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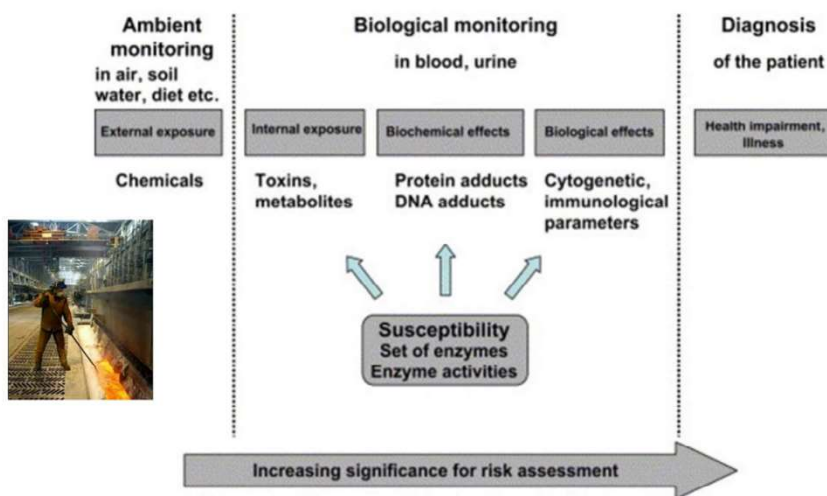
# Chemicals Uptake, toxicology

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## Toxicology and biomonitoring

From the source to the disease



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

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# Toxicology

## What is toxicity ?

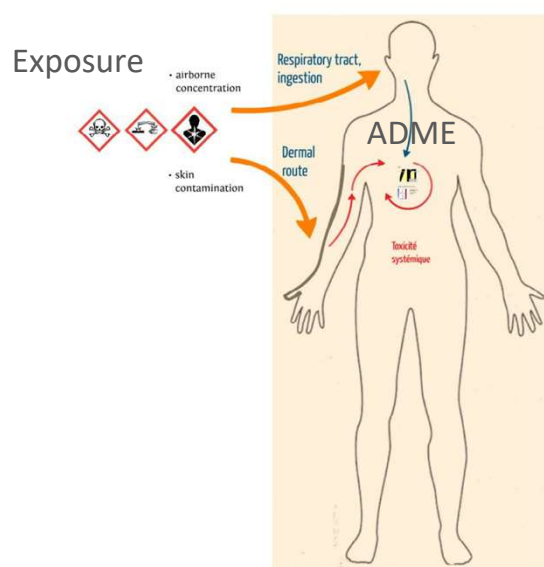
- An observable or measurable physiological alteration, reversible or not
- Observable in different species
- Against which the body's protective mechanisms are insufficient
- Which implies a dimension of quantity or dose
- The toxicity of a chemical substance depends on its degree of exposure, **A**bsorption,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

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# Toxicology



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

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## 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)

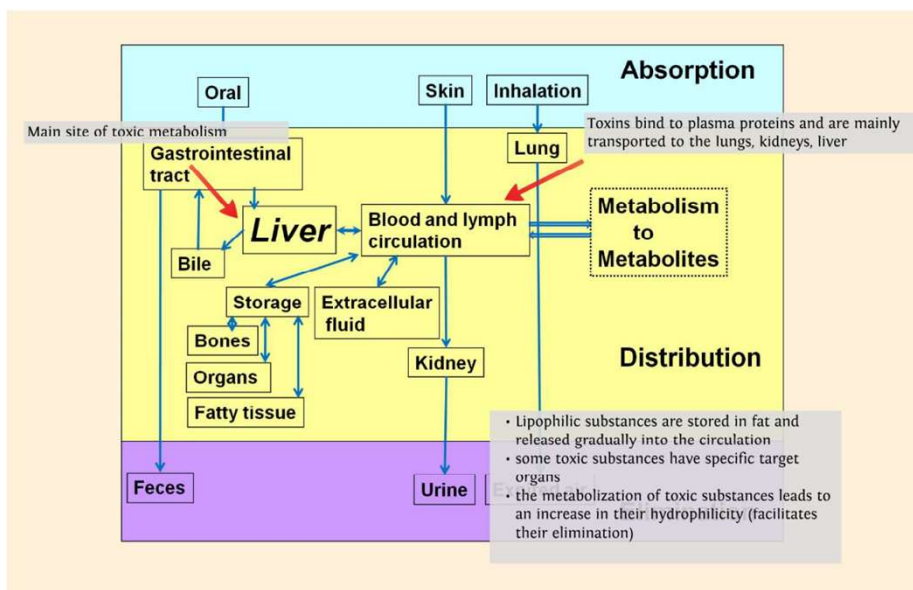
What are the possible exposure situations and health risks ?

#### 3. Composition / Hazardous Components

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

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## ADME

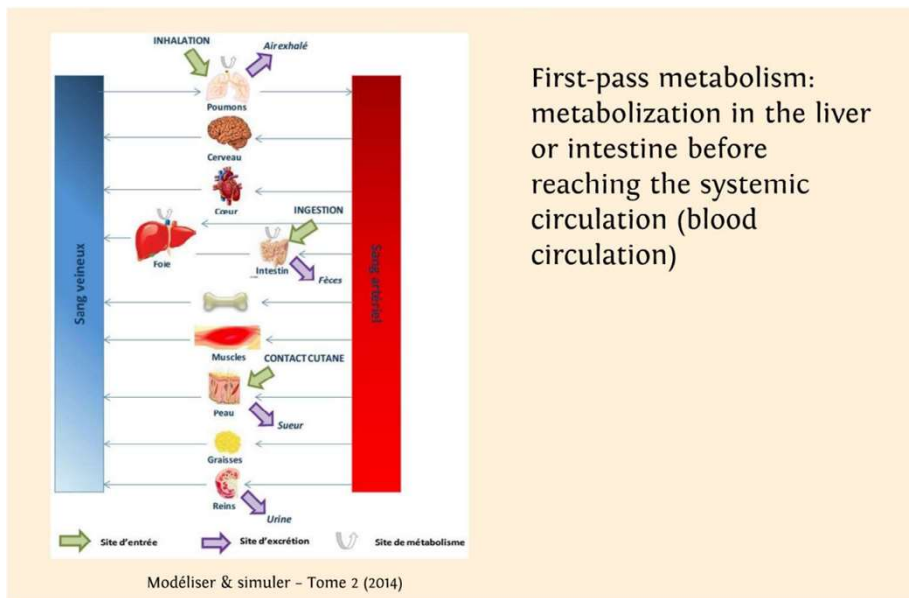


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## ADME and entry route

Ingestion,  
Inhalation and  
percutaneous



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

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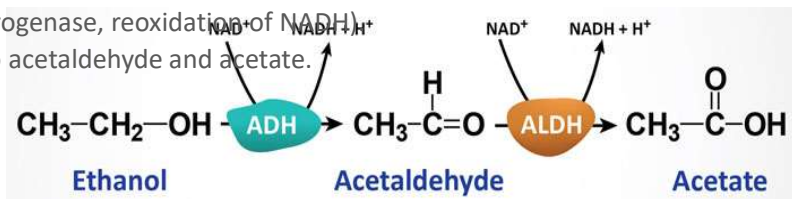
## Elimination, zero order kinetics

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.



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## 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)

Ethanol	Time after Ethanol Consumption (h)						
	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 (% of remaining)	0/60	10/60	10/50	10/40	10/30	10/20	10/10
	0	17	20	25	33	50	100

- Used in retrospective evaluation of blood alcohol [ ]

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## 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)

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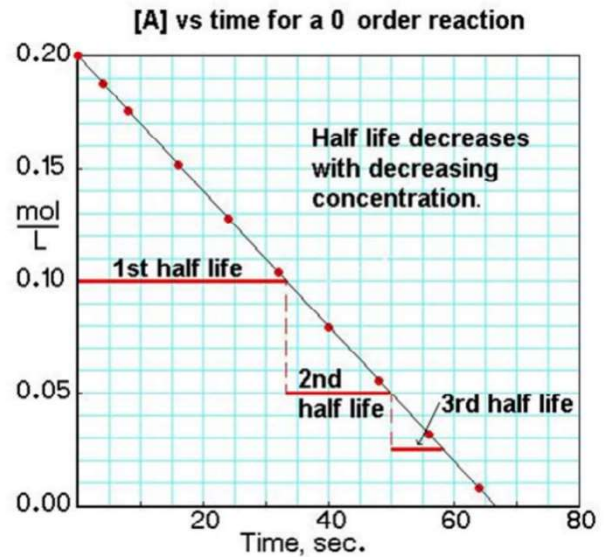
## Elimination, zero order kinetics

### Ethanol elimination

- Independent of the [ ]
- Constant speed



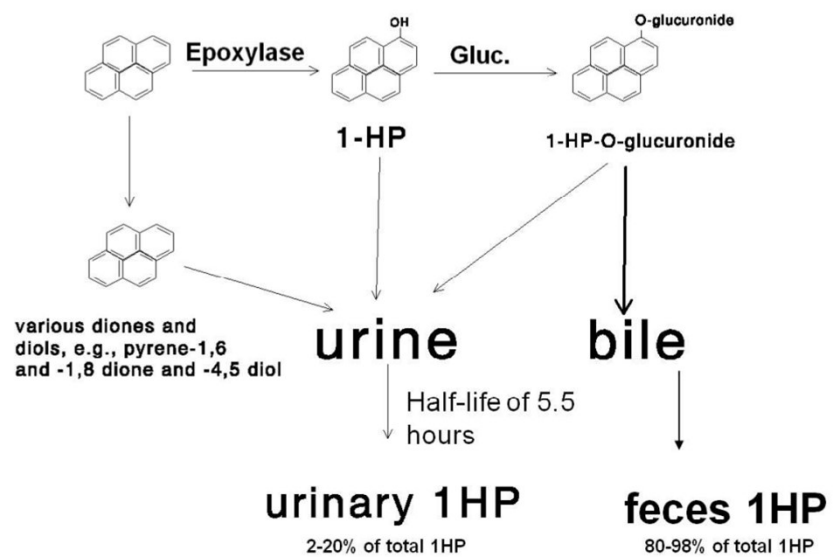
$$T_{\frac{1}{2}} = \frac{[A_0]}{2k}$$



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## Elimination, first order kinetics

Example. PAH exposures in foundries



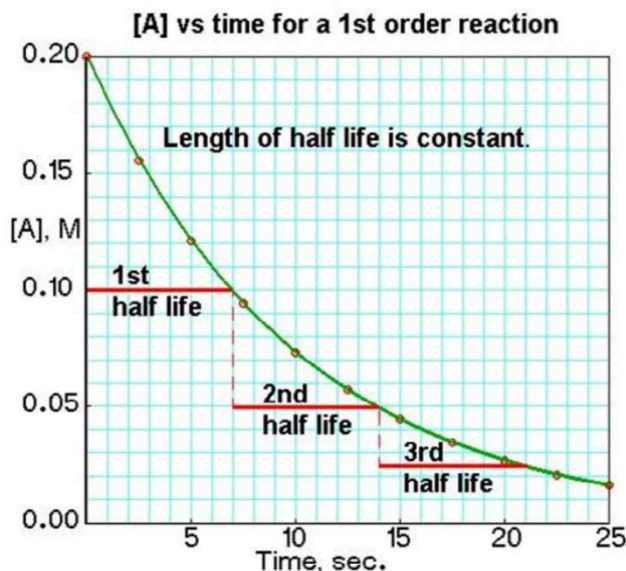
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## Elimination, first order kinetics

Elimination is proportional to the [ ] of the toxicant

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

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



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## Elimination kinetics

### Zero order elimination

A → products

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

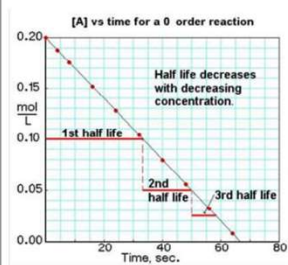
$$[A] = [A_0] - kt$$

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

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

Ex. Ethanol

No-model



\*(when [A] = [B])

### First order elimination

A → products

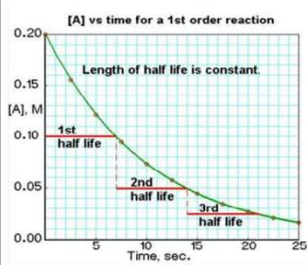
$$\text{rate} = k[A][\text{s}^{-1}]$$

$$[A] = [A_0] e^{-kt}$$

$$t_{\frac{1}{2}} = 0.693 / k$$

- Constant proportion of the agent is eliminated per unit time
  - Logarithmic process
- Ex. Most chemicals

One-compartment toxicological model



### Second order elimination

2A → or A + B → products\*

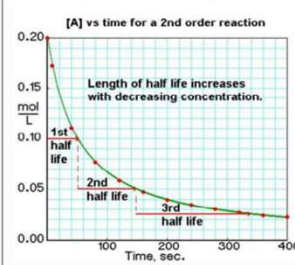
$$\text{rate} = k[A]^2 [\text{L mol}^{-1} \text{s}^{-1}]$$

$$1/[A] = 1/[A_0] + kt$$

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

- Chemical concentrations in tissues takes time to reach equilibrium with plasma
- Ex. Persistent chemicals

Multi-compartment toxicological model



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

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## Case study

### The shoe factory

Toluene is quickly absorbed and metabolized into the body (1<sup>st</sup> 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?



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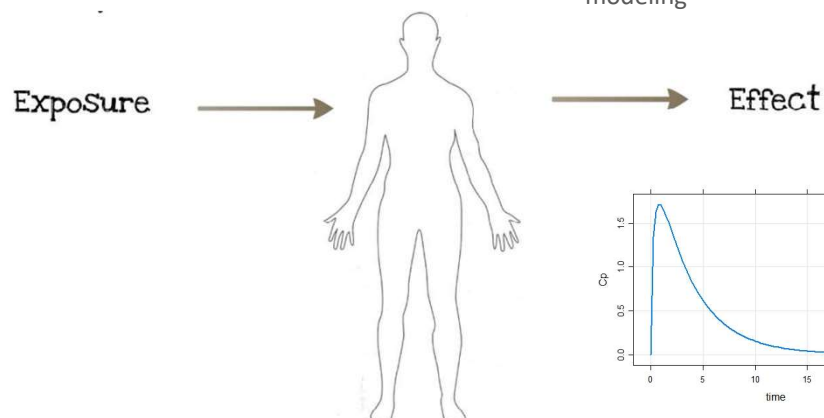
## Toxicokinetics

### 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



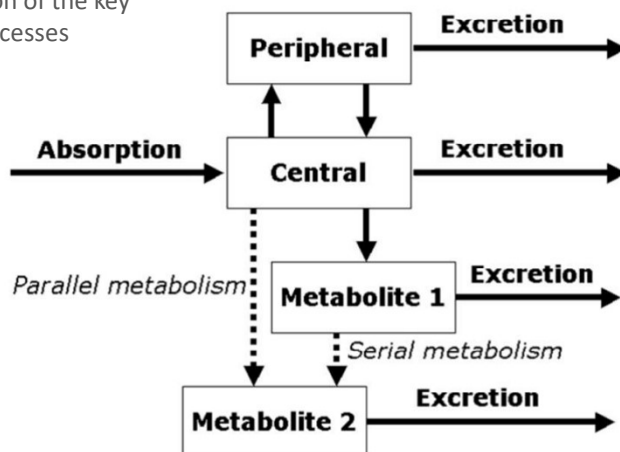
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## Toxicokinetics – modeling

TK model

- Mathematical representation of the key body compartment and processes



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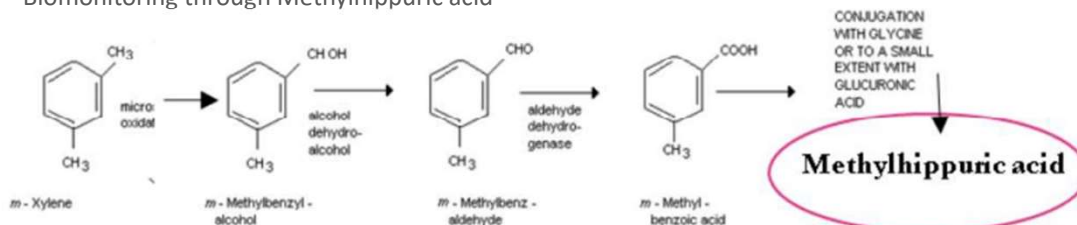
## Toxicokinetics

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

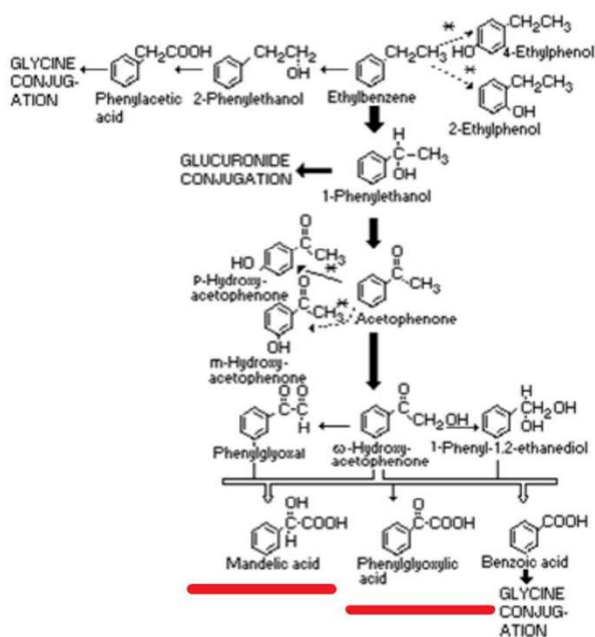


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## Toxicokinetics

### Metabolization of Ethylbenzene

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

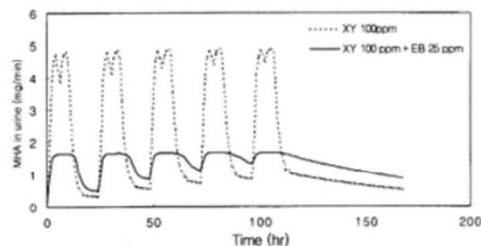


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## Toxicokinetics- biomonitoring

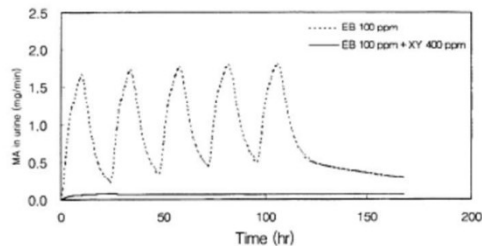
### Biomonitoring of Methylhippuric acid (MHA)

- 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



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# 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

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# 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 (biologischer arbeitstoff-toleranz wert) in Germany
- VBT (Valeurs biologiques tolérables) in Switzerland

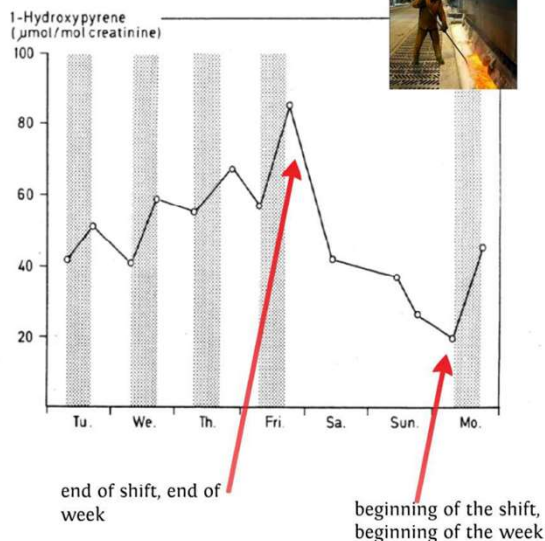
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## Biomonitoring strategy

Important factors

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



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## Biomonitoring strategy

What and When to sample ?

Determinant	Media	T1/2 [h]	Timing
<b>METALS</b>			
Lead	blood	900	not critical
Chromium	urine	7	end of shift
Cadmium	urine	20 years	not critical
Nickel	urine	24	end of shift
<b>SOLVENTS METABOLITES</b>			
N-Methylformamide	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
<b>SOLVENTS</b>			
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

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## 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:

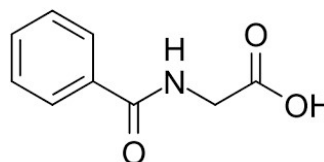
[HA]  $T_0$  (Immediately after exposure): 3.0 [g/g creatinine]

[B]  $T_4$  (4 hours after exposure) : 2.1 [g/ g creat.]

[B]  $T_{12}$  (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?



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## 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

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# 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										

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# 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										

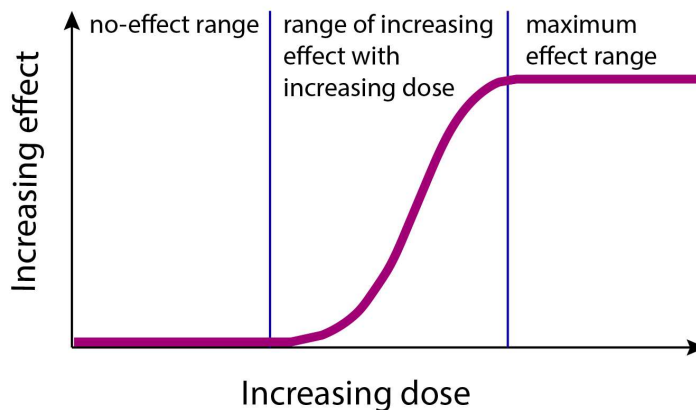
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# Dose-response relationship

## Classic relationship

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

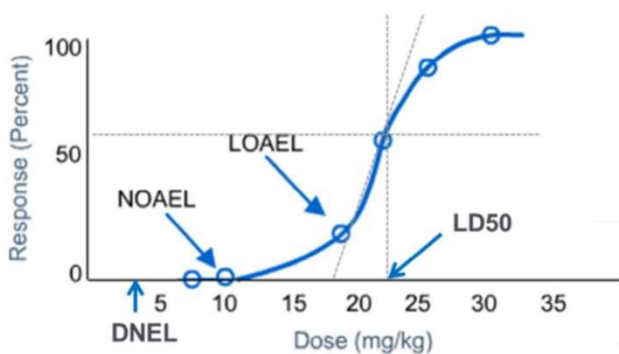


Source: Toxicology Education Foundation

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# Dose-response and thresholds

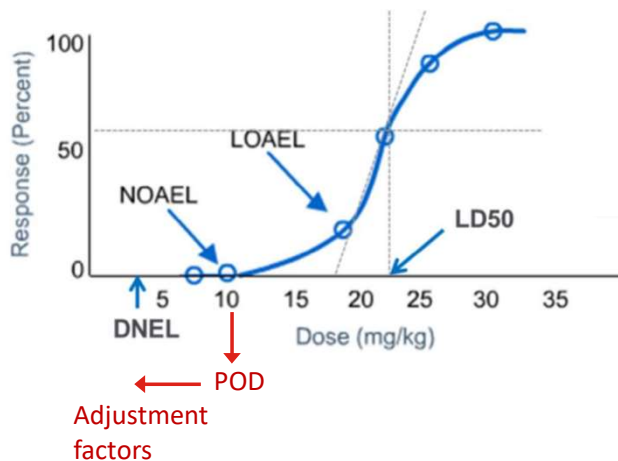


- NOAEL – No Observed Adverse Effect Level  
*Highest dose or concentration at which no effect is observed*
- LOAEL – Lowest Observed Adverse Effect Level  
*Lowest dose or concentration at which the effect is observed*
- LD – Lethal Dose 50%  
*Dose or concentration at which effects are observed in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

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## 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

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## Occupational exposure levels (OELs)

In Switzerland

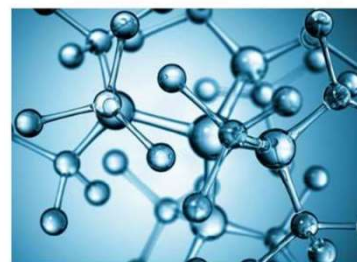
- 8h-TWA (Time Weighted Average), **VME – Valeur Moyenne d'exposition**

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



Valeurs limites d'exposition  
aux postes de travail

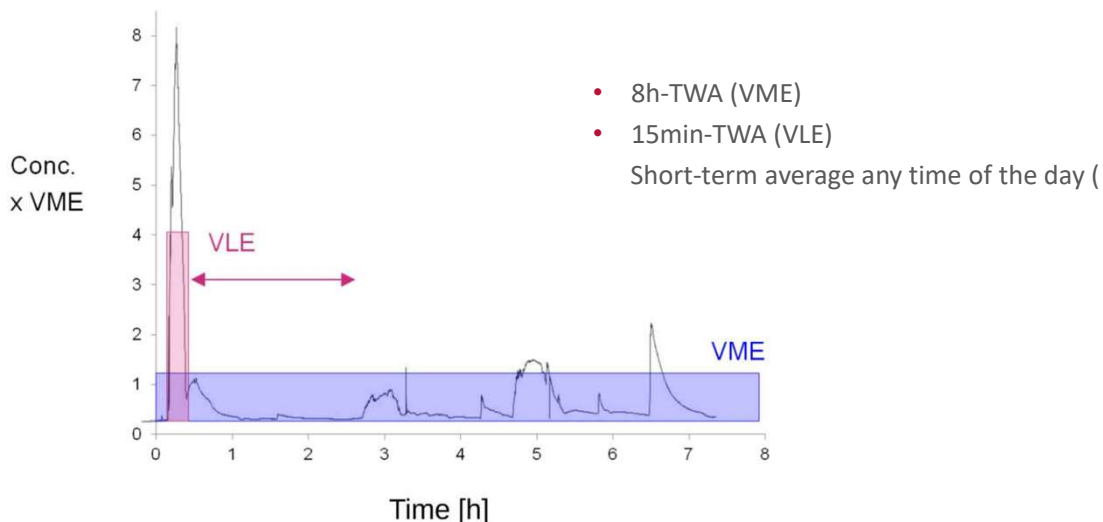


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## Occupational exposure levels (OELs)



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## VME / VLE values

Substance (no CAS)	VME		VLE		Notations R S O <sup>a</sup> B P C M Rv R <sub>h</sub> SS	Toxicité critique	Indications analytiques/ Remarques
	ml/m <sup>3</sup> (ppm)	mg/m <sup>3</sup>	ml/m <sup>3</sup> (ppm)	mg/m <sup>3</sup>			
Composés de Tétra-n-octylétain [7440-31-5] (exprimé en Sn)	0,004	0,021	0,004 <sup>a</sup>	0,021 <sup>a</sup>	R	Yeux, VRS, SNC, Nausée, Immun <sup>TC AN</sup>	
Ethane [74-84-0]	10000	12500				Arh, SNC, Formel <sup>TC</sup>	
Ethanol [64-17-5]	500	960	1000	1920	SS <sub>2</sub>	VRS, Formel <sup>TC HU</sup>	INRS, NIOSH
Ethanolamine v. 2-Aminoéthanol							
Ether de bis(chlorométhyle) v. Oxyde de bis(chlorométhyle)							
Ether bis-2-méthoxypropylique v. Oxyde de dipropylène glycolméthyle							
Ether 1-chloro-2,2,2-trifluoréthyl- difluorométhyle [26675-46-7]	10	77	80	616			
Ether 2-chloro-1,1,2-trifluoréthyl- difluorométhyle [13838-16-9]	10	77	80	616	SS <sub>2</sub>		OSHA
Ether de pétrole 30-75 sans hydrocarbures aromatiques	500	2000				SNC, VRS <sup>TC</sup> & Yeux <sup>TC</sup>	OSHA Respecter la VME du n-Hexane
Ether α,α-dichlorodiméthyle v. Oxyde de bis(chlorométhyle)							
Ether 2,2'-dichlorodiméthyle [111-44-4]	5	30	5 <sup>a</sup>	30 <sup>a</sup>	R C <sub>2</sub>	Yeux, Nausée, VR <sup>TC</sup>	NIOSH
Ether diisopropylique [108-20-3]	200	850	400	1700	SS <sub>2</sub>	Yeux, VRS, Foie, Rein, Halitose <sup>TC HU</sup>	NIOSH
Ether diméthyle [115-10-6]	1000	1910				Formel <sup>TC</sup>	
Ether diméthyle du diéthylène glycol [111-96-6]	5	27	40	216	R R <sub>12</sub> R <sub>13</sub> R <sub>37</sub> SS <sub>2</sub>	ReproM & P <sup>TC AN</sup>	

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## 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

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## Limits of the regulatory thresholds

### 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

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