CHEMICAL AND BIOCHEMICAL TOXICOLOGY

INTRODUCTION

What is toxicity?
   \textit{Answer:} Any adverse effect

Definition of the Canadian Environmental Protection Act:

- a \textbf{substance} (note, this implies a chemical material) is toxic if it is entering the environment under conditions that, or in quantities that:
  - \textit{S} can harm human health
  - \textit{S} can harm the environment
  - \textit{S} can harm the environment upon which human life depends

Chemical substances depend for their potency on many factors, including:

- water \textit{vs} lipid solubility
- charge (acid-base) properties
- ease of uptake and excretion
- rates and products of metabolism

\textit{This section of the course is intentionally built on concepts taught in first-year chemistry}
CHEMICAL AND BIOCHEMICAL TOXICOLOGY

Issues:
- qualitative and quantitative consequences of exposure
- risk assessment, safe exposure levels, and regulatory toxicology
- mechanism of toxic action
- chemical analysis and bioassays of toxicants

Definitions:
- toxicant
- toxin
- xenobiotic
- target (non-target) organism

Development of toxicity testing:
- 1800s copper arsenate used as green colouring agent for candies
- 1920s Butter Yellow used to colour butter (toxic, carcinogenic): establishment of US Food and Drug Administration
- 1960s Thalidomide: teratogenicity testing
- 1970s Organochlorines in the environment: persistent pesticides and PCBs; testing for persistence
- 1990s Environmental hormone mimics: testing programs for industrial chemicals

Not all toxic substances are anthropogenic
Natural toxins:

LYSERGIC ACID
ANAGYRINE
SAFROLE

ergotism (mouldy rye) lupine (teratogen) saffron (carcinogen)

Why organisms create toxins

- predation: snakes
- defence: plants, hornets
- competition for resources: \textit{Penicillium} sp.

In many cases, the reason is unknown, and toxicity could be incidental
Criteria for toxicity  How do we know whether:

- smoking can cause lung cancer (is that the same statement as “smoking causes cancer”?)
- sun exposure can cause skin cancer
- silicone breast implants can cause autoimmune disease
- thalidomide can cause birth defects

Bradford-Hill criteria for toxicity:
1. Strength of association (the relative risk)
2. Temporality (did exposure to X always precede effect Y?)
3. Consistency (does exposure to substance X always yield adverse effect Y?)
4. Specificity (does exposure to substance X always yield the same adverse effect Y?)
5. Exposure/response (did the severity or the percentage incidence of the response increase with increasing exposure?)
6. Biological plausibility (is there a reasonable mechanism by which agent X could have produced effect Y?)
7. Experimental evidence (toxicological experiments, often in model systems, to demonstrate the toxic effect and to support the proposed mechanism)

- Only the last criterion involves toxicological experimentation
- Complementarity of epidemiological and toxicological studies
Fundamental laws of toxicology

1. no toxicity without exposure

2. the dose makes the poison (Paraselsus)

Item (2) has the following implications:

- “non-toxic”, slightly toxic”, “very toxic” etc just mean that the dose required before an adverse effect is seen is respectively very large, moderate, and small
- dose-response behaviour should be observed

---

Dose response curves. A, human response to ethanol as a function of dose; B, percentage of mouse pups with cleft palate as a function of the maternal dose of 2,3,7,8-TCDD.
Zero exposure: a reasonable goal?
• modern analysis allows you to find almost any analyte in almost any matrix

But is it necessarily toxic?

• the smallest amount of dioxin in your diet could be deadly (?)
• fluoride is a rat poison so fluoridation of water should not be allowed (?)

Interaction between:
  Dose
  Response
  Extrapolation

CHEMICAL PROPERTIES RELATED TO TOXICITY

Lipophilicity vs hydrophilicity determines where a substance will preferentially partition

more lipophilic less lipophilic reason
$\text{CH}_3(\text{CH}_2\text{CH}_2)_4\text{CH}_2\text{OH}$ $\text{CH}_3\text{CH}_2\text{OH}$ longer alkyl chain
$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ $\text{CH}_3\text{CH}_2\text{OCH}_2\text{CH}_3$ no oxygen atom
$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+$ no charge

Some lipophilic pesticides

Chlordane (insecticide) Chlornitrofen (herbicide)
Partition Constant:

\[ K_{\text{part}} = \frac{\text{concentration of solute in solvent 1}}{\text{concentration of solute in solvent 2}} \]

Relevance:
- solvent extraction
- uptake and excretion of toxicants
- bioconcentration/biomagnification

Octanol as Solvent 1: the octanol-water partition coefficient

\[ K_{\text{ow}} = \frac{\text{concentration of solute in octanol}}{\text{concentration of solute in water}} \]

Environment Canada guideline: \( K_{\text{ow}} > 1000 \), likely to bioconcentrate

Relationship between \( K_{\text{ow}} \) and bioconcentration factor (BCF)

\[ \text{BCF} = \frac{\text{concentration of toxicant in aquatic organism}}{\text{concentration of toxicant in surrounding water}} \]

\[ \text{BCF} = K_{\text{part}} \times \% \text{ by weight of fat in the organism} \]

If octanol is a good model for fat:

\[ \text{BCF} = K_{\text{ow}} \times \% \text{ by weight of fat in the organism} \]

If fat is \(~ 5\%) \text{ of wet weight:} \quad \text{BCF} \sim 0.05 \times K_{\text{ow}} \]
Biomagnification:

- DDT in the Great Lakes back in the 1970s

<table>
<thead>
<tr>
<th>Source</th>
<th>[DDT] (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (L. Ontario)</td>
<td>0.03</td>
</tr>
<tr>
<td>Sediment</td>
<td>27</td>
</tr>
<tr>
<td>Plankton</td>
<td>400</td>
</tr>
<tr>
<td>Salmon tissue</td>
<td>8,000</td>
</tr>
<tr>
<td>Gull tissue</td>
<td>300,000</td>
</tr>
</tbody>
</table>

- PCBs in L. Ontario salmon (improvement due to phaseout)
Persistent organic pollutants (POPs)

Persistence:
- low chemical and metabolic reactivity
- often associated with halogenated compounds
- able to distribute globally if even slightly volatile
- accumulate in the polar regions

Canadian concern: Native Canadians and wildlife in the Arctic

Structure-activity relationships

Useful because they allow us to predict biological or toxicological properties of an uninvestigated substance from the known properties of related substances

- the >N-N=O functional group tends to confer carcinogenicity
- water solubilities of alkanes decrease with chain length; $K_{ow}$ values increase with chain length. $K_{ow}$ trend is opposite to water solubility trend
- graphical relationship between BCF and $K_{ow}$ allows an estimation of BCF just by measuring $K_{ow}$, provided that $K_{ow}$ and BCF values have already been determined for structurally related compounds
- structure-activity relationship for dioxin lethality (guinea pig)

<table>
<thead>
<tr>
<th>Cl subst.</th>
<th>Rel. potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,8</td>
<td>$3 \times 10^{-6}$</td>
</tr>
<tr>
<td>2,3,7</td>
<td>$3 \times 10^{-5}$</td>
</tr>
<tr>
<td>2,3,7,8</td>
<td>1</td>
</tr>
<tr>
<td>1,3,6,8</td>
<td>$6 \times 10^{-7}$</td>
</tr>
<tr>
<td>1,2,3,7,8</td>
<td>0.3</td>
</tr>
<tr>
<td>1,2,4,7,8</td>
<td>$9 \times 10^{-4}$</td>
</tr>
<tr>
<td>1,2,3,4,7,8</td>
<td>0.014</td>
</tr>
</tbody>
</table>
Pharmacokinetics and Toxicokinetics

Example:
1. $c_{(aq)} \rightarrow c_{(fish)} \quad k_1$
2. $c_{(fish)} \rightarrow c_{(aq)} \quad k_2$
3. $c_{(fish)} \rightarrow \text{metabolic products} \quad k_3$

In environmental toxicology, processes 2+3 are called **depuration**

\[
\begin{align*}
\text{rate}[1] &= k_1 . c_{(aq)} \\
\text{rate}[2] &= k_2 . c_{(fish)} \\
\text{rate}[3] &= k_3 . c_{(fish)}
\end{align*}
\]

(1) \[
\frac{dc_{(fish)}}{dt} = \text{rate}[1] - \text{rate}[2] - \text{rate}[3]
\]

\[
= k_1 . c_{(aq)} - (k_2 + k_3) . c_{(fish)}
\]

At the steady state:

(2) \[
k_1 . c_{(aq)} = (k_2 + k_3) . c_{(fish, equilib)}
\]

\[
\text{BCF} = \frac{c_{(fish, equilib)}}{c_{(aq)}} = \frac{k_1}{(k_2 + k_3)}
\]

- If the steady state has not been reached, eq. (1) must be integrated (in notes, but not tested in TOX 2000).
- A measured BCF does not necessarily represent equilibrium (example: organochlorines in Great Lakes fish)
Bioconcentration is always associated with a low rate of depuration:

\[ \text{rate[1]} \gg \{ \text{rate[2]} + \text{rate[3]} \} \]

A: Uptake and clearance of 1,3,6,8-tetrachlorodibenzo-\(p\)-dioxin by juvenile trout in water; B: uptake of mercury for different levels of mercury in the diet.
Extraction of acid-base substances from water

*Remember that you cannot extract ionic or hydrophilic solutes out of water into an organic solvent or across a lipid bilayer membrane*

**Carboxylic acid**, $\text{RCO}_2\text{H}$, $K_a \approx 10^{-5}$; $pK_a \approx 5$

- In acidic solution, pH $< 5$ exists as $\text{RCO}_2\text{H}$, non-ionic, extractable from water
- In alkaline solution, exists as $\text{RCO}_2^-$, ionic, not extractable from water

**Aliphatic amine** $\text{RNH}_2$, $K_b \approx 10^{-4}$; $pK_b \approx 4$

$pK_a$ of $\text{RNH}_3^+ \approx 10$

- pH $< 10$, $\text{RNH}_3^+$, ionic, not extractable from water
- pH $> 10$, $\text{RNH}_2$, non-ionic, extractable from water

<table>
<thead>
<tr>
<th>Functionality</th>
<th>approx. $pK_a^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-COOH</td>
<td>4</td>
</tr>
<tr>
<td>aryl-$\text{NH}_3^+$</td>
<td>4</td>
</tr>
<tr>
<td>phenolic O-H</td>
<td>10</td>
</tr>
<tr>
<td>alkyl-$\text{NH}_3^+$</td>
<td>10</td>
</tr>
<tr>
<td>alkanethiol (S-H)</td>
<td>10</td>
</tr>
</tbody>
</table>

*modified by electron withdrawing or electron donating substituents. Electron-withdrawing groups (halogens, $\text{NO}_2$, and carbonyl functions) increase the acidity of acids and reduce the basicity of bases.*
Speciation diagrams

Speciation diagram for acetic acid

- Relate diagram to Henderson-Hasselbalch equation

\[ \text{pH} = \text{pK}_a + \log\left\{\frac{[\text{basic conjugate}]}{[\text{acidic conjugate}]}\right\} \]

In bodily fluids:
- blood has pH ~ 7.4
- stomach is acidic pH 1-2
- small intestine is slightly basic pH 8-9
- urine varies, but usual pH 5-7

- Drugs that are simple carboxylic acids have pK$_a$ ~ 5
- Drugs that are aliphatic amines have pK$_a$ of their conjugate acids ~ 10
Examples:
1. Aspirin, \( \text{HC}_9\text{H}_7\text{O}_4 \), \( pK_a \approx 3.5 \). Could be absorbed from the stomach (pH \( \approx 1 \)), but not from the small intestine (pH > 9; exists as \( \text{C}_9\text{H}_7\text{O}_4^- \)). Extraction into a non-polar solvent is only possible at pH < \( pK_a \), when Aspirin exists in the molecular (non-ionic) form \( \text{HC}_9\text{H}_7\text{O}_4 \). pH is very important in determining the possible site of absorption of drugs carrying acidic or basic functional groups.

   ![Aspirin structure](image)

2. Pentachlorophenol, \( \text{C}_6\text{Cl}_5\text{OH} \), \( pK_a \approx 5.5 \). Toxic material used for wood-preserving. Not extracted from water at pH > 5.5; at this pH \( \rightarrow \text{C}_6\text{Cl}_5\text{O}^- \). If ingested, excreted in the anion form and exists in blood in anion form.

   ![Pentachlorophenol structure](image)
### QUANTITATIVE ASPECTS OF TOXICOLOGY

**THE LD<sub>50</sub> TEST**

<table>
<thead>
<tr>
<th>Substance</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;, mg/kg</th>
<th>Substance</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>3 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>alcohol</td>
<td>1.4 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salt</td>
<td>4 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>malathion</td>
<td>1.4 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aspirin</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2,4-D</td>
<td>4 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>ammonia</td>
<td>3.5 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>caffeine</td>
<td>1.3 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>DDT</td>
<td>1 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>arsenic</td>
<td>50</td>
</tr>
<tr>
<td>strychnine</td>
<td>2</td>
<td>nicotine</td>
<td>1</td>
</tr>
<tr>
<td>dioxin</td>
<td>1 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>botulinum toxin</td>
<td>1 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

What dose of DDT would be likely to be lethal to half of a group of 200 g rats?

### Qualitative descriptors of the magnitude of the LD<sub>50</sub>

<table>
<thead>
<tr>
<th>LD&lt;sub&gt;50&lt;/sub&gt;, mg/kg</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5</td>
<td>Supertoxic</td>
</tr>
<tr>
<td>5-50</td>
<td>Extremely toxic</td>
</tr>
<tr>
<td>50-500</td>
<td>Highly toxic</td>
</tr>
<tr>
<td>500-5000</td>
<td>Moderately toxic</td>
</tr>
<tr>
<td>5000-15,000</td>
<td>Slightly toxic</td>
</tr>
<tr>
<td>&gt; 15,000</td>
<td>Practically nontoxic</td>
</tr>
</tbody>
</table>

These terms are largely meaningless, because they refer *only* to lethality.
Effect of different concentrations of nicotine sulphate (in 1% saponin solution) on the common fruit fly

<table>
<thead>
<tr>
<th>Conc (mg/10 cm³)</th>
<th>No.of Insects</th>
<th>Number Killed</th>
<th>Percent Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>47</td>
<td>8</td>
<td>17.0</td>
</tr>
<tr>
<td>15</td>
<td>53</td>
<td>14</td>
<td>26.4</td>
</tr>
<tr>
<td>20</td>
<td>55</td>
<td>24</td>
<td>43.6</td>
</tr>
<tr>
<td>30</td>
<td>52</td>
<td>32</td>
<td>61.5</td>
</tr>
<tr>
<td>50</td>
<td>46</td>
<td>38</td>
<td>82.6</td>
</tr>
<tr>
<td>70</td>
<td>54</td>
<td>50</td>
<td>92.6</td>
</tr>
<tr>
<td>95</td>
<td>52</td>
<td>50</td>
<td>96.2</td>
</tr>
</tbody>
</table>

- a nice (and incidentally synthetic) data set
Linearizing the data:

- The X variable (d) is transformed into $\log_{10}(d)$
- The Y variable (p) is transformed into probit of p

Step 1: transform dose into $\log_{10}(d)$
Step 2: transform percent killed (p) into probit of p
Step 3: linear regression between X and Y gives the least squares regression line: $Y = a + bX$
Step 4: from the regression equation, substitute $Y = 5.0000$ and solve for $\log_{10}(d)$: $X = (5-a)/b$
Step 5: $LD_{50} = 10^X$

Probit line for previous data set:

$$\hat{Y} = 1.0801 + 2.8776X$$

To estimate $LD_{50}$ from the regression line:
\[
X = \frac{(5-a)}{b} = \frac{(5 - 1.0801)}{2.8776} = 1.3622
\]

Hence: \( \text{LD}_{50} = \text{antilog} (X) = 10^{1.3622} = 23 \text{ mg/10 mL} \)

This methodology can be used for most type of toxicological analysis where the phenomenon is **saturable**

*Are relative potencies always the same across the dose range?*

No; consider the curves below, that (happen) to represent equal potencies at the \( \text{LD}_{50} \):

![Graph showing two lines representing different potencies at different concentration levels](image-url)

Toxicant “A” is more potent at high concentration; “B” is more potent at low concentration

**Limitations of \( \text{LD}_{50} \) and \( \text{LC}_{50} \) tests**

1. Values obtained in one species cannot be directly
translated into other species. Example: LD$_{50}$ values of dioxin in mammals. On the basis of LD$_{50}$ dioxin is $10^3 \times$ times more potent towards guinea pigs than hamsters (both rodents).

<table>
<thead>
<tr>
<th>Species</th>
<th>LD$_{50}$, µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>guinea pig (♂)</td>
<td>1</td>
</tr>
<tr>
<td>rat (♂)</td>
<td>22</td>
</tr>
<tr>
<td>hamster</td>
<td>1100-5000</td>
</tr>
<tr>
<td>rabbit</td>
<td>115</td>
</tr>
<tr>
<td>guinea pig (♀)</td>
<td>2</td>
</tr>
<tr>
<td>rat (♀)</td>
<td>50-500</td>
</tr>
<tr>
<td>frog</td>
<td>1000</td>
</tr>
<tr>
<td>monkey</td>
<td>70</td>
</tr>
</tbody>
</table>

Remember that differential toxicity between species is what is exploited when developing biocides that are selective between target and non-target organisms.

2. Lethality is rarely an appropriate toxic endpoint to consider. But ..... you can use measures such as LD$_5$ in population toxicology to infer concentrations/doses that might be close to the NOEL

3. Modern trend in toxicology is to minimize suffering of animals. But .... some animal testing is still required by regulators
Risk and exposure

- is the substance inherently toxic? How potent?
- what is the exposure?

\[ \text{Risk} = f(\text{potency}) \times f(\text{exposure}) \]

*Example:* deaths from alcohol, dioxin and botulinum toxin

- risk benefit analysis (risk or benefit to whom?)
- the 1 in \(10^6\) criterion
- voluntary and involuntary risks

Occupational exposures

- intended to protect the healthy worker for exposure over a normal work week: threshold limit values

| Sample time-weighted TLVs, in mg m\(^{-3}\) |
|-----------------|-----------------|-----------------|
| acetone         | 1780            | CO\(_2\)         | 9000            |
| chlorine gas    | 1.5             | HCl              | 7               |
| mercury         | 0.05            | cement dust      | 10              |
Epidemiology: study of human or other populations comparing exposed (treated) vs. unexposed (control) groups

How do we know whether exposure really makes a difference (in disease incidence, for example), and whether the conclusion is statistically significant?

Synthetic data set: 1000 randomly selected individuals, 300 smokers, 700 non-smokers. 10 of the 300 smokers suffered from thromboembolism, and 8 of the 700 non-smokers.

<table>
<thead>
<tr>
<th>Table A</th>
<th>Smokers</th>
<th>Non-smokers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboembolism</td>
<td>10</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Unaffected</td>
<td>290</td>
<td>692</td>
<td>982</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>700</td>
<td>1000</td>
</tr>
</tbody>
</table>

Relative Risk (RR): probability that a given exposure will lead to toxicity compared with the probability that toxicity will occur in the absence of exposure.

\[
RR = \frac{\text{probability of disease in smokers}}{\text{probability of disease in non-smokers}}
\]

\[
= \frac{10/300}{8/700} = 2.92
\]

Smokers 2.92 times more likely to contract the disease
Odds Ratio (OR) is the ratio of the "odds" of toxicity in the exposed group to the odds of toxicity in the unexposed group.

\[
OR = \frac{\text{odds of disease in smokers}}{\text{odds of disease in non-smokers}} = \frac{10/290}{8/692} = 2.98
\]

The odds ratio and the relative risk are similar in magnitude, but only when the condition under study is relatively rare (say, <10% in either the exposed or the unexposed group).

Relative Risk (RR) is more interesting, but OR has a readily calculated 95% confidence interval (95% CI): approximately 2 standard deviations

95% CI means that if the experiment were repeated many times, OR should fall within this range 19 times out of 20

**Big question:** does the 95% CI include the value unity?

Hypothetical statistics for cancer incidence in workers exposed to two industrial chemicals, compared with unexposed controls.

**Compound A:** Odds ratio 1.79 (95% CI 1.24-3.27)

**Compound B:** Odds ratio 5.87 (95% CI 0.91-19.4)

- "With epidemiology you can tell a little thing from a big thing. What's very hard is to tell a little thing from nothing at all"  Michael Thun, Director of Epidemiology, American Cancer Society

- "No single epidemiological study is persuasive by itself unless the lower limit of its 95% CI falls above a threefold risk"  Richard Doll, Oxford University

- "... we (should be) looking for a RR of 3 or more, plus biological plausibility ..."  Marcia Angell, New England J. Med.
*Examples of large RR:* condition is very rare in the absence of exposure

- Bladder cancer among dye industry workers
- Mesothelioma among asbestos workers
- Liver cancer among workers exposed to vinyl chloride
- Sterility among male workers exposed to dibromoethane (soil fumigant)
- Phocomelia (limb foreshortening) among children whose mothers had taken Thalidomide

*Examples where epidemiology does not give clear-cut answers:* significant background incidence, low RR

- Do children born to mothers with high body burdens of PCBs have mental deficits?
- Do silicone breast implants cause autoimmune disease?
- Does living near a nuclear power plant expose children to a greater risk of leukemia?
- Does aluminum in the water supply predispose people to Alzheimer’s disease?

Even:

- does smoking increase the risk of lung cancer?
Breast implant controversy

- 1988: US FDA gains authority of regulate “devices”, orders additional testing by manufacturers

- by 1990, ~1 million American women had used these devices

- 1991: litigation against implant manufacturers

- 1992: “temporary” FDA ban → many new law suits; no epidemiological evidence

- 1993: Dow-Corning declares bankruptcy

- 1993: Case control study, one US county: 794 with implants, 1498 controls. RR = 1.06 (95% CI 0.34-2.97)

- 1994: Cohort study, 87,501 US nurses, 1183 with implants. RR = 0.6 (all types of implant, 95% CI 0.2-2.0); RR = 0.3 (silicone implants, 95% CI 0-1.9)

- 1994: Self-reported study, 395,543 US women, 11,805 with implants: RR = 1.24 (95% CI 1.08-1.41).

- 1999: Scientific “expert panel” headed by Dr. Nancy Kerkvliet of Oregon State University, “... no evidence that silicone breast implants precipitate novel immune responses or induce systemic inflammation”.
Cancer rates: overall incidence vs age-adjusted mortality

Figure 2.1
New Cases and Age-Standardized Incidence Rates (ASIR) for All Cancers, Canada, 1970-1999

Note: All cancers exclude non-melanoma skin cancer (ICD-9 173). Rates are standardized to the 1991 Canadian population.
Source: Cancer Bureau, LCDC, Health Canada

National Cancer Institute of Canada: Canadian Cancer Statistics 1999
Figure 3.2
Age-Standardized Mortality Rates (ASMR) for Selected Cancer Sites, Males, Canada, 1970-1999

Note: Rates are standardized to the age distribution of the 1991 Canadian population. See Table 7.2 for data points.
Source: Cancer Bureau, LCDC, Health Canada.

National Cancer Institute of Canada: Canadian Cancer Statistics 1999
Figure 4.2
Age-Standardized Mortality Rates (ASMR) for Selected Cancer Sites, Females, Canada, 1970-1999

Note: Rates are standardized to the age distribution of the 1991 Canadian population. See Table 8.2 for data points.
Source: Cancer Bureau, LCDC, Health Canada

National Cancer Institute of Canada: Canadian Cancer Statistics 1999
The problem of analysis in toxicology

- separation of the toxicant from the matrix
- identification of the toxicant
- quantitation of the toxicant

Case Study: Domoic acid (Atlantic provinces, 1987)

- Symptoms: vomiting, diarrhoea, ... memory loss and coma, suggested food poisoning. Chemical poisoning? Infectious disease? Natural toxin?
- Health and Welfare Canada linked the illness to eating blue mussels from PEI.
- Mouse assay (injection of mussel extract into mice) showed that mussels from one area of PEI contained a “paralytic shellfish toxin”. No evidence of heavy metals, pesticides and other possible xenobiotics.
- NRC’s Halifax laboratory fractionated the mussel extract, tested each fraction with the mouse bioassay. One aqueous sub-fraction showed high toxicity: HPLC peak not present in control mussel tissue.
- GC-MS – molar mass 312 g mol\(^{-1}\), \(C_{15}H_{22}NO_6\). Library searching of previously known compounds (Chemical Abstracts Service, Columbus, Ohio) suggested domoic acid as a possible match, confirmed by nuclear magnetic resonance spectroscopy.

\[
\text{CH}_3
\]
\[
\text{CH}_3
\]
\[
\text{CO}_2\text{H}
\]
\[
\text{CO}_2\text{H}
\]
\[
\text{N}
\]
\[
\text{H}
\]

\[
\text{H}
\]
\[
\text{H}
\]
\[
\text{CH}_3
\]
\[
\text{CO}_2\text{H}
\]

\[
\text{CH}_3
\]

[Diagram of the domoic acid molecule]
Radiation toxicology

- ionizing vs. non-ionizing radiation. Ionizing radiation can cleave chemical bonds, often with the formation of ions, radicals and solvated electrons
- α, β, γ radiation associated with nuclear decays: far more energetic than chemical reactions: 1 MeV ≈ 10^8 kJ mol\(^{-1}\) (chemical bonds <10^3 kJ mol\(^{-1}\))
  \(\alpha\) = bare He nucleus;
  \(\beta\) = energetic electron;
  \(\gamma\) = high energy electromagnetic radiation
- natural and anthropogenic sources of ionizing radiation
- main interaction in biological tissue is with water:

\[
\text{radiation} + \text{H}_2\text{O} \rightarrow \text{H}^+(\text{aq}) + \text{OH}^-(\text{aq}) + \text{e}^-\text{(aq)}
\]

- the hydroxyl radical (OH\(^-\)) is particularly damaging

\[
\text{OH}^- + \text{R-H} \rightarrow \text{R}^- + \text{H}_2\text{O} \quad \text{(RH = a lipid molecule or component of a lipid bilayer)}
\]

\[
\begin{align*}
\text{H-Ö}^- & \quad \text{H-Ö:} \\
\text{hydroxyl radical} & \quad \text{hydroxide ion}
\end{align*}
\]
**Units of radioactivity**

Number of radioactive disintegrations occurring per unit time. Each event represents the disintegration of a single atom.

1 becquerel (Bq) = 1 disintegration per second

1 curie = $3.7 \times 10^{10}$ Bq (old unit)

1 gray (Gy) = 1 J kg$^{-1}$ of the absorbing medium

A dose of ~4 Gy is lethal within days, based on actual data involving people exposed to nuclear bombs in Japan, in 1945. Lower doses are associated with cancer, with no particular target organ predominating.

For tissues:

1 sievert (Sv) = 1 J kg$^{-1}$ of tissue

Bodily organs vary in their sensitivity to radiation, so weighting factors are applied (whole body = 1.00):

**Effective Dose = Actual Dose × Weighting Factor**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>Gonads</td>
</tr>
<tr>
<td>0.12</td>
<td>Bone marrow, Colon, Lung, Stomach</td>
</tr>
<tr>
<td>0.05</td>
<td>Bladder, Breast, Liver, Oesophagus, Thyroid</td>
</tr>
<tr>
<td>0.01</td>
<td>Skin, Bone surface</td>
</tr>
<tr>
<td>0.05</td>
<td>Remainder</td>
</tr>
</tbody>
</table>
Cancer risk from radiation
Calculated risk of cancer: 1 Sv → 0.05 risk of fatality (cumulative)

Natural exposure to radiation: cosmic rays, natural radioisotopes such as $^{14}$C: ~ 2 mSv/year

Maximum effective dose rates: International Commission on Radiological Protection, with the objective of minimizing the risk of exposure to radiation.

General Public 1 mSv per year
Radiation workers 20 mSv per year (5 year average)
Pregnant Workers 0.05 mSv during confirmed pregnancy

Total risk of death from cancer in Ontario: 0.25

Compare this with risks of 1 in $10^6$ lifetimes for chemical carcinogens

Radon
• natural radioelement from decay of natural uranium
• most important isotope $^{222}$Rn, $t_{1/2} = 3.8$ days, $d = 9.7 \text{ g L}^{-1}$
• “action levels”: 4 pCi L$^{-1}$ (USA) 20 pCi L$^{-1}$ (Canada)
• no clear correlation between areas of high natural Rn and lung cancer rates
Non-ionizing electromagnetic radiation (UV)

Wavelength ranges and energies ($E = \frac{hc}{\lambda}$)

<table>
<thead>
<tr>
<th>Wavelength range (nm)</th>
<th>Region</th>
<th>Energy (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200-300</td>
<td>UV-C</td>
<td>600-400</td>
</tr>
<tr>
<td>300-325</td>
<td>UV-B</td>
<td>400-370</td>
</tr>
<tr>
<td>325-400</td>
<td>UV-A</td>
<td>370-300</td>
</tr>
<tr>
<td>400-700</td>
<td>visible</td>
<td>300-170</td>
</tr>
</tbody>
</table>
White male deaths from melanoma: US states

- Interpretation: higher sun exposure at lower latitudes
Mechanistic toxicology

- step by step understanding of the events leading from exposure to clinical signs of toxicity
- all biochemical processes are catalyzed by enzymes, but must obey normal chemical rules
- can we identify the critical target molecule with which the toxicant interacts?

Review from Biomedical section of the course

- exposure → ingestion
- distribution to target tissue
- metabolism, which may involve detoxification or bioactivation
- elimination or storage (storage implies bioaccumulation)

Two well-understood examples: Fluoroacetate and CO

Fluoroacetate ion $FCH_2CO_2^-$

- highly toxic $LD_{50}$ (rat) 0.2 mg/kg, human lethal dose 2-5 mg/kg
- symptoms: nausea, abdominal pain, seizures, coma. No known antidote
- considered as chemical warfare agent WWII but not used
- substitutes for acetate, $CH_3CO_2^-$. Target molecule is an enzyme, aconitase
- aconitase converts citrate ion to isocitrate and inhibits glucose metabolism via the citric acid cycle
Acetate $\rightarrow$ Citrate $\rightarrow$ Aconitate $\rightarrow$ Isocitrate $\rightarrow$ Acetate

Citrate $\rightarrow$ Aconitate $\rightarrow$ Isocitrate $\rightarrow$ Citrate
**Carbon monoxide**

- high affinity for hemoglobin: prevents $O_2$ uptake and distribution to the tissues
  
  \[
  -\text{CO} \quad +\text{O}_2 \\
  \text{Hb-CO} \quad \rightleftharpoons \quad \text{Hb} \quad \rightleftharpoons \quad \text{Hb-O}_2
  \]

- CO also increases the affinity of $O_2$ for hemoglobin; this makes it more difficult for a partly carboxylated hemoglobin to release $O_2$ in the peripheral tissues. As a result, 50% $O_2$ occupancy due to low $p(O_2)$ is almost without effect on a resting healthy adult, whereas 50% $O_2$ occupancy due to CO occupying the other sites brings the patient close to collapse.
Metabolism of lipophilic xenobiotics: the Phase I/Phase II system

- a strategy for solubilization
- Phase I: introducing or (less commonly unmasking) a reactive function group, often -OH or epoxide
- Phase II: conjugating a water soluble moiety to ready the xenobiotic for excretion. Common moieties are sugars, peptides or sulfate
- Phases I and II are enzyme-catalyzed reactions

role of the Cytochrome P-450 enzymes in Phase I biochemistry: iron-containing enzymes called monooxygenases

\[ \text{P-450} \]
\[ R-H + O_2 + 2[H] \quad \rightarrow \quad R-OH + H_2O \]

Bioactivation vs detoxification
• these represent the same Phase I process. The biological outcome is what varies.

substrate
\[\downarrow\]
Phase I product
\[\downarrow\]
Phase II product
\[\downarrow\]
urinary excretion

• increased toxicity (bioactivation) occurs if the Phase I product is very reactive and can attack protein, DNA, or other cellular macromolecular target. Biological effects generally follow linking the activated xenobiotic to cellular macromolecules.

• DNA damage: unless repaired may lead to cell death, cancer (damage to somatic cells) or heritable mutation (damage to germ cells)

• protein damage: homeostasis upset, but reversible if/when new protein is synthesized

• Example (not in notes): parathion (insecticide) is changed by Phase I oxidation into paraoxon, which is the active toxicant (inactivates acetylcholinesterase)

\[
\begin{align*}
\text{NO}_2 & \quad \text{P-450} \\
\text{OP}[-\text{OCH}_2\text{CH}_3]_2 \quad & \rightarrow \\
\text{NO}_2 & \quad \text{OP}[-\text{OCH}_2\text{CH}_3]_2
\end{align*}
\]

\textbf{Acetaminophen} (trade name Tylenol)
• very safe drug (high therapeutic index), but at high doses can cause liver necrosis

< 24 h  anorexia, vomiting, flu-like lethargy
24-72 h  patient feels better, but abnormal hepatic chemistry such as elevated bilirubin levels
3-5 days  hepatic necrosis and death in severe cases; peak of abnormal hepatic chemistry in sublethal cases
7-8 days  return to normal health, with no long term effects

• Un-metabolized acetaminophen can conjugate with glucuronic acid or with sulfate
• Phase I oxidation competes with conjugation; more Phase I reaction if:
  • stores of conjugating agents are depleted
  • P-450 system has been induced (alcoholics)
• Phase II conjugation competes with attack on cellular macromolecules, but is less effective if stores of glutathione (conjugating agent) are depleted

• Alcoholics induce Cytochrome P-450 2E1, which happens also to oxidize acetaminophen. [This is not the normal route for ethanol oxidation]

• 10 g (20 extra strength Tylenol): toxicity in normal adult
• 15 g (30 extra strength Tylenol): minimum lethal dose in normal adult
• 4 g (8 extra strength Tylenol): toxicity possible in severe alcoholic

• Doctors who are aware that their patients are alcoholics may have to adjust doses (up or down, depending on the biochemistry) of pharmaceutical drugs for these patients
Bioactivation by way of epoxides

Epoxides are reactive; they are deactivated by hydrolysis catalyzed by epoxide hydrolase (EH)

Epoxides also undergo (non-catalytic) ring opening with nucleophiles

$X^-$ can be an -NH$_2$ or similar group, as in a protein or DNA molecule. This allows the activated substrate to become covalently bonded to cellular macromolecules competitively with detoxification

Covalent bonding to DNA changes its shape (distorts the helix). If unrepaired, mutations may occur at the time of cell division
Example: Aflatoxin attack by cytochrome P-450

- aflatoxin causes primarily liver damage because this is the organ with the highest P-450 levels

- LD$_{50}$ data:
  - Guinea pig 1.4 mg/kg
  - Rat 8
  - Mouse $>150$
  - Chicken 15
  - Rabbit, duck, trout all <1

- organisms with high LD$_{50}$ (less susceptible) have higher levels of Phase II conjugating enzymes
Polycyclic aromatic hydrocarbons (PAHs)

- dibenz[a,h]anthracene the first chemical carcinogen identified
- DNA adducts first identified with benzo[a]pyrene
- PAHs are unreactive: require bioactivation, which is catalyzed by cytochrome P-450 1A1

Bioactivation of B[a]P is a four step process:
- P-450 catalyzed oxidation of BaP to form an epoxide at the C(7)-C(8) bond
- Opening the epoxide by epoxide hydrolase to form the 7,8-diol
- A second P-450 catalyzed epoxidation at C(9)-C(10), yielding benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE)
- Nucleophilic attack on BPDE by DNA, selectively by an -NH₂ group on guanine, to form a DNA adduct, competitively with detoxification by epoxide hydrolase and conjugation
Because BPDE is the substance that actually reacts with DNA it is sometimes called the “ultimate carcinogen”. Pure P-450 EH

- BPDE is more potent than B[a]P
Cigarette smokers have higher concentrations of B[a]P metabolites in their urine and also higher concentrations of cytochrome P-450 1A enzymes, which are induced (up-regulated) by these PAHs.

No structure-activity relationship for PAHs can be discerned. Interplay of several factors:

- initial P-450 catalyzed oxidation occurs at several sites: BaP forms epoxides at C(1)-C(2), C(2)-C(3), C(4)-C(5), and C(9)-C(10) as well as at C(7)-C(8). There is also conversion of C-H to C-OH at C(6). Only the epoxide at C(7)-C(8) appears to lead to carcinogenesis.

![BaP molecule with labeled sites]

- yields of oxidation at each site vary, within and between PAHs. Some PAHs may give negligible or zero yields of carcinogenic epoxides
- The potencies of the epoxides will be determined by their relative rates of detoxification (epoxide hydrolase) vs. their reaction with DNA
- Different DNA adducts may have different potencies with respect to forming fixed mutations.

**EROD assay**: biomarker for exposure to P-450 1A inducers
reaction catalyzed by P-450 1A1
product resorufin is fluorescent
used for monitoring pulp and paper mill effluents

Two major applications of EROD assay:

- Determine whether the livers of exposed animals (fish, for example) have higher levels of P-450 1A1 than control animals. If so, their Cytochrome P-450 levels have been induced, a sign that exposure to the relevant xenobiotics has occurred.
- Take an extract of an environmental sample (soil, sediment, effluent) and incubate it with a culture of hepatic cells (usually an immortalized cell line). After 24 h, determine the EROD activity of the cells; again, the presence of the relevant xenobiotics is signalled by an increase in EROD activity beyond that of similar untreated cells.
Glyphosate: a herbicide (Roundup™)

- not P-450 activated
- interferes with a specific enzyme on the route to biosynthesis of aromatic amino acids (phenylalanine, tyrosine, tryptophan)
- negligible mammalian toxicity and very slight toxicity to fish → ideal herbicide, since it does not affect non-target organisms
- not lipophilic
- readily degraded → not persistent
- inhibits the enzyme 5-enolpyruvyl shikimic acid-3-phosphate synthase
**Roundup-ready® crops** (Monsanto)

Concept: crop is resistant to glyphosate (Roundup®), so the field may be sprayed with glyphosate, killing the weeds without harming the crop.

Two genetic strategies for achieving herbicide resistance could be envisaged:

- introduce a gene to promote the metabolism of glyphosate
- introduce a gene to overcome the inhibition of 5-enolpyruvyl shikimic acid-3-phosphate synthesis

Roundup ready crops employ strategy (2):

- the gene for a bacterial version of 5-enolpyruvyl shikimic acid-3-phosphate synthase is introduced into the crop genome (corn, soybeans, cotton etc)
- the bacterial enzyme is not inhibited by glyphosate
- hence the plant can synthesize aromatic aminoacids even in the presence of glyphosate
- no protein synthesis; no lignin synthesis
**Sulfonylureas:** inhibitors of the enzyme acetolactate synthase (ALS), which catalyzes a key step on the route to branched chain aminoacids such as valine, leucine and isoleucine

Numerous sulfonylureas have been introduced into agriculture in the last 20 years. Almost all have the general structure:

\[
\text{aryl-SO}_2\text{-NH-CO-NH-aryl}'
\]

in which aryl is a carbocyclic aromatic ring and aryl' is heterocyclic.

Like glyphosate, sulfonylureas inhibit a specific enzyme that is needed for the biosynthesis of aminoacid → no protein synthesis

\[
\begin{align*}
\text{NH}_2 & \quad \text{(CH}_3\text{)}_2\text{CH—CHCO}_2\text{H} \\
\text{(CH}_3\text{)}_2\text{CHCH}_2\text{—CHCO}_2\text{H} & \quad \text{CH}_3\text{CH}_2\text{CH(CH}_3\text{)—CHCO}_2\text{H}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CCO}_2^- & \quad \text{CH}_3\text{CCO}_2^- \quad \text{ALS} \\
\text{O} & \quad \text{O} \quad \text{CH}_3\text{CH}_2\text{CCO}_2^- \\
\alpha\text{-ketobutyrate} & \quad \text{pyruvate} \quad \alpha\text{-aceto-}\alpha\text{-hydroxybutyrate}
\end{align*}
\]
Sulfonylureas distinguish between weed and crop when the crop is able to detoxify the herbicide

\[
\text{Ar.SO}_2\text{NH.CO.NH} \quad \text{facile O-demethylation in rice}
\]

\[
\text{Ar.SO}_2\text{NH.CO.NH} \quad \text{O-hydroxylation in corn (maize)}
\]

\[
\text{Ar.SO}_2\text{NH.CO.NH} \quad \text{hydroxylation in this ring (cereals)}
\]

These are P-450 catalyzed processes

Comparison

**Roundup ready crops:** allow the crop to synthesize aromatic amino acids even in the presence of glyphosate

**Sulfonylureas:** protected crop plants selectively metabolize the herbicide