C2003 – Toxicology

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22 November 2017

Outline

- General Characteristics of the Toxic Response
- Characteristics of exposure
- Dose – response relationship
- Absorption & routes of exposure
- Systemic toxicity
- Toxicological applications

Katerina in brief

- BSc in Biological applications & technology (UOI - GR)
  - BSc thesis: Feeding behaviour of *Atherina boyeri* in the Nestos river lagoons
- MSc in Environmental sciences – toxicology (WUR- NL)
  - MSc thesis: Effect of xenobiotics on thyroid stimulating hormone (TSH) hormone activity and synthesis
- PhD in Environmental Science – Uni of Reading (UK)
  - PhD thesis: *In vitro* bioaccessibility of emerging flame retardants present in indoor dust using simulated human fluids
- Post doc at RCX (CZ):
  - human exposure to personal care products and cosmetics

The dose makes the poison

“Alle Ding sind Gift ……
allein die Dosis macht dass ein
Ding kein Gifft ist”

Paracelsus (1493-1541)

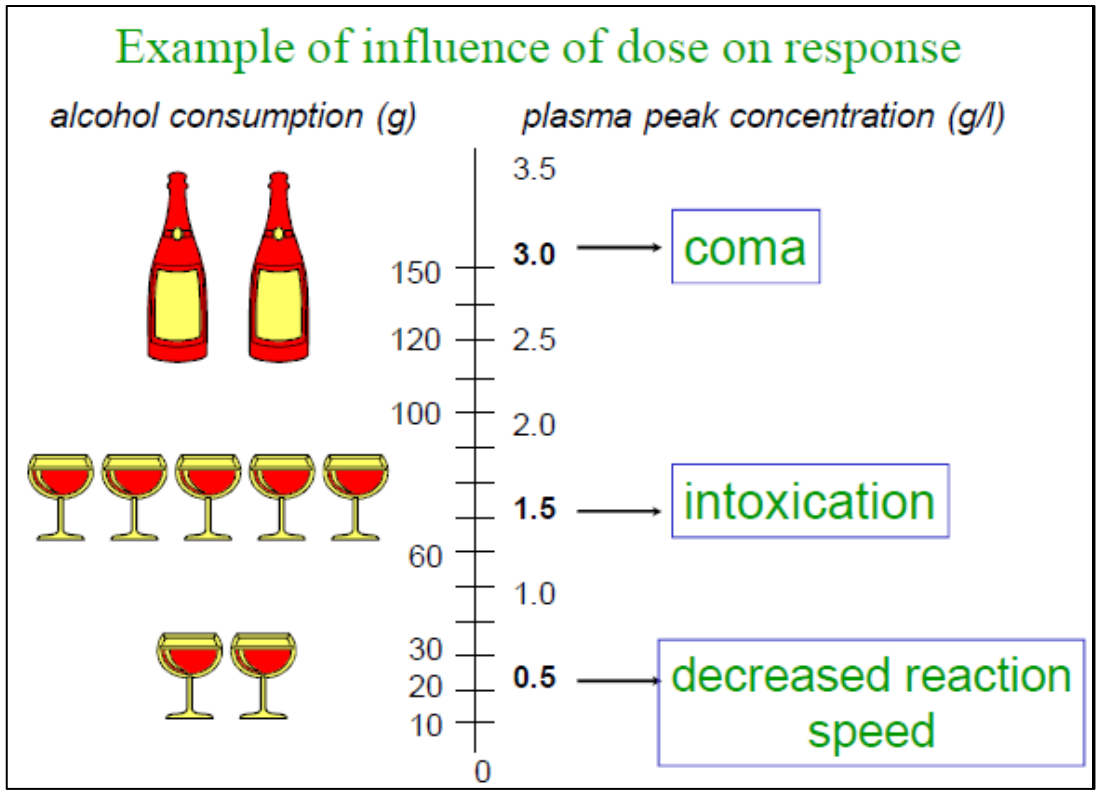
All substances are poisons,
only the dose makes a distinction
between one which is a poison
and one which is a remedy

Could you name examples of toxic substances and their biological responses/implications you are mostly familiar with?

Table 2.1
Approximate Acute LD_{50}s of Some Representative Chemical Agents

AGENT	LD_{50}, MG/KG*
Ethyl alcohol	10000
Sodium chloride	4000
Ferrous sulfate	1500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
d—Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

*LD_{50} is the dosage (mg/kg body weight) causing death in 50% of exposed animals.



Taken from CASARETT AND DOULL'S TOXICOLOGY: THE BASIC SCIENCE OF POISONS, 7th edition (2008)

To tox or not to tox?

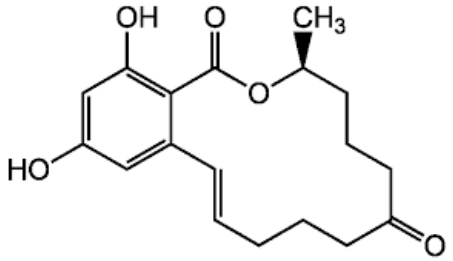
1. Strength of association (relationship between independent and dependent variables)
2. Consistency of findings (replication of results by different studies)
3. Biological gradient (strength of the dose-response relationship)
4. Temporal sequence (“cause” before effect)
5. Biologic or theoretical plausibility (mechanism of action)
6. Coherence with established knowledge (no competing hypotheses)
7. Specificity of association (cause is tightly linked to an outcome)

Short history of the chemical age

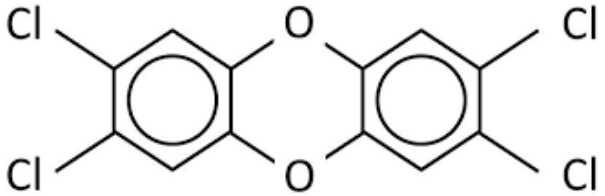
- late 18th century: industrial revolution (coal)
  (regional air pollution, chimney sweeps)
- 20th century: age of oil (petroleum based products)
- 1920s anti-knock fuels containing lead
- 1913 manufacture nitrogenous fertilizers
- trace metal contamination (e.g. mercury, cadmium)
- ≥ end of WW II: DDT and other biocides
- industrial compounds (e.g. PCBs, CFCs) ≥ 1929 & PBDEs (>1970s)

How do we define a toxic agent?

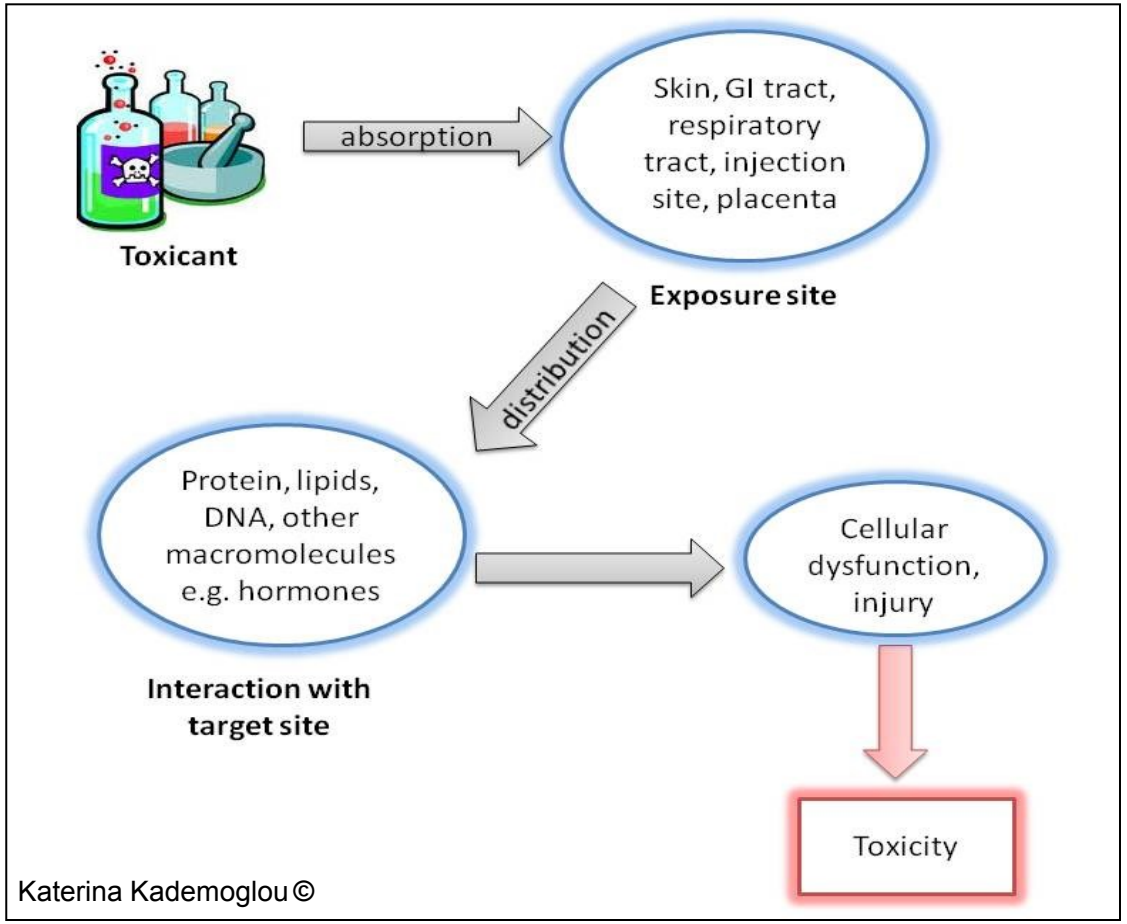
- Toxin: toxic substances that are produced by biological systems such as plants, animals, fungi, or bacteria
  - E.g zeralanone produced by a mold; log Kow = 3.6
  - hepatotoxic mycotoxin with estrogenic and anabolic activity



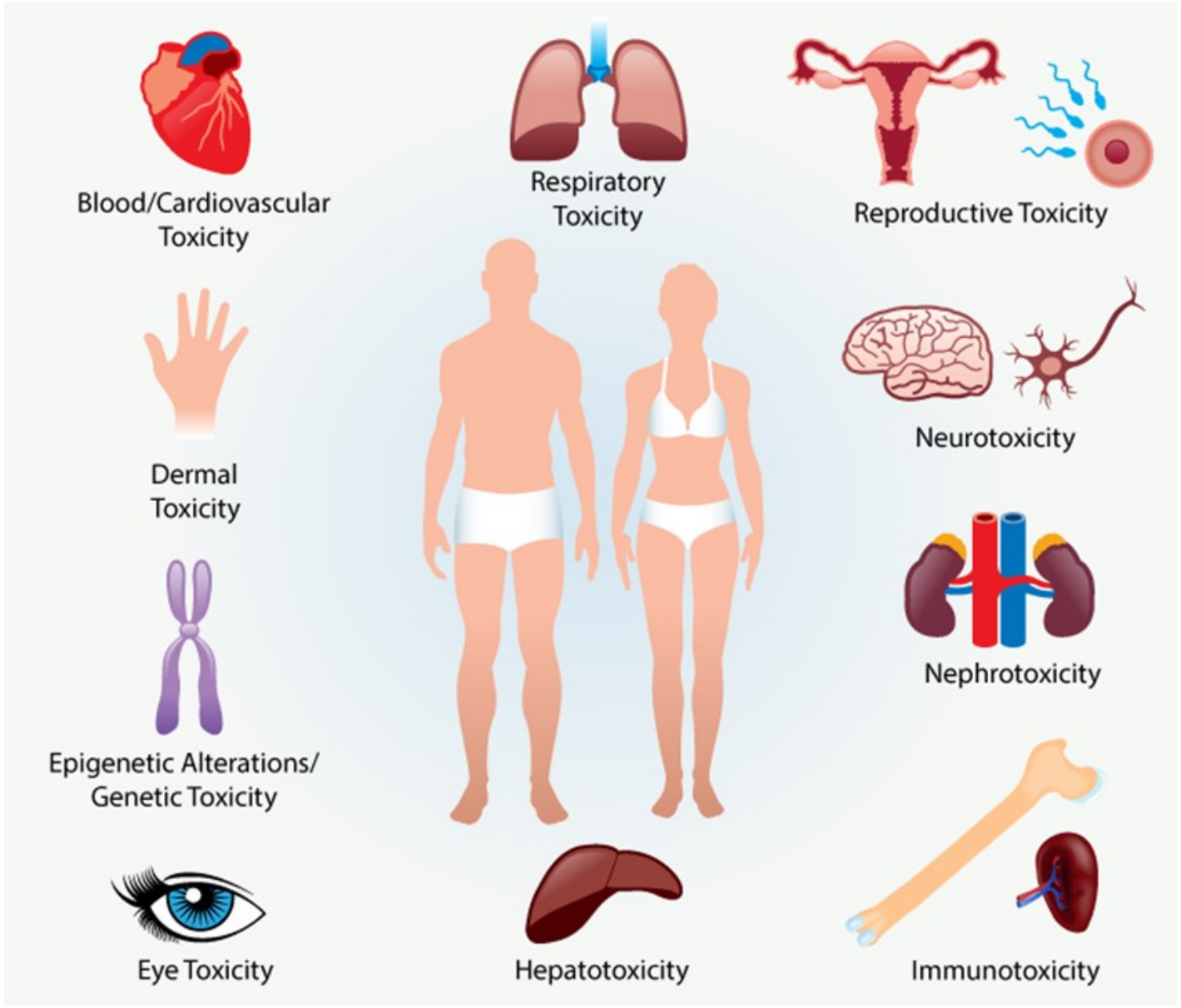
- Toxicant (or xenobiotic): toxic substances that are produced by or are a by-product of anthropogenic activities
  - E.g. dioxin; 2,3,7,8-tetrachlorodibenzo-*p* dioxin (TCDD); log Kow = 7.05
  - impairment of the immune system, development of nervous system & endocrine homeostasis



Toxicant exposure & toxicity development



- <u>Local effects</u>
  - occur at the site of first contact between the biological system and the toxicant (e.g. skin irritation)
- <u>Systemic effects</u>
  - require absorption and distribution of a toxicant from its entry point to a distant site (target organ, receptor etc.) at which deleterious effects are induced



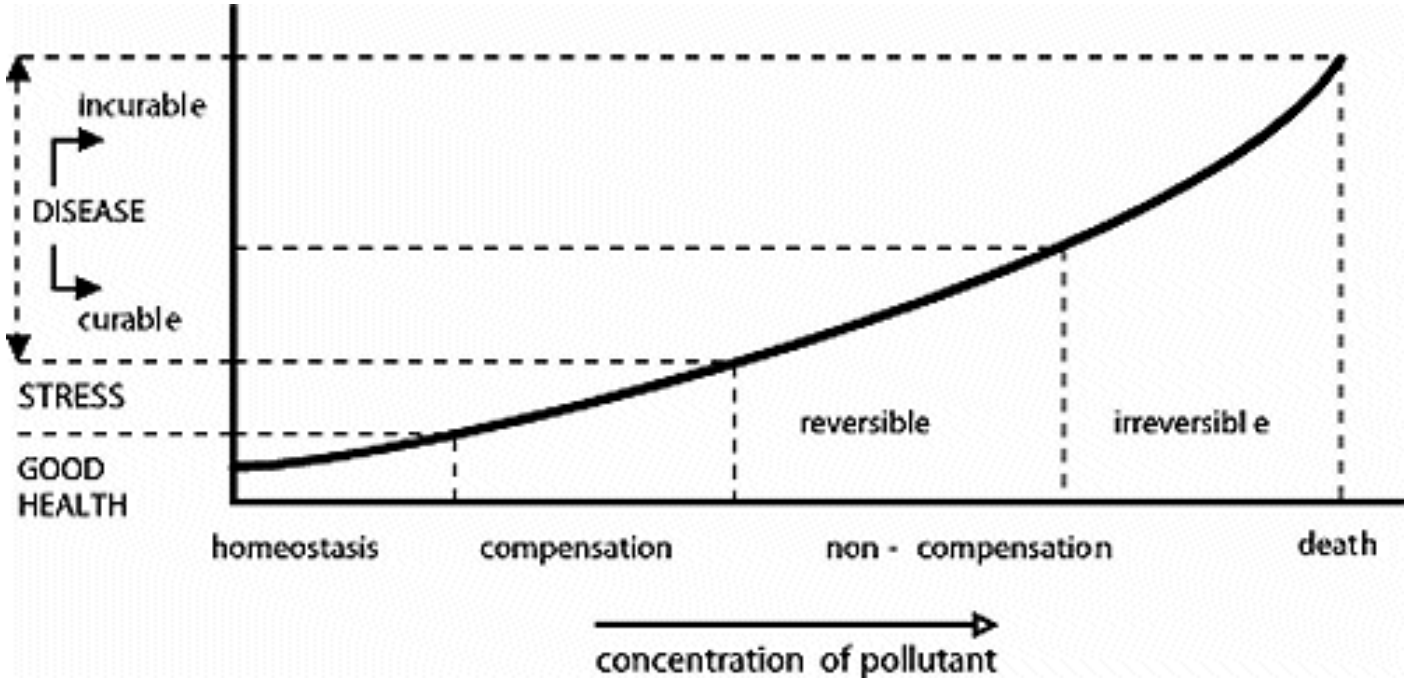
Things to consider

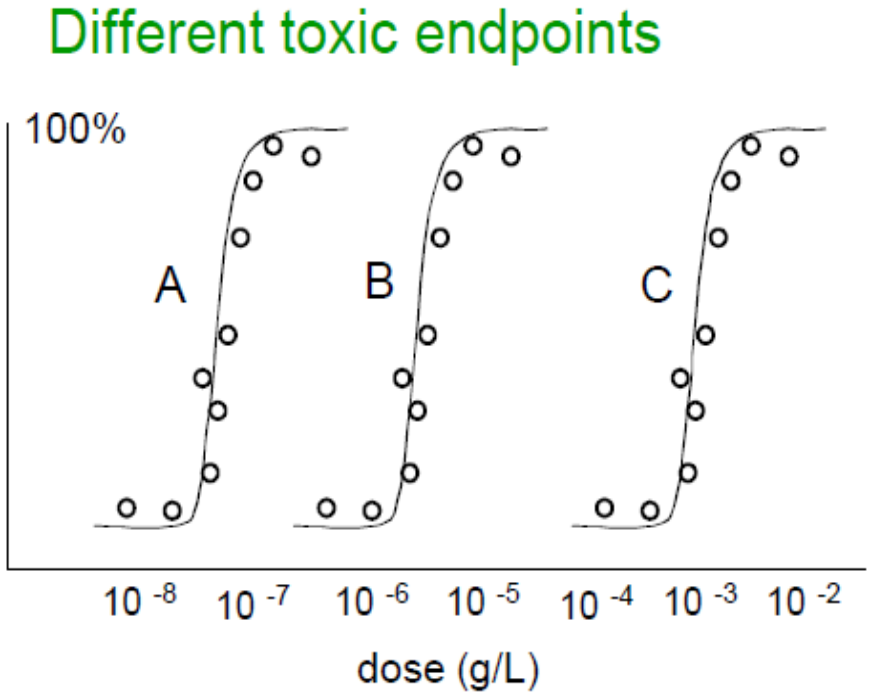
Toxicity duration:

- Chronic
- Subchronic
- Acute

Timing:
Is there a critical time during a lifetime when a chemical is most toxic (e.g., fetal development, childhood, during aging)

Health status in relation to exposure of a pollutant





E.g.
A=endocrine disruption, B=renal damage, C=death

External exposure

Absorption

Distribution

Excretion (metabolites)

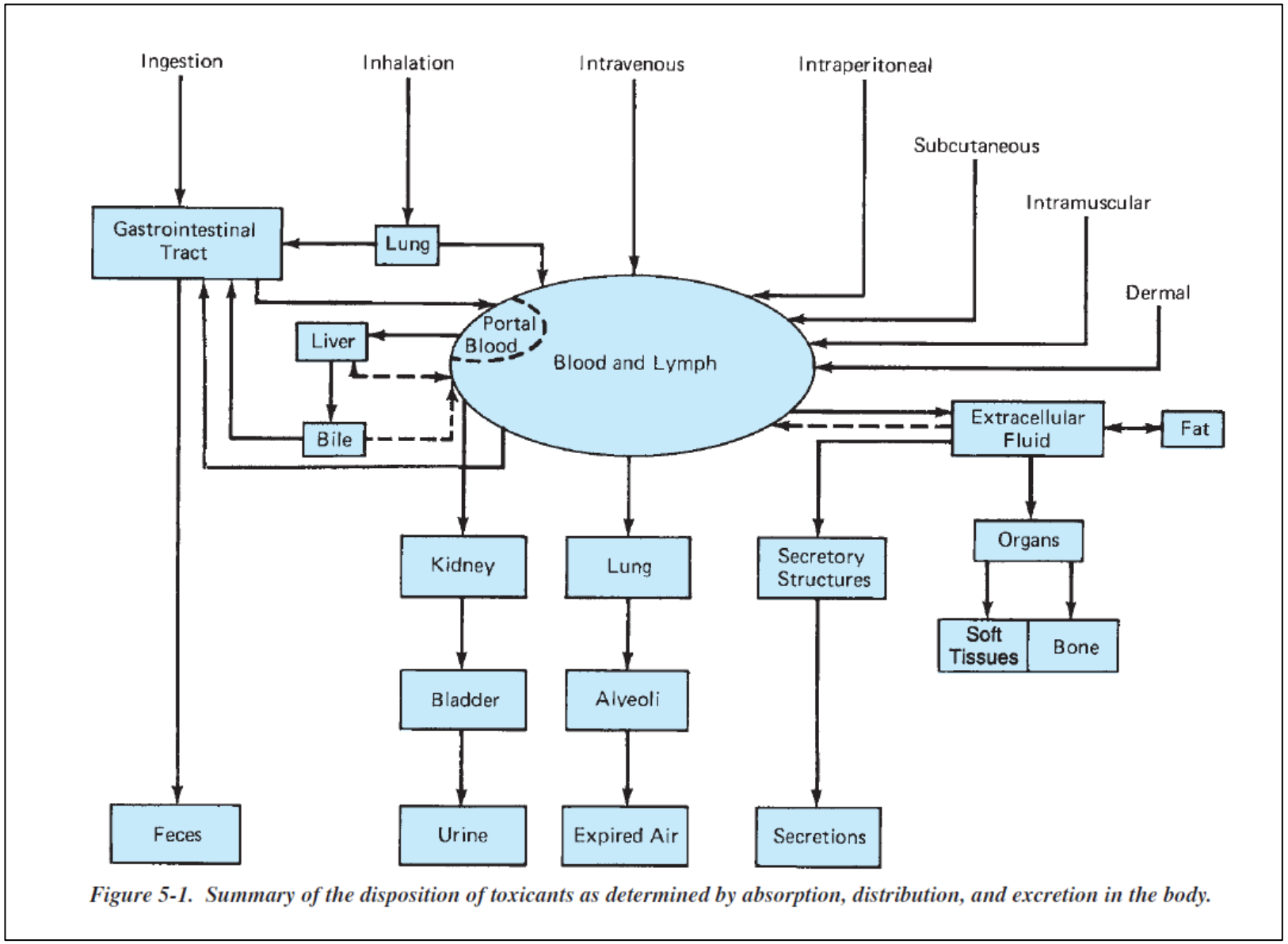


Figure 5-1. Summary of the disposition of toxicants as determined by absorption, distribution, and excretion in the body.

Absorption mechanisms & biological barriers (I)

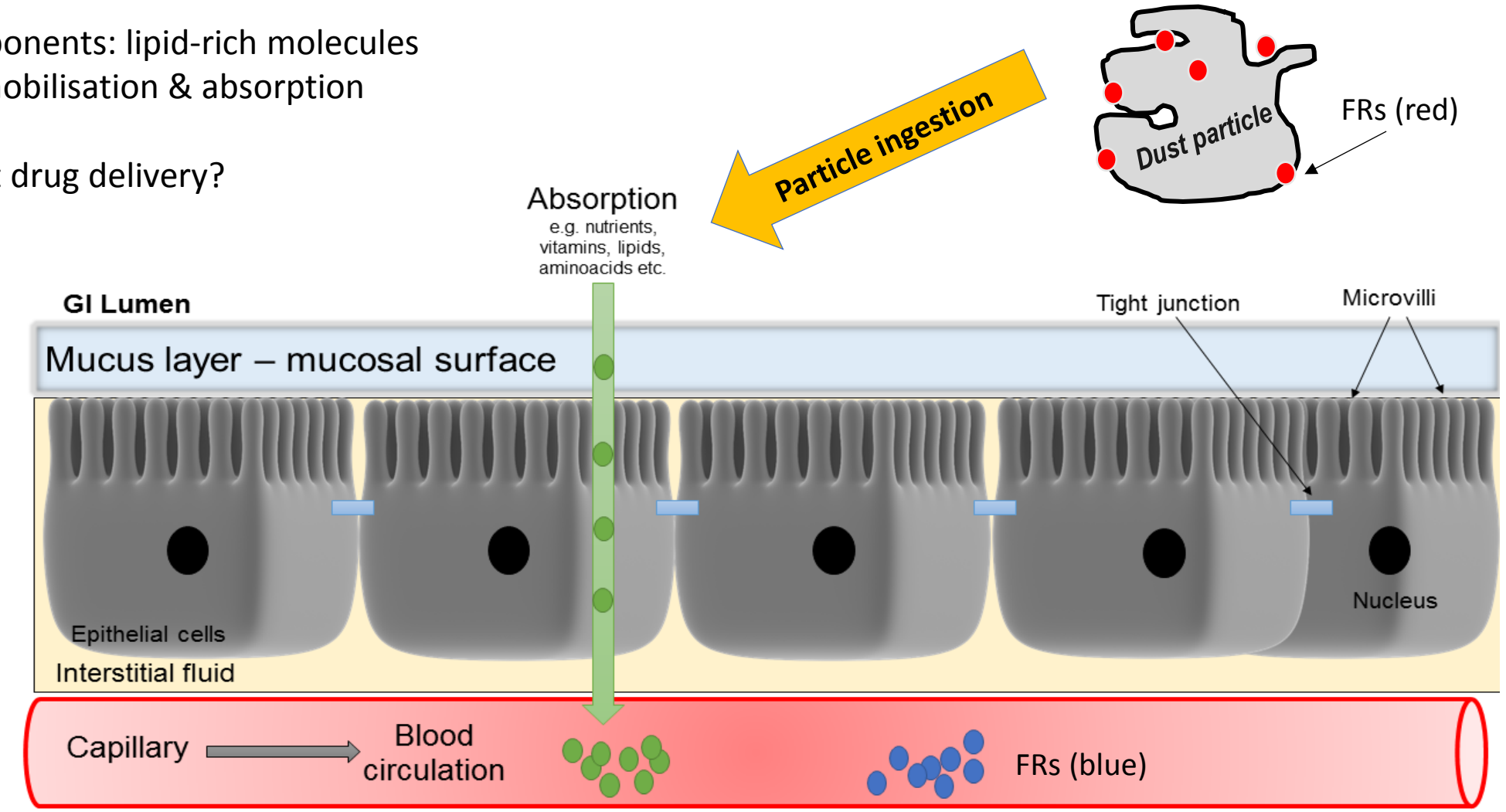
- Passive diffusion:
  - passage across (*i.e.* permeation) a biological membrane by solubilizing within the lipid bilayer
  - unforced (*i.e*. no energy expenditure)
  - Driven by: concentration gradient, hydrophobicity, MW, ionization etc.
- Active diffusion / transport:
  - transfer across membranes against a concentration gradient by a specialised carrier molecule (e.g. protein)
  - Energy-expensive process
  - Limited importance for absorption of chemicals → important in the elimination of chemicals by the liver and the kidneys

- Facilitated diffusion
- Filtration
- Endocytosis

Absorption mechanisms & biological barriers (II)

Food components: lipid-rich molecules enhance mobilisation & absorption

How about drug delivery?



Lungs – inhalation

- Toxicant absorption:
  - gases or vapours e.g. low MW PAHs
  - liquid or solid particles
- some fraction of them will undergo absorption into the bloodstream
- The rest will be either deposited locally or eliminated by exhalation even before being absorbed

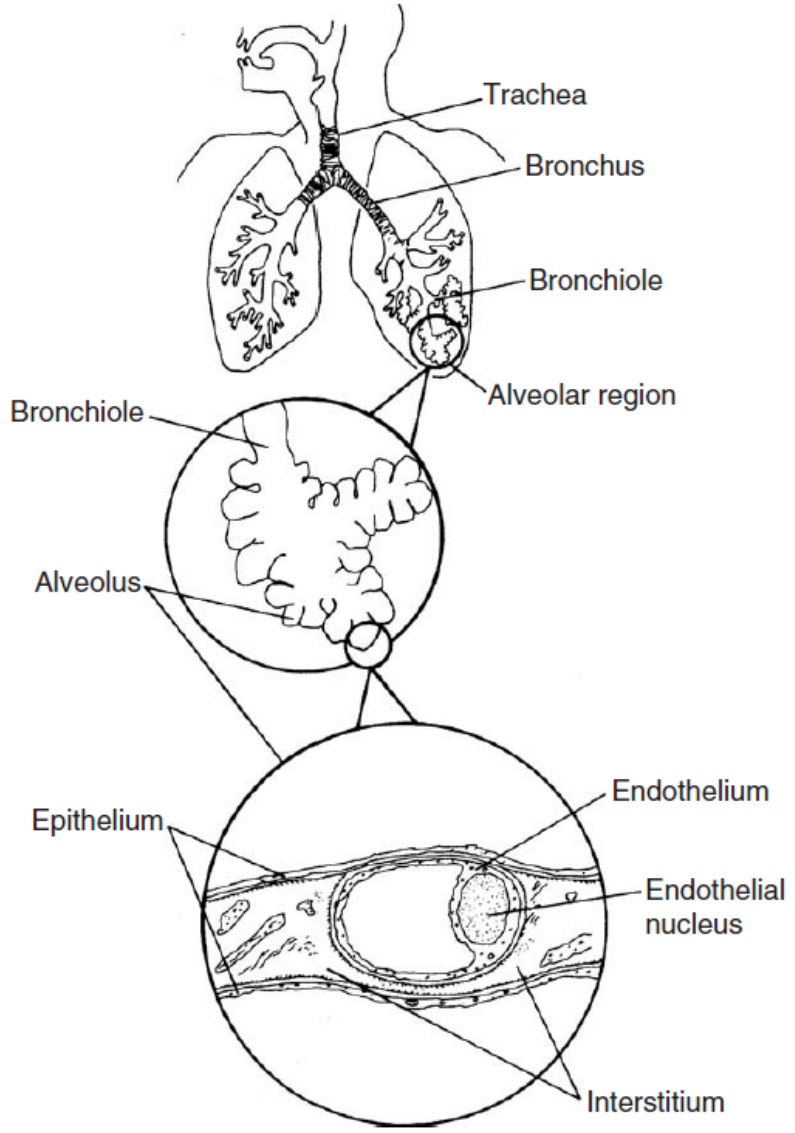


Figure 3 The anatomy of the respiratory tract from trachea to alveolus. (Reproduced from Smith RP (1992) The anatomy of the respiratory tract from trachea to alveolus. *A Primer of Environmental Toxicology*, p. 67. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

Skin - dermal uptake

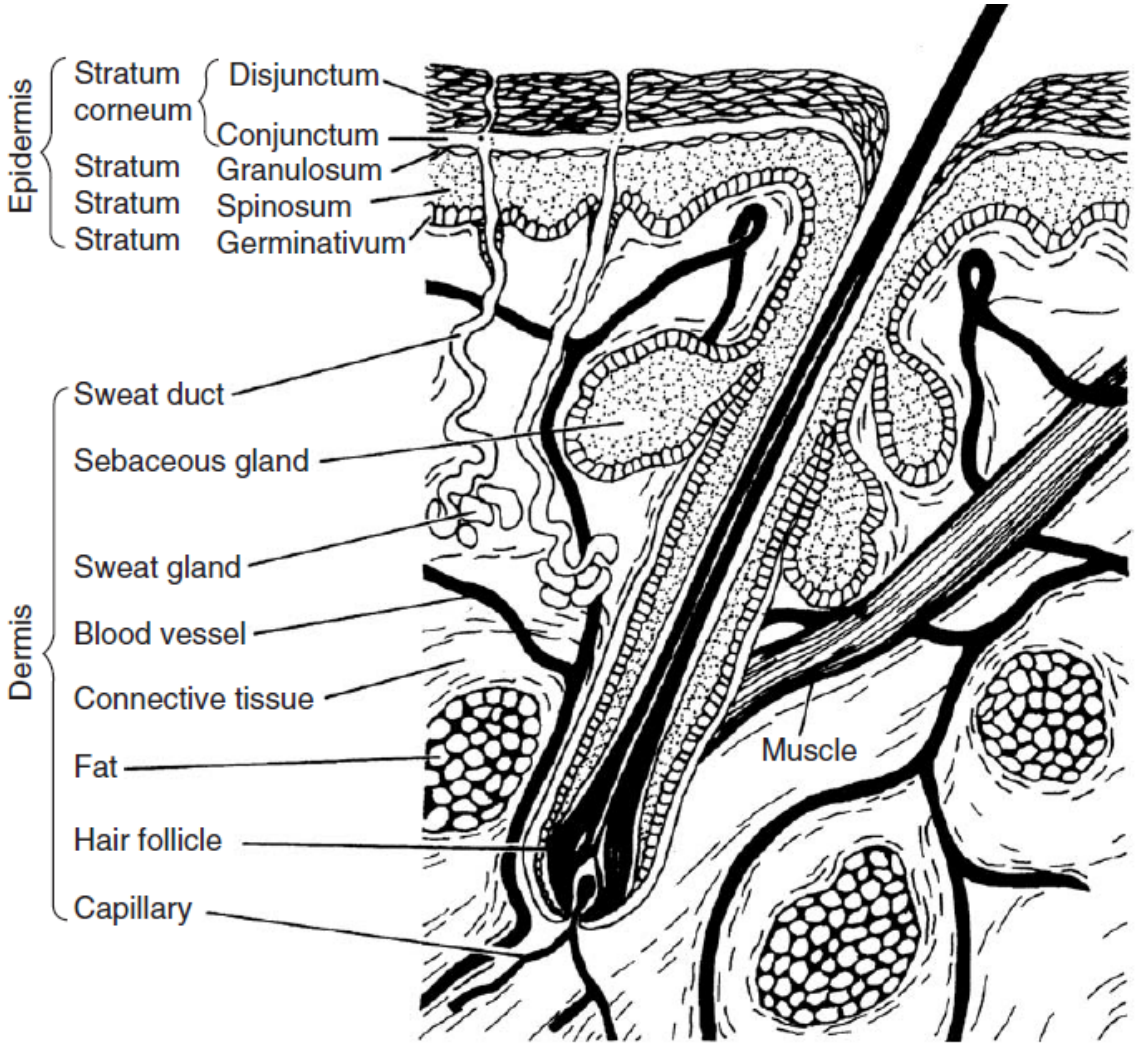


Figure 2 The organization of the skin as a biologic barrier. (Reproduced from Smith RP (1992) The organization of the skin as a biological barrier. *A Primer of Environmental Toxicology*, p. 73. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

<u>Absorbed through the skin</u>:

- organophosphate insecticides
- tetraethyl lead
- certain organic solvents
- dyes e.g. aniline are relatively

Does the environmental hazard travel throughout the body or does it stay in one place?

- Rapid vs restricted distribution:
  - Membrane permeability → tissue, organ distribution → systemic toxicity
- Accumulation & tissue storage depot:
  - protein binding, active transport, or high solubility in fat
  - in equilibrium with plasma bioavailable fraction → xenobiotic release into the circulation is eliminated

<u>However</u>:

If a toxicant accumulates at a site other than the target organ or tissue, the accumulation is likely to be protective because plasma levels and consequently its concentration at the site of action is reduced. In this case, it is assumed that the chemical in the storage depot is toxicologically inactive.

Storage in tissues

- Plasma Proteins:
  - Albumin (most abuntant plasma protein) & phenylbutazone
  - Bioavailable fraction of xenobiotic bount to plasma proteins → not availacle asap for distribution to tissues – critical point for toxicity manifestation!
- Fat tissue:
  - Pesticides, dioxins, PCBs → rapid absorption and uptake via cellular lipid bilayer
  - inert potency until fat loss, rapid mobilisation & release to blood circulation
- Liver and Kidney:
  - most important “filtration” organs of human physiology - metabolism & biotransformation
- Bone deposition→ e.g. fluoride & lead
- Placental barrier:
  - penetration by passive diffusion
  - Biotransformation
  - Maternal plasma conc. = conc. in foetus (steady state conditions)

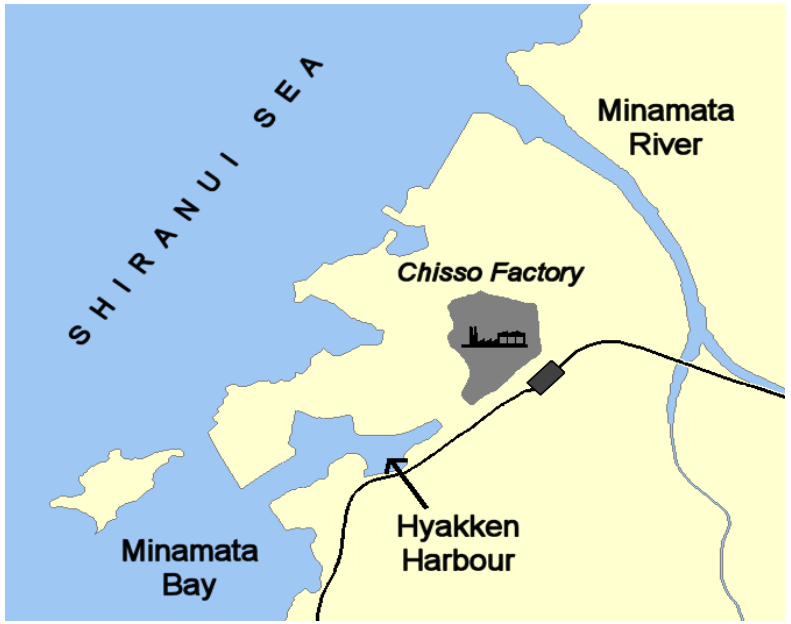
Polar bear

- seals are the preferred prey of polar bears
- Norway's arctic Svalbard archipelago:
  polar bear fat averaged 30 ug PCB/g = 10x the average for Alaska and 5x average for Canadian bears; some individuals from Svalbard 80-90 ug/g
- pregnant females hibernate
- out of 14 likely mothers, only 5 gave birth instead of the expected 11 or 12

- current standard for toxic waste:
  5 ug PCB/g lipid…..

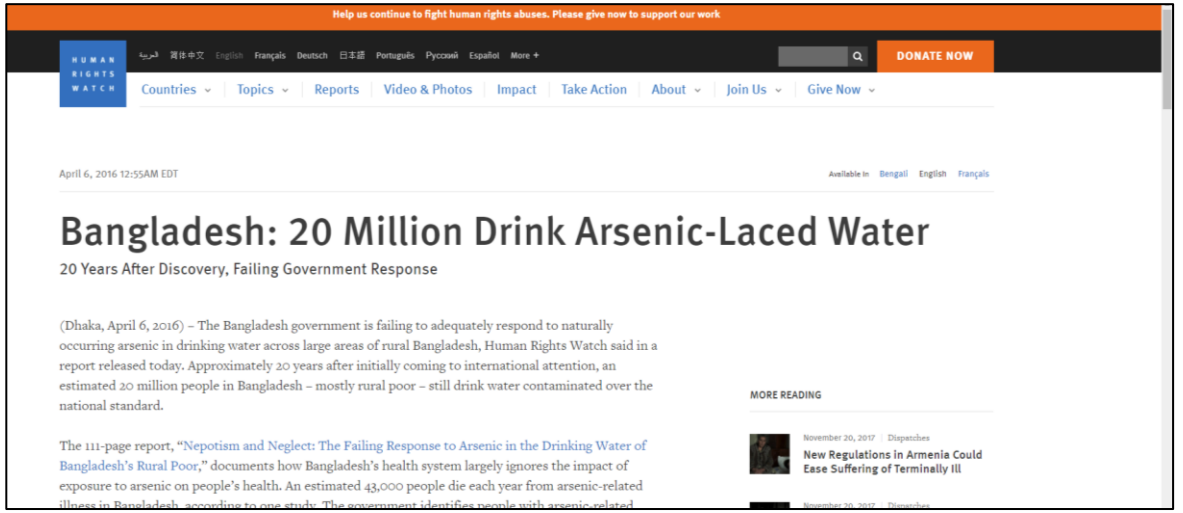
Minamata disease & neurotoxicity

- between 1932 - 1970 huge amounts of mercury were discharged into Minamata bay (SW Japan)
- methylmercury (lipophilic) accumulated in humans and food chain
- 1st: birds dropped from the sky, animals behaved strangely, cats died
- Chisso factory produced fertilizer and plastics
  - Inorganic mercury → used in PVC production
  - Methylmercury (by-product) was discharged into sea
- 17K patients & 1200 deaths



Arsenic in Bangladesh

- Up to 85 of the 125 million at risk from arsenic contaminated
- water from wells dug during 1980s and early 1990s.
- Problem unnoticed until victims began showing external symptoms of arsenic poisoning: calluses on the palms and soles of feet, leading to skin cancers



Natural toxin Aconitum spec. (Monkshood)

- Very potent poison (3-6 mg can be fatal)
- Uptake through skin (risk florists)
- cardiac arrhythmias (slowing heart rate)
- hypotension (↓ blood pressure)
- Used as an alternative medication to treat common cold symptoms as well as anxiety and fear (!)

The case of DTT

https://www.youtube.com/watch?v=QTV3XFHzvT4

Made in 1874; recognized as pesticide in 1939

- ‘Magic dust’ against malaria, dengue fever, typhus; used by military to control insects

- By the 1960s widespread agricultural pesticide

But:

- Malaysia: thatched roofs came down, cats died

- Insectivorous birds died/didn’t reproduce

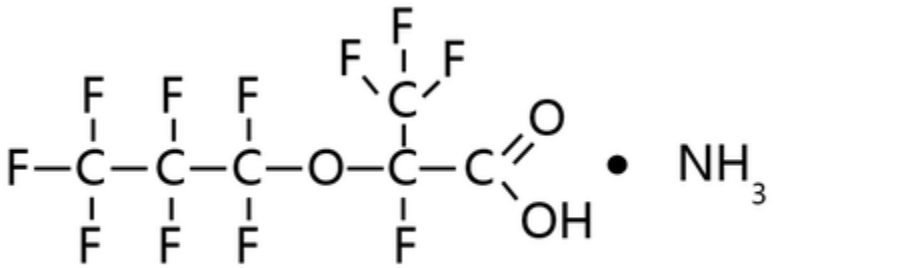
- Present in e.g. every human

- Banned in most Western countries mid 1980s; still in use in other countries

- debate about using it again (price/resistance)

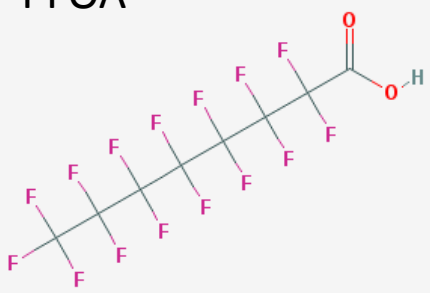
GenX in North Carolina

- DuPont introduced GenX in 2009 to replace PFOA
- GenX is associated with some of the same health problems as PFOA, including cancer and reproductive issues



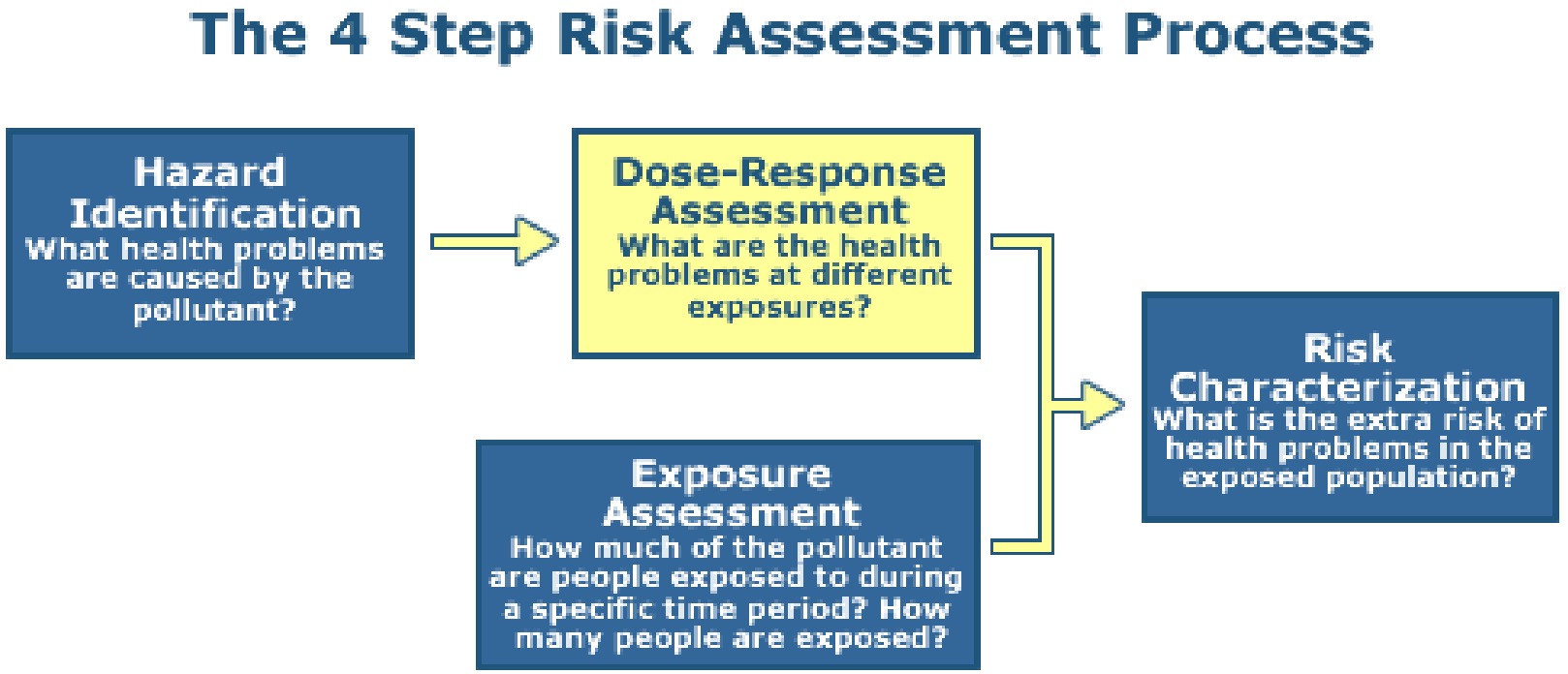
GenX Chemical Structure

PFOA



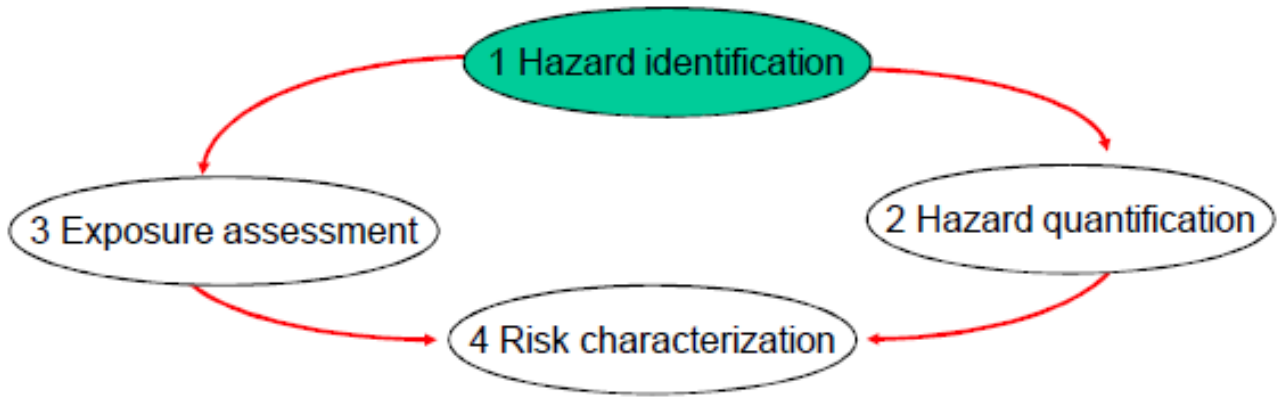
https://theintercept.com/2017/06/17/new-teflon-toxin-found-in-north-carolina-drinking-water/

High hazard & low risk – a tiered approach



https://www.epa.gov/risk/conducting-human-health-risk-assessment

Hazard Identification



-chemical properties
-structural similarities
-uses/sources
-epidemiological and experimental data
-critical effect
-target organ(ism)

Hazard identification (II)

Qualitative indicators for toxicity (I)

Type of effects

- neurotoxic (affects nervous system)
- hepatotoxic (affects liver)
- nephrotoxic(affects kidney)
- carcinogenic (induction of cancer)
- mutagenic (induction of mutations)
- teratogenic (induction development disorders)
- sensitization (allergic type reaction)
- dermal effects
- reproductive effects

Qualitative indicators for toxicity (II)

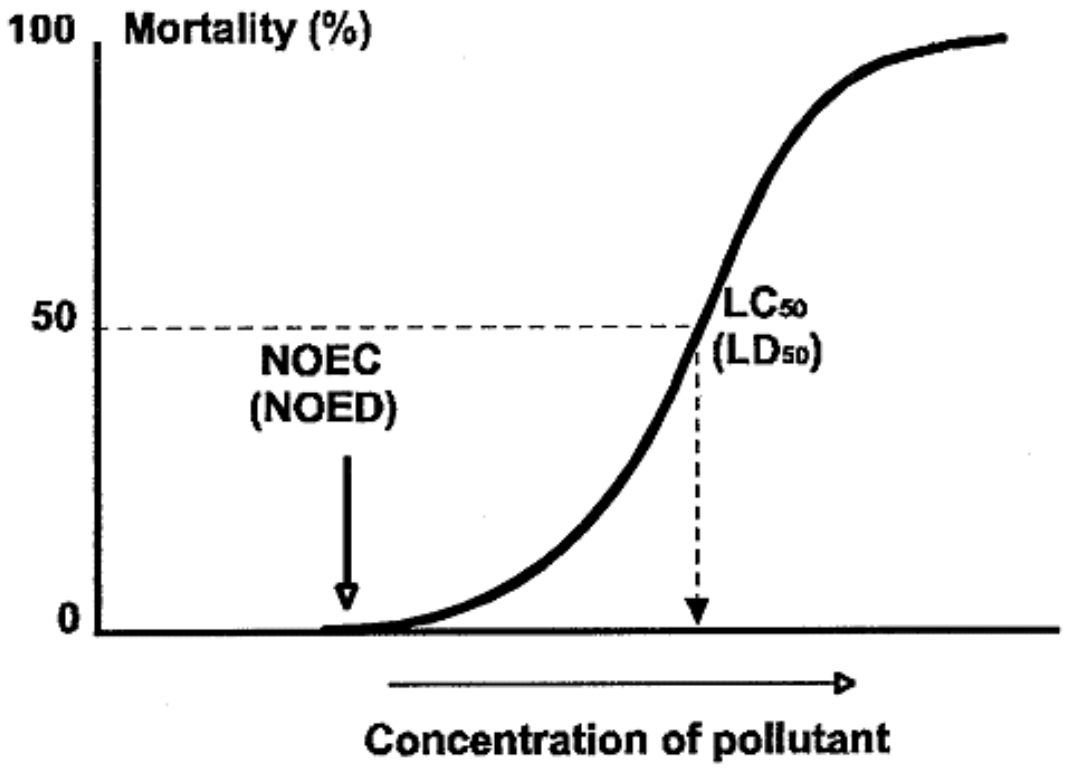
Mode of action

- inhibition of specific enzymes, e.g.
  - cytochrome oxydase (HCN,H_2S)
  - acetylcholinesterase (OP-compounds)
- specific reactions, e.g.
  - alkylation of DNA
- competition with hormones, vitamins, metabolites

Hazard quantification
(dose-response assessment)

-NOAEL, LOAEL, Benchmark dose
-route of exposure (relevant?)
-duration of exposure?
-life stage?
-extrapolation from animal to man

Dose – response relationships (I)



NOEC: highest *tested* concentration where effect is not *significantly* different from control

What's wrong?

- No ***statistically significant*** effect does not mean ***no*** effect
- Large variability in response leads to high NOECs
- Inefficient use of data (most data are ignored)
- No confidence intervals can be generated

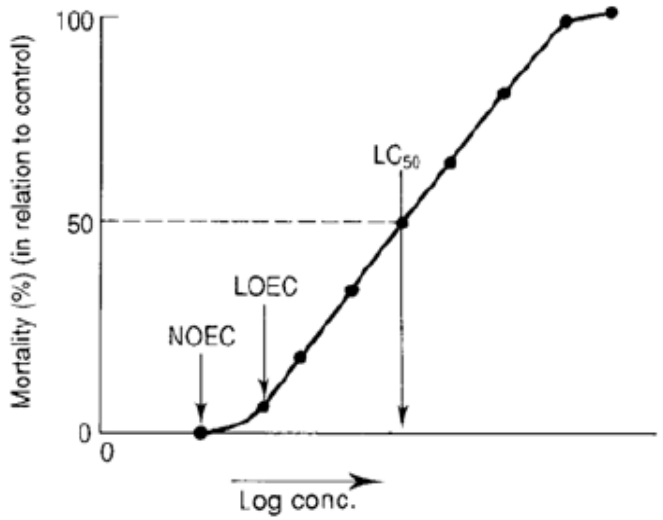


FIGURE 6.1 *Toxicity after 96 h exposure in an aquatic toxicity test. It should be noted that NOEC can be determined only where LOEC is known – otherwise there would be no indication of a concentration that can be toxic. NOEC, no observed effect concentration; LOEC, lowest observed effect concentration; LC_{50}, median lethal concentration at 96 h.*

In vivo
(whole organisms)
or *In vitro*
(cells, protein)

Definition of some exposure endpoints

ED: Effective Dose

EC: Effective concentration

ED50: Dose with 50% effect

EC50: Concentration with 50% effect

LD50: Dose that causes 50% mortality

LC50: Concentration that causes 50% mortality

NOEC: No Observed Effect Concentration

NEC: No Effect Concentration (*calculated!*)

Dose - response relationship

- Chemical A: flat response → large change in dosage is required before a significant change in response will be observed
- Chemical B: steep response → a relatively small change in dosage will cause a large change in response
- How about potency, ED50 & NEC ?

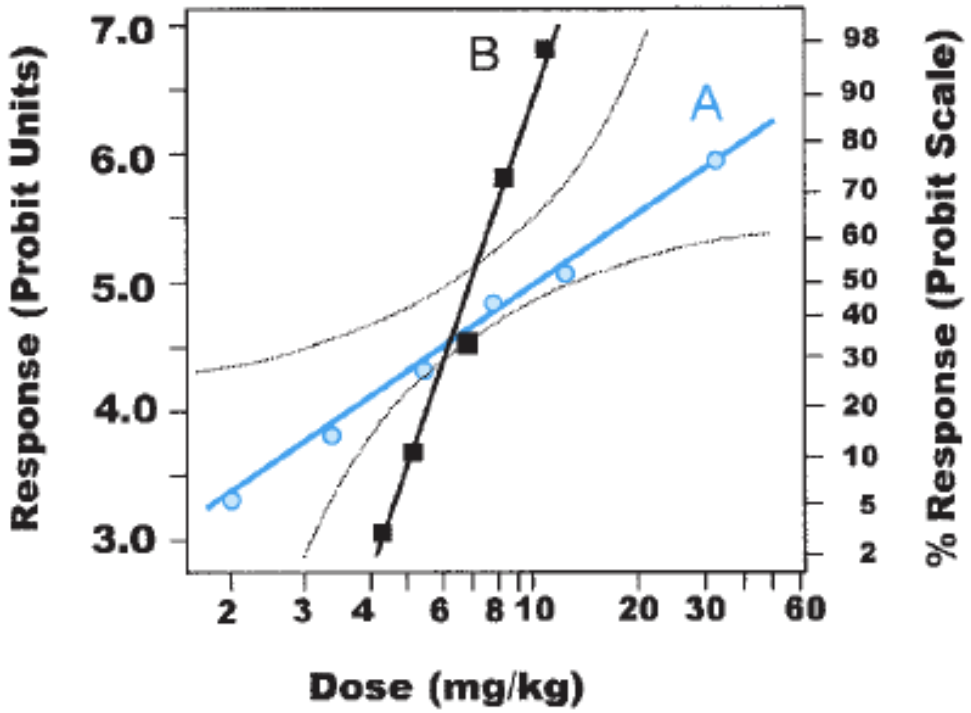


Figure 2-5. Comparison of dose–response relationship for two different chemicals, plotted on a log dose-probit scale.

Note that the slope of the dose–response is steeper for chemical *B* than chemical *A*. Dotted lines represents the confidence limits for chemical *A*.

Interactions of chemicals

The effects of 2 chemicals may produce the following responses:

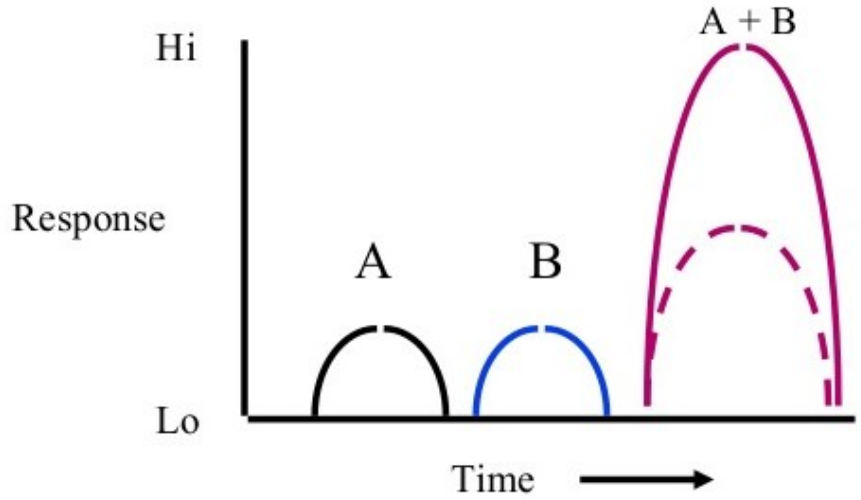
- Additive effect → 2 + 3 = 5
- Synergistic effect →2 + 2 = 20
- Antagonism → 4 + 6 = 8; 4 + (−4) = 0; 4 + 0 = 1

Chemical A &B interfere with each other's Actions/potency or one interferes with the action of the other

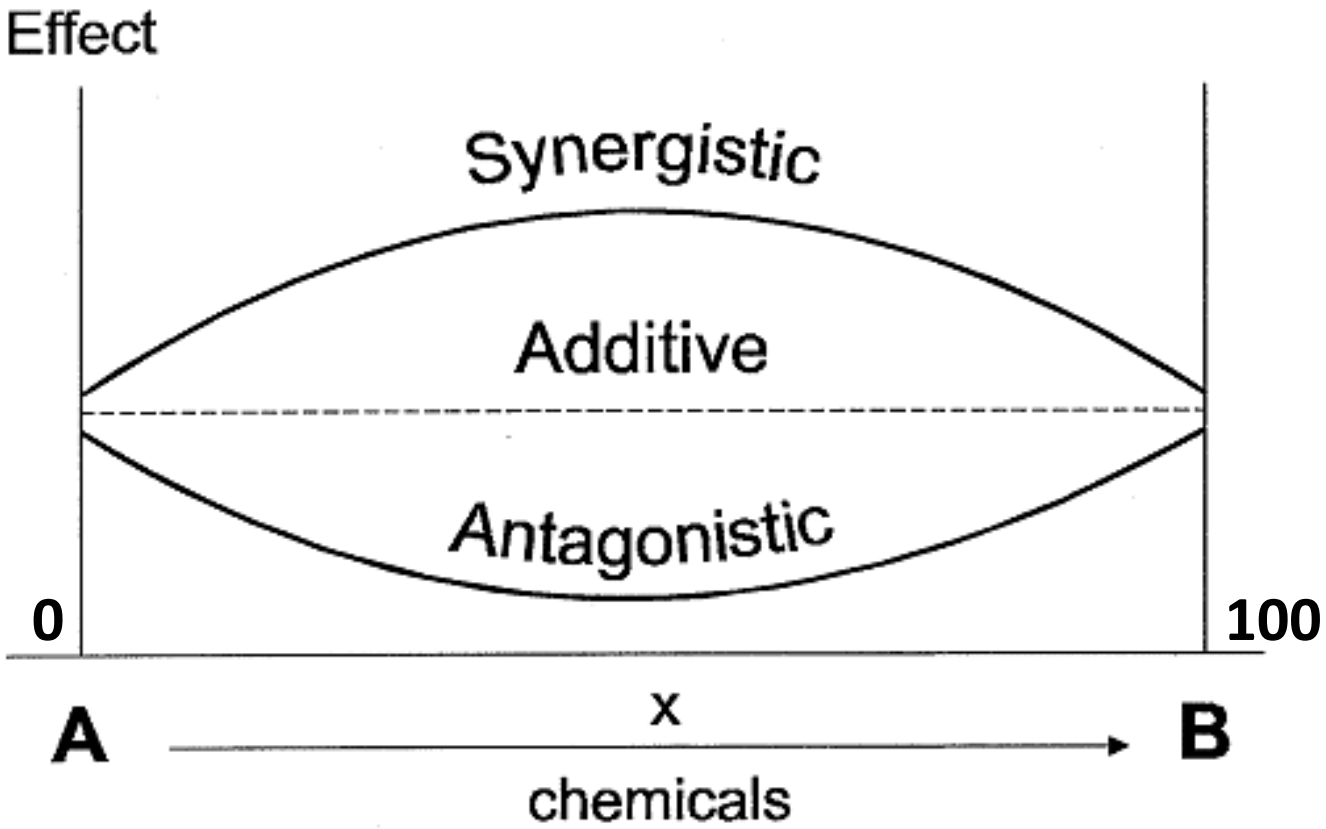
e.g tamoxifen – receptor blocker of estradiol

- Potentiation → 0 + 2 = 10

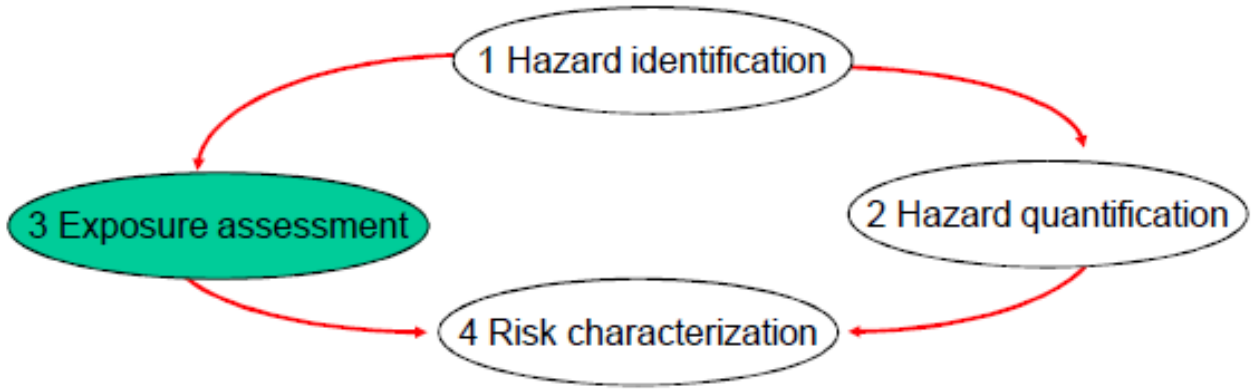
  Chemical A not toxic on a certain organ or system, but when added to another chemical makes that chemical much more toxic



The effect of two chemicals taken together is greater than the sum of their separate effect at the same doses, e.g., alcohol and other drugs


Effect
Synergistic
Additive
Antagonistic
0
100
A
x
B
chemicals

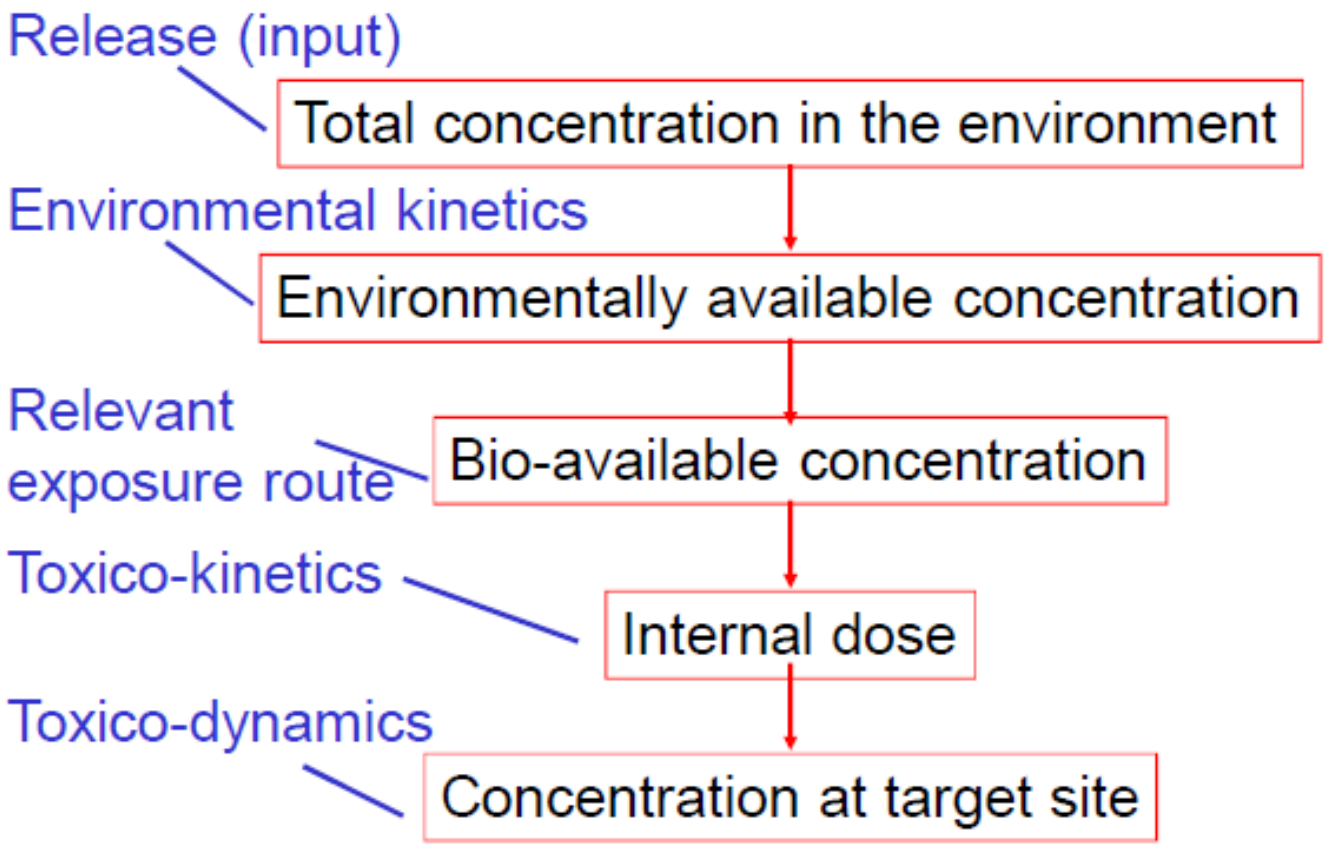
Exposure assessment



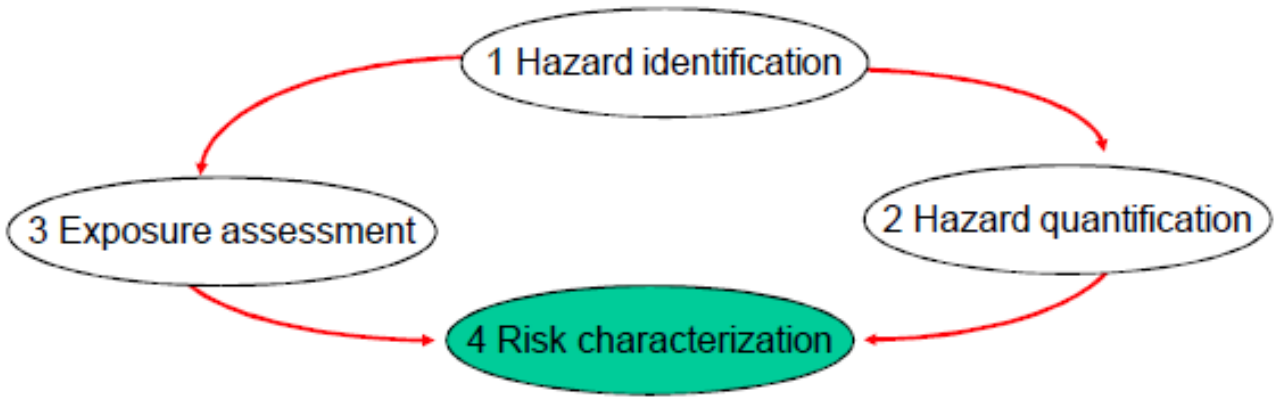
-Mains route(s) of intake/uptake
-Levels in environment (abiotic/biotic)
-Bioaccumulation, biomagnification
-Levels in food/drinking water

What is the actual exposure dose?

(important in environment and tests)

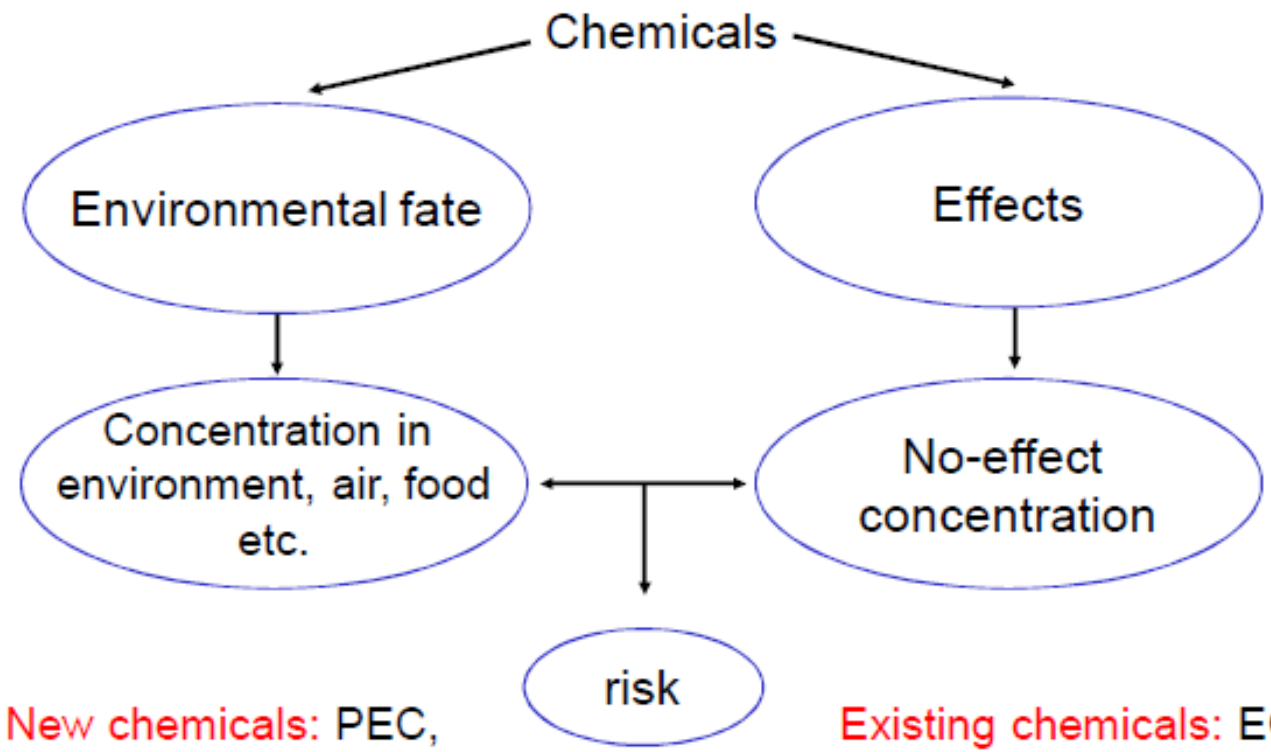


Risk characterization



-Quantitative and qualitative characterization
-Biological uncertainties
-Statistical uncertainties
-Which population (groups) are most at risk?

Risk depends on concentration (dose) and effect



New chemicals: PEC, testing and estimating (P)NEC

Existing chemicals: EC, existing info, testing NEC

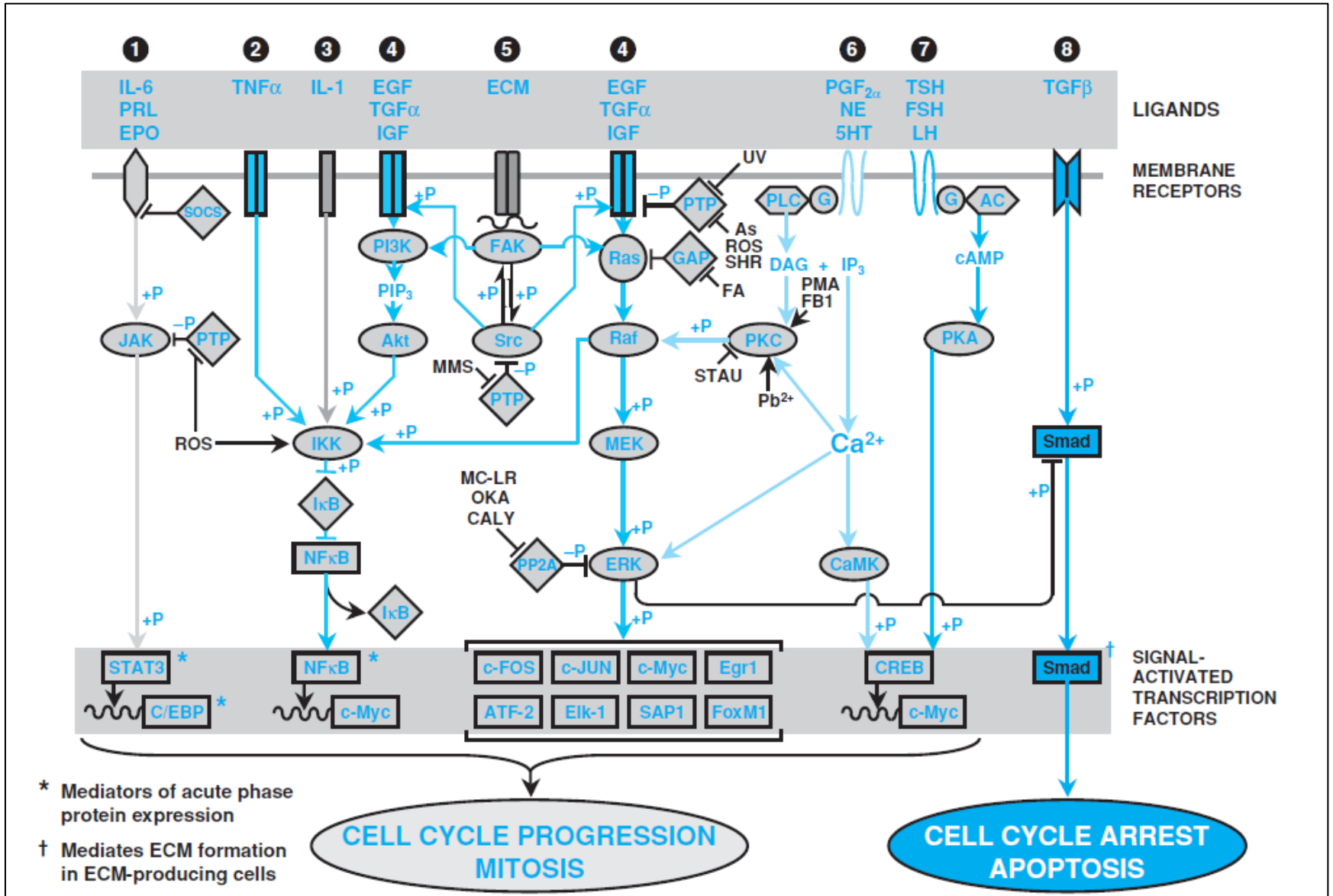


Figure 3-11. Signal transduction pathways from cell membrane receptors to signal-activated nuclear transcription factors that influence transcription of genes involved in cell-cycle regulation.