UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACOLOGICHE

PERICOLO, RISCHIO, FATTORI CHIAVE NELLE VALUTAZIONE DI SICUREZZA DEI PRODOTTI COSMETICI.

CORRADO LODOVICO GALLI

CORSO TEORICO-PRATICO DI VALUTAZIONE DELLA SICUREZZA DEI COSMETICI

ALLA LUCE DEL REGOLAMENTO 1223/2009

LUNEDI 15 APRILE - VENERDI 19 APRILE 2013

CENTRO DIDATTICO UNIVERSITA DEGLI STUDI DI MILANO VIA CELORIA, 22 MILANO

INTEGRATED RISK CHARACTERIZATION

RISK CHARACTERIZATION

✓ Hazard identification

- ❖ Inherent biological activity,

✓ Hazard assessment

- ❖ Dose-response analysis
- ❖ Assessment of relevance for humans

HAZARD IDENTIFICATION

- ❖ Identification of adverse health effects
  - ❖ Animal-based toxicological studies
  - ❖ *In vitro* toxicology data
  - ❖ Structure-activity consideration
  - ❖ Human data

HAZARD ASSESSMENT

- ❖ Quantification of adverse health effects
  - ❖ Dose-response for critical effect
  - ❖ Selection of critical data
  - ❖ Mode/mechanism of action
  - ❖ Kinetic variability
  - ❖ Dynamic variability

EXPOSURE ASSESSMENT

- ❖ Active principle
  - ❖ Dose of toxicant
  - ❖ Dose in individuals
  - ❖ Dose in special population groups
  - ❖ Max/min, chronically/occasionally

RISK CHARACTERISATION

RISK CHARACTERIZATION

La caratterizzazione del rischio richiede:

- l'uso di un intervallo di dosi adeguati negli studi tossicologici in animali standardizzati;

- la produzione da parte delle dosi di effetti significativamente osservabili, date le dimensioni ridotte del campione utilizzato in studi su animali;

- progettazione dello studio tale da fornire un numero elevato di informazioni della regione a basse dosi della curva.

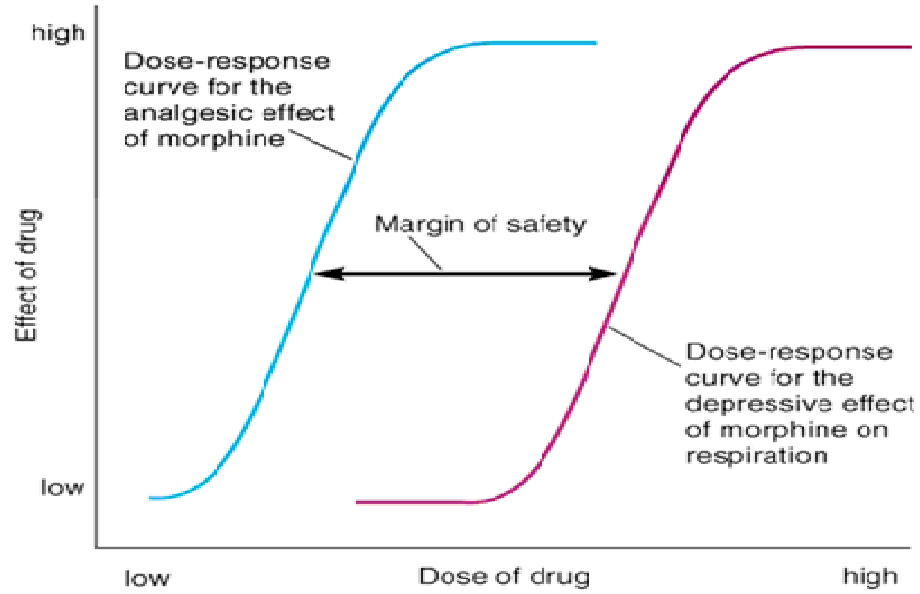
RISK CHARACTERIZATION

I punti di riferimento (RP) degli studi tossicologici sono poi utilizzati per calcolare un livello di sicurezza per l'assunzione umana:

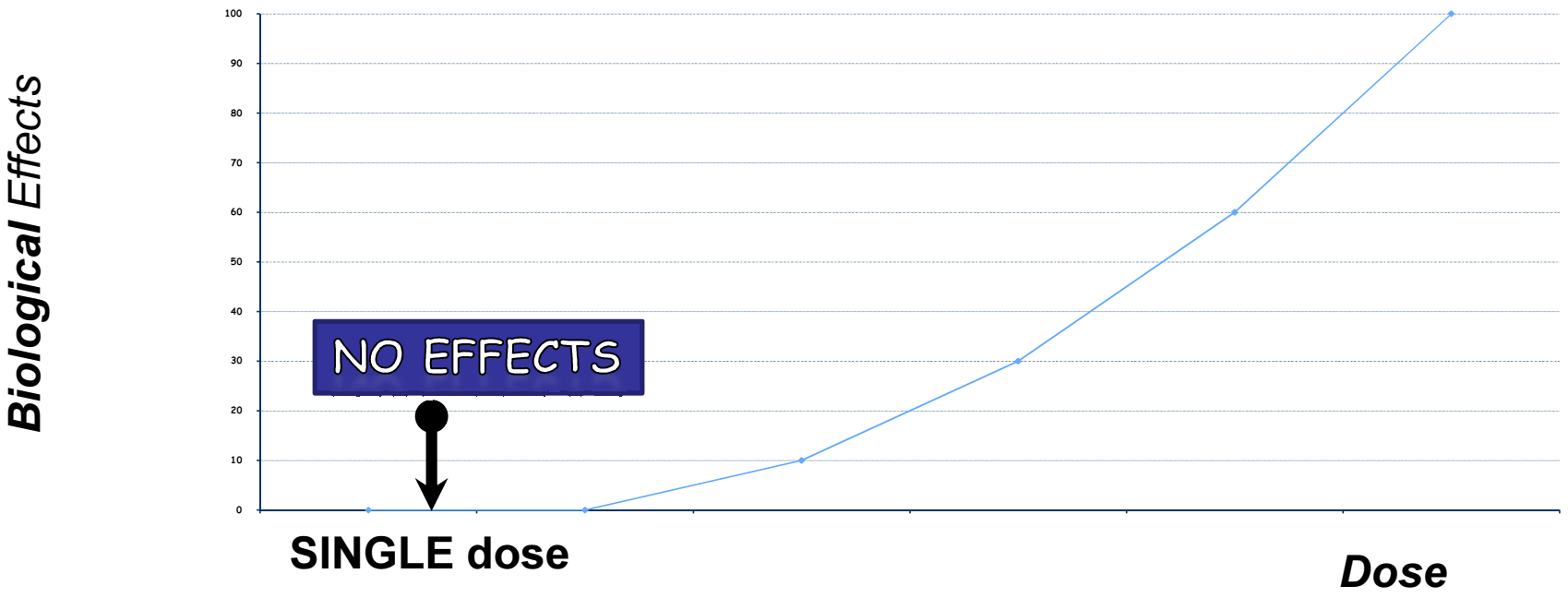
- *No-Observed-Adverse-Effect-Level* (NOAEL);
- *Benchmark Dose* (BMD).

EXPOSURE (DOSE)

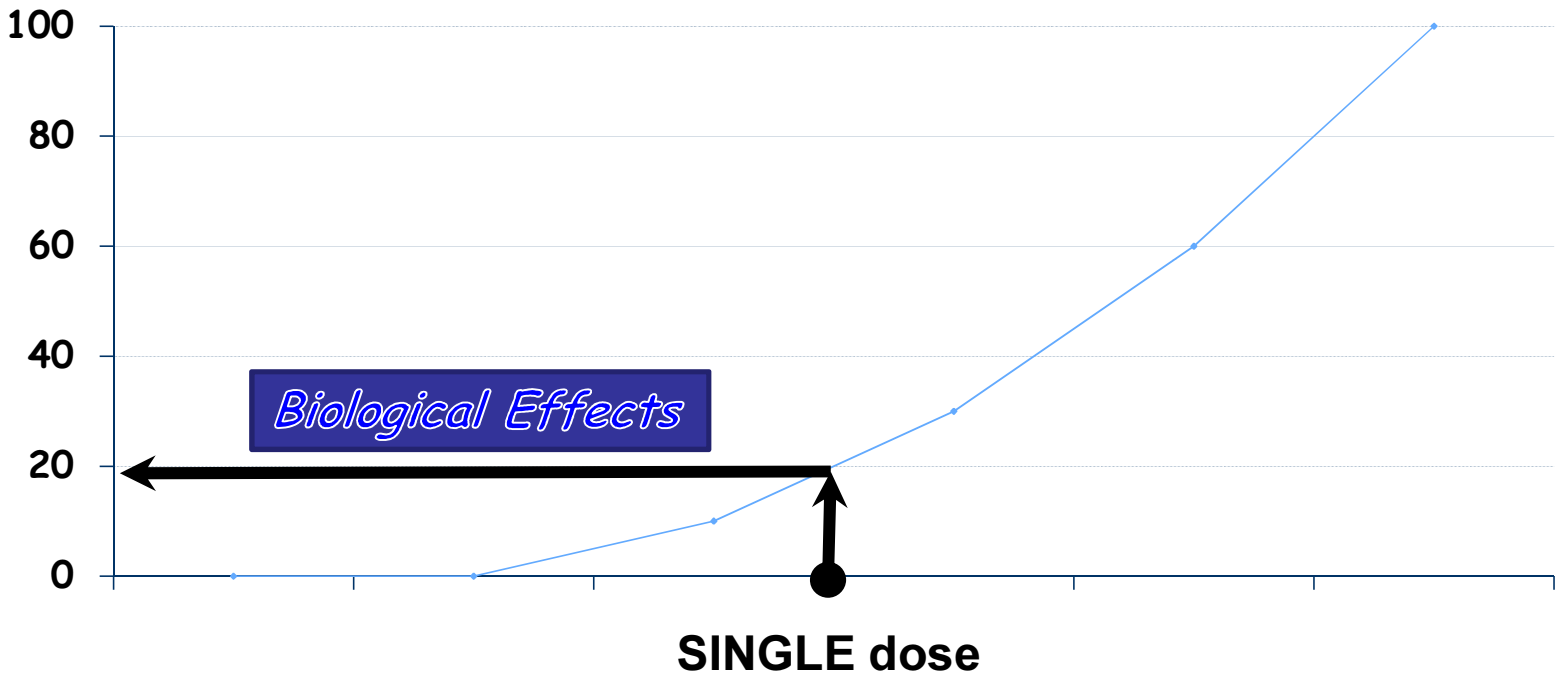
► **Dose-Response Curves for the Analgesic and Depressant Effects of Morphine**



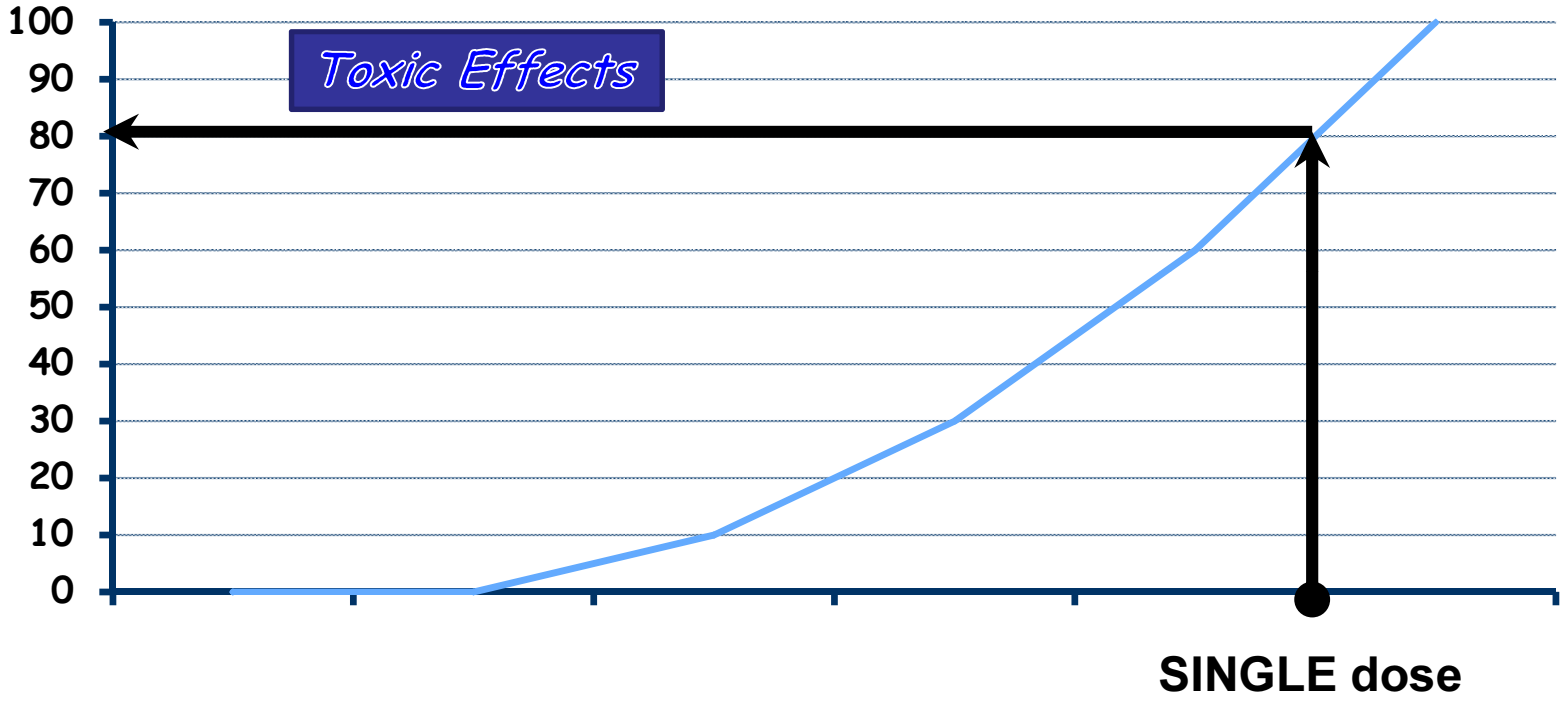
EXPOSURE (DOSE)



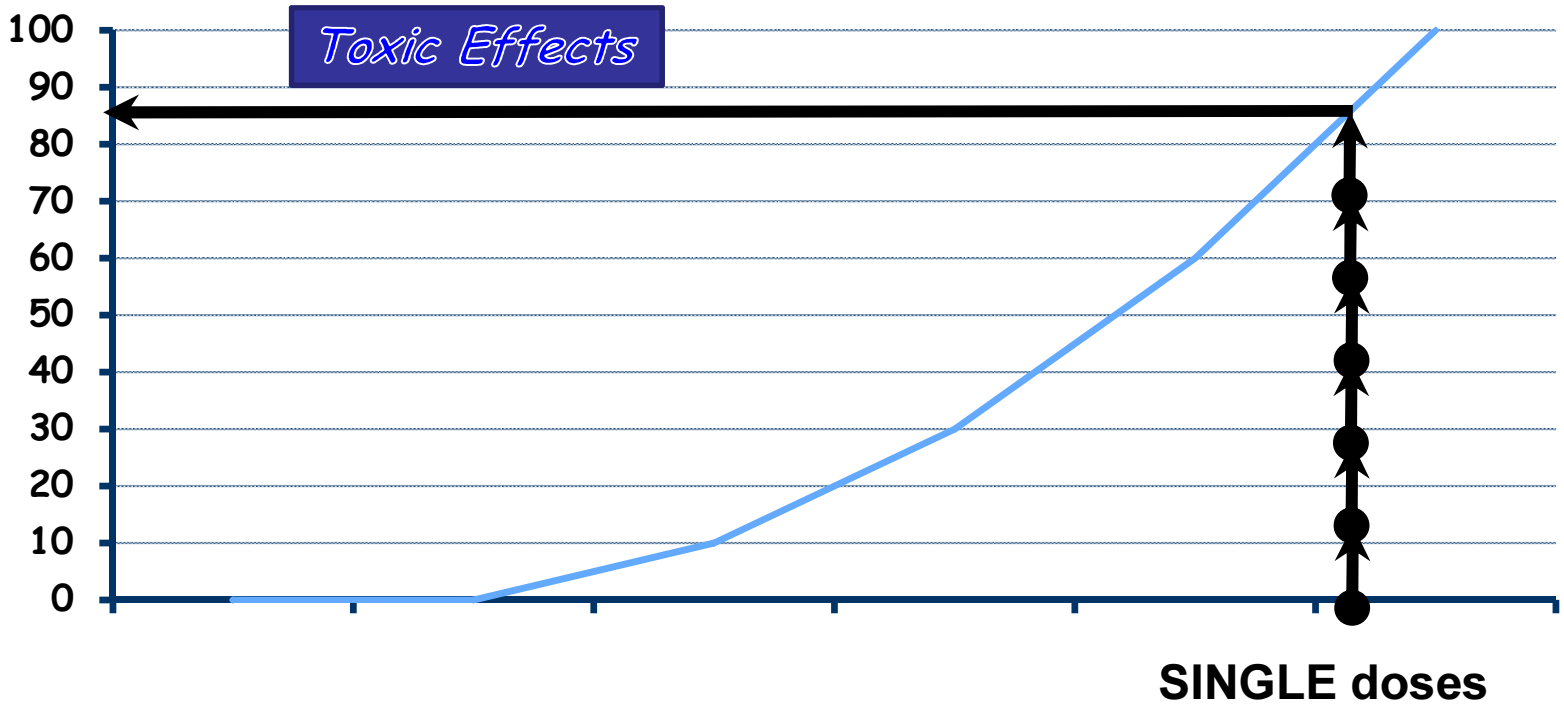
EXPOSURE (DOSE)



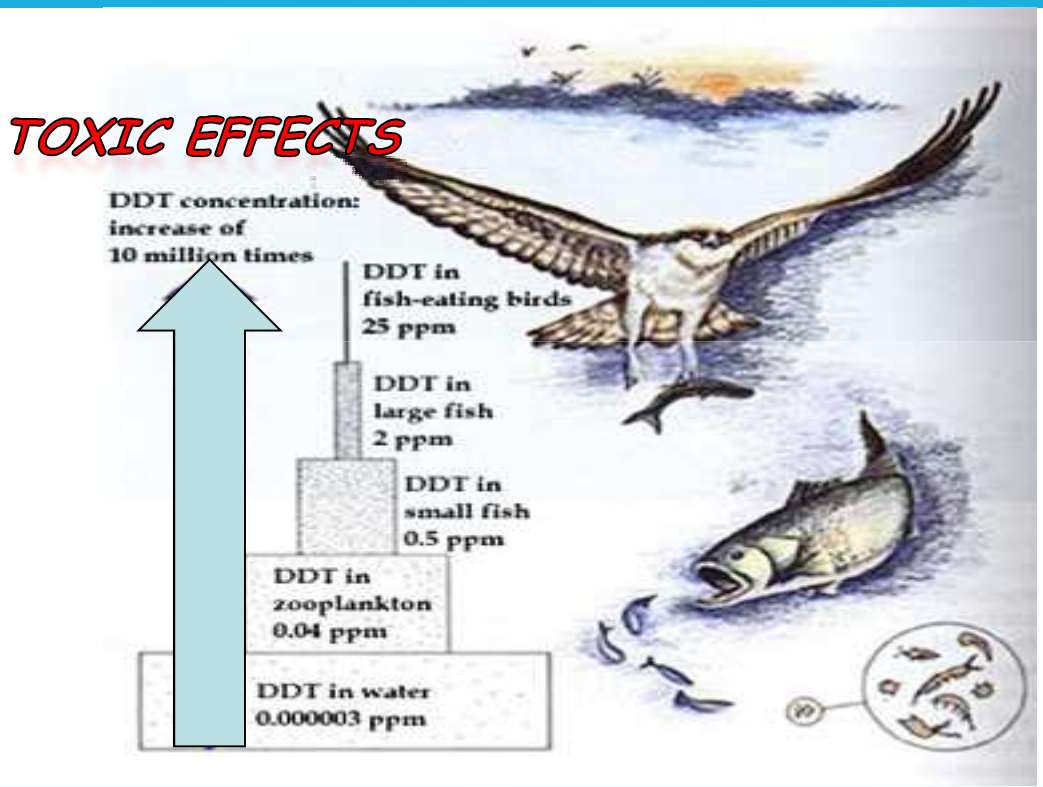
EXPOSURE (DOSE - ACUTE)



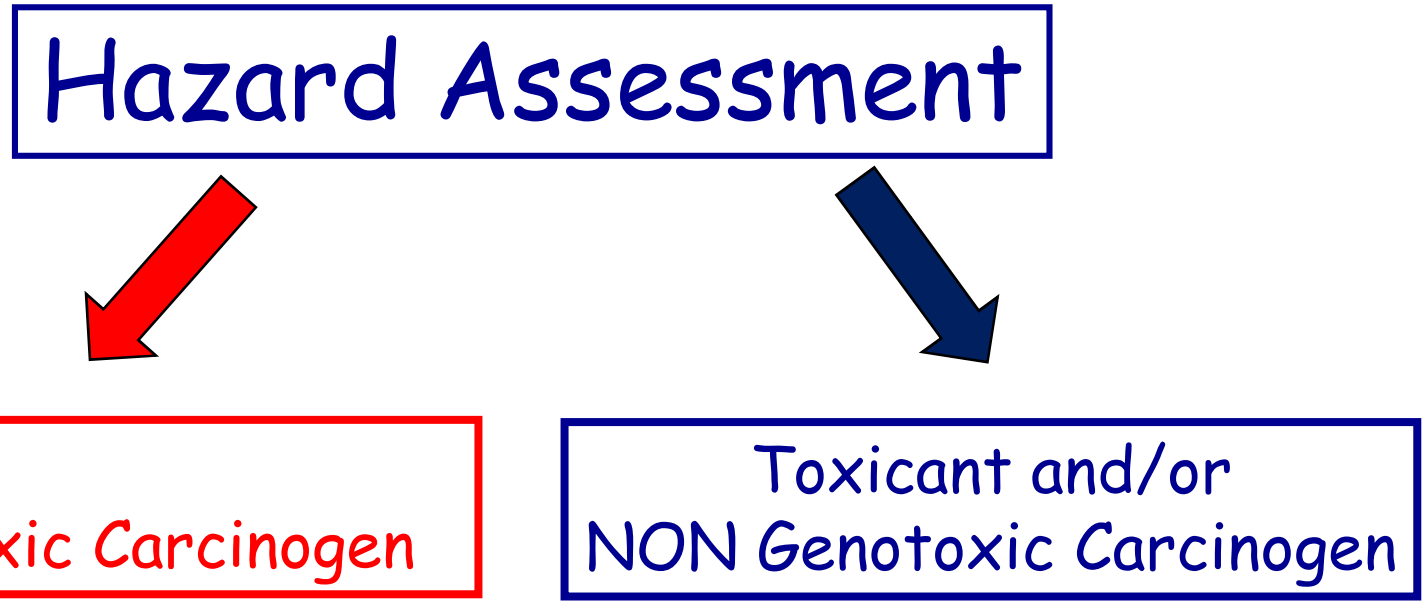
EXPOSURE (DOSE AND TIME - ADDITIVE)



EXPOSURE (DOSE - BIOACCUMULATION)



RISK CHARACTERIZATION



RISK CHARACTERIZATION

Hazard Characterization

Genotoxic Carcinogen

Toxicant and/or
NON Genotoxic Carcinogen

TOXICANT AND/OR NON GENOTOXIC CARCINOGEN
(HEALTH BASED GUIDANCE)

- ADI (Acceptable Daily Intake)
- UL (Tolerable Upper intake Level)
- T(M)DI (Tolerable Maximum Daily Intake)
- SED, XYZ ..... ecc. ecc

ADI - UL - T(M)DI

(HEALTH BASED GUIDANCE)

ADI represents the amount of a **food additive, a pesticide or a veterinary drug residue**, expressed on a body weight basis, that can be ingested daily **over a lifetime** without appreciable health risk.

UL is the maximum tolerable level of chronic daily intake of a **nutrient (vitamins, minerals)** judged to be unlikely to pose a risk of adverse effect to almost all individuals in the general population

T(M)DI represents permissible human daily exposure to those **contaminants**, expressed on a body weight basis, unavoidably associated with the consumption of nutritious foods.

HAZARD IDENTIFICATION
(HEALTH BASED GUIDANCE)

ALLOCATION

↓

ADI – ARfD -UL – T(M)DI– xxz…..

↓

TOXICOLOGICAL PROTOCOL

ANIMAL-BASED TOXICOLOGICAL STUDIES

TOXICOKINETIC

- Absorption
- Distribution
- Metabolism
- Excretion

ACUTE TOXICITY

- LD_{50} oral
- LD_{50} dermal
- LC_{50} inhalation
- Skin irritation
- Eye irritation
- Skin sensitization

GENOTOXICITY

- Mutagenesis
- Clastogenesis
- Aneuploidy

SHORT-TERM TOXICITY

- Mouse 90 day toxicity
- Rat 90 day toxicity
- Dog 90 day toxicity
- Dog 1 year toxicity

NOAEL

DEVELOPMENTAL TOXICITY

- Teratogenicity tests (Rat-Rabbit)

REPRODUCTIVE TOXICITY

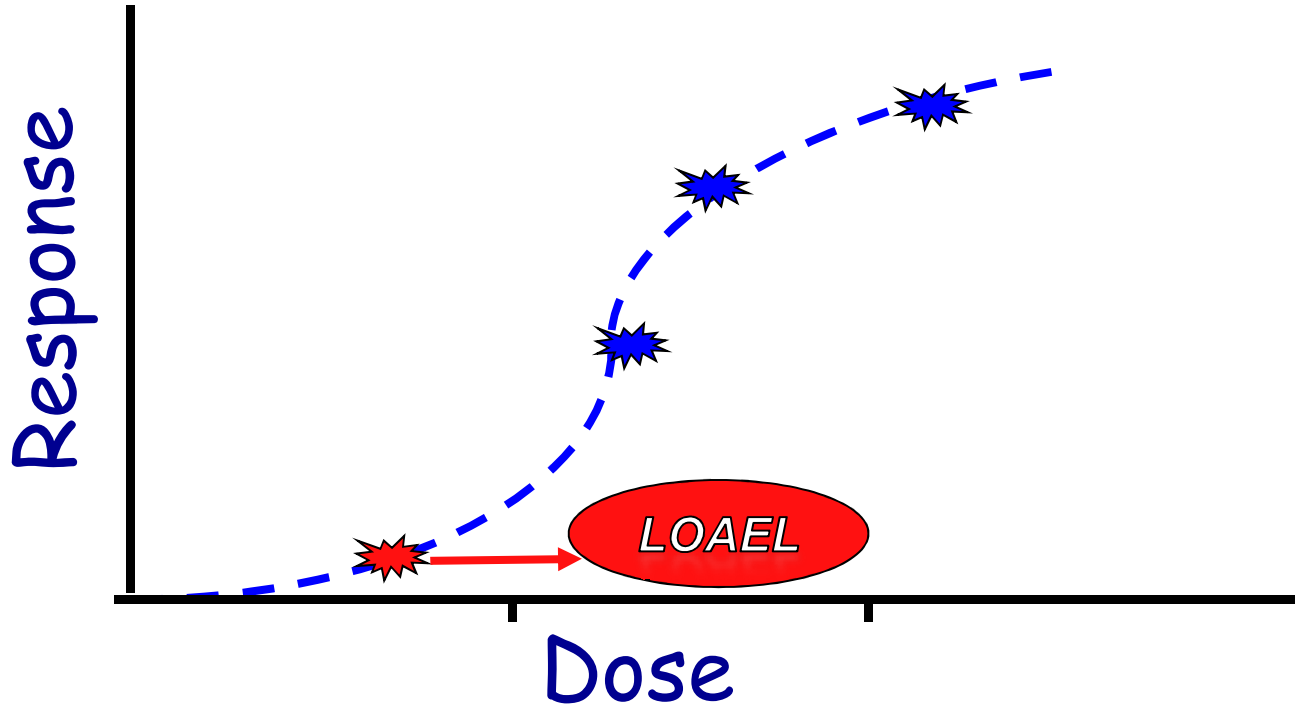
- Two generation reproductive toxicity

LONG-TERM TOXICITY AND/OR CARCINOGENICITY

- Mouse 18 months
- Rat 104 weeks

DOSE - RESPONSE FOR CRITICAL EFFECTS

(TO ESTABLISH A DOSE WITHOUT ADVERSE EFFECTS IN ANIMALS)



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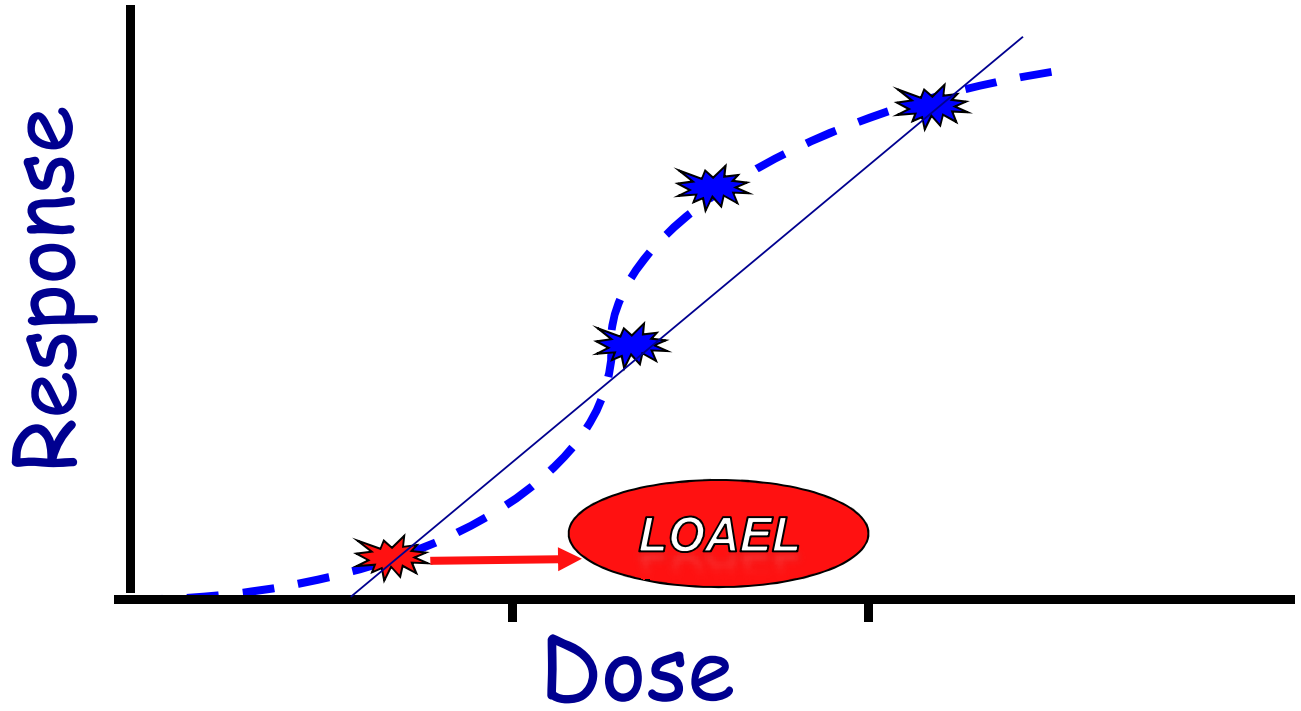
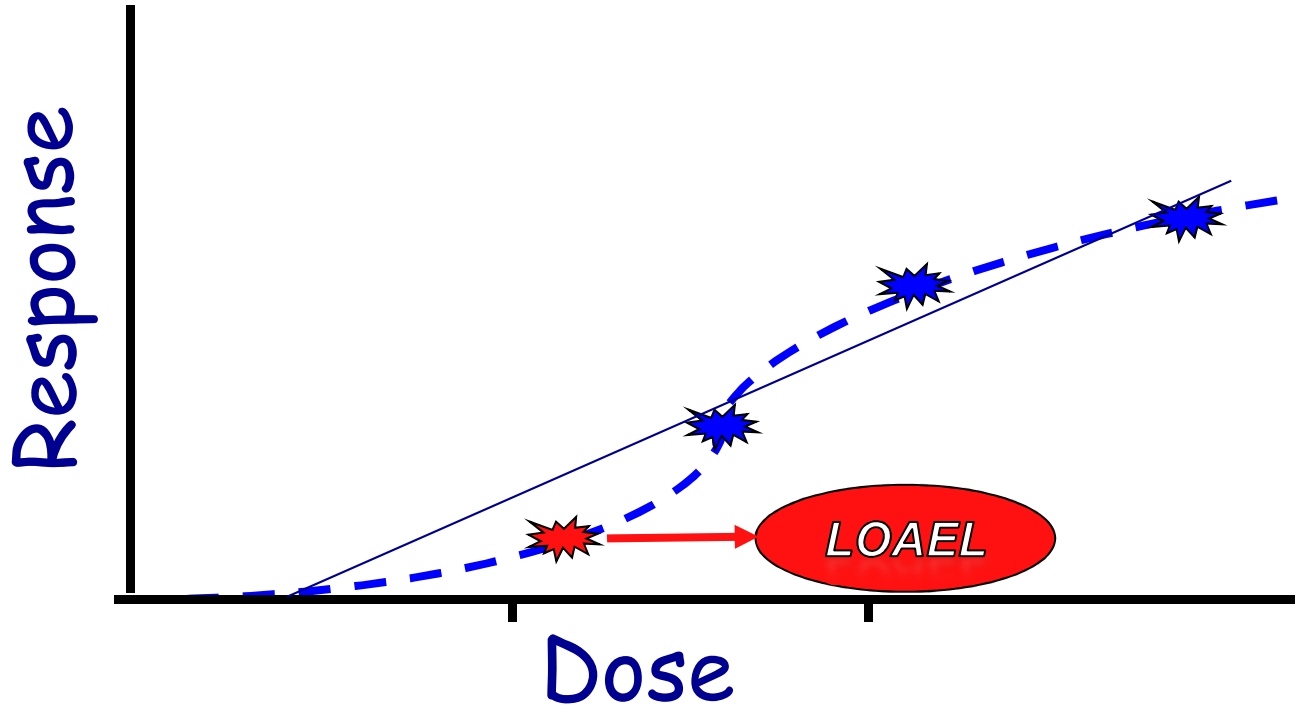


DOSE - RESPONSE FOR CRITICAL EFFECTS

(TO ESTABLISH A DOSE WITHOUT ADVERSE EFFECTS IN ANIMALS)



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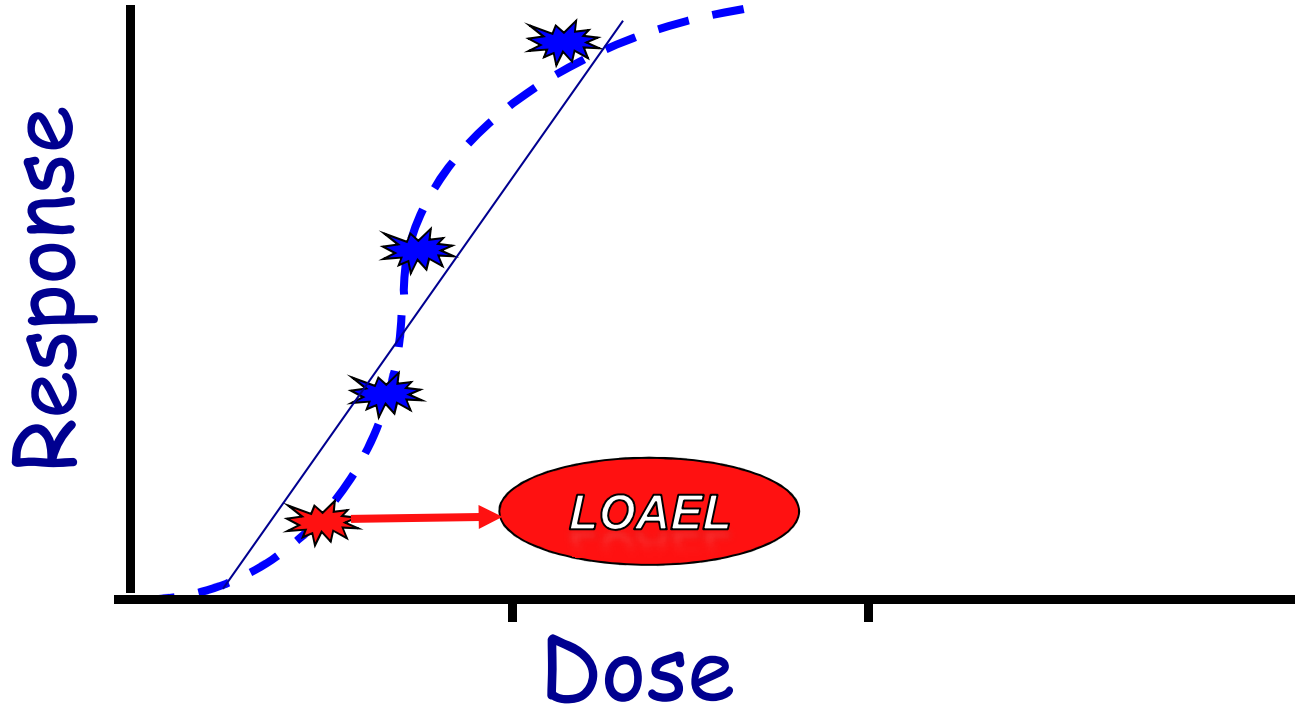

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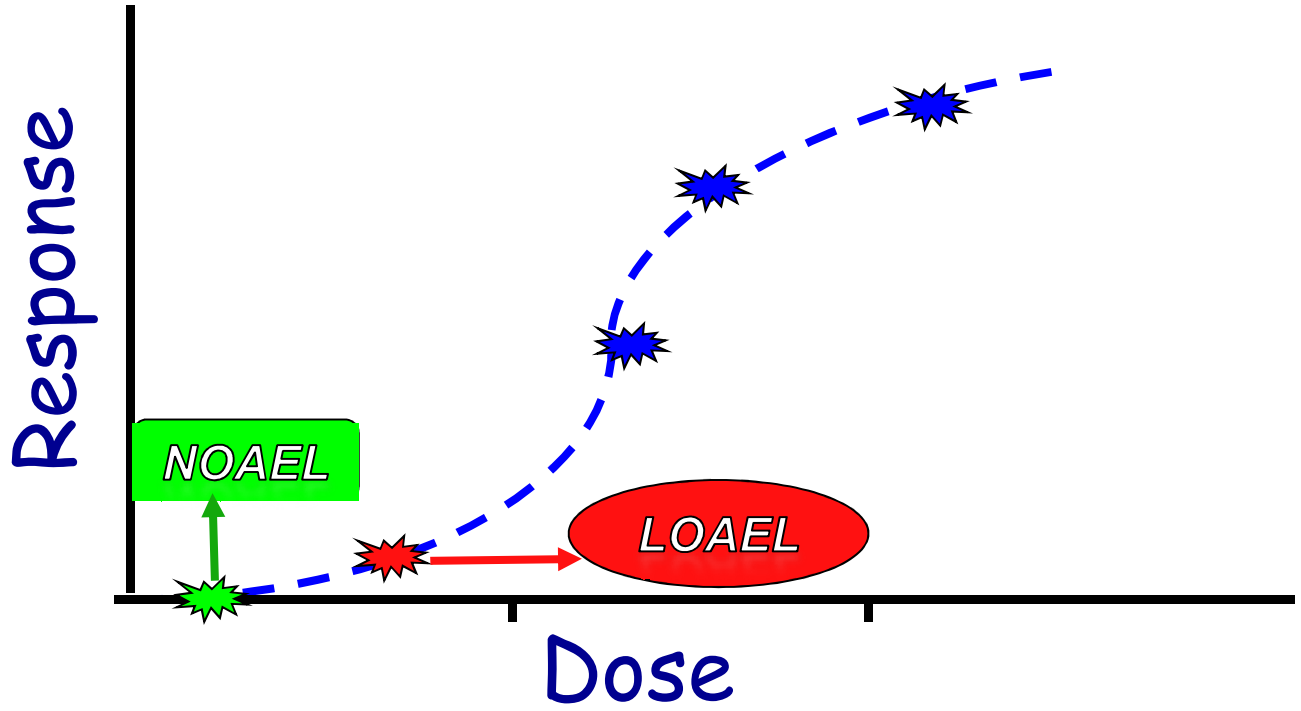
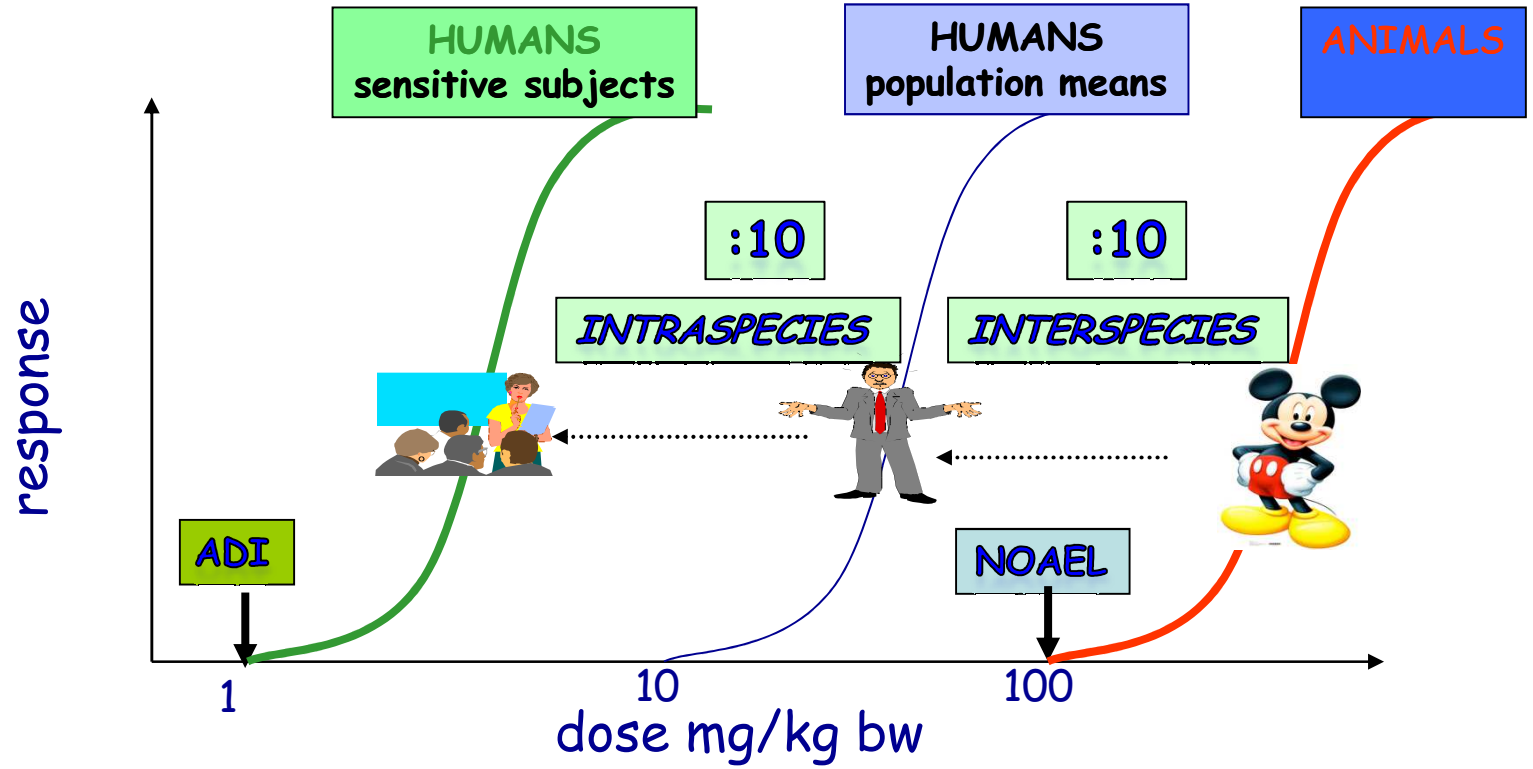


ANIMAL-BASED TOXICOLOGICAL STUDIES

(QUANTIFICATION OF ADVERSE HEALTH EFFECTS)



ADMISSIBILE DAILY INTAKE

$$ADI = \frac{NOAEL}{SF}$$

ADI = Admissible Daily Intake mg/kg b.w.

NOAEL = No Observed Adverse Effect Level (mg/kg b.w.)

SF = Safety Factor (10, 100, n)

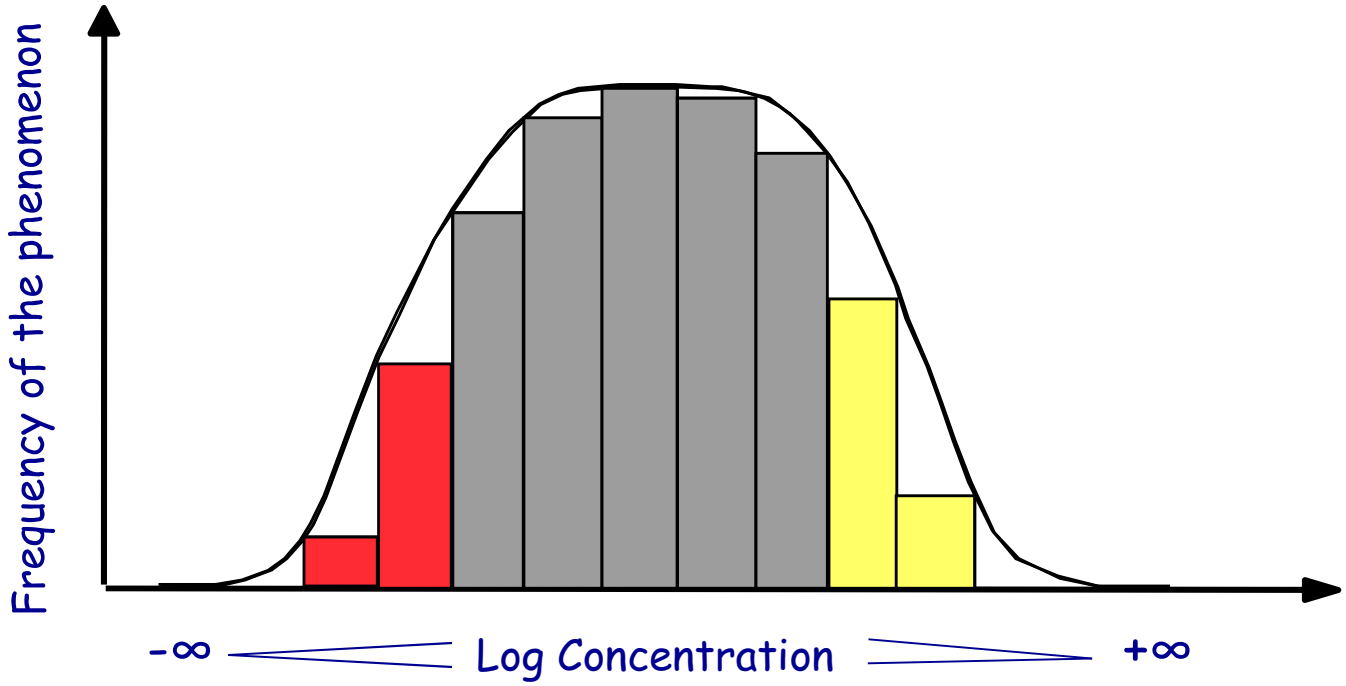
SAFETY FACTOR

Interspecies Differences 10

Interindividual Differences 10

INDIVIDUAL SUSCEPTIBILITY

(GENETIC POLYMORPHISM)



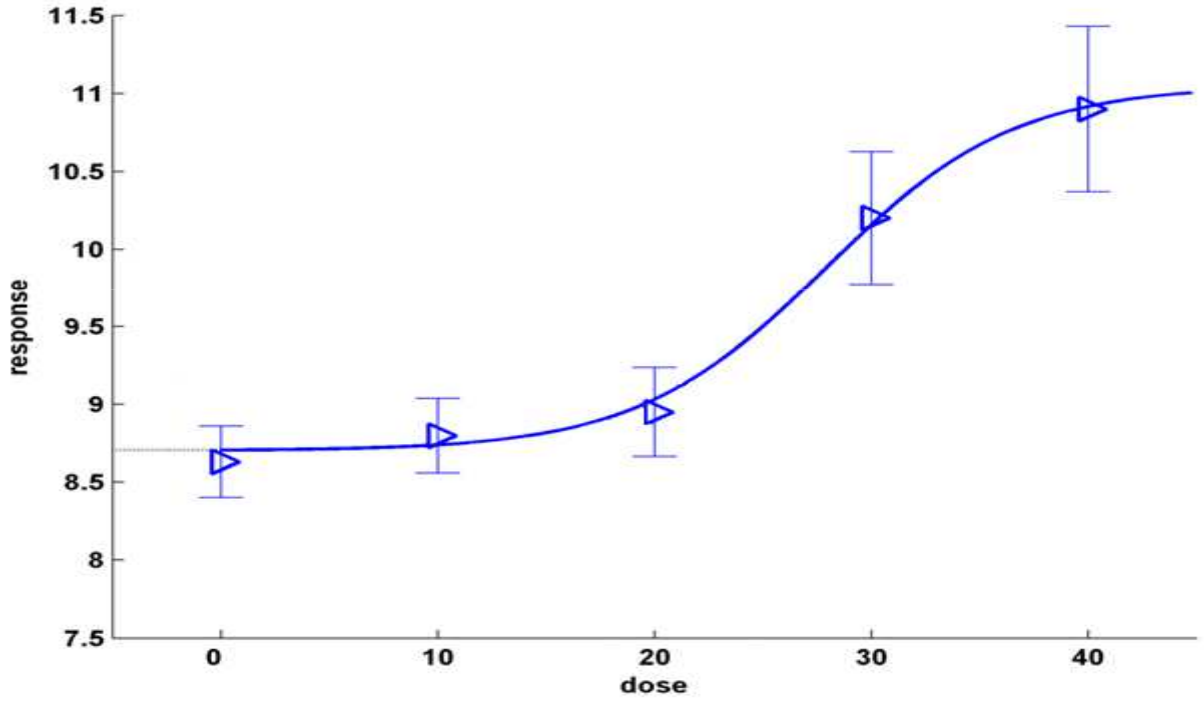
BENCHMARK DOSE - (BMD)

L'approccio BMD (*Benchmark Dose*) è applicabile ad ogni effetto biologico.

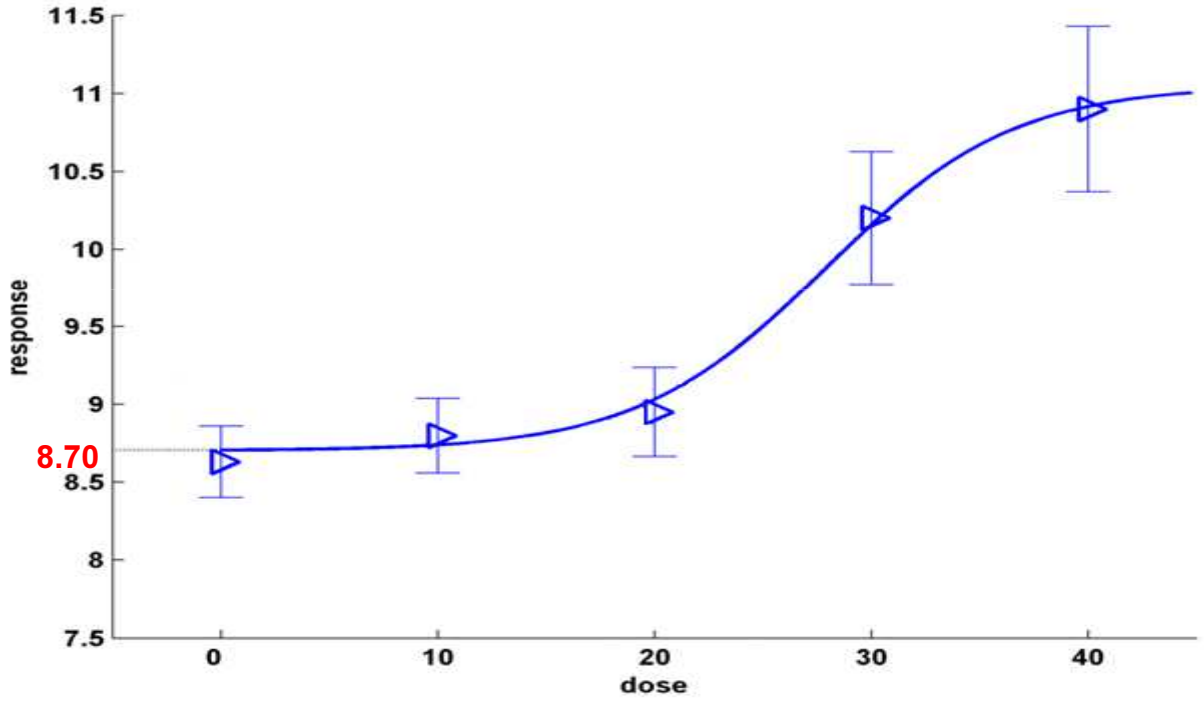
Vengono sfruttati tutti i dati disponibili per stimare la curva della relazione dose-risposta per un particolare bersaglio.

Il BMD è una dose derivata dalla curva dose-risposta stimata, associata ad uno specifico cambiamento nella risposta, il Benchmark Response (BMR).
Es. BMR=5% cambiamento del 5% della risposta relativa al background.

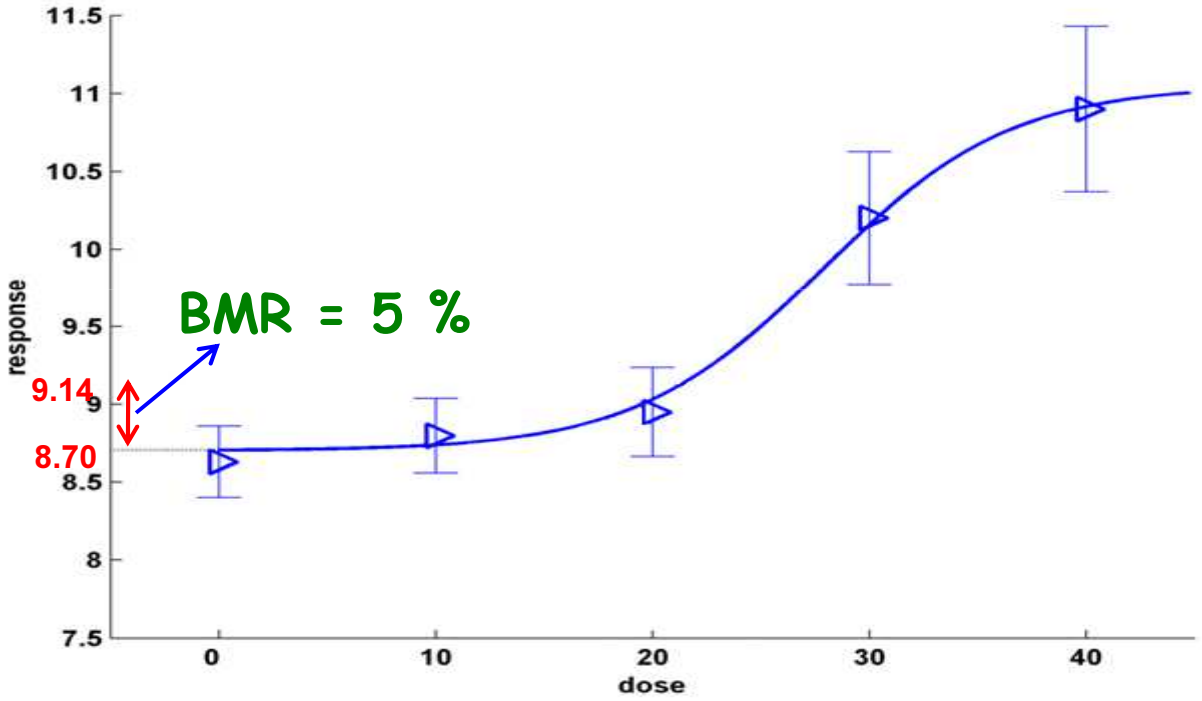
BENCHMARK DOSE - (BMD)



BENCHMARK DOSE - (BMD)



BENCHMARK DOSE - (BMD)



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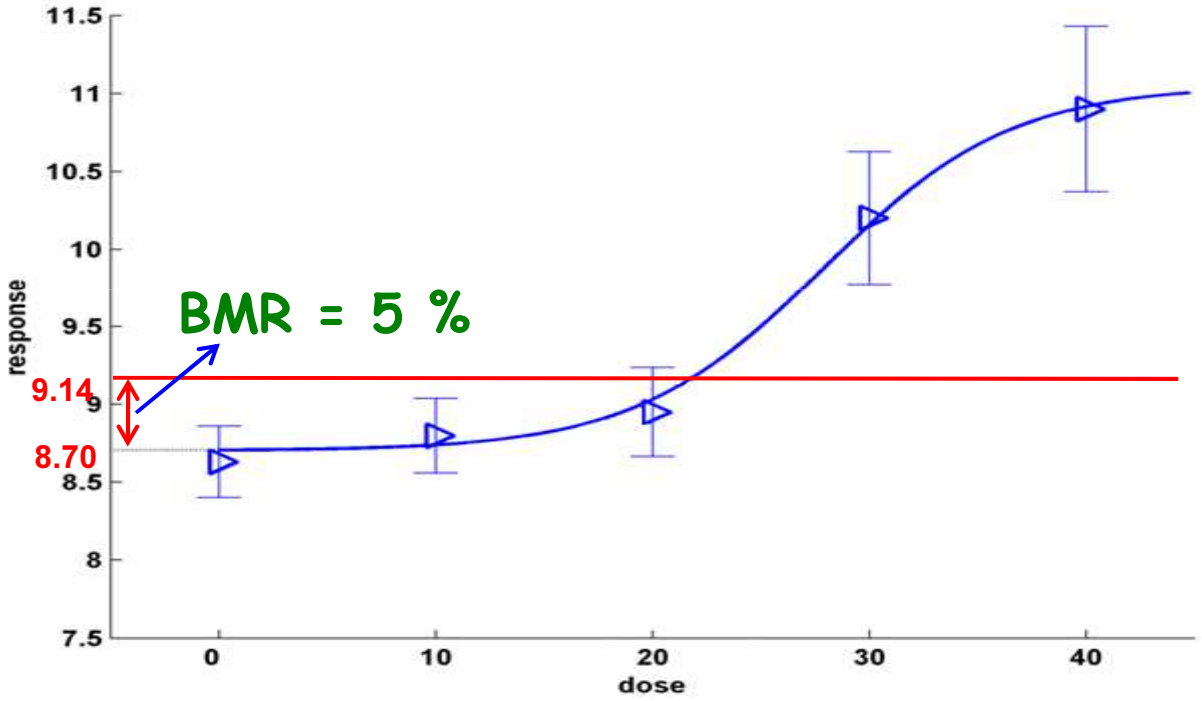

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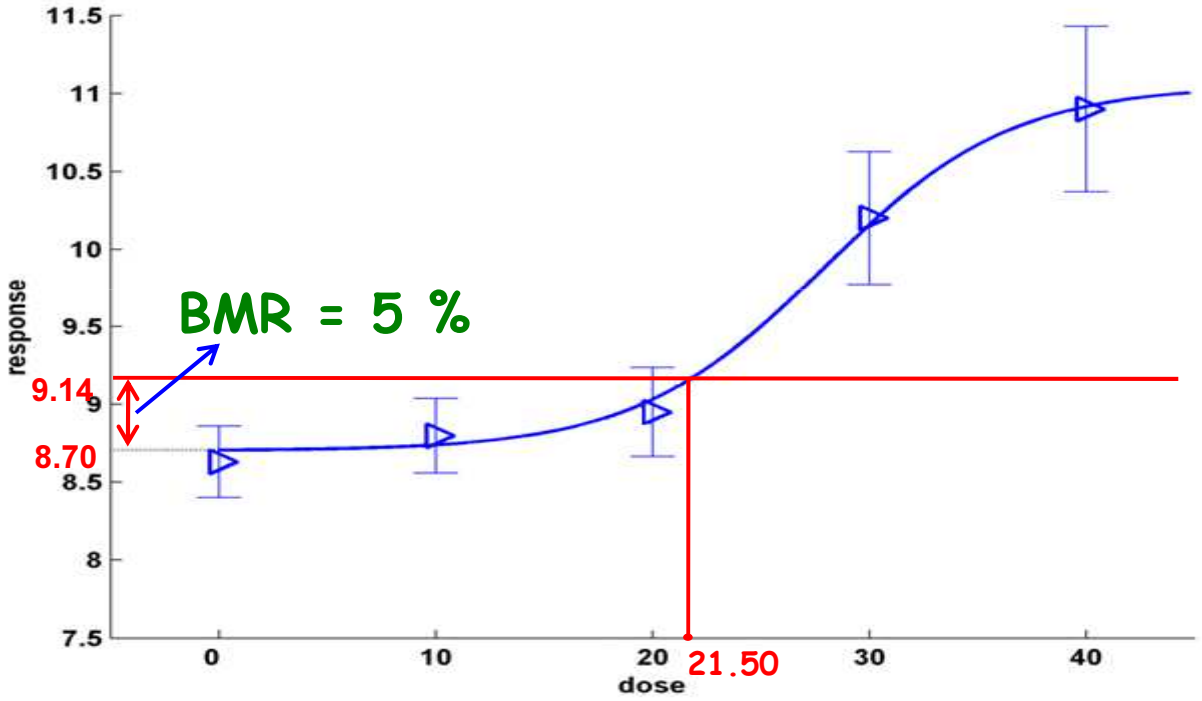
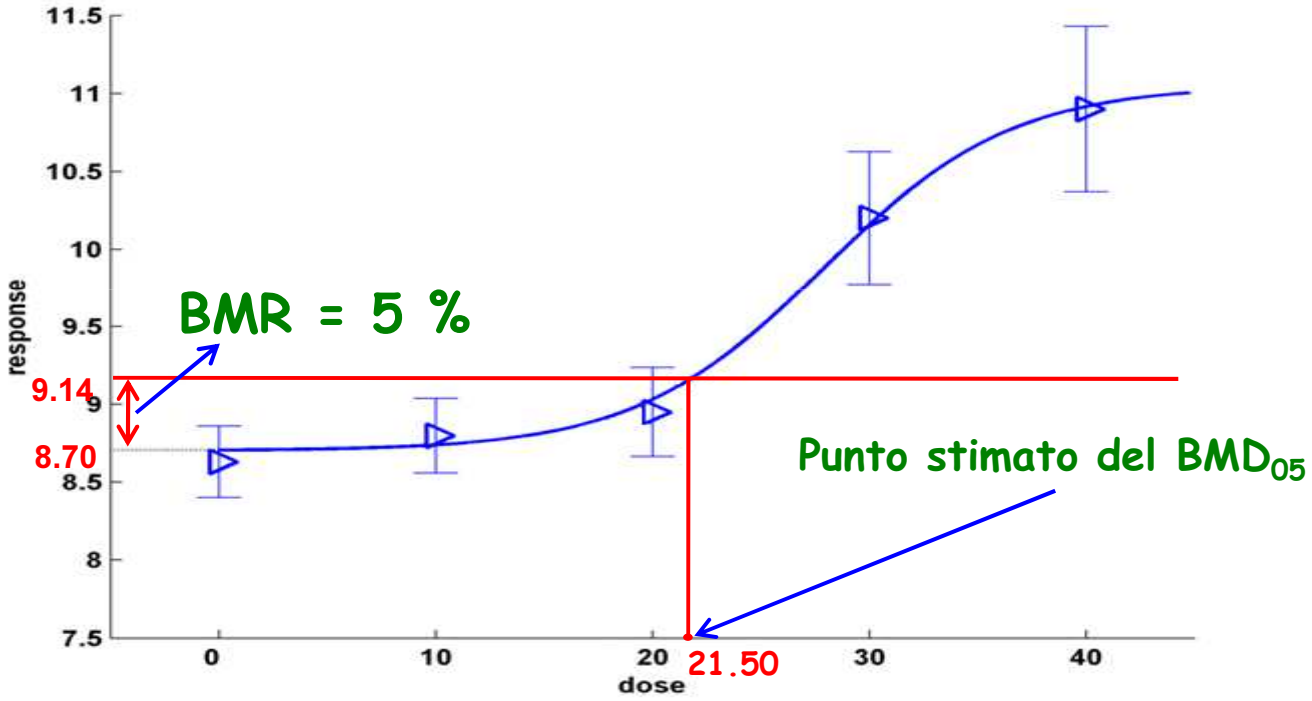


BENCHMARK DOSE - (BMD)



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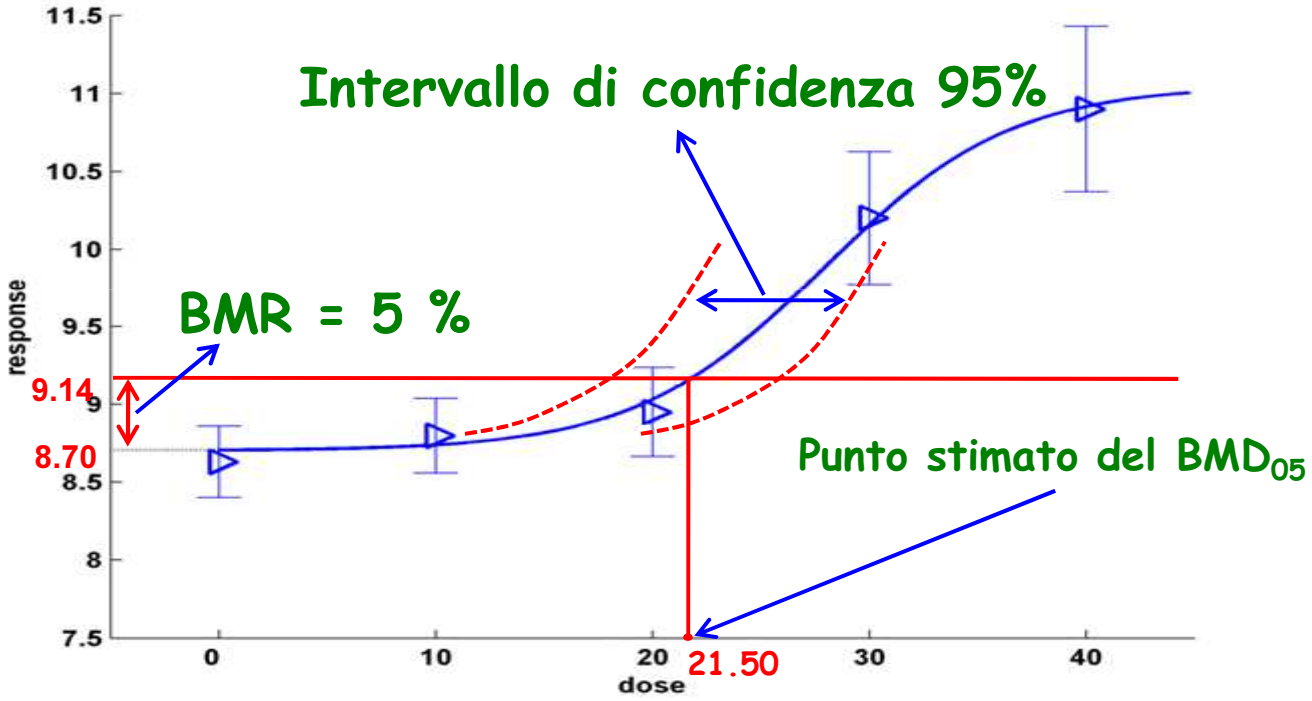

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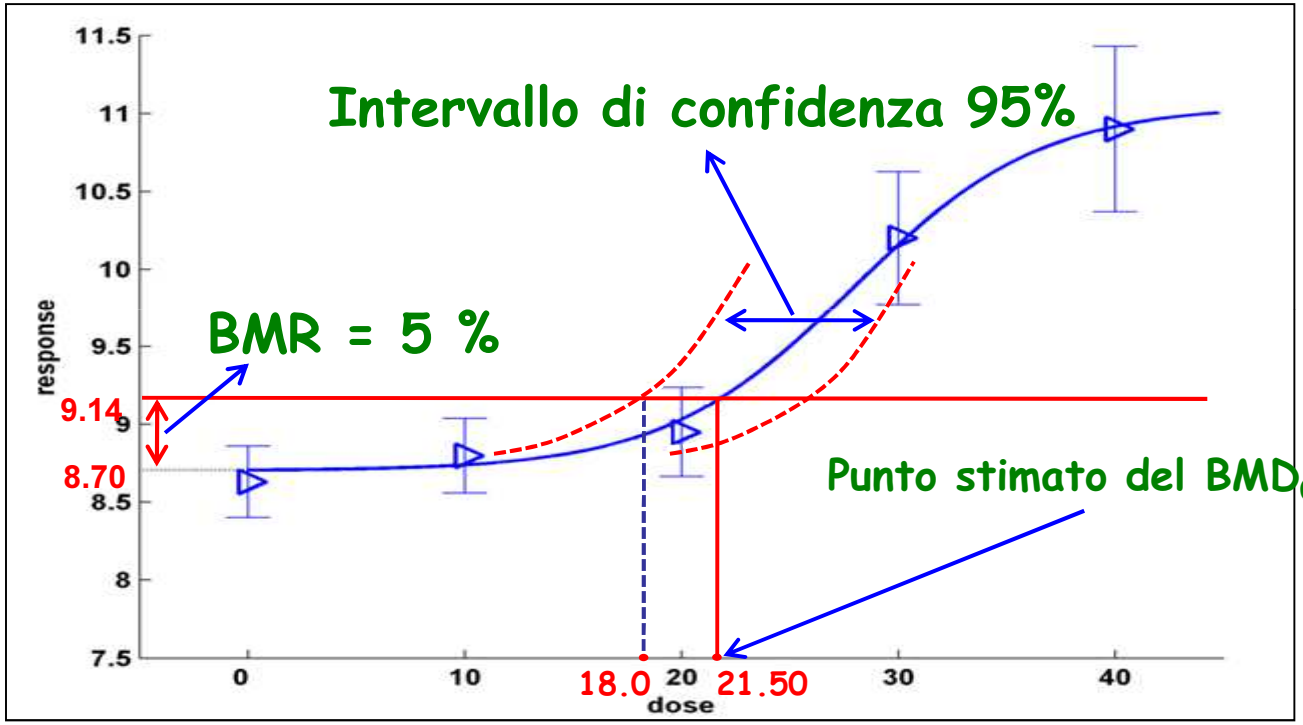
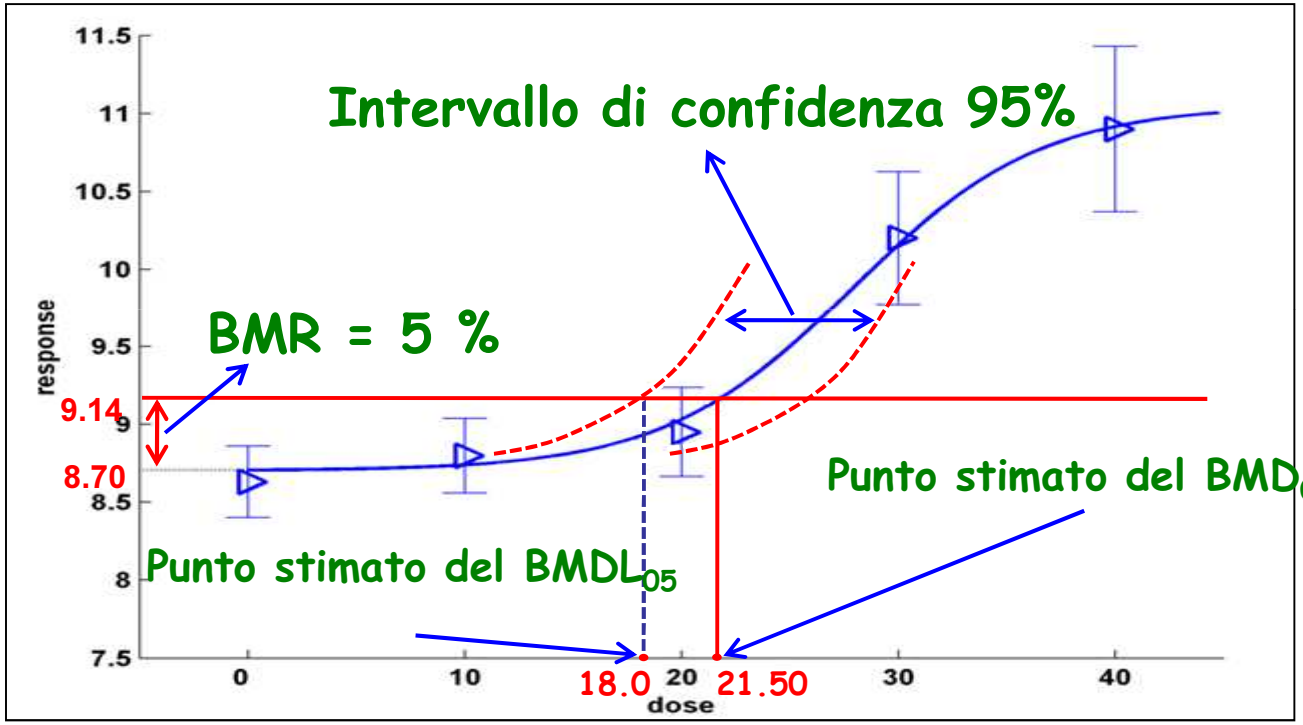
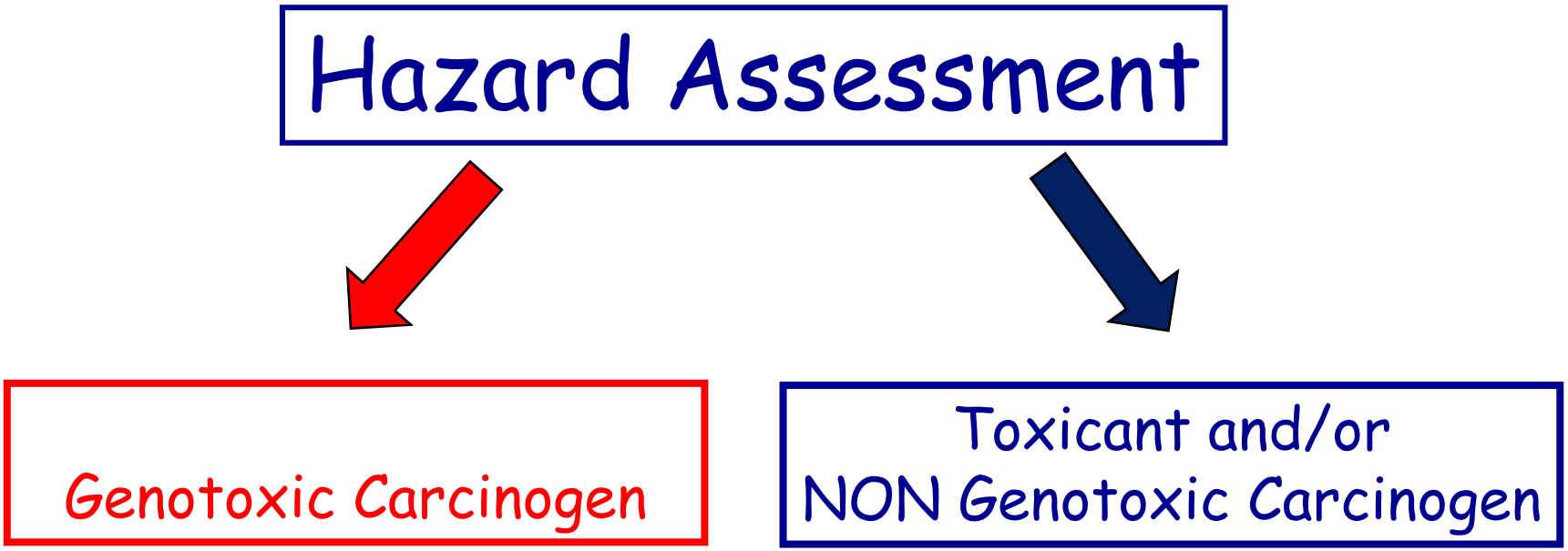


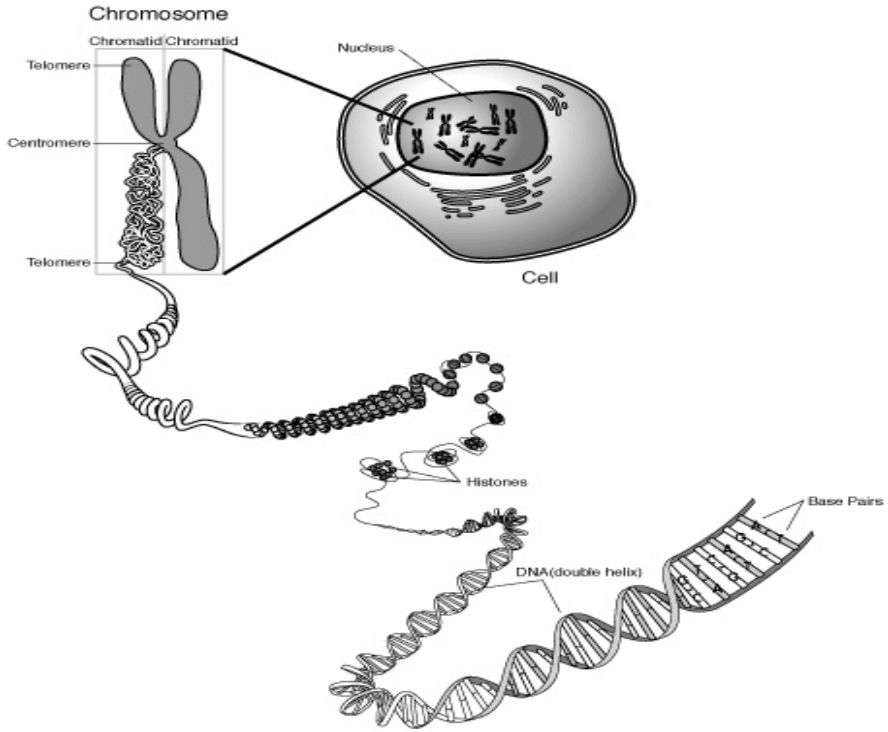
BENCHMARK DOSE - (BMD)



RISK CHARACTERIZATION

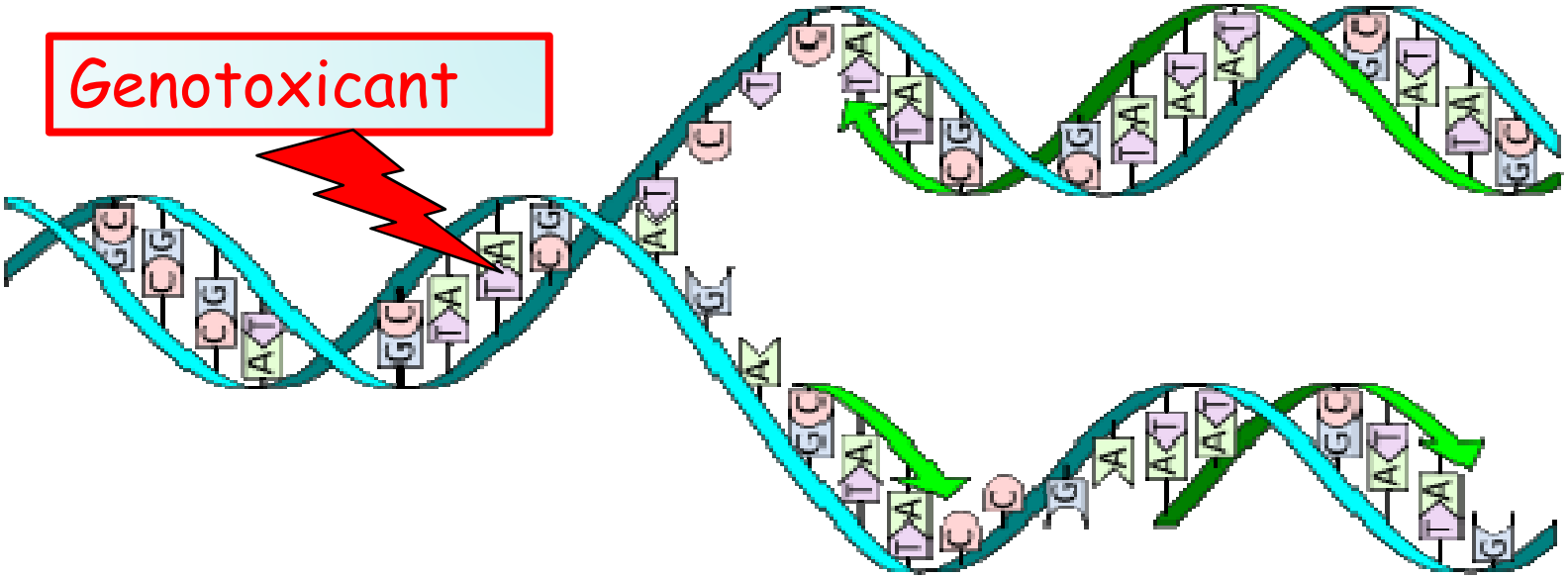


DEOXYRIBONUCLEIC ACID (DNA)

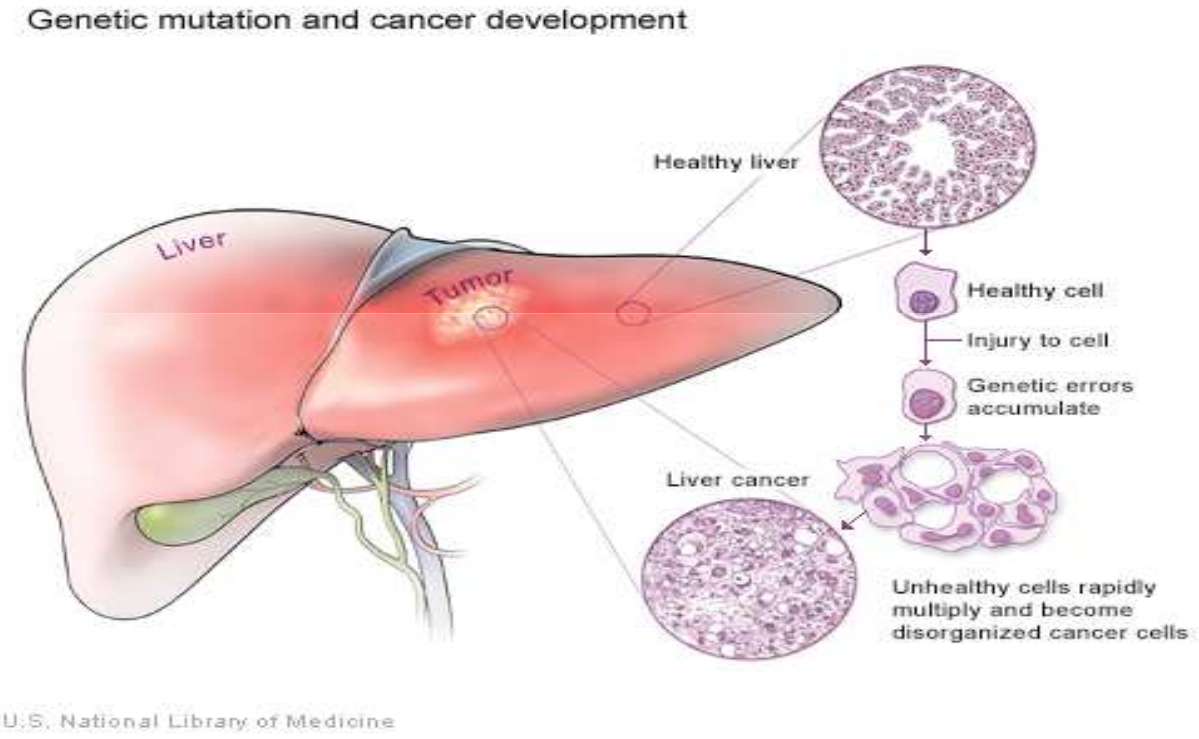


http://www.accessexcellence.org/AB/GG/chromosome.html

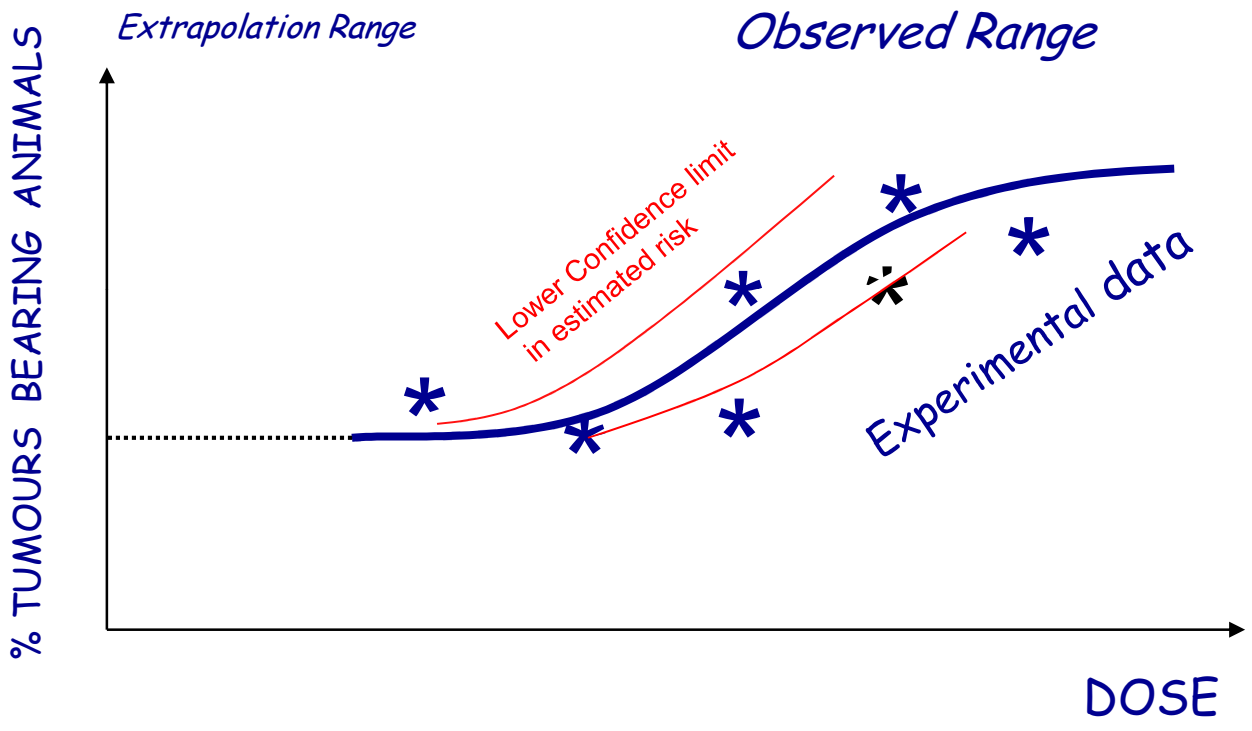
GENOTOXIC CARCINOGENS



GENOTOXIC CARCINOGENS



RISK ASSESSMENT



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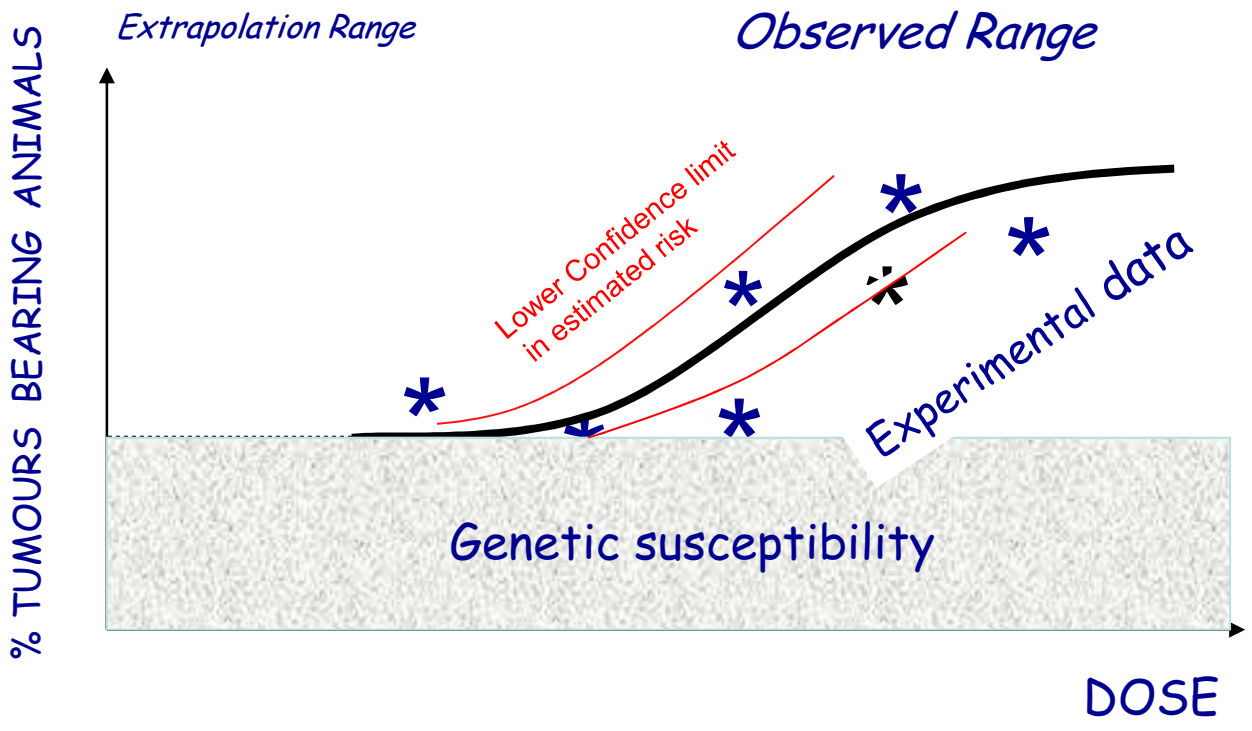
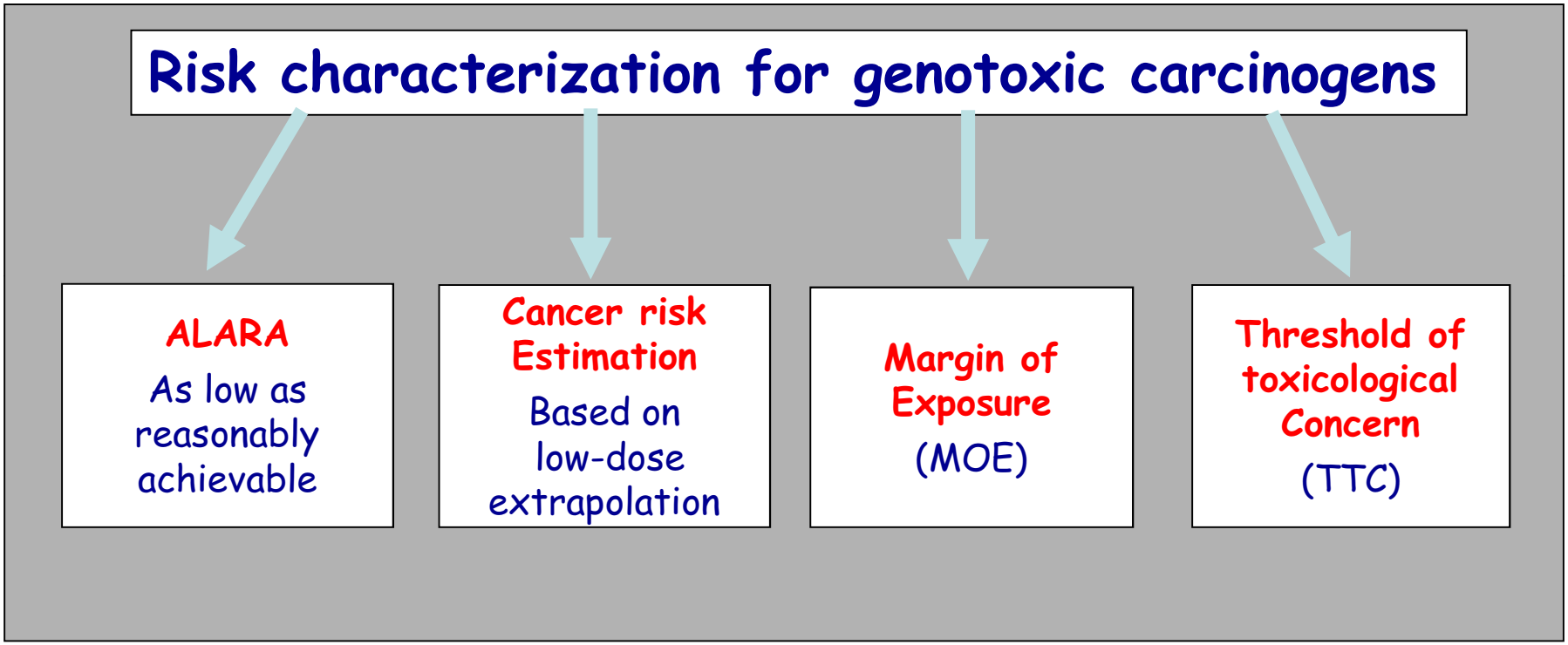


RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS



ALARA

- Based solely on **hazard identification**

- Does not take into account **human exposure**

- Does not take into account **potency**

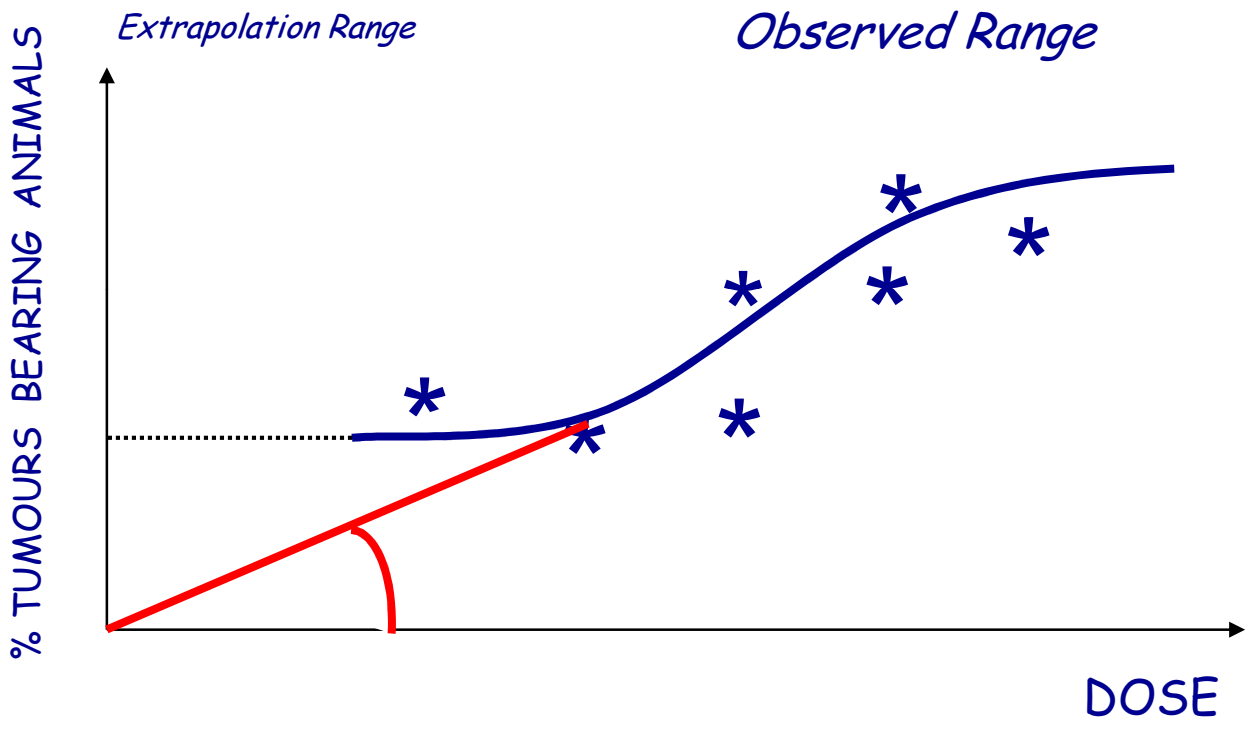
LOW-DOSE EXTRAPOLATION

Risk characterization for genotoxic carcinogens

Cancer risk Estimation

Based on low-dose extrapolation

RISK ASSESSMENT

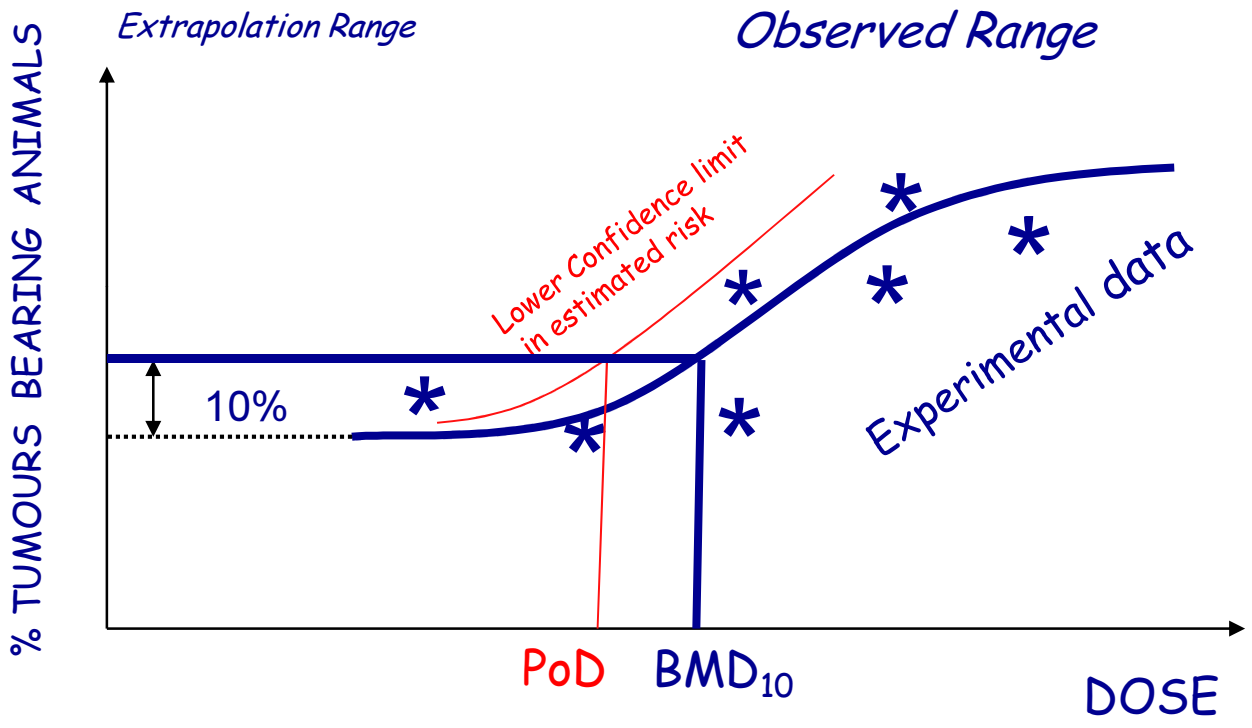


RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS

Risk characterization for genotoxic carcinogens

Margin of Exposure
(MOE)

RISK ASSESSMENT



MARGIN OF EXPOSURE (MOE)

- MoE = PoD / EXPOSURE

POD = 25 mg/kg b.w.
EXPOSURE = 0.0005 mg/kg/day

- MoE = 25 / 0.0005 = *50,000*

MARGIN OF EXPOSURE

- Species differences and human variability in the basic process of toxicokinetics and toxicodynamics are inherent in the use of data from studies in animals for human risk assessment.

- A factor of **100** fold is usually used to allow for these uncertainties in the risk assessment of non-genotoxic substances.

MARGIN OF EXPOSURE

There are additional uncertainties specifically for substances that are both genotoxic and carcinogenic:

- inter-individual human variability
- cell cycle control
- DNA repair, which influence the carcinogenic process.

x 10

MARGIN OF EXPOSURE

- The reference point is not equivalent to a NOAEL and effects can occur at lower doses.
- The dose effect relationship below the reference point, and the dose level below which cancer incidence is not increased are unknown, representing additional uncertainties.

x 10

RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS

Risk characterization for genotoxic carcinogens

Threshold of toxicological Concern

(TTC)

THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) IN RISK CHARACTERISATION

Could not be that the data requirements for risk assessment would be in relationship to human intake or exposure ?

Is there a level of exposure so low

that "risk assessment" could be based on

structural considerations alone

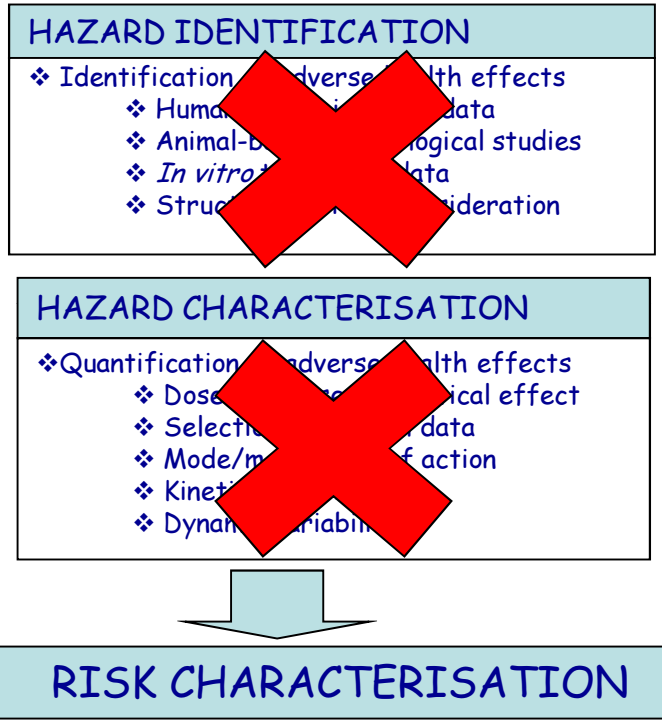
and toxicological specific data are not required ?

BENCHMARK WITH THE RISK CHARACTERIZATION PARADIGM

- **Hazard identification**
  - ❖ Inherent biological activity,
- **Hazard characterisation**
  - ❖ Dose-response analysis
  - ❖ Assessment of relevance for humans

EXPOSURE ASSESSMENT

- ❖ Levels of substance in food and diet
- ❖ Amounts of food consumed
- ❖ Intake in special population groups
- ❖ Intake in individuals
  - ❖ Max/min, regularly/occasionally



THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) IN RISK CHARACTERISATION

The threshold of toxicological concern (TTC)

is a pragmatic risk assessment tool that is based on the principle of:

establishing a human exposure threshold value for all chemicals

1.5 µg/person/day

below which there is a very low probability of an appreciable risk to human health.

TTC APPLICATIONS

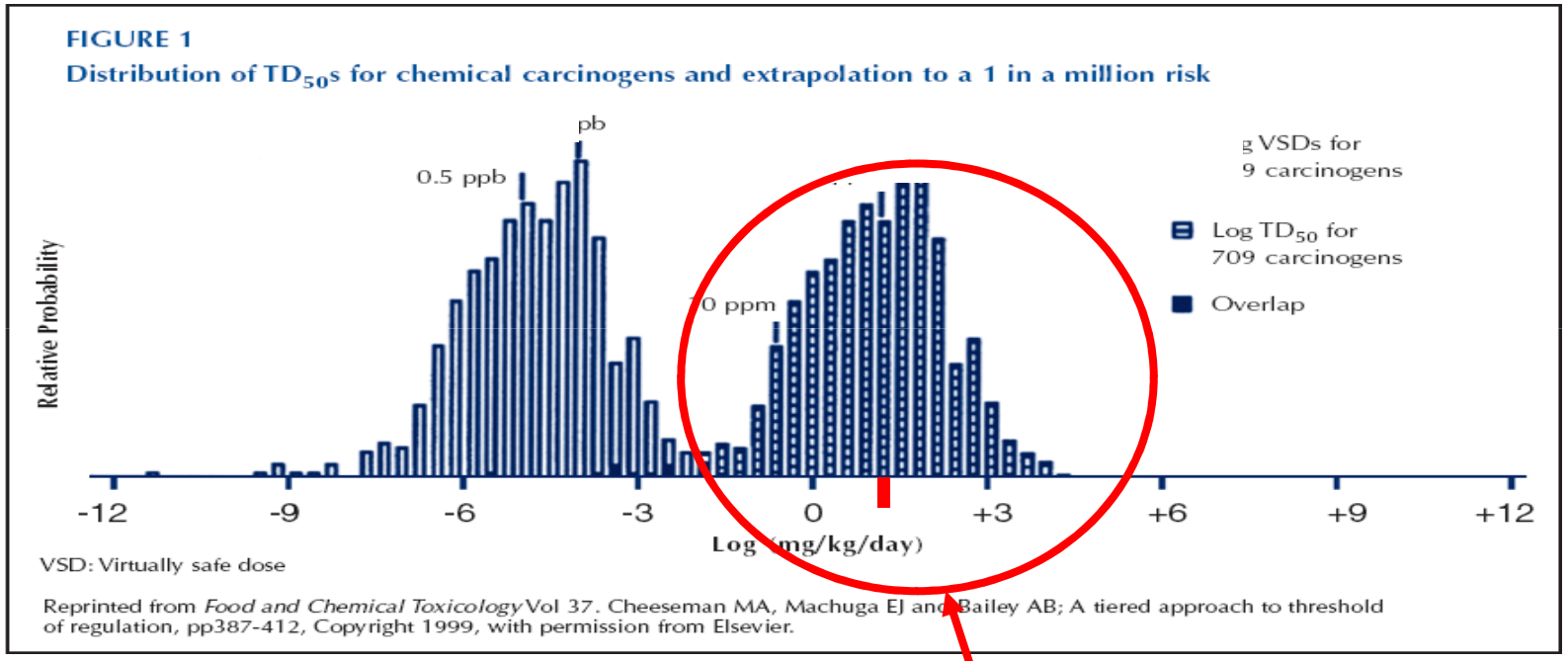
- **Migrant** substances from packaging materials (USFDA-TOR- 1993)
- **Flavourings** substances in food (WHO-JECFA 1993,1995,1999….)
- Endorsed for the risk assessment of chemicals (WHO-IPCS 1998)
- Non relevant **plant protection product metabolites** in ground water (EC-2002)
- **Genotoxic impurities** in pharmaceutical preparations (EMA 2003,2004)
- **Flavourings** substances in food (EFSA 2004)
- **Genotoxic constituents** in herbal preparations (EMA 2006)
- Suggested for **REACH** (Registr, Evaluat, Authoriz and restrict of **Ch**emical substances) (ECHA 2008)

- Suggested for application to **aquatic environmental** exposure (2005)
- Suggested for application to the **cosmetic ingredients** and their impurities (2007)
- Suggested for **prenatal developmental** toxicity (2010)
- Suggested for **mixture of substances** potentially detectable in surface water (2011)
- Suggested for risk prioritization of **trace chemicals in food**. *(2011)*

THRESHOLD OF REGULATION (TOR)
APPROACH FOR FOOD CONTACT MATERIALS

- The Threshold of Regulation(TOR) value was based on a carcinogenicity database (FDA 1995)
- Analysis of carcinogenic potencies of 343 (updated to 709) substances from 3500 experiments of the Carcinogenic Potency Database (CPDB) - Gold *et al.* (1984, 1989,1995) (Cheeseman *et al.*, 1999);
- In the CPDB the potency of each chemical was expressed in terms of the dose producing 50% tumour incidence in test animals (**TD50's**) at the end of their lifespan (corrected for background tumours in controls) in the most sensitive species and sex.

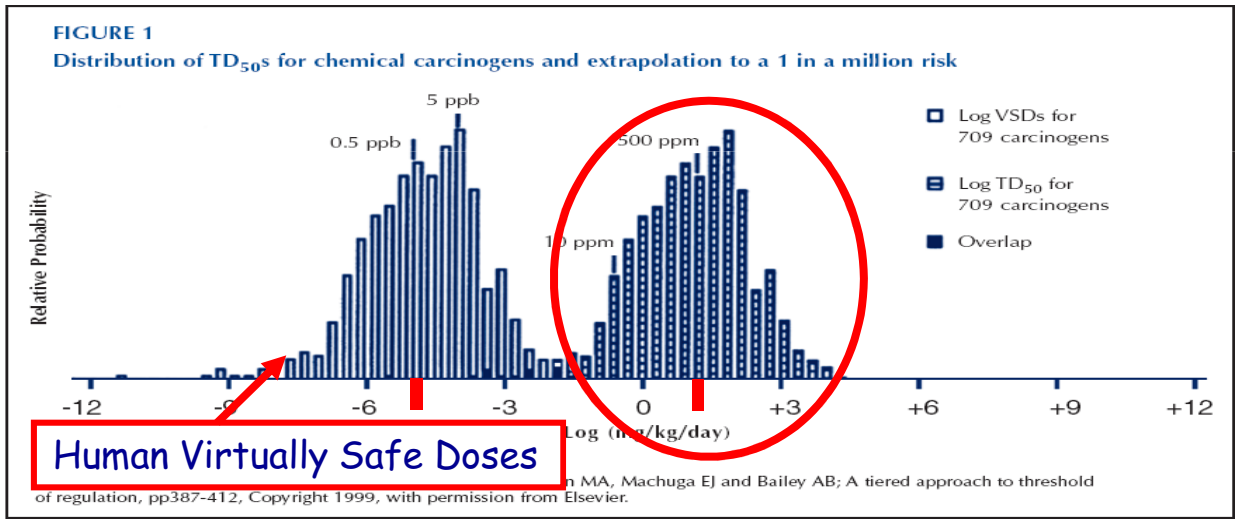
RODENT CARCINOGENICITY DATA BASE



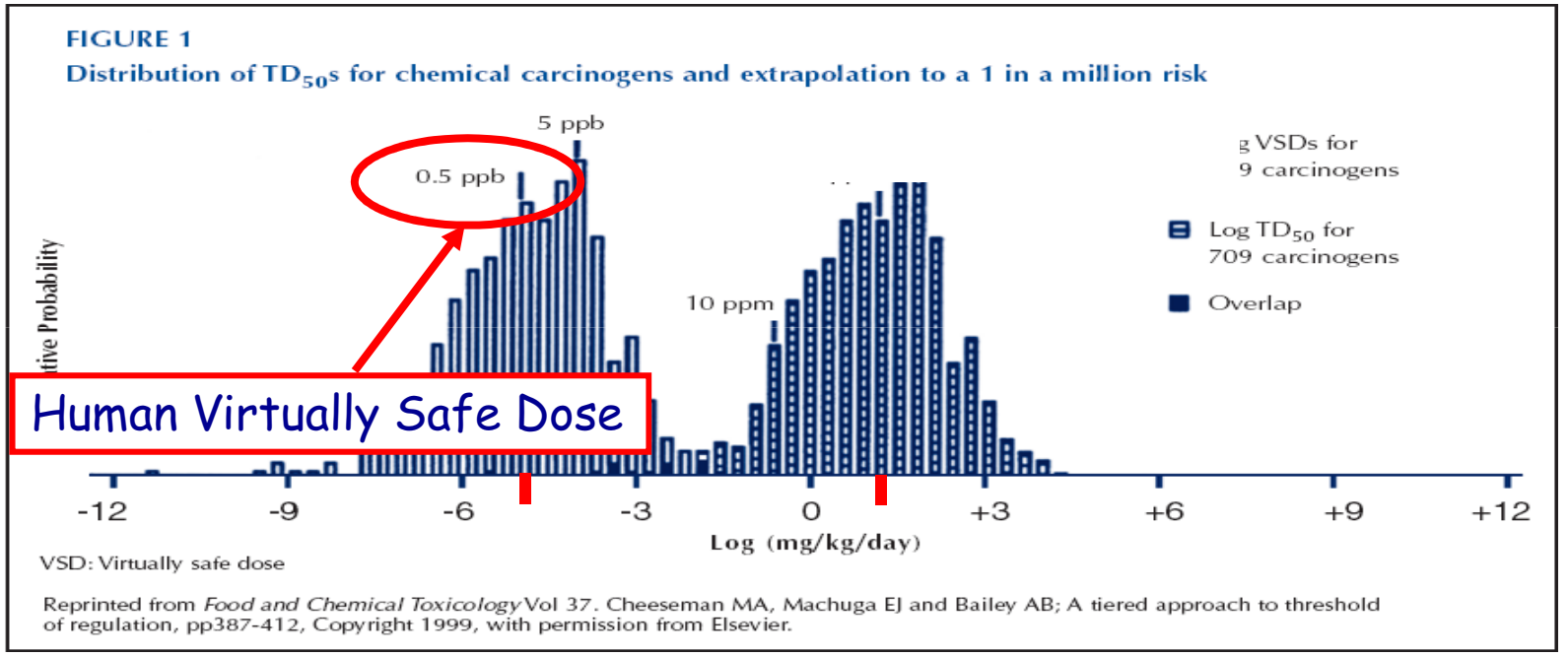
Rodent TD_{50}

RODENT CARCINOGENICITY DATA BASE

The potencies plotted as a distribution of TD50s were transformed into a distribution of exposures calculated by linear extrapolation from TD50 values to represent an estimated lifetime risk of one in a million of developing cancer or "virtually safe dose" (VSD)



RODENT CARCINOGENICITY DATA BASE



THRESHOLD OF REGULATION (TOR) APPROACH FOR FOOD CONTACT MATERIALS

- Dietary concentration of chemicals, without structural alerts for carcinogenicity, below 0.5 ppb (500 ng/kg or 500ng/L), is so negligible that it presents no public health concern:

assuming that a person consumes 1500 g of food and 1500 g of fluids daily
and the chemical is distributed evenly throughout the total diet
a daily exposure level of 1.5 µg/person/day was derived

Food contact materials with an exposure below this level are "Exempted from regulation".

- TTC principle is derived from FDA's Threshold of Regulation (TOR) approach for food contact materials.

THRESHOLD OF TOXICOLOGICAL CONCERN (TTC)

THRESHOLD IN RELATION TO STRUCTURAL CLASSES Refinement by Munro *et al.* (1996)

- Munro and coworkers (1996) evaluated the use of TTC related to other endpoints than carcinogenicity (612 compounds)
- They used structural information based on an algorithm developed in 1978 by Cramer *et al.*
- The chemicals were grouped into three structural classes based on a "decision tree" approach.
- Most sensitive species, sex, and toxicological endpoints recorded for each substance

CRAMER CLASSIFICATION TREE

NUMBER OF CHEMICALS

Class I- Substances with simple chemical structure and efficient modes of metabolism that would suggest a lower order of oral toxicity — 447

Class II – Substances that are in structural class in which there is less knowledge of the metabolism, pharmacology and toxicology, but for which there is no clear indication of toxicity — 28

Class III – Substances of chemical structure that permit no strong initial presumption of safety, or that may even suggest significant toxicity. — 137

Munro et al., 1996

THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) IN RISK CHARACTERISATION

- Munro and coworkers (1996) evaluated the use of TTC related to other endpoints than carcinogenicity (612 compounds)
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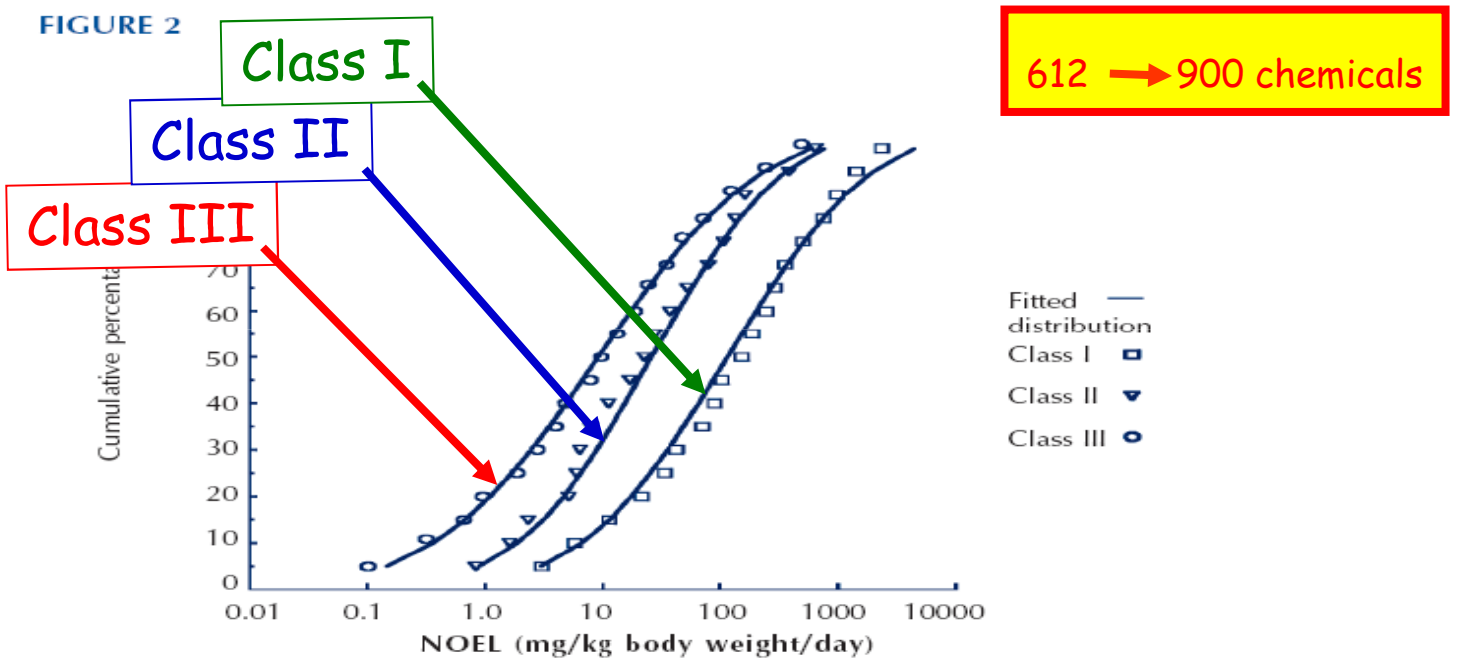
Plot of distributions of NOELs for chemicals by structural class

PLOT OF CUMULATIVE DISTRIBUTIONS OF NOELS FOR CHEMICALS BY STRUCTURAL CLASS

FIGURE 2



Reprinted from *Food and Chemical Toxicology* Vol 34. Munro IC, Ford RA, Kennepohl E and Sprenger JG; Correlation of a structural class with no-observed-effect levels: a proposal for establishing a threshold of concern, pp 829-867, Copyright 1996, with permission from Elsevier.

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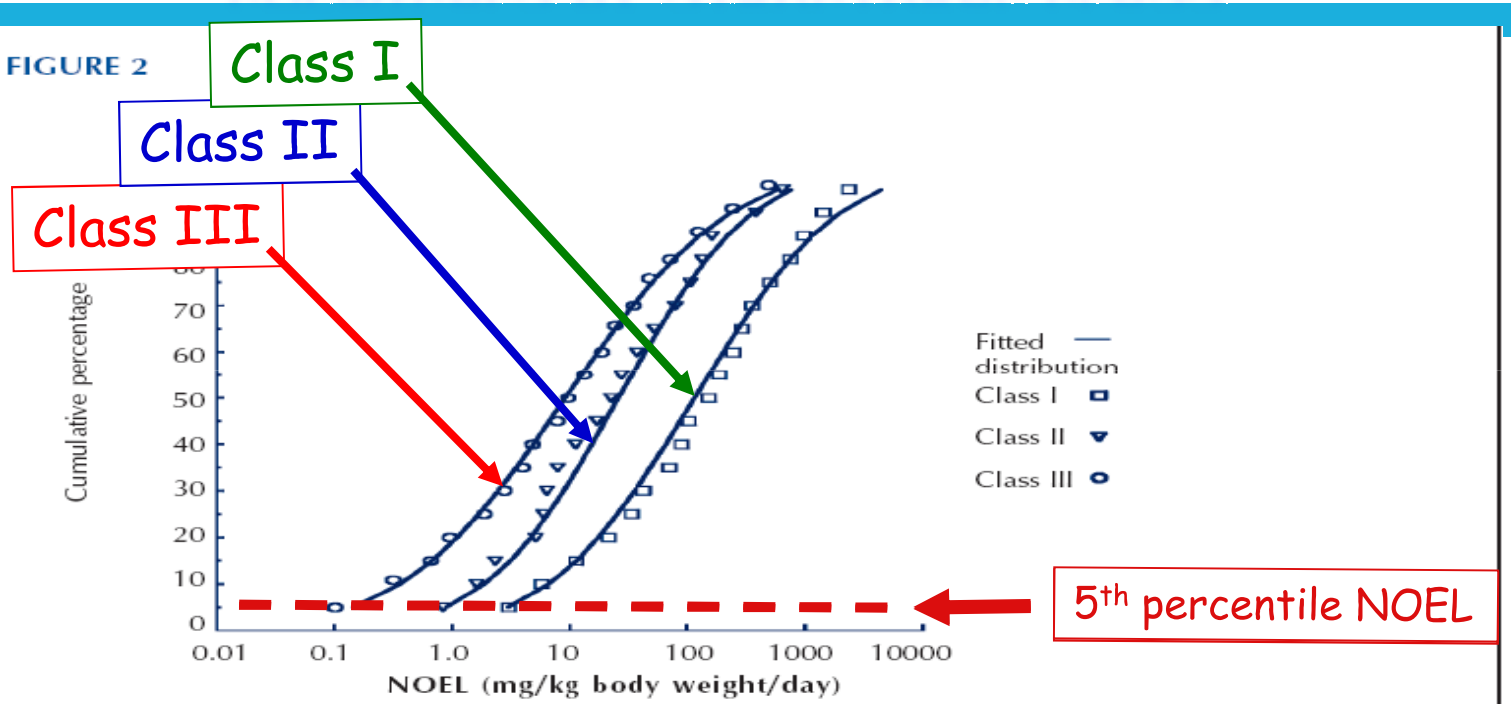

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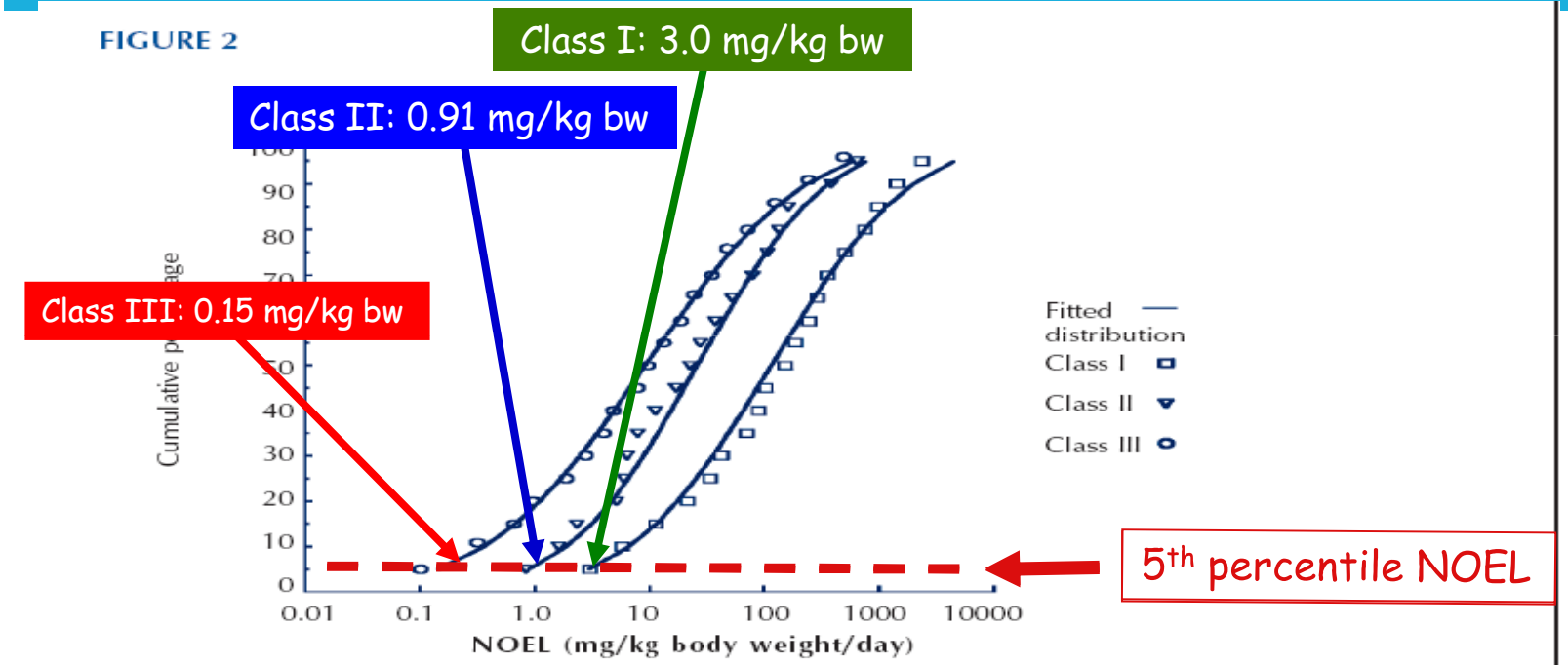

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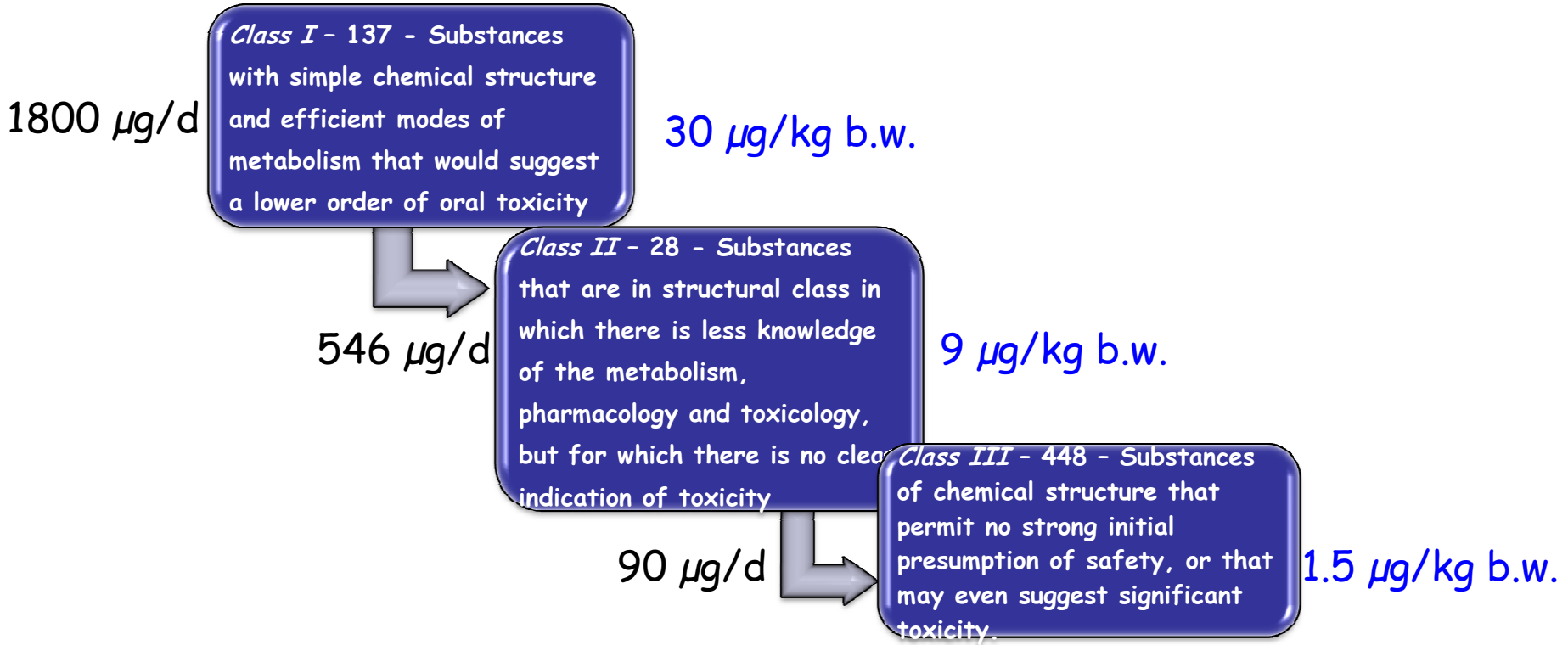
- Most sensitive species, sex, and toxicological endpoint recorded for each substance

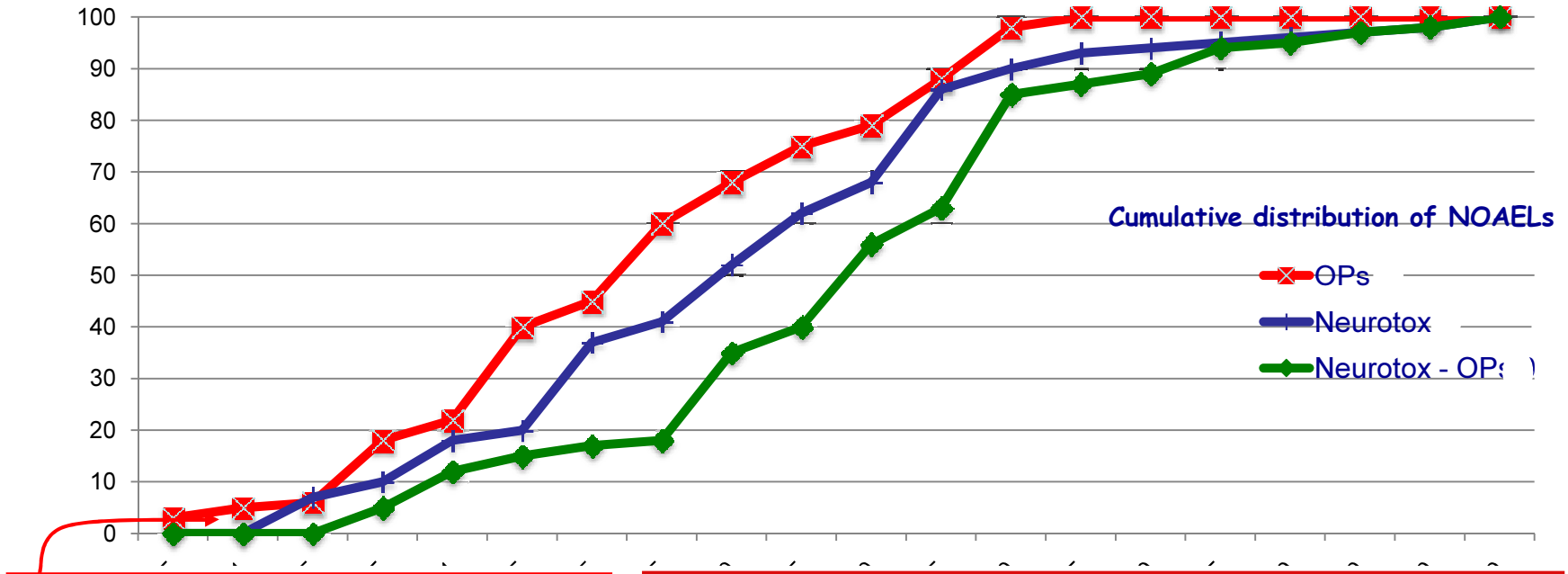
$$TTC = \frac{5^{th} \text{ percentile NOEL}}{U.F. = 100} \times 60 \text{ kg}$$

CRAMER CLASSIFICATION TREE

TTC EXPOSURE LIMITS



SUBDIVISION OF NEUROTOXICITY DATABASE INTO OPS AND NON-OPS



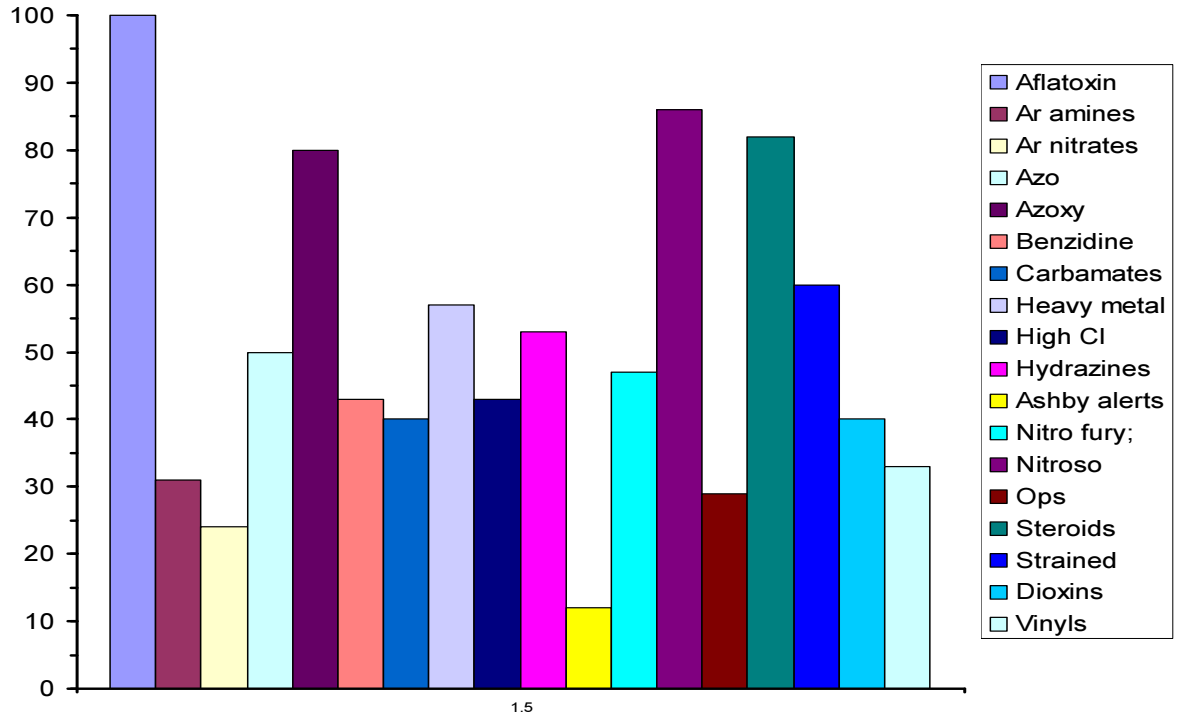
5th percentile NOEL = 30mg/kg/day

TTC = 30/100 x 60kg = 18 µg/person/day

GOLD DATA BASE CARCINOGENS

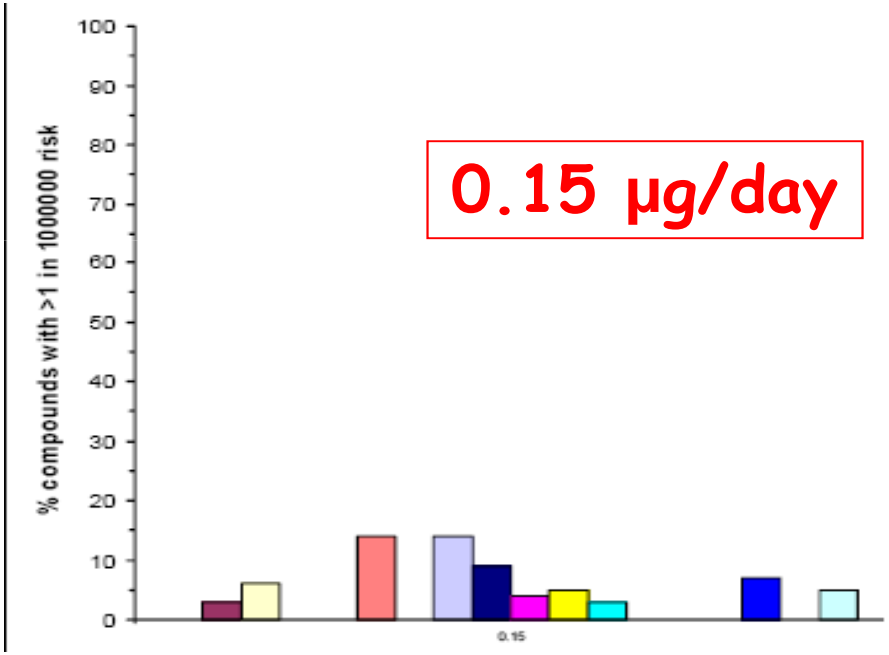
Upper bound risk for cancer lower than one in a million (calculated by linear extrapolation from the TD50)

1.5 µg/person/day intake



CARCINOGENS WITH STRUCTURAL ALERT FOR GENOTOXICTY

Upper bound risk for cancer lower than one in a million? (calculated by linear extrapolation from the TD50)

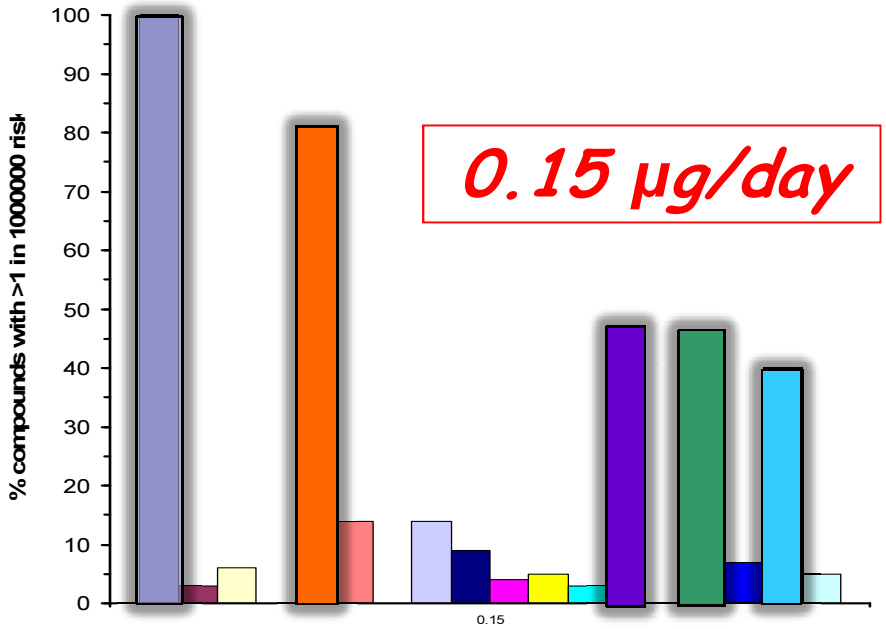


0.15 µg/day

- Recommmendation of using a TTC of 0.15 µg/day for substances with structural alerts for genotoxicity

EXCLUSION OF HIGH POTENCY CARCINOGENS

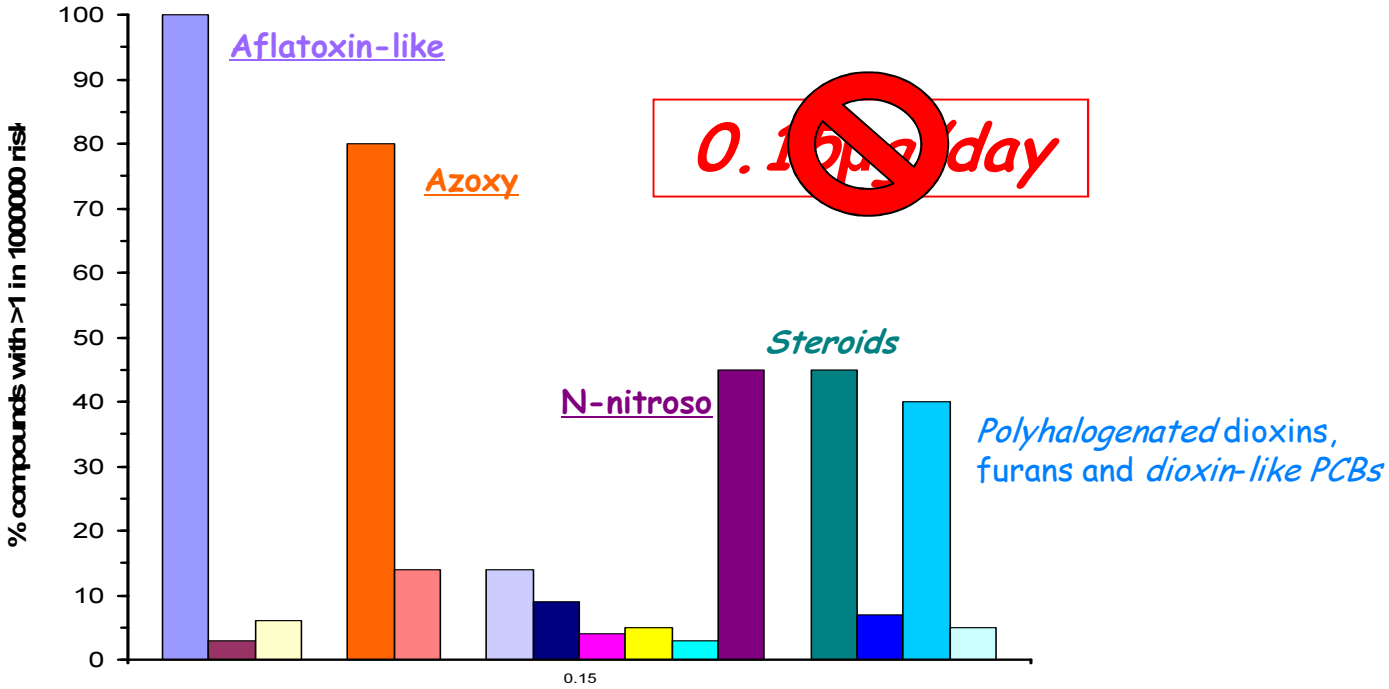
Upper bound risk for cancer of greater than one in a million (calculated by linear extrapolation from the TD50)



- Recommmendation of using a TTC of 0.15 µg/day for all other substances with structural alerts for genotoxicity which are not part of the "cohort of concern)

COHORT OF CONCERN

Upper bound risk for cancer of greater than one in a million (calculated by linear extrapolation from the TD50)



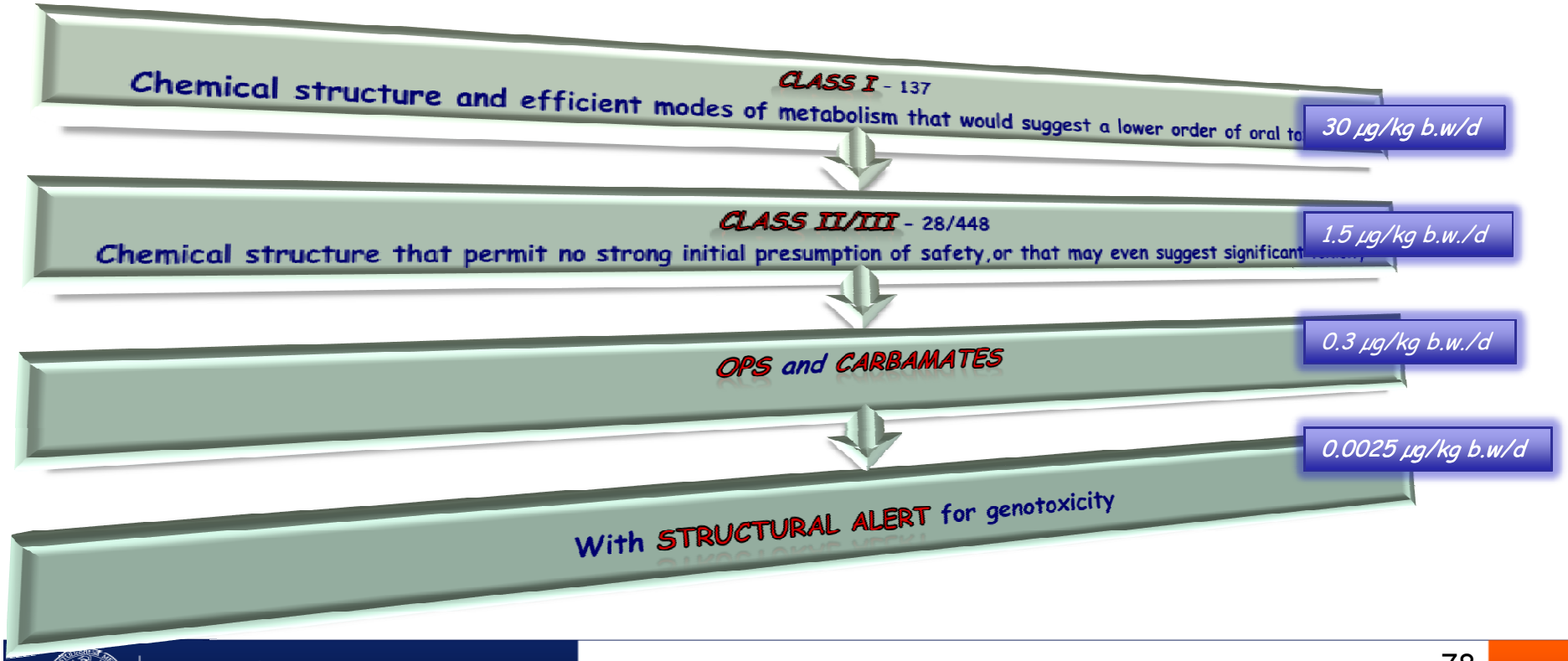
THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) IN RISK CHARACTERISATION

a TTC should NOT be considered.

- For specific structural alerts: i.e. **aflatoxin-like**, **azoxy** and **N-nitroso**-compounds (potent genotoxic carcinogens)
- **Polyhalogenated dibenzo-p-dioxins, -dibenzofurans and dioxin like PCB's** (non-genotoxic carcinogens, bioaccumulative, with very large kinetic differences between animals and humans)
- **Steroids** (potent non-genotoxic carcinogens)

- **Non essentials metals and metal containing compounds** (not included in the data base)
- **Proteins** (risk of allergenicity, not included in database)
- **High molecular weight chemicals such as polymers** (not included in database)

CONVERSION OF TTC VALUES INTO µg/kg BODY WEIGHT



REFINING THE THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) FOR RISK PRIORITIZATION OF TRACE CHEMICALS IN FOOD

PROPOSED SHORT-TERM EXPOSURE THRESHOLDS FOR POTENTIALLY GENOTOXIC CONTAMINANTS IN FOOd		
	Lifetime daily exposure	*Exposure expected not to exceed 1 year*
Chemical with strucural alerts for genotoxicity	0.15 µg/day	1.5 µg/day
Chemical with strucural alerts for genotoxicity, but negative Ames data*	1.5 µg/day	Case-by-case
*Or other data sufficient to conclude a lack of DNA reactivity		

BENCHMARK WITH THE RISK ASSESSMENT PARADIGM

HAZARD IDENTIFICATION

- Identification of adverse health effects
  - Animal-based toxicological studies
  - *In vitro* toxicology data
  - Structure-activity consideration
  - Human data

HAZARD ASSESSMENT

- Quantification of adverse health effects
  - Dose-response for critical effect
  - Selection of critical data
  - Mode/mechanism of action
  - Kinetic variability
  - Dynamic variability

EXPOSURE ASSESSMENT

- Active principle
  - Dose of nanomaterial
  - Dose in individuals
  - Dose in special population groups
  - Max/min, chronically/occasionally

RISK CHARACTERISATION

"Risk = hazard x exposure"

SYSTEMIC EXPOSURE DOSAGE (SED)

- The Systemic Exposure Dosage (SED) of a cosmetic substance is the amount expected to enter the blood stream (and therefore be systemically available) per kg body weight and per day.
- It is expressed in mg/kg body weight/day. For this definition a mean human body weight of 60 kg is commonly accepted.

MARGIN OF SAFETY (MoS)

$$MoS = \frac{NO(A)EL}{SED}$$

The MoS value is used to extrapolate from a group of test animals to an average human being, and subsequently from average humans to sensitive subpopulations.

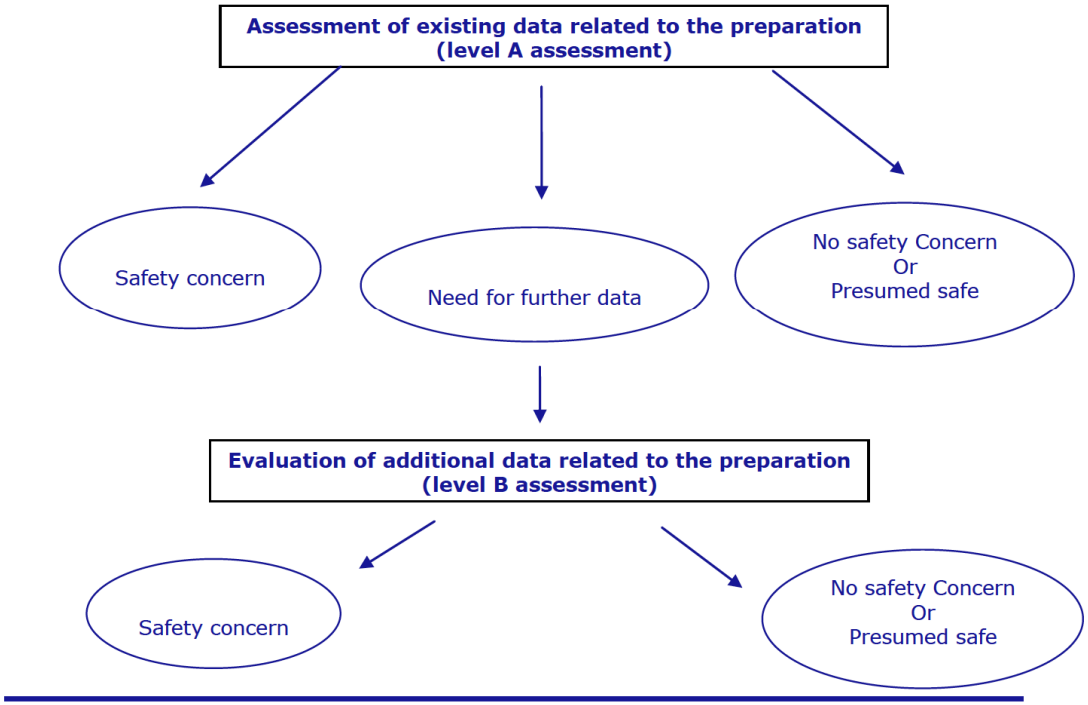
The **WHO proposes a minimum value of 100**, and it is generally accepted that the **MoS should at least be 100 to conclude that a substance is safe for use**.

TTC APPLICATIONS

- **Migrant** substances from packaging materials (USFDA-TOR- 1993)
- **Flavourings** substances in food (WHO-JECFA 1993,1995,1999….)
- Endorsed for the risk assessment of chemicals (WHO-IPCS 1998)
- Non relevant **plant protection product metabolites** in ground water (EC-2002)
- **Genotoxic impurities** in pharmaceutical preparations (EMA 2003,2004)
- **Flavourings** substances in food (EFSA 2004)
- **Genotoxic constituents** in herbal preparations (EMA 2006)
- Suggested for **REACH** (Registr, Evaluat, Authoriz and restrict of **Ch**emical substances) (ECHA 2008)

- Suggested for application to **aquatic environmental** exposure (2005)
- Suggested for application to the **cosmetic ingredients** and their impurities (2007)
- Suggested for **prenatal developmental** toxicity (2010)
- Suggested for **mixture of substances** potentially detectable in surface water (2011)
- Suggested for risk prioritization of **trace chemicals in food**. *(2011)*

SAFETY ASSESSMENT OF BOTANICALS AND BOTANICAL PREPARATIONS USE IN FOOD AS SUPPLEMENTS



EFSA Journal 2009; 7(9):280 12

SAFETY ASSESSMENT OF BOTANICALS AND BOTANICAL PREPARATIONS USE IN FOOD AS SUPPLEMENTS

- **LEVEL A: NO TESTING REQUIRED (ASSUMED PRESUMPTION OF SAFETY)**
  - **long term history of food use**
  - **absence of adverse effect at the proposed level of use**
  - **no significant increase of in intake to be expected due to the in intended levels of use as food supplement**
  - if presence of genotoxic and carcinogenic substances, MoE approach
  - if presence of otherwise toxic substances, comparison of the overall exposure with the existing safety levels (*e.g. ADI, TMDI) or Margin in of Safety approach*
- Level B: Further testing and/or data required
  - Toxicokinetics including metabolism
  - Genotoxicity testing (*in vitro testing + in vivo testing in case of (+) results)*
  - 90 days subchronic toxicity (to establish NOAEL)
  - Other studies based on previous info (target organs, structure activity…)

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SAFETY ASSESSMENT OF BOTANICALS AND BOTANICAL PREPARATIONS USE IN FOOD AS SUPPLEMENTS

- Level A: No testing required (assumed presumption of safety)
  - long term history of food use
  - absence of adverse effect at the proposed level of use
  - no significant increase of in intake to be expected due to the in intended levels of use as food supplement
  - if presence of genotoxic and carcinogenic substances, MoE approach
  - if presence of otherwise toxic substances, comparison of the overall exposure with the existing safety levels (*e.g. ADI, TMDI) or Margin in of Safety approach*
- **LEVEL B: FURTHER TESTING AND/OR DATA REQUIRED**
  - Toxicokinetics including metabolism
  - Genotoxicity testing (*in vitro testing + in vivo testing in case of (+) results)*
  - 90 days subchronic toxicity (to establish NOAEL)
  - Other studies based on previous info (target organs, structure activity…)

INTEGRATED ENVIRONMENTAL RISK CHARACTERIZATION

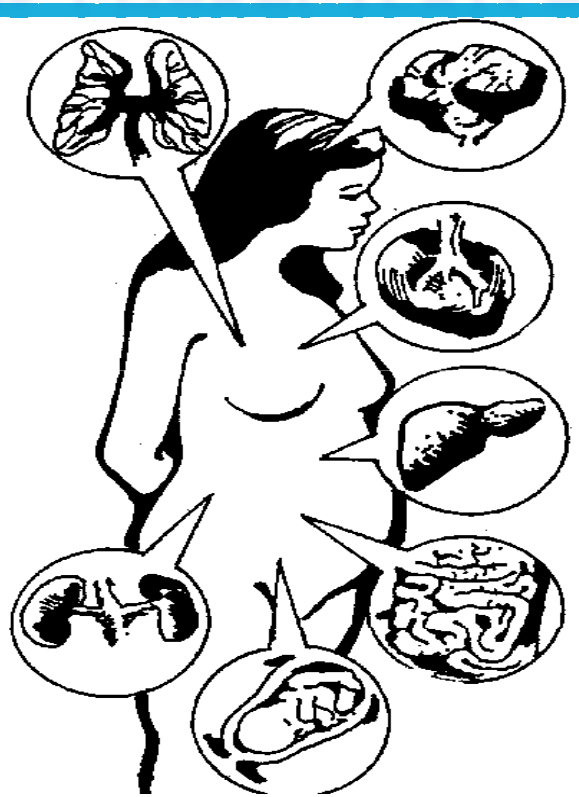
Lungs and respiratory systems:
cobalt, asbestos, sulphur oxides, ozone, nitrogen oxides, ammonia, carbon monoxide, cadmium, cigarette smoke, pesticides, animal and vegetable dusts.

Skin:
arsenic, nickel, chromium, beryllium, pesticides

Bones:
lead, strontium 90, cadium.

Cancer-causing substances:
chlorinated hydrocarbons, mercury, polycylic hydrocarbons, radioactive materials, pesticides

Kidneys:
mercury, cadmium, lead



Brain and nervous system:
lead, carbon monoxide, mercury, pesticides

Eyes:
Ultraviolet light, noxious gases

Oral cavity:
lead, mercury

Heart and circulatory system:
carbon monoxide, nitrates (in infants), pesticides, nitrogen dioxide

Liver:
Chlorinated hydrocarbons, seleniums

Digestive system:
lead, arsenic, fluoride, pesticides

Fetus:
mercury, lead, radioactive materials, pesticides

AGGREGATE AND CUMULATIVE EXPOSURE

Aggregate Risk

The likelihood of the occurrence of an adverse health effect resulting from all routes of exposure to a SINGLE SUBSTANCE.

Cumulative Risk

The likelihood of the occurrence of an adverse health effect resulting from all routes of exposure to a GROUP OF SUBSTANCE sharing a common mechanism of toxicity (MOA).

TYPES OF COMBINED ACTIONS

- Simple similar action
- Simple dissimilar action
- Interaction
  - Stronger than expected effect
  - Weaker than expected effect

SIMPLE SIMILAR ACTION

- Synonyms
  - ❖ Similar joint action

- Non-interactive (i.e. the chemicals in the mixture do not influence each other's toxicity)
- All chemicals in the mixture act by the same mechanism/mode of action (MOA) and differ only in their potencies

DOSE ADDITIVITY

TYPES OF COMBINED ACTIONS

- Simple similar action
- **Simple dissimilar action**
- Interaction
  - Stronger than expected effect
  - Weaker than expected effect

SIMPLE DISSIMILAR ACTION

- *Synonyms*
  - Simple independent action
  - Independent joint action

- **Non-interactive**
- **The Mode of Action (MOA) and, possibly, the nature and site of the toxic effect differ among the chemicals in the mixture**

RESPONSE ADDITIVITY

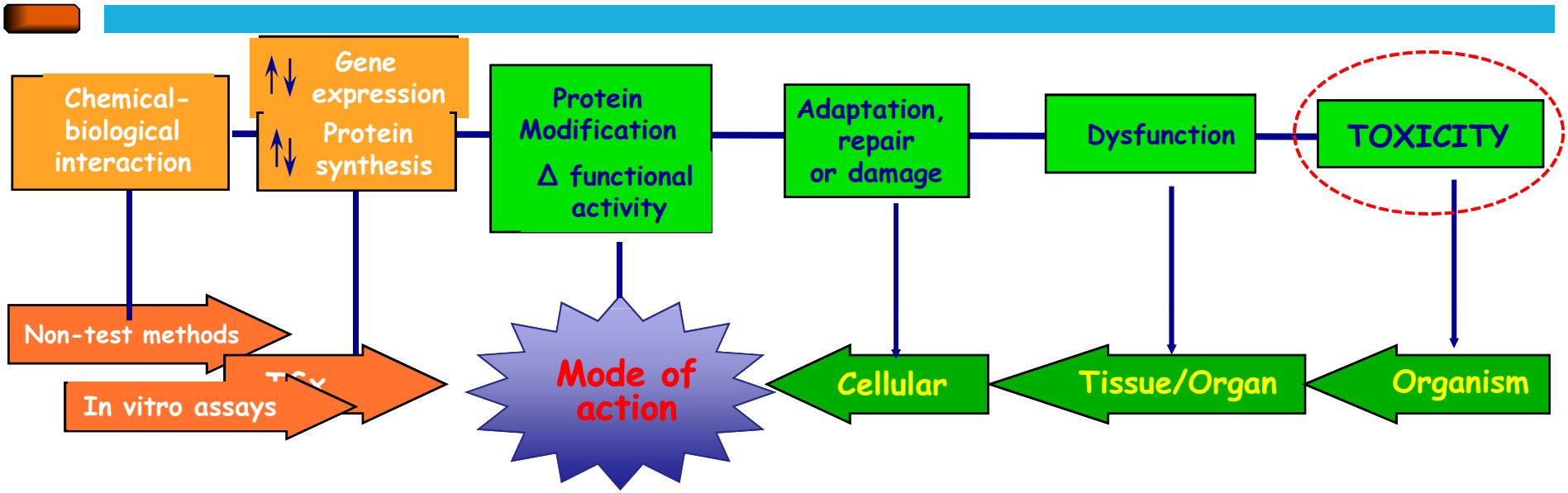
TYPES OF COMBINED ACTIONS

- Simple similar action
- Simple dissimilar action
- **Interaction**
  - **Stronger than expected effect**
  - **Weaker than expected effect**

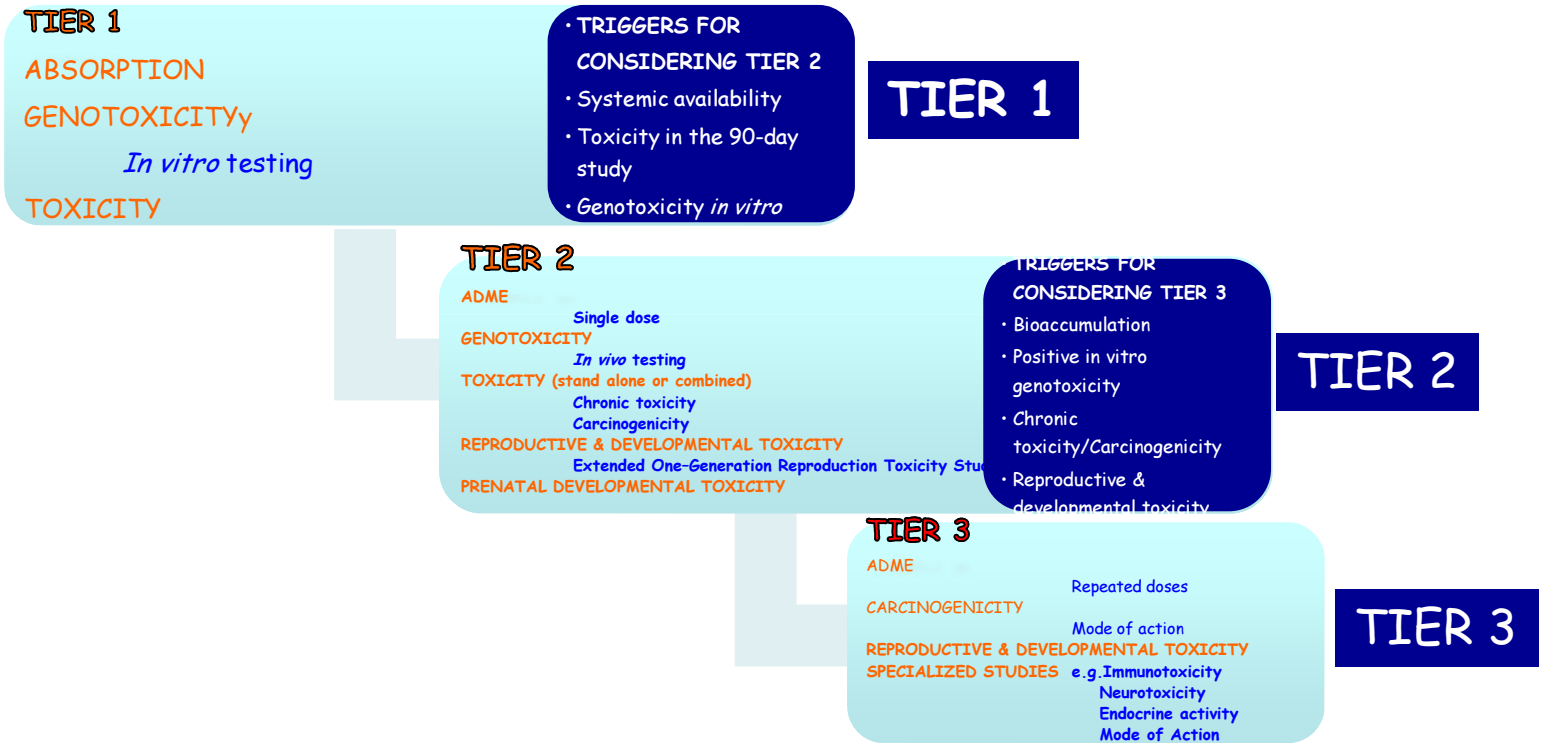
INTERACTION

Available evidence is that interaction **does not occur** at doses that are at or below the No-Observable-Adverse-Effect-Level (NOAEL)

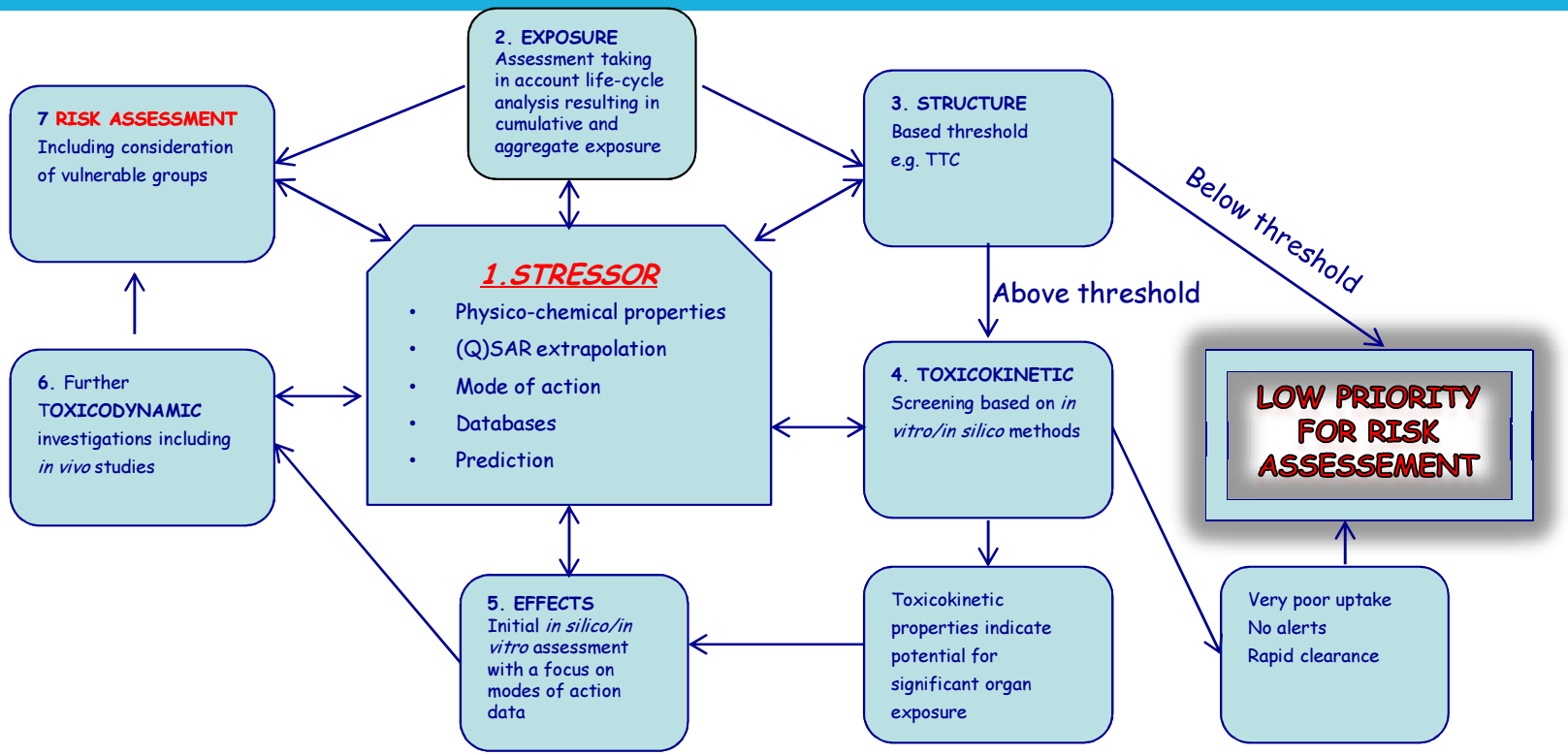
USE OF THE MOA CONCEPT



FA-TOXICITY TESTING STRATEGIES



A DYNAMIC MODEL FOR FUTURE HUMAN RISK ASSESSMENT



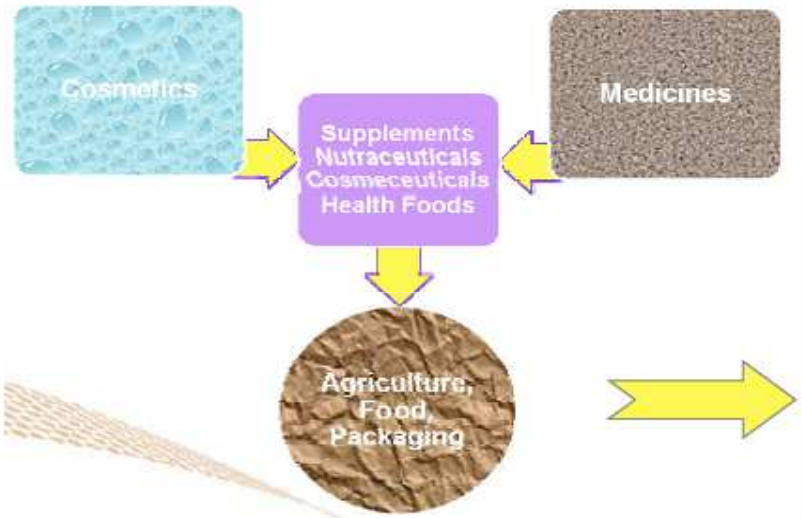
Global Sector Applications

- Cosmetics and personal care products ~60%
- Paints & coatings
- Catalysts & lubricants ~10%
- Security printing
- Textiles & sports
- Medical & healthcare
- Food and nutritional supplements
- Food packaging ~10%
- Agrochemicals
- Veterinary medicines
- Water decontamination
- Construction materials
- Electrical & electronics ~10%
- Fuel cells & batteries
- Paper manufacturing
- Weapons & explosives

2

*Source: www.nanotechproject.org/inventories/consumer/

Food related applications



- Less use of (agro)chemicals
- Safer animal feeds (e.g. detoxification of mycotoxins)
- Hygienic food processing
- Healthy food products (less fat, salt, preservatives)
- Improved bioavailability of nutrients & supplements
- Nano(bio)sensors for detection of pathogens
- Improved, 'Active' and 'Smart' packaging materials (safety, extended shelf-life)
- Coatings – hydrophobic, antimicrobial, gas barrier
- Water decontamination

ASSESSING NEW TECHNOLOGIES

CHALLENGES AND UNCERTAINTIES

- Information gaps
- Scientific publications not designed to answer risk assessment questions
- Lack of guidance documents

- Benchmark with the Risk Assessment Paradigm ?

BENCHMARK WITH THE RISK ASSESSMENT PARADIGM

HAZARD IDENTIFICATION

- Identification of adverse health effects
  - Animal-based toxicological studies
  - *In vitro* toxicology data
  - Structure-activity consideration
  - Human data

HAZARD ASSESSMENT

- Quantification of adverse health effects
  - Dose-response for critical effect
  - Selection of critical data
  - Mode/mechanism of action
  - Kinetic variability
  - Dynamic variability

EXPOSURE ASSESSMENT

- Active principle
  - Dose of nanomaterial
  - Dose in individuals
  - Dose in special population groups
  - Max/min, chronically/occasionally

RISK CHARACTERISATION

"Risk = hazard x exposure"

NANOMATERIALS IN FOOD

- Nanoparticles are being used to deliver **vitamins** or other **nutrients** in food and beverages without affecting the taste or appearance.

- These nanoparticles actually encapsulate the nutrients and carry them through the stomach into the bloodstream.

- For many vitamins this delivery method also allows a higher percentage of the nutrients to be used by the body because, when not encapsulated by the nanoparticles, some nutrients would be lost in the stomach.

NANONOMATERIALS IN FOOD PACKAGING

- Bottles made with nanocomposites that minimize the leakage of carbon dioxide out of the bottle increasing the shelf life of carbonated beverages.
- Nanosensors in plastic packaging can detect gases given off by food when it spoils and the packaging itself changes color to alert you to food gone bad.
- Plastic films are being developed that will allow the food to stay fresher longer. These films are packed with silicate nanoparticles to reduce the flow of oxygen into the package and the leaking of moisture out of the package.

NANOMATERIAL IN AGRICULTURE

- Researchers are working on pesticides encapsulated in nanoparticles:
  - these only release pesticide in an insect's stomach, which minimizes the contamination of plants themselves.

ENMS RISK ASSESSMENT PROCEDURE

CRUCIAL ISSUES

- A clear definition as to what nanoscale materials actually are (>1% of 1 – 100nm; surface area > *60 m^2/cm^3*)
- Issue of absorption
- The measure of the exposure
- Discrimination between background and engineered-NM
- The strenght (properness) of current toxicological protocol
- The appropriateness of existing methodologies to assess the potential risks
- Limited practical risk assessment experience in the food area.

THE CONCEPT OF THE DOSE

- For **CHEMICALS**, the health effects are correlated to the mass of the agent to which the individual is exposed, resulting in an accumulated mass as internal or organ dose/exposure.

- For **NANOPARTICLES** the concentration number and the resulting total surface area appear to be more reasonable parameters for doses in terms of exposure.

- Increased surface area per unit mass
  - 1 mL of nanoparticles (2.5 nm; 5 g/cm^3) has a surface of 240 m^2

THE CONCEPT OF THE EXPOSURE

- No exposure assessment without detection
  - **Diversity** NM (inorganic, organic, coated,…)
  - **Solubility, aggregation** (stability, size distribution)
  - **Matrix** (interactions, effects on size, digestion)
  - **Quality of available nanomaterials** (polydispersity, purity, conc.)
  - **Test protocols** (dispersion, reproducibility, comparability)
  - **Choice & preparation of test medium** (concentration, solvents)

PHYSICO-CHEMICAL CHARACTERISATION

- COMPREHENSIVE CHARACTERISATION NEEDED

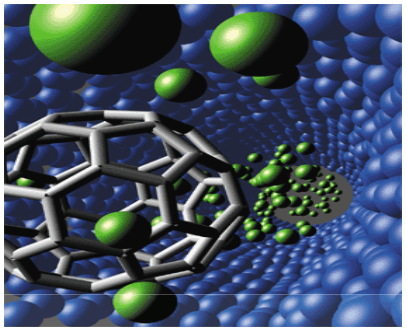
- E.g. size, size distribution, morphology, surface chemistry, catalytic activity, stability/shelf life, volume specific surface area (for dry powders).

- Concentration, dispersion medium, agglomeration-aggregation state

- Information on method of production, intended use, **batch to batch variation**

CHARACTERISATION AT SEVERAL STEPS

- Prior to use in food/feed
- As used during toxicological testing
- As used in food/feed
- As present in tissues



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- Methods used need to be carefully selected and described

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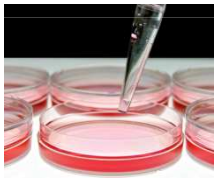

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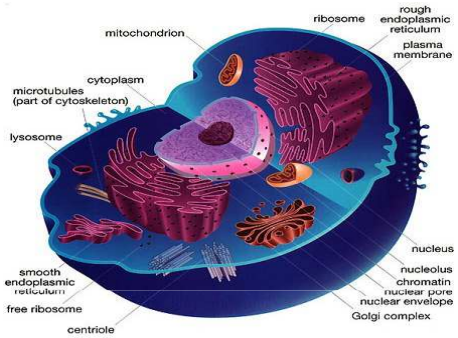
- As present in tissues



- Acknowledged that characterisation can be difficult in certain matrixes.
- Methods used need to be carefully selected and described

CHARACTERISATION AT SEVERAL STEPS

- Prior to use in food/feed
- As used during toxicological testing
- As used in food/feed
- As present in tissues

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CHARACTERISATION AT SEVERAL STEPS

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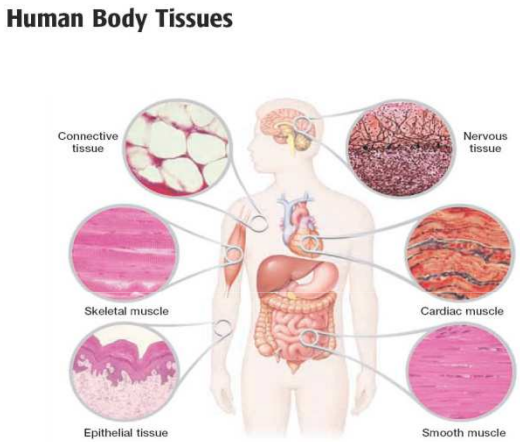

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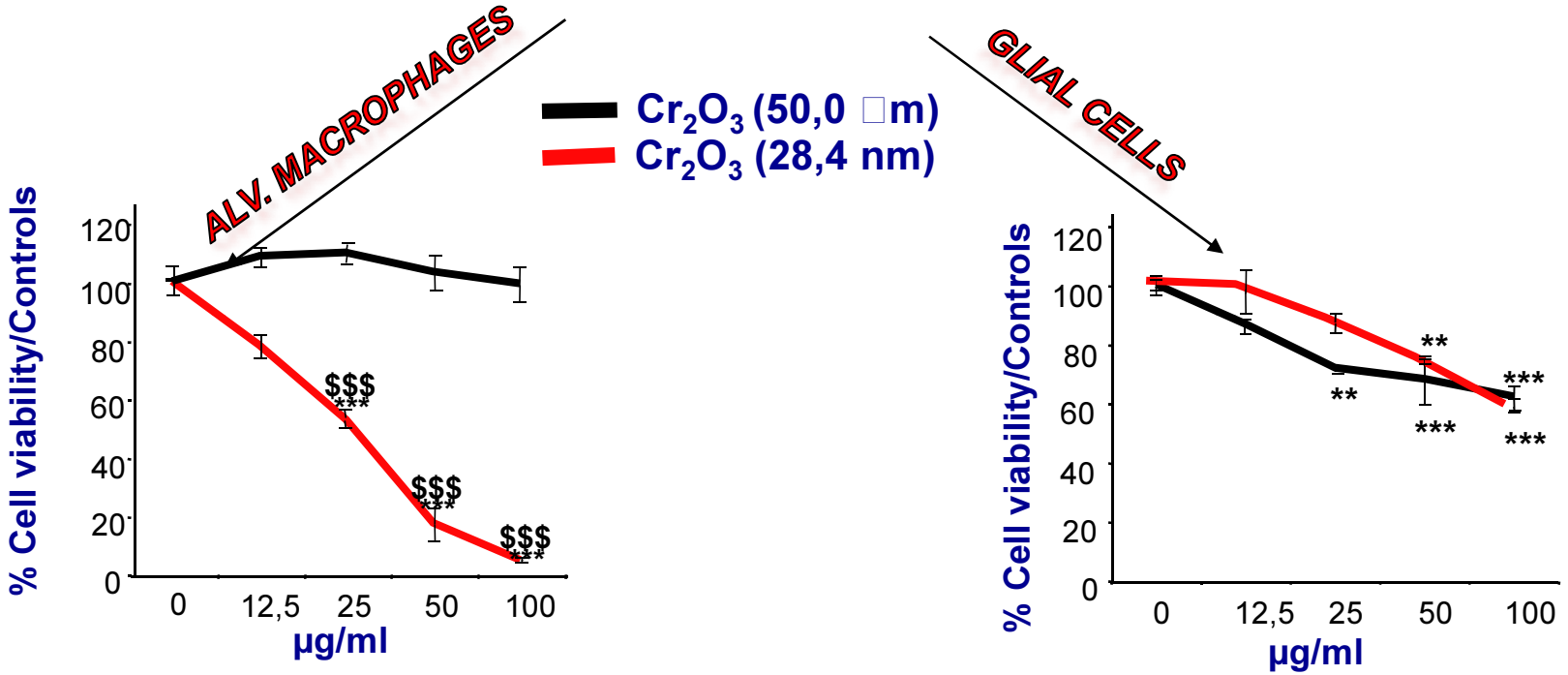
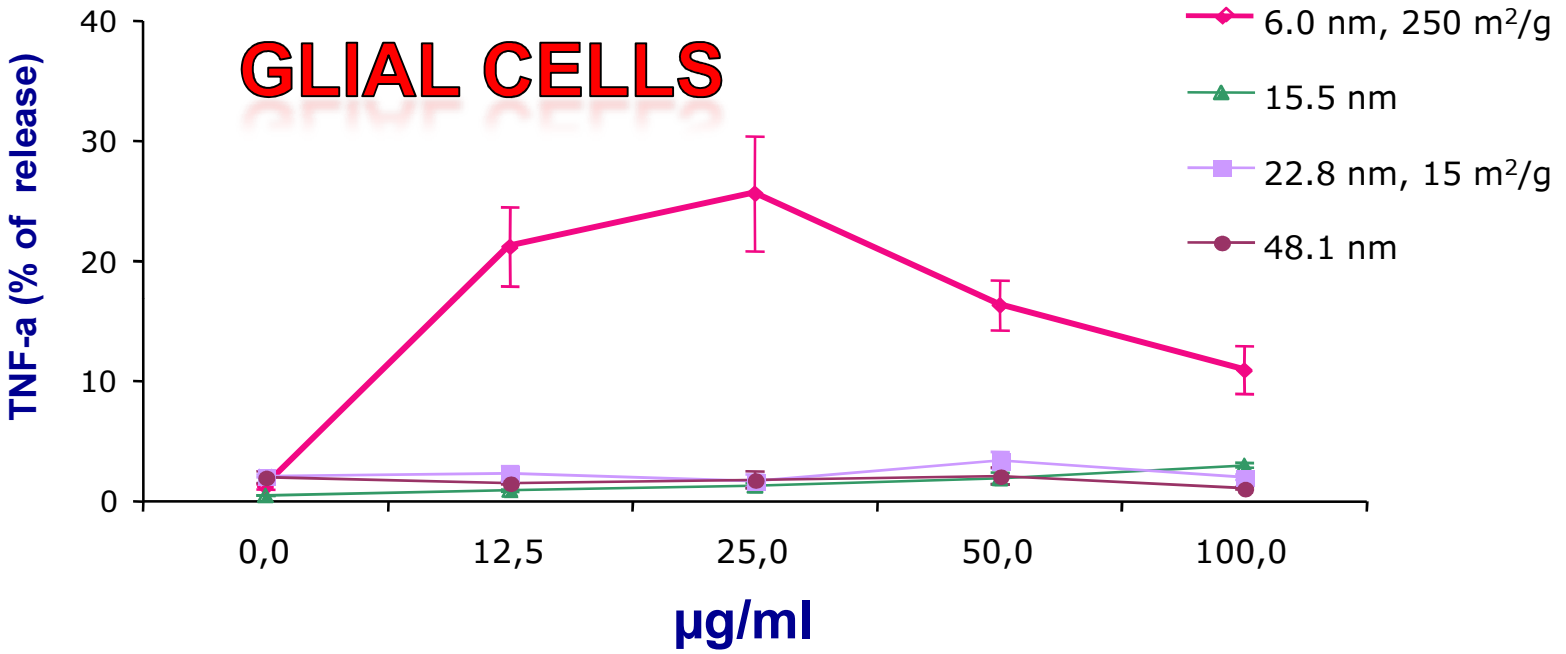
CHROMIUM OXIDE (Cr III)



*** p<0,001 vs control; ** p<0,01 vs control; $$$ p<0,001 vs Cr_2O_3 (50 mm); $$ p<0,01 vs Cr_2O_3 (50 mm).

Marinovich et al., 2007 unpublished data

TITANIA NANOPARTICLES



Marinovich et al., 2007 unpublished data

IN VITRO VS. *IN VIVO* EXPOSURE

- Rough estimates indicate that in most of these studies the nanoparticles to cell ratio was far beyond 1000:1, which largely exceeds any realistic dose in vivo.

- Generally, 2 x 10^5 nanoparticles per cell, are applied

Particle and Fibre Toxicology 2010, 7:2

KINETIC AND DYNAMIC VARIABILITY

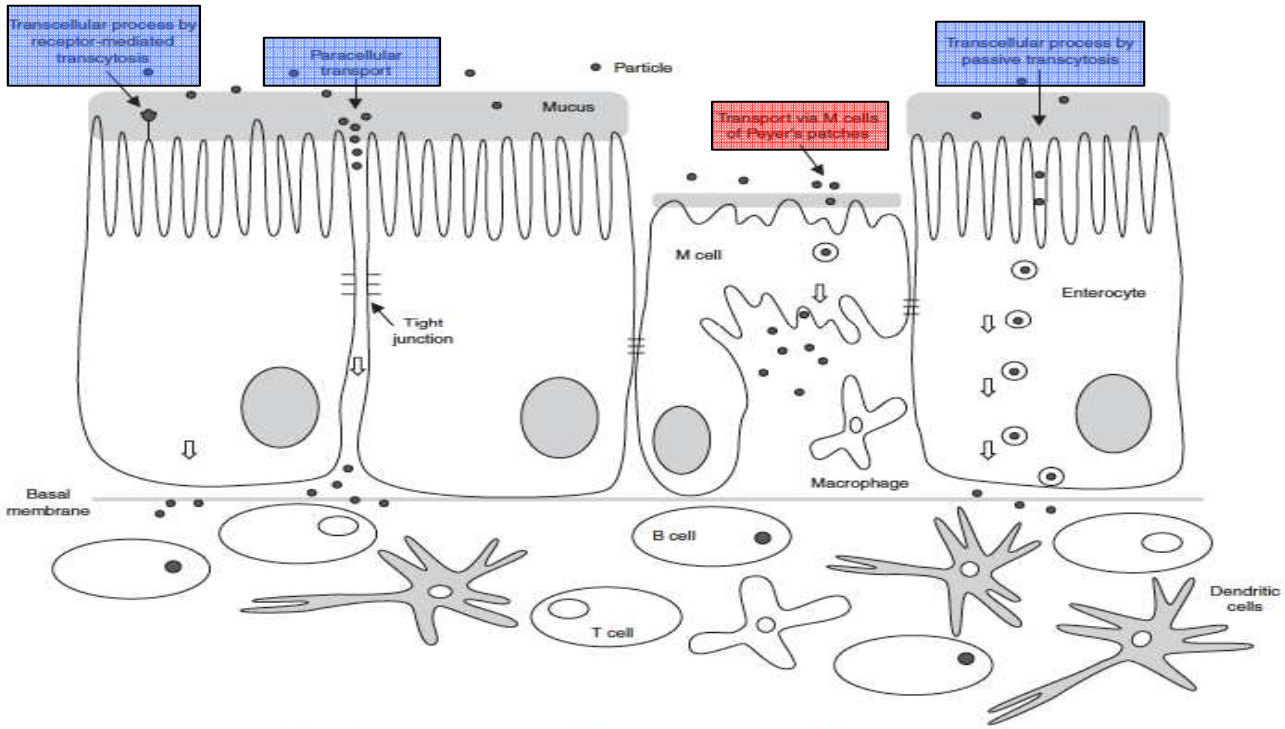
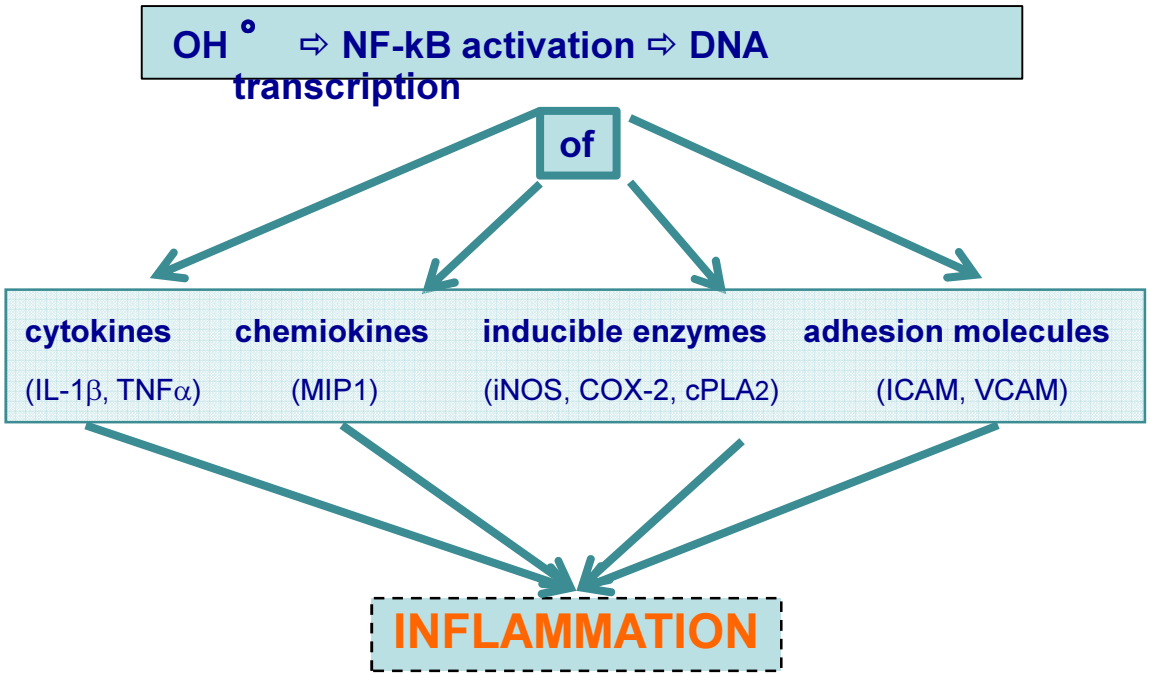


Fig. 2. The intestinal structures and routes of particle translocation across the intestinal epithelium.

Camile B. Woitiskiet al. Biodrugs 2008; 22 (4): 223-237 DRUG DELIVERY

MODE/MECHANISM OF ACTION



IN VIVO STUDIES

Species	Nanoparticle	Route	LD_{50} DOSE (g/kg)	Adverse effects/lesions	Ref.
Rat	Fullerene (C60)	po	> 2	No evidence of toxicity No effect on body weight	Mori et al., 2006
Mouse	58 nm Zn	po	> 5	Kidney, tubular dilation, casts Liver, hydropic degeneration	Wang et al., 2006
Mouse	25, 80, and 155 nm TiO_2	po	> 5	Kidney, glomerular swelling Liver, hydropic degeneration, spotty necrosis	Wang et al., 2007
Rats	Nanoscale TiO_2 T805 (primary particle 21 nm)	po	> 2.2	No evidence of toxicity No gross lesions No effect on body weight	SCCNFP, 2000
Mouse	20 nm Fe_3O_4	po, ip, iv	> 2.1, > 1.6, > 0.4	No deaths observed No histopathological lesions	Xia et al., 2005

GENERAL SCENARIOS

- **Non authorised non-nanoform of a substance not previously used in food/feed**

  comprehensive range of toxicity tests are required, following the relevant conventional guidance for the intended use.

- **Reformulation into nanoform of already authorized and approved food/feed/ingredients**

  Complementary data on the potential additional hazard of the new nanoform…….

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TOXICITY TESTING STRATEGIES

- **In *vitro* tests.**
  - In vitro digestion studies (dissolution/degradation in G.I. tract)
  - Genotoxicity and mutagenicity tests (gene mut and MN)
  - Barrier permeability (i.e. CaCo-2, M cells).
  - Immunotoxic response (i.e. whole blood assay)

- **In *vivo* tests**
  - Absorption, Distribution, Metabolism, and Excretion (ADME)
  - 90-day rodent repeat oral toxicity, considering extended endpoints (e.g. endocrine activity and immuno- and reproductive toxicity)

- **Additional tests triggered by initial results**
  - (eg. rep. dev, long term, in vivo genotox)

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EXPOSURE SCENARIO

- Anticipated exposure scenarios will influence the extent of the hazard characterisation.
  - Direct or indirect addition to food/feed
  - Certain applications may give rise to a very limited exposure (i.e.food contact material)

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EXPOSURE ASSESSMENT

- Unless information suggest otherwise estimate **worst case** exposure.

  - **Assume 100 % is in nano-form**

    - assume 100 % is absorbed as nano-form, and
    - assume 100 % is systemically available.

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EXAMPLE 1:

A FOOD PRODUCT CONTAINING NANO-STARCH OR NANO-MAYONNAISE

- The nano-sized material may be **derived from** those **natural food materials** that are digested and metabolised in the body and are not likely to be biopersistent. (i.e starch ground to a nano-form, or have been formed into nano-structures through an emulsification process).

- The risk assessment in this case, therefore, will not require any detailed toxicological assessment.

  - Nano-sizing of some materials may, however, affect their breakdown/metabolism in the body, which may lead to changes in the plasma profile of the resulting nutrients, compared to bulk form of the same materials.

    - A faster digestion of nano-starch may give rise to a greater glycemic index than the normal starch.
    - The evaluation of such nano-ingredients should, therefore, consider any major changes in digestibility, and/or the uptake of nutrients compared to the conventional bulk forms.

EXAMPLE 2:
A FOOD PRODUCT CONTAINING NANO-ß-CAROTENE

- Relates to a food additive that has been formulated in **a liposome based nano-carrier shell** that may be derived from a natural food material (e.g. a phospholipid, protein or other food polymer).

  - If the nano-carrier is digested and the contents (ß-carotene) are released in the gastrointestinal (GI) tract, the risk assessment will not be any different from the bulk form of ß-carotene.

  - If the nano-carrier is not (or is only partially) digested, and delivers the encapsulated substance to the circulatory system, the ADME properties of the encapsulated ß-carotene will be different from that of the bulk form. Therefore, the risk assessment in this case should focus on the digestibility of the nano-carrier (shell), and where applicable, ADME profile of the internal exposure encapsulated substance

EXAMPLE 3:

A FOOD PRODUCT CONTAINING NANO-TITANIUM DIOXIDE

- This example relates to the use of nano food/feed additives that are in the form of an insoluble, indigestible, and potentially biopersistent ENM.

- The ADME properties and toxicological profile of such materials may differ from their bulk equivalents. Since it is not possible to extrapolate the required information from the existing data on conventional substances, this type of application will require a detailed **physicochemical characterisation** and **toxicological assessment** with due regard to nanoparticulate nature of titanium dioxide.

  Examples include transition metals (e.g. silver, iron, titanium); alkaline earth metals (e.g. calcium, magnesium); and non metals (e.g. selenium, silicates). Food packaging is currently the major area of application of metal and metal-oxide ENMs.

EXAMPLE 4:
A FOOD PACKAGING MATERIAL CONTAINING NANO-SILVER

- In this type of application, the ENMs may be embedded, bound or dispersed in the polymer matrix. The risk assessment decisions in this case will mainly be exposure driven.

- A migration study will be needed to establish the level of migration under different food/feed storage conditions. If the data show that nano-silver does not migrate into food/feed stuffs in any significant quantity, then there will no risk to the consumer.

  It should, however, be noted that other environmental regulations (including REACH) may be applicable if there is a likelihood of potential harm to the environment after disposal of such packaging material.

MAIN UNCERTAINTIES AND CHALLENGES

- **Analytical limitations** in the measurement of nanomaterials in various matrices make assessment of toxicity and exposure data difficult.

- **More testing experience** with nanomaterials is needed to establish optimal approaches.

- **Long term oral exposure** information is missing and extrapolation from shorter exposure is not yet reliable.

- **Bioaccumulating and persistent** nanomaterials are likely to end up in the food/feed chain as contaminants.

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NO CAUSE OF CONCERNS

- Good solubility and rapid degradability
- Permanently bond in matrices
- Presence of firmly bound aggregates
- Formation of stable large agglomerates
- Modifications on surfaces (no release of particles, no reactive surfaces, etc..)

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CAUSE OF CONCERNS

- Volume of production
- Use
- Mobility in organisms
- Persistence of nanoproperties
- Bioaccumulation
- High reactivity and critical morphology
- Transformation (aging, change of surface, loss of coating)

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