WaterTOP Training School: "Risk assessment approaches for water T&O
Rome October 16-18, 2023

Fundamentals of the risk assessment procedure for single chemicals: hazard identification, dose-response relationship, exposure assessment, risk characterization

Emanuela Testai, Maura Manganelli, Simona Scardala
Istituto Superiore di Sanità
Environment and Health Dept.

From the EU web site: Drinking water (europa.eu)

High quality, safe and sufficient drinking water is essential for public health and well-being. Besides consumption, we also use it for many other purposes, such as washing, cleaning, hygiene, or watering our plants.

Most people living in the EU already enjoy very good access to high quality drinking water, thanks in part to over 30 years of EU policy on drinking water quality. **This policy ensures that water intended for human consumption can be consumed safely, leading to a high level of health protection**.

The main pillars of EU drinking water policy are to

- **protect human health by ensuring the quality of water intended for human consumption**
- ensure that drinking water quality is **controlled** through standards based on the latest scientific evidence
- secure efficient and effective **monitoring, assessment and enforcement of drinking water quality**
- provide Europeans with adequate, timely and appropriately **information**
- improve **access** to water intended for human consumption

The recast Drinking Water Directive entered into force in January 2021 is the EU's main law on drinking water. Member States have to transpose the Directive into national law and comply with its provisions by 12 January 2023.

The recast Drinking Water Directive will further protect human health thanks to updated water quality standards, tackling pollutants of concern and leading to even cleaner water from the tap for all.

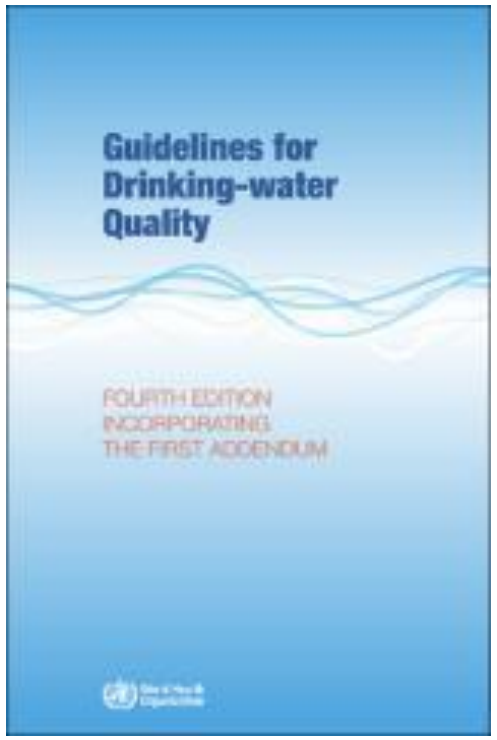
The Directive applies to

- all water, either in its original state or after treatment, intended for drinking, cooking, food preparation or other domestic purposes in both public and private premises, regardless of its origin and whether it is supplied from a distribution network, supplied from a tanker or put into bottles or containers, including spring waters

Key features of the revised Directive are:

- reinforced water quality standards, in line or, in some cases, even more stringent than the World Health Organisation (WHO) recommendations
- **tackling emerging pollutants**, such as endocrine disruptors and PFAs, as well as microplastics
- a preventive approach favouring actions to reduce pollution at source by introducing the risk-based approach
- measures to ensure **better access** to water, particularly for vulnerable and marginalised groups
- measures to **promote tap water,** including in public spaces and restaurants, to reduce (plastic) bottle consumption
- harmonisation of the quality standards for **materials and products in contact with water**
- measures to **reduce water leakages** and to increase transparency of the sector-body.

World Health Organization



WHO produces **international norms on water quality and human health in the form of guidelines** that are used as the basis for regulation and standard setting world-widefor water safety in support of public health.

The Guidelines for drinking-water quality (GDWQ) promote the **protection of public health** by advocating for the development of locally relevant standards and regulations (**health based targets**), adoption of **preventive risk management approaches covering catchment to consumer (Water Safety Plans)** and independent surveillance to ensure that Water Safety Plans are being implemented and effective and that national standards are being met.

The **fourth edition** of the World Health Organization's (WHO) Guidelines for drinking-water quality (**GDWQ**) (**published March 2022**) builds on over 50 years of guidance by WHO on drinking-water quality.
It is the product of significant updates reflecting new scientific evidence and further, provides additional explanations to support better understanding and application of the guidance. Since 1995, the Guidelines have been kept up to date through a process of "rolling revision" to ensure alignment with scientific progress and new knowledge

World Health Organization

The GDWQ provide the **scientific point of departure for standard setting and regulation**. They describe evidence-based guidance on reasonable minimum requirements of safe-practice to protect the health of consumers and progress towards improving water safety. They may also derive numerical "guidelines values" for constituents of water or indicators of water quality.

The GDWQ **describe the approaches used in deriving the guidelines**, including numerical "guideline values", and explain how the GDWQ are intended to be used.

The GDWQ include an **assessment of the health risk** presented by the various microbial, **chemical**, radiological and physical constituents that may be present in drinking-water. The GDWQ also define the criteria used to select the various constituents addressed.

The GDWQ may be accompanied by separate texts that provide **background information** substantiating or elaborating on the recommendations included in the GDWQ, and by texts that provide **guidance on good practice** towards effective implementation of the guidelines.

Contaminants and particularly Contaminants of Emerging Concern (CEC) in the aquatic compartment and in drinking water

Why are they on the spot?

Their **impact on human health and the environment** is not yet fully understood, especially when related to chronic exposure.

Why are they emerging now?

The **improved analytical methods** sensitivity has allowed to detect in the water supplies chemicals that had not previously been detected. But generally they are not routinely monitored

CEC belong to **different classes**, such as

- ✓ drugs (pharmaceuticals, antibiotics, hormones or illicit drugs)
- ✓ personal care products and cosmetic ingredients (triclosan, UV filters…)
- ✓ industrial chemicals (PFAS)
- ✓ **natural toxins** (cyanotoxins and other products such as **T&O**)
- ✓ **processing and disinfection byproducts**

Is there a common feature?

Hydrophilicity → polar/anphipathic substances and ions (charged)

- have a **high affinity for water**,
- are **poorly retained in soils**,
- **move easily** with the moving water,
- are **transported** along with it and can easily reach drinking water

→ **in other words: they are Mobile**.

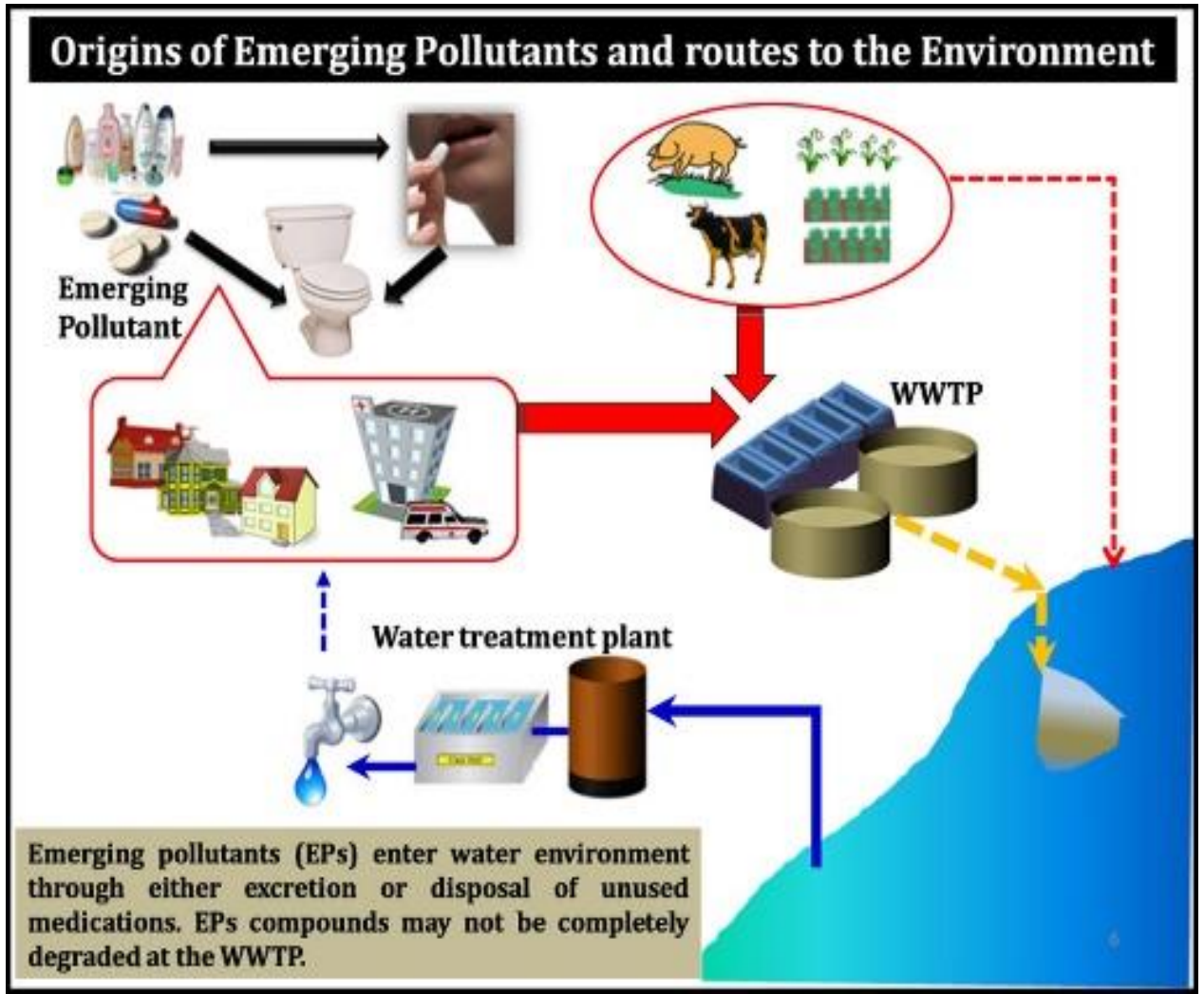
They are also poorly retained **by sorptive water treatment processes**

↓

the presence of some CEC in surface and groundwater used for drinking water production can represent a relevant issue since the **conventional drinking water treatment are not always designed to remove them** and in some cases **cost-effective technologies** applicable to drinking water treatment plants **are not available**.

CEC are currently not subject to drinking water regulations, in most cases because **regulatory limits have not been defined yet**.

In 2000, an initial list of 33 priority substances was identified under the EU Water Framework Directive (WFD).
The EU WFD (EC, 2013) listed 45 priority compounds with EQS to be respected in aquatic environments and listed others on contemporary watch list (Decision 2015/495)



The updated Watch List :

- ✓ **Estrogens** (17-Alpha-ethinylestradiol (EE2), 17-Beta-estradiol (E2), estrone (E1))
- ✓ **Macrolide antibiotics** (erythromycin, clarithromycin, azithromycin)
- ✓ Metaflumizone
- ✓ Amoxicillin
- ✓ Ciprofloxacin
- ✓ Methiocarb
- ✓ **Neonicotinoids** (imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid)

Many other chemicals are present….

For those CEC for which no legal limit have been established for DW, it is necessary to conduct a risk assessment to derive **HBGV (health based guidance values)**. This is what the chemical Group within WHO usually does to adopt the WHO DWGL (as described in the Background documents)

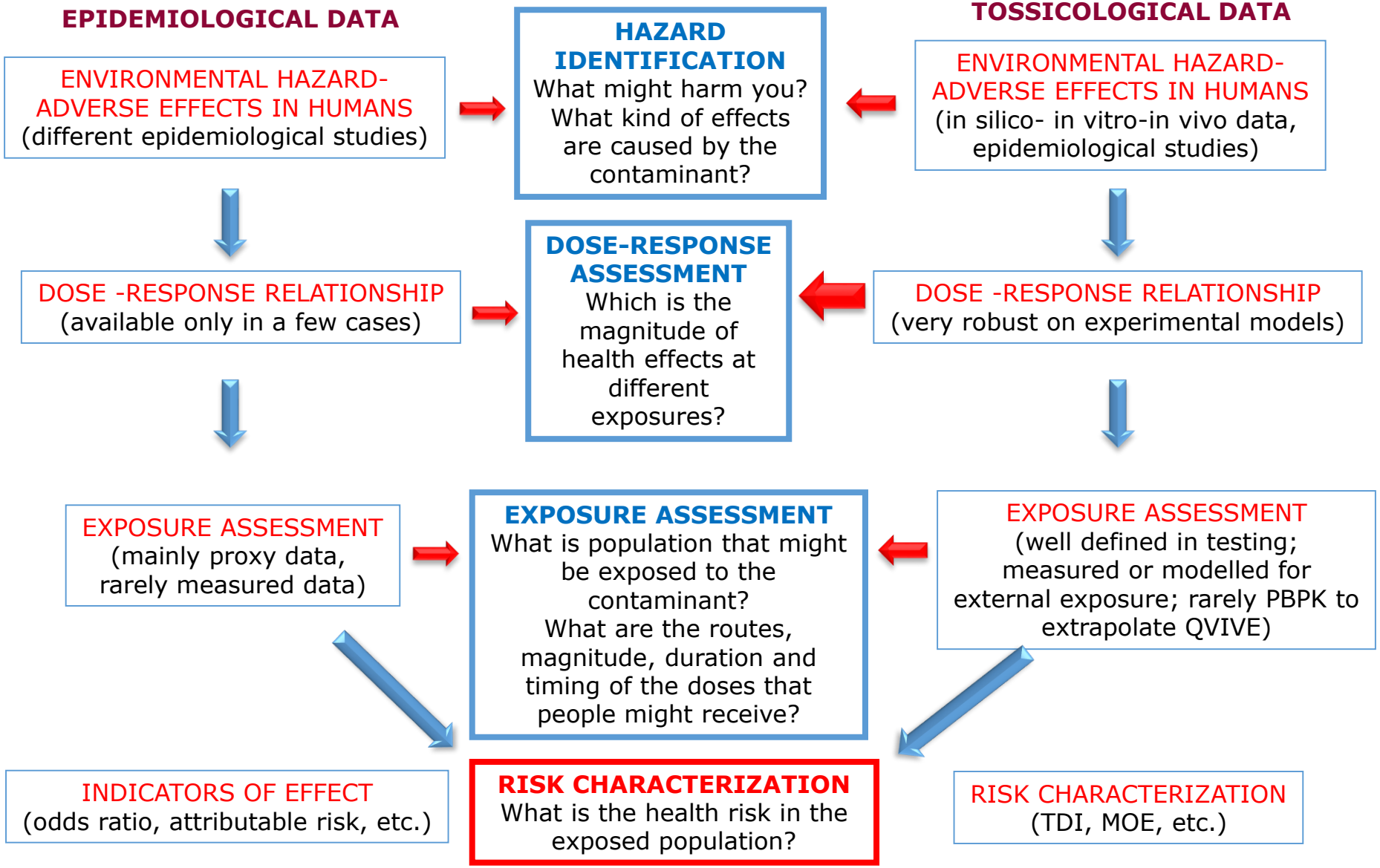
For many of the chemicals in the group of CEC (e.g. pesticides and pharmaceuticals) data on the hazard (i.e. the tox profile) to conduct a full risk assessment are often available.

The identification of exposure scenarios and exposure levels are not always that straightforward.

For many other CEC data are not good enough to perform a full RA and data gaps can be identified.

How to perform a risk assessment?

The 4 STEPs in RISK ASSESSMENT



1. *HAZARD IDENTIFICATION*

Is there a difference between **hazard and risk**?

The **hazard** is an intrinsic feature of the toxic agent.

The **risk** corresponds to the **probability** for a population of experiencing adverse effects once exposed to the **hazardous** agent.

A lion represents a significant hazard of being attacked for humans in the savana

vs

Although the hazard potential of the same lion is not changed, the risk of being attacked is highly reduced when the lion is locked in a cage

It reflects the qualitative aspects of the assessment and provides answers to the question:

Which kind of adverse effects does the chemical (stressor) induce?

1. **Hazard identification**
2. **Dose-response relationship**

} **Hazard characterization**

Mediated by toxicological research and only in rare cases by epidemiological research

The **hazard** is an intrinsic feature of the toxic agent.

The CLP classification and labeling system in Europe is based exclusively on HAZARD Reg (CE) n. 1272/2008

The risk is the probability of experiencing the adverse effects following exposure and to identify this probability, it is absolutely necessary to have the dose-response relationship

2. DOSE-RESPONSE RELATIONSHIP

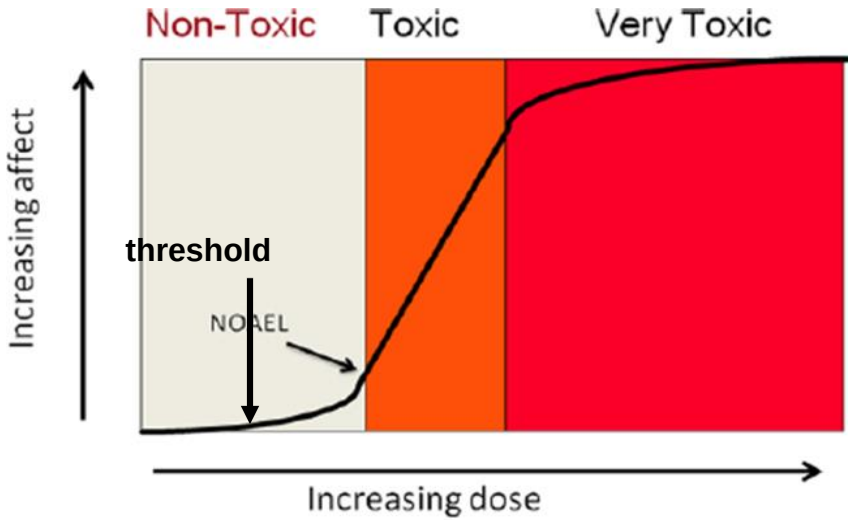
Quantitative Aspect : at which concentration/ level does the adverse effect occur?

Which is the threshold level at which no effects is observed?

Omnia venenum sunt: nec sine veneno quicquam existit. Dosis sola facit ut venenum non sit ' (Paracelso)

Misura ciò che è misurabile e rendi misurabile ciò che non lo è (Galileo)

The toxicology paradigm: the response of an organism to a chemical substance increases/decreases proportionally to the exposure dose. This determines a monotonic dose-response relationship in which the effect increases (or decreases) over the range of possible doses without changing direction



For non genotoxic chemicals a **threshold dose** exists below which adverse effects are not expected: the **threshold for adversity** has to be exceeded

By not taking into account the dose-response relationship and the subsequent comparison with real exposure levels (risk based approach), the hazard based approach (CLP) cannot be used as the only tool for making decisions in the public health field

How important is the dose? The example of hydrogen oxide

- ✓ At low doses it is non-toxic and indeed it is essential for life.
- ✓ At very high doses it alters the levels of some hormones (aldosterone, renin and angiotensin) on which our water-salt balance depends. The effects can be very serious up to death.

Based on hazard identification, water is toxic and has ED properties!

2. DOSE-RESPONSE RELATIONSHIP

The **threshold** for the effect can be achieved with :

A single exposure (acute and generally at high doses) ➡ accidents, poisonings

Repeated exposure (low doses for prolonged times; the low doses are generally non toxic if taken singularly ➡ cumulative effects)

Acute toxicity studies (i.e. identification of no acute effect level, non irritating or no sensitizing concentration; the LD50 or the yes/no answer for irritation are not useful for identifying a threshold but only for the CLP)

Repeated Toxicity Studies

Subacute, subchronic, chronic (non neoplastic effects)
Carcinogenesis studies
Reprotoxicity and development toxicity studies

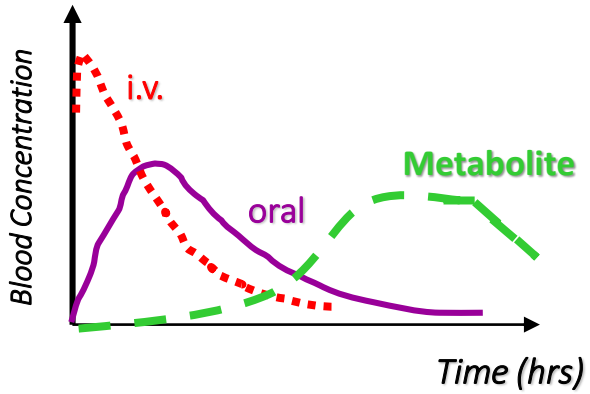
} **Multiple dose** testing; single dose are not useful to identify a threshold

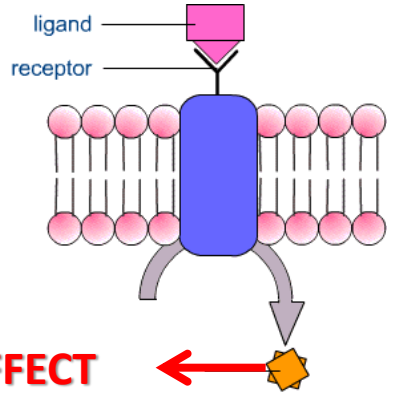
Exposure

Pharmaco/
toxico–kinetics

ADME X → X*

absorption
distribution
metabolism
escretion





EFFECT

Interaction between X o X* and the target

Pharmaco/
toxico–dynamics

How the toxicant impacts the organism

The processes integrating ADME, determining the **internal dose (biologically effective dose of the toxicant)** is usually referred to as **toxicokinetics (TK)**: how the organism handle the toxicant

The external dose is not the one causing any systemic effect. The systemic toxicity of a substance is in fact dependent on the concentration of the substance at the site of action in the target organ (internal dose).

Internal exposure (e.g. sistemically available circulating concentrations) takes into account all routes and the **TK** (metabolic fate, its distribution and excretion) including the uptake into the cells of the target organ(s) and the persistence of the chemical and or its metabolites in the body (body burden)

Toxicokinetics studies: horizontal to all kind of studies

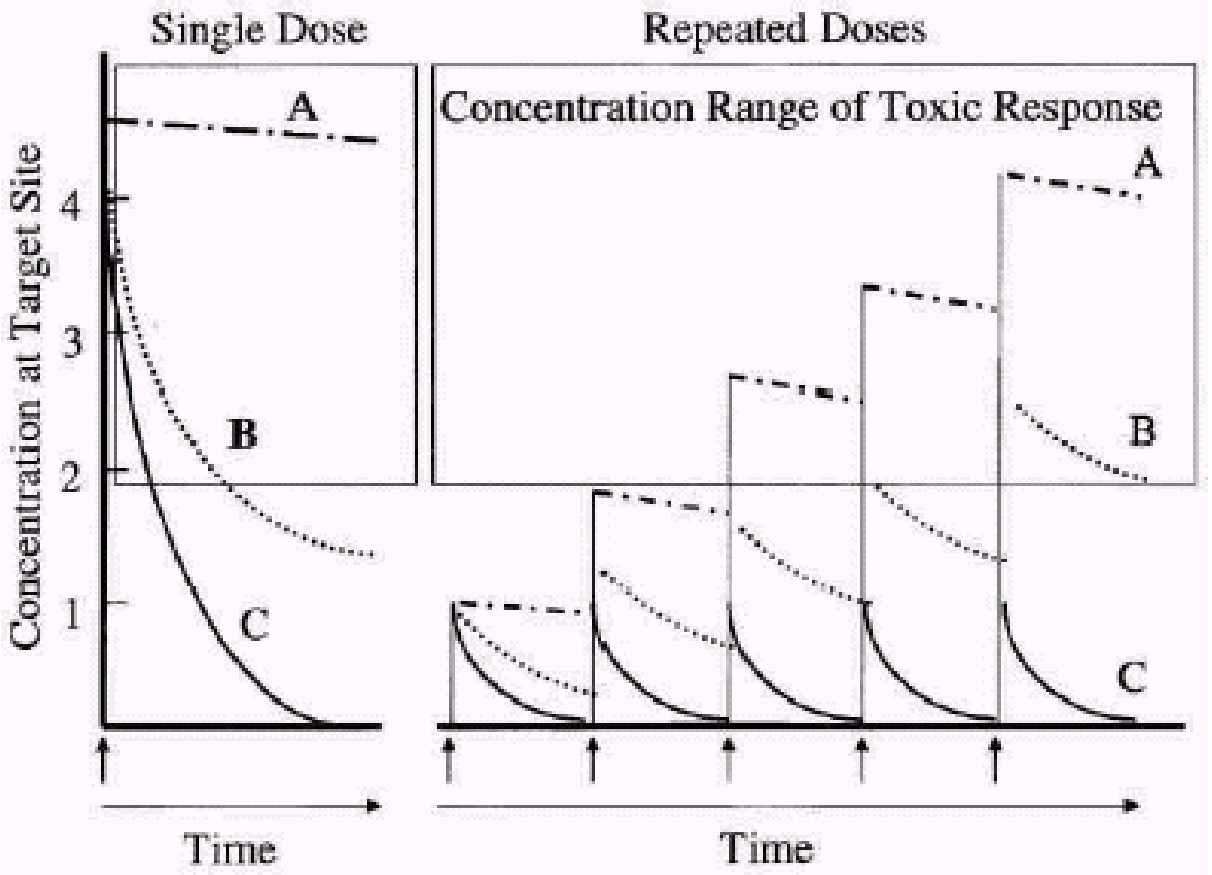
Fate of the substance within the organism (t1/2, rate and % of absorption and excretion ⇒ internal dose and bioaccumulation potential*; biotransformation, specie difference and relevance of the experimental model, doses to be used in repeated toxicity studies)

* When the **frequency/rate of administration is higher than the elimination rate**

A. Cumulative effects can be due to accumulation of the chemical after repeated exposure

Bioaccumulation of the chemical within the organism ⇒ frequency of administration is higher than rate of elimination (toxicokinetic behaviour) ⇒ the xenobiotic concentration becomes higher than the toxicity threshold

For bioaccumulating compounds, simultaneous internal exposure can occurr also when external exposure events are not simultaneous



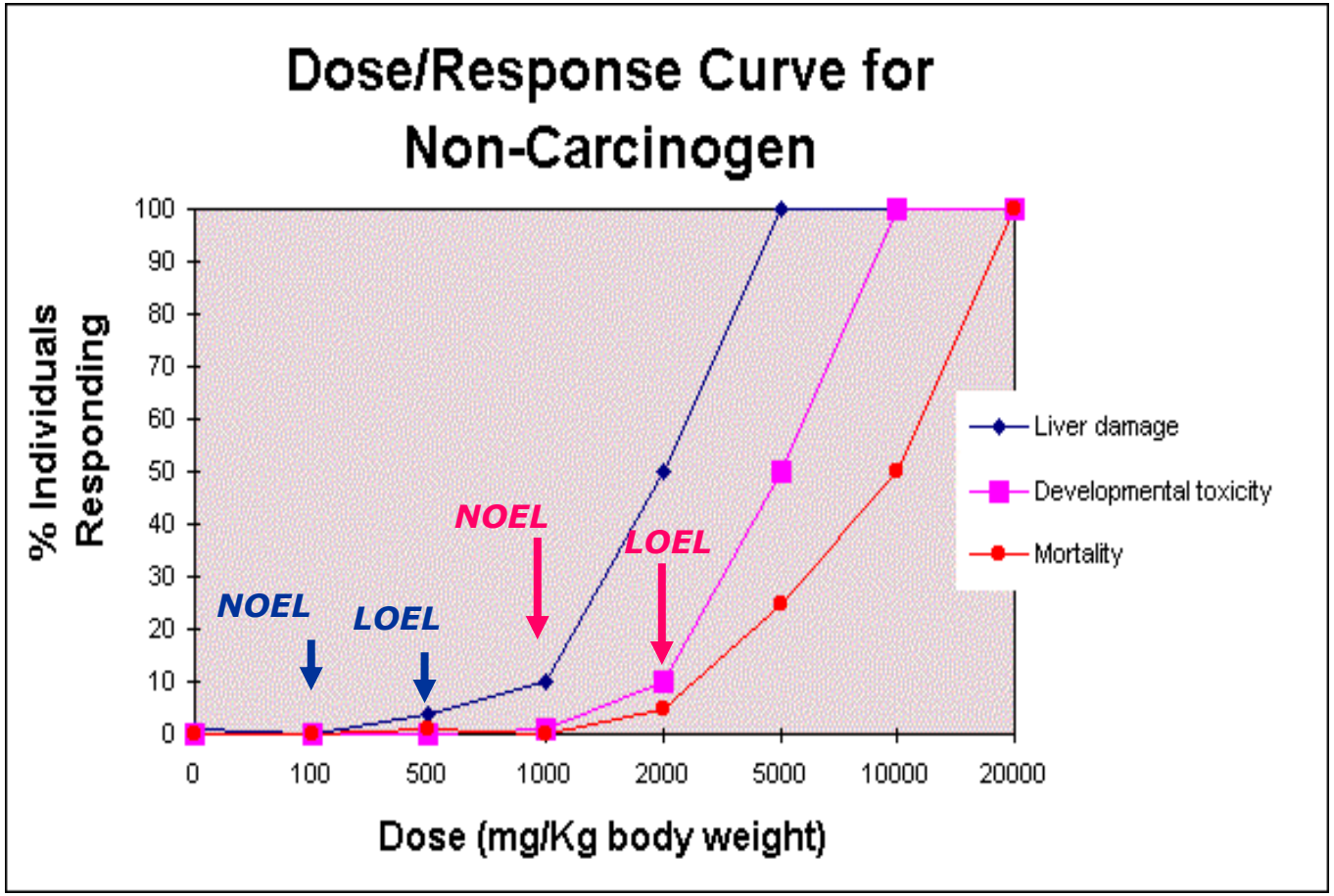
threshold for adversity

B. <u>Cumulative damages</u> ⇒ administration rate is higher than damage repair process within the organisms

It is possible to have cumulative damages without any bioaccumulation of the chemical ⇒ this is when recovery time is higher than elimination time.

Es: exposure to ethanol results in steatosis (lipid accumulation in the liver); ethanol is eliminated in a very short time, but lipids are removed from the hepatic tissue in a much longer time ⇒ outcome of chronic effects (cirrhosis) in heavy drinkers.

Dose response curves vary depending on the observed effect ⇒ threshold doses vary accordingly.



NO(A)EL - No Observed Effect Level: the highest level (exposure) at which the effect does not occur, in specific experimental conditions.

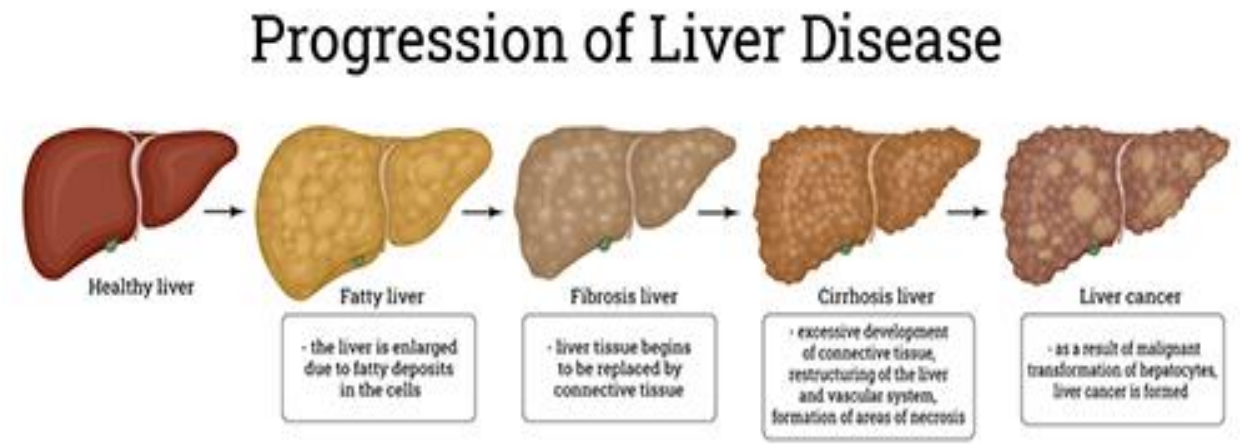
LO(A)EL - Lowest Observed Adverse Effect Level:the lowest level (exposure) at which the effect occurs, in specific experimental conditions.

The **threshold** represents the point at which the organism's ability to counteract the toxicity of a xenobiotic or to repair the damage is exceeded.

We talk about '**overcoming the threshold of adversity**' by distinguishing between **adaptive responses** and **adverse effects**

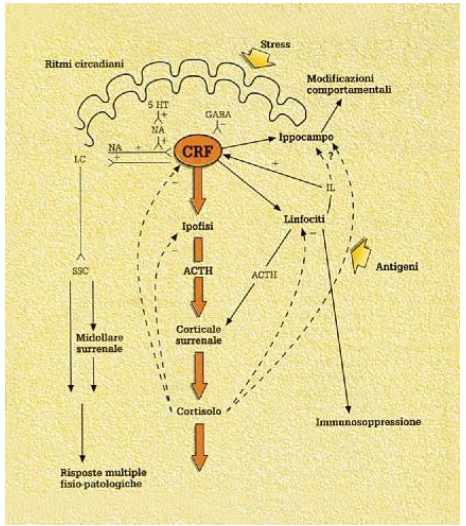
In many organs there is a reserve of 'functional capacity' that operates so that a significant reduction in performance does not occur before a significant part of the function being compromised.

The development of cirrhosis in the liver may not be apparent until 50% of the liver mass has been replaced by fibrotic tissue



This distinction is especially important for EDs

- ✓ alterations that fall **within the range of physiological variations** such as responses to a stimulus (endocrine modulation or adaptive responses) due to the **plasticity of the endocrine system**



and

- ✓ alterations which lead to the **onset of an adverse effect** (an unwanted response at the wrong times or at exaggerated levels and for too long a time, which causes the **loss of hormonal homeostasis**)

Many adaptive responses, compensatory actions and physiological processes are characterized by discrete changes in our status thanks also to the plasticity of the systems (e.g. changes in hormonal status):

- ✓ **reaction to food** (e.g. insulin secretion in response to ingestion of sugar-rich foods)

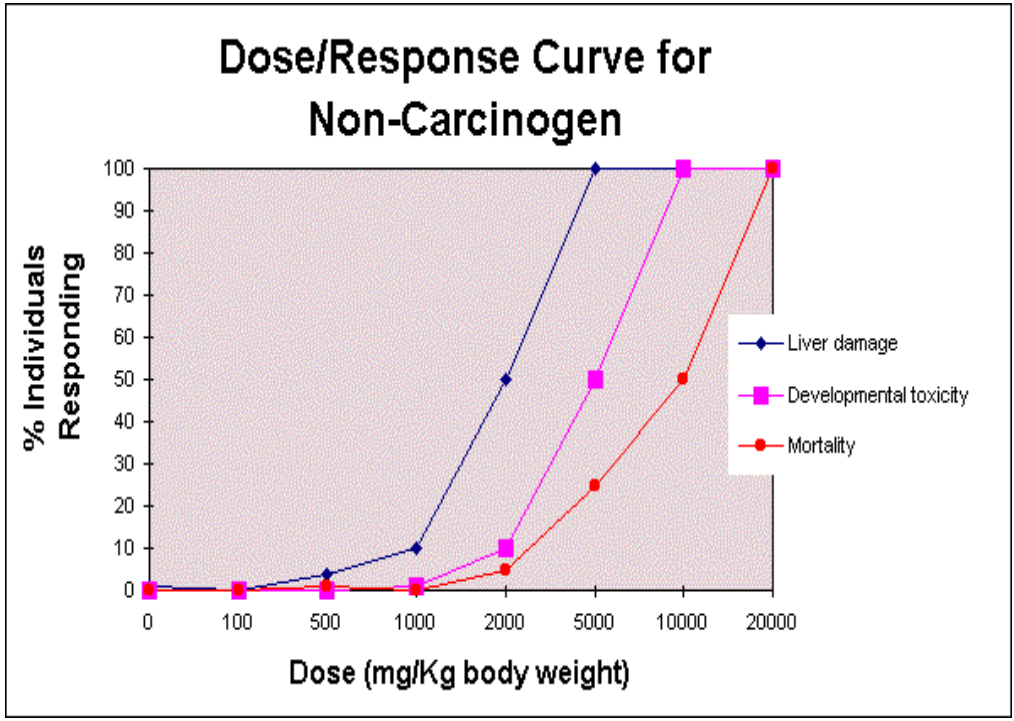
- ✓ **reaction to emergency situations** (e.g. secretion of adrenaline in case of stress or danger),

- ✓ **reactions underlying sexual behavior** (e.g. secretion of estrogen and/or testosterone)

Which is the critical adverse effect?

The effect is considered critical if:

- It is the one present at the **lowest dose**.
- It is the **most relevant** effects from a toxicological point of view (e.g. Hair loss *vs.* early marker of hepatic damage)

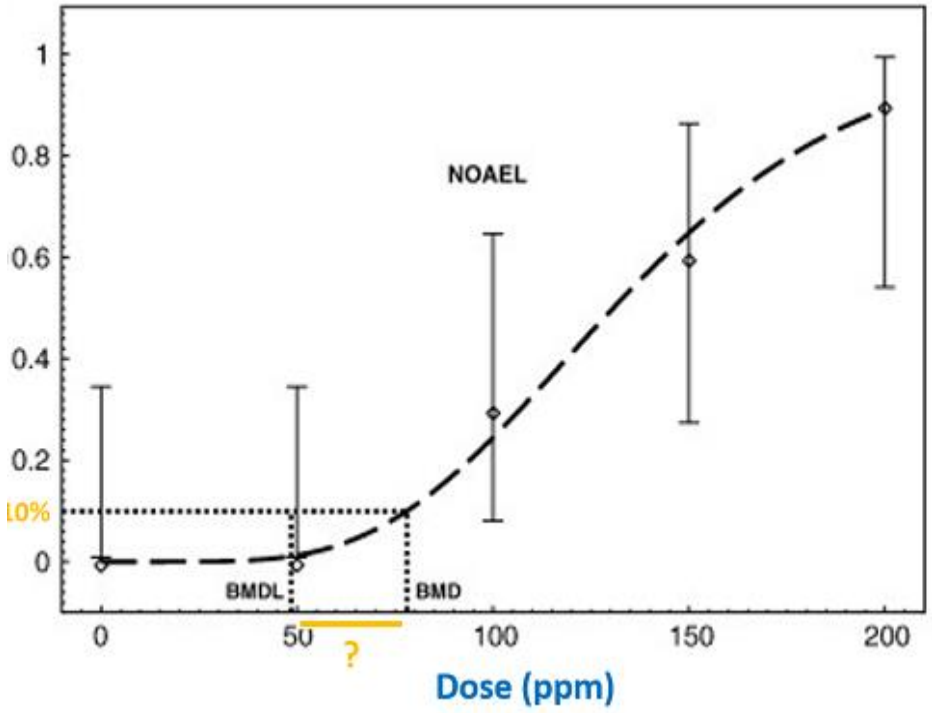


Preventing the critical effect allows to prevent all other effects, protecting human health

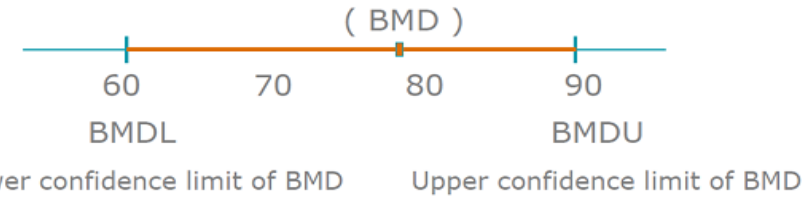
Some criteria to recognize the critical effects:

- **Toxicological relevance** of the effect (piloerection vs tremors)
- Presence of the effects with a **dose-related** trend
- Presence of related effects at higher doses (indication of **early effects progression**) : alteration of biochemical parameters followed by histopathological changes (i.e. increase in hepatic transaminases *vs* hepatocellular hypertrophy or necrosis)
- **Consistency** of the overall picture
- Presence of the effects in a **high number of treated animals**
- **Presence in both sexes**.

Beyond the NOAEL: the Benchmark Dose



1. Elaboration of the dose response curve based on the experimentl data.
2. Estrapolation of the value associated with the effects present in a specific % of animals (eg: 1, 5 o 10%) or with a % (eg: 0.1, 1, 5, 10%, depending on the effect) of the maximum effect =**BMD** o **Benchmark dose**
3. Calculation of the confidence interval of BMD : the lower limit identify the **BMDL** anc is generally used as **PoD (Point of Departure)** for the risk assesment
4. The BMDL is then divided by the appropriate AF

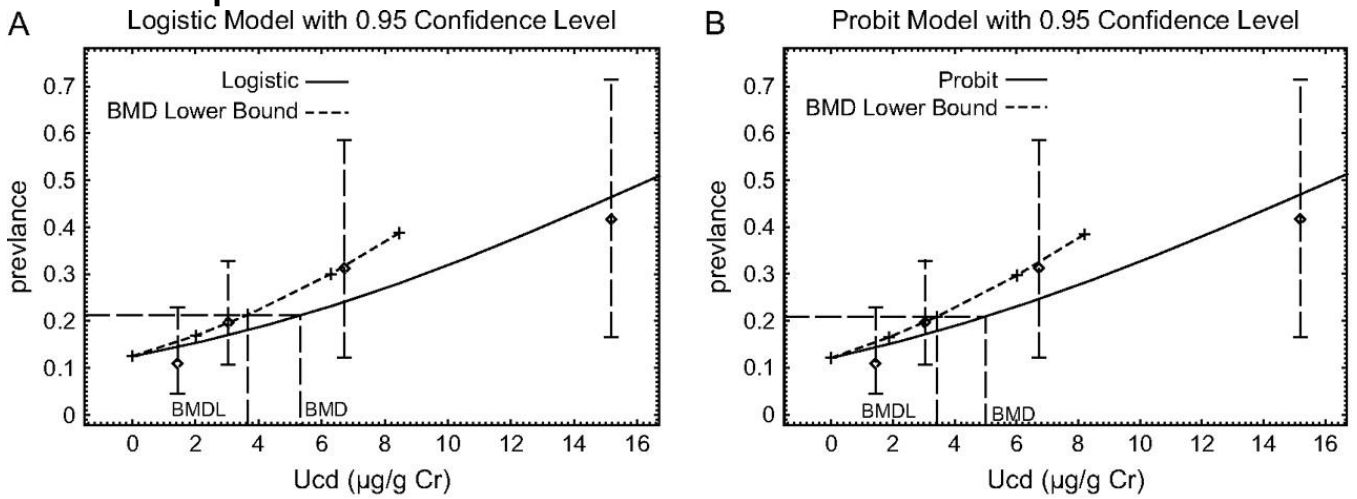


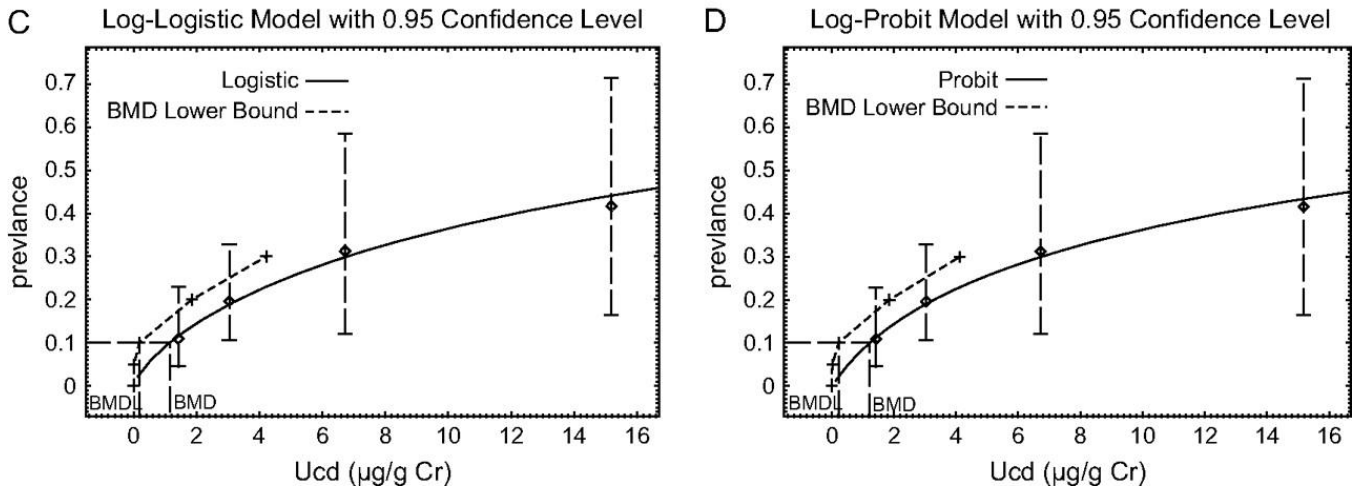
Advantages when compared to NOAEL:

- ✓ It is less dependent on the experimental design (dose spacing)
- ✓ The whole dose-response curve is considered including the slope
- ✓ Variability (biological and experimental) is considered

Disvantages:

- ✓ Need to have a number of tested doses high enough to perform a significant extrapolation
- ✓ Dependence on the model used for the extrapolation





BMD advantages

- Not limited to experimental doses
- Less dependent on dose spacing
- Appropriately accounts for variability and uncertainty resulting from study quality
- Takes into account the shape of the dose–response curve and other related information
- Corresponds to consistent response level and can be used to compare results across chemicals and studies
- Flexibility in determining biologically significant rates

BMD limitations

- Ability to estimate BMD may be limited by the format of data presented
- Time consuming
- More complicated decision making process

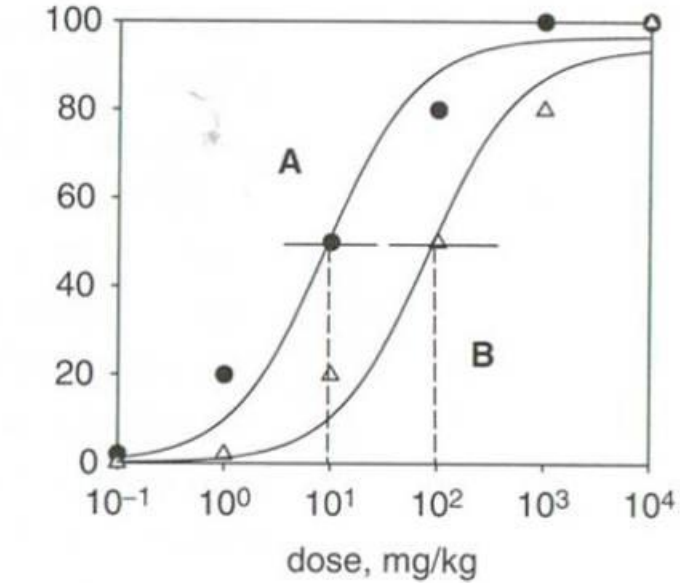
NOAEL limitations

- Highly dependent on dose selection
- Highly dependent on sample size
- Does not account for variability and uncertainty in the experimental results (e.g., does not account for study quality appropriately)
- Dose–response information (e.g., shape of dose–response curve) not taken into account
- Does not correspond to consistent response levels for comparisons across studies
- A LOAEL cannot be used to derive a NOAEL

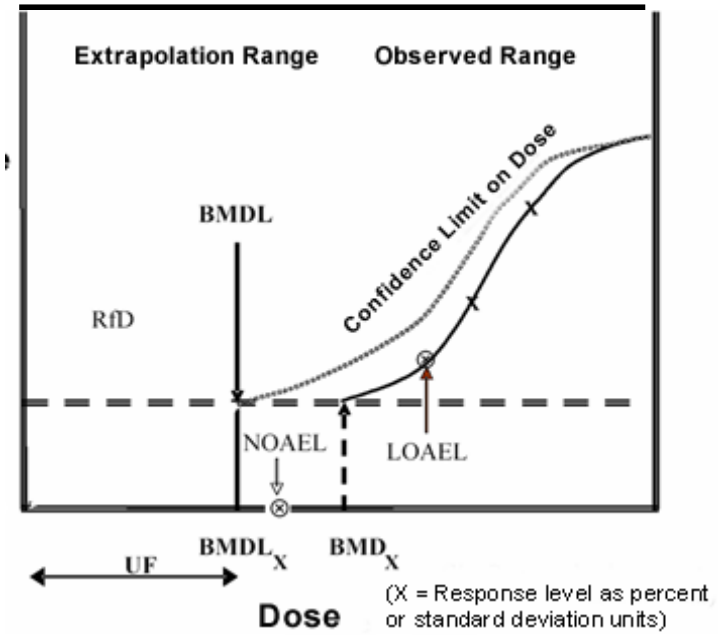
NOAEL advantages

- Can be used when data is not amenable for BMD modeling
- Easy to derive
- Has been the standard method for deriving a POD for decades (e.g., is familiar to most risk assessors)

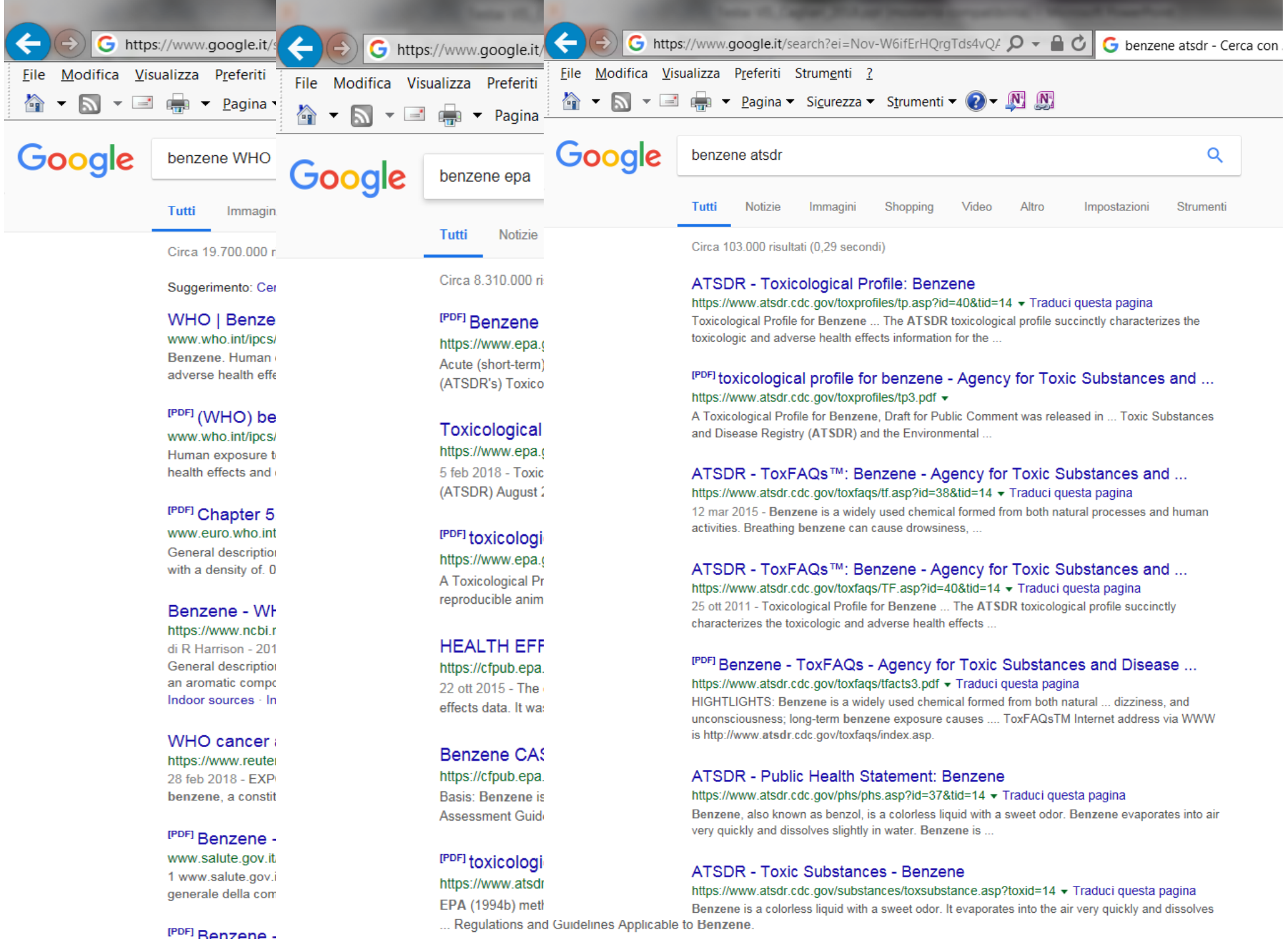
The **dose-response relationship** is fundamental to identify the reference doses (or **Point of Departure**, such as the NOAEL/LOAEL or the Benchmark Dose from experimental studies) from which to derive, through the application of appropriate uncertainty factors, reference values " health based" for human exposure:



- Acute Reference Dose or ARfD for acute/subacute exposure
- Tolerable Daily Intake or TDI or Acceptable Daily Intake (ADI), Reference Dose (RfD), DNEL for chronic exposure to a pollutant,
- Reference Concentration (RfC) for inhalation exposure



In the vast majority of cases, faced with the need to conduct a RA, you will find yourself in the position of not having to derive the health based reference values yourself, which will be available from assessments already carried out by international agencies


https://www.google.it/
File Modifica Visualizza Preferiti
Pagina
Google
benzene WHO
Tutti
Immagin
Circa 19.700.000 r
Suggerimento: Cer
WHO | Benze
www.who.int/ipcs/
Benzene. Human
adverse health effe
[PDF] (WHO) be
www.who.int/ipcs/
Human exposure t
health effects and
[PDF] Chapter 5
www.euro.who.int
General descriptio
with a density of. 0
Benzene - WH
https://www.ncbi.r
di R Harrison - 201
General descriptio
an aromatic compo
Indoor sources · In
WHO cancer
https://www.reute
28 feb 2018 - EXP
benzene, a constit
[PDF] Benzene -
www.salute.gov.it
1 www.salute.gov.i
generale della com
[PDF] Benzene -
https://www.google.it/
File Modifica Visualizza Preferiti
Pagina
Google
benzene epa
Tutti
Notizie
Circa 8.310.000 ri
[PDF] Benzene
https://www.epa.
Acute (short-term)
(ATSDR's) Toxico
Toxicological
https://www.epa.
5 feb 2018 - Toxic
(ATSDR) August
[PDF] toxicologi
https://www.epa.
A Toxicological Pr
reproducible anim
HEALTH EFF
https://cfpub.epa.
22 ott 2015 - The
effects data. It was
Benzene CAS
https://cfpub.epa.
Basis: Benzene is
Assessment Guid
[PDF] toxicologi
https://www.atsdr
EPA (1994b) metl
... Regulations and Guidelines Applicable to Benzene.
https://www.google.it/search?ei=Nov-W6ifErHQrgTds4vQ
benzene atsdr - Cerca con
File Modifica Visualizza Preferiti Strumenti ?
Pagina Sicurezza Strumenti
Google
benzene atsdr
Tutti
Notizie
Immagini
Shopping
Video
Altro
Impostazioni
Strumenti
Circa 103.000 risultati (0,29 secondi)
ATSDR - Toxicological Profile: Benzene
https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=40&tid=14 Traduci questa pagina
Toxicological Profile for Benzene ... The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the ...
[PDF] toxicological profile for benzene - Agency for Toxic Substances and ...
https://www.atsdr.cdc.gov/toxprofiles/tp3.pdf
A Toxicological Profile for Benzene, Draft for Public Comment was released in ... Toxic Substances and Disease Registry (ATSDR) and the Environmental ...
ATSDR - ToxFAQs™: Benzene - Agency for Toxic Substances and ...
https://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=38&tid=14 Traduci questa pagina
12 mar 2015 - Benzene is a widely used chemical formed from both natural processes and human activities. Breathing benzene can cause drowsiness, ...
ATSDR - ToxFAQs™: Benzene - Agency for Toxic Substances and ...
https://www.atsdr.cdc.gov/toxfaqs/TF.asp?id=40&tid=14 Traduci questa pagina
25 ott 2011 - Toxicological Profile for Benzene ... The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects ...
[PDF] Benzene - ToxFAQs - Agency for Toxic Substances and Disease ...
https://www.atsdr.cdc.gov/toxfaqs/tfacts3.pdf Traduci questa pagina
HIGHTLIGHTS: Benzene is a widely used chemical formed from both natural ... dizziness, and unconsciousness; long-term benzene exposure causes .... ToxFAQsTM Internet address via WWW is http://www.atsdr.cdc.gov/toxfaqs/index.asp.
ATSDR - Public Health Statement: Benzene
https://www.atsdr.cdc.gov/phs/phs.asp?id=37&tid=14 Traduci questa pagina
Benzene, also known as benzol, is a colorless liquid with a sweet odor. Benzene evaporates into air very quickly and dissolves slightly in water. Benzene is ...
ATSDR - Toxic Substances - Benzene
https://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=14 Traduci questa pagina
Benzene is a colorless liquid with a sweet odor. It evaporates into the air very quickly and dissolves

In the vast majority of cases, faced with the need to conduct a RA, you will find yourself in the position of not having to derive the health based reference values yourself, which will be available from assessments already carried out by international agencies

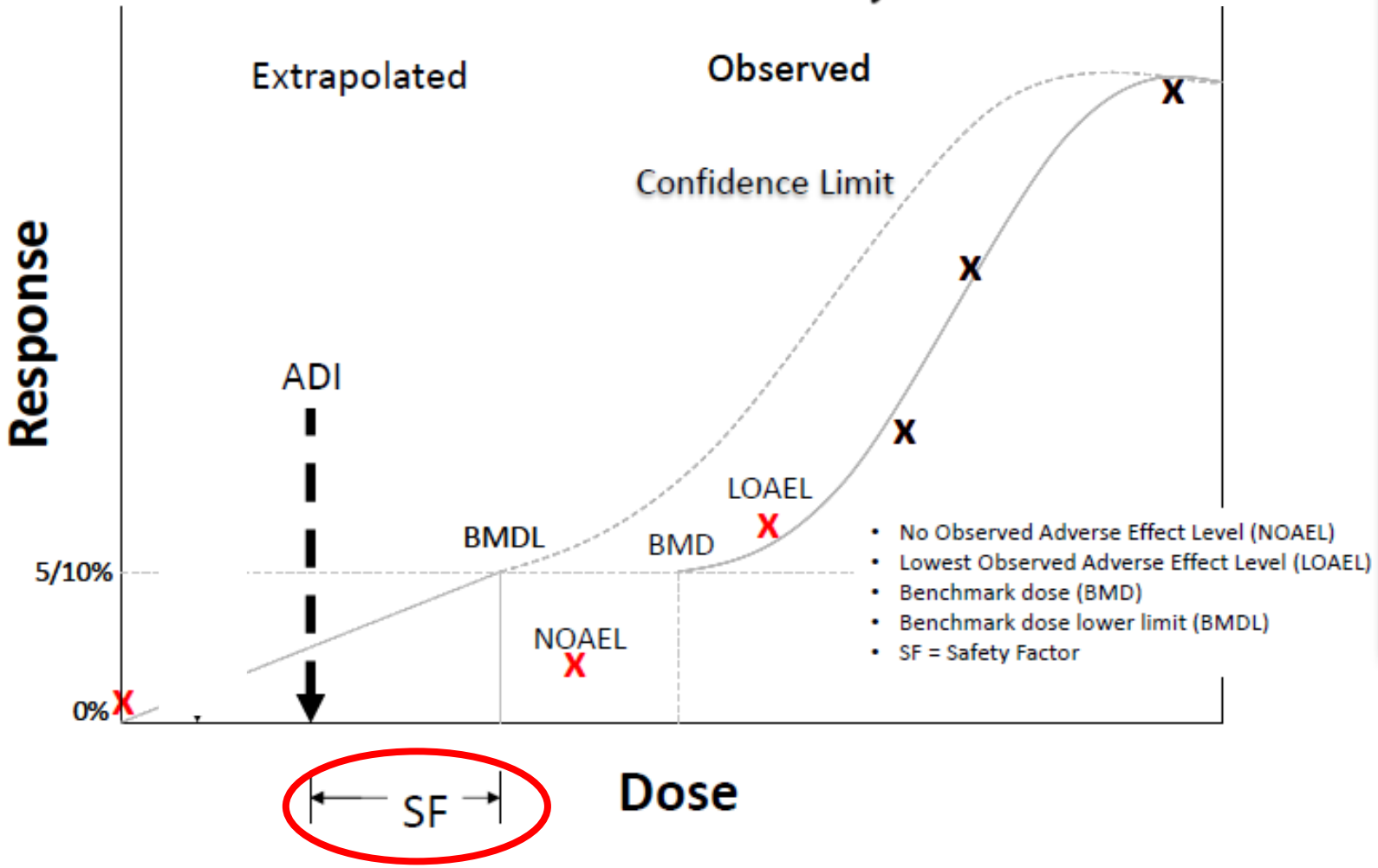
Information on how a risk assessment is conducted is essential to be able to choose the most appropriate value for the specific assessment depending on

- ✓ Time, frequency and duration of exposure (acute, subchronic and chronic)
- ✓ Route of exposure (inhalation, oral, dermal)
- ✓ Target population (children, elderly, healthy adults)

in other words with respect to the defined exhibition scenarios

Health based reference values can be very different considering the factors indicated above

HBV → ADI *(human dose)* = $\frac{\text{NOAEL } \textit{(experimental dose)}}{\text{Safety Factor(s)}}$



These values are generally 'conservative' and due to the introduction of SF have been defined to protect the population in the long term (chronic exposure), also taking into account the most vulnerable population groups.

HBV= Level of exposure below which adverse effects are assumed to have a near-zero probability of occurring in exposed populations

Reference values for chemicals characterised by a threshold (*)

ADI or TDI= acceptable/tolerable daily intake =

the daily dose below which it is assumed that adverse effects are unlikely to occur in humans exposed to the substance for the entire life span (estimated 70 years long.

$$\text{TDI or DNEL} = \frac{\text{NO(A)EL or BMDL}}{\text{AF}}$$

Lowest **NOAEL or BMDL** (critical effect/preferentially coming from chronic toxicity)

AF= variable depending on variability factors, uncertainties and severity/relevance of the observed effects.

(*) including non genotoxic carcinogens

Assessment factors (AS)
Uncertainty factors (UF)
Safety Factors (SF)

Introduction of **UF/SF/AF** in the derivation of the reference values is to take into account variability and uncertainties

Uncertainty factors :

Quality of the available studies and of the experimental results (e.g. gravity of the effects)

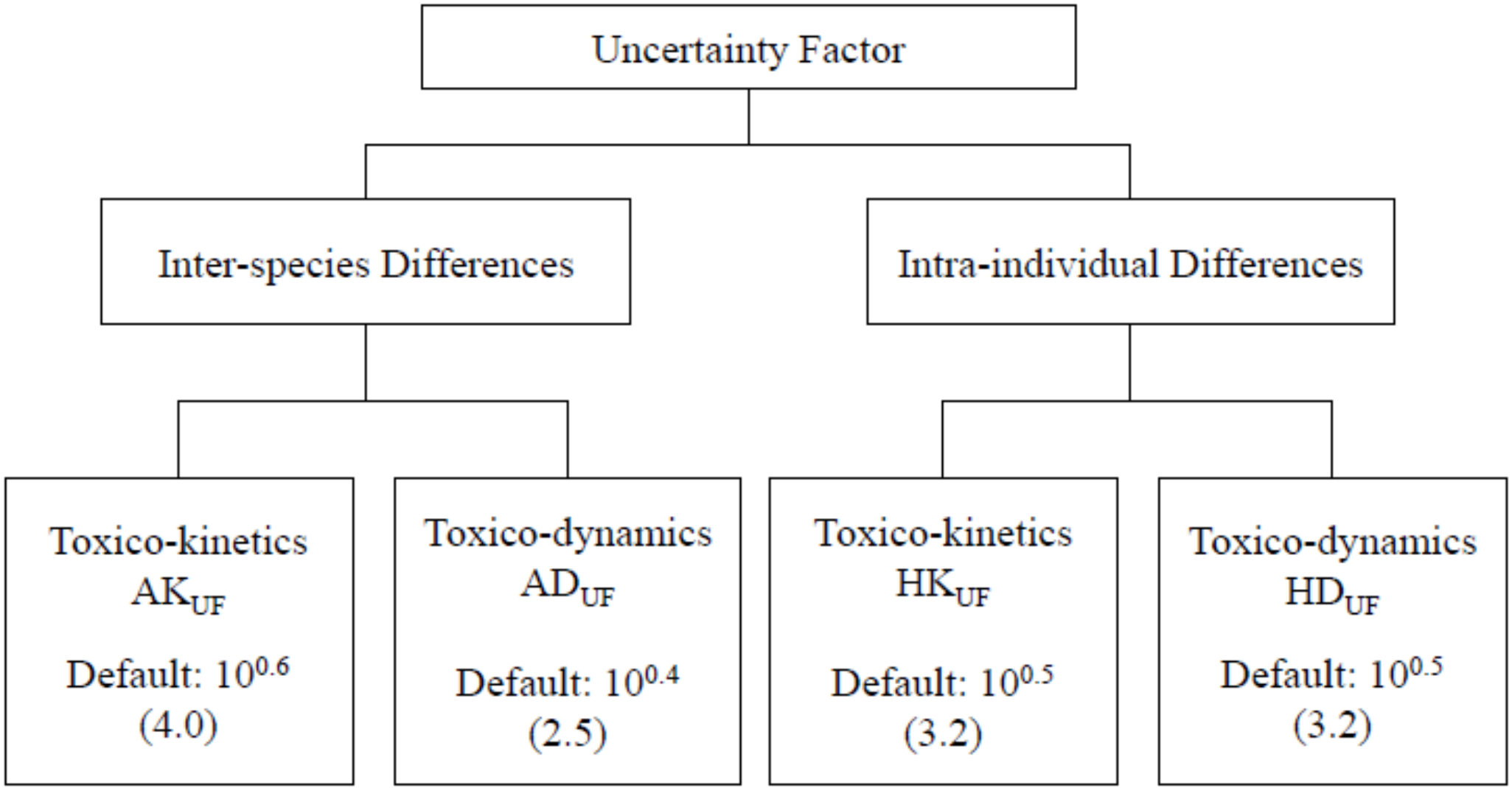
Adequacy of the experimental model (relevant animal specie, study duration, route of exposure)

Extrapolation of animal data to human and from high experimental doses to low actual exposure dose

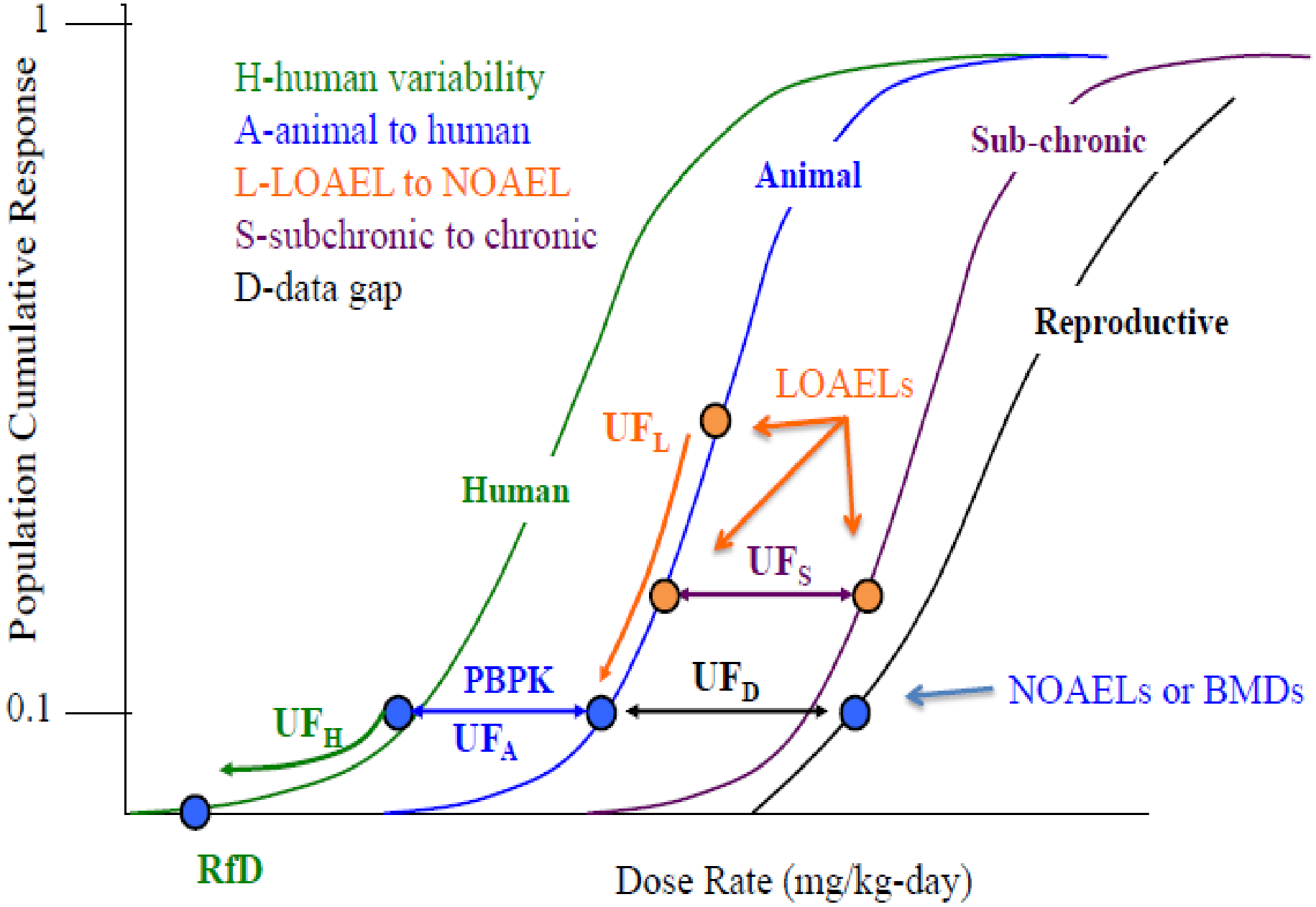
Variability factors:

Exposure (duration, dose, route): which are the groups with **higher levels** of exposure?

Susceptibility (age, patho/physiological status, genetic and/or acquired factors): which are the **more susceptible groups**, at the same levels of exposure?



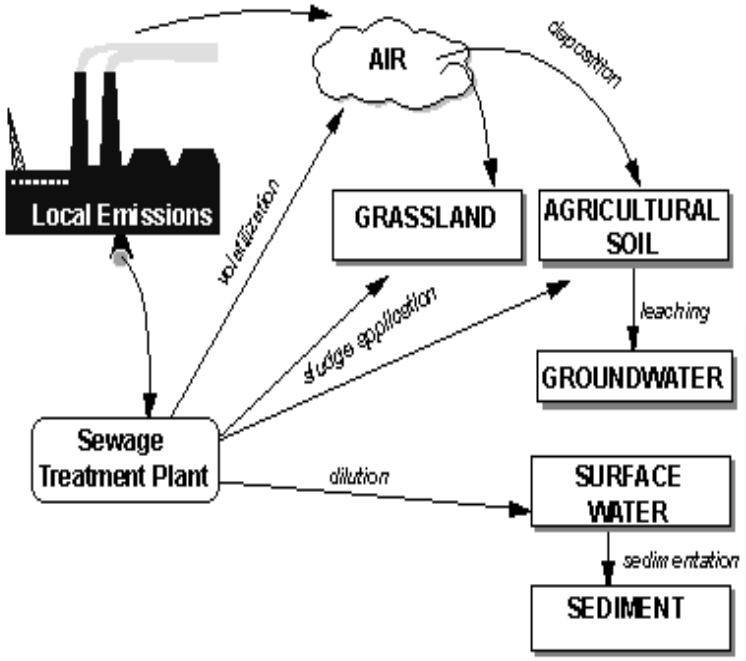
Renwick, 1991 & 1993; Health Canada, 1994; IPCS, 2005; USEPA, 2014


1
0.1
Population Cumulative Response
H-human variability
A-animal to human
L-LOAEL to NOAEL
S-subchronic to chronic
D-data gap
Animal
Sub-chronic
Reproductive
Human
LOAELs
UF_L
UF_S
UF_H
PBPK
UF_A
UF_D
NOAELs or BMDs
RfD
Dose Rate (mg/kg-day)

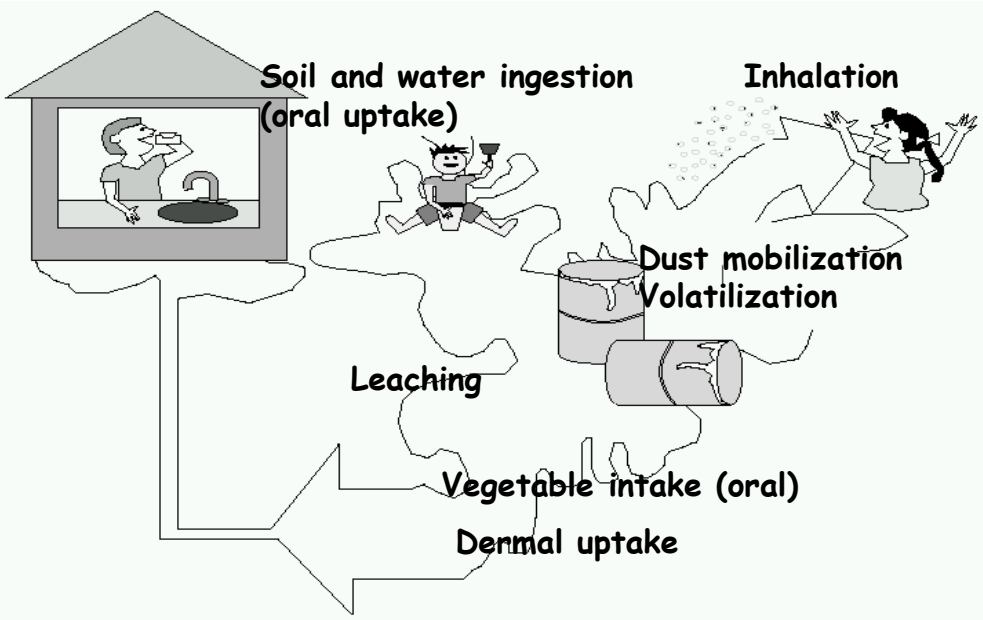
ce value,

3. EXPOSURE EVALUATION

Which is the level of exposure?



External: levels in environmental matrices
Aggregate exposure: more than one route



external → internal

Biomonitoring studies (all the routes and TK included)

4. RISK CHARACTERIZATION

What prediction can be made regarding the frequency and severity of effects in the population exposed to a specific level of the contaminant?

Reference Health based values are compared with exposure levels to estimate the likelihood of observing a toxic effect in the exposed population

RISK = HAZARD x EXPOSURE

Risk is the likelihood for an effect to occur at a specific level of exposure

Guidance Values (GV) Derivation

GV= maximum concentration acceptable in each single source of exposure (i.e.: drinking water, sea-food products, air,….)

$$GV = \frac{TDI \times body\ weight \times All.F}{daily\ intake/exposure\ (C)}$$

All.F= allocation factor= % of TDI attributable to every single source of exposure (taken the sum of them as 100%)

AN EXAMPLE

Lowest NOEL for a water **contaminant** = 0.14 mg • kg^{-1}•d^{-1} from a 2-year-chronic study in the rat (effects at LOAEL =hepatic toxicity)

AF to be used: 100 (10 x 10 for intra- and inter-species differences) No other AF is necessary (good quality of the study and data base complete Data reach compound)

$$\textbf{TDI} = \frac{\textbf{NOEL}}{\textbf{100}} = \textbf{0.0014 mg • kg}^{-1}\textbf{•d}^{-1}$$

An **additional AF** could be applied when :

- the effect observed at LOAEL gives rise to some concern (i.e: reprotoxicity)
- a NOAEL is not derived and the LOAEL value is used for the derivation

Lower assessment factors could be applied when :

- The NOAEL is derived from a study on human – the interspecies difference factor is not necessary. If the number of enrolled individuals is sufficiently high to cover possible interindividual differences also the intraspecies differences factor can be omitted.
- Data are available to demonstrate that TK parameters are similar between experimental animal and humans.
- Effects can be disregarded if mechanistic data are available to demonstrate they are specie-specific and not relevant to humans

AN EXAMPLE

Lowest NOEL for a water **contaminant** = 0.14 mg • kg^{-1}•d^{-1} from a 2-year-chronic study in the rat (effects at LOAEL =hepatic toxicity)

AF to be used: 100 (10 x 10 for intra- and inter-species differences) No other AF is necessary (good quality of the study and data base complete Data reach compound)

$$\mathbf{TDI = \frac{NOEL}{100} = 0.0014\ mg \bullet kg^{-1} \bullet d^{-1}}$$

Given the derived TDI = 0.0014 mg • kg^{-1}•d^{-1}

Considering an allocation of around 40% for DW, a partial TDI_{DW}= 0.56μg •kg^{-1}•d^{-1} is derived

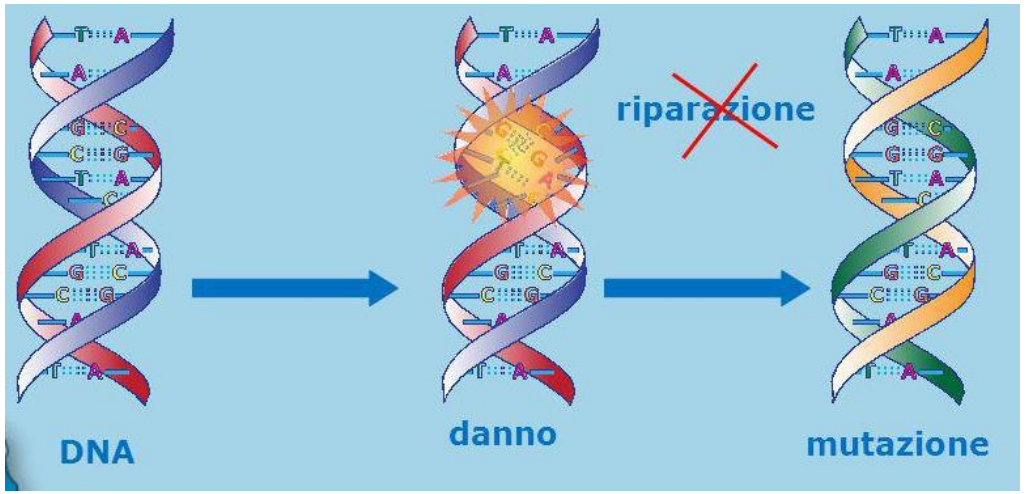
Assuming a 60kg body-weight adult and a consumption of 2L per day, the GV for DW is:

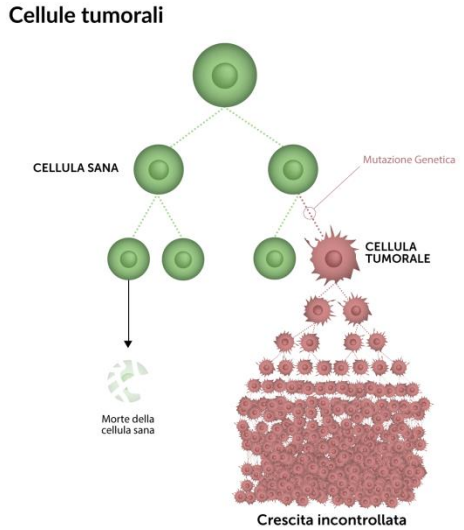
GV= 0.56x60/2=16.8 μg/L

The methodology to be used for risk assessment is different for:

- **Non-genotoxic and non-carcinogenic toxic substances, or with a known mechanism of non-genotoxic carcinogenicity**: there is a threshold, i.e. a dose below which no adverse health effects are likely to be observed.

- **Carcinogenic substances with genotoxic mechanism**: it is considered as a precaution that there is no threshold





RISK ASSOCIATED TO GENOTOXIC CARCINOGENS

Commissione Europea

The European Union uses the pragmatic and transparent **Margin of Exposure (MoE)** approach which expresses **the distance between the exposure level of interest and that associated with a minimal but detectable effect**.

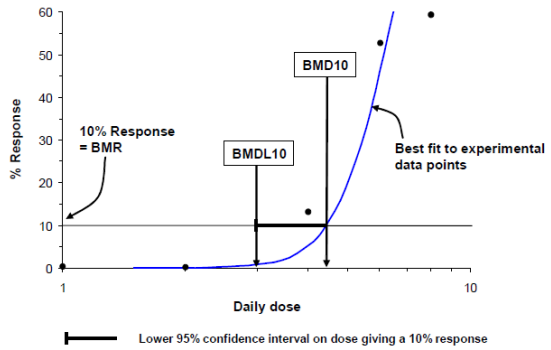
The reference dose is a **Benchmark Dose**, estimated with best fitting techniques of experimental results on the animal or, even if more rarely, on epidemiological data.

In the case of genotoxic carcinogens, the **BMDL10 is used**, i.e. the lower limit of the confidence interval of the BMD associated with a 10% increase in tumors

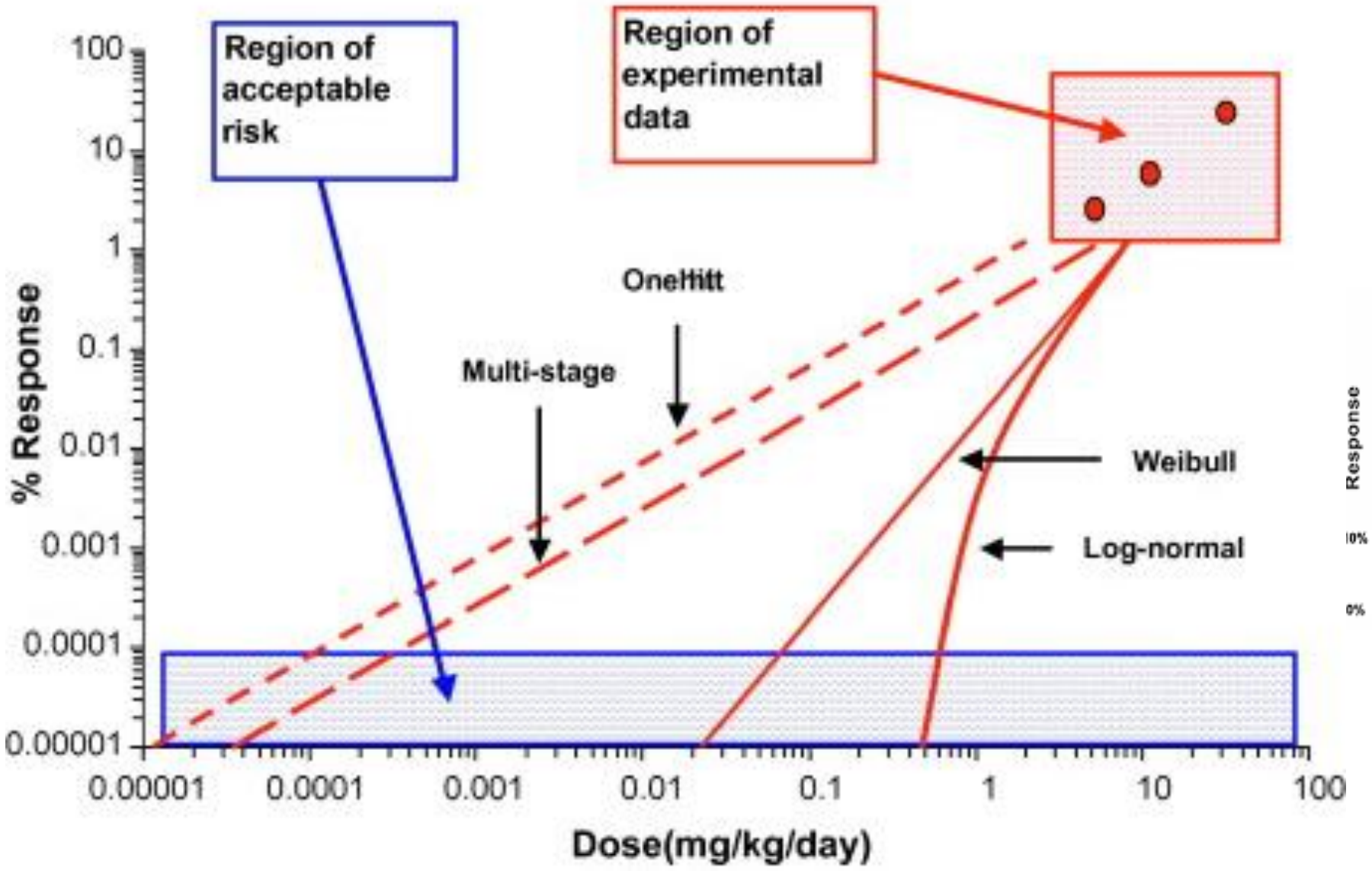
MoE = BMDL10 / Exposure

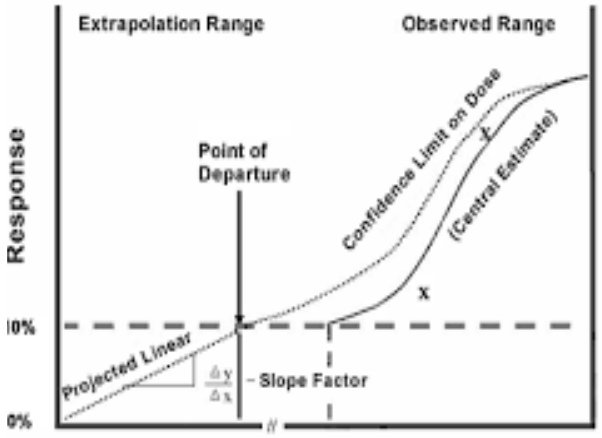
An MoE of at least 10,000 compared to the BMDL10 represents a situation of low concern or tolerable risk.

In the absence of a BMD, T25 ,which is the dose associated with an increase of tumors of 25% (puntual value as the NOAEL), and an MoE of *low concern*=25,000 is used

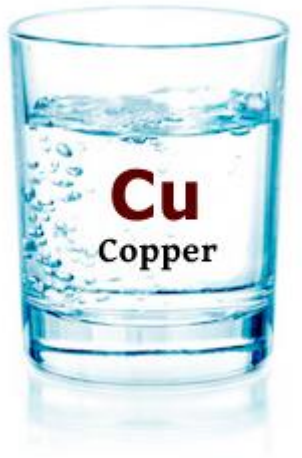


RISK ASSOCIATED TO GENOTOXIC CARCINOGENS





Copper in drinking water



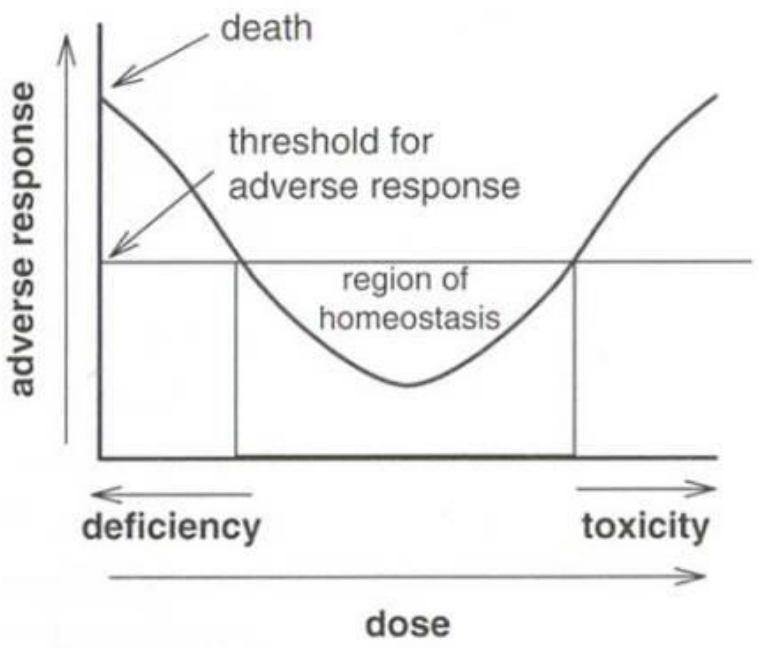
Copper is both an **essential nutrient** and a drinking-water **contaminant**. It is used to make **pipes**, valves and fittings and is present in alloys and coatings. Copper sulfate pentahydrate is sometimes added to surface water for the control of algae.

Copper concentrations in drinking-water vary widely, with the primary source most often being the corrosion of interior copper plumbing. Levels in running or fully flushed water tend to be low, whereas those in standing or partially flushed water samples are more variable and can be substantially higher (frequently above 1 mg/l).

Copper concentrations in **treated water** often increase during **distribution**, especially in systems with an acid pH or high-carbonate waters with an alkaline pH. Food and water are the primary sources of copper exposure in developed countries.

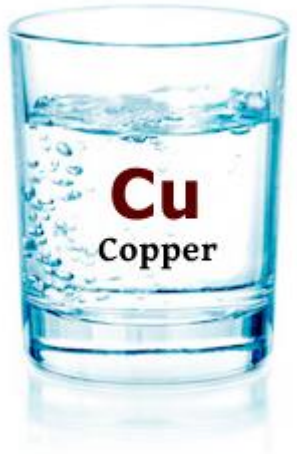
Copper in drinking water

Drinking water usually provides less than 10% of our daily copper intake.
Consumption of high levels of copper (>2 mg/L) can cause nausea, vomiting, diarrhoea, gastric (stomach) complaints and headaches. Long term exposure over many months and years can cause liver damage.
The World Health Organization suggests a minimum of 0.47mg of copper intake for every one liter of water and not more than 10 mg per day.



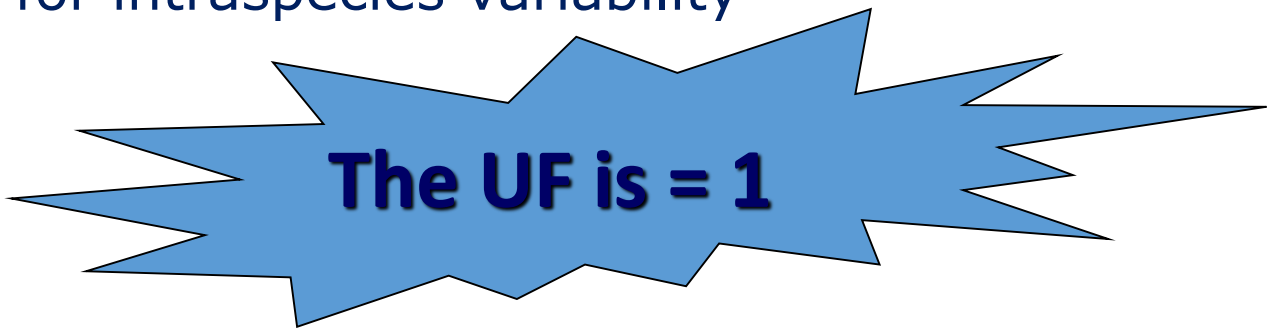
Copper effects give rise to a **non monotonic dose-response relationship (NMDR)** with a U shape

This is the case of essential trace elements (ETE)
Eg: effects due to Copper deprivation are more severe than the ones caused by high doses



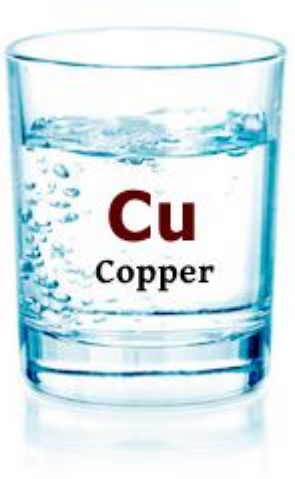
Copper: acute risk

- **Acute-short term studies** on **healthy volunteers** have been considered for NOAEL derivation (Araya *et al,* 2001 & 2003: copper was present in drinking water) ⇒ the NOAEL was based on the occurrence of g.i. symptoms (nausea):4 mg Cu/L DW.
- The **quality** of the studies has been considered good enough.
- No UF to extrapolate from animal data
- The **number of subjects** is quite high and covers the possible differences due to age (including children), gender, inter-individual differences.
- No UF to account for intraspecies variability



- WHO considers a consumption of 2L DW a day: the GV is 2mg Cu/L DW.

Copper : chronic risk



- Available repeated toxicity studies:

1. 90-days rat: $CuSO_4$ in the diet (Hébert *et al* 1993). **NOAEL= 16.3 mg Cu/kg bw/d** (external dose) based on hepatic effects (liver = target organ for repeated toxicity induced by copper).

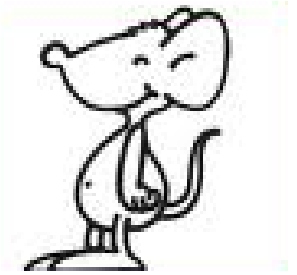
Oral Absorption= 25% ⇒ internal dose ⇒ **corrected NOAEL by multiplying for 0.25 (or ¼)=4.08 mg Cu/kg bw/d**.

The study has been carried out according to OECD TG and is of good quality.

Rat is more susceptible than mice.

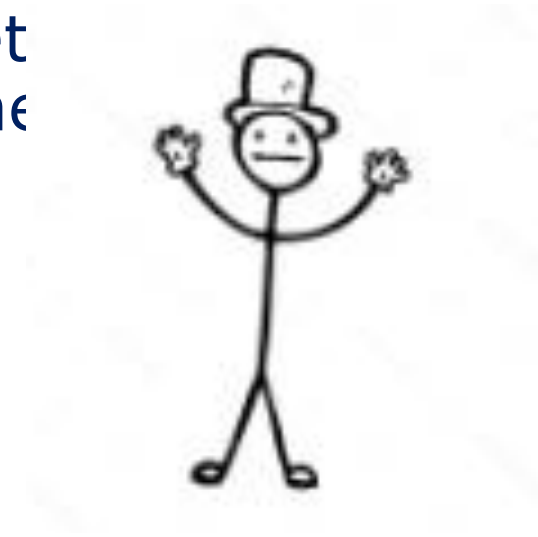
Copper : chronic risk

2. **2 generations rat** 10 weeks: dietary $CuSO_4$
   NOAEL = 15.2 –26.7 mg Cu/kg/d (OECD TG)



3. **Study on 8 volunteers** (Pratt *et al* 1985): diet supplemented with 10 mg Cu/d (considering the background consumption, total intake= 11-12 mg Cu/d) for 3 weeks ⇒ no alterations in biochemical parameters

   poor quality of the study: N° of subjects and study duration very limited. Only one dose tested



Copper chronic risk: UF identification

- Rat study =key study; human study= supporting
- UF =10 to extrapolate from animal to man generally consists of 4 (TK variability) x 2.5 (TD).
- Human copper oral absorption from human data is =25 % as in the rat. No significant ≠ in metabolism ⇒ the TK factor can be disregarded ⇒ **UF=2.5**
- UF for intraspecific variability =10.
- An additional factor =2 is needed to account for the lack of chronic toxicity

Total UF= 100 (general population)

- $\frac{\text{NOAEL}}{\text{UF}} = \frac{16 \text{ mg Cu/kg bw/}}{100} = 0.16 \text{ mg/kg} = \text{TDI or } \text{DNEL}_{\text{chronic}}$
- The TDI or $\text{DNEL}_{\text{chronic}}$ is supported by the value observed in humans
- Human NOAEL (Pratt study) :10 mg Cu/d divided per 60 kg bw =0.166 mg/kg

Current safety testing methods

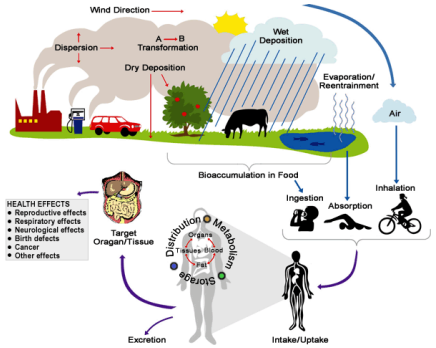
In Vitro

In Vivo

Toxicological profile

VS

Exposure



Risk assessment

The present RA paradigm generally focuses on hazard identification and characterisation as first steps.

There is a demand for changing the basis of RA, giving more focus on

1) modes of action (mechanistic approach)
2) a progressive reduction of tests using laboratory animals
3) exposure driven process

Towards the Tox21 and the EU SC document on New challenges for RA (2013)

In Europe, the Legislation is also asking for reduction in animal experiments: beside the EU Directive 2010/63, specific pieces of legislation ask for the use of alternative methods, whenever possible.

REACH (EU Reg 1907/2006)

In its Annex VI describes a general strategy to be followed by the applicant to meet data requirement

Step 1: **Collect/share all the available info**
Step 2: **Consider the specific requests**
Step 3: **Identify 'data gaps'**
Step 4: Propose a 'testing strategy' to generate missing data

…to achieve a high level of protection of human health and the environment while limiting the need for additional testing…

New test should be carried out -whenever possible- with alternative methods to animal experiments.

In Europe, the Legislation is also asking for reduction in animal experiments: beside the EU Directive 2010/63, specific pieces of legislation ask for the use of alternative methods, whenever possible.

REACH (EU Reg 1907/2006)

In its Annex VI describes a general strat… to meet data requirement

S… missing data

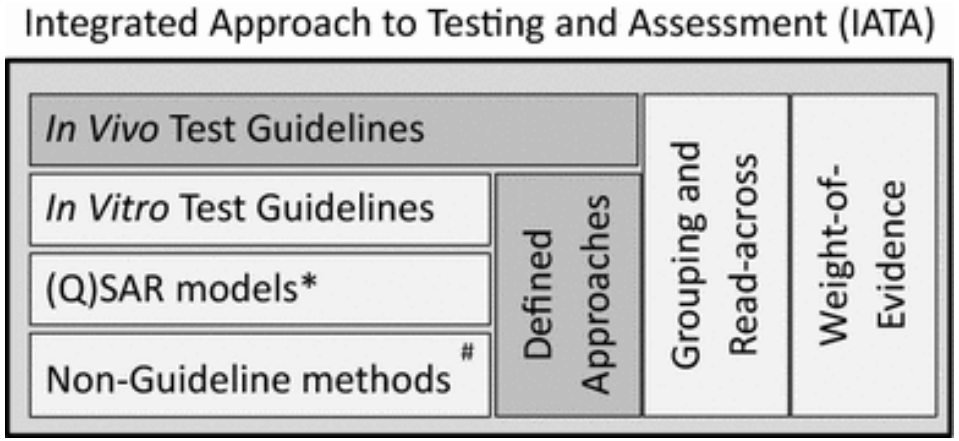
Same request is present in other regulations e.g. the biocides (528/2012) and pesticides (1107/2009) regulation to finish up with the cosmetic regulation (1223/2009) totally banning the use of animal testing conducted after 2013 to evalute the safety of ingredients and finished products.

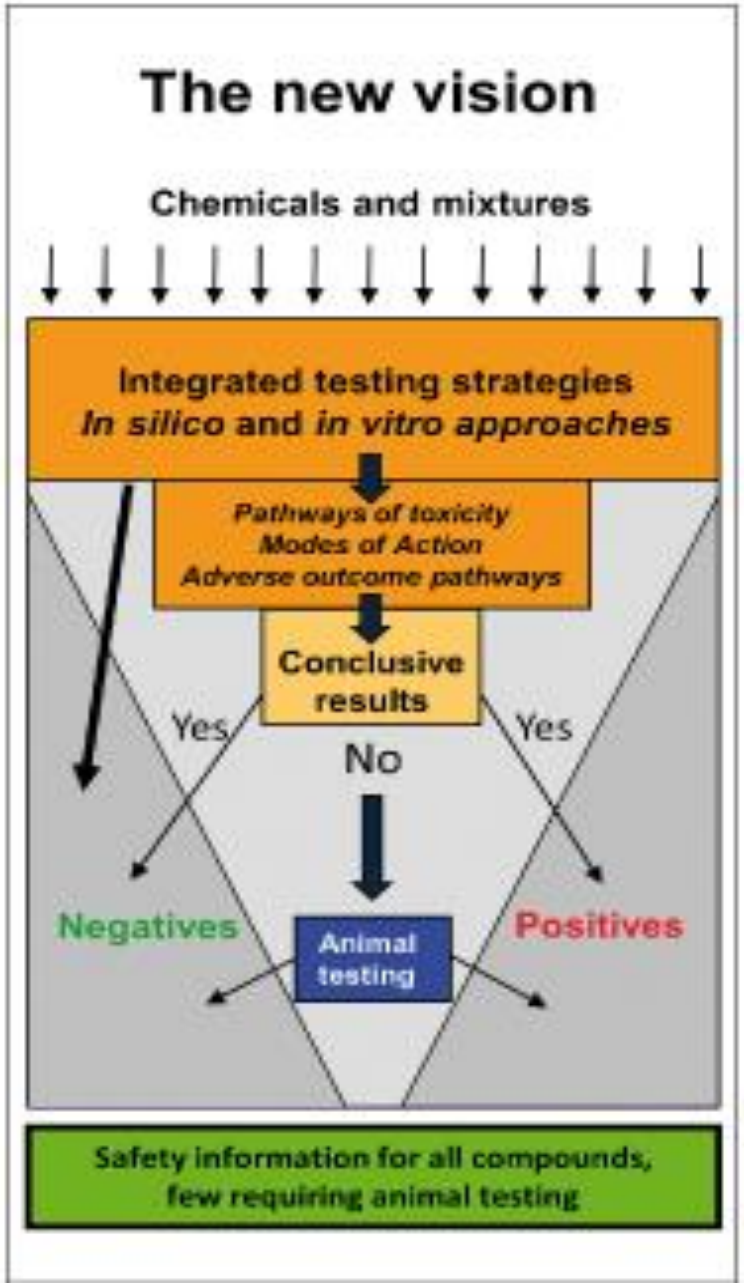
… achieve a high level of protection of human health and the environment while limiting the need for additional testing…

New test should be carried out -whenever possible- with alternative methods to animal experiments.

TESTING STRATEGIES IN AN ANIMAL FREE ENVIRONMENT (IATA)

- ✓ All the available information should/must be used (WoE) with identification of data gaps
- ✓ As the first step it is possible to apply the so called **non testing methods.**
  1. **Read across**
  2. **In silico methods**
  3. **Threshold of Toxicological Concern (TTC)**





1. Read Across : If information on structurally similar chemicals are available it is possible to apply the read across principle

The toxicological profile of chemical A is known (source chemical), scant info available for chemical B

If you can support with **in silico analysis** (e.g. SAR Structure activity relationship) the structural similarity, or with '**bridging studies**' (both in vitro and in vivo) the similarity of A and B toxicological profiles, the read across principle can be applied.

ECHA: Pratical Guidance 6
http://echa.europa.eu/documents/10162/13655/pg_report_readacross_it.pdf
http://echa.europa.eu/support/grouping-of-substances-and-read-across

OECD: GUIDANCE ON GROUPING OF CHEMICALS, SECOND EDITION Series on Testing & Assessment No. 194 (2014)
http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en

EFSA : WG on grouping on pesticides and for mixtures (2021)

In ‘grouping’ more than one ‘Source Chemical’ is used to compare and than extrapolate or interpolate data for the ‘New’ chemical under evaluation, making the estimate more robust

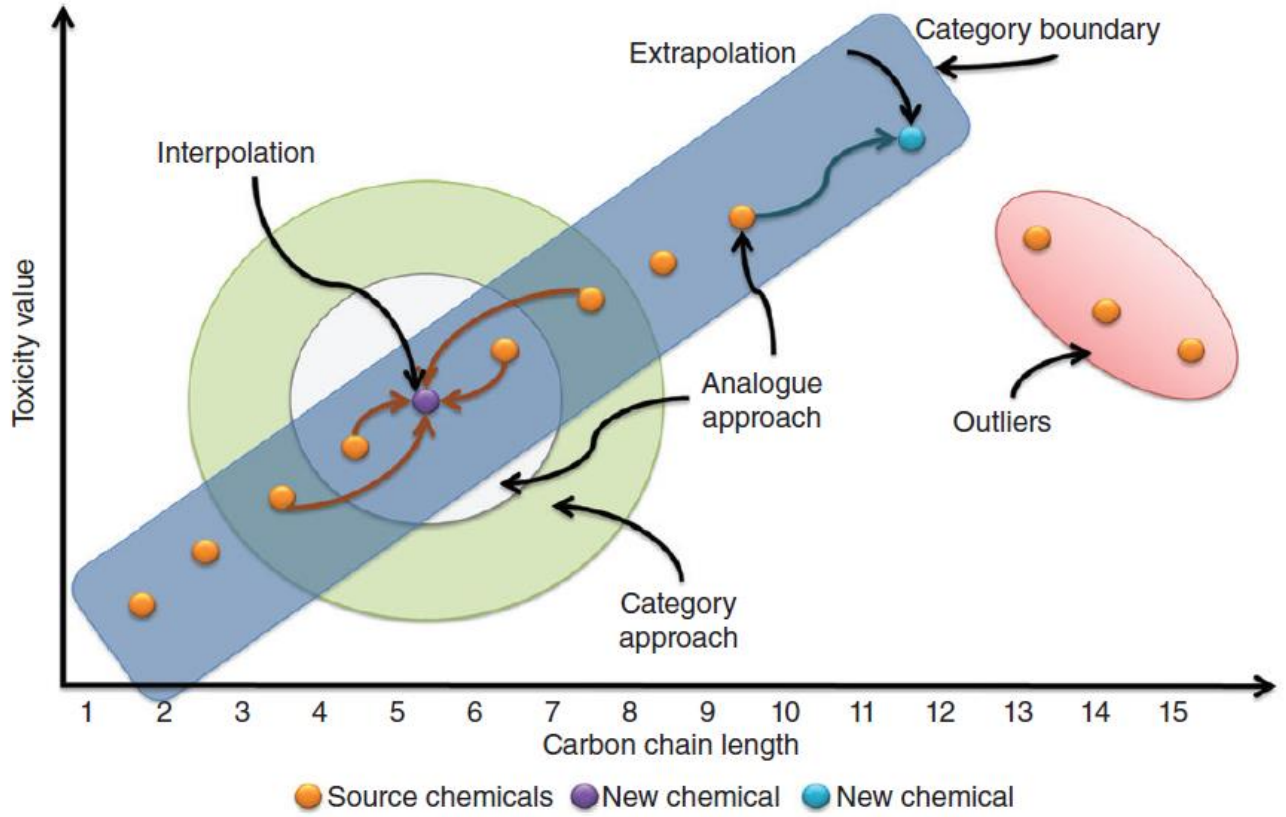
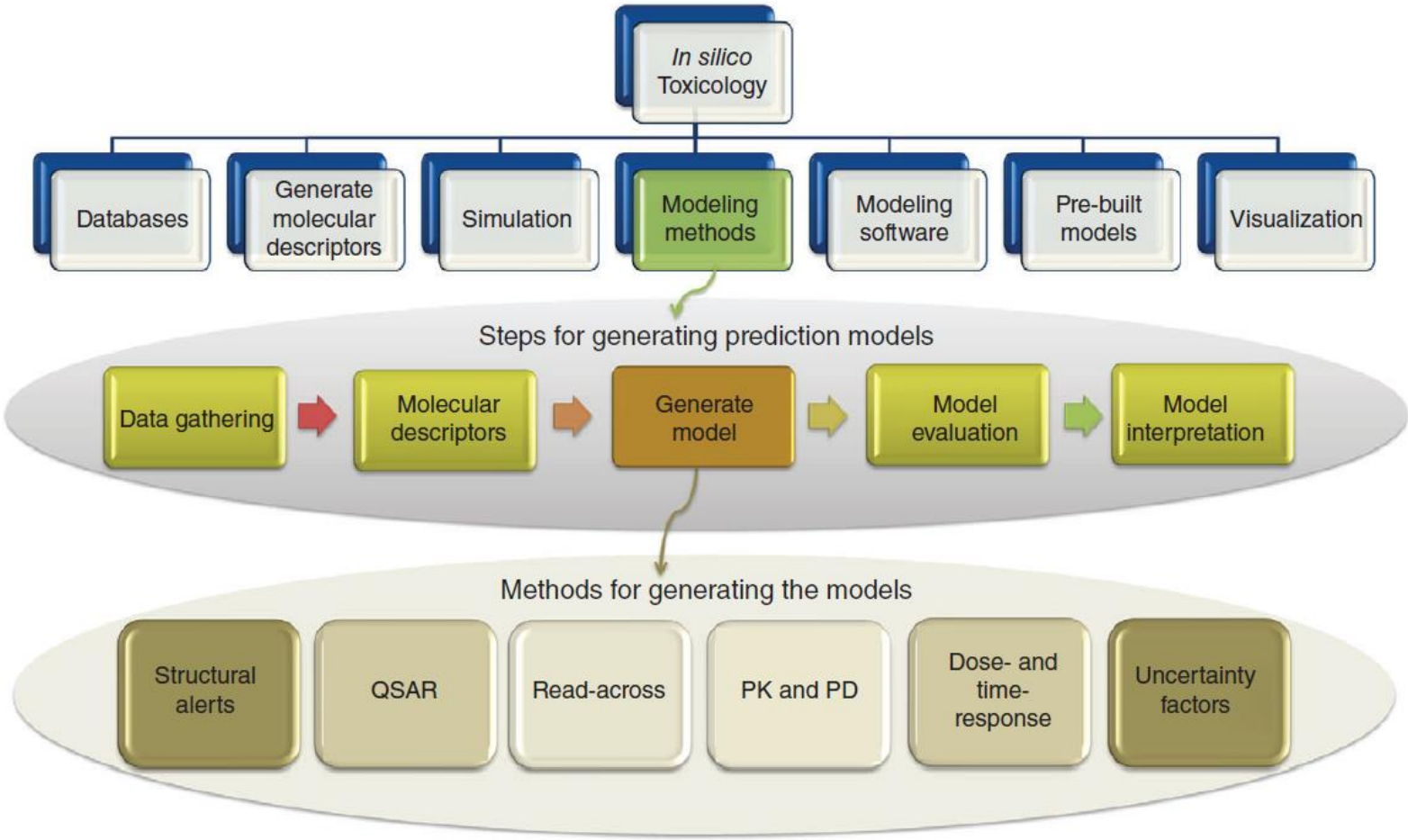


FIGURE 2 | Different approaches of read-across: analog versus category approaches, interpolation versus extrapolation, category boundary and outliers.

2. *In silico* methods

In silico toxicology uses computational methods to analyze, simulate, visualize, or predict the toxicity of chemicals. The aim is to complement existing toxicity tests to predict toxicity, prioritize chemicals, guide toxicity tests, and minimize late-stage failures in drugs design. There are various methods for generating models to predict toxicity endpoints.



The quantitative structure-activity relationship, QSAR (Quantitative Structure-Activity Relationship), is a mathematical model that quantitatively expresses the biological activity of a molecule as a function of certain chemical-physical or structural characteristics of the molecule (structure, polarity, bulk, orbitals , etc.)

Not all the methods are suitable for any chemical or group of chemicals. The applicability domain (AD) is 'a theoretical region in physicochemical space' (the response and chemical structure space) for which a QSAR model should make predictions with a given reliability.
The AD determines types of molecules and toxicity endpoints to which the model can be applied.

Even within the AD the prediction of a model is good only if the quality of the input data ig good.
The GIGO rule is always valid !

Garbage In → Garbage Out

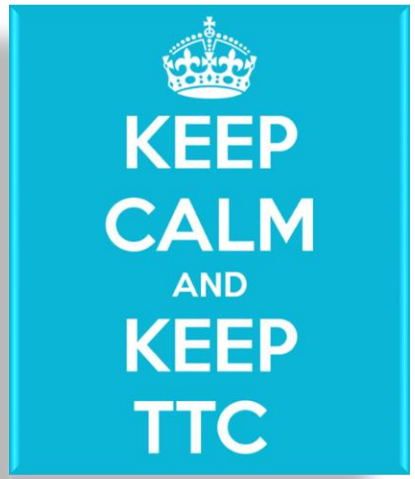
3. Threshold of Toxicological Concern (TTC)

- *The TTC approach is a **science-based pragmatic tool for screening and prioritizing chemicals** for their safety assessment when hazard data are lacking or incomplete and human exposure can be estimated.*

- It has been developed to qualitatively assess the risk of low-level substances in the diet. It can be used for an initial assessment of a substance to determine whether a comprehensive risk assessment is required or to **prioritise chemicals** that require more data over those that can be presumed to present no appreciable human health risk.

- If the chemical structure of a substance is known, health risk can be evaluated on the basis of **generic human thresholds of exposure – "TTC values"**. TTC values have been established for substances of similar chemical structure and likelihood of toxicity**, based on extensive published toxicological data**.

So in case you have to evaluate a long list of chemicals for which information are not available

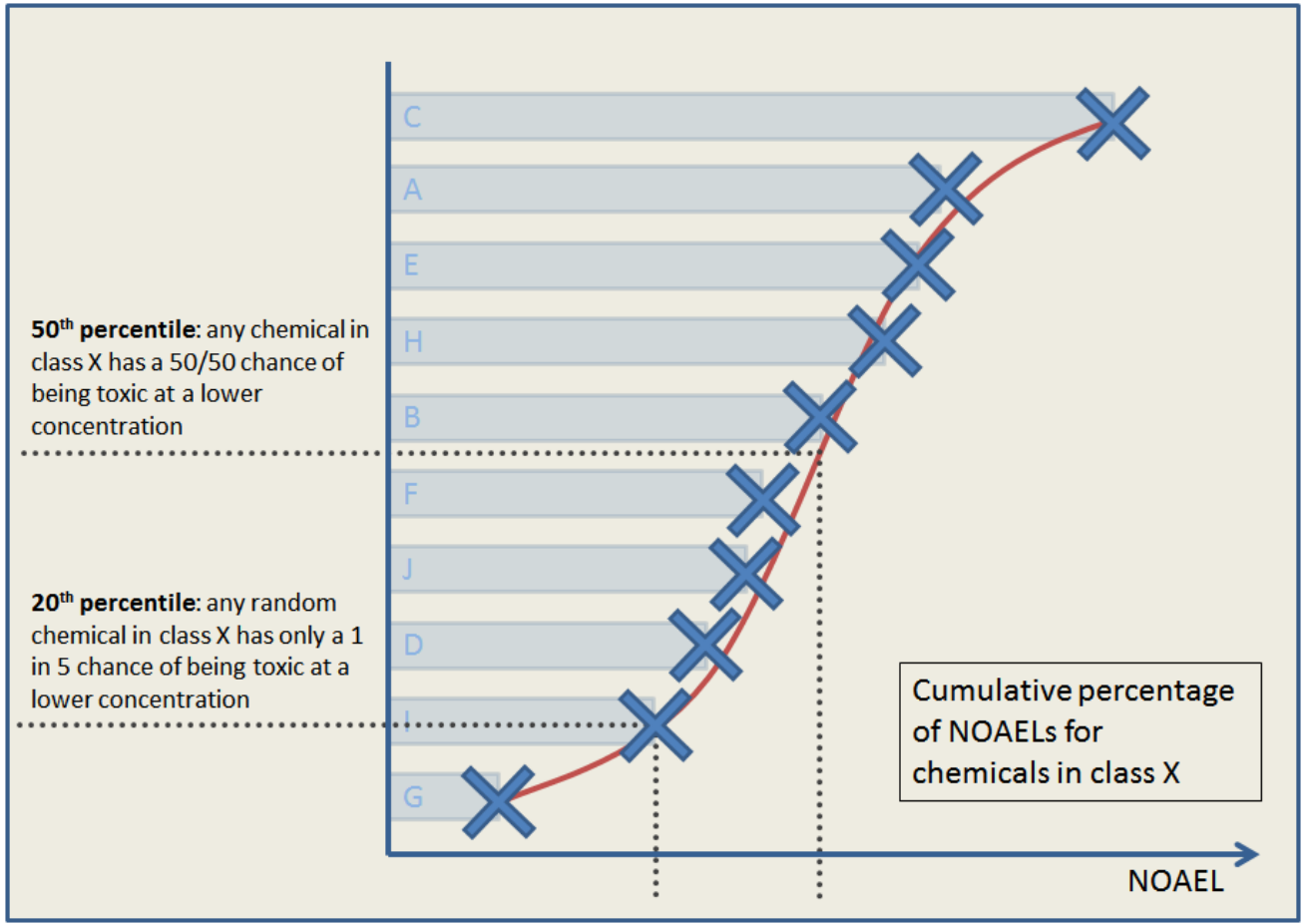


Threshold of Toxicological Concern (TTC)

There are three broad categories of low, moderate or high toxicity defined as substances in **Cramer class 1, 2 and 3** respectively, based on the chemical structure and various alerts (see specific lessons on the use of the OECD tool box)

The TTC values were determined by statistically analyzing substantial toxicological databases and other available toxicity data (NOEL, LOAEL, etc).

TTC uses distributions of NOAELs for substances. The 5th percentile value is divided by an uncertainty factor (100) to give a TTC value.



Category	Description	TTC mg/ person/day
1. Low toxicity	Substances with simple structures for which efficient modes of detoxification exist in our body	1.8
2. Moderate toxicity	Substances that are less innocuous than those in category 1, but which do not contain features suggestive of toxicity	0.54
3. High toxicity	Substances suggesting significant toxicity or containing reactive functional groups	0.09

30 µg/kg BW

1.5 µg/kg BW

The original data base was built up mainly considering oral exposure toxicity data. To account for other routes, other data bases have been developed with different TTC values.

Substances can be assessed by comparing the appropriate TTC value only with **reliable human exposure data**.
If human exposure to a substance is below the TTC value, the likelihood of adverse effects is considered to be very low.

Many substances are **outside the AD of TTC**: proteins, metals, steroids, very potent genotoxic carcinogens, aflatoxin-like chemicals, dioxine-like chemicals, etc

The TTC approach is already applied in various scientific contexts and international regulatory sectors such as in EFSA, FAO/WHO, FDA, Health Canada and EU, since it is based on scientific evidence to screen and prioritize the safety assessment of chemical substances.

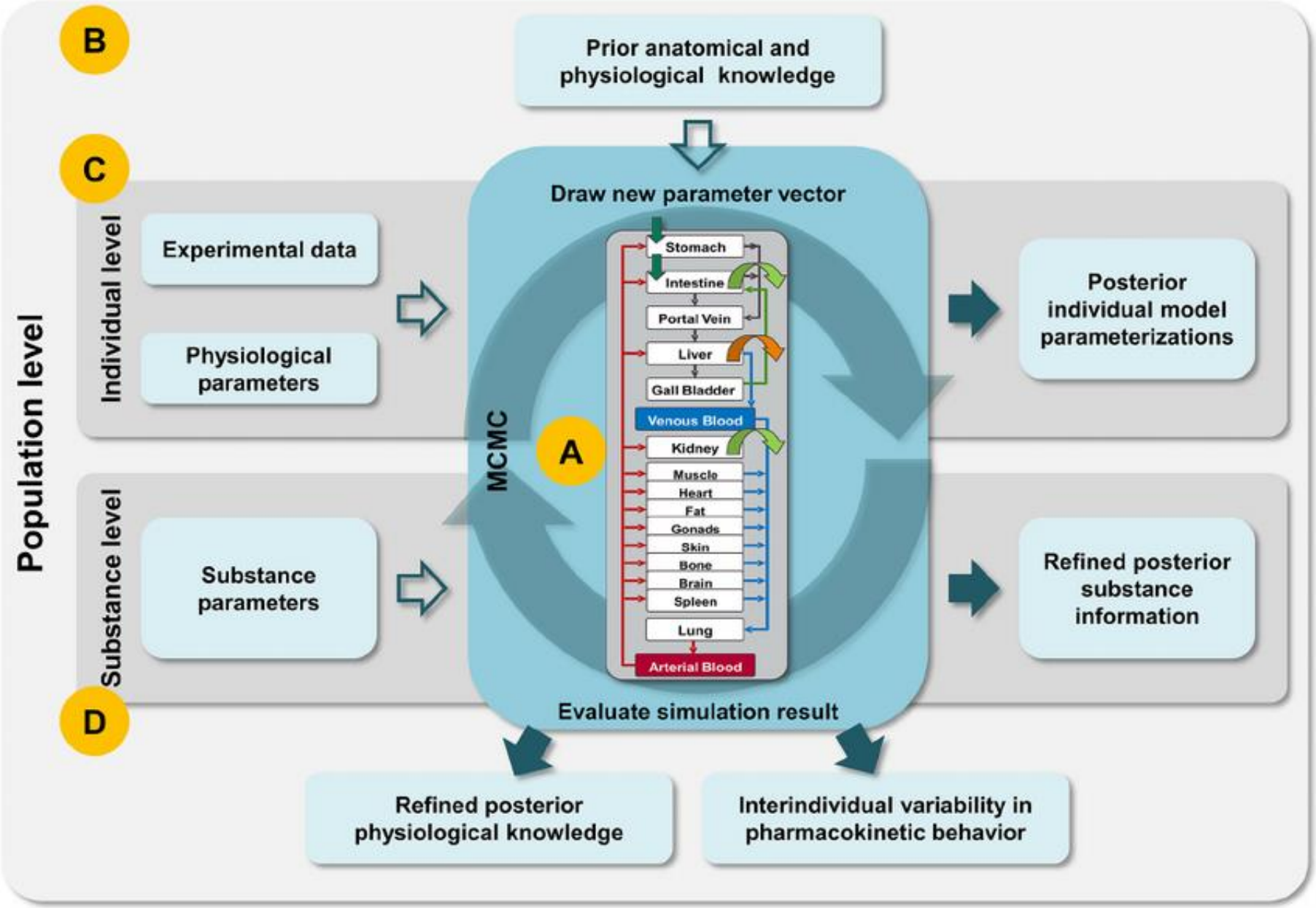
Any risk assessment as well as the TTC approach **should ideally be based on internal doses**. Therefore, when the TTC approach is applied for a DW contaminants, an adjusted internal TTC value has to be defined considering oral absorption.

This means that NOAELs in the data base should be adjusted for oral absorption so that the classical external TTC is converted into a iTTC or internal TTC.

Several attempts have been made to arrive at an iTTC by adjusting the external NOAEL (in mg/kg bw/day) by in silico estimates of oral bioavailabilty. However, the estimates were still based on external dose and not an internal exposure metric such as plasma concentration.

Further work is currently ongoing towards the development of a set of robust iTTC values that could be utilised in human safety assessment

Physiologically-based pharmaco-kinetic (PBPK) models



Limits:
Data available as input of the model are generally limited. Depending on the chemical features (mainly kinetics behaviour) specific PBPK model should be built.

Projects are going on (e.g some EFSA projects involving ISS) to implement this approch.

What if

- ✓ No information is available in the literature
- ✓ read across cannot be applied,
- ✓ no in silico method is applicable
- ✓ The chemical is out of the TTC applicability domain or the exposure exceeds the TTC value?

Consult the specific Regulation for data requirement and then the OECD web site to look for the most appropriate OECD Test Guideline

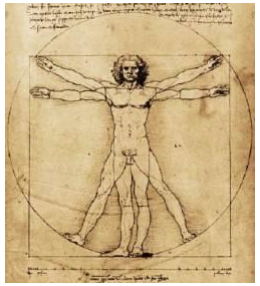
In vitro studies in RA

Limited use for risk assessment purposes ⟹ difficulties in carrying out **quantitative *in vitro* to *in vivo* extrapolation**

(QIVIVE) ⟹ translate in vitro effect concentration into human toxicologically equivalent dose

✓Need of translating information from the cell level, to organs and subsequently to organisms and to **distinguish between adaption vs. adversity**, likely identifying **actual *in vitro* markers of adversity** (Blaauboer et al, 2012) or **Key Events of AOPs**

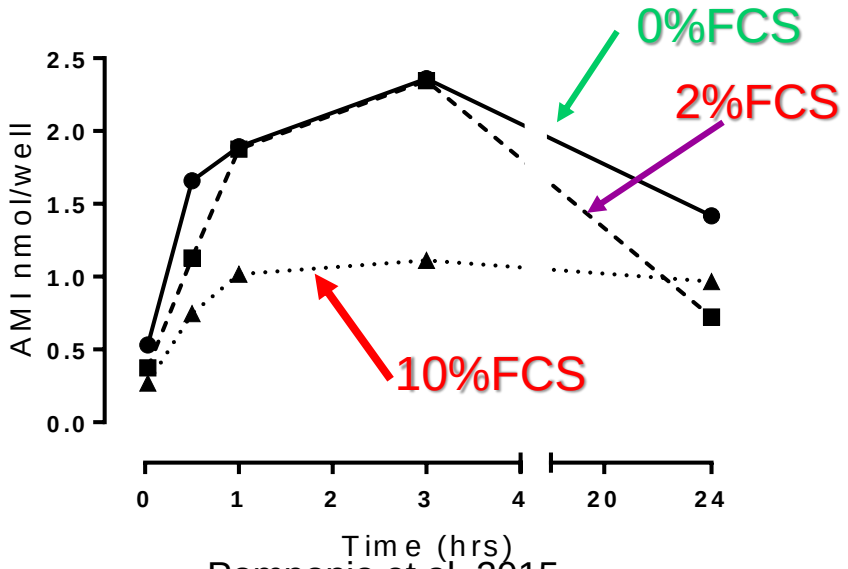
- ✓ Integrated approach: in silico and in vitro **IATA**
- ✓ Lack of information on actual cell exposure ⟹ ***in vitro* biokinetics**
- ✓ Battery KE (TD) + kinetics ⟹ **PBTK models**



Kinetics is considered the crucial body of information for the design and performance of 'traditional' in vivo toxicological tests, toxicity data interpretation, identification of internal dose…….

Biokinetics processes have been evoked to explain the in vitro/in vivo differences, but…

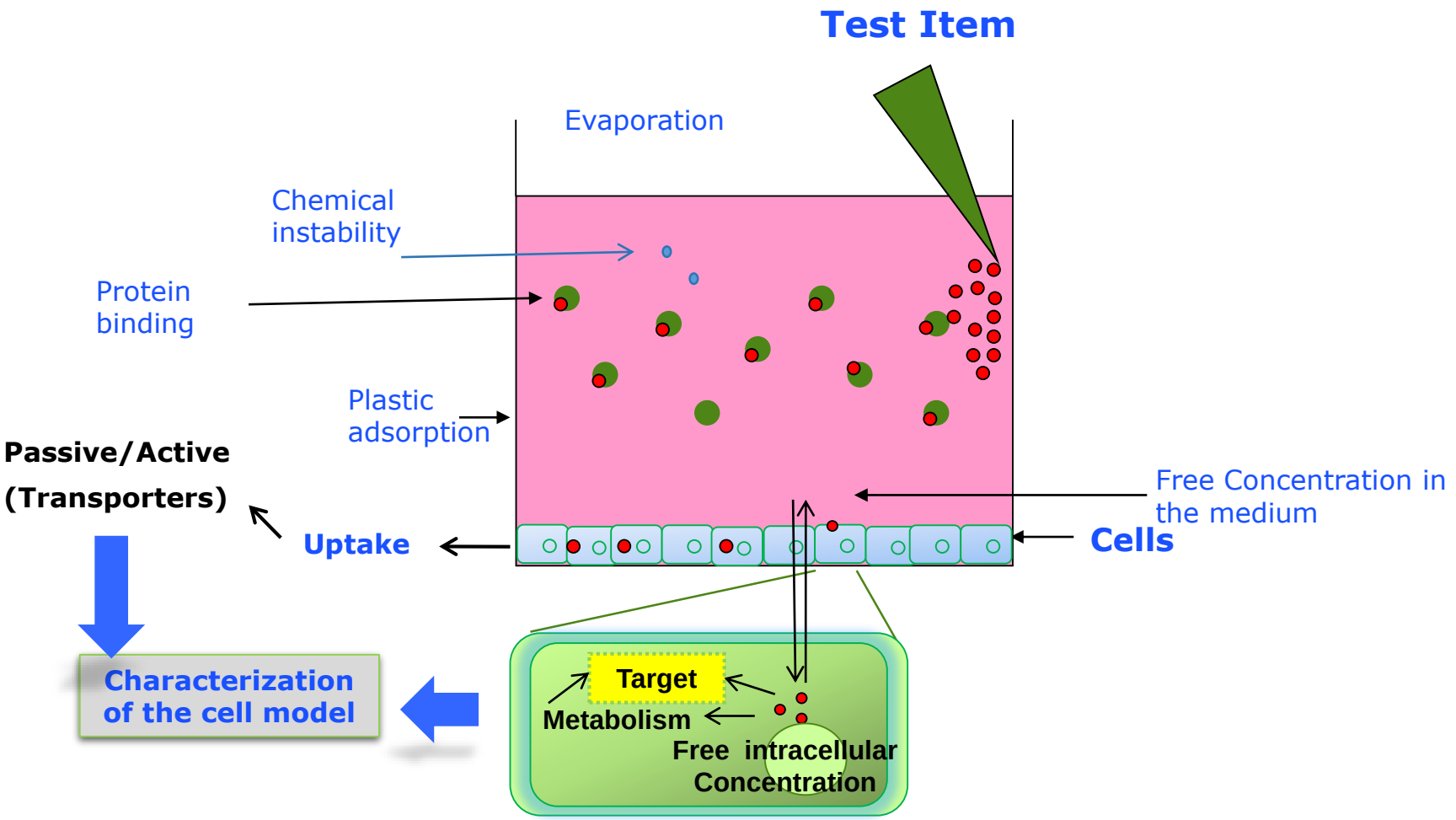
***…in vitro* the nominal applied concentration rather than the actual level of cell exposure is usually associated to the observed effects.**



Pomponio et al, 2015
Kramer et al, 2015

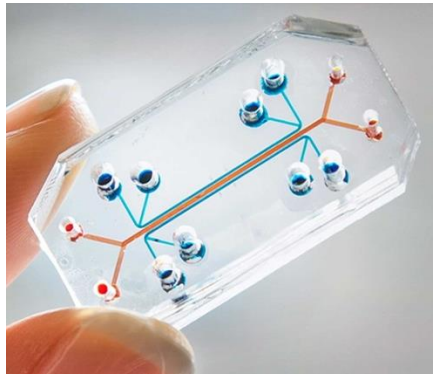
There is an urgent need to include kinetics in alternative/non animal testing strategy

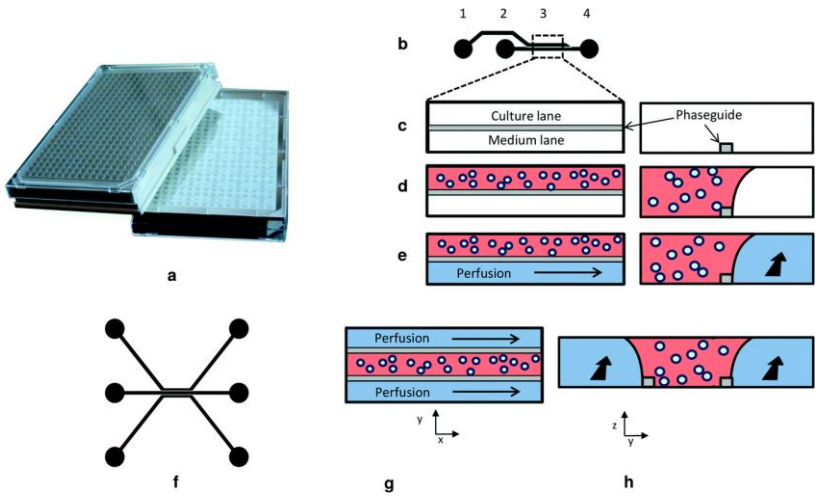
In vitro biokinetics

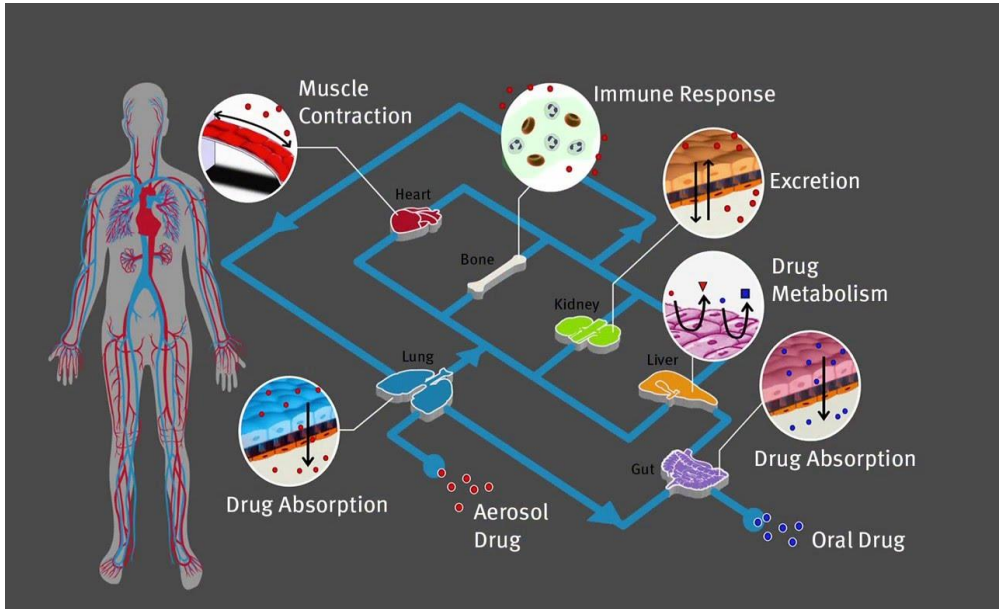


An **organ-on-a-chip** is a microfluidic cell culture device that contains continuously perfused chambers inhabited by living cells arranged to simulate tissue/organ-level physiology.

By recapitulating the multicellular architectures, tissue-tissue interfaces, physicochemical microenvironments and vascular perfusion, these devices produce **tissue and organ functionality** not possible with conventional 2D or 3D culture systems.
They also enable high-resolution, real-time imaging and *in vitro* analysis of biochemical, genetic and metabolic activities.







The derivation of a Guidance value for TCE in drinking water (WHO, 2020)

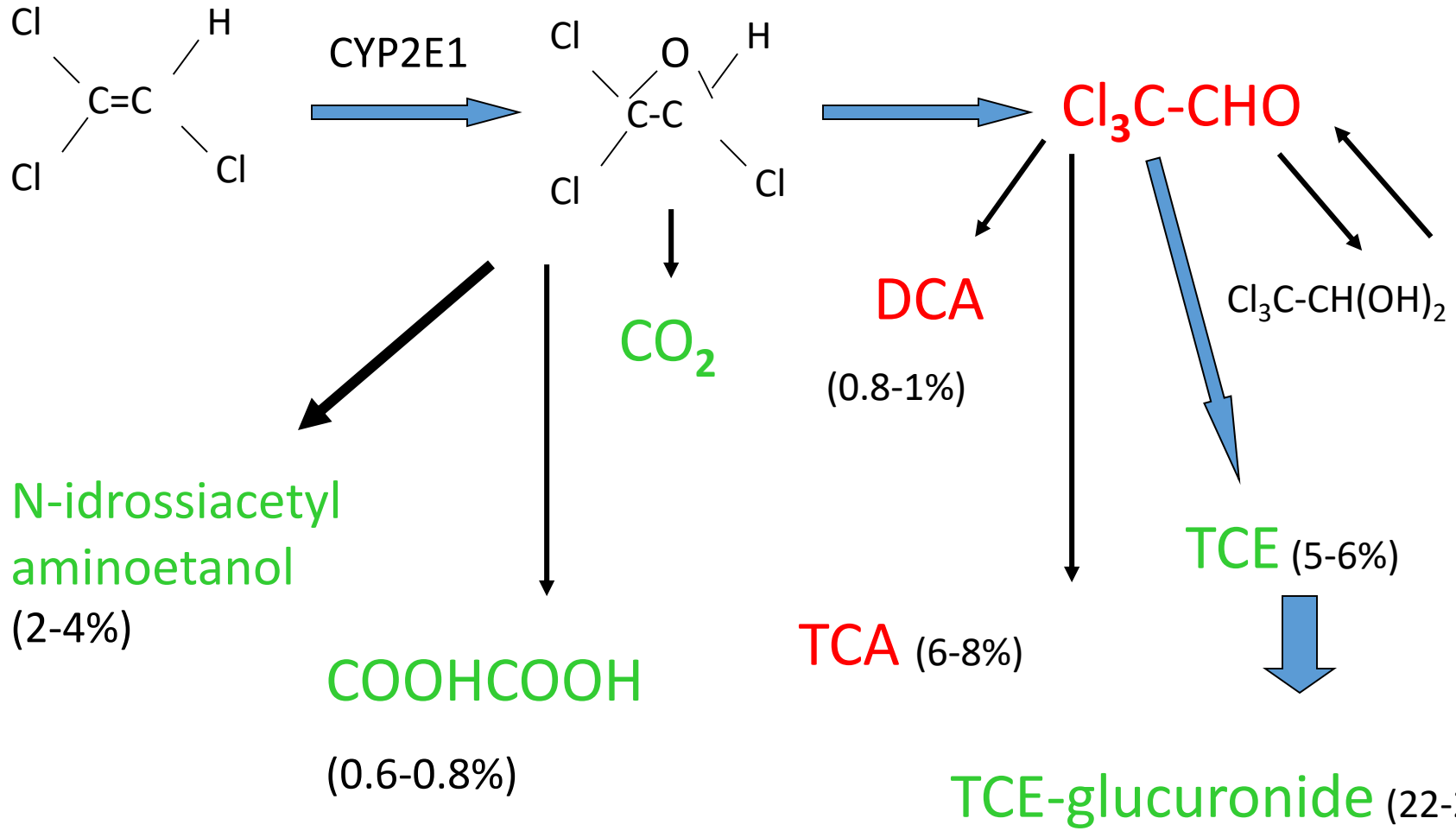
Toxicity studies have been conducted for the inhalation route in humans (occupationally exposed individuals) and in experimental animals. In contrast, the database on TCE ingestion via drinking-water is limited. Therefore, many targets of toxicity from chronic exposure to TCE largely focus on the inhalation route of exposure.

1st issue: need to know the **route specific differences**
2nd issue: need to know the **species specific differences**
3rd issue: need to know the possibility to **linearly extrapolate from high** experimental doses (or the occupational dose examined in epidemiological studies) **to low doses** in drinking water

- ✓ TCE is rapidly and well **absorbed** similarly by the oral and inhalation routes of exposure (ATSDR, 2019).
- ✓ The metabolic pathways and kinetics of excretion for oral and inhalation exposure are similar (ATSDR, 2019).
- ✓ Data for oral exposure indicate a pattern of effects similar to that of inhalation exposure.
- ✓ Differences in first-pass effects (affecting systemic bioavailability) between oral and inhalation exposures exist
- ✓ Quantitative differences in TCE metabolism between humans and rodents exist.
- ✓ Metabolite production is not linear because the oxidative pathway is saturated at the high doses at which the GST pathway starts to be active.

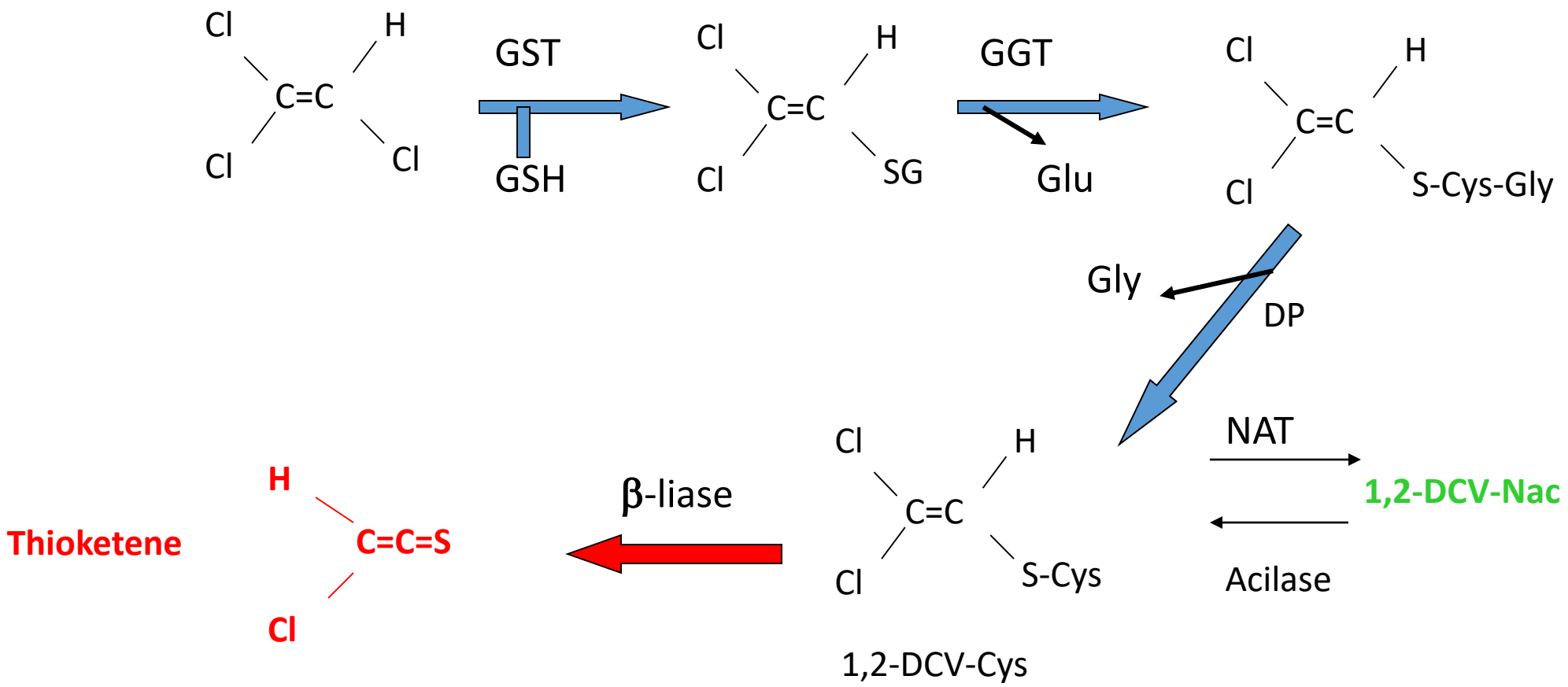
The use of PBPK modelling allows a route-to-route extrapolation (adequately accounting for TK differences), as well as estimation of the internal exposure.

TCE Oxidative metabolism



The oxidative metabolism products have the liver as the main target….however at high doses of exposure it is saturated…..

TCE Metabolism via GST



Renal tumors are mediated by Thioketene and are only relevant at very high doses of exposure (e.g. professional degreasers of aircraft and large equipment - approximately 100% males) and not for the general population

Starting from the lowest-observed-adverse-effect level (LOAEL) and no-observed-adverse-effect level (NOAEL) or benchmark dose (BMD) values, PBPK modelling was used to apply

- a route-to-route extrapolation and
- calculate an internal dose based on present understanding of the role that different TCE metabolites play and the mode of action for TCE toxicity.

PBPK model was used to calculate **an internal dose POD from a number of studies** for which plausible internal dose metrics could be determined, based on present understanding of the role that different metabolites play in TCE toxicity and the mode of action for toxicity.

The PBPK model was also used to estimate interspecies and intraspecies pharmacokinetic variability. This resulted in 99th percentile estimates of **human equivalent dose (HED99)** for the candidate critical effects.

From the three critical studies, the TDI derivation was as follows:

1.
Keil et al. (2009) from which a LOAEL of 0.35 mg/kg bw/day was identified, based on decreased thymus weight in female mice exposed to TCE in the drinking-water for 30 weeks.

- POD= HED99 = 0.048 mg/kg bw/day
- Uncertainty factor (UF) = 10 to account for use of a LOAEL
- UF = 2.5 to account for remaining uncertainty associated with interspecies toxicodynamic differences, because a PBPK model was used to characterize interspecies toxicokinetic differences
- UF = 3.2 to account for remaining uncertainty associated with human variability in toxicodynamics, because a PBPK model was used to characterize human toxicokinetic variability
- TDI = 0.048/80 = 0.0006 mg/kg bw/day

2.
Peden-Adams et al. (2006), from which a LOAEL of 0.37 mg/kg bw/day was identified and considered as the POD, based on developmental immunotoxicity effects: decreased plaque-forming cell response (at 3 and 8 weeks of age) and increased delayed-type hypersensitivity (at 8 weeks of age) in pups exposed from GD 0 until 3 or 8 weeks of age through drinking-water (placental and lactational transfer, and pup ingestion).
A BMD could not be calculated because of inadequate model fit, and no PBPK modelling was applied because of lack of appropriate models and parameters to account for fetal and pup exposure patterns.

POD =LOAEL = 0.37 mg/kg bw/day

UF = 10 to account for use of a LOAEL

UF = 10 for interspecies extrapolation (a default factor was used, because of lack of adequate toxicokinetic data to develop a PBPK model)

UF = 10 for human variability (a default factor was used, because of lack of adequate toxicokinetic data to develop a PBPK model)

TDI = 0.37/1000 = 0.00037 mg/kg bw/day

3. Johnson et al. (2003), in which pregnant Sprague–Dawley rats were administered TCE in drinking-water during GD 1–22 at concentrations ≥0.0025 ppm. Increased incidences of fetal cardiac malformations at maternal exposure levels ≥0.25 ppm (estimated maternal doses ≥0.048 mg/kg bw/day) were identified as the critical effect. A PBPK model was applied to the rat BMDL01 external dose of 0.0207 mg/kg bw/day to calculate the rat internal dose; this was converted to an HED99 of 0.0051 mg/kg bw/day.

POD= HED99 = 0.0051 mg/kg bw/day (derived from a BMDL01)

UF = 2.5 to account for remaining uncertainty associated with interspecies toxicodynamic differences, because a PBPK model was used to characterize interspecies toxicokinetic differences

UF = 3.2 to account for remaining uncertainty associated with human variability in toxicodynamics, because a PBPK model was used to characterize human toxicokinetic variability

TDI = 0.0051/8 = 0.00064 mg/kg bw/day

The TDI values fall within a narrow range of 0.0003–0.0006 mg/kg bw/day.

An overall TDI of 0.0005 mg/kg bw/day (0.5 µg/kg bw/day) was considered appropriate, being supported by multiple effects rather than an individual value. This approach is less sensitive to limitations of individual studies.

Based on the TDI as described above, the GV is:
GV= 0.5 µg/kg bw/day × 60 kg bw × 0.5 = 7.5 µg/L (rounded to 8 µg/L or 0.008 mg/L) 2 L/day

where
- 0.5 µg/kg bw/day is the TDI, as derived above
- 60 kg is the average body weight of an adult
- 0.5 is the fraction of the total daily intake that is allocated to drinking-water
- 2 L is the daily volume of water consumed by an adult.

An allocation factor higher than the default 20% factor is used, since the occurrence of TCE in food is low (see section 2.2) and human exposures to TCE overall have been declining (see section 2.5), as a result of increased environmental regulations governing TCE emissions (IARC, 2014; ATSDR, 2019)

WHO guidance on cyanotoxins

New Guideline Values for exposure via drinking-water or recreation for MCs, CYNs, STXs and ATXs

&

The 2nd edition of "Toxic Cyanobacteria in Water"

Introduction to the GVs and their derivation

(Chapter 2 in TCiW)

Emanuela Testai

Istituto Superiore di Sanità- Rome-Italy

Special session

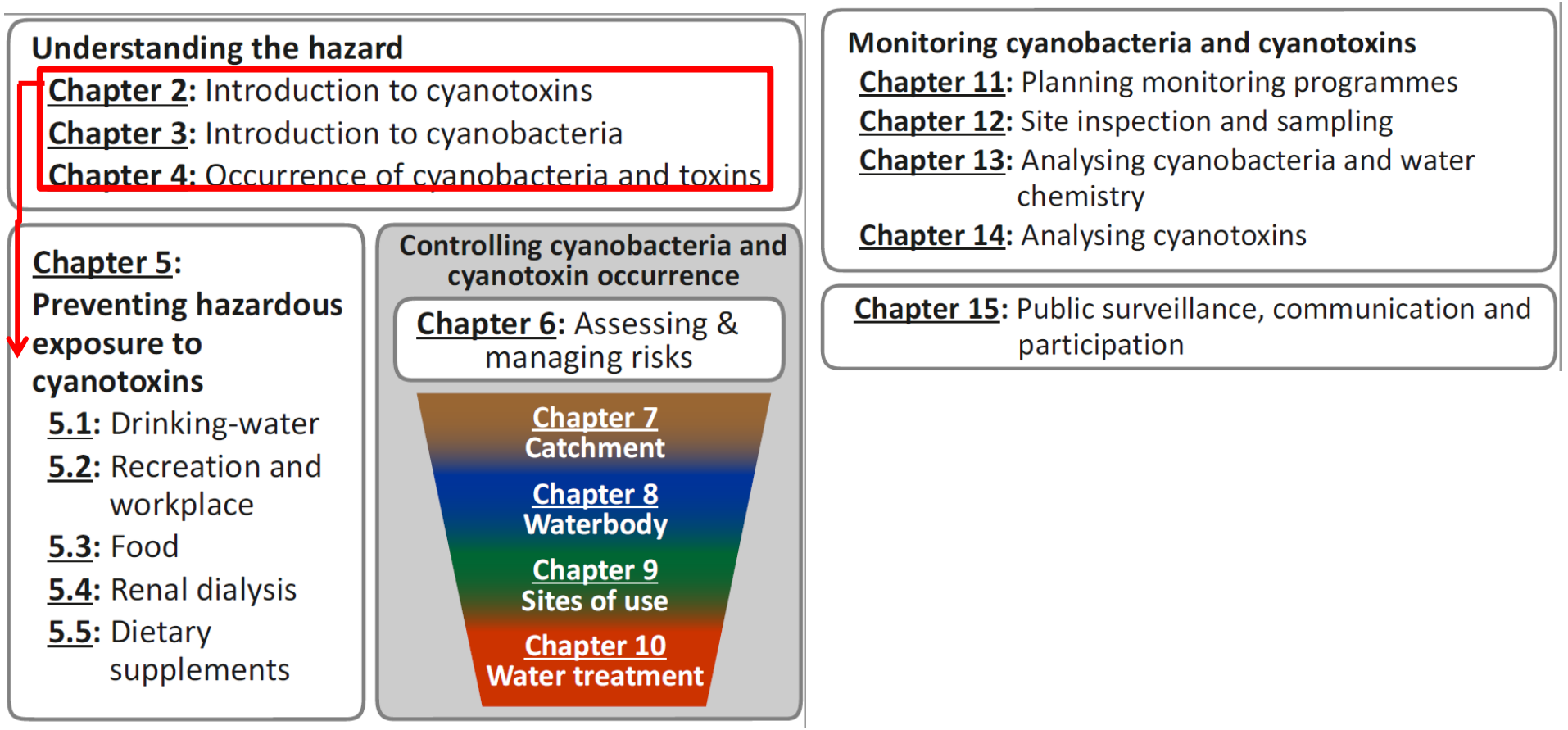
12 October, 2021

World Health Organization

ICHA 19th International Conference on Harmful Algae Mexico 2021

Introduction to the GV and their derivation

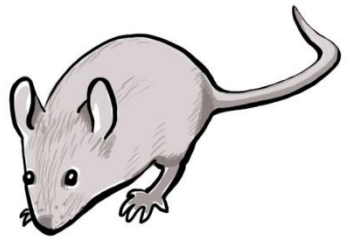
Jutta Fastner, Andrew Humpage and Emanuela Testai



How guideline values are derived for substances with a threshold for toxicity as for MC-LR

GV for lifetime exposure to drinking water

The available study covers 90 days, data on reproductive toxicity are inconclusive

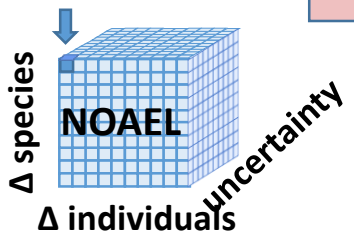


Dose with clear effect: 1000 µg / kg body weight per day

Dose with minor effect (liver): 200 µg/kg bw d

Fawell, J. K., James, C. P., & James, H. A. (1994). *Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water*. Foundation for Water Research.

Tolerable Daily Intake TDI



NOAEL: No Observed Adverse Effect Level**: 40 µg/ kg bw per d**

UF= 10x10 x10

TDI = NOAEL / 1000 =0.04 µg / kg bw per day

Guidance Values (GV) Derivation for DW

GV= maximum concentration acceptable in each single source of exposure (i.e.: drinking water, sea-food products, air,….)

MC-LR

$$GV = \frac{TDI \times body\ weight \times All.F}{daily\ intake/exposure\ (C)} = \frac{0.04 \times 60 \times 0.8}{2} = \mathbf{1\ \mu g/L}$$

All.F= allocation factor= % of TDI attributable to every single source of exposure (take the sum of them as 100%)

Beside the long term GV, WHO also derived short term values? **Why 'short term' values?**

Exposure to Cyanotoxins via drinking-water is not for 365 days a year – what about short-lived blooms?

Toledo, 2014: MCs analysed in drinking-water since 2012; results in summer 2014: **1.7 µg/L**

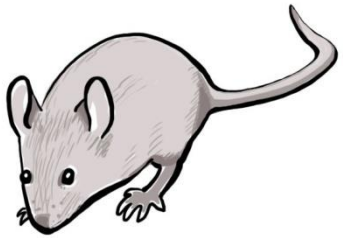
➔ ½ million people could not use water for drinking, cooking, showering;
closure of restaurants, shops ;
schools closed

➔➔ WHO: short-term guideline values are necessary to avoid mis-interpretation of lifetime GVs !

How guideline values are derived for substances <u>with</u> a threshold for toxicity as for MC-LR

GV for short term exposure to drinking water

The available study covers 90 days (short term)

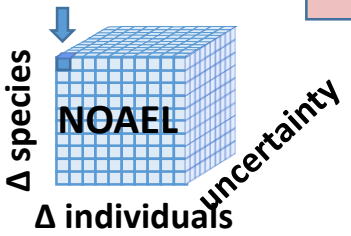


Dose with clear effect: 1000 µg / kg body weight per day

Dose with minor effect: 200 µg/kg bw d

Fawell, J. K., James, C. P., & James, H. A. (1994). *Toxins from blue-green algae: toxicological assessment of microcystin-LR and a method for its determination in water*. Foundation for Water Research.

Tolerable Daily Intake TDI



NOAEL: No Observed Adverse Effect Level**: 40 µg/ kg bw per d**

UF= 10x10 ~~x10~~

Short term Reference value= NOAEL / 100 =0.4 µg / kg bw per d

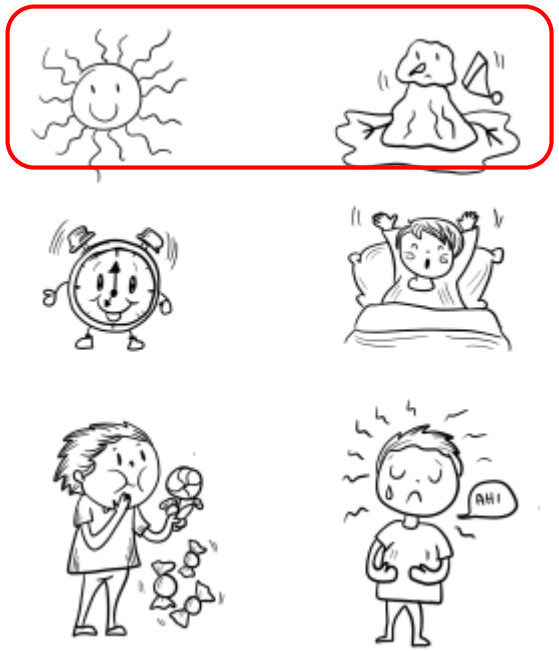
Analysis of Epi data and Human Reports

Can cases of illness support derivation?

"… *mere* ***co-occurrence of cyanotoxins and unspecific symptoms*** *(skin irritation, gastro-intestinal, etc. …) is not indicative of the known cyanotoxins having caused the symptoms…..*

Human effects ascribed to the presence of cyanotoxins in the exposure media (drinking- or recreational water, food) such as gastrointestinal illness or skin irritation may well be due to other unknown cyanobacterial metabolites or closely linked to pathogens or other substances associated with the bloom

Epidemiological studies: ➔ **Indicative value only** because (i) uncertain retrospective exposure estimates; (ii) other contaminants in surface water; (iii) demographic info is limited



Important to understand cause–effect relationships and then dose-response relationship (for the quantitative aspects)

The newly derived GV

- GVs be applied to total MCs, total CYNs and total STXs (lack of data for ≠ variants)
- Allocation factors: 80% for life time, 100% for short time and recreational
- Water intake: 2L for drinking water; 250 mL for a child as daily incidental water intake (recreational)

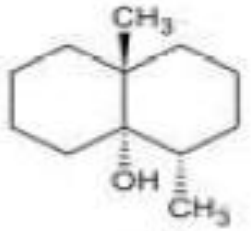
- UF :
  MC: 1000 LT, 100 ST, 100 Rec
  CYN: 1000 LT, 300 ST, 300 Rec
  STX : 3 Acute, 3 Rec
- Provisional: data base deficiency

Table 5.1 Guideline values and health-based reference values for selected cyanotoxins and exposure scenarios (WHO, 2020)

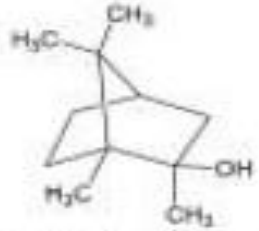
Toxin	*Exposure*[a]	*Value (µg/L)*	*Value type* [b]
Microcystin-LR	Drinking-water, lifetime	1	Provisional guideline value
Microcystin-LR	Drinking-water, short term	12	Provisional guideline value
Microcystin-LR	Recreational	24	Provisional guideline value
Cylindrospermopsin	Drinking-water, lifetime	0.7	Provisional guideline value
Cylindrospermopsin	Drinking-water, short term	3	Provisional guideline value
Cylindrospermopsin	Recreational	6	Provisional guideline value
Anatoxin-a	Drinking-water, acute	30	Health-based reference value
Anatoxin-a	Recreational	60	Health-based reference value
Saxitoxin	Drinking-water, acute	3	Guideline value
Saxitoxin	Recreational	30	Guideline value

Short-term exposure refers to about 2 weeks, until measures can be implemented to achieve concentrations < lifetime GV

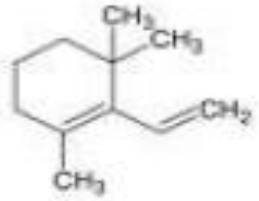
Taste & odor compounds



geosmin



2-methylisoborneol



β–cyclocitral

The terpenoids **geosmin (GEO)** and 2-methylisoborneol (**MIB**) have been associated with the global majority of waterborne T&O episodes

Data on their toxicological properties are very scant and have been mainly obtained with aquatic organisms.

It is possible to conclude only about the **lack of genotoxic potential**.

The current consensus is that these metabolites are nontoxic to humans via drinking water at environmentally relevant concentrations based on results on aquatic organisms, indicating that toxic concentration are order of magnitude higher than average environmental concentrations (up to as 700 ng/L) as well as their OTC (thus preventing water consumption and hence exposure)
The OTC of GEO can be set between 1.3–1.0 ng/L. MIB shows a similarly low OTC (6 ng/L).

Overall results appear to indicate a **low potential for inducing significant health effects** in vivo. However, most of the available results are affected by experimental design, not considering the actual exposure conditions of the test system and using poorly suitable experimental models (e.g. immortalized cell lines).

- **β–cyclocitral (or isocyclocitral)** has been identified as produced exclusively by *Microcystis*.
- By consulting ECHA public information no harmonized classification was found, but only auto-classification by registrants: more than 85% indicated it is acutely harmful if swallowed (H302), in contact with skin (H312), or if inhaled (H332). All of the registrants (100%) classified it as a skin irritant (H315) that causes serious eye irritation (H319) and as a respiratory irritant (H335). But only an oral acute toxicity study is cited and CLP is only based on hazard.

The **evaluation of β-cyclocitral toxicity with the TTC approach** (since no data are available)

- β–cyclocitral belongs to Cramer Class I (characterized by low toxicity) and therefore, levels below the threshold of 1800 µg/person per day are expected not to induce any relevant health effect in an adult of 60 Kg bw.
- About 12 µg/L can be present in dissolved form in the water after cell rupture (e.g., in senescent blooms), but peak values as high as 400–2000 µg/L were reported.
- Considering the difficulties in removing T&O compounds from the water via traditional water treatment processes, the consumption of 2 L of drinking water per day is expected to be lower than the TTC for β-cyclocitral after oral exposure although a potential risk can arise only during sporadic events.

To evaluate the quality of the studies some criteria have to be considered

The criteria for which evidence is scored relates to its **reliability, relevance and adequacy**

Reliability_– how much does the study support the evidence?
Reliability is measured by the quality of the study, the method used, the reporting of the results and the conclusion. **Klimisch et al, 1997** defined it as "***evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way that the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings.***"

To communicate the reliability the **Klimisch score** is used:
1 = reliable without restrictions (e.g. TG used and GLP compliant)
2 = reliable with restriction,
3 = not reliable
4 = not assignable.

Hills criteria are used for evaluation of causality in epidemiological data (Hill 1965)

Relevance covers the extent to which data and tests are **appropriate for a particular hazard identification and characterisation**.

Examples of issues to be considered when assessing relevance include:

- **relevance of the test material**: it should be equivalent to the submission substance identity,
- **relevance of the test method and conditions**: they should not deviate too much from the internationally approved TG, in the applicability domain of the method, species, exposure route, etc.
- **relevance of the endpoint**: the effects investigated in a study should be clearly related to the toxicity of the substance (e.g. physical effects are not relevant)
- **relevance of alternative methods**: e.g. when using (Q)SAR, read across, categories or in vitro approaches, it should be verified that they are applicable for the substance (e.g. applicability domain of the (Q)SAR models, consistency of the category, relevance of the in vitro effects).

- ✓ An *in vitro* assay result—from a test validated by the OECD and conducted in accordance with good laboratory practice—is an example of *reliable* information.

- ✓ Information on an analogue[1] chemical—that satisfies the requirements of the OECD Guidance on Grouping of Chemicals—is an example of *relevant* information.

- ✓ Information from an animal study—which was conducted according to OECD guidelines—is an example of evidence deemed *fit for the purpose* of recommending GHS[2] classification for the chemical.

1. A chemical for which acceptable evidence is available, whose properties and toxicological mode of action can be assumed to be similar to the chemical being assessed

Adequacy (or fitness for purpose) is essentially the **usefulness of information for the purpose of hazard and risk assessment**. The available data should allow clear decision making about whether the substance meets the criteria for classification and allow appropriate DNEL/PNEC values to be derived for Risk Assessment.

Quantity is also a criterion to be considered for assessing the strength of the evidence.
The overall weight of the evidence refers to more than one study/ piece of information - **the more the better** - in particular if contradictory pieces of information are encountered.

"Weight of evidence" (WOE)

It is an approach for **combining evidence** in support of a hypothesis, initially developed for medical diagnosis (in which the evidence consisted of a set of symptoms and the hypothesis was of the type "this patient has disease x" (evidence based medicine vs evidence based toxicology).

Today it is a common term used in the context of risk assessment (RA)

To start building the weight of evidence case, it is necessary to **gather all information**, from all possible sources including:
✓published literature
✓read across from chemical analogues/homologues, (Q)SAR predictions,
✓*in vitro and in vivo* studies on animal models, epidemiological data/ human experience, etc.

As a general principle, the more information the better.

Data from **old studies** which were not performed according to the current test guidelines may be less reliable or relevant since the guideline/method followed may not be in line
with more recent ones. For this reason they can be inadequate to be considered as key studies. However they could be adequate for a weight of evidence approach or as **supporting studies**. In addition they can provide sufficient information when **used in combination** with other studies i.e. to allow a weight of evidence analysis to be made.

There may be several studies available for the same test substance for the same endpoint, which are not deemed to be fully reliable. However, when **used collectively** the study results may indicate an effect at approximately the same concentration and time.
In these cases, there could be **justification for using all the studies collectively to conclude on a specific endpoint**.

Many agencies published documents on the WoE, the more recent is the one prepared by EFSA:
https://www.efsa.europa.eu/en/efsajournal/pub/4971

Conflicting results

A weight of evidence approach should be used when several studies are available which give **conflicting results**.
The weight allocated to each study will be **case-dependent** and will depend on the test method, quality of the data and the endpoint under consideration.

For **example**, the ready biodegradability test is known to be a stringent test method. If you have six poor quality studies showing a substance is not readily biodegradable and one good quality study using a test method recommended in REACH, indicating ready biodegradability, a conclusion of ready biodegradability would normally apply due to the stringency of this test method.

Possible outputs:

- The exposure values (**worst case**) are **much lower than HBV**: **no risk for the population**

- The exposure values (worst case) are **very close to HBV**: **no immediate risk**, considering the uncertainty factors used; need for some **'refinement'** of the assessment (e.g. reduction of uncertainty in exposure) after which it is decided which risk mitigation measures to apply

- The exposure values are **slightly higher than HBV**: **acceptable risk only for a limited period** (during which to remove the cause of the contamination - refer to the ARfD to exclude acute risks for an existing work; study the measures a priori in the VIS context)

- Values **much higher than HBV**: **unacceptable risk**, need for immediate measures (e.g. immediate closure of the aqueduct, withdrawal of the product from the market, negative opinion for the construction of the work)

If the control/monitoring data indicates that the limit levels established by Italian/European law have been exceeded, is there always a health risk?

The answer is yes if the legal value is also a HBV, otherwise…

An illegal value (or non-compliance) is not always synonymous of a health risk and vice versa

- ✓ The limit for all pesticides in drinking water (and groundwater) is fixed (**0.1 µg/L**).
- ✓ It is a limit that does not distinguish between different active ingredients, is not based on scientific (eco)toxicological data and is a 'political' fact defined pragmatically to obtain good water status and protect this precious resource.
- ✓ The presence of higher values is illegal but in most cases not risky (comparison with GV WHO)

This type of evaluation implies a solid knowledge of how health based values were derived (compared to legal values defined for political/practical aspects) and therefore of the mechanisms that generate an adverse effect with threshold (e.g. slope of the curve, gender differences , completeness and quality of available data), with identification of possible strengths and weaknesses. This allows us to understand the robustness of the health based values and appropriately evaluate any deviations

Critical analysis applied to any evaluation for the definition of health based values.

THANKS!