Environmental Health (3): Toxicology

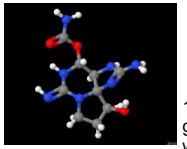
- Key concepts
  - Interdisciplinary field: studies adverse effects of chemicals on biological systems
  - All substances are potentially toxic (likelihood of human exposure is important)
  - Route of exposure is important
  - Structure of a chemical implies the relative level of toxicity and selectivity
  - Metabolic pathway modifies chemical form of substance, subsequently its toxicity
  - Basic toxicology testing is critical to risk assessment

What is toxicology?

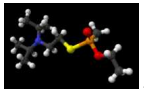
- Paracelsus (1493-1541, Physician, Alchemist) is called as "The father of toxicology"
  - "*Alle Ding sind Gift und nichts ohn Gift; alein die Dosis macht daß ein Ding kein Gift ist*"
    ("All things are poison and nothing is without poison; only the dose makes a thing not a poison")
- Definition: The scientific research to clarify the safety to human health of drugs and chemical substances.
- Core problem in medicine: any drug has both therapeutic and adverse effect on human body, which widely varies. Suppose:
  - Flu drug, to cause liver failure for 10% of users, is not acceptable.
  - New cancer drug, which can cure 80%, is acceptable even if it causes mild adverse effect for everyone

Lethal Dose 50 (LD50) of various substances

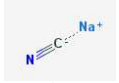
- Definition: the dose which kills a half of administered animals (mouse/rat) within a study period, in mg/kg body weight.
- The most popular indicator of acute toxicity of substances.



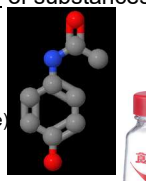
0.003 mg/kg
(saxitoxin, the best known paralytic shellfish toxin)



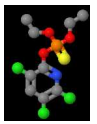
1 mg/kg (VX nerve gas, chemical weapon)



10 mg/kg
(Sodium cyanide)



500 mg/kg
(acetoaminophene)

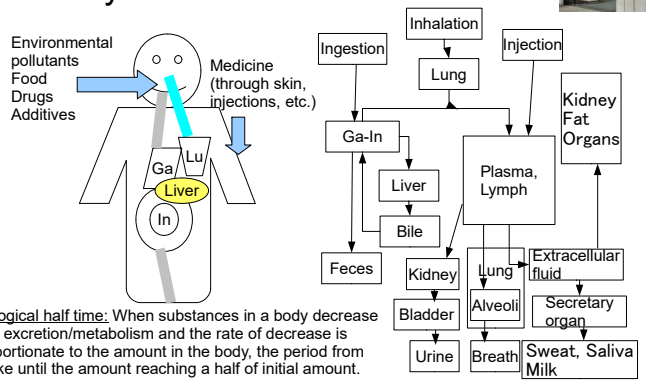


118 mg/kg
(Chlorpyrifos, insecticide)

2,400 mg/kg
(Sodium chloride)

(Note) White: hydrogen, Blue: nitrogen, Red: oxygen, Grey: carbon, Yellow: sulfur, Orange: phosphorus, Green: chlorine

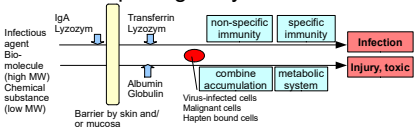
Body compartment model for dynamics of substances

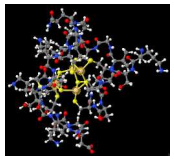


Biological half time: When substances in a body decrease with excretion/metabolism and the rate of decrease is proportionate to the amount in the body, the period from intake until the amount reaching a half of initial amount.

Biological protection system

- Via immune system
  - non-specific: phagocytosis (neutrophil, monocyte, macrophage), attack to cancer/virus infected cells (NK cell), natural immunity (IgM)
  - specific: acquired immunity (B, Helper/Killer T cells)
- Via non-immune system
  - enzymatic catabolization: fat-soluble -> water-soluble
  - metallothionein: induced by Cu, Zn, Cd (MW 6-7K)
  - superoxide elimination system: SOD, GPx, Catalase
  - DNA repairing enzyme





Cd-metallothionein

Absorption pathway

- via **gastrointestinal tract**
  - most materials absorbed from gastrointestinal organs go through portal vein to liver, then are metabolized
  - in mucosa of oral cavity, tongue surface, and mucosa of lower rectum, materials are directly absorbed
  - stomach easily absorb fat-soluble/acidulous substances
- via **lung**
  - alveoli absorbs air pollutants
  - some materials (eg. mercury) are more effectively absorbed as vapor from lung than as liquid from gastrointestinal tract
- via **skin**
  - usually low absorption efficiency due to simple diffusion
  - exceptions: sarin, tetrachlorocarbon, paraquat (herbicide)

Intra-body kinetics

- Whether a chemical substance has toxic effect or not depends on (1) sensitivity of host organ, and (2) concentration of the substance there
  - The concentration depends on intra-body kinetics composed of 4 factors (Absorption, Distribution, Metabolism, Excretion)
- Critical concentration: lowest concentration to harm tissue
- Target organ: the first organ where the substance accumulates up to critical concentration
- The highest concentration is not necessarily seen in target organ, because the sensitivity varies by organ

Target organs of toxicity

- Cadmium (Cd)
  - Chronic exposure -> Itai-itai disease (affecting bone)
  - Most cadmium accumulates liver, subsequently kidney: thus target organs are them
- Lead (Pb): hematopoietic system (bone marrow) -> decrease of hemoglobin and increase of reticulocytes
- Paraquat: lung
- Inorganic arsenic (As): No mutagenecity but carcinogenecity, probably inhibiting macrophage/NK cells

Distribution

- Distribution in the body differs by substances
  - DDT, thiopental accumulates to fat tissue
  - Inorganic mercury is more distributed to kidney, secondly liver and spleen, but methylmercury are equally distributed to any organs (incl. brain, fetus)
  - Cadmium accumulates liver and kidney (not in bone)
- Why differs by substances?
  - Host factors: various blood flow to each organ, tissue barrier (BBB, BPB)
  - Material factors: MW, fat-solubility, binding capacity with blood elements and tissue cells
    - fat-soluble substances have longer biological half-life

Metabolism

- Catabolization basically increases the excretion by increasing the polarity
- Basically reducing toxicity, but rarely the metabolic products have higher toxicity (metabolic activation)
- Stages of metabolic reaction
  - 1st phase: Increasing polarity by oxidation, reduction, or hydrolysis. In liver, most active. a kind of heme-proteins, **cytochrome P450** in hepatic microsome is most important in oxdation
  - 2nd phase: Cohesion with endogeneous substances like glucuronic acid, increasing ability of excretion
  - 3rd phase in excretion

Genetic polymorphisms of metabolic enzymes

Enzymes	Molecules	Substrate (external toxin)	Frequencies of deletion type	Effects of deletion
Cytochrome P450	CYP2C19	Mephenytoin, etc.	3% of Caucasian 20% of Japanese	More adverse effect
Alcohol dehydrogenase	ADH1	Ethanol	4-20% of Cauc. 90% of Japan.	More aldehyde production
Aldehyde dehydrogenase	ALDH2	Acetoaldehyde	Rare in Cauc. 40% of Japan.	"Flusher"
N-acetyl transferase	NAT2	Isoniazid (anti-tuberculosis)	60% of Cauc. 12% of Japan.	More adverse effect
Glutathione-S transferase	GSTM1, GSTT1, GSTP1	Epoxide	GSTM1=50%, GSTT1=38%	Cancer induction by smoking
UDP-glucuronide transferase	UGT1A1	Bilirubin	?	Crigier-Najjar syndrome
Thiopurine-methyl transferase	TPMT	Anti-leukemia, immunosuppr.	Deletion-homo 0.2-0.3% Cauc.	Suppression of bone marrow

Excretion

- Mainly from Kidney and Liver
- Excretion to urine: 25% of blood -> glomerulus -> 20% filtration (<MW 60000)
- Excretion to bile: from liver. Higher polarity materials are directly excreted into feces, lower polarity materials are cohesively coupled with glutathione or glucronic acid (after reabsorption from intestine; enterohepatic circulation), then conveyed to bile with transporters like MRP2 (Phase III)
- Other pathways of excretion: Intestine (PCB, DDT, etc.), Breastmilk (fat-soluble substances), breath, skin, saliva, tears

Classification of toxicity

- General toxicity (in terms of period to expression)
  - Acute: single exposure cause a toxic response within a short latent priod. Evaluated with LD50, LC50
  - Subacute: 1-3 months repeated exposures cause it.
  - Chronic: several months to a year exposure cause it.
  - Intergenerational: expression in the next generation
- Special toxicity (in terms of toxic responses)
  - Carcinogenecity: initiation / promotion
  - Mutagenecity: causing the mutation of genes
- Misc.
  - Reproductive toxicity, Neurotoxicity, Immune-toxicity, etc.

Evaluation of toxicity

- Target
  - human
  - experimental animal
- Types of testing (cf. OECD guideline)
  - Acute oral test: observe 2w after admin, sectio, LD50
  - Subacute: everyday admin 2-4w, sectio, NOEL
  - Chronic: Rodent+Non-rodent, at least 1yr repeatedly, NOEL, ADI, TDI
  - Misc: Carcinogenic test, Mutagenic test, Biomonitoring, etc.

Indicators of toxicity

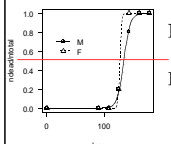
- Acute toxicity
  - LD50: lethal dose 50 = amount to kill a half
  - LC50: lethal concentration 50 = concentration to kill a half
  - ED50: effective dose 50 = amount to affect a half
- With threshold
  - Less than threshold, no toxic effect
- Without threshold
  - Within the tested doses, maxium dose with no observable effect is NOEL (or NOAEL for adverse effect)
  - The level should be adjusted by safety factor or uncertainty factor for the possible effect in the larger population or genetic variation
  - Virtually Safe Dose (VSD): setting the acceptable risk level. The amount to cause less risk than that is to be acceptable.

Reference doses (RfD)

- ADI (acceptable daily intake): For the substances to be intentionally used by human-beings, the daily intake level may have no risk even the human continue to have that level
- TDI (tolerable daily intake): For the substances which are not intentionally used but taken as environmental pollutants, the daily intake level cause no risk even the one continue to have that level
- Units are mg/kg body weight/day
  - NOAEL/NOEL/LOAEL for the most susceptible animal experiment are devided by safety factor (for ADI) or uncertainty factor (for TDI). Usually the factors are 10.

Dose-Response Relationships

- The responses of host animals may change with the dose.
- In population level, the proportion of responded animals changes with dose (toxic load). The relationship is usually S-shape. Approximated with cumulative logarithmic normal distribution.
- The dose to make 50% respond is ED50
- The dose to kill 50% is LD50
- ED50 or LD50 is estimated by probit/logit analysis.



$$\text{Probit}: F^*(X_i) = \Phi(\beta_0 + \beta_1 X_i), \Phi(z) = \int_{-\infty}^{z} \frac{1}{\sqrt{2\pi}} e^{-x^2/2} dx$$

$$\text{Logit}: F^*(X_i) = \Lambda(\beta_0 + \beta_1 X_i), \Lambda(z) = \frac{e^z}{1+e^z}$$

Calculation example: See, http://minato.sip21c.org/envhlth/dr2.R

Future Perspectives of Toxicity Testing

- Source: Collins FS, Gray GM, Bucher JR: Transforming environmental health protection. *Science*, 319: 906-7, 2008.