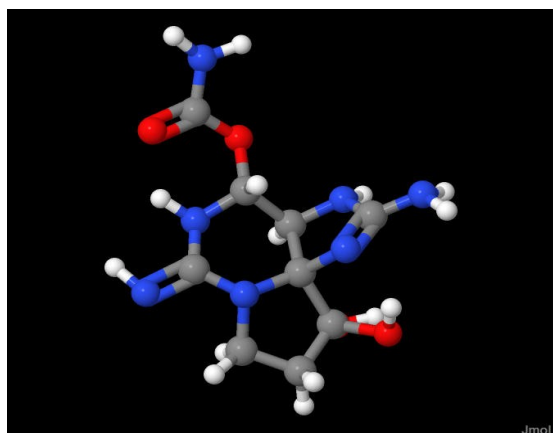


What is toxicology?

- Paracelsus (1493-1541, Physician, Alchemist) is called as "The father of toxicology"
 - *"Alle Ding sind Gift und nichts ohn Gift; allein 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")
 - See, <https://doi.org/10.1093/toxsci/53.1.2>
- Definition: **The scientific research to clarify the safety to human health of drugs and chemical substances.** (Since drugs and chemical substances constitute chemical environment, toxicology is important component of environmental health.)
- 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.
 - If the efficacy of vaccine to prevent disease occurrence is 90%, fatal adverse side effect is 0.001% and case fatality risk is 2% and prevalence is 0.1%, the vaccine is widely applicable. But if case fatality risk is 0.01%, the applicability of the vaccine with the same nature is questionable.

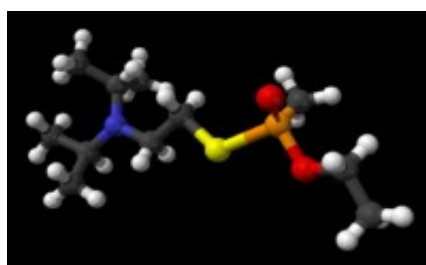
Lethal Dose 50 (LD50) of various substances

- 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.
- (FYI) Molecular model can be made by Chimera (<https://www.cgl.ucsf.edu/chimera/download.html>)

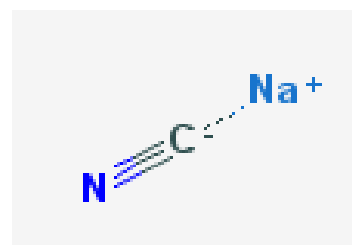


0.003 mg/kg
(saxitoxin, the best known paralytic shellfish toxin)

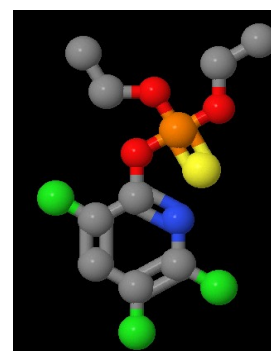
(Note) White: hydrogen, Blue: nitrogen, Red: oxygen, Grey: carbon, Yellow: sulfur, Orange: phosphorus, Green: chlorine



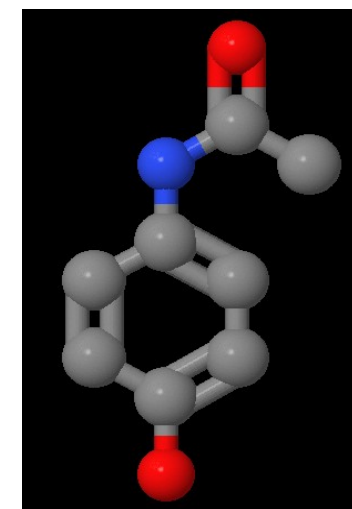
1 mg/kg (VX nerve gas, chemical weapon)



10 mg/kg
(Sodium cyanide)



118 mg/kg
(Chlorpyrifos, insecticide)

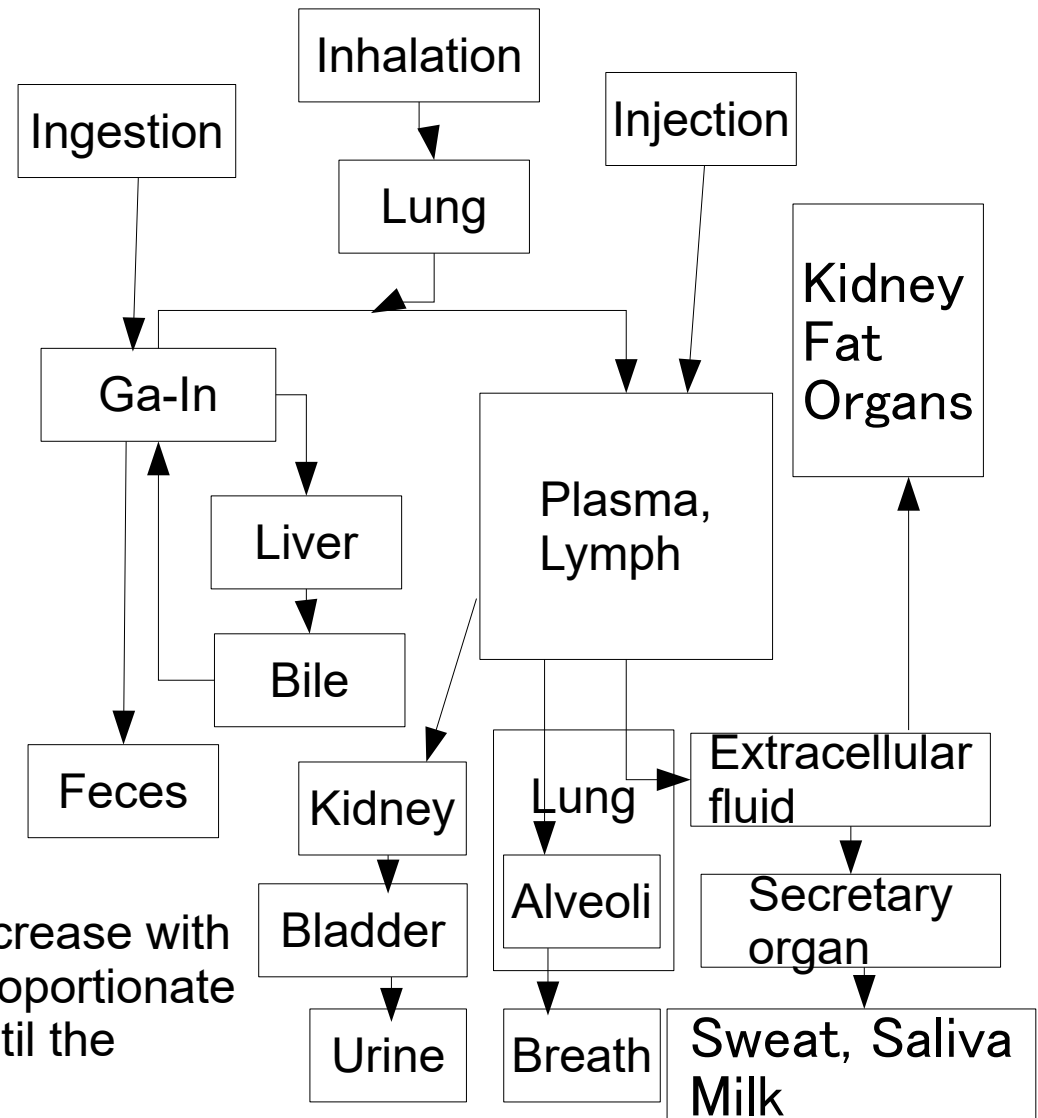
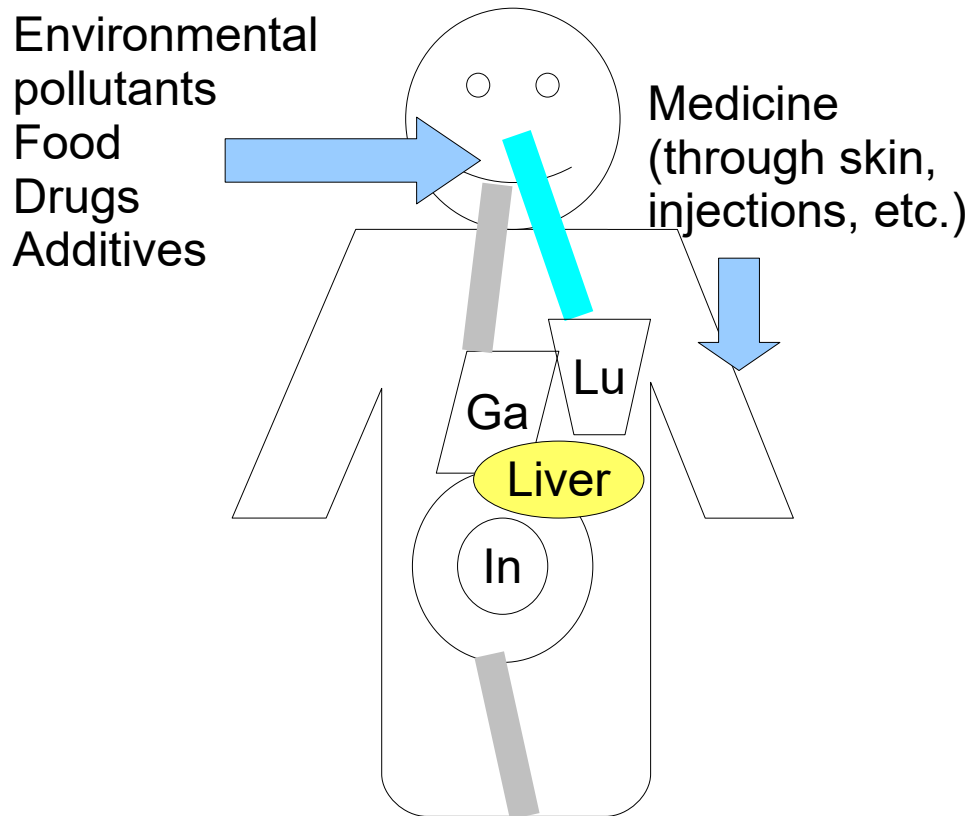


500 mg/kg
(acetoaminophene)



2,400 mg/kg
(Sodium chloride)

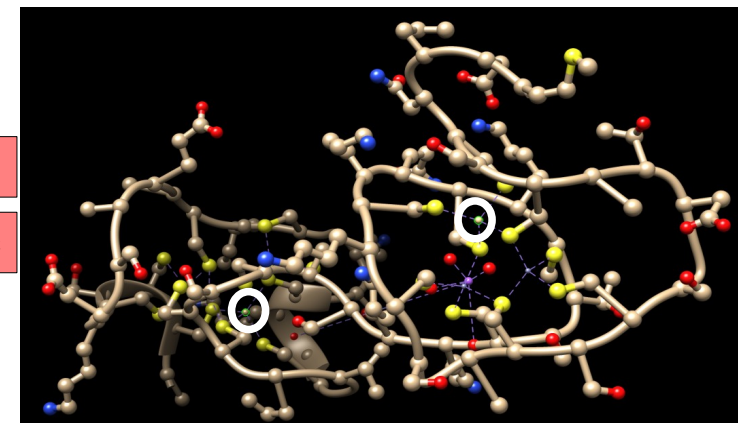
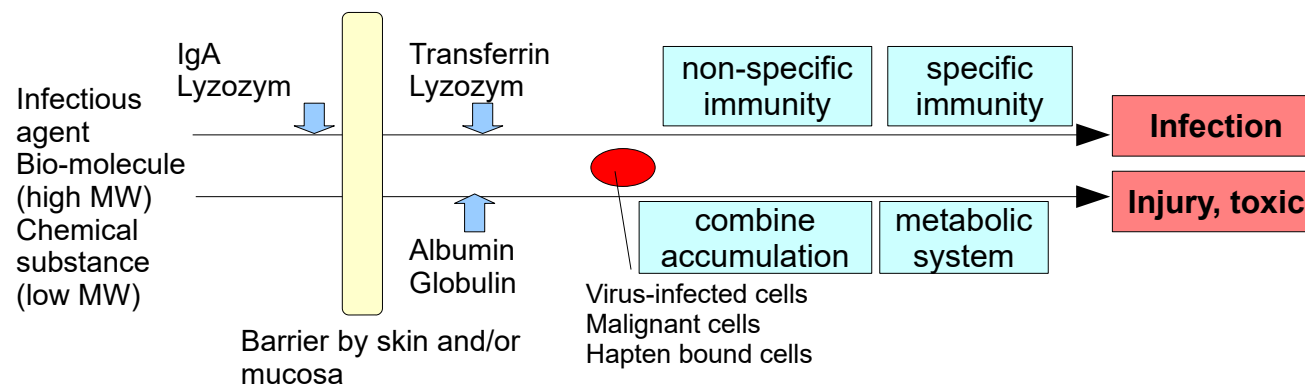
Body compartment model for dynamics of substances



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)
 - <https://doi.org/10.1016/j.mpaic.2021.06.006>
 - <https://doi.org/10.1016/j.imlet.2014.10.011>
- 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

(Data: <https://www.rcsb.org/structure/4MT2>)₄

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
 - 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
- The highest concentration is not necessarily seen in target organ, because the sensitivity varies by organ

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 oxidation
 - 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)
<https://www.lecturio.com/concepts/glomerular-filtration/>
- 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

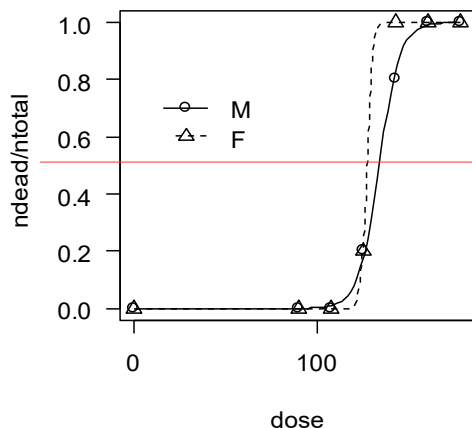
- Acute toxicity
 - LD50: lethal dose 50 = amount to kill a half
 - LC50: lethal concentration 50 = concentration to kill a half
 - ED50: effective dose 50 = amount to affect a half
- With threshold
 - Less than threshold, no toxic effect
- Without threshold
 - Within the tested doses, maximum dose with 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 divided 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. <https://dx.doi.org/10.1126%2Fscience.1154619>
- Hartung T (2009) "Toxicology for the twenty-first century" *Nature* <https://www.nature.com/articles/460208a>
- Krewski D et al. (2020) "Toxicity testing in the 21st century: progress in the past decade and future perspectives" *Arch. Toxicol.* <https://doi.org/10.1007/s00204-019-02613-4>

