: when bound, activate a receptor but only induce partial efficacy at the receptor compared to a full agonist

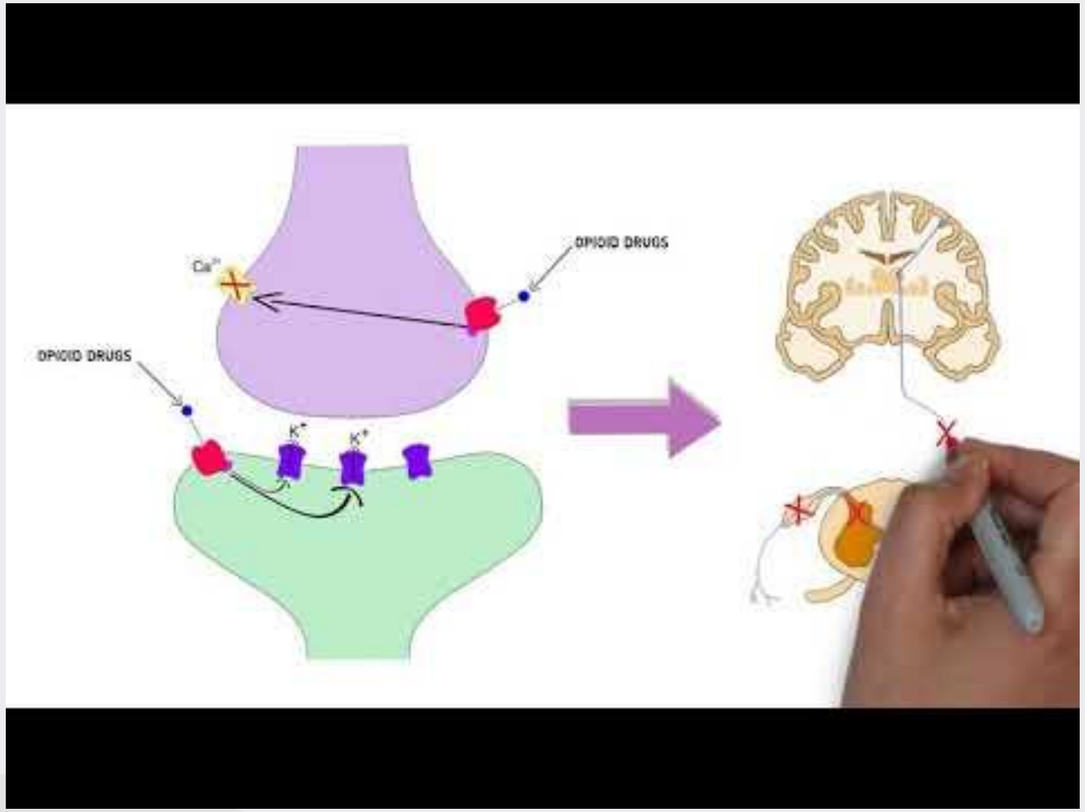


Watch this video for a visual representation of Receptor Binding and responses:



PHARMACODYNAMICS OF OPIOIDS

Pharmacodynamics studies drug-induced biochemical, physiologic, and molecular effects on the body and involves the drug binding to the receptor. This helps describe the relationship between dose and response, with the pharmacologic response depending on the drug binding to the target, among other factors. The concentration of a drug at the site of the receptor influences the effect the drug has on the body.



Overview of opioid binding to receptors on the postsynaptic nerve cell, thus reducing the sensation of pain.

Check out this video!

A+B=C

PHARMACODYNAMICS OF OPIOIDS

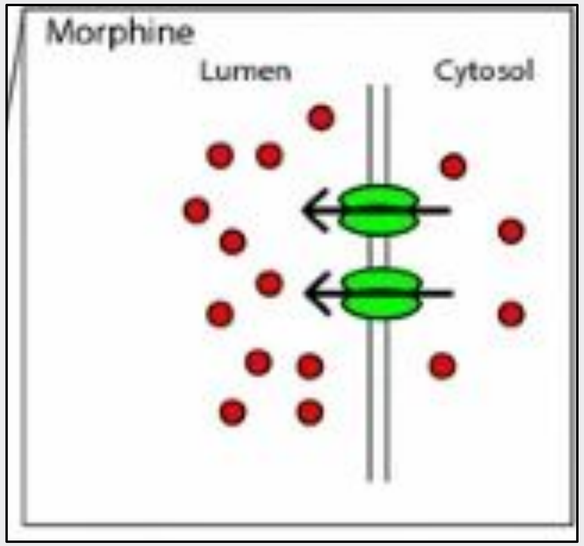
To reach effector sites in the CNS, opioids must cross the BBB and other biological membranes from the blood to the receptors on neuronal cell membranes. To cross the BBB, opioids must have the following properties:

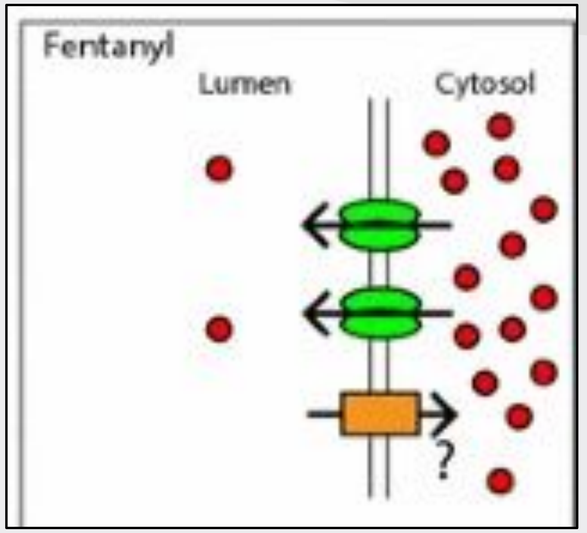
- Proper molecular size
- Correct ionization states
- Lipid solubility
- Protein binding

Diagram showing schematic of selected opioids penetrating the **blood-brain barrier**.

The ability of the BBB to act as a permeable barrier is reliant on transport proteins in the membrane (green)

A diagram indicating the relative brain penetrance of selected opioids. Red dots represent specific drug molecules





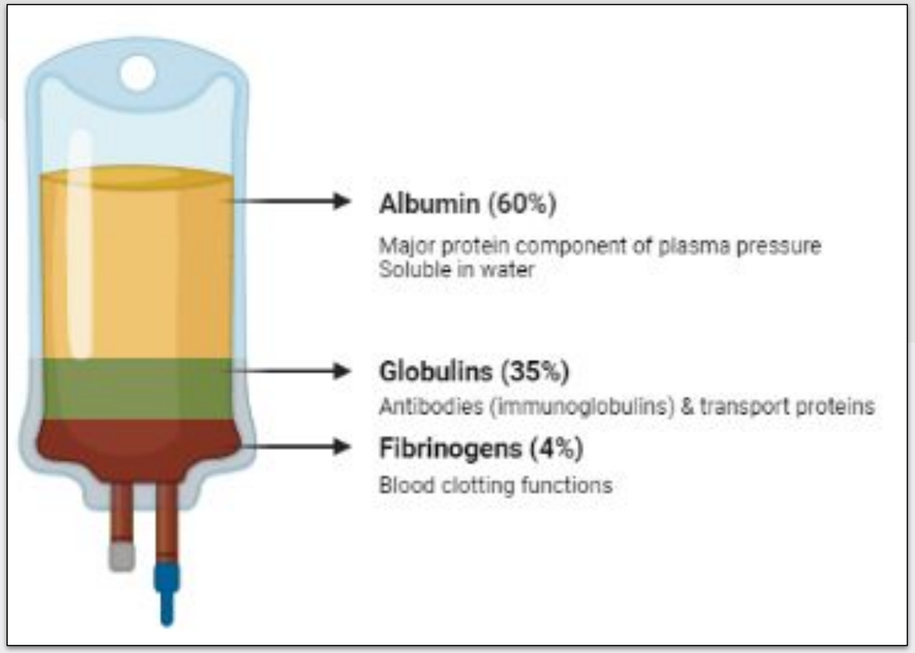
Fluids and Barriers of the CNS, 2017, 14, 32.
volum

PHARMACODYNAMICS OF OPIOIDS

Of the properties, **lipid solubility** and **ionization** are the most important in determining the rate of access to the CNS and thus, the rate of receptor binding.

- Nonionized drugs: 1000-10,000 times more lipid soluble than the ionized form (depends on the pKa of the opioid & the pH of the environment)

- Plasma protein binding affects opioid distribution since only the unbound (free) drug is able to diffuse across membranes
    - Major plasma proteins opioids bind to include:
        - **Albumin**
        - **α1-acid glycoprotein**



Created with **BioRender**

PHARMACODYNAMICS OF OPIOIDS

Properties of pharmacodynamics includes receptor classification, *mu* receptor binding, & intrinsic activity (agonists/antagonists).

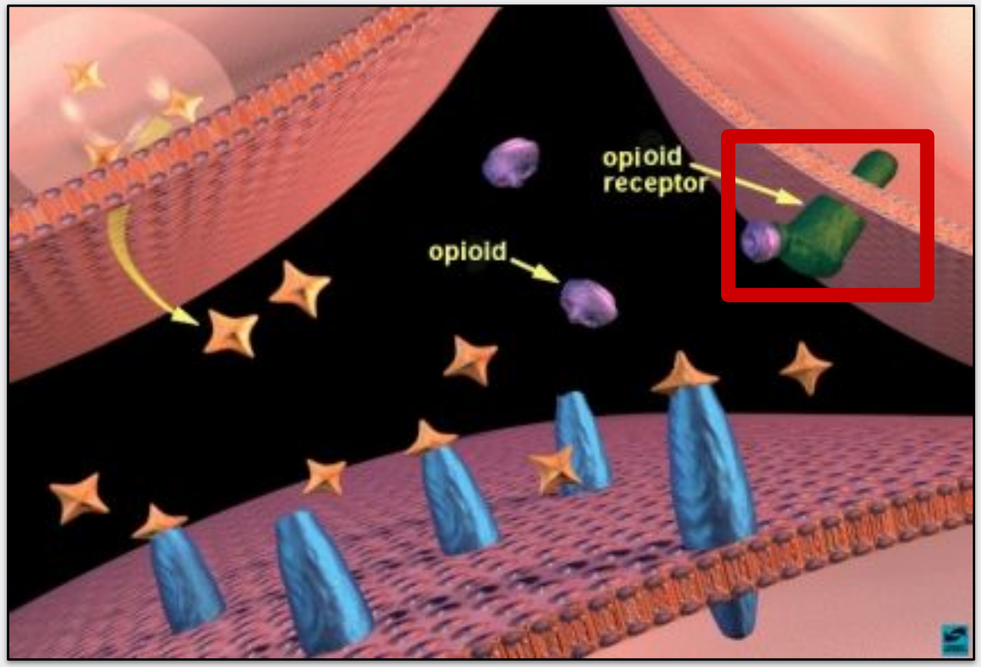
Receptor Classification

Based on their endogenous ligands that bind:

1. **Mu (MOR):** euphoria, stress-coping, most important and common opioid receptor

1. **Delta (DOR):** anxiolytic, positive effect

1. **Kappa (KOR):** dysphoria, stress, negative effect



Receptor Binding: *mu*

The *mu* opioid receptor serves two functions:

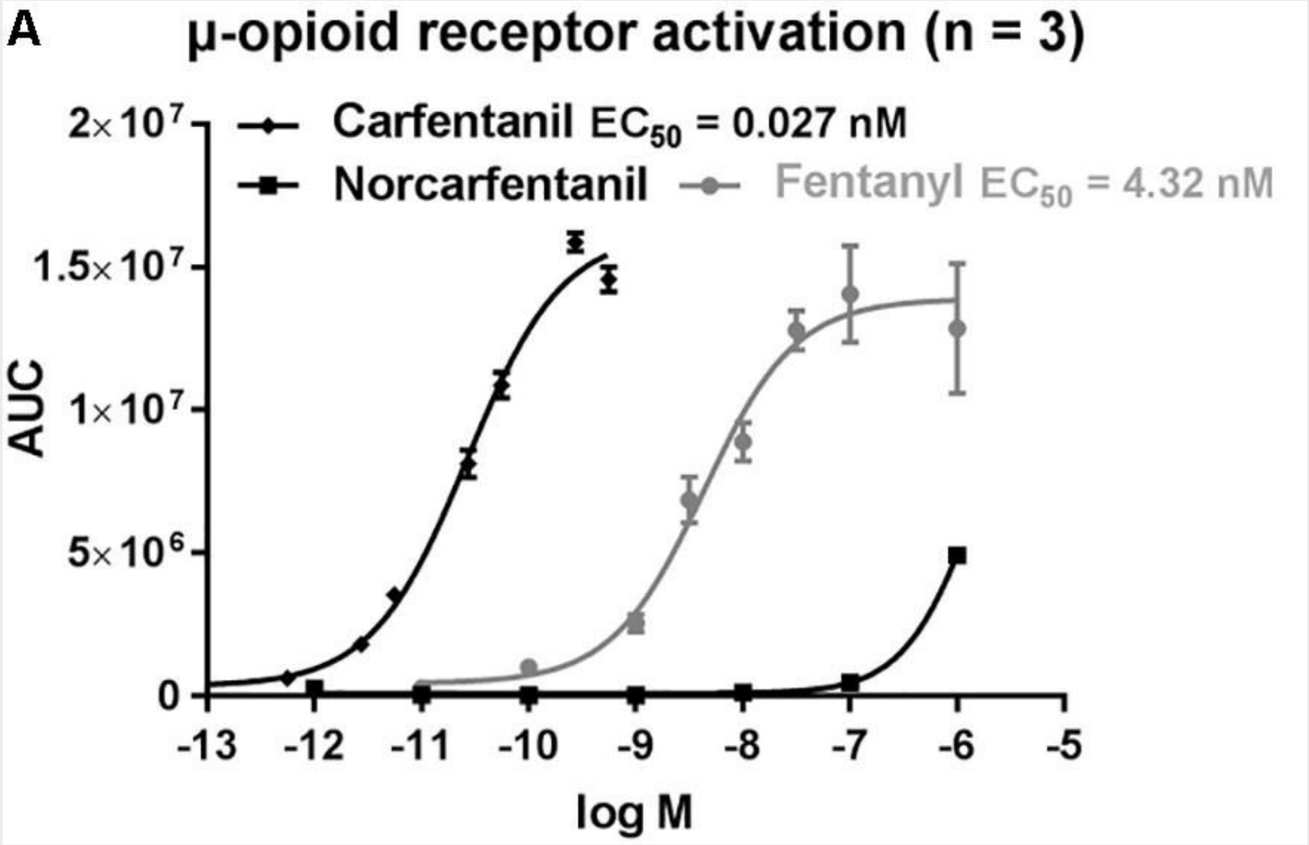
1. **Recognition:** only the L-isomers of opioids exhibit analgesic activity
2. **Biological activity:** the strength of the binding (affinity) correlates with analgesic potency

(ex.) Binding affinities of morphine and fentanyl have been calculated using the equilibrium inhibition constant (K_i) (the lower the value, the higher the binding affinity for the mu receptor):

Fentanyl = 1.6 nM
Morphine = 5.7 nM

A+B=C

μ-opioid receptor activation by fentanyl, carfentanil and norcarfentanil



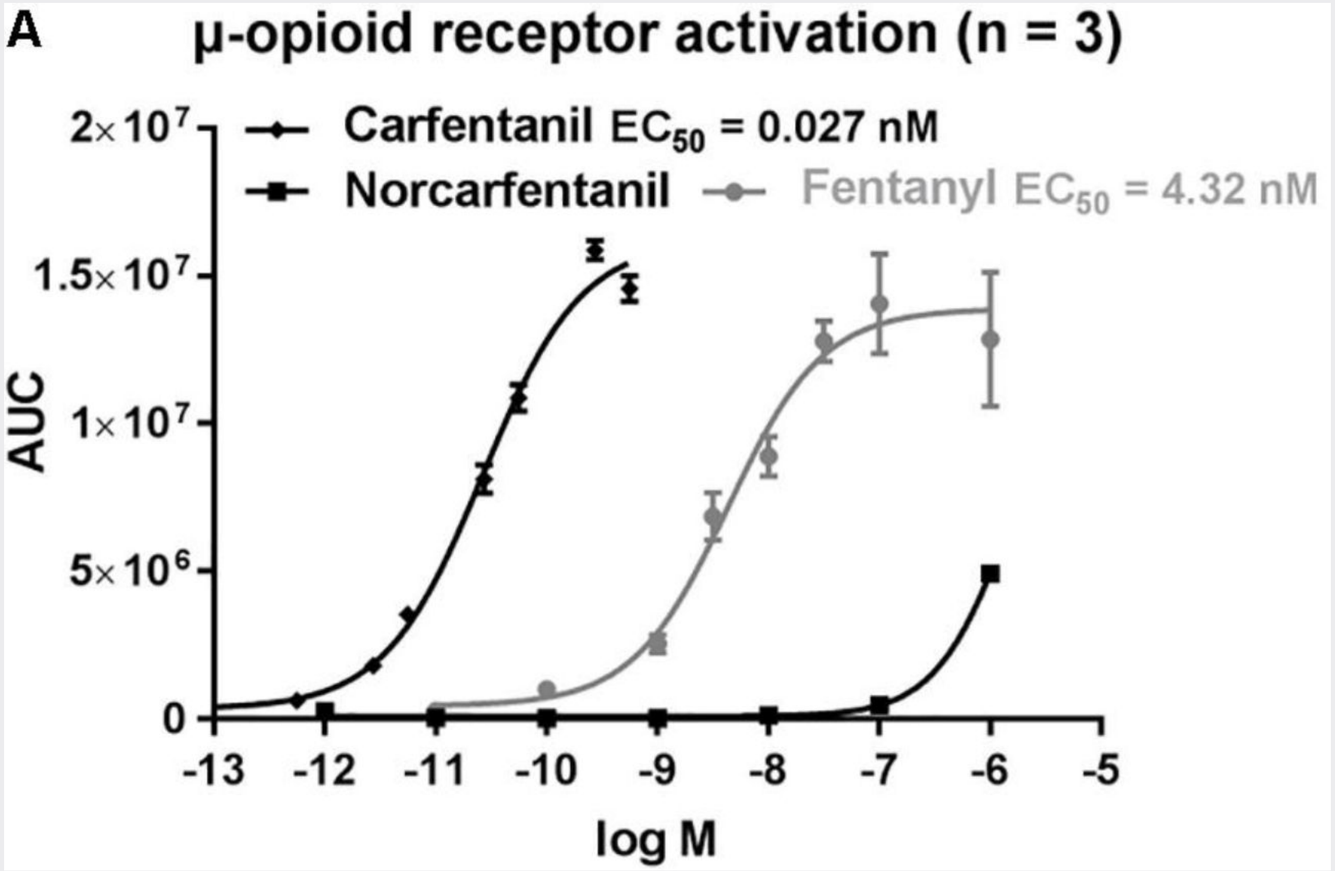
Front. Pharmacol., 15 May 2018

The concentration-dependent curves EC50 values were determined for carfentanil [EC50 = 0.027 nM] and fentanyl [EC50 = 4.32 nM] as a measure of relative potency

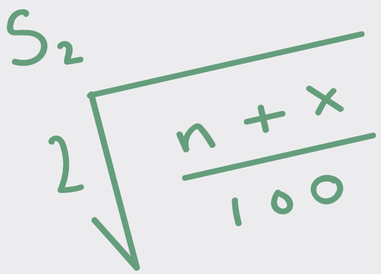
Norcarfentanil, the major metabolite of carfentanil, was only able to generate low opioid activity at a high concentration (1 μM) = not very potent

A+B=C

QUESTION #5



A+B=C



06

PHARMACOKINETICS OF OPIOIDS

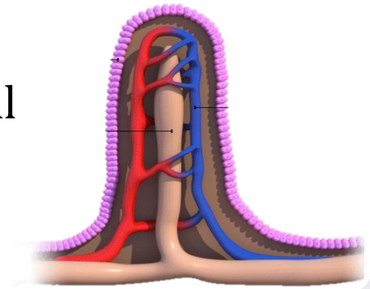
Absorption
Distribution
Metabolism
Excretion

OVERVIEW OF PHARMACOKINETICS

1

ABSORPTION

Refers to the way a drug is absorbed from a pharmaceutical chemical formulation into the bloodstream



2

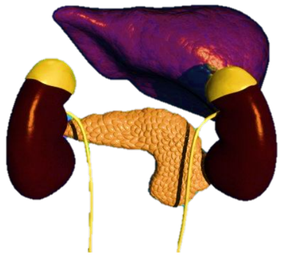
DISTRIBUTION

Reversible transfer of a drug from one location in the body to another
(ex.) fat tissues to gut lumen

3

METABOLISM

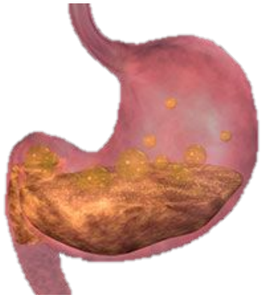
Chemical reactions that break down drugs into chemical compounds that are easier to eliminate in the body (termed "*metabolites*")



4

EXCRETION

Process of removing a drug and its metabolites from the body
(ex.) through the kidneys



A+B=C

ABSORPTION OF OPIOIDS

The proportion of opioids (active drug) that enters the body systemically is defined as "**bioavailability**", which is partially attributable to the first pass metabolism.

First pass metabolism occurs when the drug is metabolized directly by the liver from the GI tract before it reaches the bloodstream, thus avoiding **absorption** and affecting the rate of absorption.

Opioid (Route)	Absorption	Distribution	Metabolism		Excretion	
	BIOAVAILABILITY	Vd (L/kg)	Major Metabolism Enzyme(s)	Active Metabolite	Urine (%)	Half-Life (hours)
Codeine (PO)	53	3-6	CYP3A4 & 2D6	Morphine	90	3
Fentanyl (TDS)	N/A	4-6	CYP2D6	None	75	20-27
Oxycodone IR (PO)	60-87	2.6	CYP3A4 & 2D6	Oxymorphone	19-64	7-9
Morphine IR (PO)	<40	4	UGT	M6G	90	2-4

EXAMPLE: OXYCODONE

- Oxycodone acts directly on a number of tissues unrelated to its pain relief with a bioavailability of 60-87% when ingested orally
- Compared to codeine (53%), the bioavailability of oxycodone is significantly higher, suggesting it has an increased potency

A+B=C

DISTRIBUTION OF OPIOIDS

- **Distribution** refers to the movement of a drug between the blood and several tissues throughout the body, often described in terms of **volume of distribution (Vd).**
- Opioids with a higher Vd value (i.e., more able to cross the BBB) are usually more lipophilic and more likely to distribute faster/more in & out of the BBB, with quicker and shorter pain relief.

Opioid (Route)	Absorption	Distribution	Metabolism		Excretion	
	BIOAVAILABILITY	Vd (L/kg)	Major Metabolism Enzyme(s)	Active Metabolite	Urine (%)	Half-Life (hours)
Codeine (PO)	53	3-6	CYP3A4 & 2D6	Morphine	90	3
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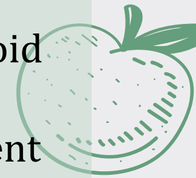
Morphine IR (PO)	<40	4	UGT	M6G	90	2-4

EXAMPLE:
Codeine has a **Vd of 3-6 L/kg**, showing significant distribution of the drug into tissues

Fentanyl has an **intravenous Vd of 3-8 L/kg** and an **oral Vd of 25.4 L/kg**.

Fentanyl is classified as a fully synthetic opioid that can cross both the BBB and placenta, making it one of the most addictive and potent synthetic opioids to date.



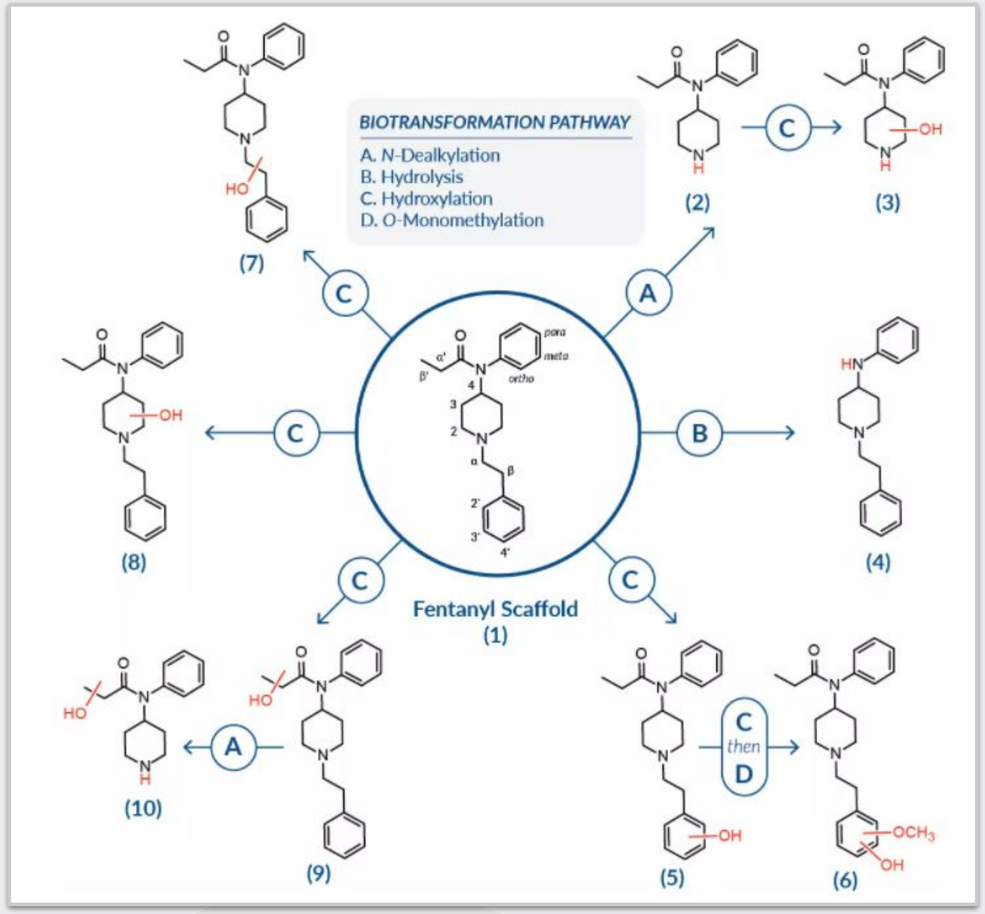
A+B=C

METABOLISM OF OPIOIDS

The **metabolism** is the process of the chemical modifications of the drug into different metabolites for easier elimination. This process occurs in two phases:

Phase 1 Metabolism (chemical reactions like oxidation, reduction, hydrolysis, CYP pathway), and

Phase 2 Metabolism (reactions characterized as conjugation reactions catalyzed by transferase enzymes).



This figure describes the proposed routes of metabolism for **fentanyl** & **designer (synthetic) fentanyls.** The fentanyl scaffold is in the centre of the figure and clearly identifies the different transformation pathways the drug can undergo, including:

A. *N*-Dealkylation
B. Hydrolysis
C. Hydroxylation
D. *O*-Monomethylation

Most of these metabolites have the potential to undergo further transformations to yield additional metabolites of fentanyl.

Extensive metabolism of each individual opioid is described later in this module.

A+B=C

EXCRETION OF OPIOIDS

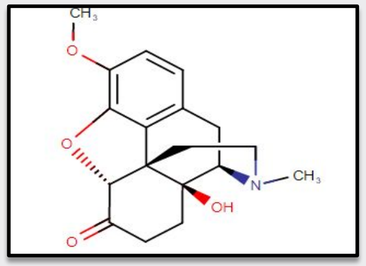
The majority of opioids are excreted as metabolites through the kidneys (methadone is excreted in the bile). Each opioid has its own elimination half-life (T½), which dictate the clearance rates of each drug. In general, when opioids are given at a steady state, opioids reach a steady state after four half-lives.

Opioid (Route)	Excretion	
	Urine (%)	Half-Life (hours)
Codeine (PO)	90	3
Fentanyl (TDS)	75	20-27
Oxycodone IR (PO)	19-64	7-9
Morphine IR (PO)	90	2-4
Hydrocodone IR (PO)	26	3.3-4.4
Methadone (PO)	<10	7-59

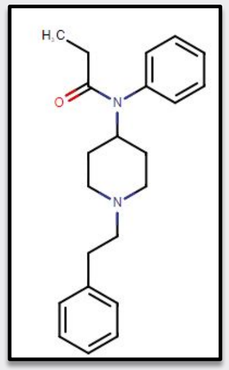
EXAMPLE:

Fentanyl has an excretion value of 75% with a T½ of 20-27 h.
Oxycodone has an excretion value of 19-64% with a T½ of 2-4 h.

This shows that depending on the structural composition of the opioid drug, its clearance rates are dependent on how 'excretion-ready' its metabolites are in the body.

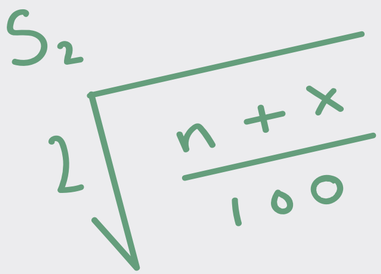


Oxycodone



Fentanyl

A+B=C



07

METABOLISM OF OPIOIDS

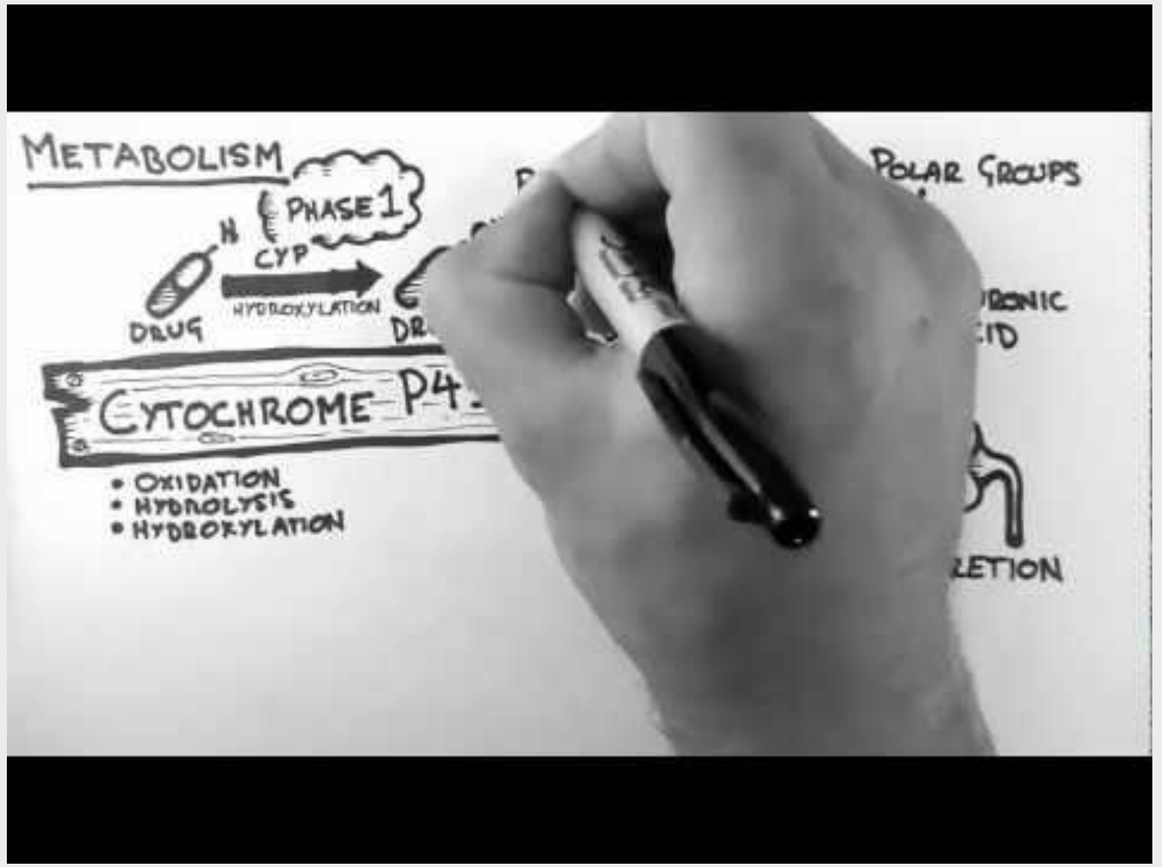
Phase 1 & Phase 2 Metabolism

07

METABOLISM OF OPIOIDS

To understand the biochemical aspects of opioid metabolism further, **watch the video** below.

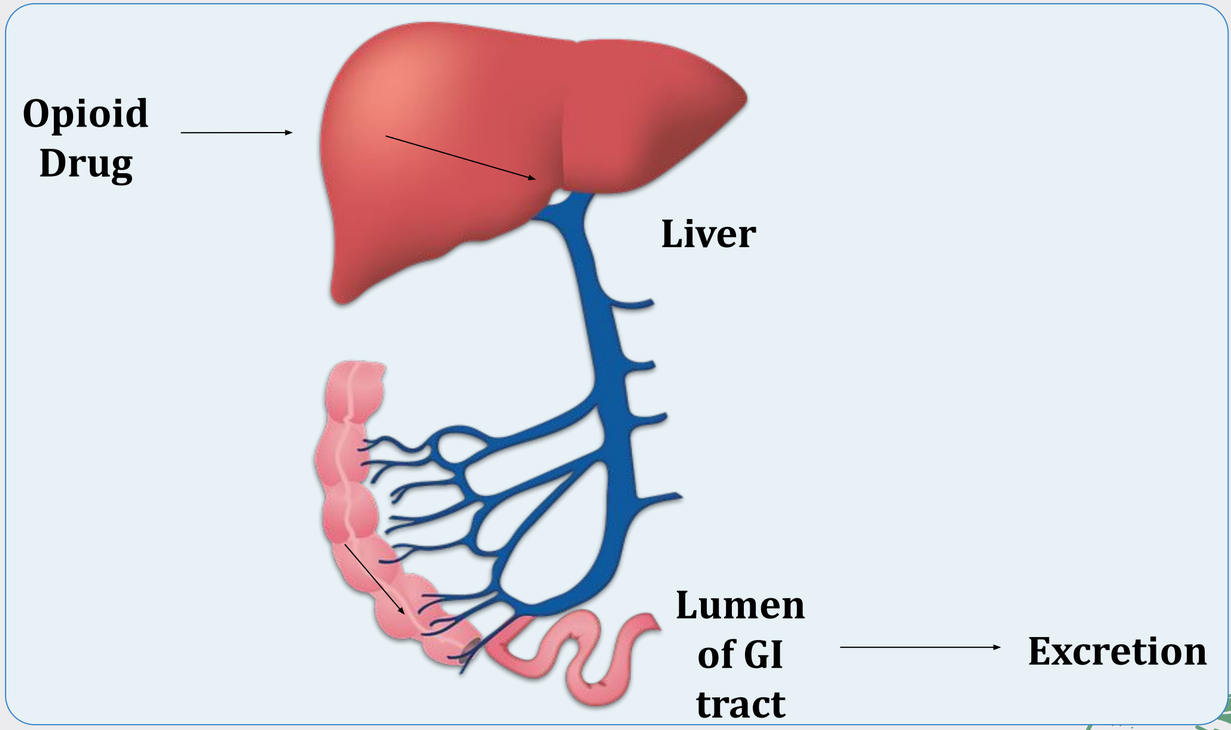
NOTE: Keep an eye out for specific examples used in the video, as they will be useful for the rest of the lesson!



A+B=C

METABOLISM OF OPIOIDS

- **Metabolism:** process of biotransformation where drugs are broken down for elimination purposes
- Most opioids undergo first-pass metabolism in the liver before entering systemic circulation (i.e., bloodstream)
- **First-Pass Metabolism:** reduces bioavailability of the drug
- Metabolism of opioids take place in the liver, creating enzymes for the breakdown of the compounds



A+B=C

METABOLISM OF OPIOIDS

PHASE 1 METABOLISM

- **Oxidation** or **hydrolysis** of opioid drugs
- Involves the cytochrome P450 (CYP) enzymes -- allow for *N-*, *O-*, and *S*-dealkylation
- Each opioid has an interaction with the specific enzyme, indicating that drug metabolism is dependent on the enzymes that metabolizes it

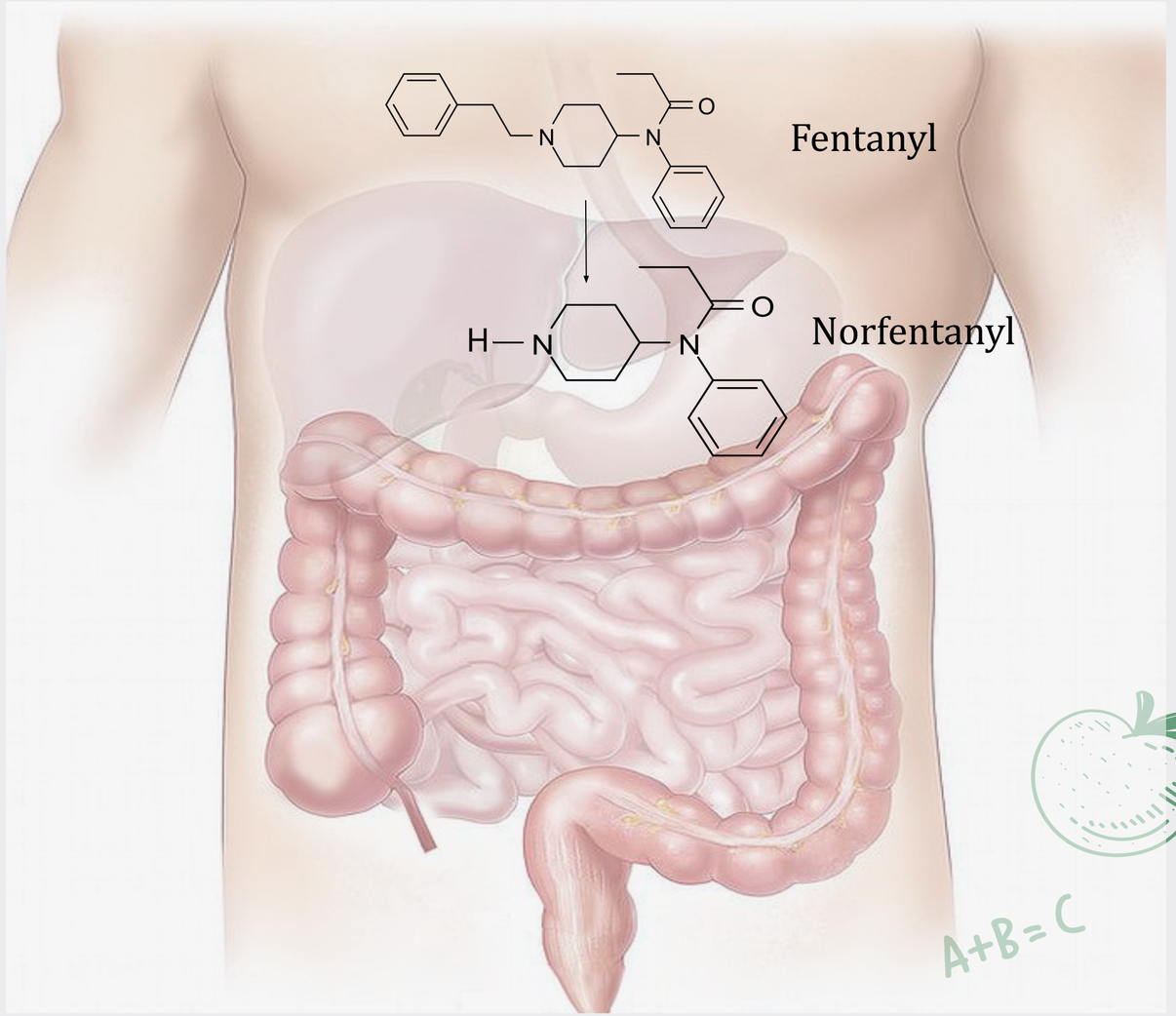
Opioid	Phase 1 metabolism
Morphine[12]	None
Codeine[13]	CYP2D6
Hydrocodone[14]	CYP2D6
Oxycodone[11]	CYP3A4
	CYP2D6
Methadone[15]	CYP3A4
	CYP2B6
	CYP2C8
	CYP2C19
	CYP2D6
	CYP2C9
Tramadol[16]	CYP3A4
	CYP2D6
Fentanyl[10]	CYP3A4
Hydromorphone[17]	None
Oxymorphone[18]	None

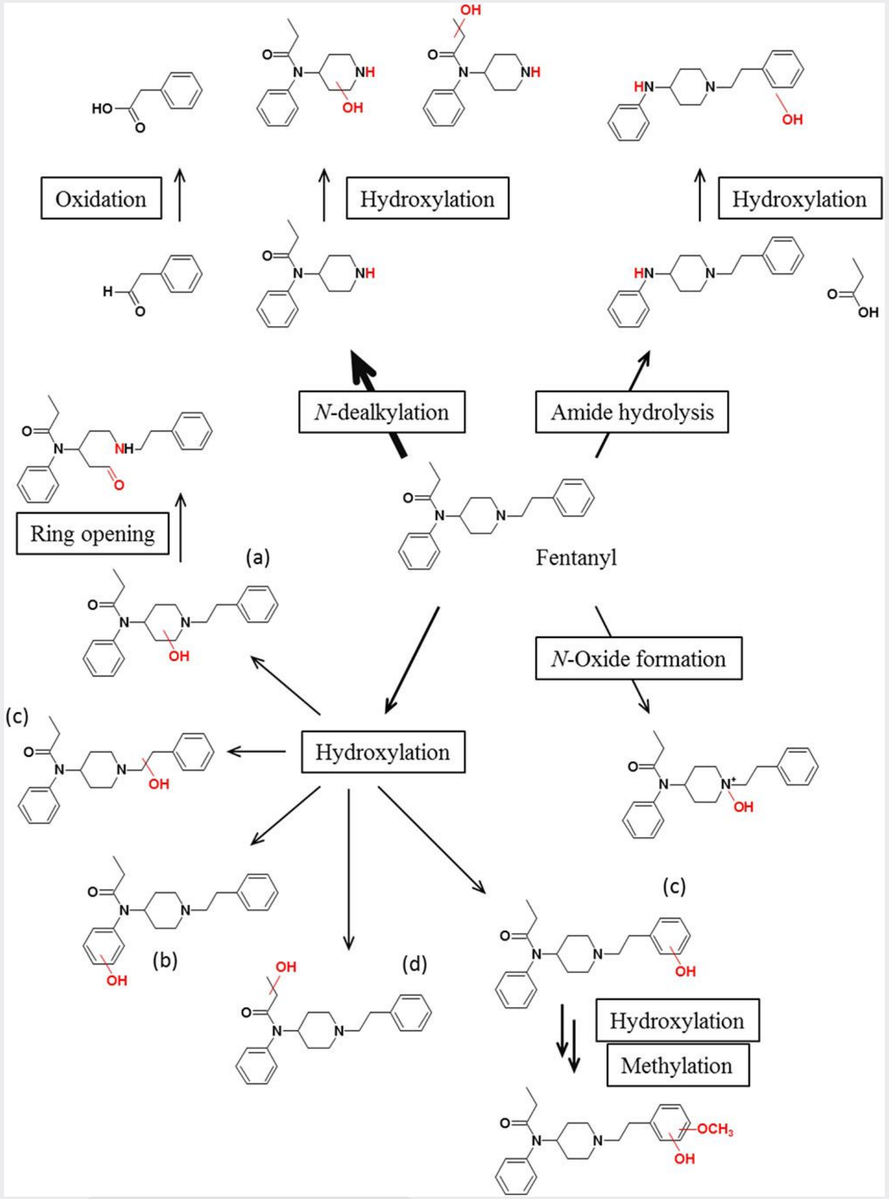
Mayo Clin Proc. 2009 Jul; 84(7): 613–624

A+B=C

METABOLISM OF SYNTHETIC OPIOIDS:
FENTANYL

- Major metabolism reaction for fentanyl is *N*-dealkylation to norfentanyl
- Since most of the fentanyl is metabolized via First Pass, the metabolites formed from fentanyl possess no pharmacological activity
- Fentanyl is a high extraction drug: rapidly and extensively cleared from the blood by the liver
  - Insensitive to changes in hepatic clearance when CYP3A4 is altered
- Thus, systemic fentanyl elimination remains unknown and requires more research on metabolism in liver, intestine, & other organs





Front. Pharmacol., 05 April 2019

The metabolites of FENTANYL

- Fentanyl / carfentanil are mostly converted to norfentanyl by CYP3A4-mediated *N*-dealkylation (nontoxic & inactive metabolite)

- No Phase 2 Metabolism
- Depending on the administration route, 30-90% of fentanyl is excreted via first-pass metabolism

A+B=C

$$S_2 \sqrt{2} \frac{n+x}{100}$$

08

OPIOIDS IN BIOLOGICAL FLUIDS

Urinalysis
Blood Plasma
Saliva

OPIOIDS IN BIOLOGICAL FLUIDS

The detection of opioids in biological fluids is key for the analysis of drug-impaired legal cases, often sampling **urine** or **blood** for opioid metabolites. Urinalysis testing of opioids commonly detects the metabolites of heroin and codeine (morphine).

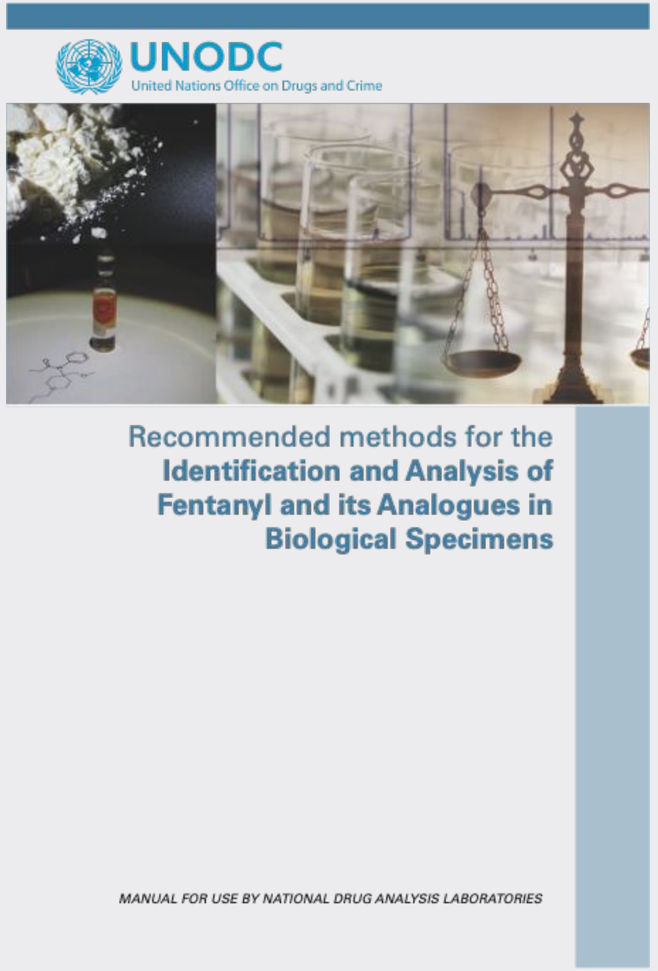
Drug		Detection in URINE	Detection in BLOOD	Detection Limits
SEMI-SYNTHETIC	Hydromorphone	**2-4 days**	**2-3 days**	Detection varies across different immunoassays with reported range of 20-50% cross-reactivity for morphine
	Hydrocodone	**6-24 hours**	**24 hours**	Most widely abused prescription drug, exhibits variable detection using immunoassays that show equivalent detection to morphine
	Oxycodone	**2-4 days**	**26-28 hours**	Detected using immunoassays, no detection at concentrations more than 50-fold higher than morphine (oxymorphone = 100% cross-reactivity, well detected)
SYNTHETIC	Fentanyl	Not easily detected **(24-72 hours)**	Not easily detected **(5-48 hours)**	Not detected by morphine/oxycodone-specific immunoassays, but detected via fentanyl-specific immunoassays; confirmation tests required (GC-MS, LC-MS)
	Carfentanil	Not enough research	Not enough research	Detected in animals with immunoassays and LC-MS-MS

A+B=C

OTHER MATRICES FOR OPIOIDS

Since fentanyl and other opioids are metabolized differently, the amount of time they can be detected in the body differs, with the detection of opioids in the saliva ranging from 24 to 48 hours and up to 90 days in hair follicles.

OPIOID	DETECTION IN SALIVA	DETECTION IN HAIR
Heroin	Only in the first 5 hours after the last dose	Up to 90 days
Hydrocodone	First 12-36 hours after the last pill was ingested	Up to 90 days
Oxycodone (Oxycontin)	Detectable within minutes up to 48 hours	Up to 90 days
Fentanyl	1-4 days	Up to 90 days
Methadone	Detectable after 30 minutes and up to 2 days	Up to 90 days



A+B=C

Post-mortem distribution of acetyl fentanyl

Table 4 Postmortem concentrations of mepirapim and acetyl fentanyl in body fluid and solid tissue specimens obtained from the deceased

Specimen	Mepirapim (ng/mL or g)	Acetyl fentanyl (ng/mL or g)
Heart whole blood	587 ± 42	212 ± 15
Femoral vein whole blood	554 ± 46	170 ± 9
Urine	309 ± 44	169 ± 15
Cerebrum	2740 ± 185	649 ± 34
Cerebellum	2690 ± 316	688 ± 28
Pons	3300 ± 162	821 ± 5
Medulla oblongata	1710 ± 41	489 ± 22
Lung	2720 ± 339	448 ± 28
Myocardium	3120 ± 332	1180 ± 82
Liver	6300 ± 405	416 ± 31
Pancreas	2400 ± 325	987 ± 43
Kidney	5410 ± 574	1140 ± 140
Adrenal gland	1580 ± 194	481 ± 26
Spleen	3610 ± 287	1150 ± 32
Psoas major muscle	792 ± 122	281 ± 11

Data are presented as the mean ± SD ($n = 5$ each)

The highest concentrations of acetyl fentanyl among these specimens were found in the myocardium, followed by the spleen and kidney at 1140–1180 ng/g.

The urinary level of acetyl fentanyl was close to those in femoral vein and heart whole blood, which suggested that death occurred before sufficient excretion and metabolism of the drug.

Moreover, the concentrations in urine were lower than those in the liver and brain, which suggested that death was rapid

A+B=C

Forensic Toxicol. 2019; 37(1): 27–33.

Post-mortem distribution of drugs and metabolites - case study

A 21-year-old male was found dead at home with a note stating that he had taken carfentanil with suicidal intentions. A foil bag and plastic bag labeled "C.50" were found at the scene (see Figure 1 for evidence)

Blood, urine and vitreous, obtained during autopsy, tested to detect compounds based on their μ-opioid receptor activity rather than their chemical structure.
All extracts showed strong opioid activity

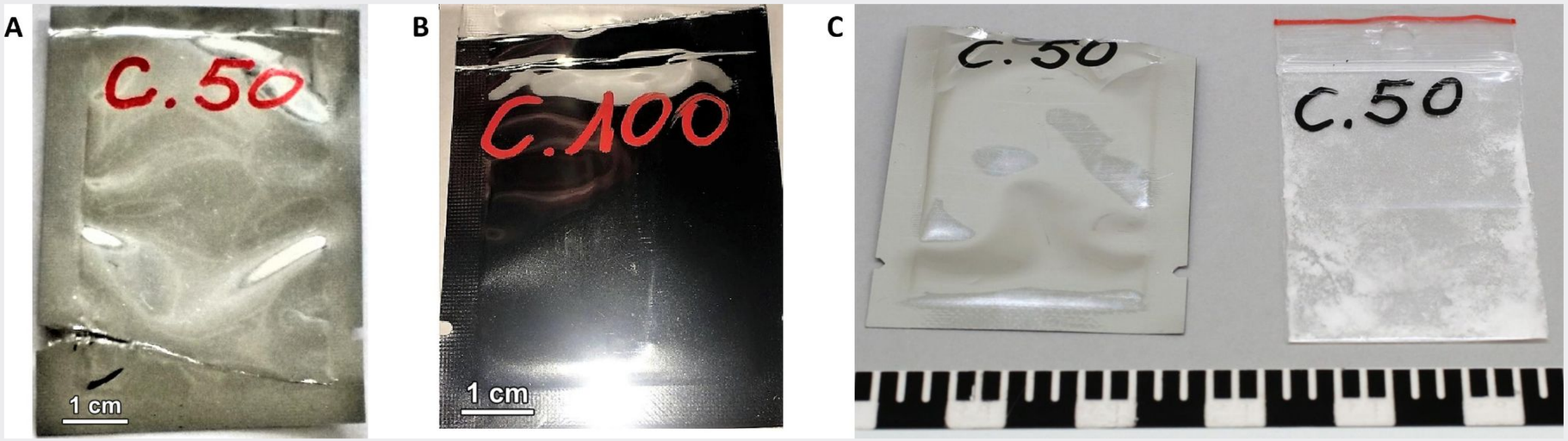


FIGURE 1. (A) Foil bag found at the scene. **(B)** Foil bag obtained by the Belgian Early Warning System on Drugs. **(C)** Foil bag and plastic bag found in a fatality in Norway [image used with kind permission of the National Criminal Investigation Service/Photo (Norway)] (Vevelstad and Drange, 2017).

A+B=C

Front. Pharmacol., 15 May 2018

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

09

CAN-LII CASE STUDY

R. v. Macdonald

2021 BCSC 371 (CanLII)

TIMELINE OF CASE

Incidence Date:
June 24, 2016

R. v. Macdonald

10:50 am

Time of the Accident

On June 24, 2016, a Honda Civic and a Dodge Ram collided with both vehicles airbags deployed. 9-1-1 was called.

10:58 am

Police Arrive at Scene

Constable Eric Parent (RCMP) was the first to arrive to the scene at 10:58 am. The officer noted the accused was not walking straight along the side of the highway, and had trouble keeping his balance. The DRE examination noted intoxicated actions.

11:19 am

Hospital Observations

The accused was taken to the hospital and was admitted at 11:30 am. Dr. Bartnell found a green powder on the accused's nostrils, lips, and clothing. The doctor found the accused to have slow movements and difficulty with a finger-to-nose test. Eye contact was poor, failed to answer questions, and yawned every 3-5 minutes, indicating intoxication.

Shortly after 11:30 am

Biological Sample Collected

Dr. Bartnell ordered urine screening tests on the accused. The accused was unable to urinate after 3 attempts, forcing a catheter to be arranged for sample collection. The samples were then analyzed at the hospital.

1:10 pm

Discharged from Hospital

Once Dr. Bartnell assessed the urinalysis, the accused was discharged from the hospital at 1:10 pm.

A+B=C

FROM CRIME SCENE TO SCREENING LAB

CRIME SCENE

POLICE STATION

LAB

During this stage, the police officer detects the impairment, initiates an oral and physical drug test on the roadside, and then evaluates the individual via a Drug Recognition Expert (DRE). 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.

FROM CRIME SCENE TO SCREENING LAB

CRIME SCENE

POLICE STATION

LAB

12-Step DRE Protocol:

1. Breath Alcohol Test
2. Interview of the Arresting Officer
3. Preliminary Examination and First Pulse
4. Eye Examinations
5. Divided Attention Psychophysical Tests
6. Vital Signs and Second Pulse
7. Dark Room Examinations
8. Examination for Muscle Tone
9. Check for Injection Sites and Third Pulse
10. Subject's Statements and Other Observations
11. Analysis and Opinions of the Evaluator
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**

A+B=C

FROM CRIME SCENE TO SCREENING LAB

R. v. Macdonald

CRIME SCENE

POLICE STATION

LAB

DRE ASSESSMENT:

Constable Eric Parent (RCMP) was the first to arrive to the scene at 10:58 am. The officer noted the accused was not walking straight along the side of the highway, and had trouble keeping his balance. Constable Parent engaged in conversation with the accused, but noted his speech was slow and confused, as if he just woke up. Upon examination, the accused had great difficulty with the finger-to-nose test and lacked responses when asked them.

Take a closer look at the highlighted regions!

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

Incidence Date:
June 24, 2016

CASE STUDY: R. v. Macdonald

CRIME SCENE

POLICE STATION

LAB

TOXICOLOGICAL EVIDENCE

- Jacqueline Mack (Forensic Toxicologist) employed by the RCMP was given 2 vials of blood and one vial of urine for analysis
- Tests on the vials of **both blood and urine** confirmed the presence of diazepam (Valium), nordiazepam, temazepam, and fentanyl in the accused system (consistent with the hospital's analysis)
- Fentanyl found in the blood was at very low concentrations, not a suitable level for an accurate concentration reading
- Overall, Mack found the accused to have high levels of central nervous system depressants in his system that would slow down brain activity and induce a longer reaction time / inappropriate reaction to situations.

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