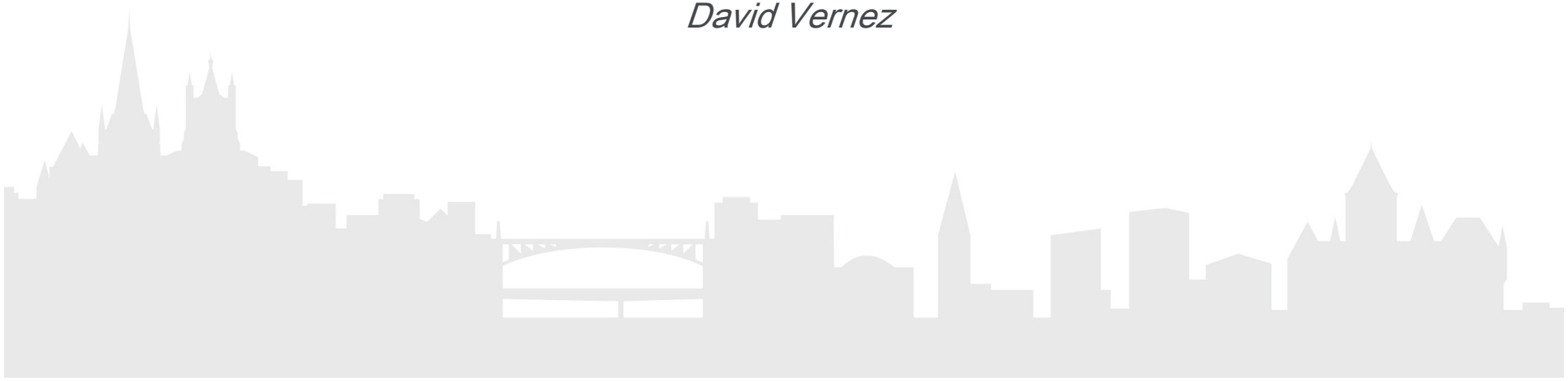
unisanté

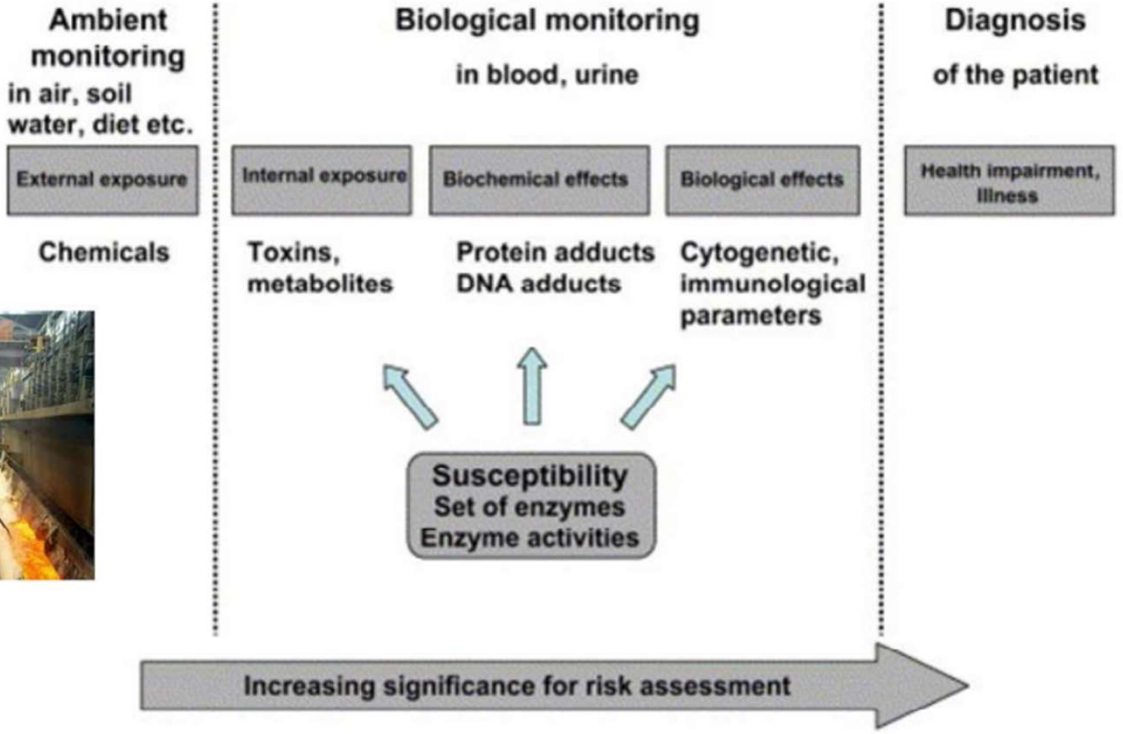
Chemicals
Uptake, toxicology

David Vernez



Toxicology and biomonitoring

From the source to the disease



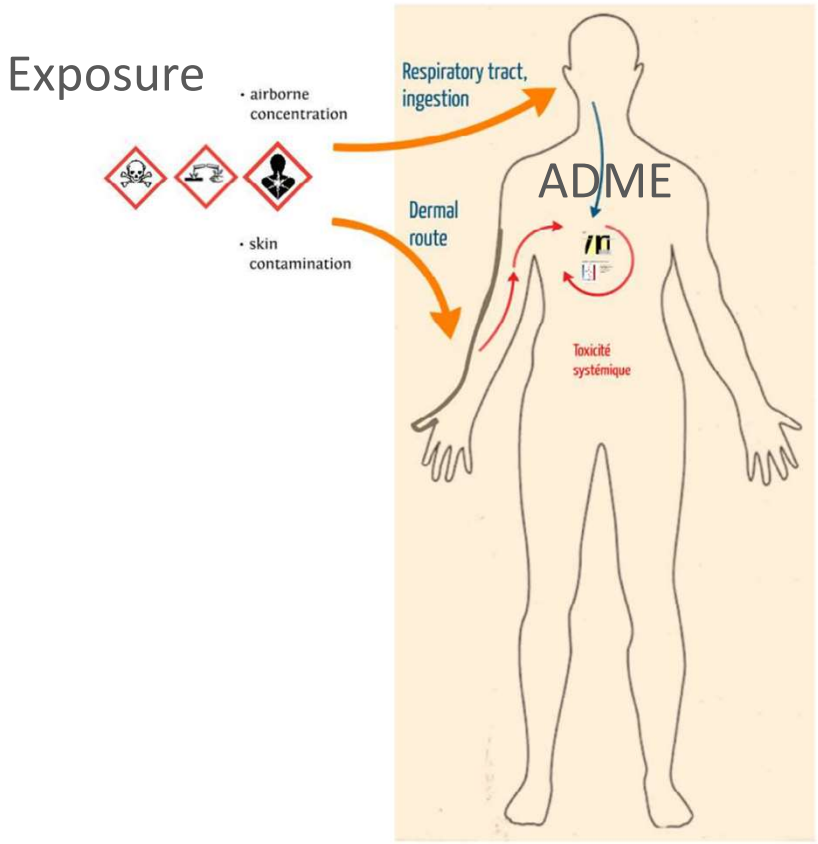
Scheme of ambient and biological monitoring (modified acc. DFG, 2002; Angerer and Gündel, 1996)

Toxicology

What is toxicity ?

- An observable or measurable physiological alteration, reversible or not
- Observable in different species
- Against which the body's protective mechanisms are insufficient
- Which implies a dimension of quantity or dose
- The toxicity of a chemical substance depends on its degree of exposure, **A**bsorption, **D**istribution, **M**etabolism and **E**xcretion (ADME)

Toxicology



- Ultimately, the relevant toxic dose is the concentration/quantity of a toxic substance/metabolite that will reach a target organ

Case study

The shoe factory

Mrs Blue works part-time in a shoe factory, which allows her to devote part of her time to her hobby, painting...
To glue the soles, she uses an adhesive containing toluene. The premises where she works are dilapidated, and the company's safety officer is concerned about her exposure.

Question (2.2a)

What are the possible exposure situations and health risks ?

3. Composition / Hazardous Components

Chemical Name	CAS No.	WT%
Grafted Chloroprene Rubber	-	10 – 30 %
Toluene	108-88-3	70 – 90 %

Case-study solution

Question 2.2a

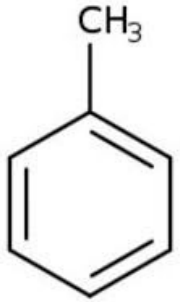
Chloroprene rubber

- *solid material, stable and inert*
- *possible presence of sensitizing agents (impurities or degradation products)*

→ *Possible skin allergies*

Toluene

- *MW 92 g/mol, liquid, LogKow 2.7*
- *Vp: 3 kPa (21°C), solubility in water 0.58g/l (25°C), LIL 1.2%*
- *reprotoxicant, ototoxic, irritant, Central Nervous System (CNS)*
- *OEL-8h (Switzerland): 50 ppm (190 mg/m^3)*

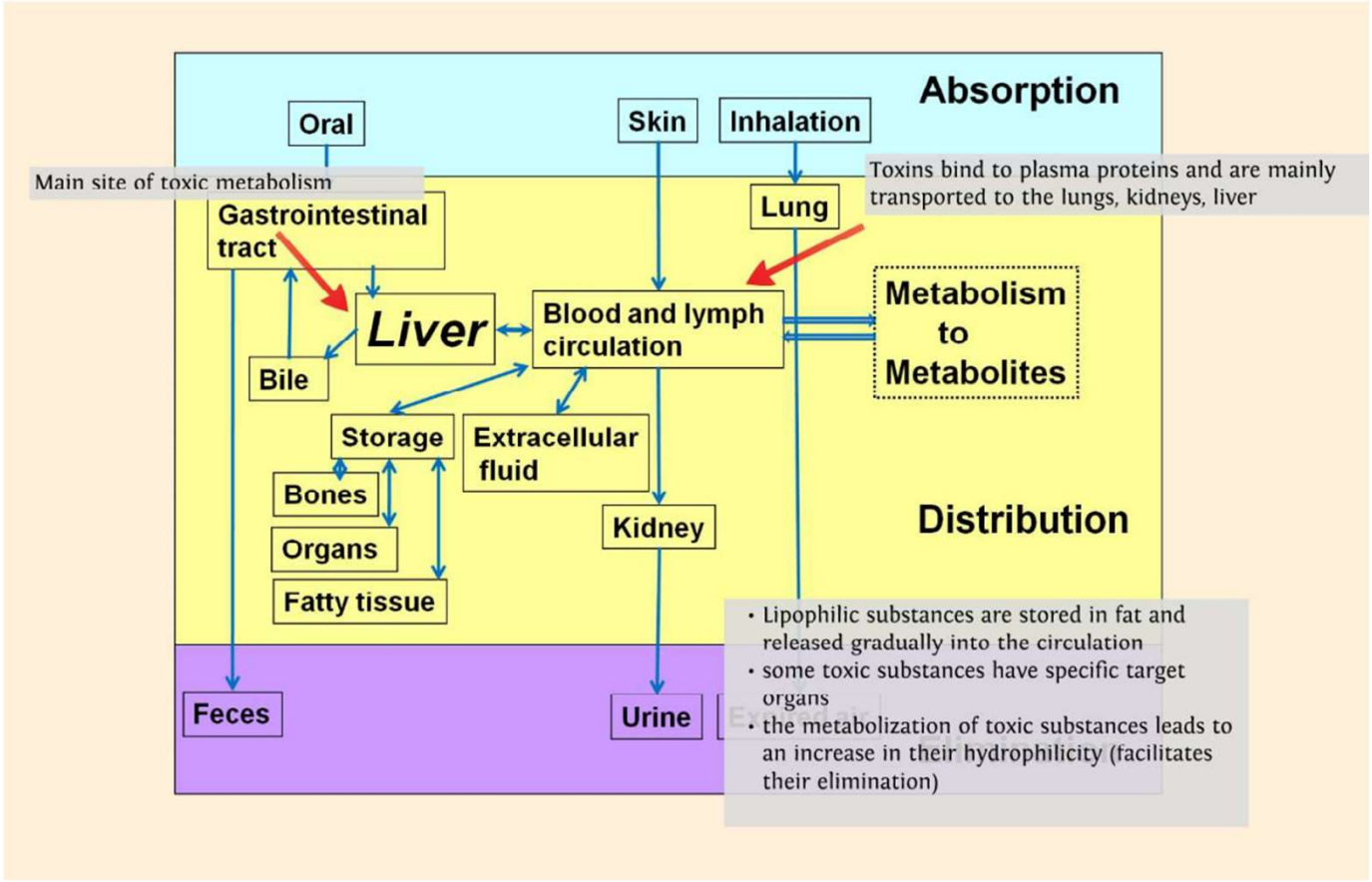


→ *Exposure through inhalation*

→ *Possible skin permeation*

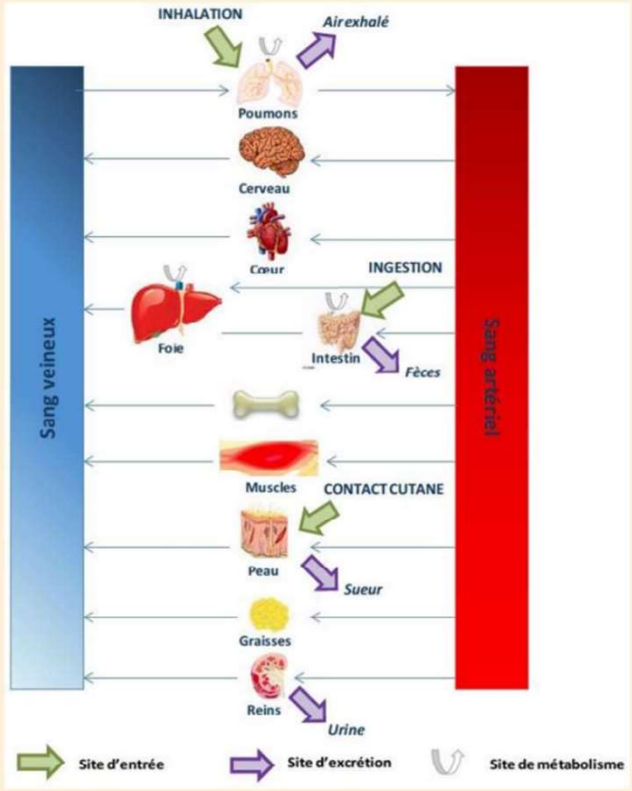
→ *Toluene is present in many varnishes and paints, possible domestic exposure*

ADME


Absorption
Oral
Skin
Inhalation
Main site of toxic metabolism
Toxins bind to plasma proteins and are mainly transported to the lungs, kidneys, liver
Gastrointestinal tract
Lung
Liver
Blood and lymph circulation
Metabolism to Metabolites
Bile
Storage
Extracellular fluid
Bones
Organs
Fatty tissue
Kidney
Distribution
Feces
Urine
• Lipophilic substances are stored in fat and released gradually into the circulation
• some toxic substances have specific target organs
• the metabolization of toxic substances leads to an increase in their hydrophilicity (facilitates their elimination)

ADME and entry route

Ingestion, Inhalation and percutaneous



Modéliser & simuler – Tome 2 (2014)

First-pass metabolism: metabolization in the liver or intestine before reaching the systemic circulation (blood circulation)

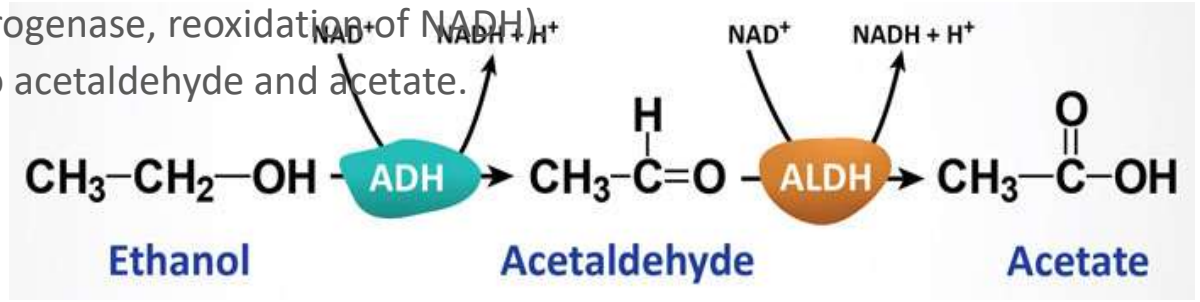
Elimination, zero order kinetics

The rate of elimination is independent of the concentration of the toxic substance

- A constant amount of the toxicant is eliminated per unit of time

Example: ethanol

- Mainly enzymatic (alcohol dehydrogenase ADH1…7)
- Limiting factor (alcohol dehydrogenase, reoxidation of NADH)
- Successive decomposition into acetaldehyde and acetate.



Zero order kinetics, ethanol

General kinetics

- The body eliminates about 0.015 (0.01-0.06) g/100 mL blood/hour (one beer)
- Example: ethanol removal after 6 beers (60 ml)

	Time after Ethanol Consumption (h)						
Ethanol	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Ethanol eliminated (ml)	0	10	10	10	10	10	10
Ethanol remaining (ml)	60	50	40	30	20	10	0
Ethanol eliminated	0/60	10/60	10/50	10/40	10/30	10/20	10/10
(% of remaining)	0	17	20	25	33	50	100

- Used in retrospective evaluation of blood alcohol [ ]

Zero order kinetics, ethanol

Inter-individual kinetic variability

- Body mass
- Physical activity

Genetic polymorphism of ADH

- Proportion of ADH alleles varying by ethnicity

	Fréquence (%)				
	*ADH 2*1*	*ADH 2*2*	*ADH 2*3*	*ADH 3*1*	*ADH 3*2*
Caucasoïdes américains	> 95	< 5	< 5	50	50
Caucasoïdes européens	85	< 15	< 5	60	40
Asiatiques (Japonais)	15	85	< 5	95	5
Afro-Américains	85	< 5	15	85	15

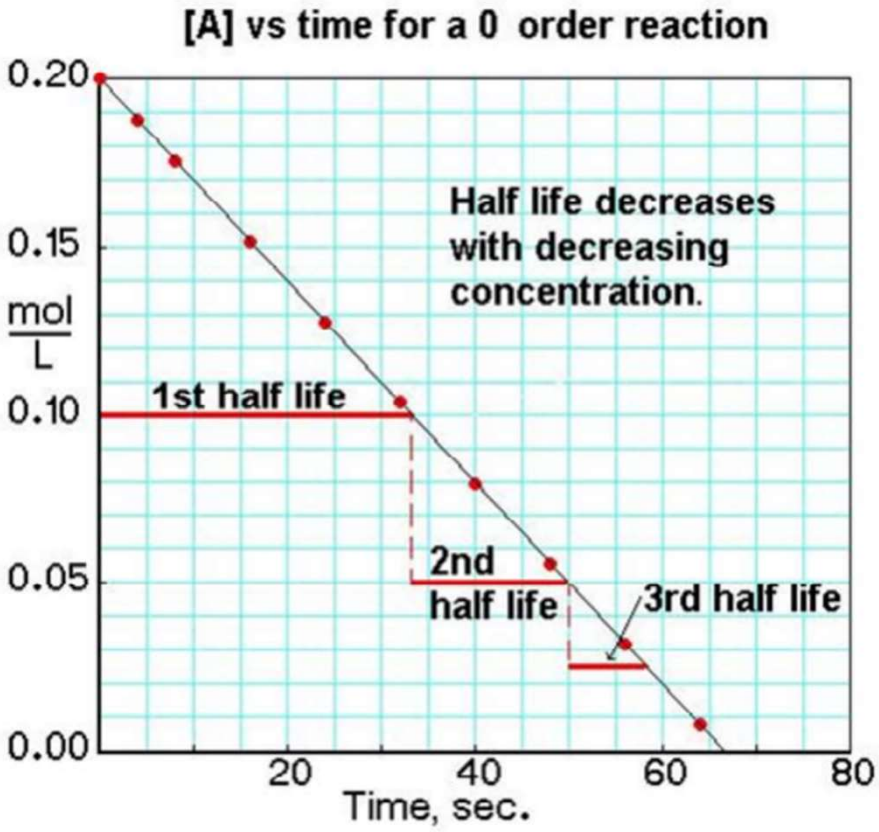
ADH allele frequency (%) by ethnicity (from Borson and Li, 1986)

Elimination, zero order kinetics

Ethanol elimination

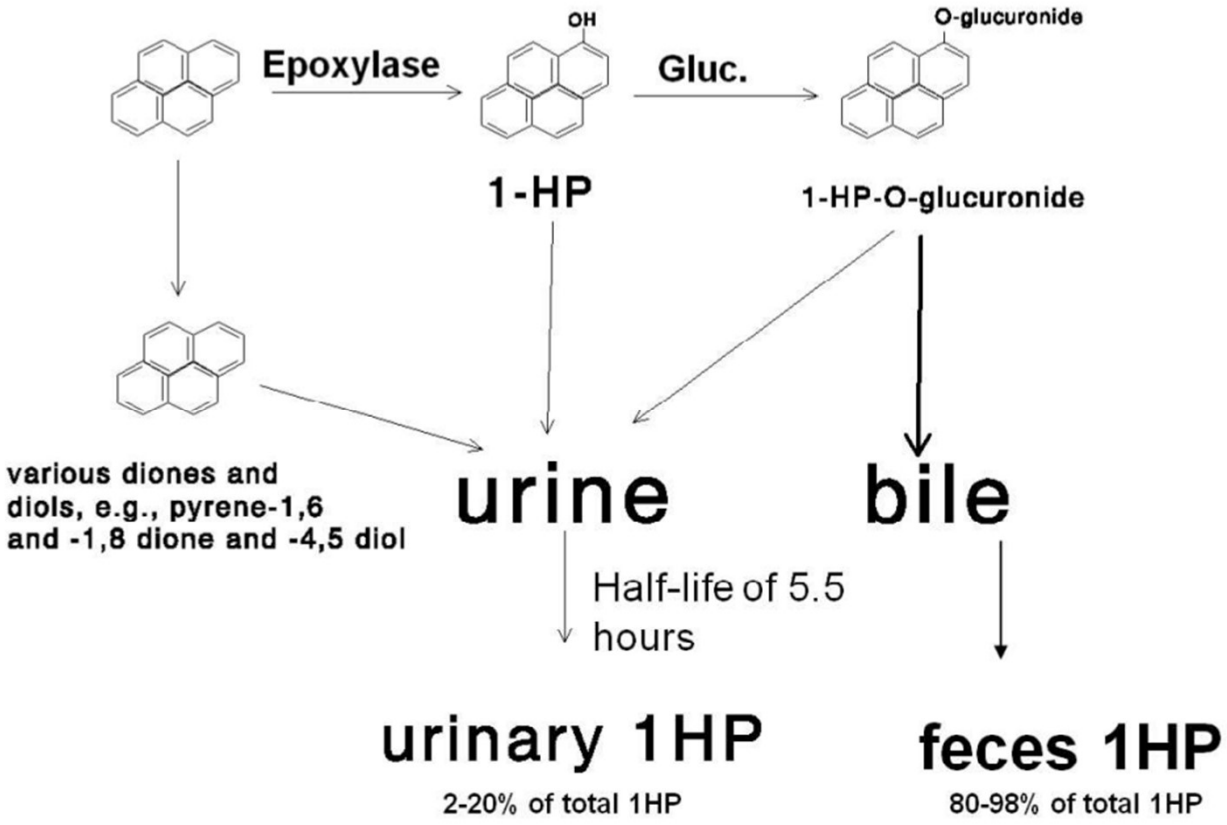
- Independent of the [ ]
- Constant speed

$$T_{\frac{1}{2}} = \frac{[A_0]}{2k}$$



Elimination, first order kinetics

Example. PAH exposures in foundries

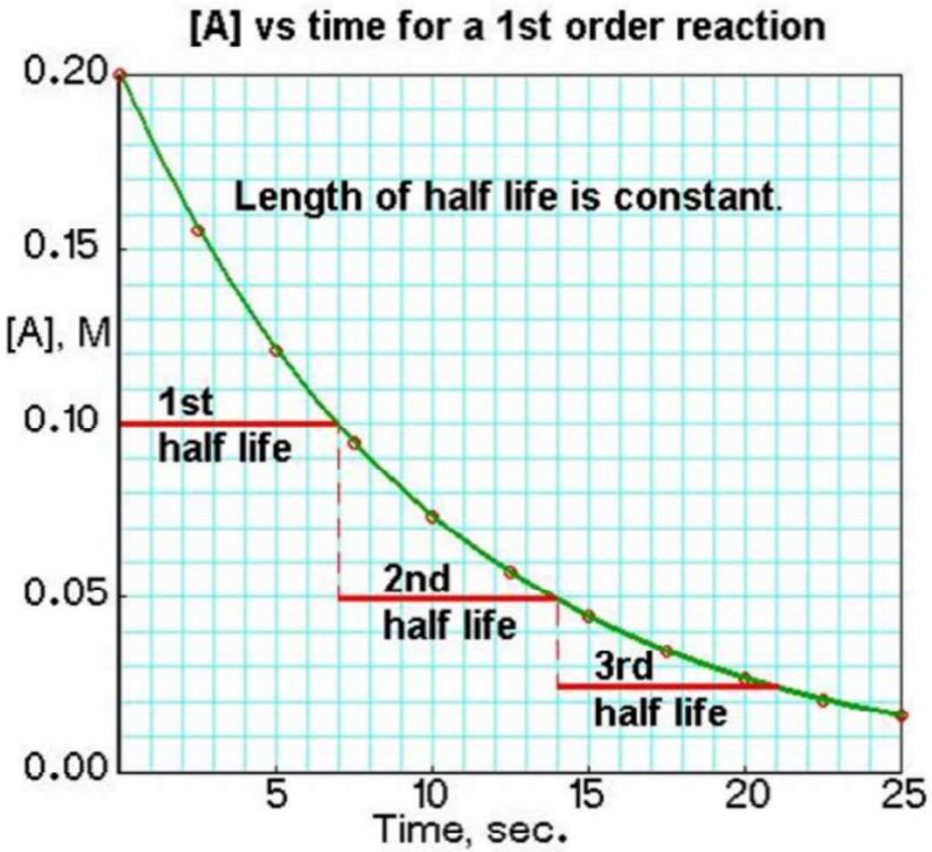


Elimination, first order kinetics

Elimination is proportional to the [ ] of the toxicant

- A constant proportion of the toxicant is eliminated per unit of time

$$T_{\frac{1}{2}} = \frac{\ln k}{2}$$



Elimination kinetics

Zero order elimination

$A \rightarrow$ products

$rate = k\ [mol\ L^{-1}\ s^{-1}]$

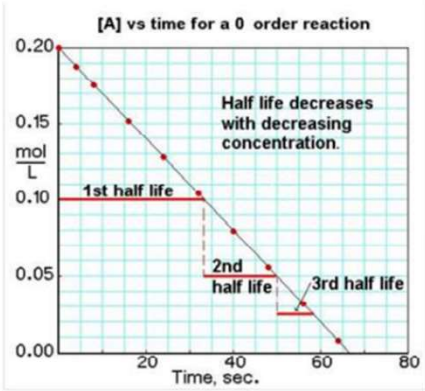
$[A] = [A_o] - kt$

$t_{½} = [A_o] / 2k$

- Constant elimination
- Independent of amount of chemical in the body

Ex. Ethanol

No-model



*(when [A] = [B])

First order elimination

$A \rightarrow$ products

$rate = k[A]\ [s^{-1}]$

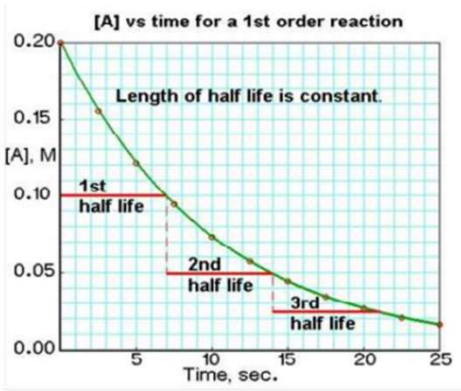
$[A] = [A_o]\ e^{-kt}$

$t_{½} = 0.693 / k$

- Constant proportion of the agent is eliminated per unit time
- Logarithmic process

Ex. Most chemicals

One-compartment toxicological model



Ref: http://www.chem.purdue.edu/gchelp/howtosolveit/Kinetics/Halflife.html#0Order

Second order elimination

$2A \rightarrow$ or $A + B \rightarrow$ products*

$rate = k[A]^2\ [L\ mol^{-1}\ s^{-1}]$

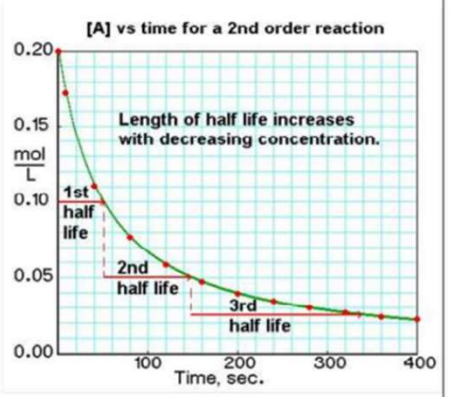
$1/[A] = 1/[A_o] + kt$

$t_{½} = 1 / k\ [A_o]$

- Chemical concentrations in tissues takes time to reach equilibrium with plasma

Ex. Persistent chemicals

Multi-compartment toxicological model



Case study

The shoe factory

Toluene is quickly absorbed and metabolized into the body (1st order kinetic). Its half-life in the blood is about 1,5 hours.

Question (2.2b)

What would be the toluene concentration (in ppm) in the exhaled air of Mrs Blue at 9 pm if he had, when leaving her work at 5 pm, 15 ppm of toluene in her exhaled air?

Case-study solution

Question 2.2b

Exhaled air concentration will reflect the blood concentration and follows the same kinetics due to the rapid air-blood exchange in the lungs

Using the first order kinetic equation

Half life $t_{1/2}$ = 1.5 h, Time interval Δt = 4 h

kinetic constant $k = \ln 2 / t_{1/2} = 0.462\ h^{-1}$

concentration decrease

$A = A_0 \cdot e^{-kt}$

$A = 15 \cdot e^{-(0.462 \times 4)}$ = **2.36 ppm**

Using the half life of the substance

An interval of 4h corresponds to between 2 and 3 Half life

The concentration ranges between **1.9 and 3.7 ppm**

Blood and exhaled air concentration will decrease quickly after exposure

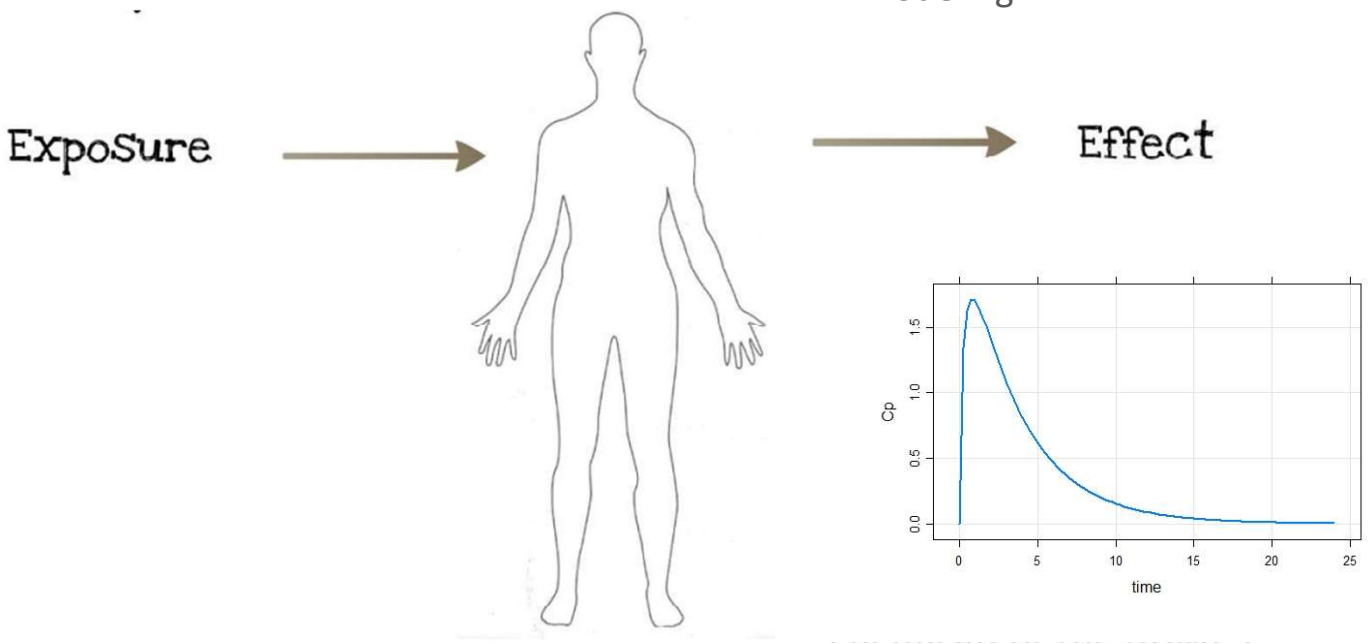
Toxicokinetics

Toxicokinetics

- Description of the behavior and fate of the pollutant in the body

Modelling

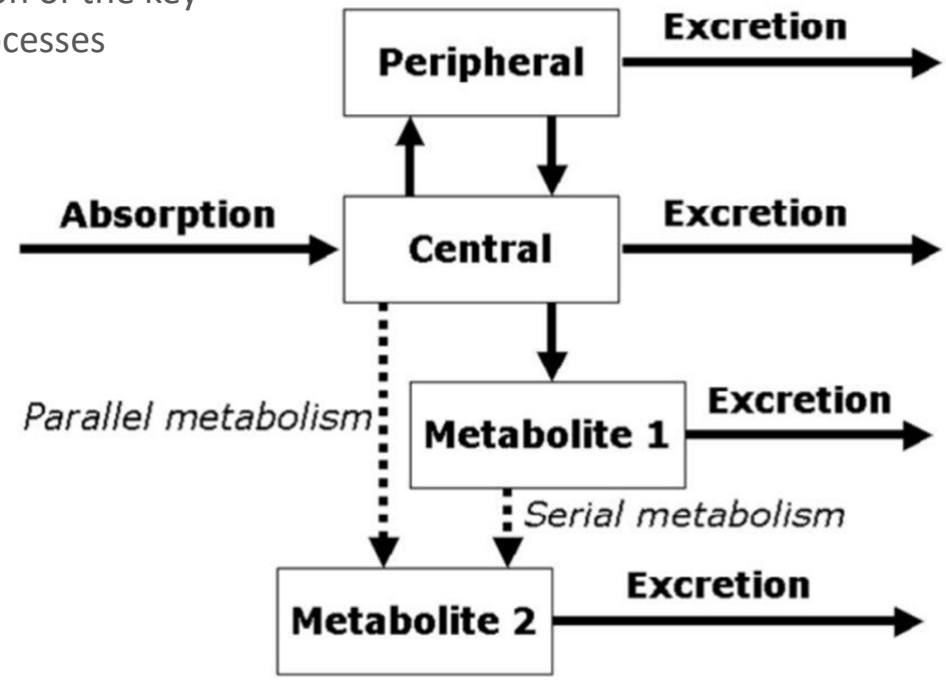
- Mathematical modeling of the ADME process.
- TK – toxicokinetic modelling
- PBPK – Physiologically-Based Pharmacokinetic modeling



Toxicokinetics – modeling

TK model

- Mathematical representation of the key body compartment and processes



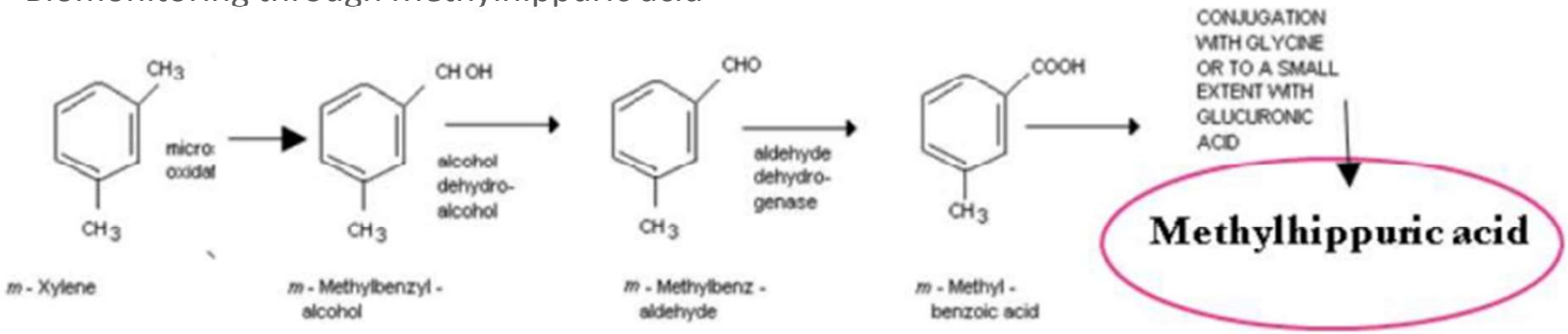
Toxicokinetics

Example in the metal industry

- Co-exposure to xylene and ethylbenzene
- Similar health effects of the two solvents (CNS effects)
- Interferences when co-exposed to both solvents

Metabolization of Xylene

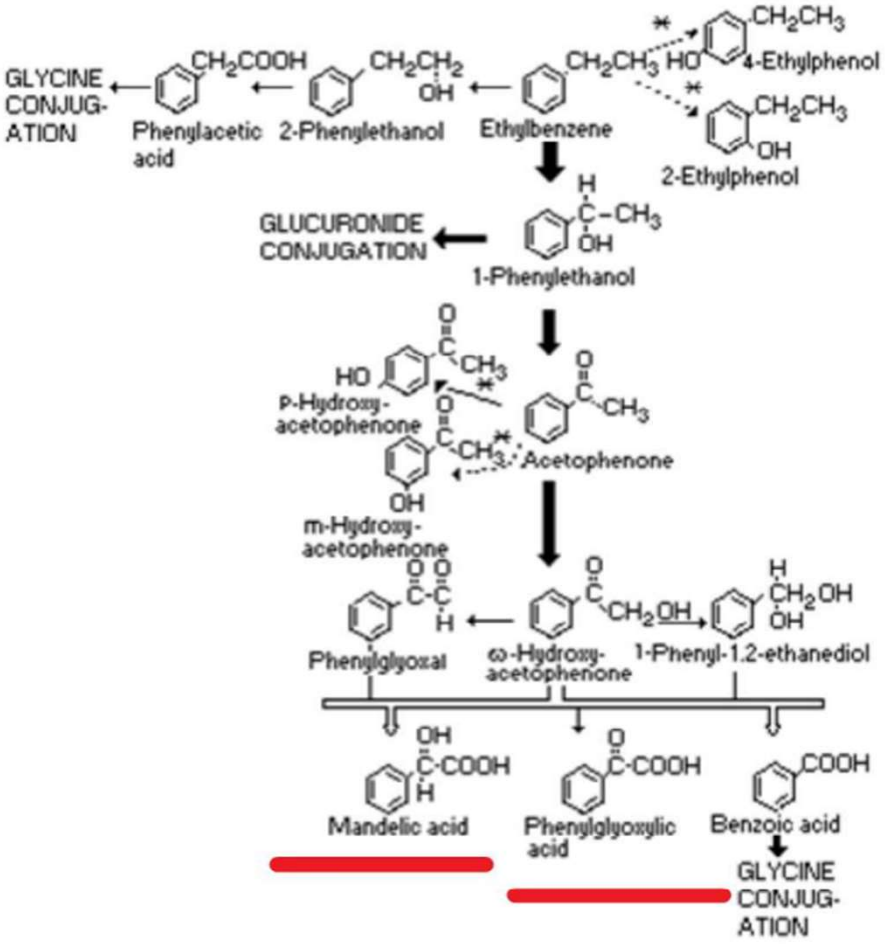
- Biomonitoring through Methylhippuric acid



Toxicokinetics

Metabolization of Ethylbenzene

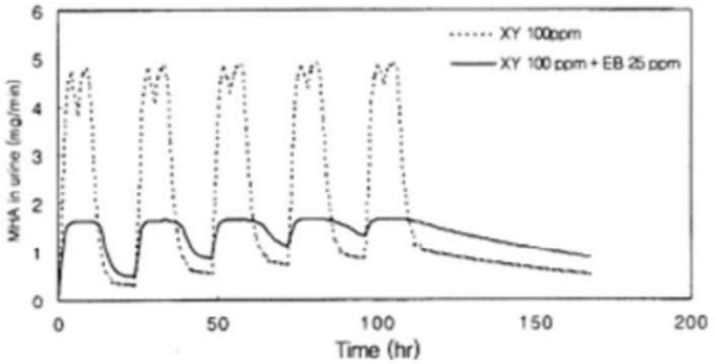
- Biomonitoring through Phenylglyoxyl acid and Mandelic acid
- Same enzymatic process



Toxicokinetics- biomonitoring

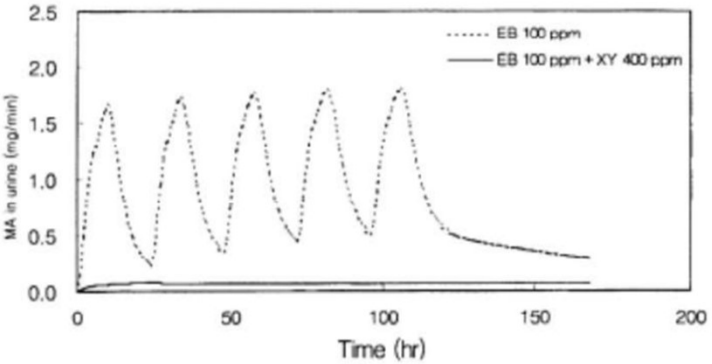
Biomonitoring of **Methylhippuric acid (MHA)**

- After (one week) exposure to 100 ppm Xylene (XY)
- After (one week) co-exposure to 100 ppm Xylene (XY) and 25 ppm Ethylbenzene (EB)
- Biomonitoring will underestimate XY exposure



Biomonitoring of **Mandelic acid (MA)**

- After (one week) exposure to 100 ppm Ethylbenzene (EB)
- After (one week) co-exposure to 400 ppm Xylene (XY) and 100 ppm (Ethylbenzene)
- Biomonitoring will underestimate EB exposure



Biomonitoring

Internal dose indicators

- Unchanged substance (metal, solvent) in blood, urine, exhaled air…
- Metabolites (organic compounds) in blood, urine, saliva
- Biological determinant adducted to DNA of hemoglobin

Interest of biomonitoring

Takes into account

- Physical effort
- All entry routes (skin, ingestion, inhalation..)
- Personal protection
- Fluctuations of the environment
- Constitution: fat, muscle,…
- Metabolic differences

Biomonitoring

Disadvantages

- High inter-individual variability
- Possible interferences(alcohol, medication,…)
- Sometimes invasive
- Does not detect exposure peaks
- Limited to a few substances
- Sometimes difficult to interpret

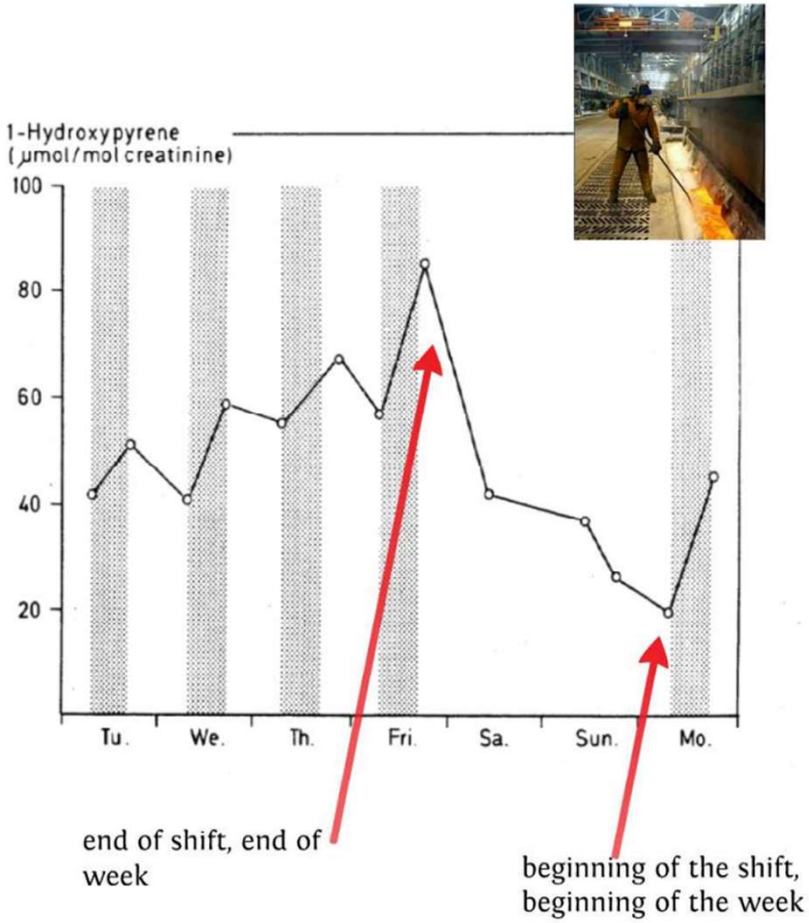
References values

- BEI (biological exposure indices) in the USA
- BAT (biologicher arbeitstoff-toleranz wert) in Germany
- VBT (Valeurs biologiques tolérables) in Switzerland

Biomonitoring strategy

Important factors

- Half-life of the biological indicator
- Exposure level
- Exposure variability
- Individual variability (diuresis/creatinine)
- Timing of sampling



Biomonitoring strategy

What and When to sample ?

Determinant	Media	T1/2 [h]	Timing
METALS			
Lead	blood	900	not critical
Chromium	urine	7	end of shift
Cadmium	urine	20 years	not critical
Nickel	urine	24	end of shift
SOLVENTS METABOLITES			
N-Methlyformamide	urine	4	end of shift
2,5-Hexadione	urine	15	end of shift
Trichloroethanol	blood	12	end of shift
Trichloroacetic acid	urine	80	end of shift at end of week
SOLVENTS			
Styrene	blood	0.5-4	end of shift
	blood	70	next morning
Methyl Ethyl ketone	urine	4	end of shift
Perchloroethylene	exhaled air/blood	96	prior to last shift of workweek

Case study

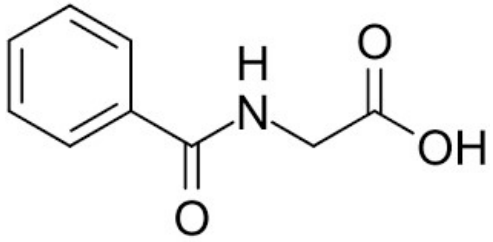
The shoe factory

Mrs Blue exposure was followed by biomonitoring. Urine samples were collected after the end of the daily work shift and measured for hippuric acid (a metabolite of toluene), with the following results:

[HA] T_0 (Immediately after exposure): 3.0 [g/g creatinine]
[HA] T_4 (4 hours after exposure) : 2.1 [g/ g creat.]
[HA] T_{12} (12 hours after exposure) : 1.4 [g/ g creat.]

Question (2.2c)

Characterize the kinetics of this decay. What will be the potential implications of such kinetics on the follow-up strategy of the Mrs Blue?



Case-study solution

Question 2.2c

During the first 4 hours, the concentrations decreased by a factor 0.7 (2.1/3). During the next period, it decreased by a factor similar 0.67 (1.41/2.1), although the period was two times longer 8h. The half-life therefore increases with decreasing concentration, indicating a second-order like decrease.

We can calculate the kinetic rate using two points

$1/[A] = 1/[Ao] + kt$

$kt = 1/[A] - 1/[Ao]$

$12 \cdot k = 1/1.41 - 1/3$

kinetic constant k = $\mathbf{3.13\ 10^{-2}}$ $Lmol^{-1}\ s^{-1}$

The complete elimination of the organism is thus very slow, it is thus necessary to take samples at the end of the working week (and end of working shift.

Assessing toxicity

Structural analogy

- Similar biological and chemical structures can produce similar responses (QSAR)

In vitro evaluation

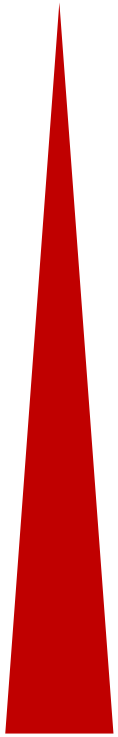
- Mutagenicity test (Ames)
- Cell culture (target organ)
- Human and animal tissues

Animal experimentation

- Acute and chronic tests in rats, mice and dogs
- Choice of animal based on similarities to humans (absorption, metabolism)
- Importance of the route of entry

Human epidemiological data

- Descriptive (disease prevalence), retrospective (exposure to a particular substance
- Health effects measured in exposed populations



Resources, representativeness

Toxicological profile

Minimal requirements according to REACH (EU Chemical Regulation)

- Prior to marketing

Study type	study duration (months, ca.) 1	2	3	4	5	6	7	8	9	>9
Production volume > 1 t/a, Annex VII										
Skin irritation or corrosion in vitro										
Eye irritation in vitro										
Skin sensitization										
Mutagenicity: in vitro gene mutation study in bacteria										
Acute toxicity by oral route										

Study type	study duration (months, ca.) 1	2	3	4	5	6	7	8	9	>9
Production volume > 10 t/a, Annex VIII										
Skin irritation in vivo										
Eye irritation in vivo										
Mutagenicity: in vitro cytogenetic study in mammalian cells or in vitro micronucleus test										
Mutagenicity: in vitro gene mutation study in mammalian cells										
Acute toxicity by inhalation or by dermal route										
Repeated dose toxicity: 28 days (**most appropriate route of administration**)										
Reproduction toxicity: Screening test										

Toxicological profile

Minimal requirements according to REACH (EU Chemical Regulation)

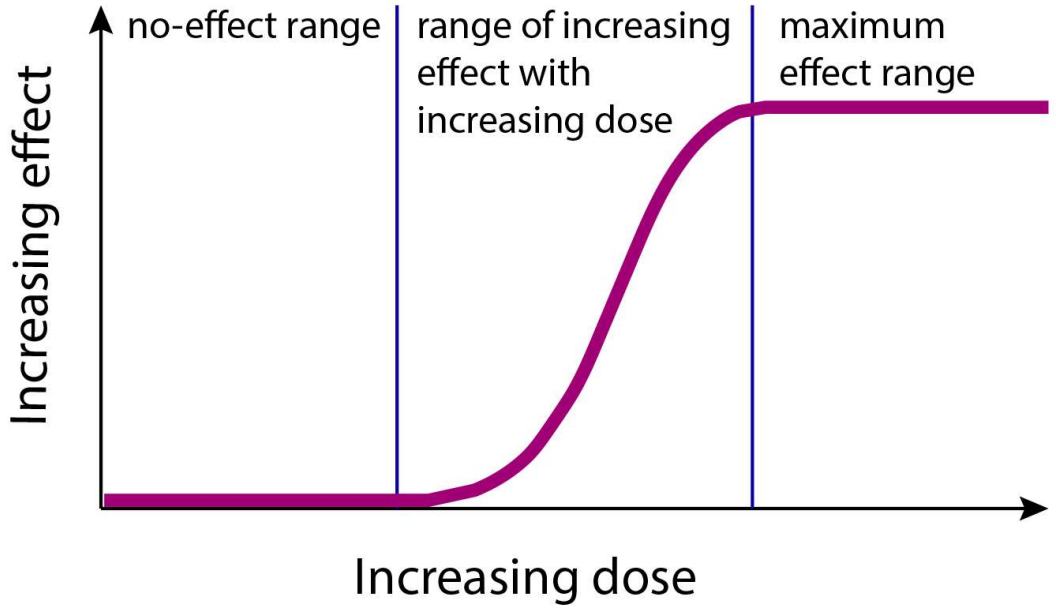
- Large production volume

Study type	study duration (months, ca.)									
	1	2	3	4	5	6	7	8	9	>9
Production volume > 1000 t/a, Annex X										
Mutagenicity: second in vivo somatic cell genotoxicity study										
Repeated dose toxicity: ≥ 12 months										
Specific toxicological studies (neurotoxicity, immunotoxicity etc.)										
Further reproduction toxicity studies										
Carcinogenicity study										

Dose-response relationship

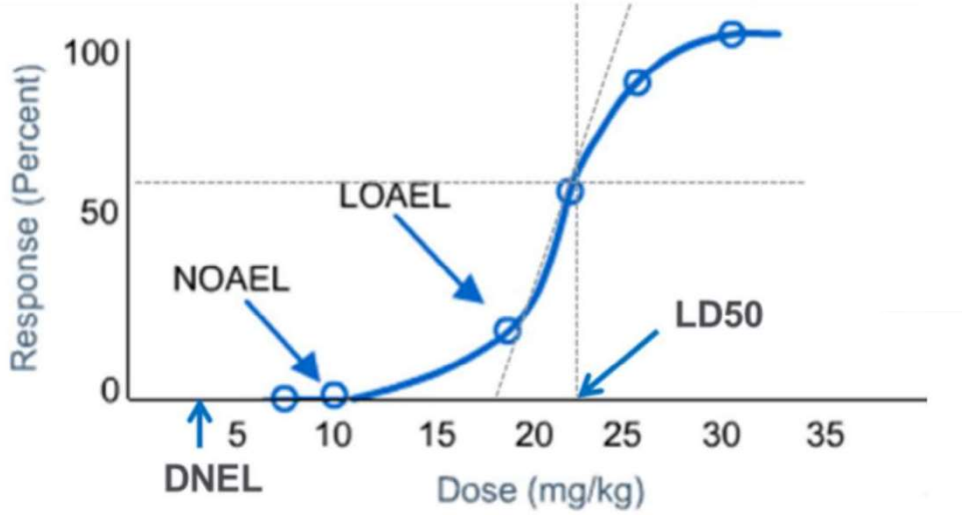
Classic relationship

- An increase in dose leads to an increase in effect (mortality, morbidity) in the exposed population



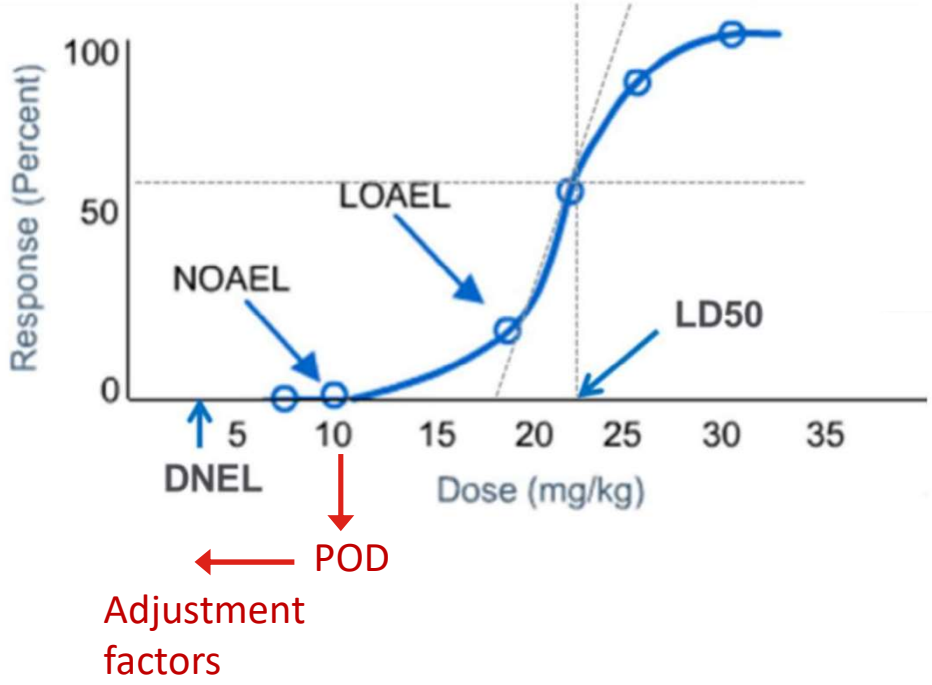
Source: Toxicology Education Fundation

Dose-response and thresholds



- NOAEL – No Observed Adverse Effect Level
  Highest dose or concentration at which no effect is observed
- LOAEL – Lowest Observed Adverse Effect Level
  Lowest dose or concentration at which the effect is observed
- LD – Lethal Dose 50%
  Dose or concentration at which effects are observed in 50% of the exposed individuals
- DNEL – Derived no effect Level
  Dose or concentration threshold used in regulation and prevention (includes safety factors)

Dose-response and thresholds



Instances

- The Scientific committee on Occupational exposure limits (SCOEL)
- National Committees
- In Switzerland: MAK Commission

Approach

- Prioritization of substances
- Data analysis
- Identification of critical effect
- Identification of a POD (Point of Departure)
- Assignment of adjustment factors

Occupational exposure levels (OELs)

In Switzerland

- 8h-TWA (Time Weighted Average), VME – Valeur Moyenne d'exposition

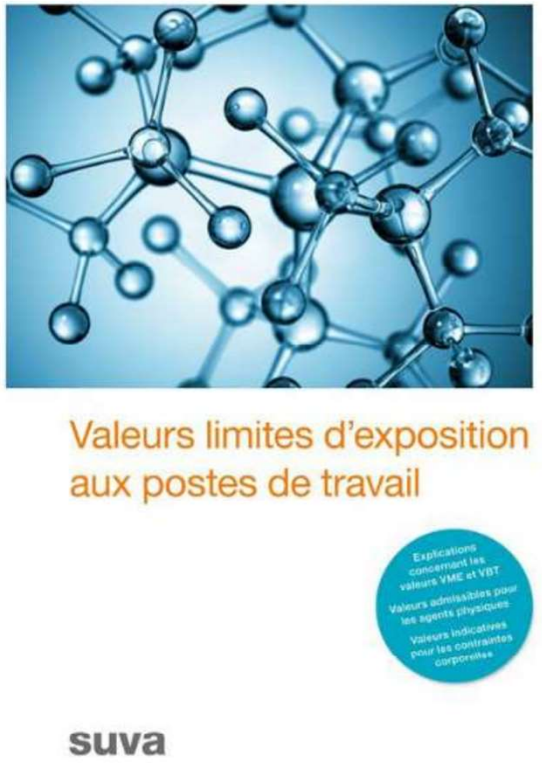
  Average concentration in the air of workstations of a given pollutant which, according to the current knowledge, does not endanger the health of the vast majority of workers exposed to it, for a duration of 42 h per week, at a rate of 8 hours per day, for long periods

- 15min-TWA, *VLE – Valeur Limite d'exposition*

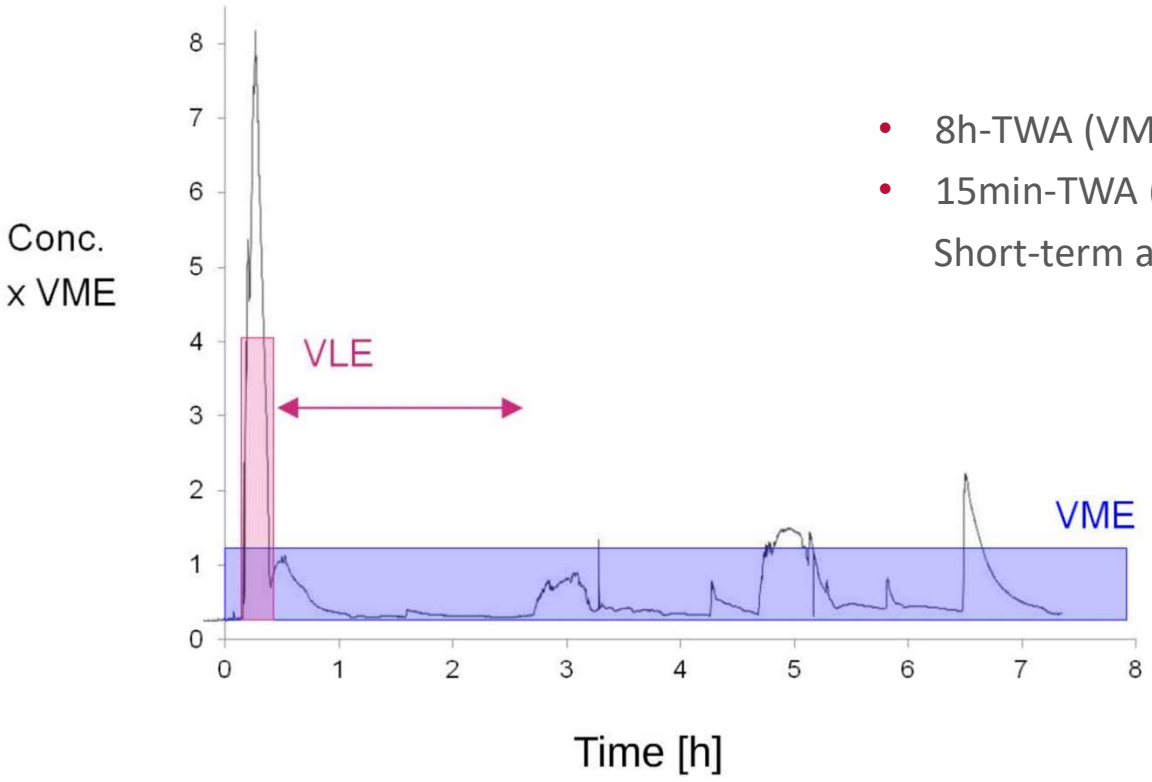
  Short period exposures

  Limitation in intensity, time and frequency

- VBT – tolerable biological values
- Threshold for physical agents



Occupational exposure levels (OELs)



- 8h-TWA (VME)
- 15min-TWA (VLE)

  Short-term average any time of the day (

VME / VLE values

Substance [no CAS]	VME ml/m³ (ppm)	VME mg/m³	VLE ml/m³ (ppm)	VLE mg/m³	Notations R S O[B] B P C M R_F R_O SS	Toxicité critique	Indications analytiques/ Remarques
Composés de Tétra-n-octylétain [7440-31-5] (exprimé en Sn)	0,004	0,02 i	0,004#	0,02 i#	R	Yeux, VRS, SNC, Nausée, Immun[TC AN]	
Ethane [74-84-0]	10000	12500				Arh, SNC, Formel[TC]	
Ethanol [64-17-5]	500	960	1000	1920	SS_C	VRS, Formel[TC HU]	INRS, NIOSH
Ethanolamine v. 2-Aminoéthanol							
Ether de bis(chlorométhyle) v. Oxyde de bis(chlorométhyle)							
Ether bis-2-méthoxypropylique v. Oxyde de dipropyléneglycolméthyle							
Ether 1-chloro-2,2,2-trifluoréthyl-difluorométhylique [26675-46-7]	10	77	80	616			
Ether 2-chloro-1,1,2-trifluoréthyl-difluorométhylique [13838-16-9]	10	77	80	616	SS_C		OSHA
Ether de pétrole 30–75 sans hydrocarbures aromatiques	500	2000				SNC, VRS[TC] & Yeux[TC]	OSHA Respecter la VME du n-Hexane
Ether α,α-dichlorodiméthylique v. Oxyde de bischlorométhyle							
Ether 2,2'-dichlorodiéthylique [111-44-4]	5	30	5#	30#	R C_3	Yeux, Nausée, VR[TC]	NIOSH
Ether diisopropylique [108-20-3]	200	850	400	1700	SS_C	Yeux, VRS, Foie, Rein, Halitose[TC HU]	NIOSH
Ether diméthylique [115-10-6]	1000	1910				Formel[TC]	
Ether diméthylique du diéthylèneglycol [111-96-6]	5	27	40	216	R R_{F3}* R_{O3}* SS_B	ReproM & P[TC AN]	

VME / VLE values

Substance	Paramètre biologique	VBT	Substrat d'examen	Prélèvement	Remarques
2-Ethoxyéthanol	Acide éthoxyacétique	50 mg/l (480,3 µmol/l)	U	c, b	
2-Ethoxyéthylacétate	Acide éthoxyacétique	50 mg/l (480,3 µmol/l)	U	c, b	
Ethylbenzène	Ethylbenzène	1,5 mg/l (14,1 µmol/l)	B	b	
	Acide mandélique + acide phénylglyoxylique	2 g/g créatinine	U	b	
Composés fluorés inorganiques et acide fluorhydrique	Fluorures	7 mg/g créatinine (41,6 nmol/mmol créatinine)	U	b	X
	Fluorures	4 mg/g créatinine (23,87 nmol/mmol créatinine)	U	d	X
Halotane	Acide trifluoroacétique	2,5 mg/l (12,6 µmol/l)	S	c, b	
Hexachlorobenzène	Hexachlorobenzène	150 µg/l (52,7 µmol/l)	P/Se	a	X
n-Hexane	2,5-Hexanedione + 4,5-Dihydroxy-2-hexanone	5 mg/l	U	b	N
2-Hexanone	2,5-Hexanedione + 4,5-Dihydroxy-2-hexanone	5 mg/l	U	b	N
Isopropanol	Acétone	25 mg/l (0,4 mmol/l)	U	b	
	Acétone	25 mg/l (0,4 mmol/l)	S	b	
Isopropylbenzène (Cumène)	2-Phényl-2-propanol	50 mg/g créatinine (41,5 µmol/mmol créatinine)	U	b	
Lindane (γ-1,2,3,4,5,6-Hexachlorocyclohexane)	Lindane	25 µg/l (85,9 nmol/l)	P/Se	b	
Manganèse et ses composés inorganiques	Manganèse	20 µg/l (364 nmol/l)	S	c, b	Q

Limits of the regulatory thersholds

Substances without exposure limits

- Known substances: > 20 Mio
- Industrial substances: 15’000-35’000
- Substances with exposure limits in Switzerland: 620
- Tolerable biological values in Switzerland (VBT): 45

Other limitations

- Almost all workers
- Hypersensitivity below the threshold
- Existence of a residual risk in absence below the threshold (e.g direct carcinogen)
- Limitation to healthy workers (age, gender, health status)
- Cumulative exposure
- New knowledge