FORENSIC TOXICOLOGY:
FROM CRIME SCENE TO VIRTUAL LAB

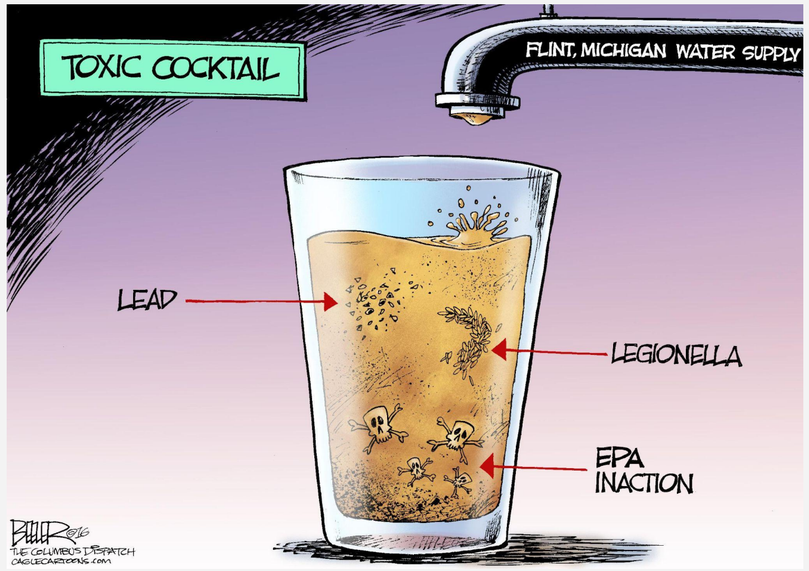
MODULE 1

CHAPTER 5: HEAVY METALS

WHAT'S AT STAKE

Fighting for safe drinking water in Flint, Michigan

Flint, USA water crisis!



What happened to… Flint Michigan water crisis

Lead-Laced Water In Flint: A Step-By-Step Look At The Makings Of A Crisis

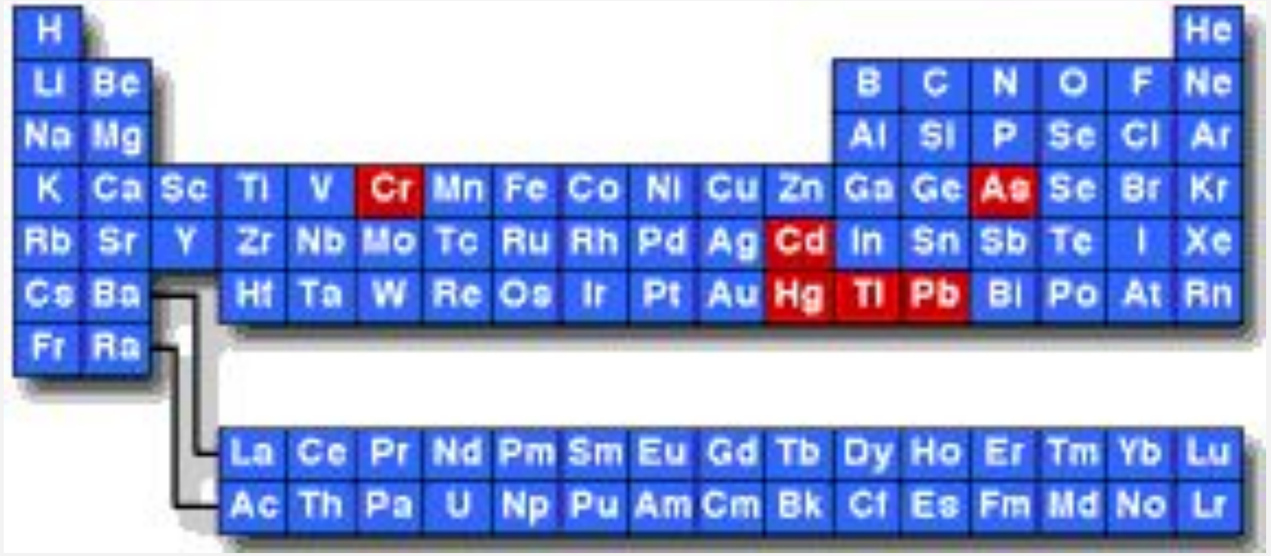
FLINT MICHIGAN WATER CRISIS

Flint, Michigan switched its water supply in April of 2013. The decision was made to build a pipeline and connect to a new system

This resulted in **hundreds of millions** of dollars of damaged infrastructure, caused deadly bacterial outbreaks that killed **at least 12 people**, and exposed **thousands of children** to **high levels of lead** in their drinking water.

Flint switched the water supply back in 2015, and lead content was **below the federal limit** by 2017

TABLE OF CONTENTS

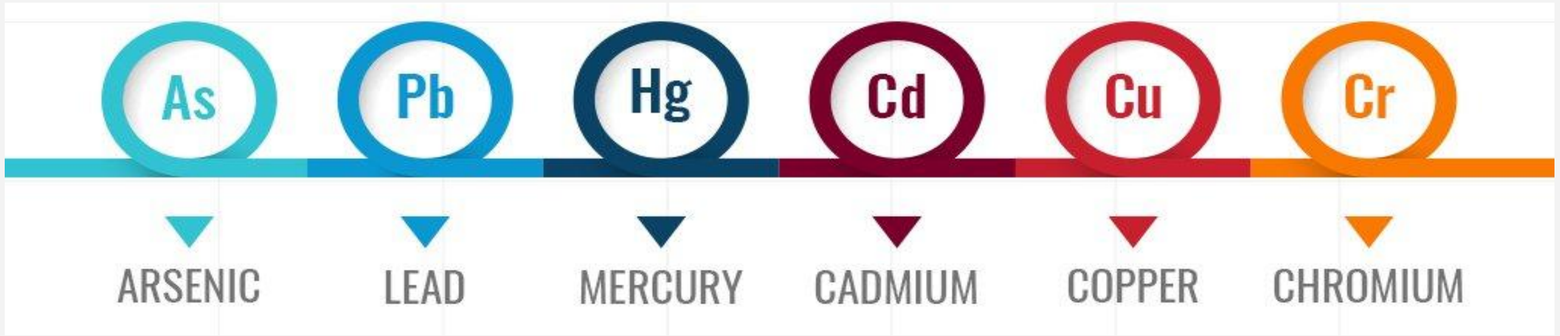


01. INTRODUCTION

01.

WHAT ARE HEAVY METALS?

The term heavy metal refers to any metallic chemical element that has a relatively high density, is exogenous, and is toxic or poisonous at relatively low concentrations.



Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed.

01.

Antimony

WHERE ARE HEAVY METALS FOUND?

Cadmium

Sb
Antimony

Flame retardant, batteries, pigments, ceramics, and glass

Cd
Cadmium

By-product of zinc refining, batteries, coatings, pigments, stabilizers for PVC, alloys and electronic compounds

Cr
Chromium

Metal alloys, and pigments for paints, cement, paper, and rubber

Copper

Cu
Copper

Pipes, algicides, wiring, motors

Pb
Lead

Batteries, petrol additives, alloys, pigments and compounds, cable sheathing, shot and ammunition, old paint

Lead

Hg
Mercury

Batteries, lamps, thermometers, and dentistry

01.

SOURCES OF ENVIRONMENTAL POLLUTION

Common household products, agricultural and industrial products, and waste disposal

Runoff from industrial activities accumulates in bodies of water

Chimneys are one of the main sources of atmospheric pollution
Examples: burning of fossil fuels, engine exhaust, smelting etc.

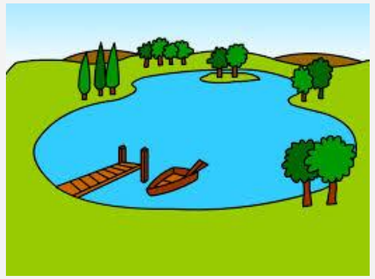
01.

BIOACCUMULATION OF HEAVY METALS

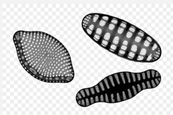
Industrial activities...

Heavy Metals

Bioaccumulation is an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment



...put heavy metals into the environment...



.... which are taken up by phytoplankton ...

... which are eaten by small fish...

...who are eaten by bigger fish....

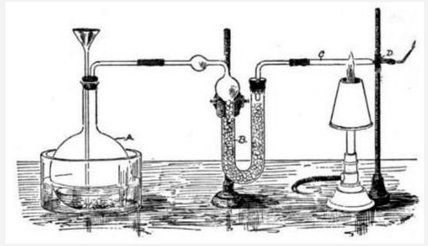
...and finally consumed by humans

Compounds accumulate in living things any time they are taken up and stored faster than they are metabolized and excreted

02. HISTORICAL AND CURRENT USES

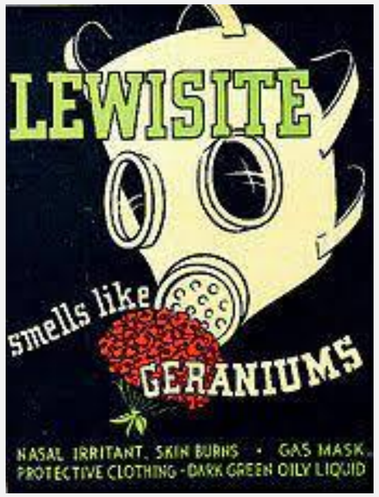
02.

History of Arsenic



1836
English chemist James Marsh perfected a sensitive and specific chemical test for arsenic

1940
In 1940, Germans developed an organic blistering war gas containing arsenic, which was known by the code name of Lewisite.



1250
German scholastic Albertus Magnus credited with the discovery of Arsenic

1900
In Frankfurt, Germany, a pharmacologist named Paul Ehrlich became preoccupied with arsenic. Ehrlich, was convinced that arsenic could be used therapeutically as a treatment for diseases such as syphilis

1960
A solution of one-percent potassium arsenite (Fowler's Solution) was used as a general tonic and in the treatment of psoriasis. It was still being recommended in through the 1960's, although the harmful effects of this solution were known

02.

RECENT CASE OF ARSENIC POISONING

A 50-year-old man present with a six-week history of diarrhea and vomiting, and also reported general malaise, abdominal tenderness, mild fever, numbness in arms and legs and blurring of vision

Results from a vast array of medical tests came back normal. Further testing was scheduled for the following morning, however the patient was found deceased.

The man thought he may have been poisoned as he had felt unwell following a meal

Samples of liver, urine, blood and hair were collected, and and tested for arsenic, lead, mercury, and thallium using ICP-MS

02.

RECENT CASE OF ARSENIC POISONING

Toxicological Data

Patient's Levels

Blood: 7.0 umol/L
Urine: 64.5 umol/L
Liver: 39 ug/g
Hair: 11ug/g

Reference range

Blood: <0.135 umol/L
Urine: <0.25 umol/L
Liver: <0.5 ug/g
Hair: <0.5 ug/g

Concentrations of lead, mercury and thallium were within reference limits but arsenic was grossly elevated in all samples

In order to conclude that arsenic poisoning is a result of intentional poisoning, **three specific criteria** must be satisfied:

1. That arsenic is present in toxic concentrations in tissues ✓
2. Its presence could not be accounted for by alternative incidental possibilities ✓
3. The observed symptoms are consistent with previously reported fatal cases ✓

The victim's hair was 25 mm long representing around the last **10 weeks** of his life. Arsenic concentrations were high along this length suggesting he had been poisoned over this entire time period

In this case, all three conditions were satisfied and so it was concluded that death was the result of intentional fatal arsenic poisoning

02.

MONITORING HEAVY METALS IN ENVIRONMENT

1 Sample Collection

2 Sample Preparation

3 Sample Analysis

World Health Organization (WHO) recommended safe limits of heavy metals in soil and wastewater

Metal	Hg (ppm)	Cd (ppm)	Pb (ppm)	Cr (ppm)	Ni (ppm)
Soil	0.05	0.003	0.1	0.1	0.05
Wastewater	0.001	0.003	0.01	0.05	0.02

03. HEAVY METAL EFFECTS ON THE BODY

03.

Hg

WHAT IS MERCURY?

Mercury exists in three forms:

80
Hg
Mercury
200.592

Elemental

silvery, shiny, volatile liquid gives of a colourless, odourless vapour at room temperature

Inorganic

compounds formed when elemental mercury combines with other elements such as sulphur, chlorine or oxygen to create compounds known as mercury salts

$H_3C—Hg^+$

Organic

compounds, such as methyl mercury, that are formed when elemental mercury combines with carbon

Mercury can change from one form to another in the environment.
For example, some types of bacteria and fungi can change mercury into its most toxic form, methyl mercury

03.

Hg

MERCURY EXPOSURE

Common Mercury Exposure Routes			
	Elemental	Inorganic	Organic
Inhalation	High	Low	Low
Oral	Low	Med	High
Dermal	Low	Med	Low

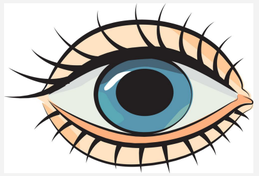
- Pure elemental mercury (quicksilver or Hg) is liquid at room temperature. If ingested, has very low toxicity because it is not absorbed by the GI tract and is eliminated completely in the stool.
- If quicksilver is agitated or heated, it turns into a vapour which is readily absorbed by inhalation and is highly toxic to the lungs and CNS.

03.

Hg

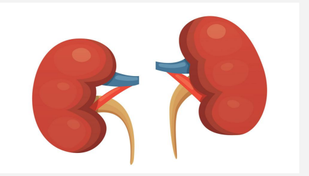
EFFECTS OF MERCURY ON THE BODY

Nervous system is the primary target

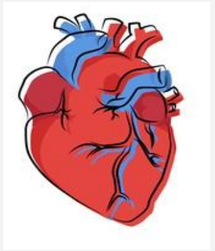


Mercury and its compounds can cause impaired **vision** and **hearing**

No positive role in the body



Depending upon the specific exposure, the **kidneys, liver** and **lungs** are also important targets



Possible harmful effects on the **cardiovascular, immune** and **reproductive** systems

High doses of mercury can be **fatal** to humans

During pregnancy, mercury compounds cross the placental barrier and can interfere with the development of the fetus, and cause attention deficit and **developmental delays** during childhood

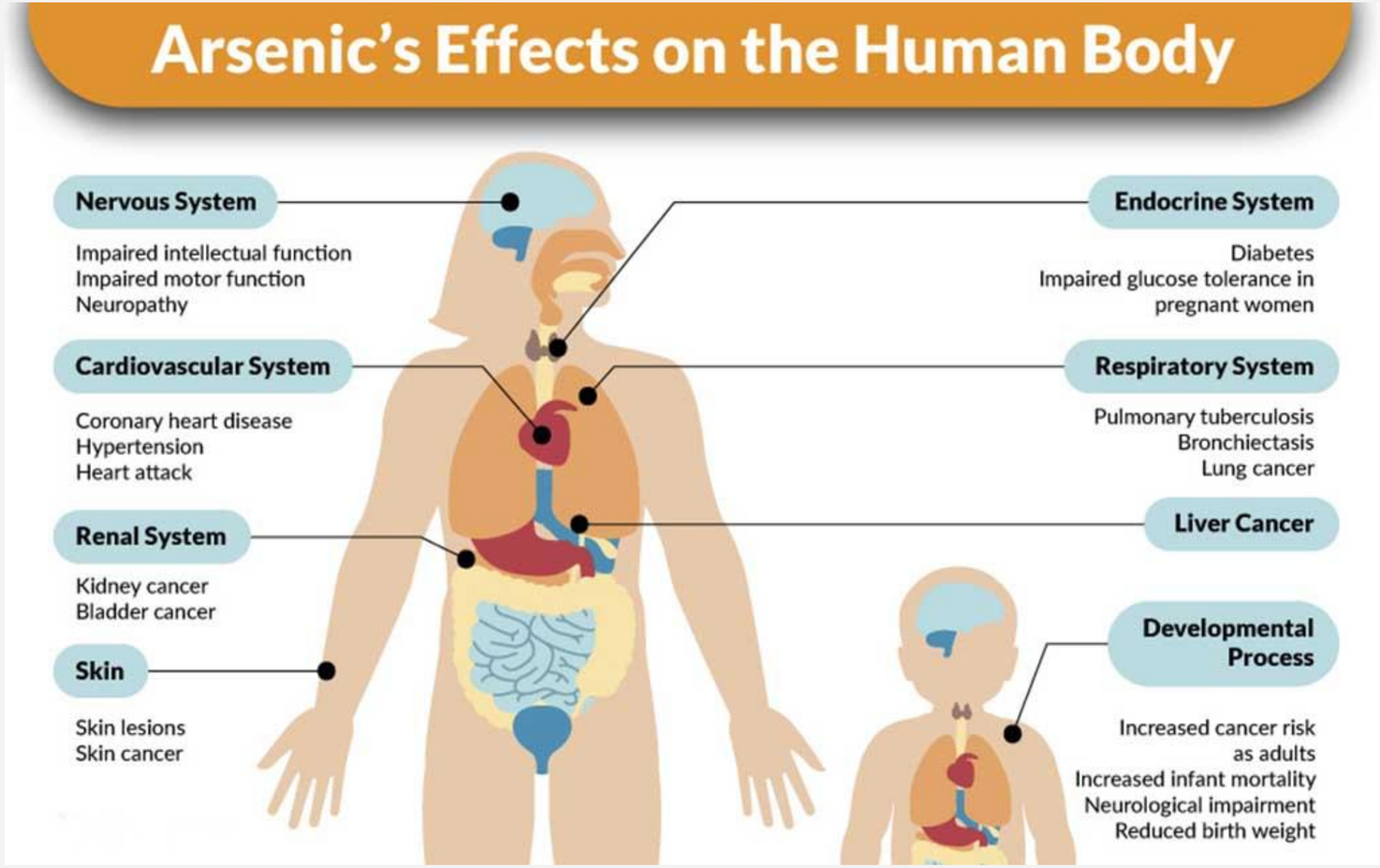
03.

As

Acute Effects

- Vomiting
- Abdominal pain
- Diarrhea
- Numbness and tingling of the extremities
- Muscle cramping
- Death (in extreme cases

Long Term Effects



03.

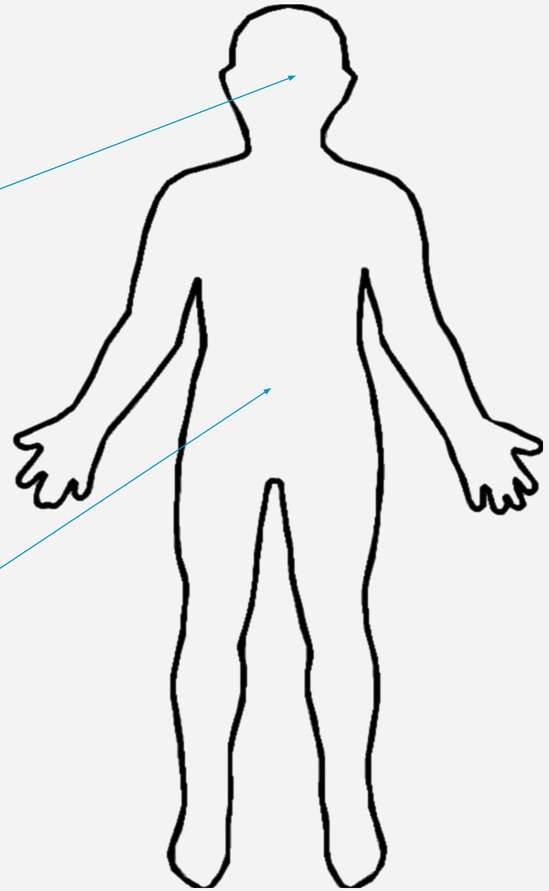
LEAD POISONING Pb

Lead buildup in the body causes serious health problems

Symptoms

Headaches
Irritability
Reduced sensations
Aggressive behaviour
Difficulty sleeping

Abdominal pain
Poor appetite
Constipation
Anemia



Additional Complications for Children:

Lead is more harmful to children as it can affect developing nerves and brains

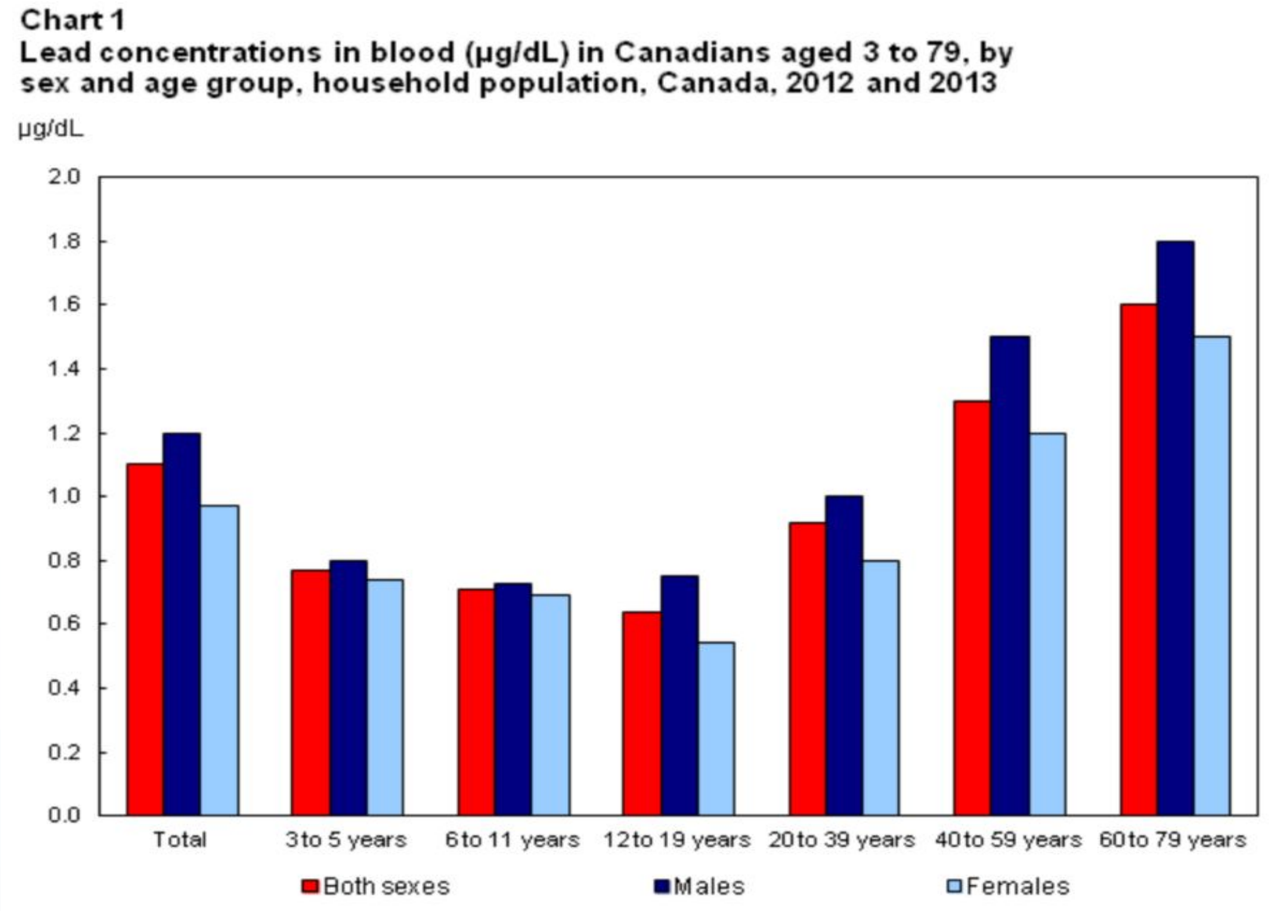
- Loss of developmental skills
- Behaviour, attention problems
- Hearing loss
- Kidney damage
- Reduced IQ
- Slowed body growth

04. HEAVY METALS IN BIOLOGICAL FLUIDS

04.

Pb

HEAVY METAL CONCENTRATION IN BLOOD

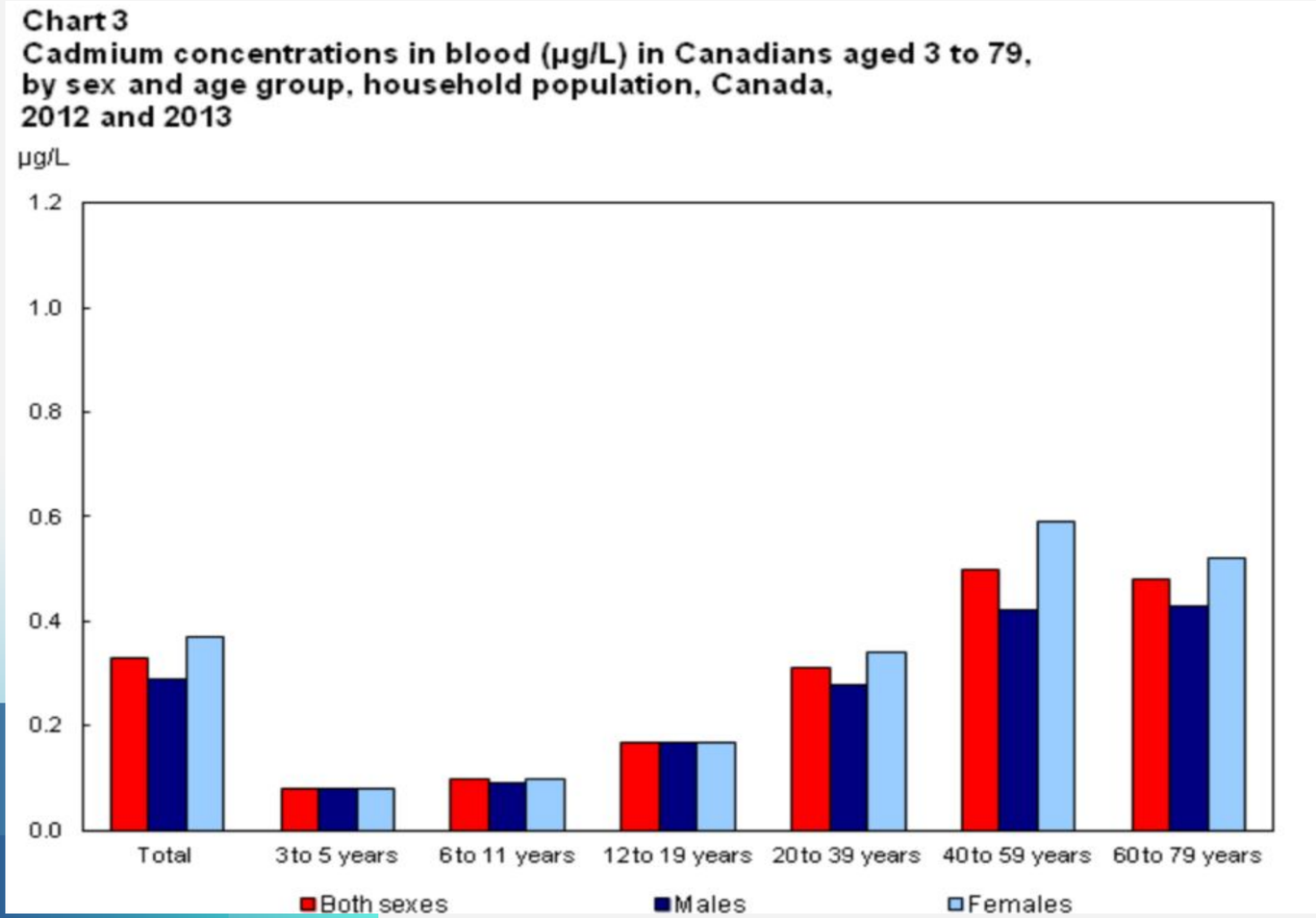


- 96% of Canadians aged 3 to 79 years had detectable levels of lead in their blood.
- The average concentration of blood lead was 1.1 ug/dL
- Males had a significantly higher blood lead concentration compared with females
- THE oldest age group, 60 to 79 years, had the highest LEAD level

04.

Cd

HEAVY METAL CONCENTRATION IN BLOOD

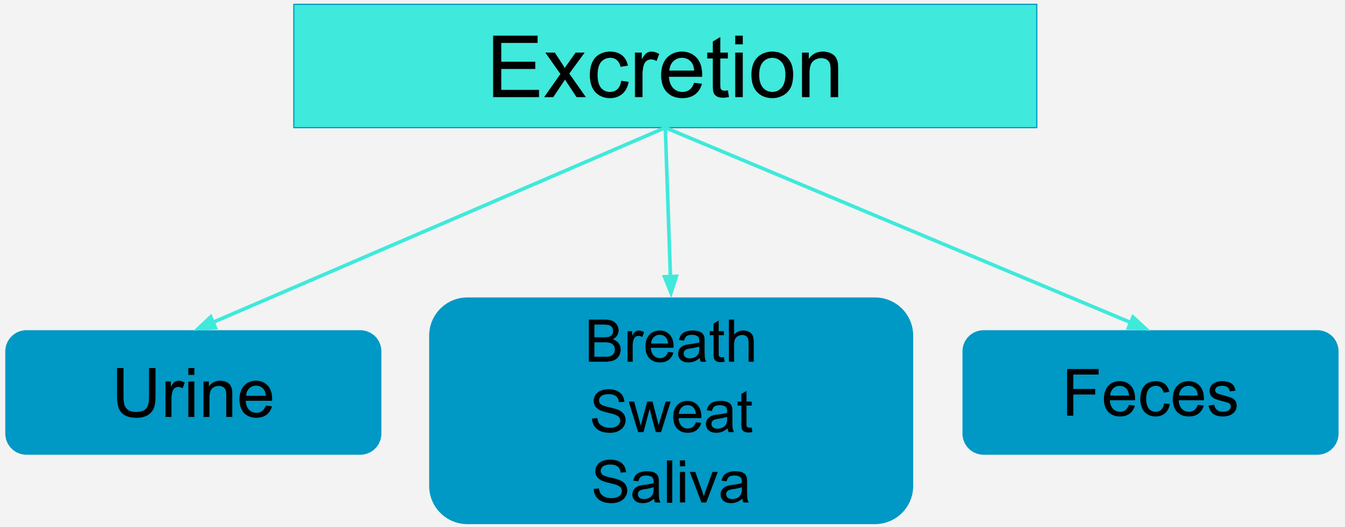


- The cadmium in the blood of 85% of the Canadian population aged 3 to 79 years.
- The average blood cadmium concentration was 0.33 ug/L.
- There was a difference between males and females
- Average concentrations of cadmium in blood tended to be higher in the older age groups.

04.

MERCURY IN BIOLOGICAL FLUIDS

Hg



Urine and feces are the main pathways of excretion, although a small amount of inhaled mercury can be eliminated in the breath, sweat, and saliva.

Excretion is **dose-dependent** and **biphasic**: initially rapid then followed by slow excretion

04. MEASURING HEAVY METALS IN BIOLOGICAL FLUIDS

URINE

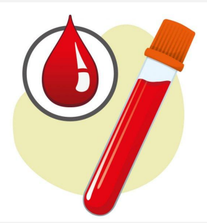
Neutron Activation Analysis
NAA
Atomic Absorption
HGAAS
Atomic Emission
DCP-AES
X-Ray Fluorescence
XRF
Mass Spectrometry
ICP-MS
Colorimetric Photometry

TISSUE

Atomic Absorption
HGAAS
Gas Chromatography
GC-ECD

HAIR

Atomic Absorption
HGAAS
Mass Spectrometry
ICP-MS



BLOOD

Atomic Absorption
FAAS
GFAAS
HGAAS
Gas Chromatography
GC-ECD
Mass Spectrometry
ICP-MS
HPLC-ICP-MS
Anodic stripping voltammetry
ASV

04.

WORKFLOW: DETECTION OF METALS IN BIOLOGICAL FLUIDS

Blood Sample Collection

- 3 mL blood collected in an EDTA tube
- EDTA tube inverted 8-10x to prevent clotting
- Specimens stored at 4 degrees C

Digestion of Whole Blood

- Concentrated nitric acid added to blood sample
- Microwave digestion
- Digestion stopped when colourless solution was obtained and evaporated to dryness
- Diluted to 25 mL with DI water

Determination of Metal Concentration

The standard solutions for metal ions used to build a calibration stock solutions

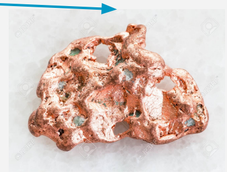
Analysis

Samples are analyzed using a specific method,, and statistical testing is used to compare the samples to the standards to determine the concentration of the selected heavy metals present in the sample

Antimony

Copper

Lead



Cadmium

Urine Sample Collection

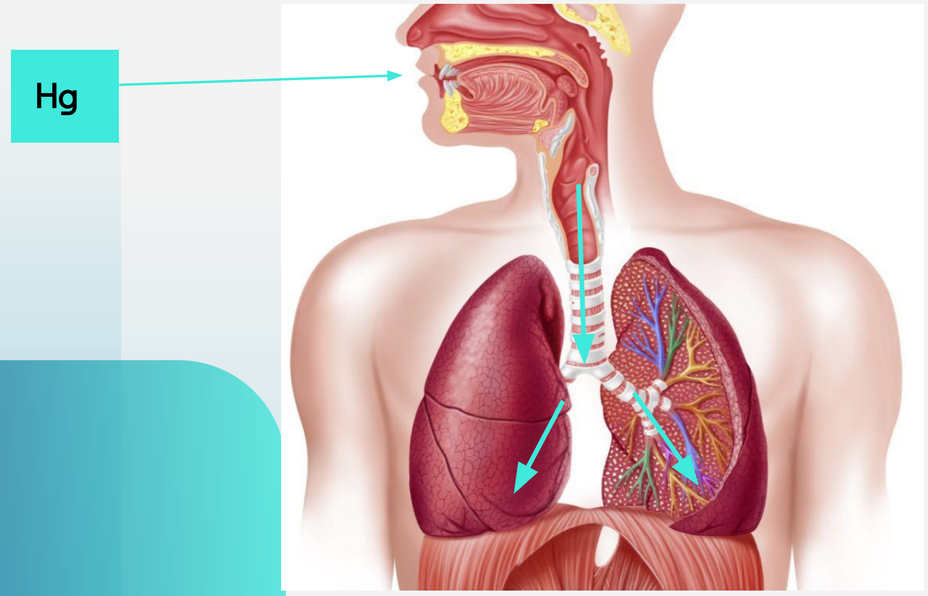
- Clean plastic container used for sample collection
- Collected into 10 mL aliquot tube
- Stored at 4 degrees C

Digestion of Urine

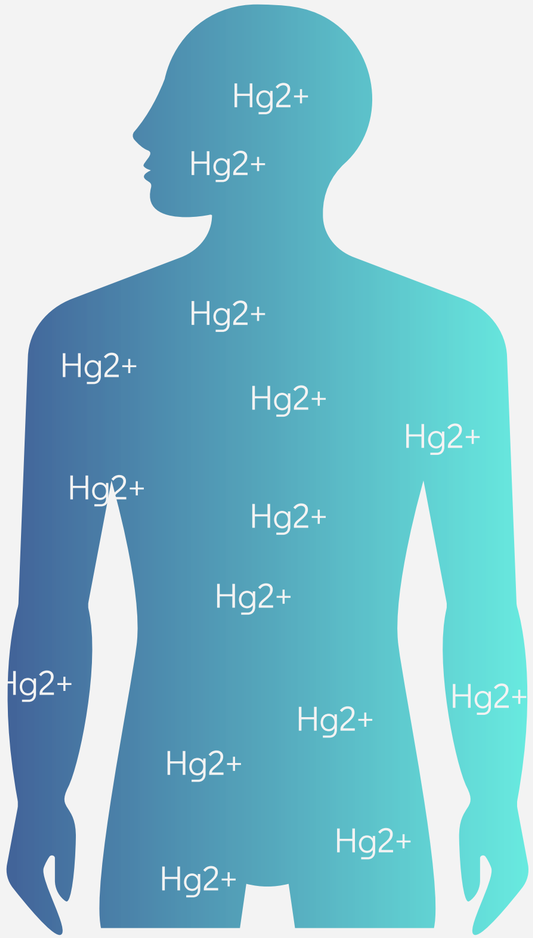
- Concentrated nitric acid added to urine sample
- Microwave digestion
- Digestion stopped when colourless solution was obtained and evaporated to dryness
- Diluted to 25 mL with DI water

05. HEAVY METAL METABOLISM

05. METABOLISM OF ELEMENTAL MERCURY Hg



- Elemental mercury is poorly absorbed through ingestion (<0.01% of dose)
- Inhaled mercury vapour is readily absorbed at a rate of ~80% in the lungs, and quickly diffused in the blood and distributed to all organs of the body



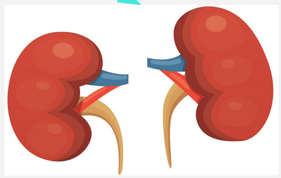
Absorbed elemental mercury is oxidized to the ionic mercury form (Hg 2+) in the RBCs and tissues, a process that takes several minutes ONLY!

05.

METABOLISM OF ELEMENTAL MERCURY Hg

Deposition

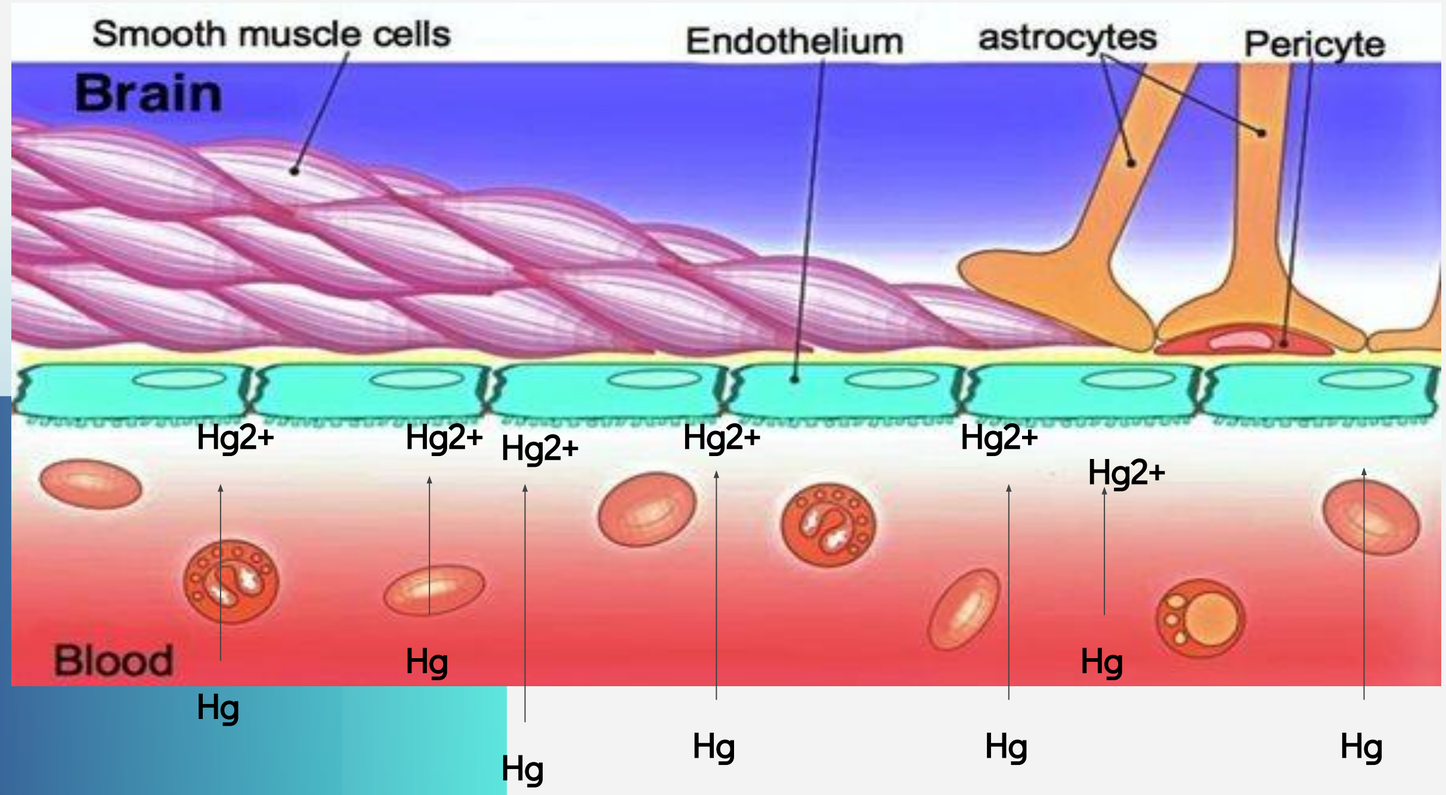
The primary organs of mercury deposition following inhalation exposure to elemental mercury vapor are the brain and kidney



With time after exposure, the greater proportion of the body burden of mercury is found in the kidney

Biological half-life: estimated to be **~30-60 days** in the body. Half- life in the brain is not entirely clear, but estimated to be as long as **~20 years**

05. METABOLISM OF ELEMENTAL MERCURY Hg

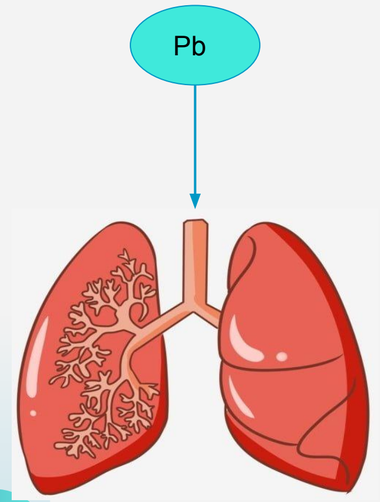


Inhaled mercury vapor accumulates in the CNS
Can cross the blood-brain barrier and
blood-placenta barrier as well as the lipid bilayers of cellular and intracellular organelle membranes

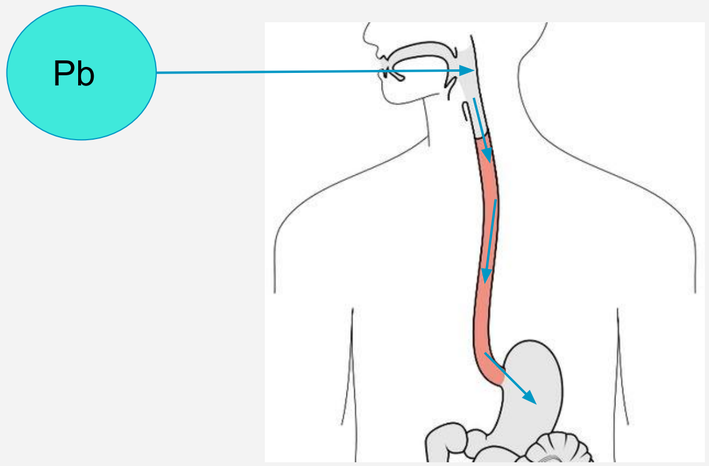
- Elemental mercury vapor is rapidly oxidized to ionic mercury
- Hg remains as vapor in the blood for a short time, which is long enough for a significant amount of mercury vapor to penetrate the blood-brain barrier before it is oxidized
- Mercury molecules can then be oxidized and accumulate in the brain
- The oxidized form will not effectively cross the blood-brain barrier.

05. METABOLISM OF LEAD Pb

Small particles of inorganic lead can be **absorbed** through the **respiratory tract**....



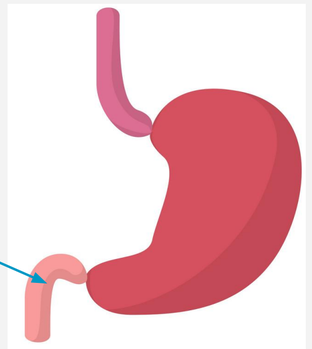
...while larger particles are removed by the mucociliary cells and transported to the oropharynx and then **swallowed**



Amount of lead taken up by the GI tract depends on several factors such as:

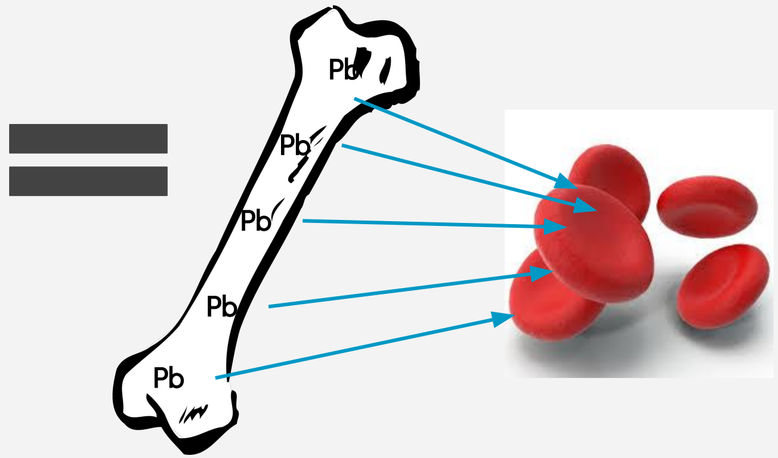
- Age
- Nutrition
- Diet
- Physiological characteristics of the metal in the medium ingested

Absorption of lead is mainly occurs in the duodenum

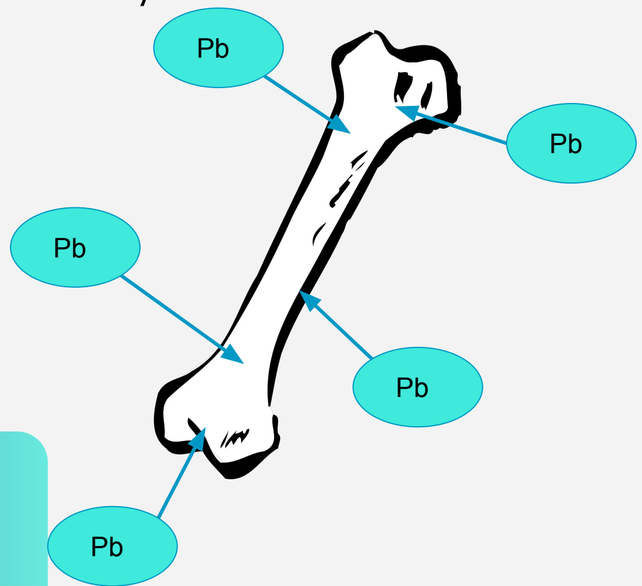


05. METABOLISM OF LEAD Pb

Lead is **distributed** throughout the body and is **route independent**.



It is **distributed** mostly to the **bones**

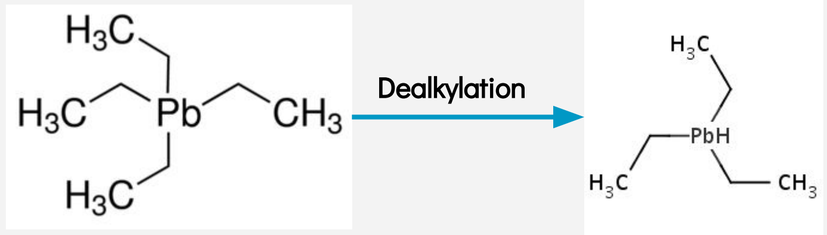


Pregnancy, menopause, lactation, and osteoporosis can increase **bone resorption**, thus also **increasing the lead in the blood**. Lead can primarily be found in **red blood cells**

05. METABOLISM OF LEAD Pb

Metabolism of Organic Lead

Alkyl compounds are actively metabolized through an oxidative dealkylation in the liver, and catalyzed by the cytochrome p-450



Tetraethyl lead

Triethyl lead

Exposure to tetraethyl lead results in excretion of ethyl lead, diethyl lead, and inorganic lead through the urinary route

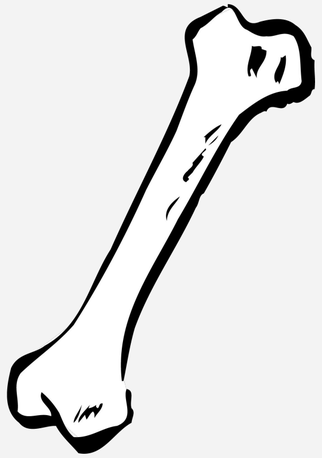
Metabolism of Inorganic Lead

Forms complexes with:

- Protein ligands (e.g., albumin)
- Non-protein ligands (e.g., sulfhydryl)
- Proteins in the cytosol
- Proteins in the cell nucleus

05. METABOLISM OF LEAD Pb

70% **of lead excretion occurs via the urine**



Lesser amounts are excreted via the **feces**, and almost negligible amounts are excreted through the **sweat, hair** and **nails**

Half-life in bones: **years to decades**

Half-life in blood and soft tissue: **1-2 months**

06. CanLii CASE STUDY

06.

100002351830 (Re), 2016 CanLII 34067 (CA VRAB)

Background

- The Applicant is a retired RCMP officer, who applied for a disability pension for the condition of **lead poisoning**
- As a firearms instructor, he was required to go down range to mix metal plates and curtains. The range had inadequate filters to control the **lead gases** resulting from **shot and ammunition.**

Issue

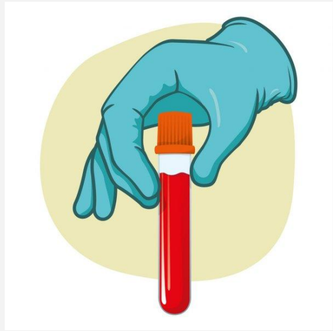
The issue before the Panel is whether the Applicant's claimed condition arose out of, or is directly connected, with RCMP service

06.

100002351830 (Re), 2016 CanLII 34067 (CA VRAB)

Blood Test Evidence

- The appellant's blood lead level (BLL) in January of 1996 was **38.33 ug/dL**
- Blood testing from January to July of 1996 identified **elevated lead levels** in the Applicant's blood
- Elevated blood lead levels for 6 months is considered "**chronic**"
- The panel concluded the applicant has a service-related chronic condition of **lead poisoning**



BLOOD LEAD LEVELS

Normal: <5 ug/dL
Moderately toxic: 50-70 ug/dL
Severely toxic: >70 ug/dL

06.

100002351830 (Re), 2016 CanLII 34067 (CA VRAB)

Neuropsychological Evidence

2012: Dr. Fisher, psychologist, conducted neuropsychological testing on the Appellant and found the results to be "**not inconsistent with**" frontal-subcortical and limbic deficits associated with **lead exposure.**

2014: Dr. Roberts, neurologist, concluded that the cognitive changes identified with neuropsychological testing were consistent with **lead exposure.** However, he questioned if the future progression of symptoms could "suggest an **alternate explanation**" for cognitive decline

The Decision

The Panel concluded the Applicant had a service-related chronic condition of **lead poisoning** based on **blood test results** in 1996 and **neuropsychological testing**

07. LIST OF REFERENCES

07.

LIST OF REFERENCES

1. Flint Water Crisis | NRDC
2. What happened to... Flint Michigan water crisis - Toronto | Globalnews.ca
3. Cartoons: Flint, Michigan water crisis – East Bay Times
4. Flint Water Crisis: A Step-By-Step Look At What Happened : The Two-Way : NPR
5. Here's what you need to know about the Flint water crisis | CTV News
6. Nerdfighteria Wiki - The Science of Flint's Water Crisis
7. Heavy Metals - Lenntech
8. Heavy Metals - Lenntech
9. Arsenic: A Murderous History | Dartmouth Toxic Metals
10. Marsh test - Wikipedia
11. Lewisite - Wikipedia
12. Fowler — Toxipedia
13. Homicidal arsenic poisoning
14. Levels of heavy metals in wastewater and soil samples from open drainage channels in Nairobi, Kenya: community health implication | Scientific Reports
15. Mercury and Human Health - Canada.ca
16. 1. How does mercury affect our health?
17. Metabolic Effects of Toxic Metals
18. Lead, mercury and cadmium concentrations in Canadians, 2012 and 2013
19. Human Exposure and Health Effects of Inorganic and Elemental Mercury
20. Brief guide to analytical methods for measuring lead in blood
21. TOXICOLOGICAL PROFILE FOR ARSENIC
22. Evaluation of some heavy metals concentration in body fluids of metal workers in Kano metropolis, Nigeria
23. Human Exposure and Health Effects of Inorganic and Elemental Mercury
24. How Pathogens Penetrate the Blood-Brain Barrier
25. Heavy metal pollution in the environment and their toxicological effects on humans - ScienceDirect
26. CDC Lead Biomonitoring Summary
27. 100002351830 (Re), 2016 CanLII 34067 (CA VRAB)