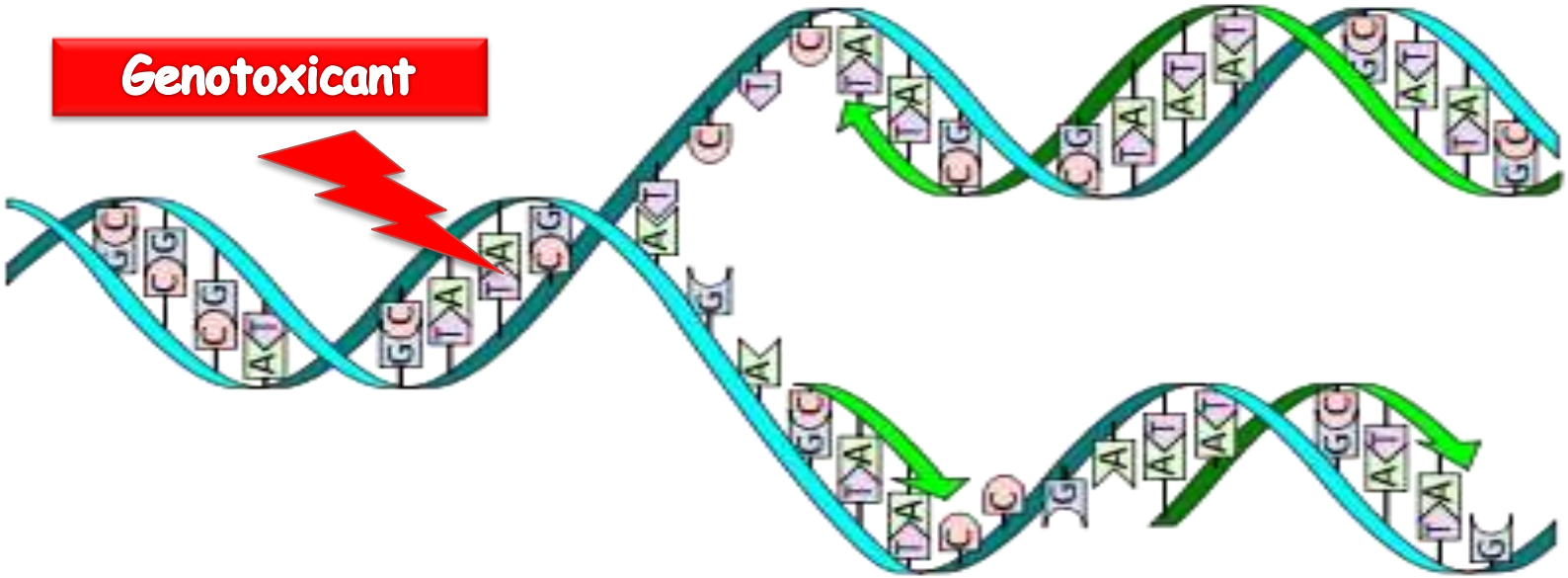
RISK ASSESSMENT

Hazard Assessment

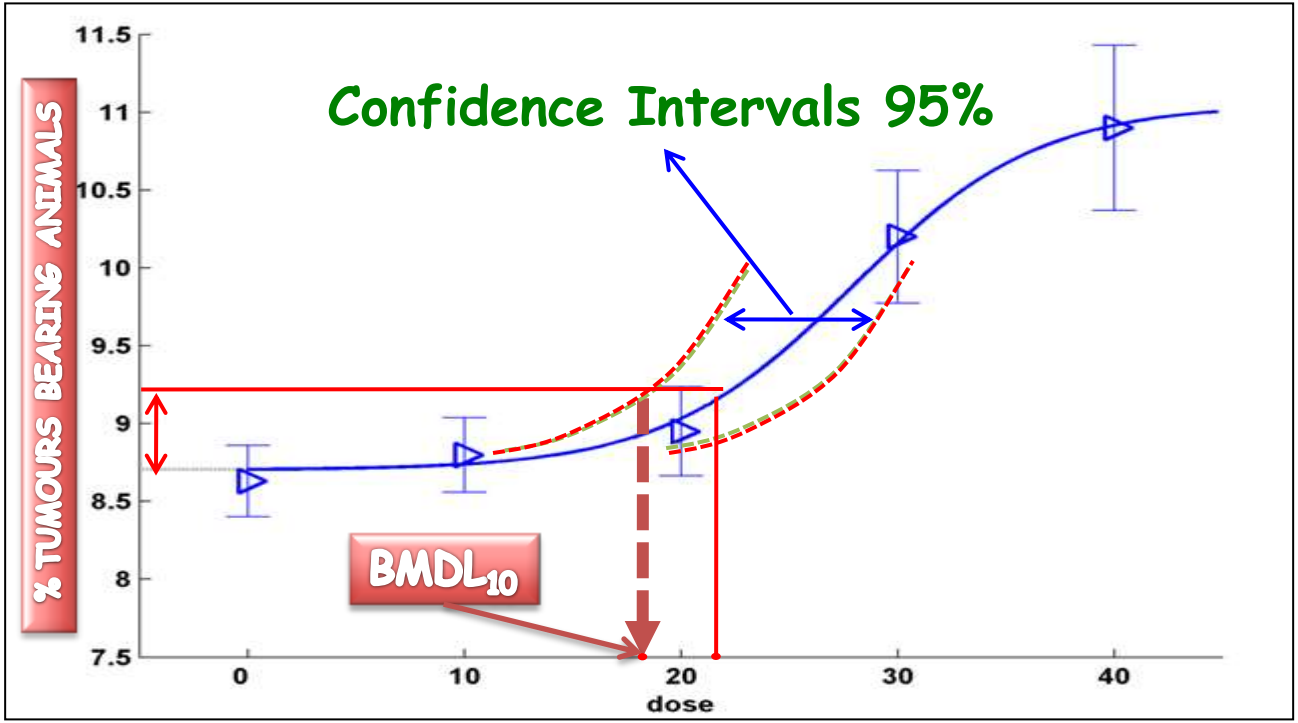
Genotoxic Carcinogen

Toxicant and/or
NON Genotoxic Carcinogen

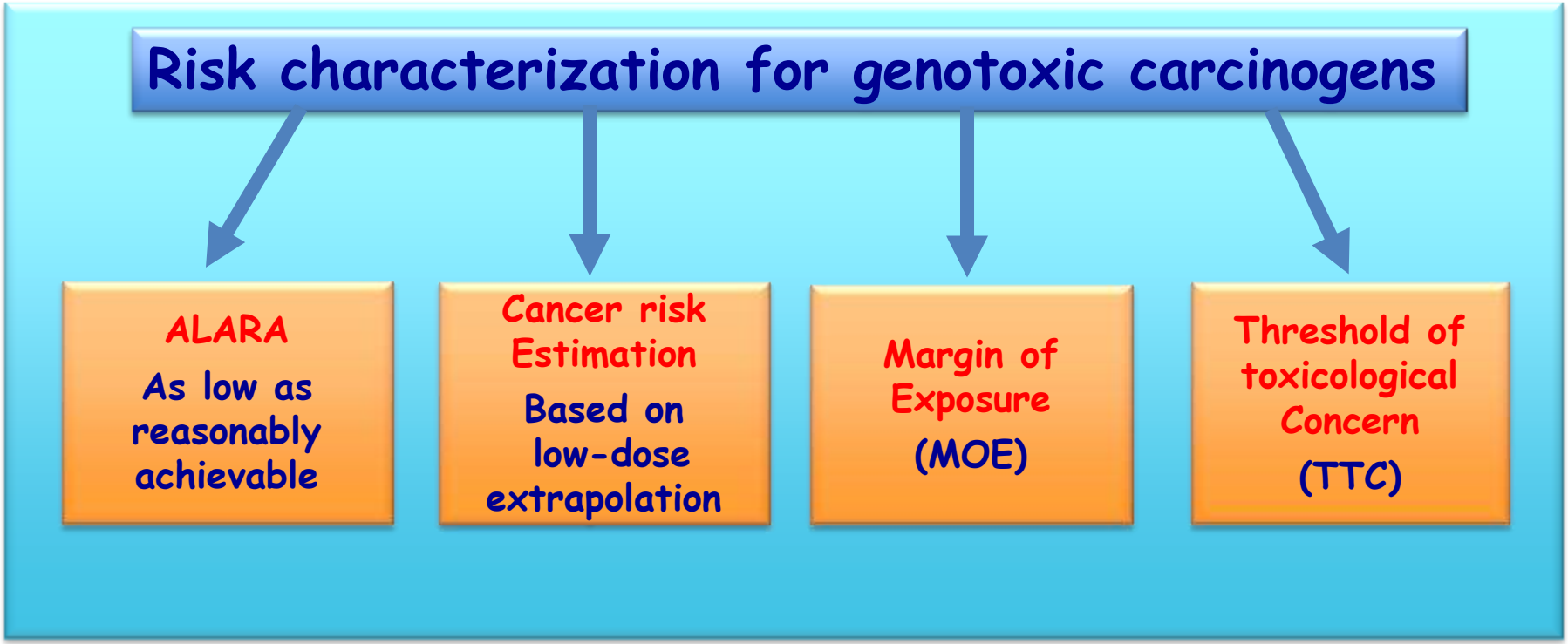
GENOTOXIC CARCINOGENS



BENCHMARK DOSE - (BMD)



RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS



RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS

Risk characterization for genotoxic carcinogens

ALARA

As low as reasonably achievable

ALARA

- Based solely on **hazard identification**
- Does not take into account **human exposure**
- Does not take into account **potency**

RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS

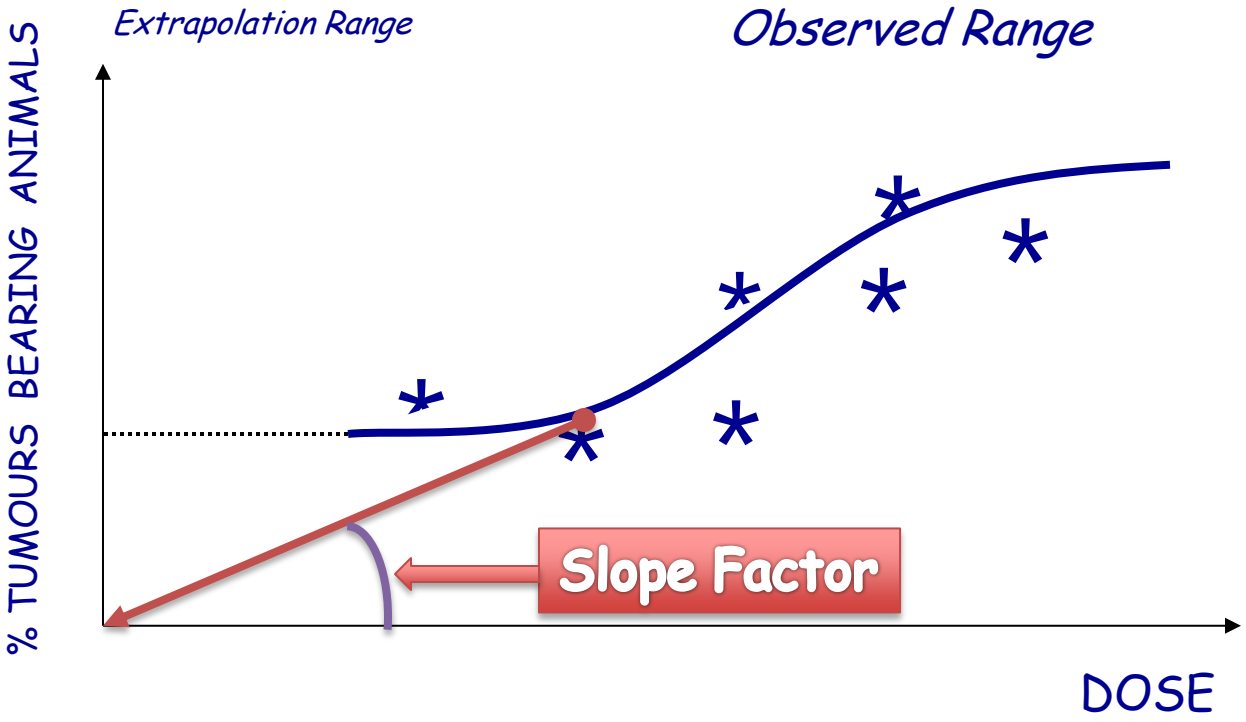
Risk characterization for genotoxic carcinogens

↓

Cancer risk Estimation

Based on low-dose extrapolation

RISK ASSESSMENT



RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS

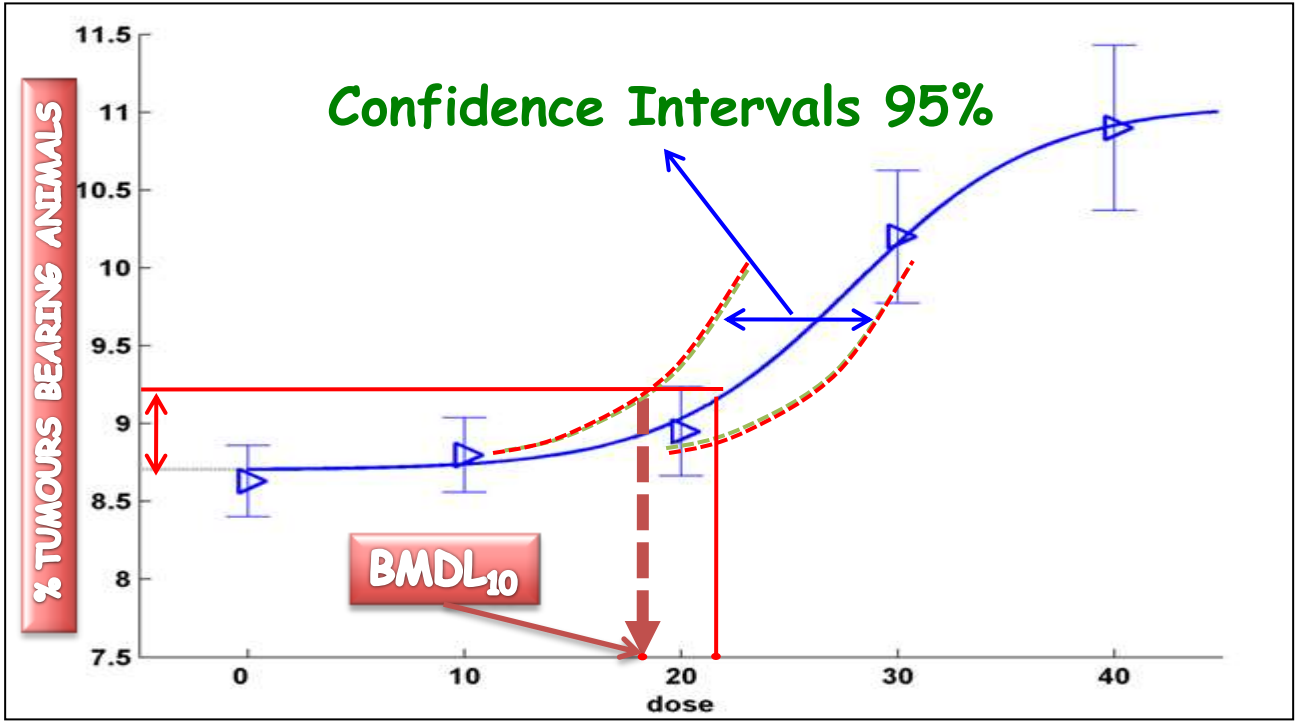
Risk characterization for genotoxic carcinogens

↓

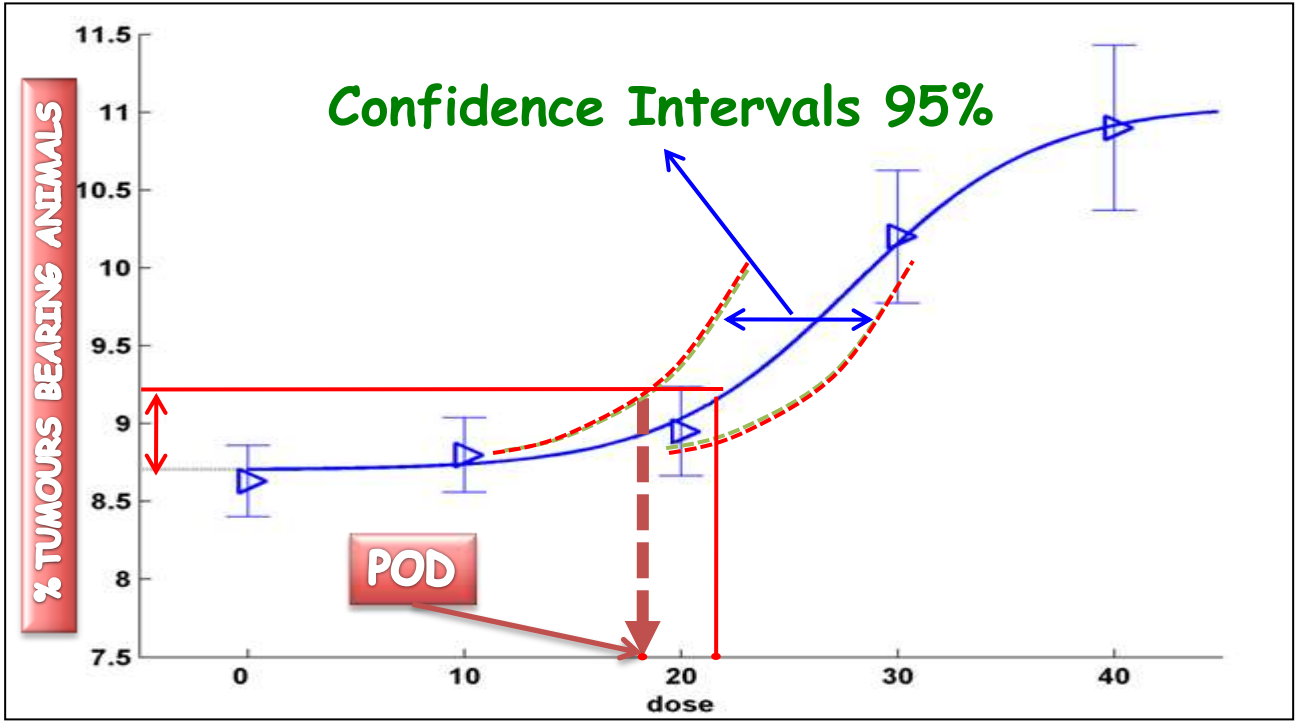
Margin of Exposure (MOE)

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACOLOGICHE E BIOMOLECOLARI

BENCHMARK DOSE - (BMD)



BENCHMARK DOSE - (BMD)



MARGIN OF EXPOSURE (MOE)

- MoE = BMDL10 or PoD / EXPOSURE

BMDL10 or PoD → 25 mg/kg b.w.

EXPOSURE → 0.0005 mg/kg/day

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

MARGIN OF EXPOSURE: > 10,000

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

- 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.

MARGIN OF EXPOSURE: > 10,000

x 10

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.

MARGIN OF EXPOSURE: > 10,000

x 10

- 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.

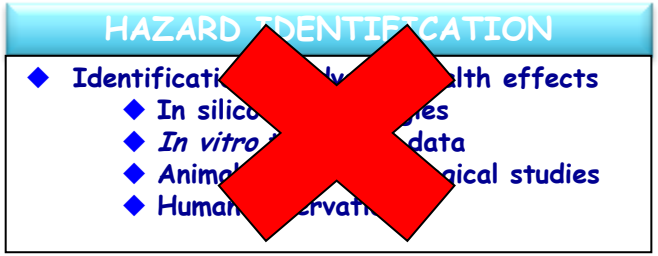
RISK CHARACTERIZATION
AND FORMULATION OF ADVICE TO RISK MANAGERS

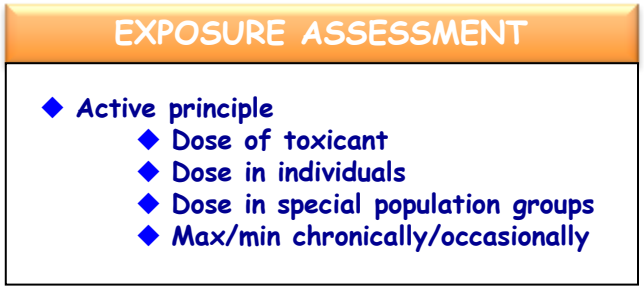
Risk characterization for genotoxic carcinogens

→ **Threshold of toxicological Concern (TTC)**

RISK ASSESSMENT

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





RISK CHARACTERISATION

RISK PRIORITIZATION

EXPOSURE ASSESSMENT

- Dose of toxicant
- Dose in individuals
- Dose in special population groups
- Max/min chronically/occasionally

CHEMICAL STRUCTURE

- Structural information based on an algorithm developed in 1978 by Cramer
- Chemical grouped in three classes

RISK PRIORITIZATION

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.

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 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.

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** (**R**egistr, **E**valuat, **A**uthoriz and Restrict of **Ch**emicals) (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 **(unknown)** chemicals in food. *(2011)*

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** (612/900…… compounds)
- Structural information based on an algorithm developed in 1978 by Cramer *et al.* were used
  - The chemicals were grouped into three structural classes based on a "decision tree" approach that consists of 33 questions each of which is answered by "yes" or "no. Each answer leads to another question or to a final classification into one of the three classes

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

137

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

27

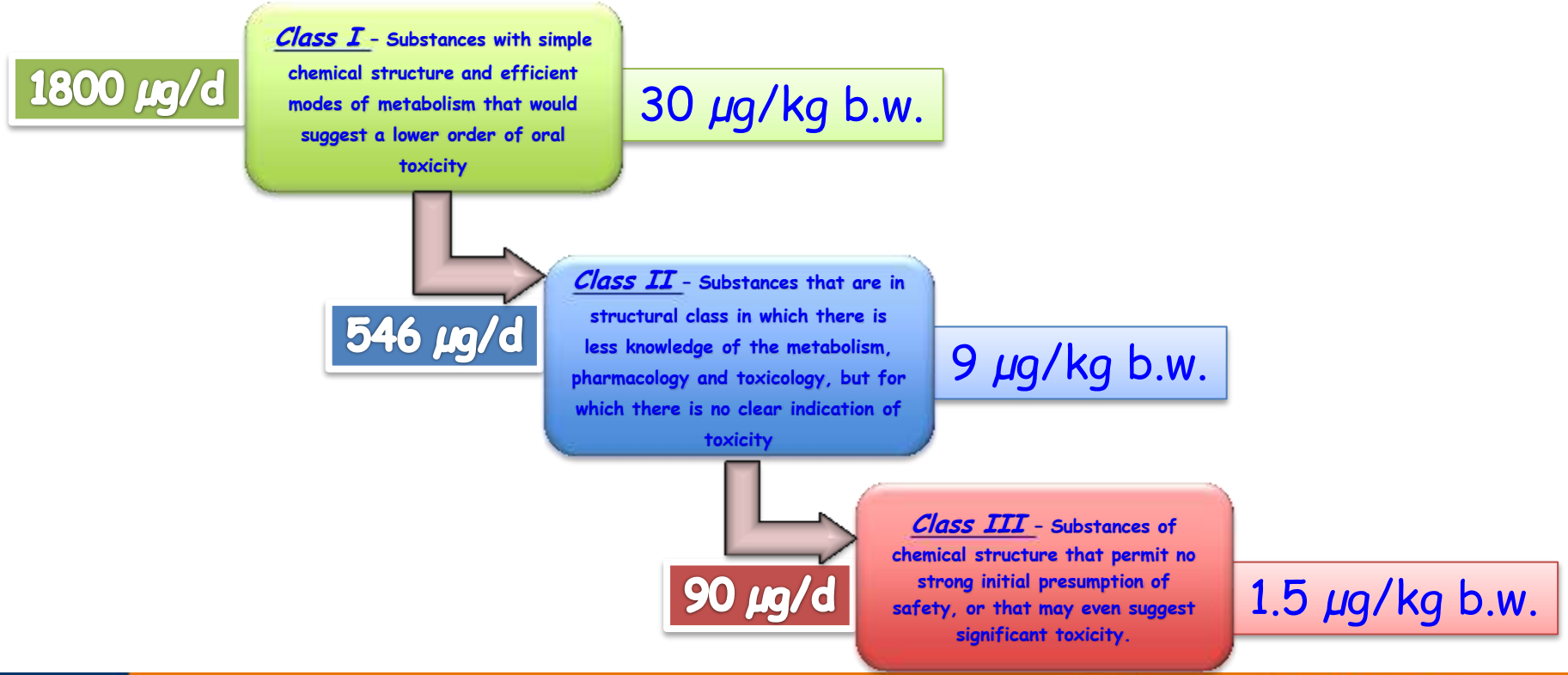
Class III - Substances of chemical structure that permit no strong initial presumption of safety, or that may even suggest significant toxicity.

448

Munro et al., 1996

CRAMER CLASSIFICATION TREE

TTC EXPOSURE LIMITS



CRAMER CLASSIFICATION TREE

TTC EXPOSURE LIMITS

30 µg/kg b.w.

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

1800 µg/d

9 µg/kg b.w.

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

546 µg/d

1.5 µg/kg b.w.

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

90 µg/d

0.3 µg/kg b.w.

Organo Phosphates and Carbamates

18 µg/d

0.0025 µg/kg b.w.

With structural alert for genotoxicity

0.15 µg/d

THRESHOLD OF TOXICOLOGICAL CONCERN (TTC)

COHORT OF CONCERN (COC)

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)*

- **Inorganic chemicals, metals and organometallics** (not included in the data base)
- **High molecular weight chemicals such as polymers** (not included in database)
- **Nanomaterial** (not included in database)
- **Radioactive substances** (not included in database)
- **Organo-silicon compounds** (not included in database)
- **Proteins** (not included in database and……..risk of allergenicity)

MARGIN OF SAFETY
AND
SYSTEMIC EXPOSURE DOSAGE

MARGIN OF SAFETY (MoS)

$$MoS = \frac{NOAEL}{SED}$$

- 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**.

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.

DERMAL ABSORPTION REPORTED AS A PERCENTAGE OF THE AMOUNT OF SUBSTANCE APPLIED

$$SED = A\ (mg/kg\ bw/day) \times C\ (\%)/100 \times DAp\ (\%)/100$$

SED (mg/kg bw/day) = Systemic Exposure Dosage

A* (mg/kg bw/day) = Estimated daily exposure to a cosmetic product per kg body weight, based upon the amount applied and the frequency of application: see the calculated relative daily exposure levels for different cosmetic product types.

C (%) = the Concentration of the substance under study in the finished cosmetic product on the application site

DAp (%) = Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real- life conditions

*data given

SED CALCULATION RESULTING FROM EXPOSURE TO A SUBSTANCE USED IN PRODUCTS FOR PRACTICES OF TATTOO AND PERMANENT MAKEUP

Maximum daily amount of product applied	=	1g
Average percentage of chemical in permanent makeup	=	0,00072 %
Dose of the substance	=	0,072 mg
Cutaneous absorption (worst case 100%)	=	100 %
Absorbed substance	=	0,072 mg
Adults body weight average	=	60 kg

SED = ($1*10^3$ mg/d * 0,00072/100 * 100/100) / 60kg = 0,00012 mg/kg p.c.

SED CALCULATION RESULTING FROM EXPOSURE TO A SUBSTANCE USED IN PRODUCTS FOR PRACTICES OF TATTOO AND PERMANENT MAKEUP

$$MoS = \frac{NOAEL = 5\ mg/kg\ b.w.}{SED = 0.00012\ mg/kg\ b.w.} = 41.166$$

COSMETIC PRODUCT FORMULATED WITH 100% OF THE DYE CONTAINING ONLY THE MAXIMUM ALLOWED LEVEL OF EACH HEAVY METAL CONTEMPLATED BY LAW.

Metal	ppm
Antimony	10
Arsenic	5
Cadmium	5
Cobalt	70
Chromium (III)[1]	100
Chromium (VI)	25
Mercury	1
Nickel	200
Lead	20

(1) Amount of chromium (III) potentially including from impurities of chromium (VI) that can reach a maximum value of 25 ppm. The data is valid for cosmetic products that do not contain dyes, chromium-based.

PRESENCE OF CHROMIUM IN COSMETIC PRODUCTS

CROMO (VI)

NOAEL: 2,5 mg/kg p.c./giorno

Una RfD cronica orale di 0,003 mg di cromo(VI)/kg p.c. al giorno è stata calcolata e verificata dall'Environmental Protection Agency americana EPA-USA per i sali solubili di cromo(VI). La RfD è basata su un NOAEL per effetti sistemici nel ratto esposto a 2,5 mg di cromato di potassio in acqua da bere per 1 anno in uno studio condotto da MacKenzie et al. (1958).

ASSORBIMENTO ORALE: 3%

Nell'uomo solo una piccola frazione (0,5-3%) del cromo ingerito viene assorbito a livello gastrointestinale (Christensen, 1995; Paustenbach et al., 1996).

PRESENCE OF CHROMIUM IN COSMETIC PRODUCTS

CHROMIUM (VI)

Product type	Amount theoretically present in cosmetic product (ppm)	max % theoretically present in cosmetic product	% dermal absorption	SED (mg/kg b.w./day)	NOAEL (mg/kg b.w./day)	MoS (NOAEL/SED)
Facial make-up	25	0.0025	3	6.37×10^{-6}	2.5	392465
Eye shadow	25	0.0025	3	2.50×10^{-7}	2.5	10000000
Mascara	25	0.0025	3	3.12×10^{-7}	2.5	8012821
Eyeliner	25	0.0025	3	6.25×10^{-8}	2.5	40000000
Lipstick	25	0.0025	3	7.12×10^{-7}	2.5	3511236
Powdered fard	25	0.0025	3	6.25×10^{-6}	2.5	400000
All products				1.39×10^{-5}	2.5	179856

PRESENCE OF CADMIUM IN COSMETIC PRODUCTS

CADMIO

$BMDL_5$: 0,004 mg/kg p.c./giorno

L'EFSA Contaminant Panel ha calcolato il valore di $BMDL_5$ che produce un cambiamento specifico dei livelli urinari di B2M (beta-2-microglobulina) pari a 4 µg di cadmio/g creatinina nelle urine. Per rimanere al di sotto di 4 µg cadmio/g creatinina nelle urine, è stato calcolato che l'assunzione media giornaliera di cadmio nella dieta non dovrebbe superare 1,44 µg/kg di peso corporeo (The EFSA Journal, 2011).

ASSORBIMENTO CUTANEO: 0,8%

L'assorbimento cutaneo considerato è molto basso tra 0,3-0,8% (ATSDR, 1999).

PRESENCE OF CADMIUM IN COSMETIC PRODUCTS

CADMIO

Make-up	Quantità max di metallo presente nel cosmetico (ppm)	% max di metallo presente nel cosmetico	% di assorbimento dermico	SED (mg/kg p.c./ giorno)	$BMDL_5$ (mg/kg p.c./ giorno)	MoS ($BMDL_5$/ SED)
Fondotinta	5	0.0005	0.8	$3.40x10^{-7}$	0.004	11765
Ombretto	5	0.0005	0.8	$1.33x10^{-8}$	0.004	300000
Mascara	5	0.0005	0.8	$1.67x10^{-8}$	0.004	240000
Matita	5	0.0005	0.8	$3.33x10^{-9}$	0.004	1200000
Rossetto	5	0.0005	0.8	$3.80x10^{-8}$	0.004	105263
Polveri per il viso	5	0.0005	0.8	$3.33x10^{-7}$	0.004	12000
Tutti i prodotti				$7.44x10^{-7}$	0.004	5714

PRESENCE OF NICKEL IN COSMETIC PRODUCTS

NICHEL

NOAEL: 2,2 mg/kg p.c./giorno

In uno studio di due generazioni su ratti, è stato identificato un NOAEL di 2,2 mg di nichel per kg di peso corporeo al giorno per tutti gli end-point studiati, tra cui la letalità post-impianto/perinatale (SLI, 2000; UE, 2004).

ASSORBIMENTO CUTANEO: 0.2%

Studi *in vitro* su pelle umana hanno indicato un assorbimento cutaneo di nichel di meno del 2% che si riduce all'1% considerando la quantità di nichel fissata dalle cellule dello strato corneo della cute (HEALTH RISK ASSESSMENT GUIDANCE FOR METALS - HERAG 01, 2007; Tanojo et al., 2001).

PRESENCE OF NICKEL IN COSMETIC PRODUCTS

NICHEL

Make-up	Quantità max di metallo presente nel cosmetico (ppm)	% max di metallo presente nel cosmetico	% di assorbimento dermico	SED (mg/kg p.c./ giorno)	NOAEL (mg/kg p.c./ giorno)	MoS (NOAEL/ SED)
Fondotinta	200	0.02	0.2	3.40×10^{-6}	2.2	647059
Ombretto	200	0.02	0.2	1.33×10^{-7}	2.2	16500000
Mascara	200	0.02	0.2	1.67×10^{-7}	2.2	13200000
Matita	200	0.02	0.2	3.33×10^{-8}	2.2	66000000
Rossetto	200	0.02	0.2	3.80×10^{-7}	2.2	5789474
Polveri per il viso	200	0.02	0.2	3.33×10^{-6}	2.2	660000
Tutti i prodotti				7.44×10^{-6}	2.2	29730

Fischer et al. reported that 5% of a sensitized population reacted to 0.44 μg Ni/cm^2 and 10% of a sensitized population reacted to 1.04 μg Ni/cm^2.

INTEGRATED ENVIRONMENTAL RISK CHARACTERIZATION

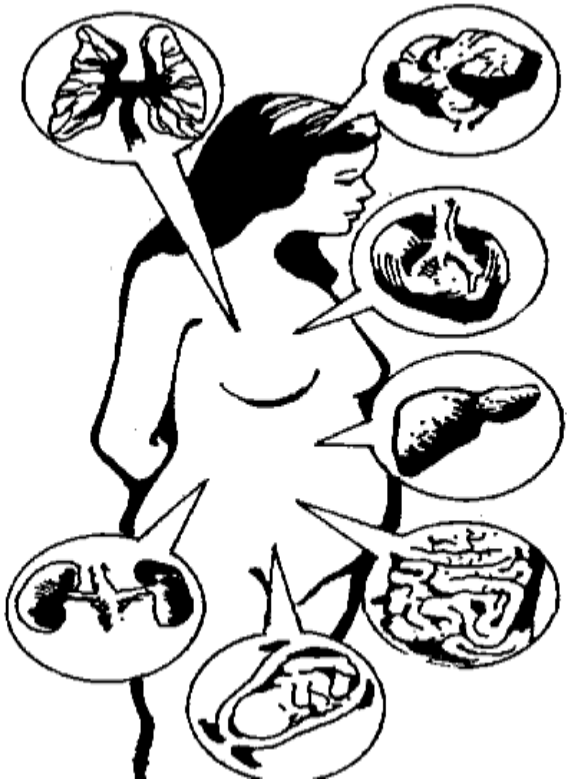
Lungs and respiratory systems:
cobalt, asbestos, sulphur oxides, ozone, nitrogen oxides, ammonia, carbon monoxide, cadmium, cigarette smoke, pesticides, animal and vegetable dusts.

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 Mode of Action (MoA).

TYPES OF COMBINED ACTIONS

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

SIMPLE SIMILAR 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

- 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

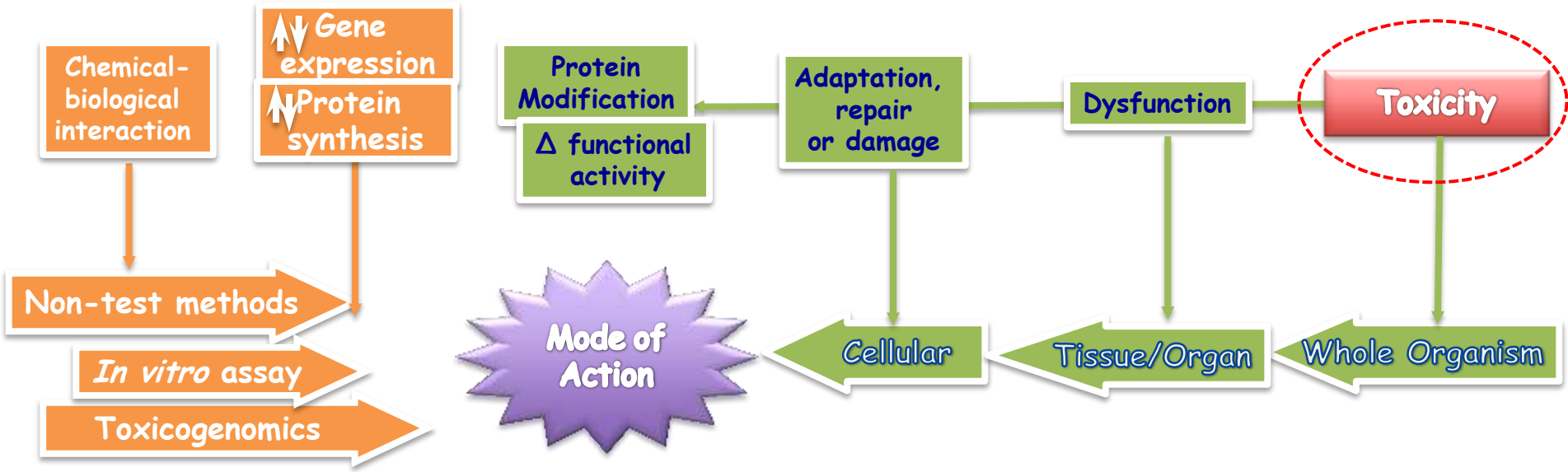
TYPES OF COMBINED ACTIONS

- 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



TOXICITY TESTING STRATEGIES

TIER 1

- ABSORPTION
- GENOTOXICITY

  In vitro testing
- TOXICITY (28-day/90-day study)

TRIGGERS FOR CONSIDERING TIER 2

- Systemic availability
- Toxicity in the 28/90-day study
- Genotoxicity *in vitro*

TOXICITY TESTING STRATEGIES

TIER 2

- **ADME**
  - Single dose
- **GENOTOXICITY**
  - *In vivo* testing
- **TOXICITY (stand alone or combined)**
  - Chronic toxicity
  - Carcinogenicity
- **REPRODUCTIVE & DEVELOPMENTAL TOXICITY**
  - Extended One-Generation Reproduction Toxicity Study
- **PRENATAL DEVELOPMENTAL TOXICITY (Teratogenicity)**

TRIGGERS FOR CONSIDERING **TIER 3**

- Bioaccumulation
- Positive in vivo genotoxicity
- Chronic toxicity/Carcinogenicity
- Reproductive & developmental toxicity

TOXICITY TESTING STRATEGIES

TIER 3

- ADME

  Repeated doses

- CARCINOGENICITY

  Mode of action

- REPRODUCTIVE & DEVELOPMENTAL TOXICITY

  Endocrine Disruptor?

- SPECIALIZED STUDIES

  Immunotoxicity

  Neurotoxicity

  Endocrine activity

  Mode of Action