1st Summer School on Environmental applications of Advanced Oxidation Processes, Department of Civil Engineering University of Salerno, (*Fisciano/Italy, June 15-19, 2015*)

Risk assessment of pollutants in different environmental matrices.

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TABLE OF CONTENTS

- Toxicology: basic principles;
- Regulatory toxicology: toxicological evaluation;
- Risk assessment procedure;
- New challenges in Risk assessment;
- Case studies: from dietary exposure; from the environment.

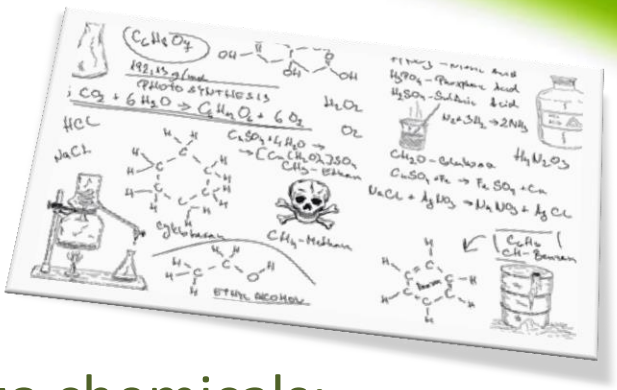
Toxicology

- Before **new** industrial and consumer substances/products can be developed and marked, **toxicology** is needed to make sure that they can be used safely;
- For substances **already** produced, used and present in the environment, toxicology helps to determine whether or not a risk is present and how to mitigate that risk for the population and the environment;

Evaluation of possible adverse effects on humans and possible risk for animals and the environment due to exposure to the substances/products in the workplace, home or environment

Toxicology

..anyone can become a toxicologist in two easy lessons, each of which takes ten years...(A.J.Lehman)

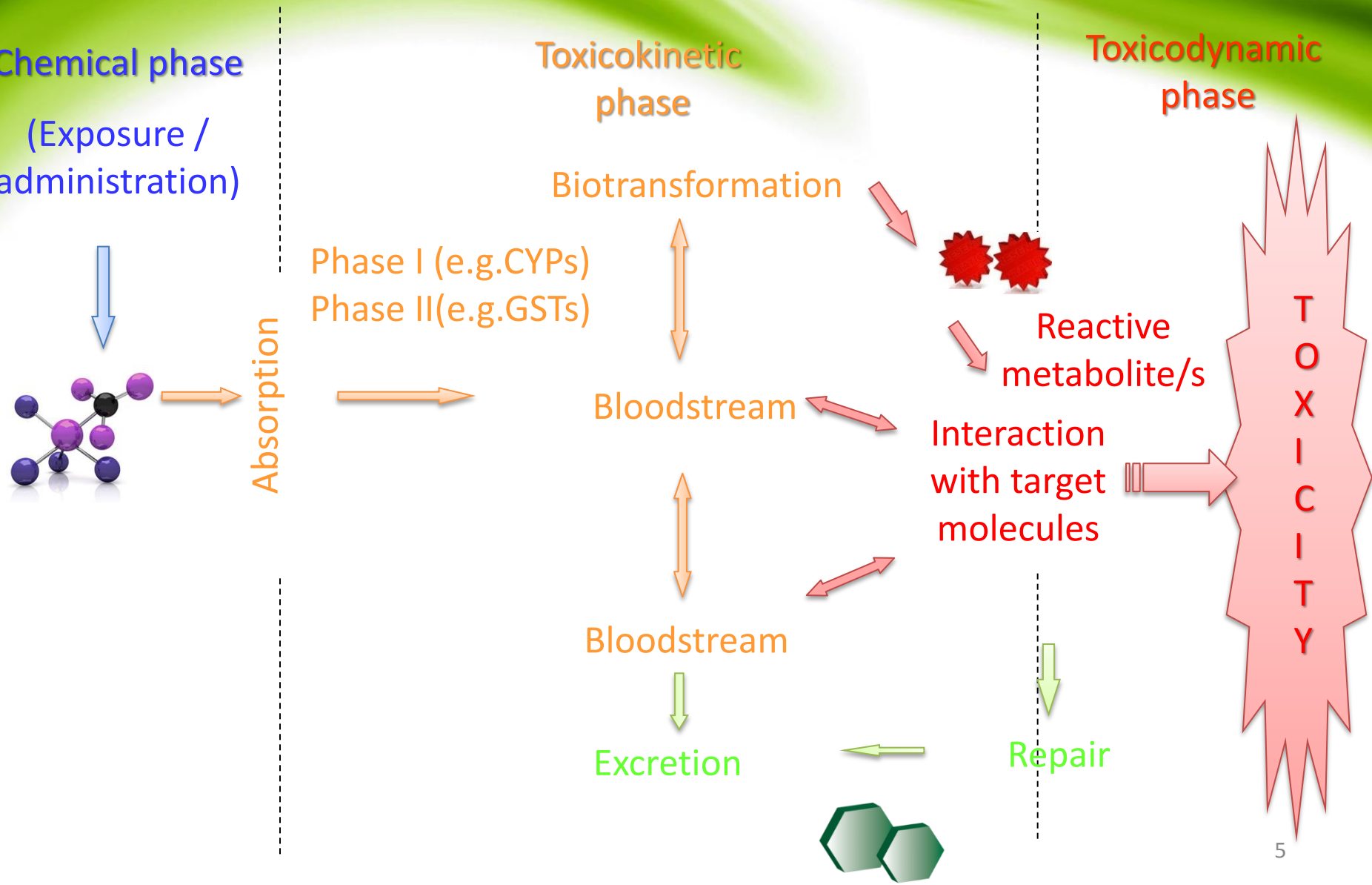


Interdisciplinary science to study the adverse effects of chemicals on living systems, including:

- Mechanisms of action and exposure to chemicals;
- Recognition, identification, quantification of hazards from occupational exposure to chemicals;
- Development of standards/regulations to protect humans and the environment from adverse effects of chemicals.

From phenomena observation and characterization... . testing (analysis , understanding, deduction)... to the quantification of the events...

From the exposure to the target site



"The dose makes the poison"

The basic principle:

"All substances are poisons…the right dose differentiates a poison or a remedy" → it is the dose that makes the poison

- **Challenge for the toxicologist**: to identify the dose(s) at which a specific substance can cause adverse effects;
- Differences between **"toxic"** or **not**: all substances are toxic, it is only the degree and type of toxicity different among all the agents.

Just because a chemical is present, does not mean there is a risk in the **amount** present

The dose concept

By definition:

is the amount of a substance administered at one time.

Other parameters are needed to characterize the exposure to xenobiotics:

- the number of doses;
- frequency;
- total time period of the treatment.

i. A better means to allow for comparison of **effectiveness /toxicity** is the amount of a substance administered on a **body weight basis**

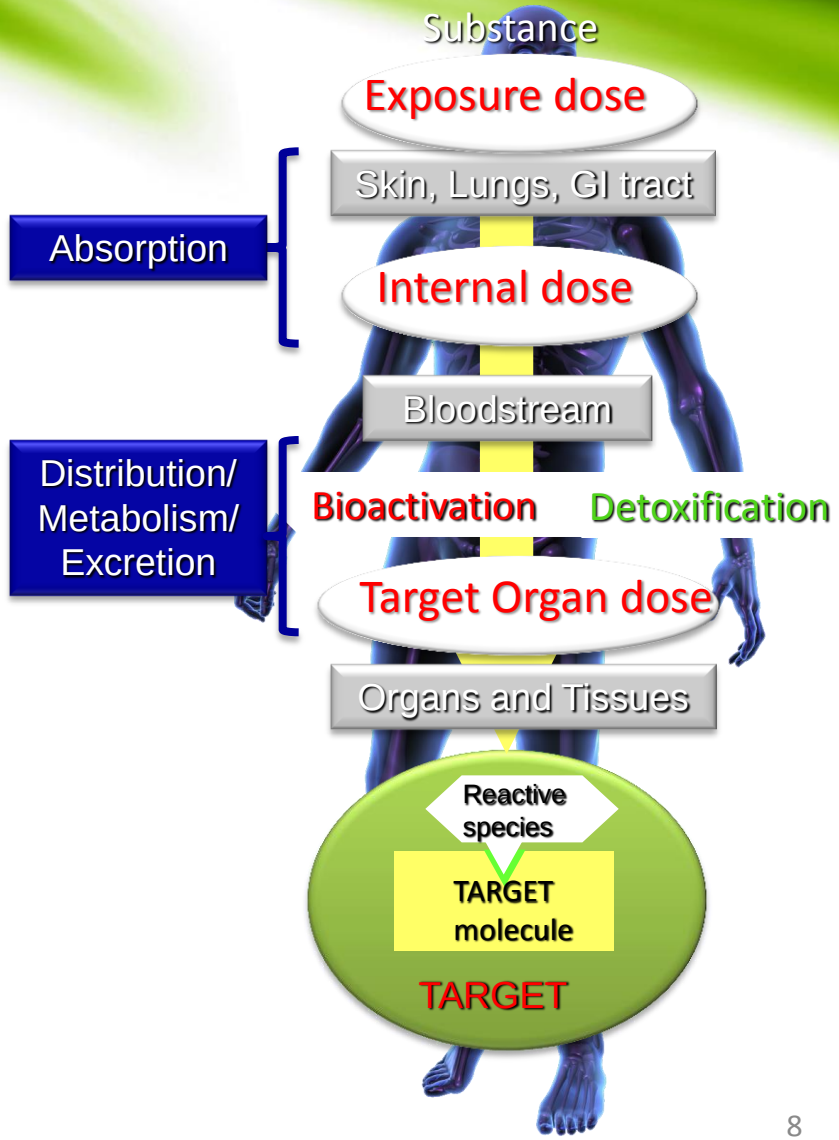
mg/kg =mg of substance per kg of bw

ii. the **time** over which the dose is administered. Important for several day or chronic exposures. Commonly used time unit = one day .

Usual dosage unit = **mg/kg/day**

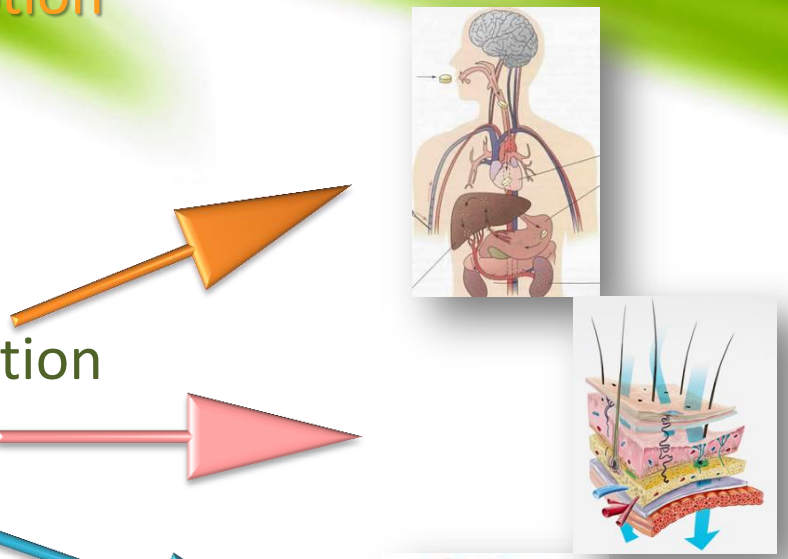
Relevant toxicologically doses

- The amount of a substance to which an **individual/population is exposed** (occupational/environmental): **Exposure dose**;
- The amount **absorbed into the organism**. The bioavailable dose that can cause the effect far from the exposure site: **Internal dose**;
- The amount able to **reach the target** site(s) and to cause the effect (adverse or effective): **Target organ/ biologically effective dose**



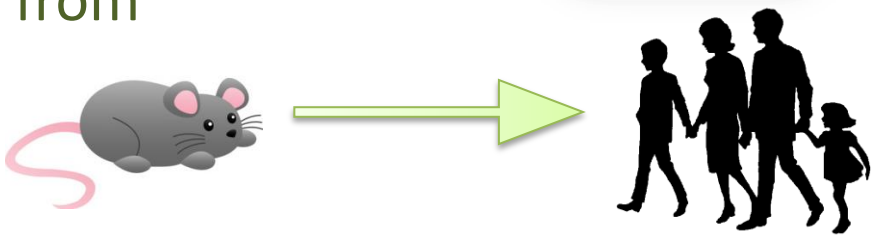
Exposure/absorption measurment

Quantification: exposure *vs* absorption



- Various routes of exposure /absorption (e.g. oral, dermal, inhalation);

- Level of uncertainty in extrapolation the effects of absorbed dose from animals to humans



The dose and its variable

Many variables are connected with the dose and consequently with the effects that occur at those doses:

- **Amount**: the magnitude of the dose;
- **Frequency**: how often (daily, weekly)
- **Duration**: how long? (acute, sub-chronic, chronic)
- **Route of exposure**: the way to be exposed (oral, dermal)
- **Individual variability/susceptibility**:
  subject characteristics (age, sex); health conditions (asthma)

The occurrence of the possible adverse effects due to the exposure to a chemical is evidenced during different toxicological studies carried out, taking into account all these variables.

Risk identification on humans, animals and environment

Regulatory toxicology

Regulatory toxicologists evaluate the *toxicity tests* that is done on chemicals, contaminants and consumer products or *preexisting information*.

They must ask questions such as:

- ? What responses are considered “adverse”?
- ? To what doses are the consumers most likely to be exposed?
- ? They must define the risk associated with each chemical, and the level of risk that the public will accept.

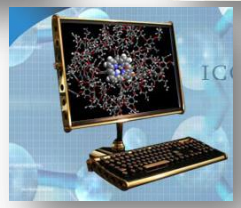
The analysis of the nature and magnitude of risk is called

RISK ASSESSMENT

Toxicological studies

Accurate predictions of effects of chemicals on humans depend upon scientific studies.

Most **toxicological studies** are empirical in nature, and are performed on experimental animals (***in vivo***) or ***in vitro*** test systems (i.e., cell culture or other systems to mimic the results in part of an organism) or on the basis of ***in silico*** predictions.

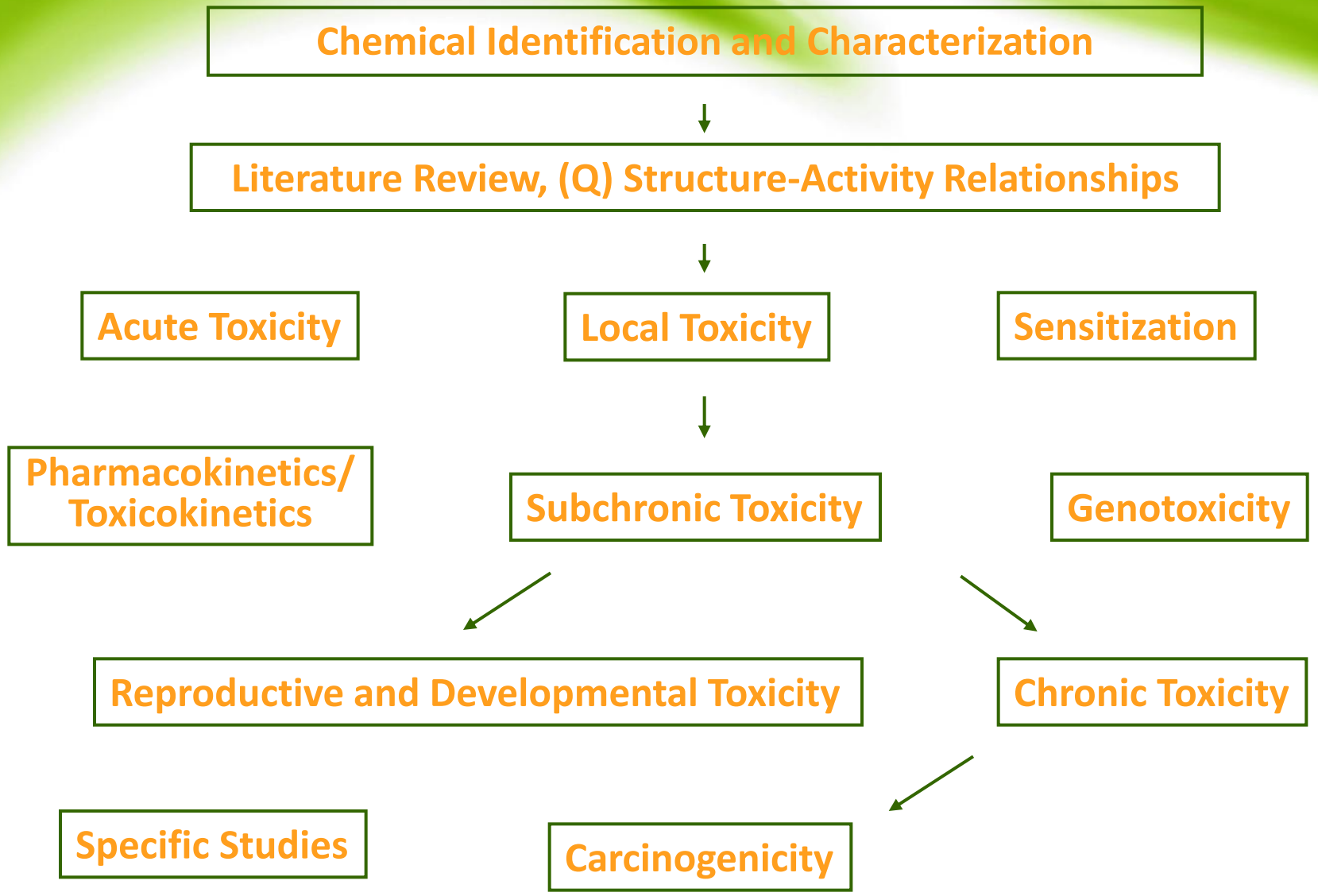


Since the results are often used for regulatory purposes, the goal of such studies is to predict effects in humans.

To achieve this goal, scientists need to understand the differences between experimental (animals or *in vitro* systems) and real conditions (in humans) in the way that they process xenobiotics, as well as the applicability to humans of results obtained.

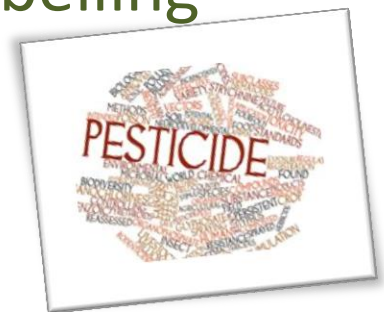
Toxicological evaluation

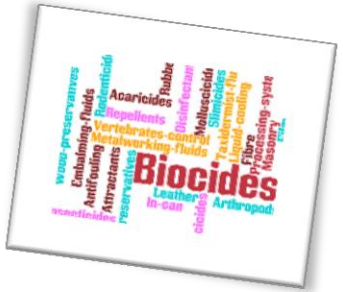
A Typical Safety Evaluation Program



INFORMATION REQUIRMENTS: ACTIVE SUBSTANCE

1. Identity of active substance
2. Physical and chemical properties
3. Further information (use, function, harmful organisms controlled, MoA)
4. Analytical Methods of detection and identification
5. Toxicological and Metabolism studies
6. (Residues in or on treated products, food and feed)
7. Fate and behaviour in the environment
8. Ecotoxicological studies
9. Literature data
10. Classification and labelling





5. TOXICOLOGICAL ENDPOINTS – core for a.s.

5.1 Studies on absorption, distribution, metabolism and excretion in mammals by oral (5.1.1) and other routes (5.1.2);

5.2 Acute toxicity:
oral (5.2.1), dermal (5.2.2), inhalation (5.2.3);
skin (5.2.4) and eye (5.2.5) irritation;
skin sensitisation (5.2.6); phototoxicity (5.2.7);

Important for C&L (Classification and Labelling)

5.3 Short-term toxicity:
oral 28-day study(5.3.1), oral 90-day study(5.3.2), other routes (5.3.3);

Identification of a NOAEL (No Observed Adverse Effect Level) and a LOAEL (Low Observed Adverse Effect Level).
Preliminary to long term repeated toxicity studies and relevant for Reference values definition.

5.4 Genotoxicity testing:
In vitro studies (5.4.1), in vivo studies in somatic cells (5.4.2), in vivo studies in germ cells (5.4.3);

5. TOXICOLOGICAL ENDPOINTS – core for a.s.

5.5 Long term toxicity and carcinogenicity

Identification of a **NOAEL** and **LOAEL** and major effects and target organ. **Relevant** for **Reference values** definition.

5.6 Reproductive toxicity:

Generational studies(5.6.1), Developmental toxicity studies (5.6.2);

Identification of a **NOAEL** and a **LOAEL** and major effects. **Relevant** for **Reference values** definition.

5.7 Neurotoxicity studies:

Neurotoxicity studies in rodents(5.7.1), Delayed polyneuropathy studies (5.7.2);

5.8 Other toxicological studies:

Toxicity studies of metabolites (5.8.1), Supplementary studies on the active substance (5.8.2), Endocrine disrupting properties (5.8.3); if necessary → mechanistic studies

5.9. Medical data

RISK ASSESSMENT

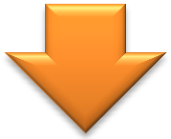
Risk assessment process

"A process intended to **calculate** or **estimate** the **risk** to a given target organism, system or (sub)population, including the identification of attendant **uncertainties**, following **exposure** to a particular agent, taking into account the inherent characteristics of the **agent** of concern as well as the characteristics of the specific **target system**"
(joint OECD/IPCS survey)

Risk Assessment:
Comparison of **exposure** with **effects**
Final outcome: **Risk characterization**

What is risk assessment?

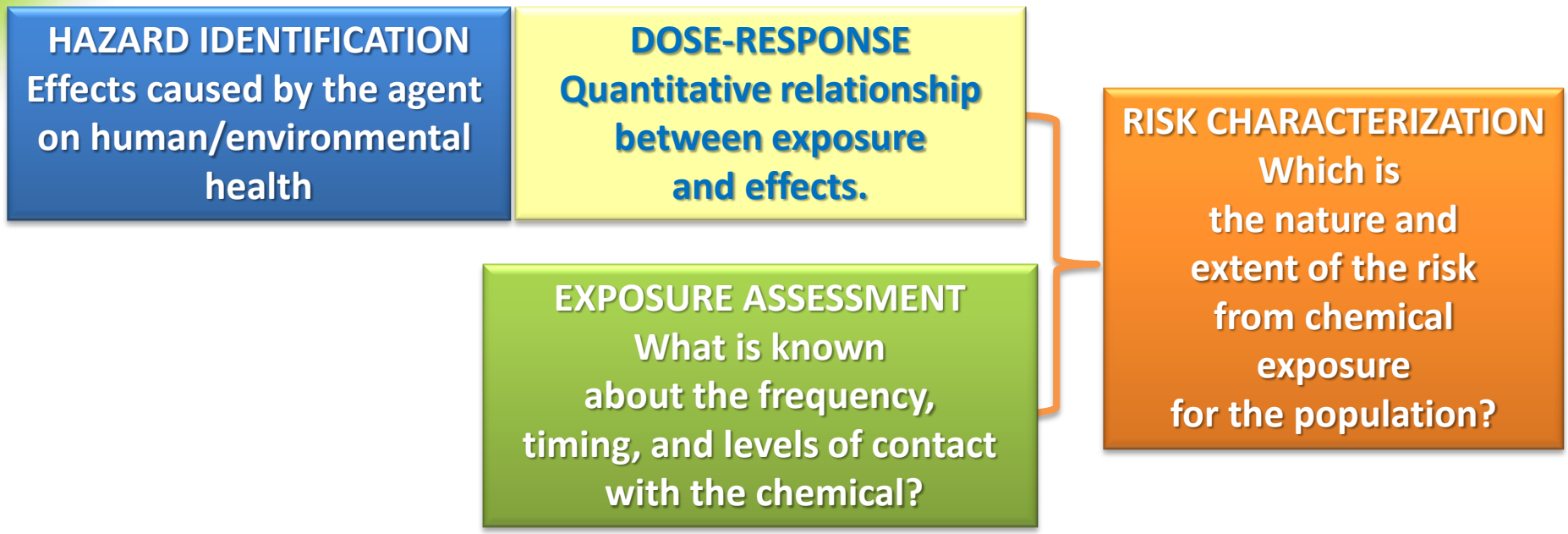
- Definition of Hazard : Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub) population is exposed to that agent.
- Definition of Exposure : Concentration or amount of a particular agent that reaches a target organism, system or (sub) population in a specific frequency for a defined duration.
- Definition of Risk: The probability of an adverse effect in an organism, system or (sub) population caused under specified circumstances by exposure to an agent.



$$R = H_{(HAZARD)} \times E_{(EXPOSURE)}$$

Toxicological Risk Assessment

A 4 Phases Process starting from data/info acquisition:



The procedure is defined at EU level: Commission Directive 93/67/EEC; Council Regulation (EEC) 793/93; TGD Technical Guidance Document on Risk Assessment, 2003; Commission Regulation (EC) No. 1488/94, and used by WHO, EPA, FDA, EFSA…

HAZARD IDENTIFICATION

I. Hazard identification

The hazard identification reflects the **qualitative aspects of the assessment** and provides answers to the question:

Which kind of adverse effects does the toxic agent induce?

Hazard: is an intrinsic feature of the toxic agent.	**Risk**: corresponds to the probability for a population of experiencing adverse effects once exposed to the toxic agent.




How can hazard be identified?

- Acute toxicity studies on rats
  (single administration, oral & dermal LD_{50}, inhalation LC_{50})
- Dermal and Eye Irritation (usually on rabbits)
- Sensibilization studies on guinea pig or LLNA on mice
- Mutagenicity test
- Repeated toxicity studies

(ex: R48 Danger of serious damage to health by prolonged exposure;
H373: May cause damage to organs through prolonged or repeated exposure)

- Carcinogenicity
- Reproductive/development toxicity studies

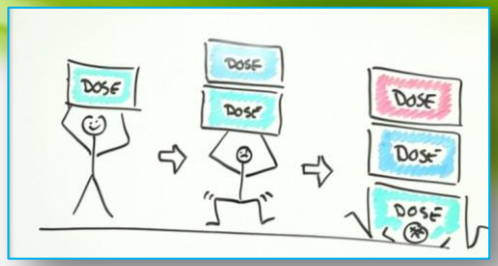
Classification and labeling for these endpoints must be performed according to **regulation:** On hazard basis and not risk

e.g. CLP Regulation (EU) n. 1272/2008

DOSE-RESPONSE

II. Dose-response Relationship

Quantitative Aspect



At which concentration/ level does the adverse effect occur?

The threshold for the effect can be achieved with :

Single exposure: acute and generally at high doses
→ accidents, poisonings.

Repeated exposure: low doses for prolonged times; doses generally non toxic if taken singularly
→ cumulative effects

Toxicological relevant compound → threshold dose

- Several adverse effects are caused by a substance only in the case the **toxicological relevant compound** (parent or toxic metabolite/s) reaches the **threshold concentration at the target site/organ**.
- Such a concentration is correlated to the **exposure level** (external) but mainly to the **actual exposure** (internal) of the organism (humans or animals).
- Therefore, the **threshold concentration changes** in relation to →

  i. ≠ exposure route,
  ii. ≠ species

  due to ≠ toxicokinetics, ≠ mechanism of action

TOXICOKINETIC

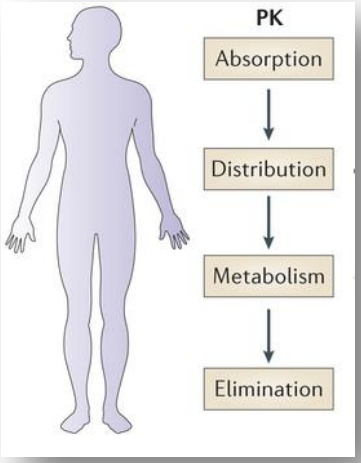
Absorption, **D**istribution, **M**etabolism and/or **E**xcretion

The main routes of entries:
GI mucosa (oral); Lung epithelium (inhalation); Skin (dermal)

The main routes of excretion:
urinary ; faecal (via the bile); expired air; tears; sweat; breast milk

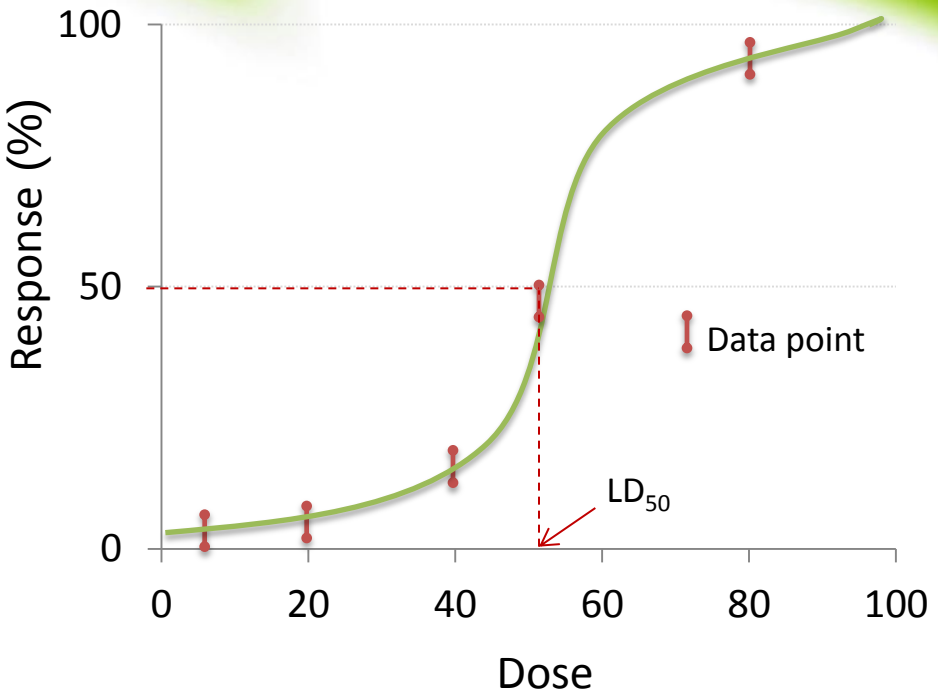
INTERNAL → At what concentration the chemical is present in different organ/tissue and in the target site?

- Which the time-dependent fate of the chemical within the body?
- Which is the concentration of the tox relevant chemical species?

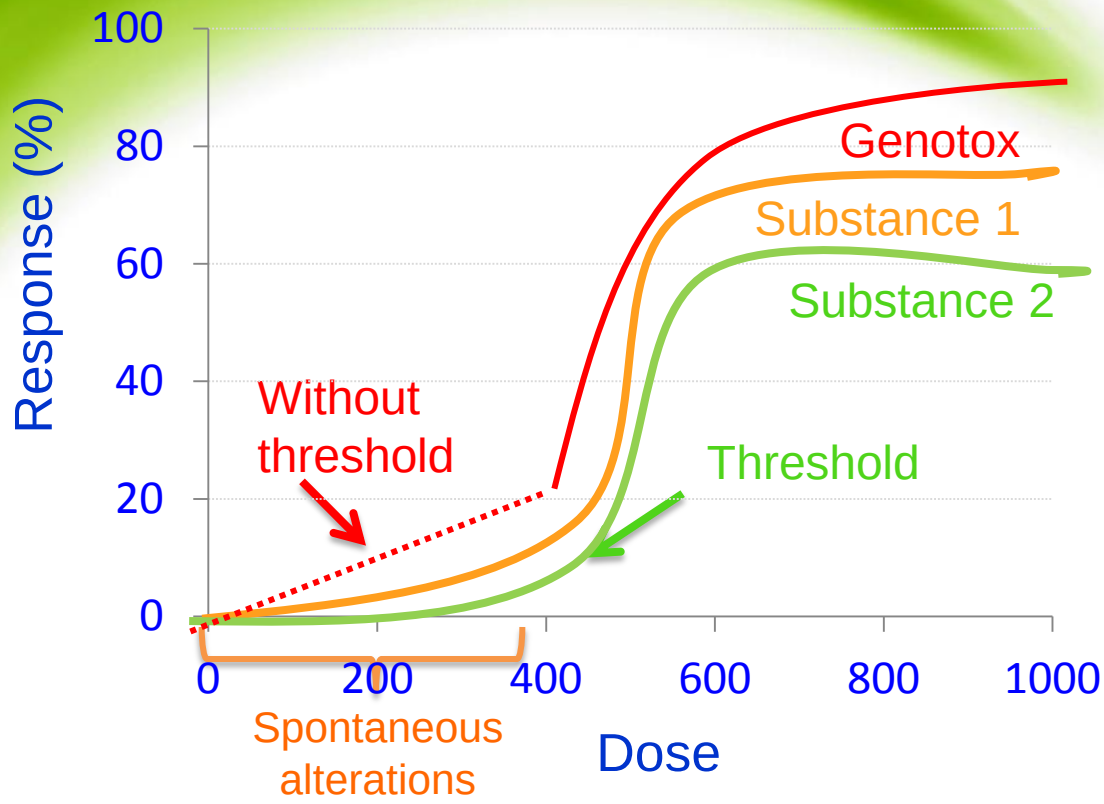


Dose-response graph

- The data coming from the toxicological studies are plotted on a graph.
- The dose is plotted against the number or portion of animals exhibiting a specific response



Responses to different agents *vs* increasing dose



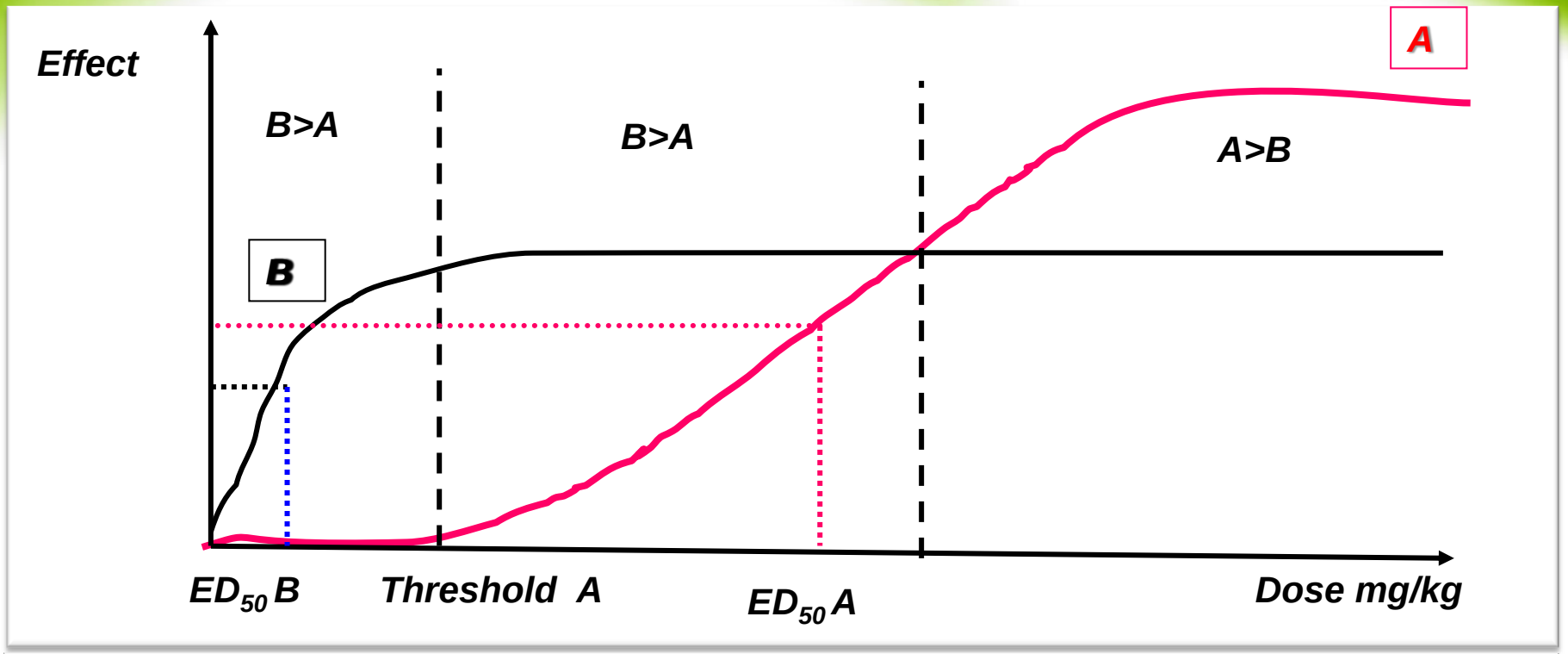
The extent and nature of the effects related to:

- dose;
- route of exposure;
- characteristics of the individual;
- time course and duration of the administration/ exposure;
- spacing between doses

Critical variable in determining whether/how or not adverse effects occur.

Threshold dose

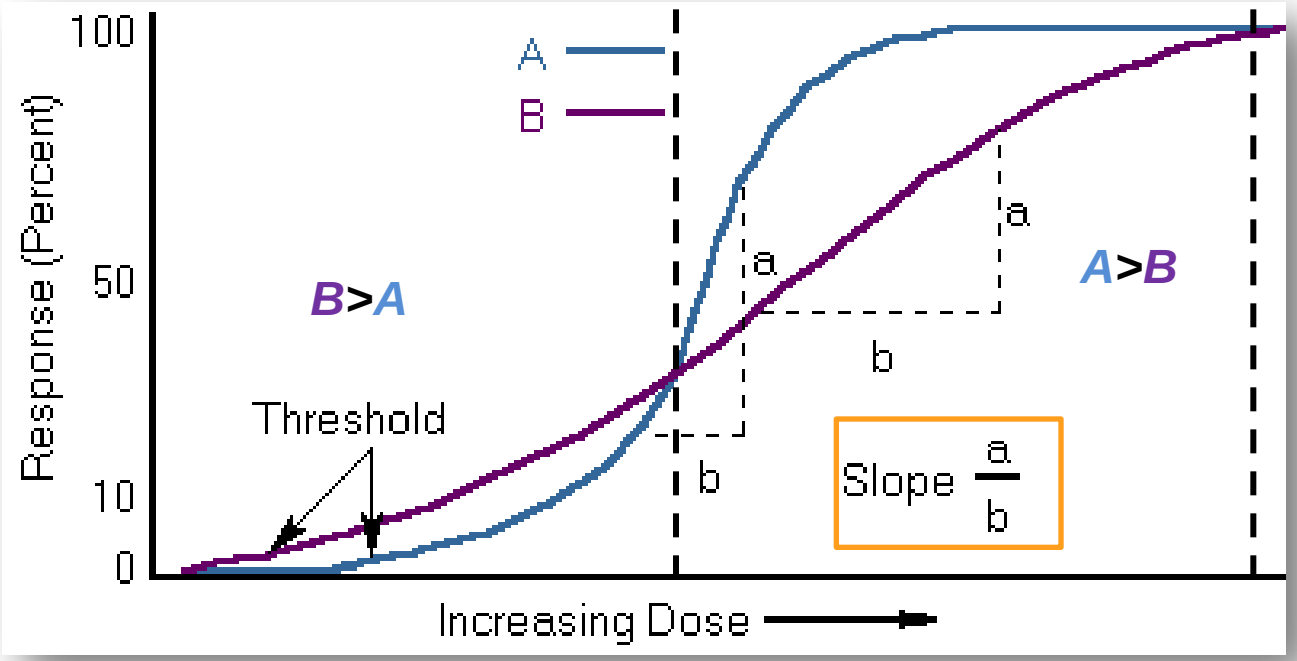
The dose below which there is no effect (dependent on the endpoint).



Between dose and response: a linear relationship or with a threshold…
Dose vs response curves can take many different shapes.
Different relative potency of two compounds defined by different shapes and slopes.

Dose response curve: shape and slope

Slope= steep curve → small dose variations induce great differences in the effects.



Threshold dose = specific for endpoint and substance.

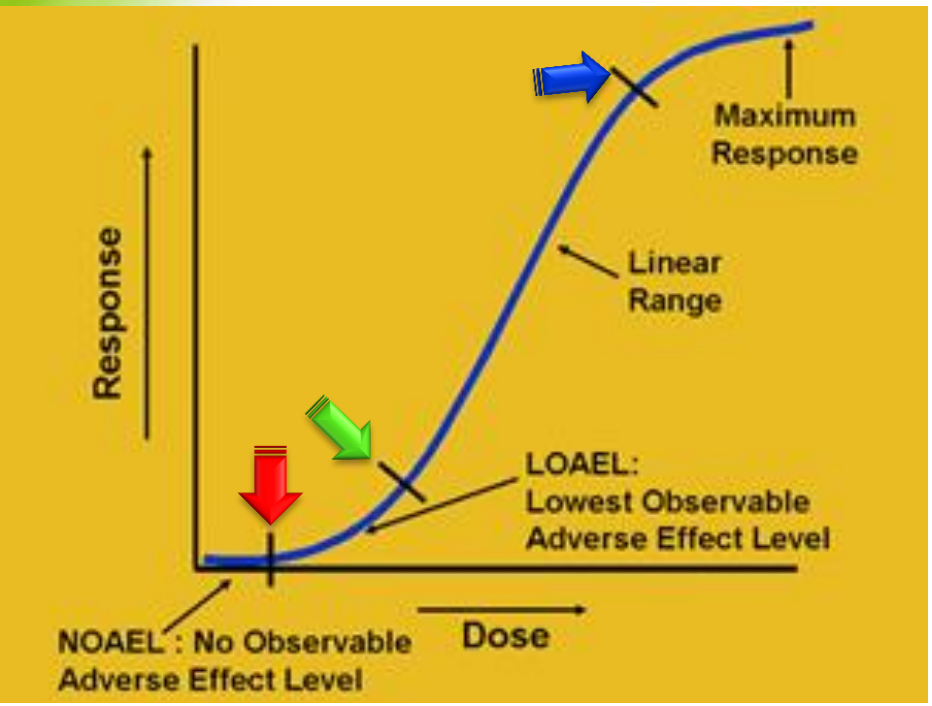
Threshold dose and toxic effects

- A ***systemic effect*** is defined as an effect that is normally observed distant from the site of first contact, i.e., after having passed through a physiological barrier (mucous membrane of the gastrointestinal tract or of the respiratory tract, or the skin) and becomes systemically available.

- A ***local effect*** is an effect that is observed at the site of first contact, caused irrespective of whether a substance is systemically available.

All available information regarding systemic toxicity and local effects needs to be evaluated and, where possible, **dose descriptors** N(L)OAEL, Benchmark Dose (BMD), etc. need to be established.

Dose descriptors/Reference Point (RP)



NOAEL:
(No Observed Adverse Effect Level):

The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and the control.

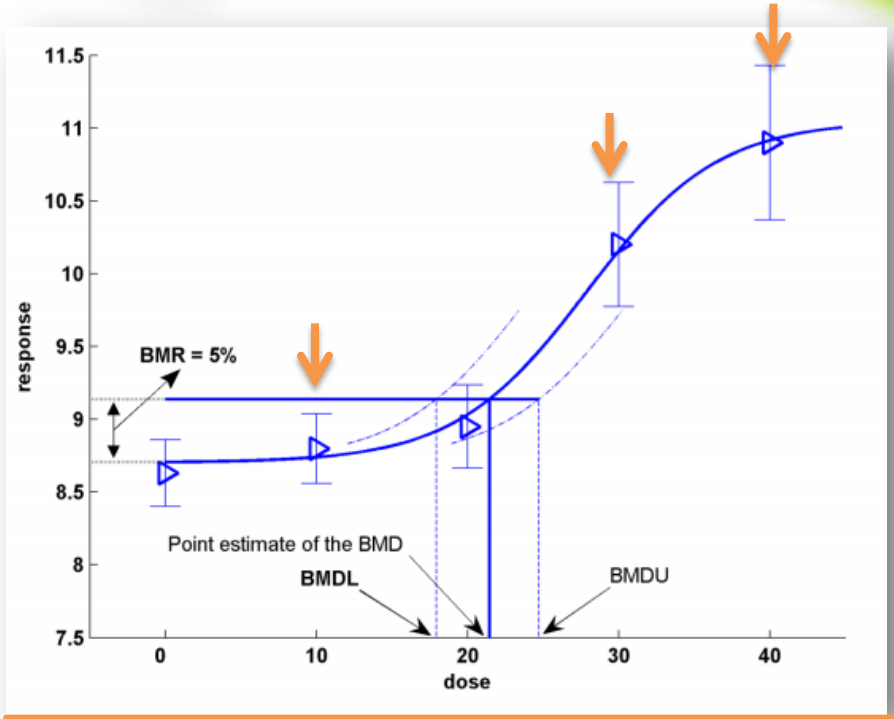
LOAEL:
(Lowest Observed Adverse Effect Level)

The lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

Dose descriptors/ Reference Point (RP)

Benchmark Dose (BMD): is a dose level, derived from the estimated dose-response curve, associated with a specified change in response. BMDL is the BMD‟s lower confidence bound, and this value is normally used as the RP.

- A more quantitative alternative to the first step in the dose-response than the current NOAEL/LOAEL process.
- It is likely that there will continue to be endpoints that are not opened to modeling and for which a NOAEL/LOAEL approach must be used .
- In some cases a combination of BMDs and NOAELs



Triangles: observed mean responses +confidence intervals; BMD (point estimate); Dashed curves: upper and lower 95% confidence limits

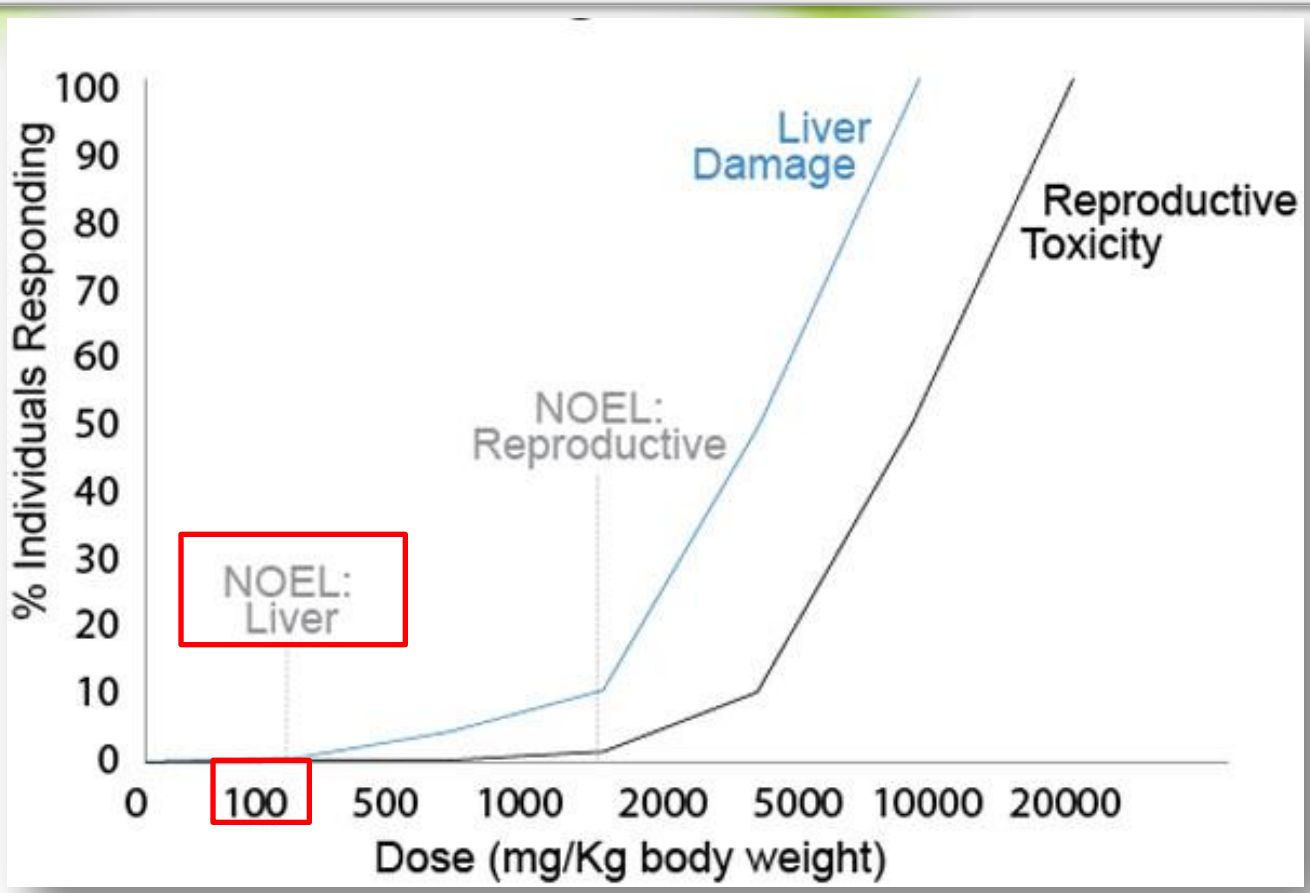
Which is the critical effect?

What questions are addressed within the test evaluation?

- Is it the one present at the **lowest dose**? **n°/sex of animals?**
- Is it the **most relevant effect** from a toxicological point of view (e.g. hair loss *vs* early marker of hepatic damage)
- Is the difference an **effect of treatment**?
- **Dose –response**? Due to outlier? Within **historical control** range? **Biological plausibility**? **Consistency** of the overall picture?
- Presence of **related effects** at higher doses or in the following times (indication of early effects) : alteration of biochemical parameters followed by histopathological changes (i.e. increase in hepatic transaminases *vs* hepatocellular hypertropy or necrosis)
- Can the treatment related effect be defined as an **adverse effect**?

Which is the critical effect?

For each endpoint → identification of a key study (data on ≠ species, ≠ exposure route, ≠ results from valid studies)



Preventing the critical effect we can avoid the other effects and thus we can get a total prevention for human health

EXPOSURE ASSESSMENT

III. Exposure assessment

Which is the level of exposure?

External

- At **which concentration** the chemical is present in different matrices or environment (diet/air/water/working place/consumer products)?
- Which is the preferred **route(s) of exposure**?
- Which the **pattern of exposure**? Working hours, environmentally, dietary.
- Which is the **toxicologically relevant species**? (e.g., parental or metabolite or degradation products).

III. Exposure assessment

Internal

At what concentration the toxicologically relevant compound is present in different organ/tissue and in the target site?

ADME

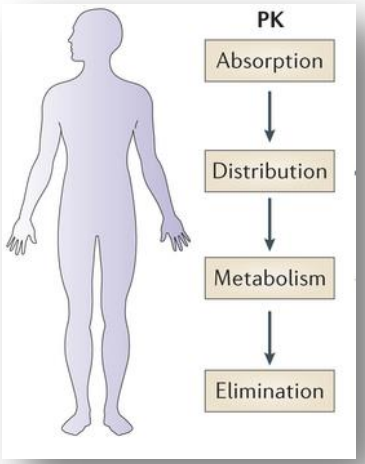
- Biomarkers of exposure
- Biomonitoring studies and “body-burden” measurements

The most difficult dose to quantify: target organ or biologically effective dose (e.g. the dose that actually reaches the kidney).

Generally it cannot be directly measured. It is usually calculated on the basis of information gathered from TK studies.

ADME studies: Parameters to be derived

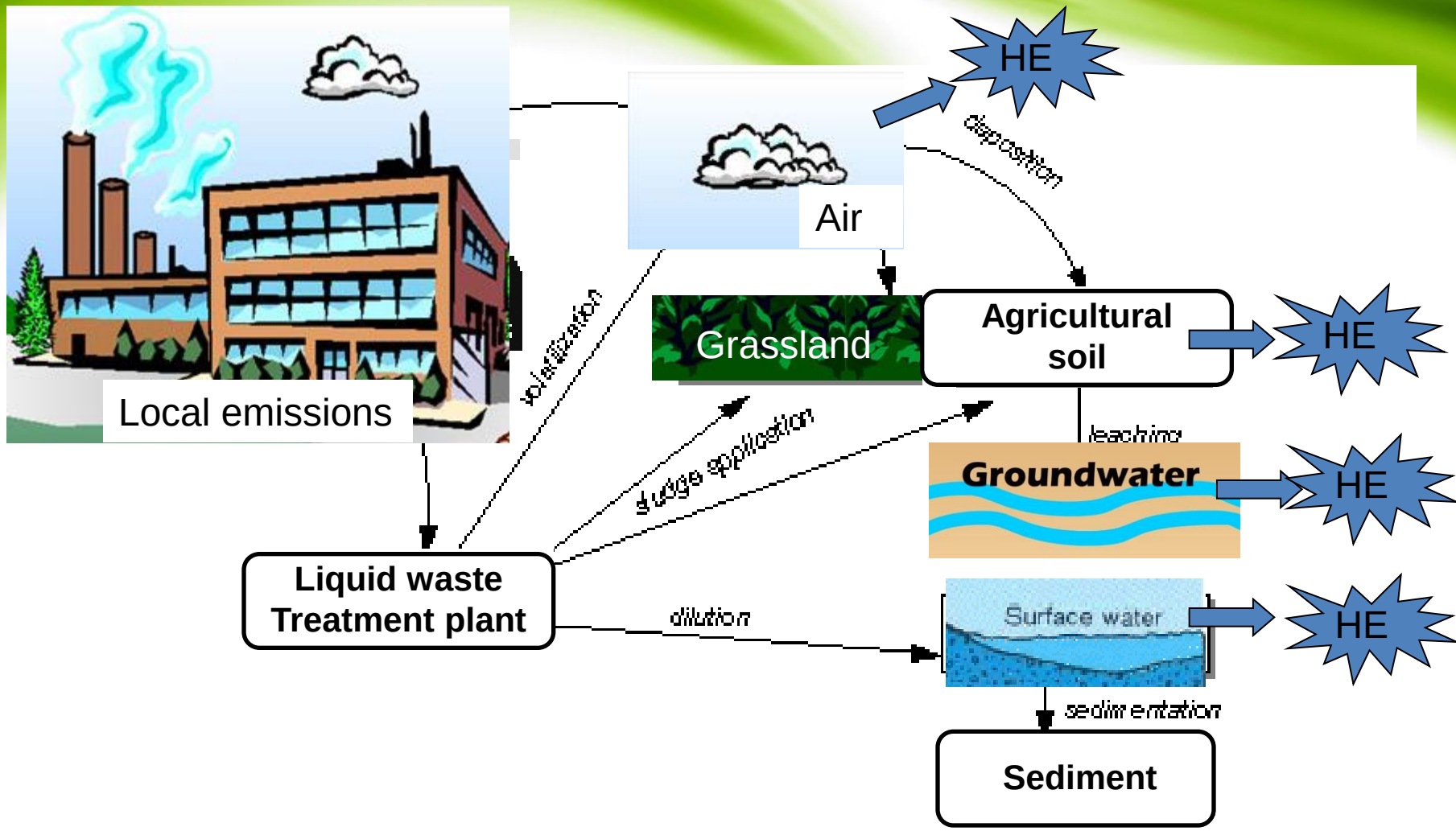
- % Oral Absorption;
- % Percutaneous Absorption (if not available default values derived on the basis of MW and LogP);
- % Inhalation Absorption (if not available: route/route extrapolation);
- Bioaccumulation Potential



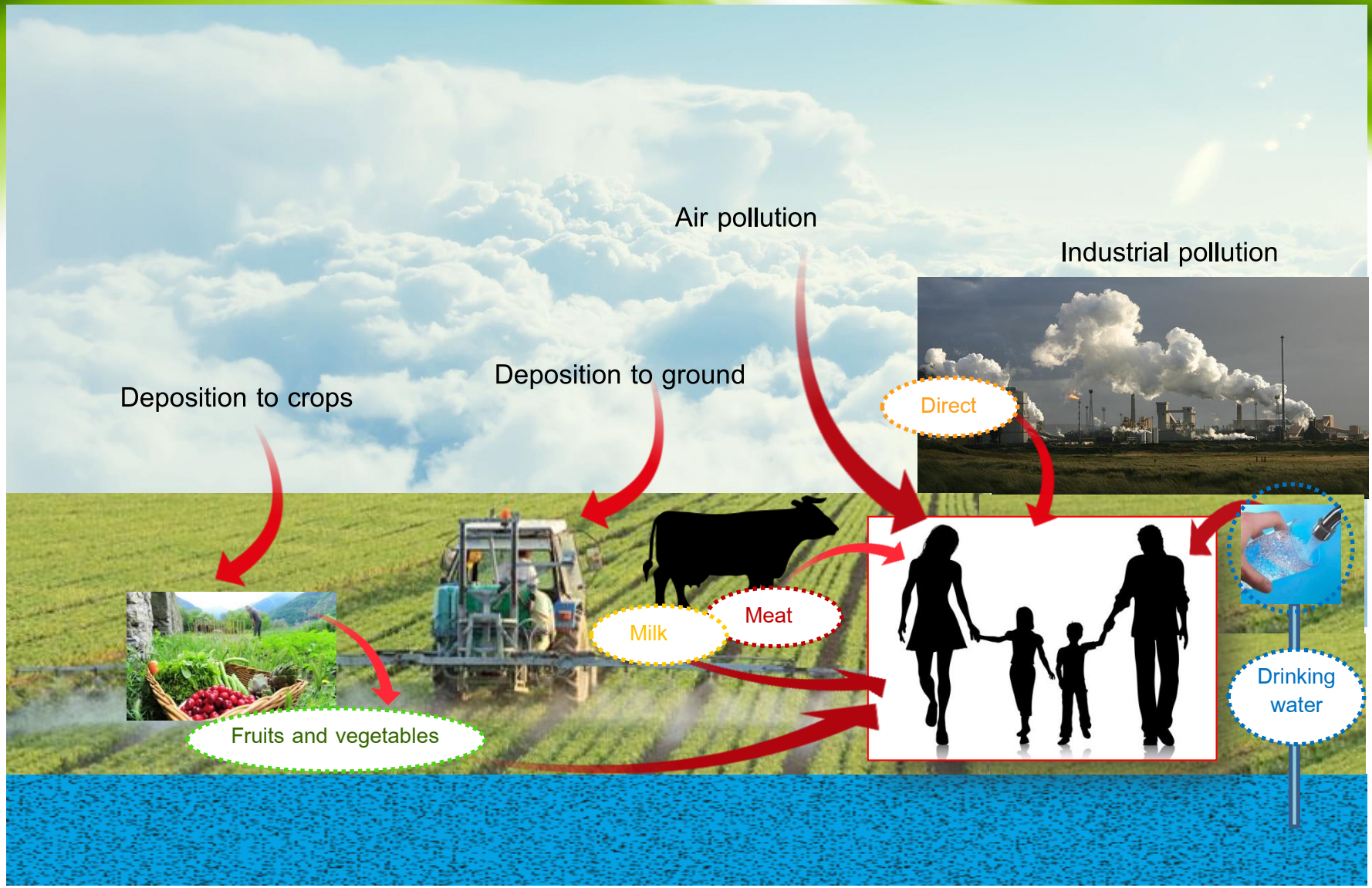
Exposure pattern

Consumers; Workers, Operators, Bystanders; Indirect exposure

Source of Exposure



Pathways of exposure



Exposure estimation

Xenobiotic exposure in humans can be measured in **two ways**, either through **direct monitoring** by measuring biomarkers from individuals or **developing models** to assess exposure.

- **Measuring Biomarkers**

  Biomarkers, or biological markers, are chemicals/metabolites that can be measured in **body fluid**, such as urine, blood, saliva, and other body fluids.

- **Modeling**

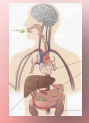
  It can be an alternative to estimate exposure to the direct measurements of biomarkers and less time consuming and expensive. It is a basically a mathematical equation that inputs **known variables** to asses exposure levels.

  The four main components of a model are **environment, agent, host, and time**.

Exposure estimation: models

Occupational exposure model

Consumer exposure model

Oral 

Dermal 

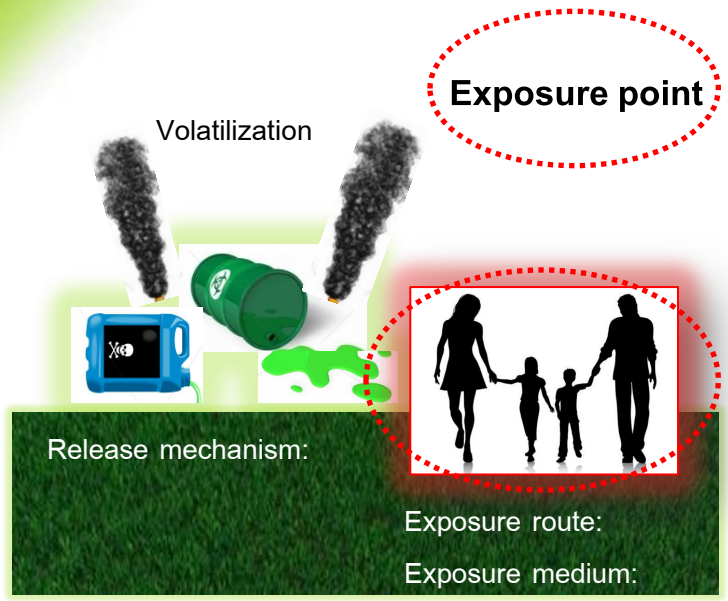
Inhalatory 

Four components that may be used in the **model equation**:

1. **Environment**: partition gradient, physical area;
2. **Agent**: chemical properties (e.g.vapor pressure, physical state, concentration);
3. **Host**: health, age, exposure pathway;
4. **Time**: length of exposure.

Exposure estimation

Scenario of exposure



Description/
Characterization

Model

- Substance
- Source
- Release
- Distribution
- Deposition
- Disappearance
- People
- Behaviour
- Anthropometrics

Formula conversion

Model variables

Exposure Factors

Data to be insert into the model

Exposure estimation: data quality

Body weight and height

Substance/formulation

physiologic data

use descriptions

dust and soil uptake

food consumption

time patterns

house and room characteristics

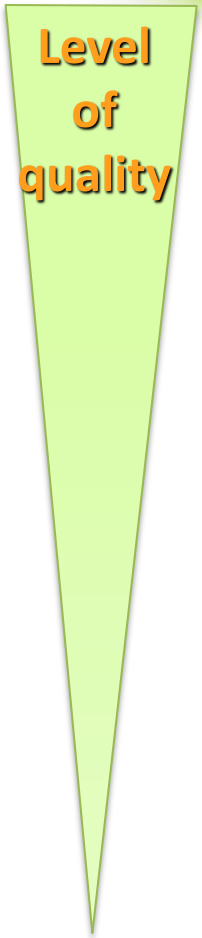
room ventilation

dermal uptake data

hand to mouth

emission rates

migration of substances in material



RISK CHARACTERIZATION

IV. Risk characterization

Which is the probability to have an effect and at what extent in the exposed population?

Having conducted the **hazard assessment** for all relevant human health **endpoints** and populations and the **exposure estimation**; a **quantitative risk characterization** is carried out.

Dose-response relationship data are compared with information of the extent of exposure to estimate the probability to observe the toxic effect within the population.

IV. Risk characterization: Reference values (RfV)

Reference values are established for a given **critical effect**, and are **specific to a substance**, a **duration of exposure** (acute, subchronic /chronic) , a route of exposure (oral, inhalation, etc.).

Exposures above the RfV are not necessarily dangerous because a large **margin of safety** is allowed in their calculation but every effort should be made to keep the level of exposure below these values.

The **RfV** is an oral or dermal dose derived from:

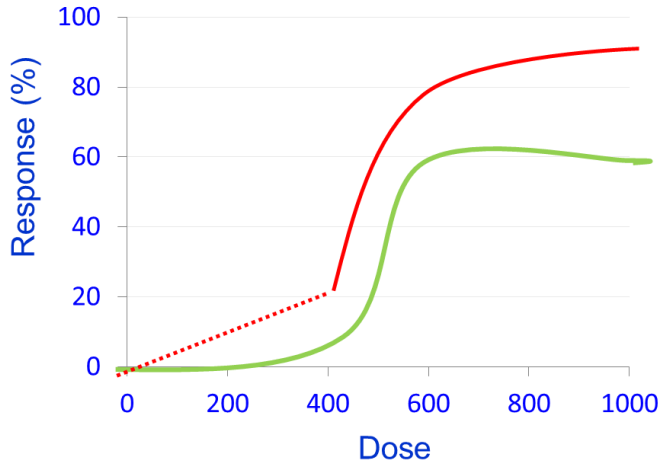
- the **NOAEL, LOAEL or BMD**
- by application of generally order-of-magnitude **assessment/uncertainty/safety factors** (Afs/Ufs/Sfs).

Mode of Action (MoA)

Before deriving RfV:

? the substance exerts its effects by a **non-threshold MoA** (non-threshold mutagens or carcinogens);

? it is **possible to derive a threshold**



Mode of Action (MoA) 1

There is a generally assumption that there is no threshold for safe exposure to substances which may cause cancer by mutation on DNA. If the substance exerts its effects entirely or partly by a non-threshold MoA (e.g. for mutagenicity, carcinogenicity) a RfV cannot be derived and for these effects semi-quantitative approach has to be followed where relevant or a qualitative approach for risk characterization:

e.g. Margin Of Exposure (MoE): to consider possible safety concerns arising from the presence in food of substances which are both genotoxic and carcinogenic.
It is a ratio of two factors: the dose at which a small but measurable adverse effect is first observed and the level of exposure to the substance considered.

Non-threshold MoA → MoE approach

- Many substances are considered non-threshold compounds. The advice given by the risk assessor to the risk manager was to reduce exposure to a level that is as low as reasonably achievable. To overcome this, EFSA proposed the **margin of exposure (MoE)** approach.
- The **MoE approach** is not confined to genotoxic/carcinogenic compounds, but when the data are insufficient or inappropriate to establish a RfV.
- It uses a reference point (ex. $BMDL_{10}$:benchmark dose lower confidence limit 10% which is an estimate of the lowest dose which is 95% certain to cause no more than a 10% cancer incidence), taken from an animal study (but also from human data) corresponding to a dose that causes a low but measurable tumourigenic response. **This reference point is then compared with exposure estimates in humans**.
- EFSA considers that a **MoE of 10,000 or higher** (on $BMDL_{10}$), would be of low concern for human health, to be considered as a low priority for risk management action. 10,000 = 100 (inter+intraspecies)x100 (uncertainties in the nature of the carcinogenic process and the reference point.

EFSA SC (2005).EFSA Journal, 282 :1-31

Mode of Action (MoA) 2

If the substance exerts its effects by a **threshold MoA**, the derivation of RfV on the basis of the Reference Point is required. RfV must be derived for **the most critical effect(s)**:

it is often wise to focus on the most sensitive population;
regulatory efforts are generally made to keep exposures below the population threshold, which is defined as the lowest of the thresholds (dose below which no adverse effect is expected to occur), of the individuals within a population.

Reference values derivation/ Reference Point (RP) selection:

- Usually the study in the **most sensitive** and **relevant species** resulting in the most **relevant lowest RP** (e.g.: NOAEL(C)s, LOAEL(C)s, BMDs) will be selected for establishing the relevant RfV;

- A comparison of the relevant RP for RfV derivation for different **time-frames** provides useful information on the influence of exposure duration on the severity and spectrum of toxicity. An assessment of the entire data package is of high scientific value, as it helps in elucidating time-dependency of toxicity.

General rule: if several relevant NOAELs (or others) are available the one that would result in the lowest RfV for a given time-frame should be chosen. However the lowest RP may not always provide the lowest RfV as it depends on the **Assessment Factors** that will be used for its derivation.

Expert judgement

Reference values derivation/ Uncertainty factors

- Uncertainty factors (UFs) (also called assessment factors, safety factors, adjustment factors or extrapolation factors) are used to derive health-based guidance values (RfV) by extrapolating from experimental animal data to humans;

- The setting of the overall UF is a critical step that involve a high level of expertise (with clear, scientifically based justification);

- UFs are intended to cover the variability and uncertainty arising out of such an extrapolation.

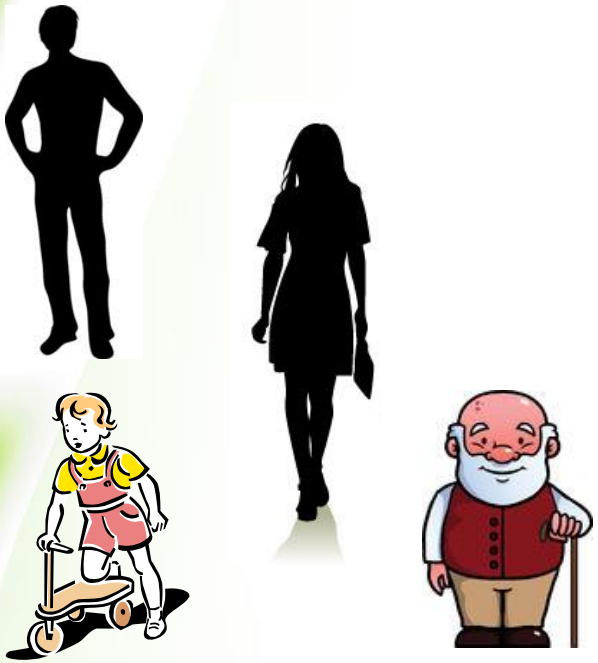
Variability factors:

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

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

When the more vulnerable groups are protected, all the population is protected

Variability :



Interindividual differences in susceptibility due to genetic make up (enzyme polymorphism)

Intraspecies differences due to: age, gender, pathological status (liver/kidney/lung diseases), altered metabolic status due to exposure to contaminants (induction/inhibition of enzymes)

Uncertainty:

- **Quality** of the available studies and of the experimental results: NOAEL vs LOAEL; relevance; data base consistency;
- **Adequacy** of the experimental model: relevant animal specie, study duration;
- **Extrapolation** of animal data $\rightarrow$ human
  high experimental doses $\rightarrow$ low actual exposure dose

Overall UFs

- In the absence of chemical-specific data on kinetics and/or dynamics, it is recommended to use overall **default UFs**;
- If available and relevant, **chemical-specific data** on kinetics and/or dynamics and in general **experimental results** **should be used**.

1. Intra/inter-species extrapolation

Default uncertainty factor of 100:
10 for inter-species variability x
10 for intra-human variability

$UF_{inter\text{-}species}$ = TK:4 x TD:2.5 (WHO approach)

- for inter-species variability in toxicokinetics: 4.0
- for inter-species variability in toxicodynamics: 2.5
- for intra-human variability in toxicokinetics: 3.16
- for intra-human variability in toxicodynamics: 3.16

(EFSA approach)

EFSA Journal 2012;10(3):2579

An allometric scaling (to correct differences in the basal energetic metabolism: it changes among species)

2. Other UFs

- **Deficiencies in the data available for the assessment:**

  When additional data cannot be obtained or requested, the use of an **additional UF** to take account of the **deficiency of a database** should be considered on a case-by-case basis and justified. It is not possible to propose a default value for this UF, as it will be directly dependent on the dataset available.

  ✓**Extrapolation for duration of exposure**

  Extrapolation from subchronic to chronic study duration in rodents: EFSA recommends the use of an UF of 2.

  ✓**Accounting for the absence of a NOAEL**

  The LOAEL approach might be used and an additional UF will be needed (to be determined on a case-by-case basis).

- **Severity and nature of the observed effect:**

  the need for an extra UF to allow for the **severity of an effect** is exceptional(case-by-case basis).

  Ex. An irreversible and particularly severe toxicological effect: this should be a trigger to consider the finding in more detail before choosing an appropriate UF.

Overall UFs

Basis for AF	Factor	Source of uncertainty
Human (Intraspecies)	1-10	variability in human populations, (variation in sensitivity between members of the same species)
Animal (Interspecies)	1-10	differences in responses between animals and humans
Subchronic to Chronic	1-10	when chronic data are unavailable and a 90- day study is used
LOAEL to NOAEL	1-10	when LOAEL instead of NOAEL is used
Data Gaps	3-10	applied for "Incomplete" data bases (i.e., adequacy of studies or database, missing studies)
Nature and severity of effect	1-10	
Modifying Factor		usually applied for differences in absorption rates, tolerance to a chemical, lack of sensitive endpoint, or other toxicokinetics/dynamic parameters. The default value is 1.

To summarize:

IDENTIFICATION OF CRITICAL EFFECTS:
Systemic and route specific effect; most relevant species and study; dose-response

THRESHOLD IDENTIFIED

DOSE RESPONSE ASSESSMENT

Identification of a Reference Point
Select relevant NOAEL, LOAEL

MODIFICATION OF
Reference Point
Determination of absorption, bioavailability

APPROPRIATE UFs
Chemical specific;
Default values+additional UFs

REFERENCE VALUES

NO THRESHOLD IDENTIFIED

MoE approach

Different Reference Values: some examples

- *"The **ADI** (Acceptable Daily Intake) of a chemical is the estimate of the amount of a substance in food or drinking water, expressed on a bw basis, than can be ingested daily over a lifetime without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation"* (WHO, 1997). Established for food additives and pesticide residues.
- **Tolerable Daily Intake (TDI)** is an estimate of the daily intake of a chemical contaminant which can occur over a lifetime without appreciable health risk.

Guidance Values (GV): Maximum acceptable concentration in each exposure source (e.g.: drinking water, fish…):

$$(TDI \times bw \times AF)/C$$

AF= allocation factor: fraction of the TDI allocated to each source;
C= daily consumption

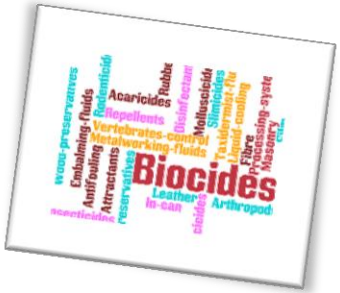
- **DNELs (Derived No-Effect Levels)** for threshold effects
- **AEL Accepted exposure level**

Different Reference Values: some examples

- Acceptable Operator Exposure Level (AOEL)
  is the maximum amount of a.s., expressed on a b.w. basis,
  to which an operator may be exposed
  without any adverse health effects.

- For highly acutely toxic compounds
  the reference value is
  the Acute Reference Dose (ARfD).
  Daily consumption for a limited period of time.

- Acute AEL



- Sub-chronic DNEL

Derivation of ADI as an example

It is a health-based exposure limit to be used for comparison with estimated/measured exposure levels for assessing the risk also of the sensitive groups of population arising from the application of pesticides.

Normally based on Reference points (i.e. N(L)OAELs) that
are obtained in **long-term animal toxicity studies**.

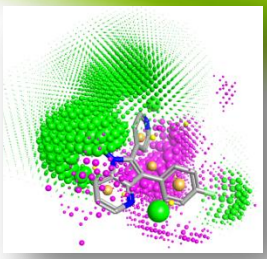
$$ADI = \frac{N(L)OAEL}{UF}$$ (inter x intra x expdur x exprt x dose-resp)

Risk assessment: a nonstop evolution process

- To evaluate current approaches and **alternative methodologies to the derivation of RfV**. The basic considerations apply to a wide number of areas of chemical risk assessment. Evaluations have to be reviewed to determine **studies** and **endpoints** utilized to derive RfV, **UFs** applied and any aspects routinely debated during peer review.
- **Alternative approaches** to the **NOAEL** have to be evaluated. Particular consideration was given to the Benchmark Dose (BMD) approach.
- **Alternative approaches** to the use of the **default 100 fold UFs** to address uncertainties in extrapolating between animal data and human exposures.

What are 3Rs alternatives?

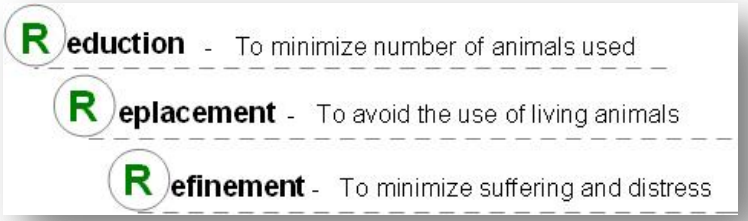
- Efficient use of existing information;



- (Q) Structure-activity relationships, mathematical models, computer simulations;

- Grouping of chemicals, Read Across;
- *In vitro* methods: isolated organs
  tissue slices
  tissue cultures
  cell cultures
  subcellular fractions;

- Lower animals; animal welfare



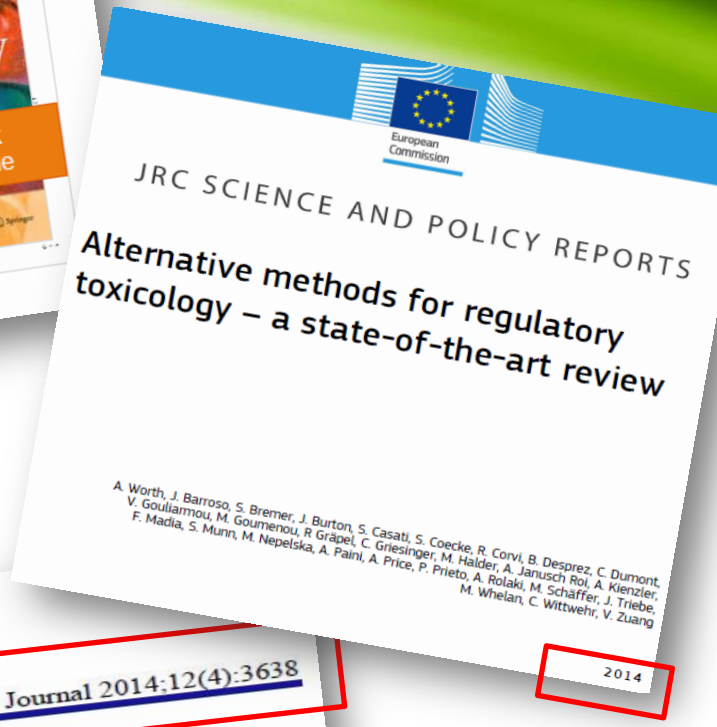
New challenges in risk assessment

State of the art


Archives of Toxicology
May 2011, Volume 85, Issue 5, pp 367-485

Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010

Sarah Adler, David Basketter, Stuart Creton, Olavi Pelkonen, Jan van Benthem, Valérie Zuang, Klaus Ejner Andersen, Alexandre Angers-Loustau, Aynur Aptula, Anna Bal-Price, ... show all 55


European Commission

JRC SCIENCE AND POLICY REPORTS

Alternative methods for regulatory toxicology – a state-of-the-art review

A. Worth, J. Barroso, S. Bremer, J. Burton, S. Casati, S. Coecke, R. Corvi, B. Desprez, C. Dumont, V. Gouliarmou, M. Goumenou, R Gräpel, C. Griesinger, M. Halder, A. Janusch Roi, A. Kienzler, F. Madia, S. Munn, M. Nepelska, A. Paini, A. Price, P. Prieto, A. Rolaki, M. Schäffer, J. Triebe, M. Whelan, C. Wittwehr, V. Zuang

2014


efsa
European Food Safety Authority

EFSA Journal 2014;12(4):3638

SCIENTIFIC REPORT OF EFSA

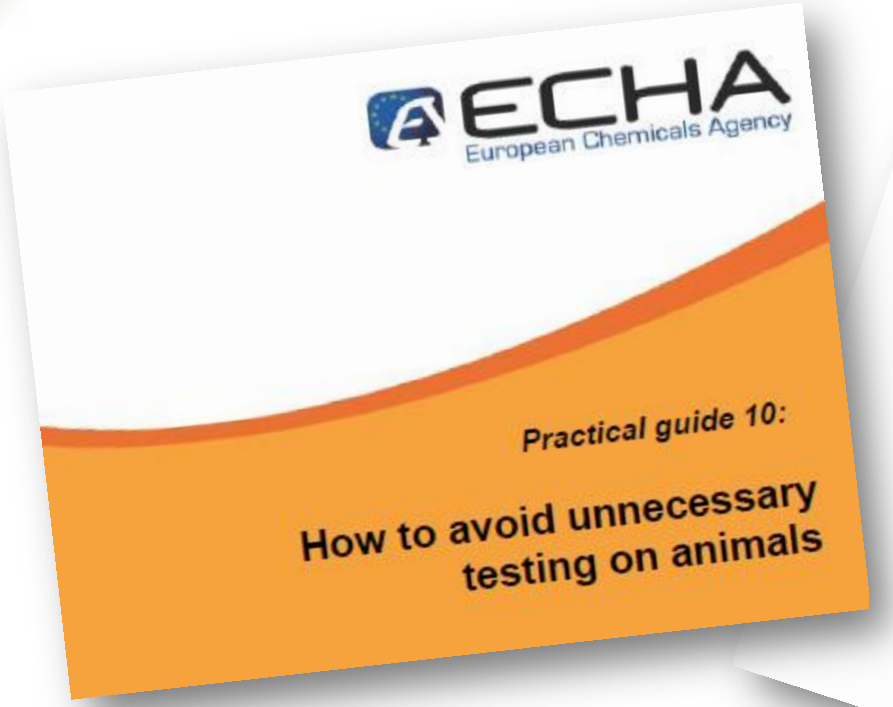
Modern methodologies and tools for human hazard assessment of chemicals[1]

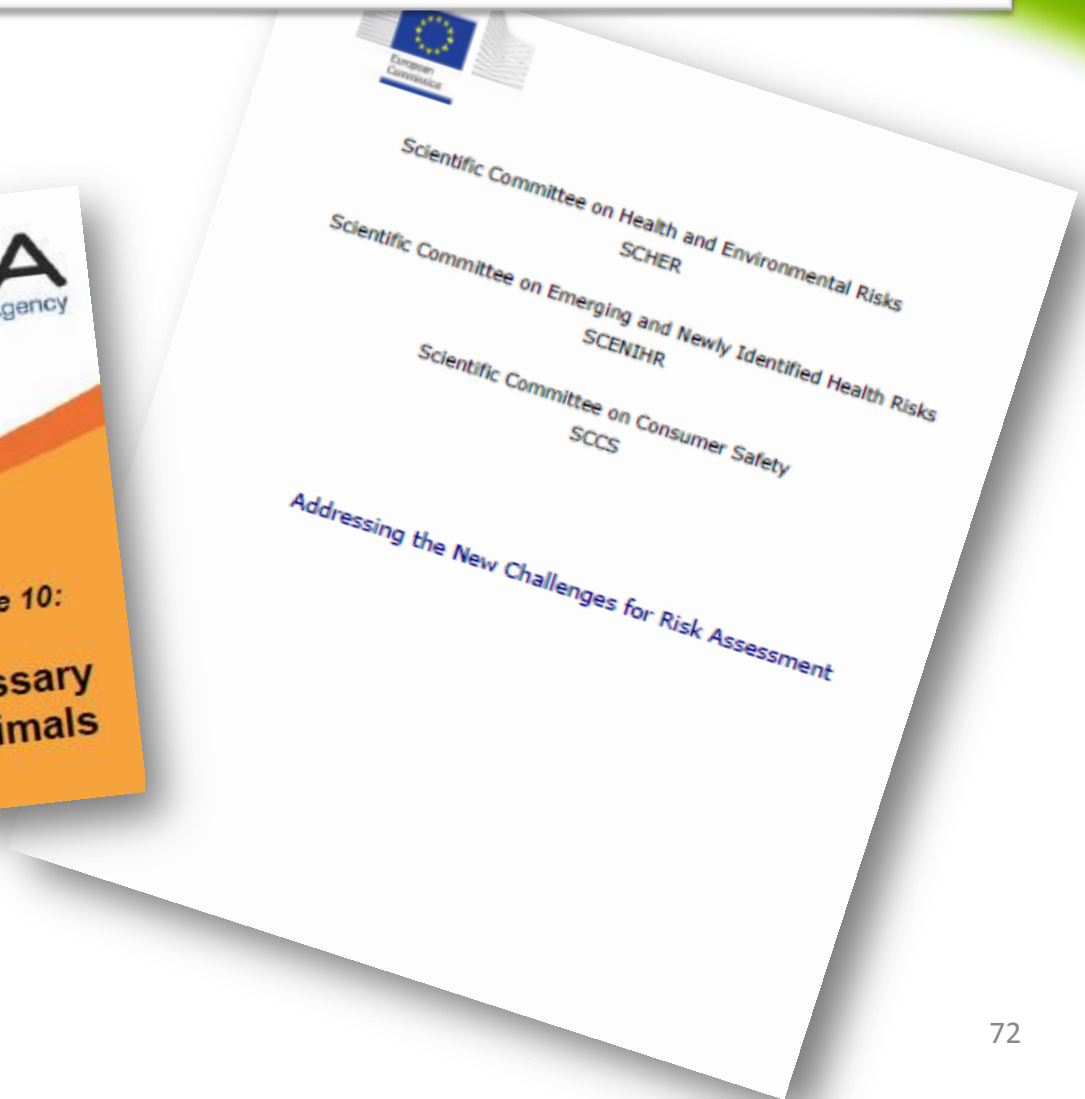
European Food Safety Authority[2,3]

European Food Safety Authority (EFSA), Parma, Italy

New challenges in risk assessment

What are scientists doing to advance safety assessment of chemicals without relying on animal testing?





New challenges in risk assessment

☺ **Exposure-based approaches such as TTC**
(Threshold of Toxicological Concern) for prioritization:
"Toxicity testing in the 21st century: a vision and a strategy";

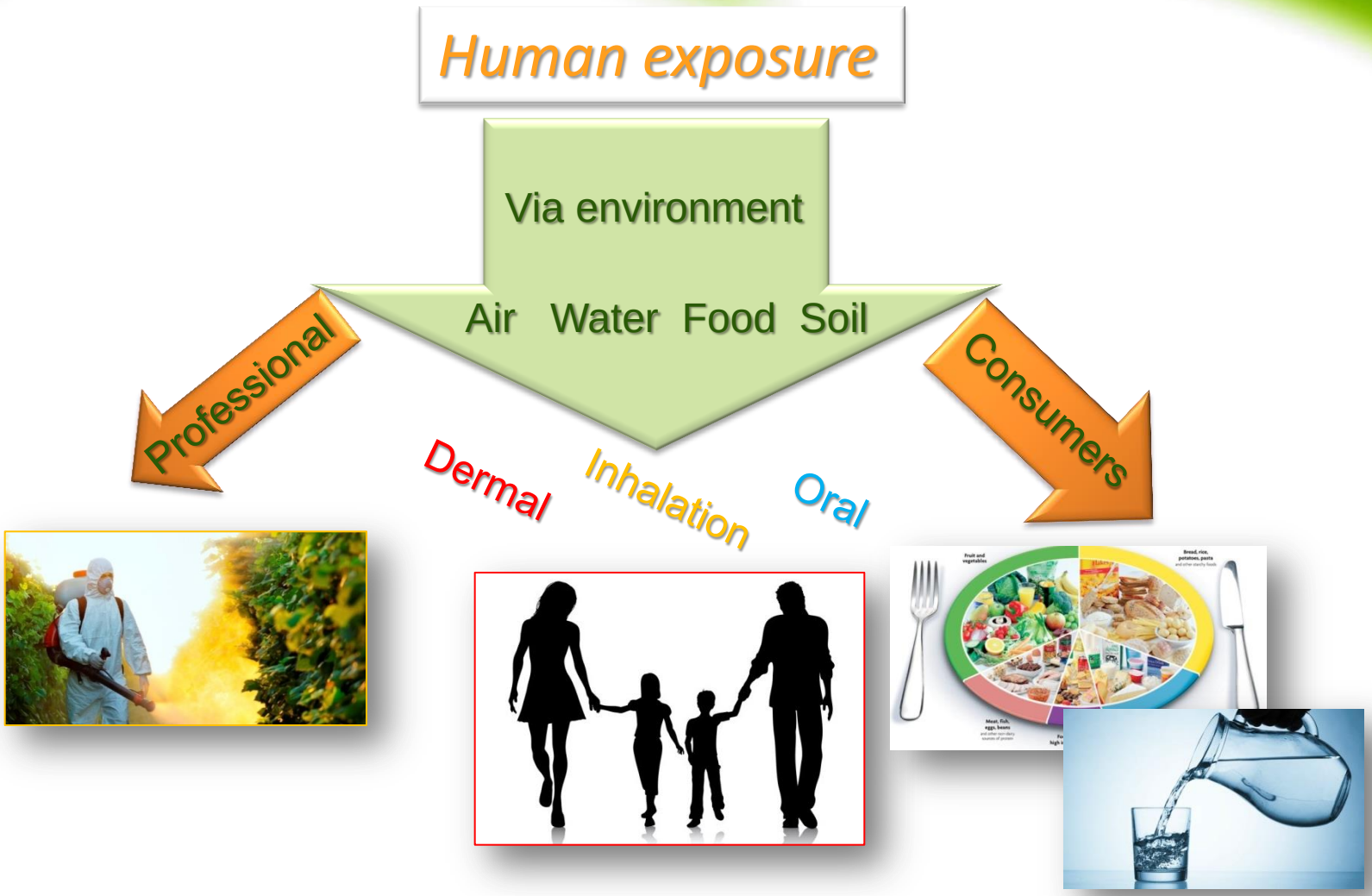
The **Threshold of Toxicological Concern (TTC)** is a principle which refers to the possibility of establishing a human exposure threshold value, below which there is no appreciable risk to human health.

- For each structural classes of chemicals by considering extensive databases of toxicity data (by the oral route) generated in the past.
- Requires reliable exposure assessment and confidence in data evaluation.

CASE STUDIES

Exposure pattern

Consumers; Workers, Operators, Bystanders;
Indirect exposure



1. Pesticides: dietary exposure assessment

Dietary exposure to pesticide residues =

Food consumption X Pesticide residues levels in food

Human Health Risk Assessment

Dietary exposure

Reference values (ADI; ARfD)

1. Pesticides: dietary exposure assessment

- **Acute exposures:** calculated over a period of **one day**;
- **Chronic exposure:** assessed as the average **daily exposure** of an individual over **their lifetime**;
- It considers the whole of the EU or national population. EU Regulation requires particular attention to protection of **vulnerable groups** including pregnant women/unborn children, infants and children →
  by conducting **specific exposure assessments** for vulnerable groups,
  or by assessing the **overall population** (separated results
  for vulnerable groups);

- Consideration of **all plant and animal commodities** in the form they are consumed (raw and/or processed) when they are expected to contain residues of the pesticide in question, and all foods that contain those commodities;
- EU regulation requires that account should be taken **of known cumulative and synergistic effects** where scientific methods to assess such effects are available;

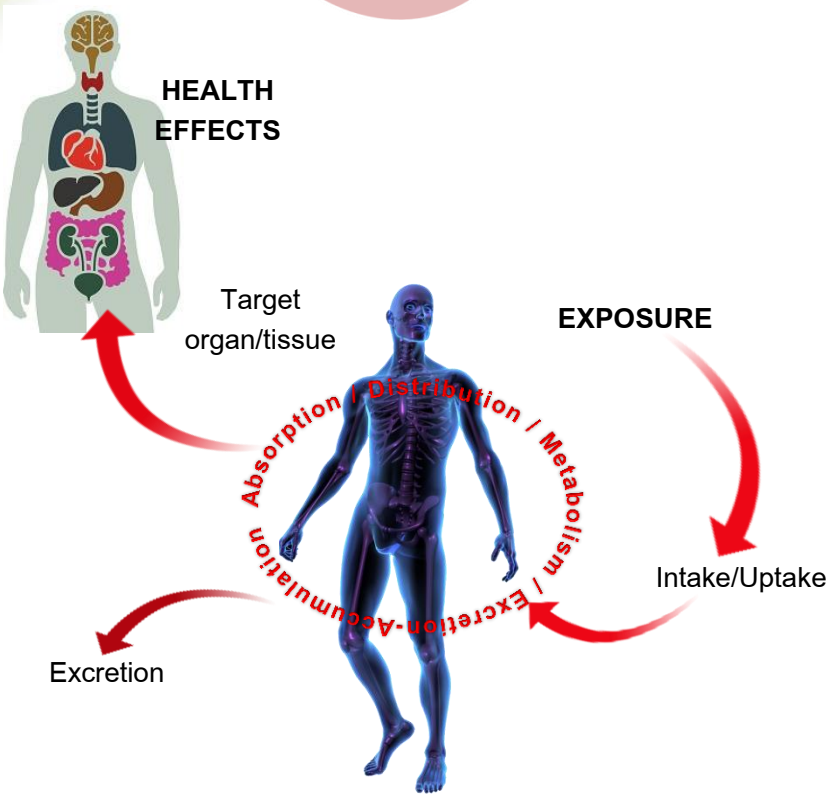
1. Pesticides: dietary exposure assessment

- Available residue data are combined with dietary information to estimate potential residue intake by consumers. The consumer is considered to be adequately protected when estimated dietary intake of pesticide residues does not exceed the acceptable daily intake (ADI) or the acute reference dose (ARfD).
- The health impact of pesticides in food is estimated by comparing dietary exposure to toxicological levels of concern (RfVs).
  Exposure assessments combine data on concentrations of a pesticides present in food with the quantity of those foods consumed.
- Theoretical Maximum Daily Intake (TMDI); International Estimated Daily Intakes (IEDIs); National Estimated Daily Intakes (NEDIs).
- The risk assessment methodology developed for this specific risk assessment task is based on internationally recognized methodologies → EFSA model for chronic and acute risk assessment

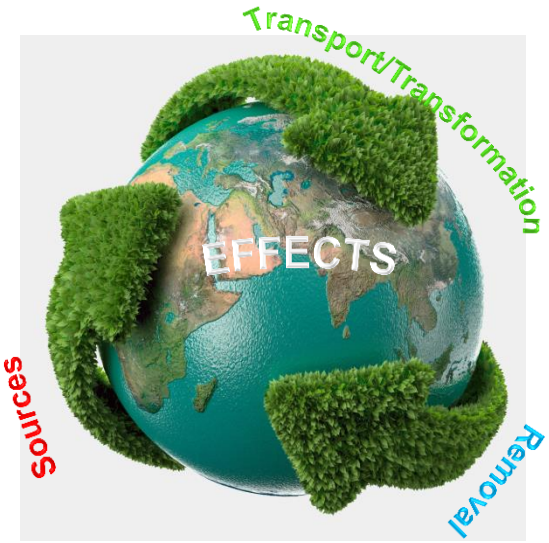
EFSA journal 2012; 10(10):2839

Human *vs* Environmental Health

Human Health Risk Assessment



ENVIRONMENTALhealth



Environmental Health Risk Assessment

Environment and health :

- **Key points:** some **characteristics** of environmental pollution and the variety and heterogeneity of the possible **exposure scenarios** for the people living in polluted areas.
- The process for **estimating exposures** to environmental pollutants is one of the most important **sources of uncertainty**, limiting the ability to quantify the role of the environment as a determinant of human health.
- **Analytical process**: the characterization of environmental exposure must follow an integrated multi-disciplinary approach: characterization of the **sources of emission/release**; **distribution** of sources of contamination; **time-frame** of contamination;
- **Transport , environmental fate and bioavailability of contaminants**: **Physico-chemical properties** play a key role in the analysis of the environmental fate of pollutants and transport.

The **aim** is to describe: **What compartments** of the ecosystem are most affected; the factors that influence the **environmental concentrations** of a contaminant , its **bioavailability** and its potential to bioaccumulate and to produce break down products (secondary pollutants).

Environmental fate and behaviour

Required data for the estimation of exposure, for example:

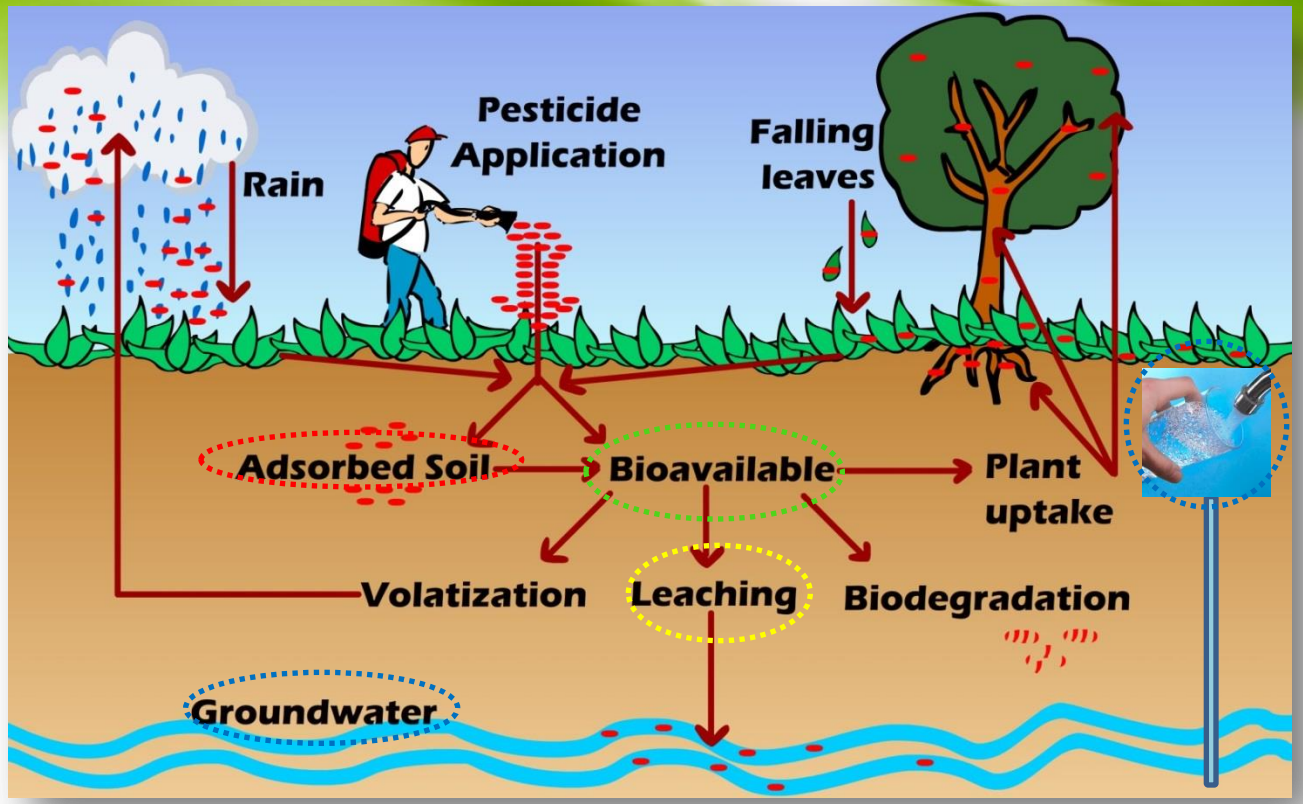
- **Source :** Levels of the substance in a product, its release, use.
- **Fate**: Identification of release , distribution , adsorption and desorption , degradation.
- **Contact**: behavior of people exposed , contact time , frequency , duration (e.g.: children crawling, playing on the ground, sucking toys).
- **Routes** by which pollutants enter ecosystems (e.g. surface waters).

The **physico-chemical properties** of the pollutants and the characteristics of abiotic compartment determine how long and how the substance is or move from/to a given compartment:

Molecule properties:
mass, charge, solubility, vapor pressure, partition coefficients;

Properties of the compartments:
pH, organic matter, soil quality, fine particulate , temperature, etc.

2. Pesticides: in the environment



Persistence and Bioaccumulation of Persistent Organic Pollutants (POPs); http://dx.doi.org/10.5772/56418

Application Rate Approach: Plant Protection Products

- Soil exposure is directly dependent on application rate (as defined by the GAP, Good Agricultural Practices).
- Also determines quantity available to be transported via other pathways.
- Run-off: movement in water over a slopping surface.

2. Pesticides: in the environment

Environmental fate data:

- Generally relates to the calculation of **predicted environmental concentrations (PECs)** in all relevant compartments. This can involve relatively simple calculations for **soil** or **water** via spray drift, or more complex modelling using agreed European tools such as the FOCUS models to simulate long term leaching behavior to **groundwater**.

- Soil **metabolites** which may leach into groundwater must also be assessed as for the active substance.

Metabolites which are found to occur at **significant levels (>0.1 µg/l)** must also be considered for **toxicological relevance** in a stepwise approach according to the approaches in the
"Relevance of metabolites in groundwater, SANCO/221/2000 – rev.10, 25/02/2003"

2. Pesticides: relevant metabolite in groundwater

Sequential assessment of the relevance of metabolites:

- Step 1 (fate and behaviour expert): Exclusion of degradation products of no concern, like CO2 or an inorganic compound, not containing a heavy metal;
- Step 2 (fate and behaviour expert): Quantification of potential groundwater contamination. FOCUS groundwater models and scenarios have to be used with data on degradation and sorption of metabolite as input;

If a metabolite is predicted in groundwater at a concentration higher than 0.1 µg/L, it has to be identified as relevant or not by toxicologists.

- Step 3 (human toxicology expert):
  Hazard Assessment : Identification of relevant metabolites.
  → Screening for biological activity,
  → Screening for genotoxicity,
  → Screening for toxicity.

If a compound is considered relevant, the threshold of 0.1 µg/L is of reference.
If a compound is considered not relevant with respect to hazard assessment, the exposure assessment (Step 4) has to be evaluated:

2. Pesticides: relevant metabolite in groundwater

- **Step 4** (fate and behaviour/toxicology expert): *Exposure assessment-TTC approach*. For non-relevant metabolite a **TTC of 0.75µg/L** can be considered for GW. If the **PECgw** is < of this limit, **no concern is expected**. **If not**, the Step 5 has to be taken into account.

- **Step 5** (human toxicology expert): *Refined risk assessments for non-relevant metabolites*.
  Non-relevant metabolites with **0.75 < PECgw<10 µg/L** require a **refined assessment** of their potential toxicological significance risk for consumers.

  Case-by-case basis:

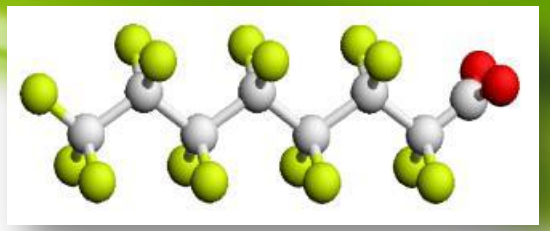
For example: for a metabolite found in animals the acceptable limit may be defined starting from the ADI values derived for the parent compound.
The MAC (Maximum Acceptable Concentration) can be evaluated as:

$$MAC = \frac{ADI \times bw}{C_w \times LF}$$

where:

ADI= Acceptable daily intake; *bw* = 60 kg for an adult; C_w = daily water consumption (2L); LF = location factor of 10% (% ADI attributable to drinking water)

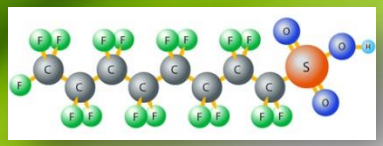
3. Perfluoroalkylated substances (PFAS)



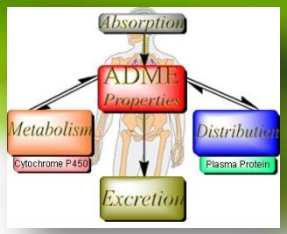
Environment and health

- **Perfluoroalkylated substances (PFAS):** is the collective name for a vast group of fluorinated compounds, including **PFOA** is perfluorooctanoic acid and is sometimes called C8 or C-8, perfluorooctane sulfonate **(PFOS)** .
- **PFAS** have been widely used in **industrial and consumer applications** including stain- and water-resistant coatings for fabrics and carpets and clothing (GORE-TEX®), oil-resistant coatings for paper products approved for food contact, fire-fighting foams, floor polishes, and non-stick surfaces on cookware (Teflon®).
- However, consumer products made with fluoropolymers and fluorinated telomers, including Teflon® are not PFOA. Rather, some of them may contain trace amounts of PFOA and other related perfluorinated chemicals as impurities. Therefore, a number of different PFAS have been widely found in the environment

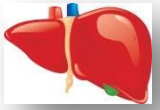
3. Perfluoroalkylated substances (PFAS)

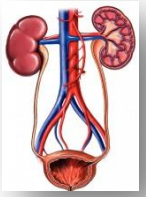


Physico-chemical and kinetic characteristics



- vP, TvB, BB and T (that is very persistent , very bioaccumulative and toxic). The bioaccumulation potential depends on the level of exposure and can hardly be traced to a single value of BAF (bioaccumulation factor).
- Data on the proportion between body-burden and age are conflicting .
- The potential for bioaccumulation of PFAS is not related to the partition in lipid tissue and adipose tissue, but
  - ? rapid oral absorption
  - ? relevant protein binding in plasma , accumulation in the liver, kidney, absence of biotransformation processes
  - ? slow elimination with re-absorption at renal level





3. Perfluoroalkylated substances (PFAS)

Reference values for PFOS e PFOA according to different Regulatory agencies

	PFOA	PFOS
EFSA	*1,5 µg/kg bw*	*0,15 µg/kg bw*
U.S. EPA	*0,4 µg/L*	*0,2 µg/L*
UK	*3,0 µg/kg bw*	*0,3 µg/kg bw*

PFOS TDI in food chain

EFSA Journal 2008; 653: 7-131
EFSA Journal 2012;10(6):2743

- From a subchronic study in *Cynomolgus monkeys*, the *Scientific Panel on Contaminants in the Food Chain (CONTAM)* identified 0.03 mg/kg b.w. per day as the lowest no-observed-adverse-effect level (NOAEL) and considered this a suitable basis for deriving a Tolerable Daily Intake (TDI).
  End-point: Biochemical alterations (lipid metabolism, tyroid hormones);
- The CONTAM Panel established a TDI for PFOS of 150 ng/kg b.w. per day by applying an overall uncertainty factor (UF) of 200 to the NOAEL.
  An UF of 100 was used for inter and intra-species differences and an additional UF of 2 to compensate for uncertainties in connection to the relatively short duration of the key study and the internal dose kinetics.

TDI: 150 ng/kg bw/day

(0.03 mg/kg : 200)

PFOA TDI in food chain

EFSA Journal 2008; 653: 7-131
EFSA Journal 2012;10(6):2743

- The CONTAM Panel noted that the 95% lower confidence limit of the benchmark dose for a 10% increase in effects on the liver ($BMDL_{10}$) values from a number of studies in mice and male rats were in the region of 0.3 - 0.7 mg/kg b.w./day.
  Therefore, the Panel concluded that the lowest $BMDL_{10}$ of 0.3 mg/kg b.w./day was an appropriate point of departure for deriving a TDI.
- The Panel established a TDI for PFOA of 1.5 µg/kg b.w./day by applying an overall UF of 200 to the $BMDL_{10}$.
  An UF of 100 was used for inter- and intra-species differences and an additional UF of 2 to compensate for uncertainties relating to the internal dose kinetics.

TDI: 1,5 µg/kg bw/day

(0,3 mg/kg : 200)

The CONTAM Panel noted that the indicative human average and high level dietary exposure for PFOA of 2 and 6 ng/kg b.w. per day, respectively, are well below the TDI of 1.5 µg/kg b.w. per day.

4. Persistent Organic Pollutants (POPs)

PCB

- Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants widely used as industrial chemicals, particularly in the electrical industry and for the manufacture of paints, plastics and adhesives. They have been in use since the beginning of the 1930's;

- It is a mixture of compounds containing the biphenil structure with varying numbers (1 to 10) and arrangements of chlorine atom attached. PCB'S are generally used as a mix of isomers;

- Due to PCBs' environmental toxicity and classification as a persistent organic pollutant (POP), PCB production was banned some years ago.

4. PCBs properties

PCB

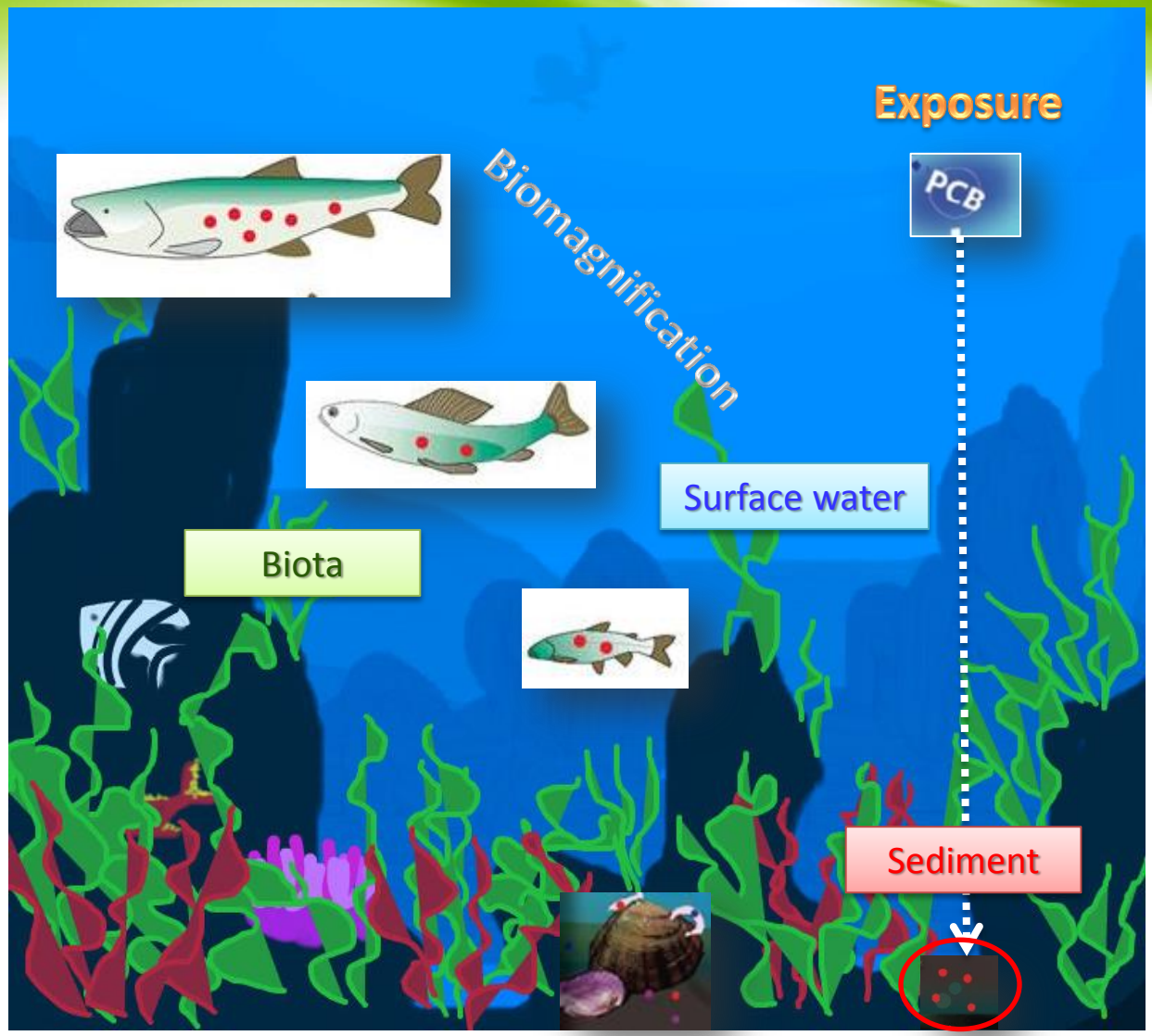
- Chemical properties:
  I. lipophilic (easily mixed with oil and fat): high Log Kow (6.0-8.2);
  II. very stable;
  III.low aqueous solubility:
  48.6 (PCB 47), 5.97 (PCB 99), 1.28(PCB 153) $*10^{-6}$;
  IV. low vapor pressure;

- Persistent in the environment;

- Bioaccumulation & bioconcentration effects;

- Accumulation in human fat tissue:
  i.e. humans: 2300 ng/g, human breast milk: 1200 ng/g.

4. PCBs: bioaccumulation and biomagnification

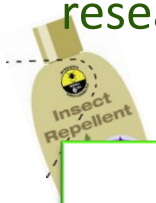


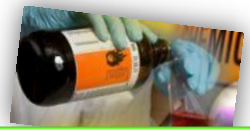
4. PCBs: contaminated site Risk Assessment

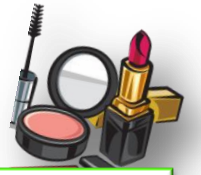
- PCBs can be carried in the atmosphere, changing phases depending on temperature and vapor pressure, to contaminate surface water or soil;
- Living organisms : important source of POPs, contaminating plants and small organisms that are then consumed by larger animals. Progression up the food chain, with each animal consuming greater quantities of ontaminated species on the lower levels of the food chain, magnifies the concentration of the contaminant consumed (biomagnification);
- Routes of exposure: dermal (skin absorbed), respiratory (inhaled), and gastrointestinal (ingested);
- Pathways of exposure: through soil ingestion (oral); through vegetable and food consumption (oral); through inhalation of indoor air (inhalation);
- Assessing human exposure: i. measurement of the actual body burden through biomonitoring (sampling and measuring body fluids or body tissue). For several reasons, however, these measurements often offer limited possibilities and are only used in higher tier Risk Assessments (i.e. importance of reliability of the data); ii. by using a so-called exposure model. These exposure models enable calculation of the rate contaminants enter the human body, blood stream, or target organs.

Risk assessment: lessons learned

- Consistent/transparent: to ensure objectivity and rationality;
- easily understood by all the interested parties; flexible enough to deal with complex situations; reproducible;
- based on the best scientific evidence available at the time, well-documented and supported with references to the scientific literature/data and other sources, including expert opinion;
- regularly reviewed and updated when additional new information becomes available;
- complemented by decisions/actions based on available information; taking into account uncertainties (gaps in knowledge) and assumptions made, in order to evaluate the effect of these on the final risk estimate and priorities for future research.



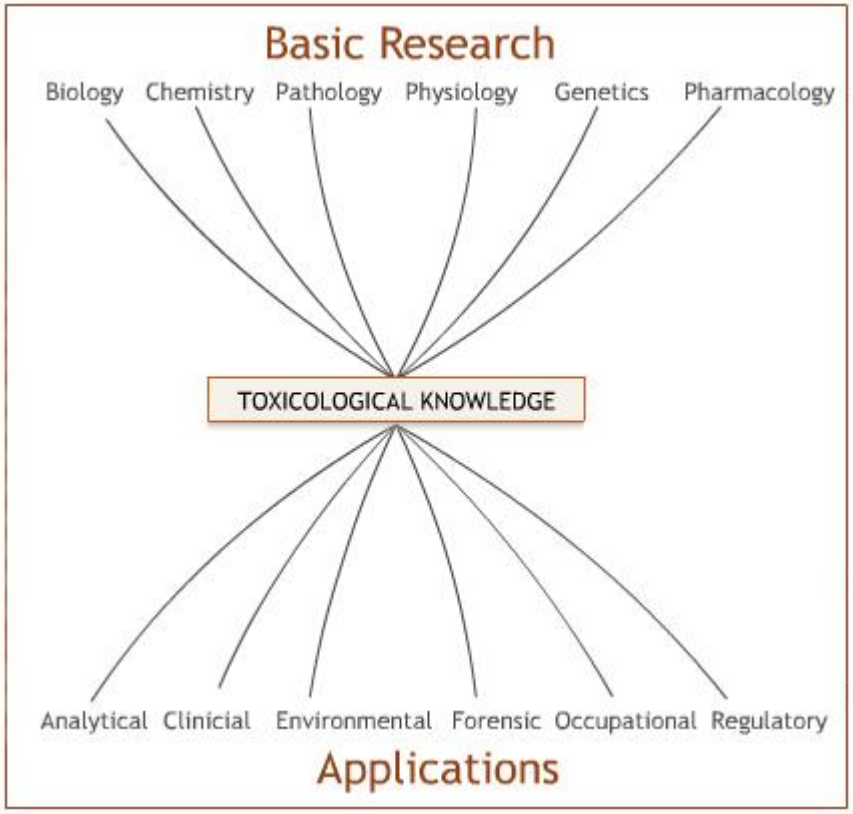


Fundamental principles of Risk Assessment apply to

Plant Protection Products, Biocides, Chemicals within REACh, Cosmetics, Pharmaceuticals, Environmental Contaminants…

What to do now?

You have now been introduced
to some of the basic principles of toxicology.



Some useful web sites

OECD: *http://www.oecd.org*

Test Guidelines Series on Testing and Assessment;

EFSA: *http://www.efsa.europa.eu/en/panels/pesticides.htm;*

EPA: *http://www.epa.gov/*

Echa: *http://echa.europa.eu/home*

European commission:
http://ec.europa.eu/sanco_pesticides/public/?event=homepage;

CircaBC:
https://circabc.europa.eu/faces/jsp/extension/wai/navigation/container.jsp;

ISS: *http://www.iss.it/*

JRC- EURL ECVAM: *http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam*

Thank you very much for Your Attention!!