General aspects:
- Toxicological Risk: dose-response
- Toxicology vs. epidemiology
- Physiological effects on man
- Environmental effects

Prof. Attilio Citterio
Dipartimento CMIC “Giulio Natta”
http://iscamap.chem.polimi.it/citterio/education/course-topics/
• As of August 2011, ~30 million organic and inorganic substances were documented (indexed by the American Chemical Society’s Chemical Abstracts Service in their CAS Registry; excluding bio-sequences such as proteins and nucleotides)

• Of these chemicals, ~10 million were commercially available.
What does Toxicology Means?

- Evaluate the **HAZARD** (danger) of new/old substances
- Propose measures for preventing and contain the risk associated with the **EXPOSURE**
- Calculate the **RISK = f (hazard , exposure)**

E.g.

We handle (or we are exposed) to many chemicals in our everyday lives.

**Gasoline** is a good example. We know that it is toxic if ingested or inhaled, but if we take care to limit our exposure to it and handle it safely, we take little risk in using it.
Definitions

Hazard
The inherent toxicity of a substance, based on appropriate models (*in-vivo, in-vitro, in-silico*) or information from human epidemiologic studies

Exposure
- *Route of entry:*
  - Oral = Ingestion by mouth
  - Dermal = Skin exposure
  - Inhalation = Absorbed by lungs
  - Ocular = Eye exposure
- *Probability of contact;*
- Use of safety equipment (individual: e.g. gloves, collective: e.g. cabinet);
- ....

Risk
The probability of adverse effect due to exposure of a chemical substance IN PARTICULAR CIRCUMSTANCES
On 11 March 2013 the full ban on animal testing for cosmetic products within the European Union entered into force.
Legislation related to toxicity and risk of chemicals
http://www.hse.gov.uk/chemicals/index.htm
Example: Acrylamide vs. N-vinyl-Formamide

Polyacrylamide, used in papermaking, oil recovery, personal care, water treatment

Highly toxic, causes CNS paralysis  ~ $ 1/kg  capacity > 500,000 t/y  
MW = C₃H₅NO

Poly(N-vinyl formamide), many of the same uses, hydrolyzed to polyvinyl amine.

Acute oral, > 1400 mg/Kg, not a neurotoxin  ~ $ 4.50/kg
Two Alternative Acrylamide Synthesis

a) \[ \begin{align*} &+ \text{NH}_3 + 1.5\text{O}_2 \rightarrow \text{NH}_2\text{CN} + \text{H}_2\text{O} \\ &\text{(Cu catalyst or enzyme)} \end{align*} \]

b) \[ \begin{align*} &\text{H}_2\text{O} \rightarrow \text{NH}_2\text{C}=\text{O} \\ &\text{Cu catalyst or enzyme} \]

Enzymatic route newest, greenest approach

Process green, Product not
N-Vinylformamide Synthesis

HCN, an inherent hazard, raises costs

Product green, Process not
Essential versus Nonessential Nutrients

Essential elements are elements like nitrogen, phosphorus, magnesium and zinc. They are necessary to life, but they are also toxic at high concentrations.

Non-essential elements are elements like mercury, tin, arsenic, cadmium, boron, lithium and nickel. At lower concentrations they may be acceptable, but eventually they will become toxic.
Exposure to Exogenous Agents

- Hazard Identification
- Exposure Assessment
- Effects Assessment
- Dose-response
- Risk Characterization
Toxicity and Risk

Toxicity from Chemicals and Risk Assessment

Toxicity
Effects on Safety

Toxicological and epidemiological studies

Concentration in environment
Box Model

Risk assessment
Cost
Actions
Toxicity Risk Assessment

• Identify toxins

• Correlate entity of exposure to effects on health
  ▪ Evaluation dose-response
  ▪ Evaluation of exposure

• Evaluate the levels of acceptable risk
  ▪ Quantify dead/diseases per million

• Estimate cost of controls
  ▪ Economic costs of control vs. social costs or benefit
Health Effects of Pollutants

- **Acute Toxicity**: Short term exposure to high concentrations
- **Chronic Toxicity**: Long term exposure to low concentrations
  - Dose-response relationship
    - Threshold like
    - Non threshold like
• **Dose**: combination of environmental concentration and exposure time

Dose

- **Acute**
  - High concentration, Low exposure time

- **Chronic**
  - Low concentration, High exposure time

Effect
Exposure Time

It is NOT equal to the residence time in the box model!

**Exposition Time** ≡ time of contact with toxin

- **Acute**: short time exposure (normally high concentration)
- **Chronic**: long time exposure (normally low concentration). It is NOT equal to the residence time in the box model!

**Dose-response relationship:**

- Threshold type
- Non threshold type
**Toxicity Measure**

**LD$_{50}$**: the dose that produces 50% mortality in a test population.

*E.g. LD$_{50}$ can be expressed in milligrams of substance per kilogram of test animal body weight (mg/kg).*

**NOAEL**: No Observable Adverse Effect Level

**DNEL**: the Derived No Effect Level = \[
\frac{\text{NOAEL}}{\text{Assessment factors}}
\]

- **Human assessment**
- **Environmental assessment**

**ADI**, Acceptable Daily Intake
Population Dose-Response

Attilio Citterio

Many

Few

Number of individuals

Resistant individuals

Sensitive individuals

Minimal effect

Average effect

Majority of individuals

Maximal effect

Mild

Extreme

Response to SAME dose
Response Curve with and without Threshold

- **Sigmoid Response Curve**
- **Threshold**
- **Dose (intensity of stimulus)**
- **% of population/response**
- **(man damage)**

Response curve with threshold

Response curve without threshold
Dose-Response

Increasing Dose

- Interval without effect
- Interval where the effect increases
- Interval with maximum effect

Increasing Response

threshold

sigmoid

Casarett e Doull, Cap. 2, pp. 18-27
Timbrell Cap. 2 (pp. 7-25)
Exposure to Ozone (cat)

Difference in mortality of cats under ozone and control, %

Interpretation with threshold value

Interpretation without threshold

ozone concentration, ppm

0.0 0.1 0.2 0.3 0.4 0.5 0.6
Some Acronyms

- **NEL**, No Effects Level
- **NOEL**, No Observed Effects Level
- **NOAEL**, No Observed Adverse Effects Level
- **ADI**, Acceptable Daily Intake
  - AWI, AMI, etc.
- **TDI**, Tolerable Daily Intake
- **MCL**, Maximum Contaminant Level (SDWA)
- **SF, UF, MF**: safety, uncertainty, modifying factors
Pollutants and Effects

- Relation between high concentrations of pollutants and respiratory problems, *morbidity*
- Relation between high concentrations of pollutants and daily dead, *mortality*
- Relation between high concentrations of pollutants and mean life, *cancer risk*
- Risk population
Dose = C × V
- C: Pollutant concentration, mass/volume normally a time function
- V: volume of inhaled air
dV/dt, inhalation rate is function of the activity of the organism

Integrated Dose = ∫ C \left(\frac{dV}{dt}\right) dt

Exposure = ∫ C dt
Assessment of Effects on Health
Correlation between exposure and specific response

- Toxicological studies on animals
  - expose animals (cat, mouse) to pollutant of concern in controlled conditions and see the effects, then extrapolate the results to man

- Epidemiologic studies
  - analyze the effects on populations with similar characteristics unless exposition to pollutant of concern

- In vitro studies
  - verify on model system the relationship exposure/response

- In silico studies
  - predict the effects based on structural models
Toxicity Evaluation

*In-vivo models*

*In-vitro models*

*In-silico models*
“Toxicological” Studies

Clinical/laboratory experiments:

❖ Advantages
  • Controlled change of exposure
  • Controlled environment of individuals

❖ Disadvantages
  • Small samples
  • Short testing periods
  • Not humanoid subjects
Epidemiology Basics

**Measures of disease occurrence**

- *Cumulative Incidence*, CI, the proportion of healthy individuals who get the disease
  
  - CI = Number of individuals who get the disease during a period / number of individuals in the population

Disadvantages:

- Wide dimension of individuals sample
- Possible long term studies
- Absence of control on exposure and environment
- Unknown history, behavior of individuals
- Mobility of individuals
Toxic Effects

When toxic compounds enter the body, they may have a range of effects.

• At low levels, there may be no effect. This "threshold" may be considered a "safe" level of exposure.

• Chemicals may have acute effects, the result of a high dose in a short period of time. Most animal experiments are simple acute toxicity studies.

• Chemical may also have chronic effects, caused by a low-level dose over a long period
  • Acute / chronic
  • Reversible / irreversible
  • Immediate / delayed
  • Idiosyncratic - hypersensitivity
  • Local / systemic
  • Target organs
Acute mammalian toxicity criteria differentiate compounds based upon a common measure of short term exposure toxicity, the median lethal dose or concentration (LD$_{50}$ or LC$_{50}$), through oral, dermal, and respiratory routes. These values were derived from the GHS criteria [GHS, Chapter 3.1: Acute Toxicity. 2009, United Nations].

<table>
<thead>
<tr>
<th>Acute Mammalian Toxicity</th>
<th>Very high</th>
<th>High</th>
<th>Moderate</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral LD$_{50}$ (mg·kg$^{-1}$)</td>
<td>≤ 50</td>
<td>&gt; 50 - 300</td>
<td>&gt; 300-2000</td>
<td>&gt; 2000</td>
</tr>
<tr>
<td>Dermal LD$_{50}$ (mg·kg$^{-1}$)</td>
<td>≤ 200</td>
<td>&gt; 200 - 1000</td>
<td>&gt;1000-2000</td>
<td>&gt; 2000</td>
</tr>
<tr>
<td>Inhalation LC$_{50}$ (vapor/gas) (mg·L$^{-1}$)</td>
<td>≤ 2</td>
<td>&gt; 2 - 10</td>
<td>&gt; 10 - 20</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>Inhalation LC$_{50}$ (dust/mist/fume) (mg·L$^{-1}$)</td>
<td>≤ 0.5</td>
<td>&gt; 0.5 – 1.0</td>
<td>&gt; 1.0 - 5</td>
<td>&gt; 5</td>
</tr>
</tbody>
</table>

Acute mammalian toxicity criteria differentiate compounds based upon a common measure of short term exposure toxicity, the median lethal dose or concentration (LD$_{50}$ or LC$_{50}$), through oral, dermal, and respiratory routes. These values were derived from the GHS criteria [GHS, Chapter 3.1: Acute Toxicity. 2009, United Nations].
## EPA Threshold-Based Criteria for Toxicity

<table>
<thead>
<tr>
<th>Endpoint (LOAEL, NOAEL)</th>
<th>High</th>
<th>Moderate</th>
<th>Low</th>
<th>Very Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral (mg/kg-bw/d)</td>
<td>&lt;50</td>
<td>50-250</td>
<td>&gt;250-1000</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>Dermal (mg/kg-bw/d)</td>
<td>&lt;100</td>
<td>100-500</td>
<td>&gt;500-2000</td>
<td>&gt; 2000</td>
</tr>
<tr>
<td>Inhalation (vapor, mg/L/d)</td>
<td>&lt;1</td>
<td>1-2.5</td>
<td>&gt;2.5-20</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>Inhalation (dust, mg/L/d)</td>
<td>&lt;0.1</td>
<td>0.1-0.5</td>
<td>&gt;0.5-5</td>
<td>&gt; 5</td>
</tr>
</tbody>
</table>

- Chemicals with data
- Considers exposure route
- Examples of threshold-based criteria:
  - *Acute toxicity*
  - *Acute aquatic toxicity*
  - *Bioaccumulation*
  - *Repeated dose toxicity*
  - *Reproductive & developmental toxicity*
Health Hazard Endpoints

Acute Toxicity
- Oral (LD$_{50}$), Dermal (LD$_{50}$), Inhalation (LC$_{50}$)
- Skin Irritation or Corrosion
- Eye irritation or Corrosion
  - Serious Eye Damage
- Sensitization
  - Dermal and Respiratory
- Genotoxicity/Mutagenicity
  - Germ Cell Mutagenicity
- Carcinogenicity

Reproductive Toxicity
Specific Target Organ Toxicity - Single Exposure
Specific Target Organ Toxicity - Repeated Exposure
Aspiration Hazard

1See : Appendix A to 2012 OSHA HazCom Standard (1910.1200) and GHS (5th edition; 2013) for more detailed information on each endpoint
Human Toxicity by Ingestion

\[ INGTP_i = \frac{C_{i,w}}{C_{tot,w}} \frac{RfD_i}{RfD_{tot}} \]

or

\[ INGTP_i = \frac{C_{i,w}}{C_{tot,w}} \frac{LD_{50}}{LD_{50,tot}} \]

\[ I_{ING} = \sum_i INGTP_i \cdot m_i \]

<table>
<thead>
<tr>
<th>Compd.</th>
<th>RfD, mg/kg/d</th>
<th>LD$_{50}$, (rat) mg/kg</th>
<th>P$_{vap}$ at 25°C, mbar</th>
<th>RfC, mg/m$^3$</th>
<th>LC$_{50}$, g/m$^3$ 4h (rat)</th>
<th>INHTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>toluene</td>
<td>0.08</td>
<td>636</td>
<td>38</td>
<td>5</td>
<td>49</td>
<td>1.0</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>0.06</td>
<td></td>
<td></td>
<td>76</td>
<td></td>
<td>0.64</td>
</tr>
<tr>
<td>hexane</td>
<td>28,700</td>
<td>200</td>
<td>0.2</td>
<td></td>
<td></td>
<td>7.2</td>
</tr>
</tbody>
</table>

RfD and RfC data available on the IRIS website. [http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList](http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList)


(search for the compound, select it, then select Toxicity Effects on the left)
Basic Assumptions

(a) The response is causally related to the compound administered

(b) The response is a function of the concentration at the site of action

(c) The concentration at the site of action is related to the external dose

(d) An interaction at the site of action initiates a proportional response

(e) The crucial interaction involves reversible formation of a receptor-toxin complex
Effects on Health from Air Pollution Exposure

- **Respiratory System**
- **Circulatory System**
- **Central nervous System, Organs**
- **Carcinogenic Aerosol**
- **Ionizing Radiations**

Aerosol particles introduced in the respiratory system (particle diameter, µm):

- > 10
- 10
- 6
- 3
- 1
- < 1

Halohydrocarbons (liver)

- Mercury (Hg)
- Lead (Pb)
- Cadmium, Chrome, Arsenic, Nickel (nose)
- Iodine-131, (thyroid)
- NOx, SOx, CO (threat)
- Cadmium (Cd) (heart)
- Asbestos (lungs)
- Mercury (kidney)
- Strontium-90 (bone)
Respiratory Diseases

- **Bronchitis**
  - Inflammation of bronchial ducts

- **Pulmonary Emphysema**
  - Destruction of alveolar sacs

- **Silicosis**
  - Internal covering of lungs with particles
Effects on Respiratory System

• Smell
  • i.e.: Hydrogen sulfide

• Mucous irritation, inflammation
  • i.e.: Ozone, Asbestos

• Mutagen agents
  • i.e.: Radioactive particles, Nanomaterials
Effects on Circulatory System

• Blood Poisoning by CO
  • Oxygen uptake is limited
  
  \[ \text{CO} + \text{hemoglobin} \rightleftharpoons \text{CO}\cdot\text{Hb} \] (carboxyhemoglobin)
  • CO fits in the site of Hb normally used to transport oxygen to body tissues
  • The exposure to CO is cumulative and reversible

• Lead Poisoning
  • Red blood cells transport Lead along the body
Effects of CO Exposition

**Percent of carboxyhemoglobin vs. Exposure (h)**

- **Dead**: 600 ppmv CO
- **Coma**: 300 ppmv CO
- **Vomiting, collapse**: 100 ppmv CO
- **Heart Arrhythmia**: 100 ppmv CO
- **Faint, reduced mental capacity**: 30 ppmv CO
- **No symptom**: 15 ppmv CO

The graph shows the percentage of carboxyhemoglobin as a function of exposure time for different CO concentrations.
Heavy Metals:

- **Lead**
  - cerebral damages, anemia, bones, immune system

- **Mercury**
  - CNS attack, immune system, cell membranes

- **Cadmium**
  - kidney, heart, CNS centers of odor, cancer promoter

- **Arsenic**
  - cell metabolism

- **“Aluminum”**
  - stomach, bones, cell metabolism
Carcinogens

Materials inhaled or ingested which induce cellular mutations

- Heavy metals
  - Arsenic, Chrome, Cadmium
- Volatile Organic Solvents
- Polycyclic aromatic compounds
- Aromatic amines
- Dioxins
- ..........
Carcinogenicity demands further deliberation.

- Many chemicals have the potential to cause cancer
- Only a relatively few are known human carcinogens
    - “reasonably expected” to cause human cancer = 174 agents
    - “known” to cause human cancer = 49 agents
  - [http://monographs.iarc.fr/monoeval/crthgr01.html](http://monographs.iarc.fr/monoeval/crthgr01.html)
    - 75 agents known to cause human cancer
- High-dose animal studies identify potential carcinogens, but the results may not apply to humans, especially at low doses.
- The carcinogenic effects of naturally occurring chemicals is often overlooked.
Dr. Bruce Ames, author of the **Ames mutagenicity test**, suggest we take a more reasonable approach to carcinogens.

- HERP index (Human Equivalent Rodent Potential) ranks carcinogens in our environment according to the dose that caused cancer in rodents.

http://pubs.acs.org/hotartcl/chas/96/julaug/ames/ames.html
### EPA Carcinogenicity Criteria for Hazard Designation

<table>
<thead>
<tr>
<th>Carcinogenicity</th>
<th>Very high</th>
<th>High</th>
<th>Moderate</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carcinogenicity</strong></td>
<td>Known or presumed human carcinogen (equivalent to GHS Category 1A and 1B)</td>
<td>Suspected human carcinogen (equivalent to GHS Category 2)</td>
<td>Limited or marginal evidence of carcinogenicity in animal (and inadequate evidence in humans)</td>
<td>Negative studies or robust mechanism based SAR</td>
</tr>
</tbody>
</table>

These criteria mirror the classification approach used by the International Agency for Research on Cancer (IARC),* and incorporate the Globally Harmonized System (GHS) classification scheme.** Authoritative lists can supplement these criteria.

** GHS, Chapter 3.6: Carcinogenicity. 2009, United Nations.
Other Toxicity Criteria for Hazard Designations

- Mutagenicity/Genotoxicity
- Reproductive and Developmental Toxicity
- Reproductive and Developmental Toxicity (including Developmental Neurotoxicity)
- Neurotoxicity
- Repeated Dose Toxicity
- Respiratory and Skin Sensitization
- Eye and Skin Irritation / Corrosivity
- Endocrine Activity
**Radiation Toxicity**

- **Radioisotopes**: an unstable form of an atom (with propensity to “decay” spontaneously)
- **Nuclear symbol**:

\[
\begin{array}{cc}
\text{Mass Number} & A \\
\text{Atomic Number} & Z \\
\text{Symbol} & n(\pm) \\
\text{Electrical charges} & (\text{electrons in excess or less than the neutral atom}) \\
\text{Number of atoms aggregates with chemical bonds} & (\pm) \\
\end{array}
\]

\[
\text{Es.: } {}_1^1\text{H} \quad {}_{12}^{12}\text{C}^{3+} \quad {}_{127}^{53}\text{I}^-
\]

- **Alfa and Beta particles, Gamma rays**: high-energy products of radioactive decay
- **Half-life**: time to reduce to an half the mass of radioisotope by decay
Receptors: Molecular Targets of Chemical Compounds

Receptors

- Ion Channel Receptors
- Carrier Proteins
- G-Protein Coupled Receptors
- Tyrosine-Kinase Receptors
- Ah Receptors
- Steroid Hormone Receptors

Usually proteins:

- Located on outside of cell wall, or inside cell
- Interact with ligands
Receptors Important in Pharmacology

- **Agonists and antagonists of neurotransmitters:**
  - *cholinergic receptors*: acetylcholine;
  - *nicotinic receptors*: skeletal muscle, autonomic ganglia;
  - *muscarinic receptors*: smooth muscle, heart, exocrine glands

- **Adrenergic receptors**: dopamine, endorphins, encephalins, histamine

- **Hormone receptors**: Insulin, cortisone (glucocorticoids), estrogen, progesterone, testosterone, prostaglandins

- **Drug receptors**: Benzodiazepines
Receptors Important in Toxicology

- Ah (TCDD) receptor
- Steroid receptors

Reversible formation of a receptor-toxin complex

\[ R + T \rightleftharpoons_{k_1}^{k_{-1}} R-T \]

R = receptor
T = toxic compound
R-T = receptor-compound complex

\( k_1 \) and \( k_{-1} \) are rate constants for formation and dissociation (respectively) of the complex R-T
Dose-Response Relationship

LD\(_{50}\)  \(\text{LC}_{50}\)  \(\text{ED}_{50}\)
Common Dose-response Models

- **Normal:**

- **Logistic:**

- **Modified Logistic:** (e.g. Seefeldt et al. 1995)

- **Gompertz:**

- **Exponential:**

\[
y_{i,j} = \frac{1}{\sqrt{2\pi\sigma}} e^{\frac{(x-\mu)^2}{2\sigma^2}}
\]

\[
y_{i,j} = \frac{1}{\left(1 + e^{\beta_1(dose_i - \beta_0)}\right)}
\]

\[
y_{i,j} = C + \frac{D - C}{1 + e^{(-B(dose_i - 1))}}
\]

\[
y_{i,j} = \beta_0 e^{-\beta_1(dose)}
\]

\[
\begin{align*}
y_{i,j} &= \beta_0 e^{-\beta_1(dose)} \\
y_{i,j} &= \beta_0 \left[1 - e^{-\beta_1(dose)}\right]
\end{align*}
\]
Acute Toxicity Studies

**Single dose** - rat, mouse (5/sex/dose), dog, monkey (1/sex/dose)
- 14 day observation
- In-life observations (body wt., food consumption, clinical observations)
- Necropsy

**Repeated dose studies** - rat, mouse (5-10/sex/dose), dog, monkey (2/sex/dose)
- In-life observations
- Necropsy
- Histopathology
- Clinical pathology (optional)
## Acute LD$_{50}$ Values vs. Toxicity

<table>
<thead>
<tr>
<th>Chemical</th>
<th>LD$_{50}$ (mg/kg)</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride</td>
<td>4000</td>
<td>Slightly toxic</td>
</tr>
<tr>
<td>DDT</td>
<td>100</td>
<td>Moderately toxic</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>5</td>
<td>Highly toxic</td>
</tr>
<tr>
<td>Strychnine</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dioxin</td>
<td>0.001</td>
<td>Super toxic</td>
</tr>
<tr>
<td>Botulinum Toxin</td>
<td>0.00001</td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Dose (mg/kg body weight)</td>
<td>Species</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Practically nontoxic</td>
<td>15 000</td>
<td></td>
</tr>
<tr>
<td>Slightly toxic</td>
<td>10 000</td>
<td>Mouse</td>
</tr>
<tr>
<td></td>
<td>5 000</td>
<td></td>
</tr>
<tr>
<td>Moderately toxic</td>
<td>4 900</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Highly toxic</td>
<td>250</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Extremely toxic</td>
<td>13</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Supertoxic</td>
<td>3</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>Duck</td>
</tr>
</tbody>
</table>
## Species Differences in the Acute Toxicity of Dioxin*

<table>
<thead>
<tr>
<th>Species</th>
<th>LD$_{50}$ (µg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea-pig</td>
<td>0.5-2</td>
</tr>
<tr>
<td>Rat</td>
<td>20 -100</td>
</tr>
<tr>
<td>Mouse</td>
<td>114-284</td>
</tr>
<tr>
<td>Rabbit</td>
<td>10-115</td>
</tr>
<tr>
<td>Chicken</td>
<td>25-50</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>&lt; 70</td>
</tr>
<tr>
<td>Dog</td>
<td>&gt;30-100</td>
</tr>
<tr>
<td>Hamster</td>
<td>5051</td>
</tr>
</tbody>
</table>

*Dioxin: 2,3,7,8-tetrachlorobenzodioxin: TCDD*
Other Factors

- Interval of exposure
- Interaction may be not reversible
- Repair or removal of complex R-T may be important
- Response may be multi-step, binding of T to R may not be the rate-limiting step
- Assumes normal (Gaussian) distribution
- Uniform population - no significant inter-individual differences in response
- Dichotomous (quantal) response, e.g. tumor frequency
## Effect Factors (EF) and Hazard Risk factors after TRGS 440 *(GER 2001)*

<table>
<thead>
<tr>
<th>Risk phrases</th>
<th>Hazards</th>
<th>effect factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>R45, R46, M1, M2, K1, K2</td>
<td>carcinogenic or mutagenic</td>
<td>50,000</td>
</tr>
<tr>
<td>R26, R27, R28 or LGW &lt; 0,1 mg/m³</td>
<td>highly toxic</td>
<td>1,000</td>
</tr>
<tr>
<td>R32, R60, R61, RE1, RE2, RF1, RF2</td>
<td>potential reproduction toxicity or teratogenic, formation of highly toxic gases in contact with acids</td>
<td>1,000</td>
</tr>
<tr>
<td>R35, R48/23, R48/24, R48/25, R42, R43</td>
<td>highly corrosive, high chronic toxicity, potentially sensitizing</td>
<td>500</td>
</tr>
<tr>
<td>R23, R24, R25, R29, R31, R34, R41, H</td>
<td>toxic, generation of toxic gases in contact with water or acids, cauterizing for eyes, skin absorption</td>
<td>100</td>
</tr>
<tr>
<td>R33, R40, K3, M3, pH &lt; 2 or pH &gt; 11,5</td>
<td>risk of cumulative effects, potentially irreversible damages, suspected mutagenic or carcinogenic effects</td>
<td>100</td>
</tr>
<tr>
<td>Not tested sufficiently</td>
<td>= No LGW, no risk phrases</td>
<td>100</td>
</tr>
<tr>
<td>R48/20, R48/21, R48/22, R62, R63, RE3,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R20, R21, R22</td>
<td>chronically harmful, suspected reproduction toxicity or teratogenic effects</td>
<td>50</td>
</tr>
<tr>
<td>R36, R37, R38, R65, R67</td>
<td>harmful</td>
<td>10</td>
</tr>
<tr>
<td>other risk phrases or LGW &gt; 100 mg/m³</td>
<td>irritating, narcotic</td>
<td>5</td>
</tr>
</tbody>
</table>
Toxicity Trends in Similar Molecules

DL_{50} (mouse, oral) 54.3 mmol/kg  133 mmol/kg  12.3 mmol/kg  13.9 mmol/kg

DL_{50} (mouse, oral) 43.5 mmol/kg  29.8 mmol/kg  2.74 mmol/kg  1.20 mmol/kg
Toxicity Trends in Reaction Products

\[ \text{Effect factor } > 1000 \]
\[ \text{Effect factor } > 100 - 1000 \]
\[ \text{Effect factor } > 10 - 100 \]
\[ \text{Effect factor } 0 - 10 \]
Sub Acute Toxicity

- 28 week study (3 doses and control)
- Species – rat (10/sex/dose), dog or monkey (2/sex/dose)
- In-life observations
- Clinical pathology
- Necropsy
- Histopathology
Sub Chronic Toxicity

- 13 week study +/- 4 wk recovery (3 doses and control)
- Species - rat (10/sex/dose), dog or monkey (2/sex/dose)
- In-life observations (+/- ophthalmology)
- Clinical pathology
- Necropsy
- Histopathology
Chronic Toxicity

- 1 year study +/- 4-13 wk recovery (3 doses and control)
- Species - rat (10-15/sex/dose), dog or monkey (2-3/sex/dose)
- In-life observations including ophthalmology
- Necropsy
- Histopathology
Endocrine Disrupting Compounds

Any exogenous agent that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function. Specifically:

Any exogenous chemical that interferes with the production, release, transport, binding, action, or elimination of natural hormones responsible for the maintenance of homeostasis and regulation of development.

The WHO/IPCS definition is characterised by three elements: a chemical can be defined an ED;

1. if it shows an adverse effect in an intact organism (generally from in vivo animal testing);
2. if it is able to interfere with the endocrine/hormonal system (mechanistic data show the substance can act via an endocrine/hormonal mode of action); and
3. if a plausible link can be established between the endocrine mode of action and the adverse effect observed for the substance.
The EU List of Potential Endocrine Disruptors

- **Category 1**: Substances for which endocrine activity have been documented in at least one study of a living organism. These substances are given the highest priority for further studies. Category 1 contains 194 substances.
- **Category 2**: Substances without sufficient evidence of endocrine activity, but with evidence of biological activity relating to endocrine disruption.
- **Category 3a and 3b**: Substances for which there are no indications of endocrine-disrupting properties or which cannot be evaluated due to a lack of data.

Evaluations are based on various endocrine modalities: the androgen (A), the oestrogen (E), the thyroid (T) and the (S) steroidogenesis modalities (often referred to as EATS modalities) (OECD 2012*; EFSA 2013**)

Endocrine Disruptors

**At Least 4 Modes of Action**

- Serving as steroid receptor ligands.
- Modifying steroid hormone-metabolizing enzymes.
- Perturbing hypothalamic pituitary release of trophic hormones.
- Miscellaneous or unknown.

**Chemicals: Wide Variety**

- Pesticides
- Herbicides
- Fungicides
- Plasticizers
- Surfactants
- Organometals
- Halogenated PAHs
- Phytoestrogens
Prototypical Exo-estrogens: Note the Diverse Chemical Structures

**Endogenous ligand**

17β-estradiol

Zearalenone

**Synthetic estrogen**

Diethylstilbesterol

o.p’-DDT

Bisphenol-A

Genistein
Specific Examples of Endocrine Disruption

<table>
<thead>
<tr>
<th>Natural Products</th>
<th>Environmental pollution</th>
<th>Industrial products</th>
<th>Pharmaceutical</th>
<th>Complex mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein</td>
<td>DDT</td>
<td>Bisphenol-A</td>
<td>Etinyl estradiol</td>
<td>Effluents</td>
</tr>
<tr>
<td>Naringenin</td>
<td>Kepone</td>
<td>Nonionic surfactants</td>
<td>Diethylstilbestrol</td>
<td>Sediment extracts</td>
</tr>
<tr>
<td>Coumestrol</td>
<td>PCBs/HO-PCBs</td>
<td>Phthalate esters</td>
<td>Gestodene</td>
<td>Air particulate matter</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>PAHs and dioxins</td>
<td>Endosulfan</td>
<td>Norgestrel</td>
<td>Tissue extracts</td>
</tr>
</tbody>
</table>

*DDT = dichlorodiphenyltrichloroethane, PCBs = polychlorinated biphenyls, HO-PCBs = hydroxylated PCBs


**Tributyltin**
Causes imposex and intersex in gastropod mollusks.
Neogastropods have separate sexes but it was observed that many female dogwhelk from some areas of the UK had a penis-like structure behind the right tentacle. This was also seen later in other gastropods in the eastern US. These gastropods also had a vas deferens (sperm duct) and a convoluted gonoduct. The term “imposex” was coined to describe the superimposition of male characters onto females. It was demonstrated that levels of imposex were elevated close to marinas, a feature attributed to the presence of anti-fouling paints.
Carcinogens and Carcinogenicity Studies

- 2 years (3 doses and control)
- Species - rats and mice (50/sex/dose)
- In-life observations
- Clinical pathology (rats, optional)
- Necropsy
- Histopathology

Evaluation Issues

- Survival
- Body weight
- Variability of endpoints
- Pathology Working Group

- MTD
- Statistics vs. biology
- Dose-response
- Mechanistic Factors
Human Health Protection

- Health and Safety at work
  - Short-term exposure limit (STEL)
  - Time-Weighted Average (TWA)
- Specific quality goals
- Monitoring and Bio-monitoring

Occupational Toxicology

Multidisciplinary field:
- Routes of exposure & uptake
- P450 metabolism & characterization of toxic metabolites
- Phase II metabolism & identification of urinary metabolites
- Characterization of DNA adducts
- Identification of target tissue
### Example:

<table>
<thead>
<tr>
<th>Element</th>
<th>Requirements (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In dry brittle, powder like or pliable toy material</td>
</tr>
<tr>
<td>Aluminum</td>
<td>5625.00</td>
</tr>
<tr>
<td>Antimony</td>
<td>45.00</td>
</tr>
<tr>
<td>Arsenic</td>
<td>3.80</td>
</tr>
<tr>
<td>Barium</td>
<td>4500.00</td>
</tr>
<tr>
<td>Boron</td>
<td>1200.00</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.30</td>
</tr>
<tr>
<td>Chromium(III)</td>
<td>37.50</td>
</tr>
<tr>
<td>Chromium(VI)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cobalt</td>
<td>10.50</td>
</tr>
<tr>
<td>Copper</td>
<td>622.50</td>
</tr>
<tr>
<td>Lead</td>
<td>13.50</td>
</tr>
<tr>
<td>Manganese</td>
<td>1200.00</td>
</tr>
<tr>
<td>Mercury</td>
<td>7.50</td>
</tr>
<tr>
<td>Nickel</td>
<td>75.00</td>
</tr>
<tr>
<td>Selenium</td>
<td>37.50</td>
</tr>
<tr>
<td>Strontium</td>
<td>4500.00</td>
</tr>
<tr>
<td>Tin (Organic Tin 0.9, 0.2, 12)</td>
<td>15000.00</td>
</tr>
<tr>
<td>Zinc</td>
<td>3750.00</td>
</tr>
</tbody>
</table>
Occupational Exposure Limits

- **TLV**, Threshold Limit Value
- **TWA**, Time-Weighted Average \(8\text{h/d}\text{ay}, 40\text{h/week}\)
  - Used for contaminants with longer-term or chronic
    - Lead, carcinogens, etc.
  - “Integrated” exposure over the shift
    - Single sample TWA
    - Multiple samples can be combined

\[
TWA = \frac{C_1 T_1 + C_2 T_2 + \ldots + C_n T_n}{T_1 + T_2 + \ldots + T_n}
\]

- **TLV-C**, Ceiling level
- **STEL**, Short-term exposure limit
- **IDLH**, Dangerous to Life and Health
### Example of TWA, STEL, and IDLH Value for Some Chemicals (in ppm)

<table>
<thead>
<tr>
<th>Substance</th>
<th>TLV TWA</th>
<th>TLV STEL</th>
<th>IDLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>-</td>
<td>25C</td>
<td>2,000</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>10</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Acetone</td>
<td>500</td>
<td>750</td>
<td>2,500</td>
</tr>
<tr>
<td>Acrolein</td>
<td>-</td>
<td>0.1C</td>
<td>2</td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>2</td>
<td>-</td>
<td>85</td>
</tr>
<tr>
<td>Ammonia</td>
<td>25</td>
<td>35</td>
<td>300</td>
</tr>
<tr>
<td>Arsine</td>
<td>0.05</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.5</td>
<td>2,5</td>
<td>500</td>
</tr>
<tr>
<td>Boron Trifluoride</td>
<td>-</td>
<td>1C</td>
<td>25</td>
</tr>
<tr>
<td>Bromine</td>
<td>0.1</td>
<td>0.2</td>
<td>3</td>
</tr>
<tr>
<td>1,3 – Butadiene</td>
<td>2</td>
<td>-</td>
<td>2000</td>
</tr>
<tr>
<td>Butane</td>
<td>1000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>n-Butyl Acrylate</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>n-Butyl Alcohol</td>
<td>20</td>
<td>-</td>
<td>1400</td>
</tr>
<tr>
<td>Butyl Mercaptan</td>
<td>0.5</td>
<td>-</td>
<td>500</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>5000</td>
<td>30000</td>
<td>40000</td>
</tr>
<tr>
<td>Carbon Disulfide</td>
<td>10</td>
<td>-</td>
<td>500</td>
</tr>
<tr>
<td>Carbon Monoxide</td>
<td>25</td>
<td>-</td>
<td>200</td>
</tr>
<tr>
<td>Carbon Tetrachloride</td>
<td>5.1</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

Data from 2005 Threshold Limit Values & Biological Exposure Indices, copyright 2005 by the American Conference of Governmental Industrial Hygienists (ACGIH). IDLH values extracted from the NIOSH Pocket Guide to Chemical Hazards, 2004 published by the National Institute for Occupational Safety and Health (NIOSH). “C” indicates Ceiling Limit.
TWA Example

Assume 4 exposure samples

1. 1 hour, 10 ppm
2. 3 hours, 20 ppm
3. 3 hours, 30 ppm
4. 1 hour, 100 ppm

\[ TWA = \frac{(1 \times 10) + (3 \times 20) + (3 \times 30) + (1 \times 100)}{(1 + 3 + 3 + 1)} = 32 \text{ ppm} \]

(straight average = 40 ppm)
Interactions

• **Additivity**  Chemicals A, B, C…N are all toxic
  Potency of mixture = Sum of potencies of constituents
  \[ \text{Effect}_{\text{total}} = \text{Potency}_A \times \text{Dose}_A + \text{Potency}_B \times \text{Dose}_B \\
  + \text{Potency}_C \times \text{Dose}_C + \ldots + \text{Potency}_N \times \text{Dose}_N \]

• **Synergism**  Potency of the whole is greater than the sum of the potencies of the individual constituents
  \[ \text{Effect}_{\text{total}} >> \text{Potency}_A \times \text{Dose}_A + \text{Potency}_B \times \text{Dose}_B \ldots \\
  + \ldots + \text{Potency}_N \times \text{Dose}_N \]

• **Potentiation**  One constituent is toxic, the other is not. Potency of the combination is greater than the potency of the active constituent
  \[ \text{Effect}_{\text{total}} >> \text{Potency}_A \times \text{Dose}_A \text{ where } \text{Potency}_B = 0 \]

• **Antagonism**  Potency of the whole is less than the sum of the potencies of the individual components
  \[ \text{Effect}_{\text{total}} << \text{Potency}_A \times \text{Dose}_A + \text{Potency}_B \times \text{Dose}_B \ldots \\
  + \ldots + \text{Potency}_N \times \text{Dose}_N \]
Routes of Exposure

- Airborne (dusts, fibers, particulate matter, aerosols, gases)
- Waterborne (solution, suspension, emulsion)
- Foodborne (solids, liquids)

**Portals of Entry**

- Skin (percutaneous)
- Respiratory tract (inhalation)
- Gastrointestinal tract (ingestion)
- Trans placental
- Injection

**Experimental equivalents**

- Skin-painting
- Intra-tracheal instillation
- Oral gavage
- Administration to dam
- Injection
Exposure Assessment

Chemical Evidences of Exposure

- Urine metabolites, or in air
- Residuals in tissues, included blood (sampling)
- Covalently bonded macromolecular adducts
  - Adducts to DNA
  - Adducts to proteins
- Antibody
The Major Structures of the Skin

- Stratum corneum (10-15μm)
- Living epidermis (>100μm)
- Dermal vasculature
- Eccrine gland
- Arrectores pilorum muscle
- Subcutaneous fatty tissue
- Subcutaneous vasculature

Diagram highlights:
- Hair shaft
- Sebaceous gland
- Eccrine gland duct

Effect on Skin of Contact with Solvents

- Acetone
- 600 µmol Ethyl Acrylate
- 60 µmol TPGDA
- 1.25 µmol TPA
Exposition Phases

- Absorption
- Distribution
- Metabolism
- Elimination
Mechanisms of Uptake

- Passive diffusion
- Filtration
- Carrier-mediated transport
  - Facilitated diffusion
  - Active transport
- Engulphment
  - Pinocytosis
  - Phagocytosis
Engulphment

The cellular mechanism that involves the internalization of extracellular components (solid and liquids)
Factors Influencing Route and Uptake Rate

• Properties of the chemical
  – Size and shape
  – Similarity to “endogenous” molecules
  – Solubility
    • Hydrophilicity / hydrophobicity
    • Lipophilicity / lipophobicity
    • Partition coefficient: $K_{ow}$
      • Log10 of [octanol]/[water]
  ▪ Charge
    • Ionization state
Each relevant organ or tissue is a compartment

Material flows into compartment, partitions into and distributes around compartment, flows out of compartment usually in blood

If blood flow rates, volume of compartment and partition coefficient are known, can write an equation for each compartment

Assuming conservation of mass, solve equations simultaneously – can calculate concentration (mass) in each compartment at any time
Example of a Model

Air inhaled

Lungs

Rest of body

Liver

Metabolism

Kidney

Urine

Venous blood

Arterial blood
Biomarker Types

- Air Levels
- Body Burden
- Internal Dose of Reactive Metabolite
- Early Biologic Effects
- Disease
- External Exposure Assessment
- Susceptibility Biomarkers
- Biomarkers of Exposure
- Susceptibility Biomarkers
- Biomarkers of Effect
Please note that these categorizations are NOT exact - they are just meant to provide a framework for understanding. (E.g., DNA adducts may be considered a measure of early effect rather than of exposure.)

**Biomarkers of Exposure**

- **Body Burden**
  - Exhaled breath
  - Blood or urinary levels
- **Internal Metabolite Dose**
  - Blood metabolite levels
  - Protein adducts
  - Urinary metabolite levels
- **Biologically-Effective Dose**
  - DNA adducts

**Biomarkers of Effect**

- Sister chromatid exchanges
- Micronuclei
- Chromosomal damage

**Biomarkers of Susceptibility**

- Breathing rate
- Enzyme genotype (DNA sequence) or phenotype (activity)
  - P450s
  - Glutathione S-transferase
  - Epoxide hydrolase
  - DNA repair enzymes
R² represents the % variation in the biomarker (e.g., tissue dose) that can be explained by exposure.

**High R²**
- external exposition is linearly related to dose
- low inter-individual variability in uptake & metabolism
- biomarker is specific
- sample size is adequate

**Low R²**
- MORE DIFFICULT TO INTERPRET
- external exposure is not linearly related to dose
- high inter-individual variability in uptake & metabolism
- biomarker is non-specific
- sample size is inadequate
Biomarker Choice

The choice is dependent upon a number of factors:

- Time frame (short or long-term effects? latency of disease?)
- Is exposure constant or highly variable over mins/hrs/days/weeks/months?
- Number of subjects?
- Budget/time constraints?
- Toxic metabolite known?
- Mechanism of toxicity known or not?
- Route of exposure (just inhalation, or also dermal/oral)?
- Inter-individual differences in uptake (protective equipment) or metabolism?
- Source of exposure (just work?)
- What (bio)monitoring techniques are available?
Ambient Monitoring vs. Bio-monitoring

**External Monitoring**
- Can compare to old records
- Lower cost
- Less skilled labor
- Larger number of samples
- Easier to obtain (air)
- Just inhalation exposure
- Just work exposures
- Short time period (mins-hrs)
- Air levels of compound

**Exposure Biomarkers**
- New techniques, little historical data
- Higher cost
- Highly skilled labor
- Smaller number of samples
- Harder to obtain (blood, urine, tissue)
- All routes of exp (inhalation, dermal, oral)
- Reflects ALL exposures (hobbies, diet)
- Short to long time periods (mins-weeks)
- Tissue levels of reactive metabolite
  - Accounts for personal habits (hand washing, use of protective equipment)
  - Inter-individual differences in metabolism (both activation & detoxification) and repair
Biomarker Choice

- Good relationship between biomarker and exposure
  - High $R^2$
  - No need for a biomarker - choose whichever is cheaper/easier (generally the external measure)

- Poor relationship
  - Low $R^2$
  - Looks like 2 distinct populations - indicative of differences in either
    - uptake (protective equipment)
    - metabolism
    - repair
  - Biomarkers helpful in this case
Proteins used

- **Hemoglobin (Hb)**
  - 120 day lifespan of the red blood cell
  - 150 \( \text{mg/mL} \) blood

- **Albumin**
  - 20-25 day half-life
  - 18-25 \( \text{mg/mL} \) blood

- **Target tissue protein**

- Protein adducts can be used to predict the average blood concentration (blood dose) of the reactive metabolite over the lifespan of the protein. Regardless of which protein is used, the predicted dose should be the same.
Benzene

- 48 billion kilo produced in the world in 2016
- Human exposures due to cigarette smoking, gasoline & processing of petroleum products
- Highest exposures occur in countries such as China and Turkey
- Causes cancer in rodents and hematotoxicity and leukemia in exposed humans
- Metabolite(s) responsible for benzene toxicity are still unknown; possibilities include:
  - Benzene oxide (BO)
  - 1,2-or 1,4-benzoquinone
  - Hydroxybenzoquinone
  - Trans,trans-muconaldehyde
  - Reactive oxygen species
Benzene Metabolism

- Benzene
- Benzene oxide
- t,t-muconaldehyde
- Muconic acid
- Phenol
- Catechol
- Hydroquinone
- Epoxide hydrolase
- Dihydriodiol dehydrogenase
- S-phenyl mercapturic acid
- GST/GSH conjugate
- Reaction with macromolecules
Example of Exposure Evaluation: Alcohol Ethoxysulphates, AES

Typical Distribution of Ethoxylate Adducts:

\[
\text{CH}_3(\text{CH}_2)_x\text{O(CH}_2\text{CH}_2\text{O})_n\text{SO}_3\text{Na}
\]

\(x = 7 – 15\), typically 11.

90-40% of the carbon chains are linear, the remainder being mono-branched 2-alkyl isomers, predominantly 2-methyl.

\(n\) ranges from 0-8.

Used as detergent/cleaning agent. Ethoxylation of alcohols is carried out by base catalyzed reaction with ethylene oxide. The \(n\) average for the important sulphation grades is 1-3 moles EO per mole alcohol. Toxic dioxane must be removed.

<table>
<thead>
<tr>
<th>Oligomer distribution, %m/m, of (\text{RO(CH}_2\text{CH}_2\text{O})_n\text{H}) where (n=)</th>
<th>3</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.1</td>
<td>23.5</td>
<td>42.9</td>
</tr>
<tr>
<td>1</td>
<td>9.1</td>
<td>12.8</td>
<td>20.3</td>
</tr>
<tr>
<td>2</td>
<td>11.9</td>
<td>15.6</td>
<td>14.9</td>
</tr>
<tr>
<td>3</td>
<td>12.9</td>
<td>13.4</td>
<td>8.8</td>
</tr>
<tr>
<td>4</td>
<td>11.8</td>
<td>10.1</td>
<td>5.1</td>
</tr>
<tr>
<td>5</td>
<td>10.3</td>
<td>7.5</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>7.9</td>
<td>5.0</td>
<td>1.9</td>
</tr>
<tr>
<td>7</td>
<td>6.5</td>
<td>4.0</td>
<td>1.4</td>
</tr>
<tr>
<td>8</td>
<td>4.8</td>
<td>2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>9</td>
<td>3.9</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>2.9</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>11</td>
<td>1.9</td>
<td>0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>12</td>
<td>1.3</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>13</td>
<td>0.7</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>14</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>15</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Average EO Number

| Average EO Number | 3.1 | 2.1 | 1.0 |

Source: Hera 2003
Direct Skin Contact from Hand-washed Laundry

\[ \text{Exp}^{\text{sys}} = F^1 \times C \times K_p \times t \times S^{\text{der}} \times n / BW \]

- \( F^1 \) % weight fraction of substance in product
- \( C \) product concentration in mg/ml:
  - 20% (0.2) [AISE Internal data]
- \( K_p \) dermal penetration coefficient
  - 1.62 x 10^{-4} \text{ cm} \cdot \text{h}^{-1} [Black et al. 1979]
- \( t \) duration of exposure or contact
  - 10 min (0.167h) [AISE/HERA Tab 2002]
- \( S^{\text{der}} \) surface area of exposed skin
  - 1980 cm\(^2\) [TGD, 1996]
- \( n \) product use frequency (tasks per day)
  - 3 [AISE/HERA 2002]
- \( BW \) body weight
  - 60 kg

\[ \text{Exp}^{\text{sys}} = [0.2 \times (10 \text{ mg/ml}) \times (1.62 \times 10^{-4} \text{ cm/h}) \times (0.167h) \times 3 \times (1980 \text{ cm}^2)] / 60 \text{ kg} = 5.4 \mu g \cdot \text{kg}^{-1} \text{ bw/day} \]

* the dermal penetration coefficient calculated from the dermal flux (0.39 \mu g/cm\(^2\)) which was determined in an in vivo dermal penetration experiment conducted by Black and Howes according to the following algorithm: \( K_p = \frac{\text{dermal flux/exposure time} \times \text{concentration of solution}}{10 \text{ mg/cm}^3} = 1.62 \times 10^{-4} \text{ cm/h} \)
Chemistry-based Data Mining & Exploration:

- **Databases**
  - Chemical(s) of concern
  - Chemical specific data

- **Structure-searchable**
  - Structural analogs

- **Data Mining**
  - Property analogs

- **Structure-Activity Relationships**
  - Biological/mechanistic analogs
Search by: Chemical Name CAS Registry #

Integrated Risk Information System (IRIS)

Getting Started with IRIS

- An overview of the web site
- What is IRIS?
- How does EPA decide which substances to add or update?

Using the IRIS Database

- Advanced Search in IRIS
- Compare IRIS Values
- Download IRIS

A to Z List of IRIS Substances

The substances in IRIS are listed in alphabetical order or by last significant revision date. You can click on the first letter of the substance name to bring you to the section where the substance appears, or use your browser's "Find" command to search for a substance name or Chemical Abstract Services Registry Number (CASRN).

(To search the IRIS database, use Advanced Search)

http://cfpub.epa.gov/ncea/iris/index.cfm
EPA’s Chemical Data Islands

- National Toxicology Program
- National Library of Medicine
- PubChem Chemical Structures
- European Chemicals Bureau (SIDS)

- Chemical Names
  - CAS Registry Nos.

- Toxicogenomics
- Ecotox, Aster, Teratox

- Pesticides
  - Actives
  - Other Ingredients
  - Pesticides

- Drinking water contaminants
- IRIS
- Air

- High Production Volume Information System
- Tasca Substance Registry List

- Green Chemistry Chemical Structures
Reports: NTP is converting study reports into an electronic format which can be accessed from the website. These reports are made available as soon as they have been converted.

Data Searches: The NTP has been loading study information into databases and has developed applications to access this data from the web. There are two types of data mining searches:

- All types of data - search provides a way to find the various types of studies conducted on a test agent and has options to mine that data if it is available in electronic format.
- Biostat pathology data mining search provides a way to access the pathology databases. It is also possible to search the historical control database and to view the Pathology Code tables used by the NTP

Pathology Tables for Peer Review: To inform the public in a timely manner, the NTP makes available information on the pathology, body weight changes and survival of completed 13-week and 2-year studies as soon as possible after the appropriate reviews of the data have been made.

Summaries/Associations: The NTP has put together sets of summaries and associations based on frequently asked questions.

Strategy for Compounds Evaluation

1. In Silico screening
   a) Epi-suite (US EPA)
   b) Toxtree (JRC)
   c) Demetra/Caesar (EU funded prj)

Toxtree descriptors:
- MW
- LogP
- Vapor Pressure
- Water Solubility
- Lipid Solubility
- Melting Point
- Surface Tension
PubChem is a component of National Institutes of Health (NIH) Roadmap Molecular Libraries Initiative. PubChem contains information on substances, compound structure and bioactivity data. PubChem is integrated with Entrez, the main research engine of NCBI.

- 3 main database: PC Substance; PC Compound; PC BioAssay.
Entrez with PubChem ...

- MEDLINE Abstracts
- 3D Structures
- Small Molecule Structures
- Term Frequency Statistics
- VAST Structure Similarity
- Activity Profile Similarity
- Chemical Structure Similarity

Bioactivity
Assay
Results
Toxicological Data Banks

ACToR Draws from Diverse Data Sources

ToxRefDB

PubChem

NLM

ToxCast

ToxMiner

Application 2

OPPIN

OPPTS

DSSTox

Substance Registry System
Correlating Domain

Data Mining
- Relational data models
- Toxicological description
- Data standards
- Data integration
- Summary activities

QSAR Modeling
- Chemical properties
- Structural descriptors
- Chemical similarity metrics
- Statistical associations

Predictive Toxicology

Biological Profiling
- HTS assays
- Toxicogenomics
- Metabolomics
- Mode-of-action

Toxico-chemoinformatics
- Chemical genomics
- Chemical diversity
- Chemical neighborhoods
REACH, Art. 1: “The purpose of this Regulation is to ensure a high level of protection of human health and the environment, including the promotion of alternative methods for assessment of hazards of substances…”

Art. 13: “…In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across)…”

Use of expert system tools for predicting toxicity
Episkin: Reconstructed Human Skin Model

TARGET: human skin artificial multilayer

OECD 431

*in vitro* method where test substance is applied to stratum corneum of the epidermal model at different time of exposure.

Detection throughout mitochondrial activity analysis

Replacement for the *in vivo* corrosivity test for hazard identification and classification corrosive potential
Chromosomal Aberration

**TARGET:** human PBMC (*Peripheral Blood Mononuclear Cell*)

**OECD 473**

Mutagen-induced aberrations include:

- Gaps
- Breaks
- Dicentric chromosomes
- Ring Chromosomes
Before assessing impact of a pollutant, we need to find out where it goes.

Need to define:
1. $K_{ow}$
2. $K_{oc}$
3. Henry’s Law Constant
4. Multimedia Compartment Model
Multimedia Compartment Model Formulation

**Multimedia compartment model**

**Model Domain Parameters**
- surface area - 10^4 - 10^5 km^2
- 90% land area, 10% water
- height of atmosphere - 1 km
- soil depth - 10 cm
- depth of sediment layer - 1 cm
- multiphase compartments

**Processes modeled**
- emission inputs, $E$
- advection in and out, $D_A$
- intercompartment mass transfer, $D_{i,j}$
- reaction loss, $D_R$

## Multimedia compartment model input data

<table>
<thead>
<tr>
<th>Environmental Property</th>
<th>Unit</th>
<th>Spreadsheet Location</th>
<th>Benzene</th>
<th>Ethanol</th>
<th>PCP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>g/mole</td>
<td>C6</td>
<td>78.11</td>
<td>46.07</td>
<td>266.34</td>
</tr>
<tr>
<td>Melting Point</td>
<td>°C</td>
<td>C7</td>
<td>5.53</td>
<td>115</td>
<td>174</td>
</tr>
<tr>
<td>Dissociation Constant</td>
<td>log pKₐ</td>
<td>C8</td>
<td></td>
<td></td>
<td>4.74</td>
</tr>
<tr>
<td>Solubility in Water</td>
<td>g/m³</td>
<td>C11</td>
<td>1.78E+2</td>
<td>6.78E+5</td>
<td>14</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>Pa</td>
<td>C12</td>
<td>1.27E+4</td>
<td>7.80E+3</td>
<td>4.15E-3</td>
</tr>
<tr>
<td>Octanol-Water Coefficient</td>
<td>log Kₗₐw</td>
<td>C13</td>
<td>2.13</td>
<td>-0.31</td>
<td>5.05</td>
</tr>
<tr>
<td>Half-life in air</td>
<td>hr</td>
<td>C33</td>
<td>1.7E+1</td>
<td>5.5E+1</td>
<td>5.50E+2</td>
</tr>
<tr>
<td>Half-life in water</td>
<td>hr</td>
<td>C34</td>
<td>1.7E+2</td>
<td>5.5E+1</td>
<td>5.50E+2</td>
</tr>
<tr>
<td>Half-life in soil</td>
<td>hr</td>
<td>C35</td>
<td>5.5E+2</td>
<td>5.5E+1</td>
<td>1.7E+3</td>
</tr>
<tr>
<td>Half-life in sediment</td>
<td>hr</td>
<td>C36</td>
<td>1.7E+3</td>
<td>1.7E+2</td>
<td>5.50E+3</td>
</tr>
</tbody>
</table>

*PCP = Pentachlorophenol
### Multimedia compartment model input data

<table>
<thead>
<tr>
<th>Chemical (emission scenario)</th>
<th>Total mass (kg)</th>
<th>Air</th>
<th>Water</th>
<th>Soil</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene (a)</td>
<td>$1.98 \times 10^4$</td>
<td>99.59</td>
<td>0.29</td>
<td>0.12</td>
<td>$1.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>Benzene (b)</td>
<td>$1.41 \times 10^5$</td>
<td>4.48</td>
<td>95.17</td>
<td>5.5×10^{-3}</td>
<td>0.35</td>
</tr>
<tr>
<td>Benzene (c)</td>
<td>$6.86 \times 10^4$</td>
<td>20.61</td>
<td>1.61</td>
<td>77.78</td>
<td>5.8×10^{-3}</td>
</tr>
<tr>
<td>Ethanol (a)</td>
<td>$4.56 \times 10^4$</td>
<td>92.87</td>
<td>3.85</td>
<td>3.28</td>
<td>2.9×10^{-3}</td>
</tr>
<tr>
<td>Ethanol (b)</td>
<td>$7.35 \times 10^4$</td>
<td>0.22</td>
<td>99.7</td>
<td>7.8×10^{-3}</td>
<td>0.08</td>
</tr>
<tr>
<td>Ethanol (c)</td>
<td>$7.84 \times 10^4$</td>
<td>0.92</td>
<td>5.64</td>
<td>93.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Pentachlorophenol (a)</td>
<td>$2.07 \times 10^6$</td>
<td>0.26</td>
<td>2.56</td>
<td>97.07</td>
<td>0.11</td>
</tr>
<tr>
<td>Pentachlorophenol (b)</td>
<td>$4.59 \times 10^5$</td>
<td>7.2×10^{-5}</td>
<td>96.19</td>
<td>0.03</td>
<td>3.78</td>
</tr>
<tr>
<td>Pentachlorophenol (c)</td>
<td>$2.39 \times 10^6$</td>
<td>2.9×10^{-4}</td>
<td>0.54</td>
<td>99.44</td>
<td>0.02</td>
</tr>
</tbody>
</table>

- a) 1000 kg/hr emitted into the air compartment
- b) 1000 kg/hr emitted into the water compartment
- c) 1000 kg/hr emitted into the soil compartment
$K_{ow}$ and Bioaccumulation

1-octanol

$K_{ow} = \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_{ow}$</th>
<th>$\log K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>0.18</td>
<td>-0.74</td>
</tr>
<tr>
<td>benzene</td>
<td>148</td>
<td>2.17</td>
</tr>
<tr>
<td>toluene</td>
<td>537</td>
<td>2.73</td>
</tr>
<tr>
<td>decachlorobiphenyl</td>
<td>182,000</td>
<td>8.26</td>
</tr>
</tbody>
</table>

$K_{ow}$ data available from CRC Handbook of Chemistry and Physics. Estimates from ChemDraw or from [http://www.vcclab.org/lab/alogps/start.html](http://www.vcclab.org/lab/alogps/start.html)
Estimation of $EC_{50}$

\[ \log EC_{50} = a + b \cdot \log K_{ow} \]

- A key parameter in the assessment of environmental risk and in the prediction of the fate of chemicals in the environment.
- Describes the hydrophobicity or hydrophilicity of a compound.
- It can be used to estimate $EC_{50}$ for *simple organisms* because $K_{ow}$ is the basis of correlations to calculate bioaccumulation and toxicity.
- Log $K_{ow}$ can be estimated using several programs such as KowWIN Program (atom/fragment contribution method).
  
  [http://www.epa.gov/opptintr/exposure/docs/episuite.htm](http://www.epa.gov/opptintr/exposure/docs/episuite.htm)

- **Group contribution methods:**
  
  Studies have been performed on the relationship between toxicity and chemical structure for several compounds.
# Henry’s Law Constant

![Diagram of Henry's Law](image)

**Hints:**

\[ H = \frac{2479}{10^C} \]

**Divide by 101,325 Pa/atm**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>H (25 °C), mol·kg⁻¹·bar⁻¹</th>
<th>H (25°C), Pa·m³·mol⁻¹</th>
<th>H (25°C), atm·m³·mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexane</td>
<td>0.00060</td>
<td>170,000</td>
<td>1.7</td>
</tr>
<tr>
<td>toluene</td>
<td>0.16</td>
<td>677</td>
<td>6.7 × 10⁻³</td>
</tr>
<tr>
<td>benzene</td>
<td>0.18</td>
<td>557</td>
<td>5.5 × 10⁻³</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>0.36</td>
<td>290</td>
<td>2.9 × 10⁻³</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>6.4</td>
<td>16</td>
<td>1.6 × 10⁻⁴</td>
</tr>
<tr>
<td>1-propanol</td>
<td>140</td>
<td>0.73</td>
<td>7.2 × 10⁻⁶</td>
</tr>
<tr>
<td>ethanol</td>
<td>200</td>
<td>0.51</td>
<td>5.0 × 10⁻⁶</td>
</tr>
</tbody>
</table>


Multi-Compartmental Models

EXAMPLE:
Estimate the four Z factors for DDT, and then calculate the concentration of DDT in each of the phases. If 1 mole of DDT were released into the environment, what % of the DDT would go into each phase? Assume the following data:

- $K_{oc} = 2.04 \times 10^5 \text{ m}^3/\text{ton}$
- $V_{\text{air}} = 10^{10} \text{ m}^3$
- $V_{\text{water}} = 7 \times 10^6 \text{ m}^3$
- $V_{\text{soil}} = 9 \times 10^3 \text{ m}^3$
- $V_{\text{sediment}} = 2 \times 10^4 \text{ m}^3$
- $H = 9.57 \times 10^{-6} \text{ atm m}^3/\text{mol}$

See: Life cycle assessment file
Books on Toxicity and Eco-toxicity of Chemicals

Endocrine Disruption:
- Theo Colborn, Dianne Dumanoski, John Peterson Myers *Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival?—A Scientific Detective Story*.

Toxicity and Eco-toxicity:
- Mark Cronin, Judith Madden, Steven Enoch, David Roberts *Chemical Toxicity Prediction; Category Formation and Read-Across* 2013 SBN: 978-1-84973-384-7