UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACOLOGICHE E BIOMOLECOLARI

RISK ASSESSMENT:

APPROACHES ON EMERGING FOOD TOXICOLOGICAL ISSUES

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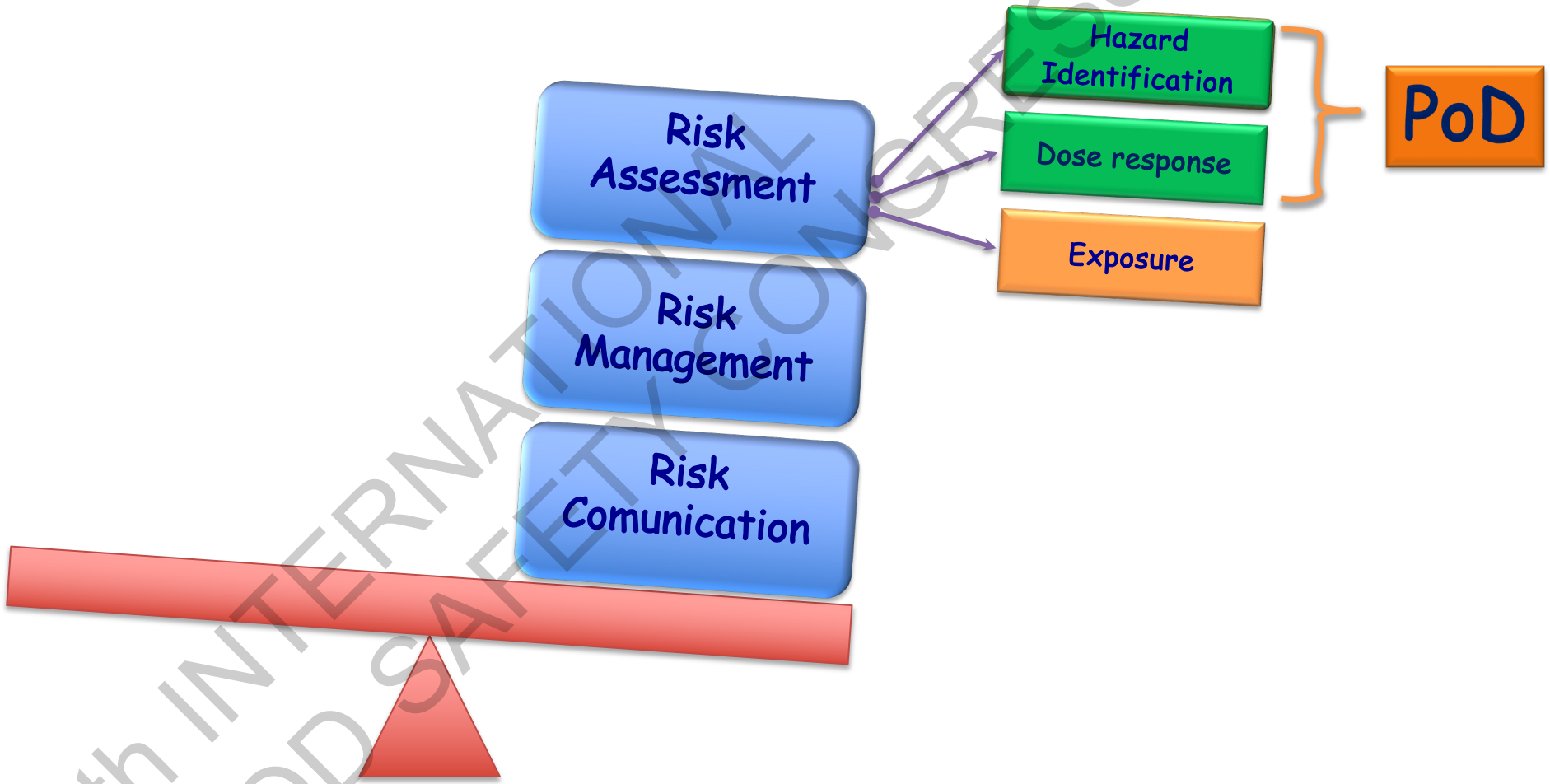
FOOD SAFETY: CURRENT TOPICS

7TH INTERNATIONAL FOOD SAFETY CONGRESS

GRAND CEVAHIR HOTEL & CONVENTION CENTER, ISTANBU

NOVEMBER 3-4, 2022

RISK ANALYSIS and RISK ASSESSMENT



LINES OF EVIDENCES and POINT of DEPARTURE

PoD

HBGV

POINTS OF DEPARTURE or REFERENCE POINTS

Toxicants or Non DNA reactive-Carcinogens

- No-Observed-(Adverse)-Effect-Level (NO(A)EL)
- Benchmark Dose (BMD)

DNA reactive-Carcinogens

- Benchmark Dose (BMD)
- TD25
- TD50

HAZARD ASSESSMENT

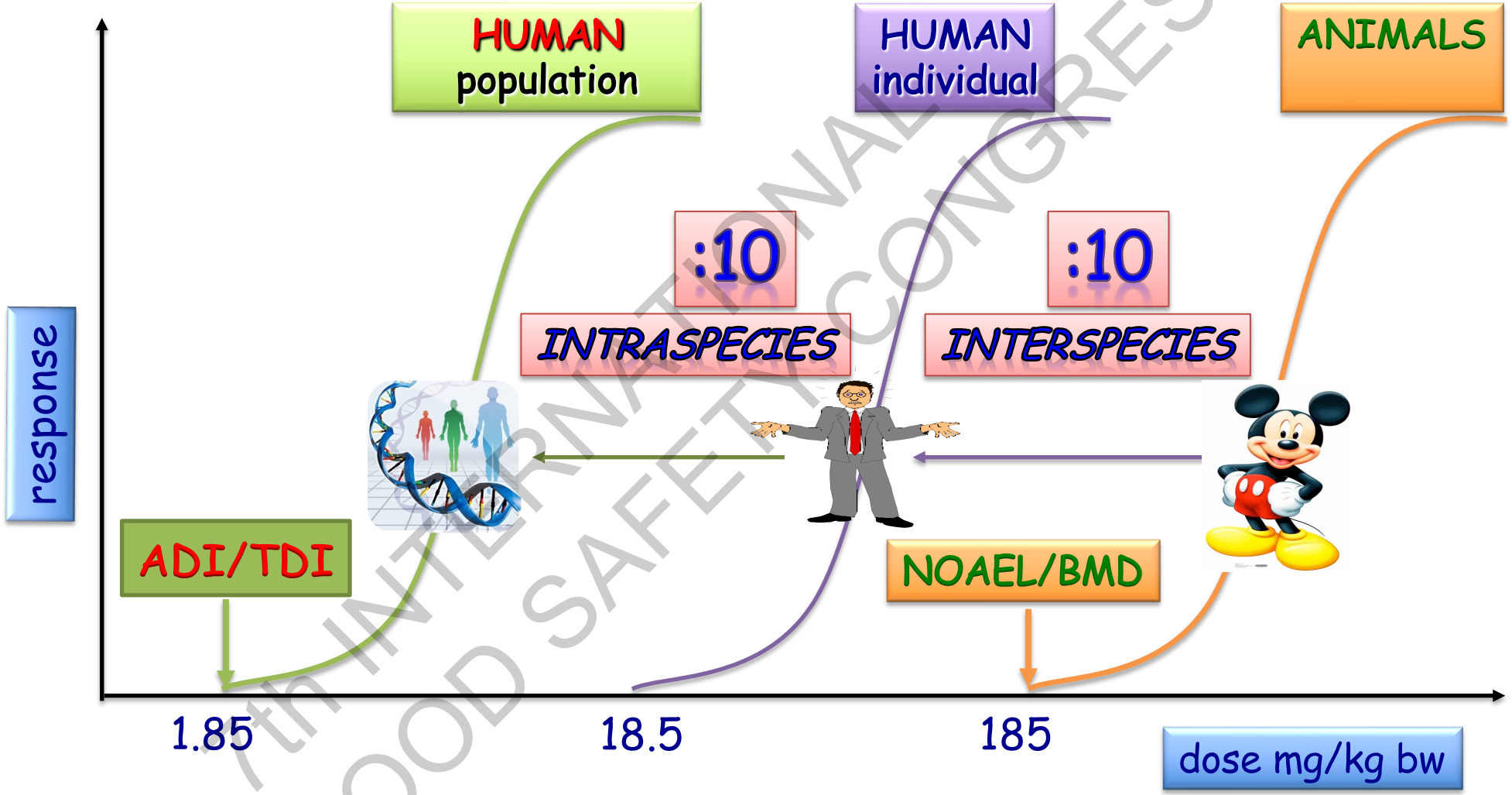
HEALTH BASED GUIDANCES VALUES

ADI (Acceptable Daily Intake)

TDI/TWI (Tolerable Daily/Weekly Intake)

ANIMAL-BASED TOXICOLOGICAL STUDIES

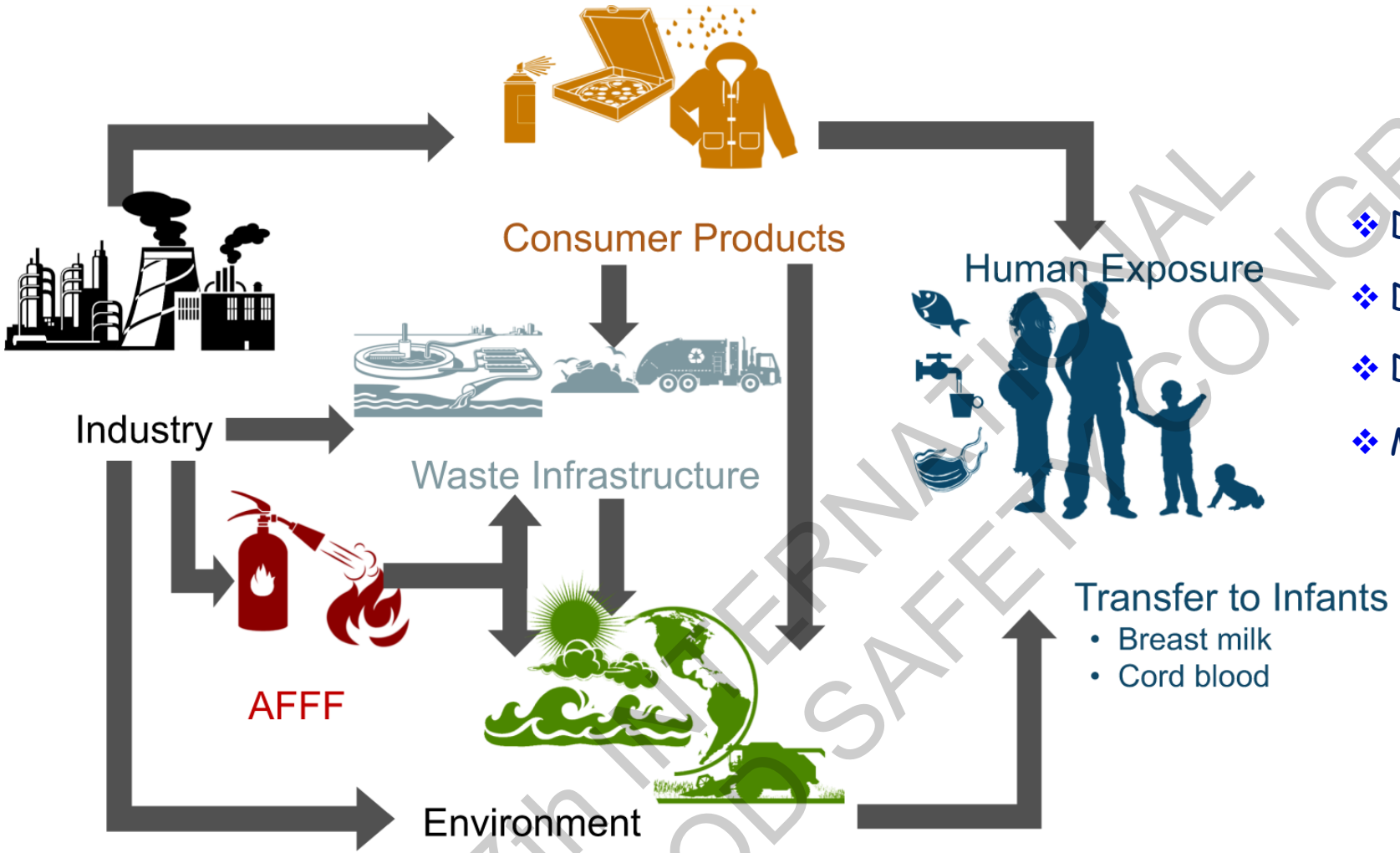
(quantification of adverse health effects)



LINES of EVIDENCES and WEIGHT of EVIDENCES

EXPOSURES

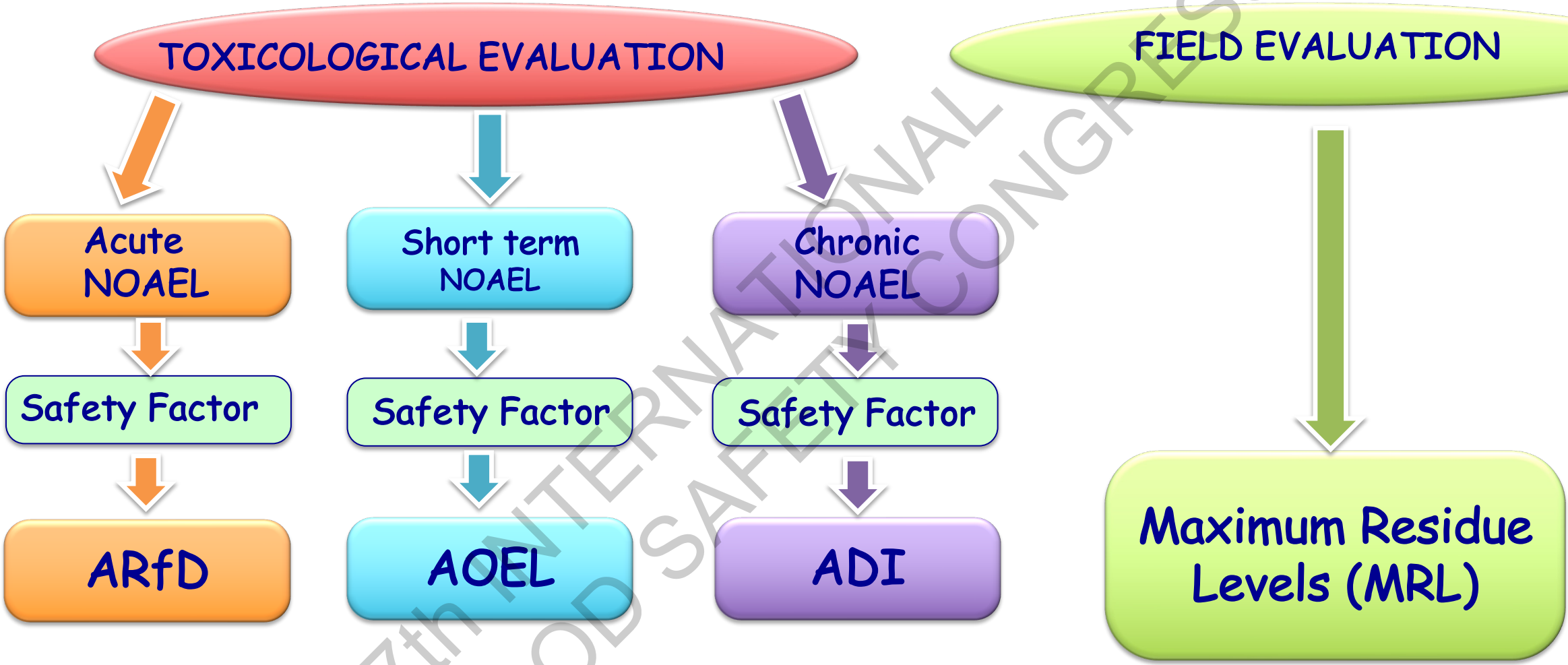
INTEGRATE RISK ASSESSMENT



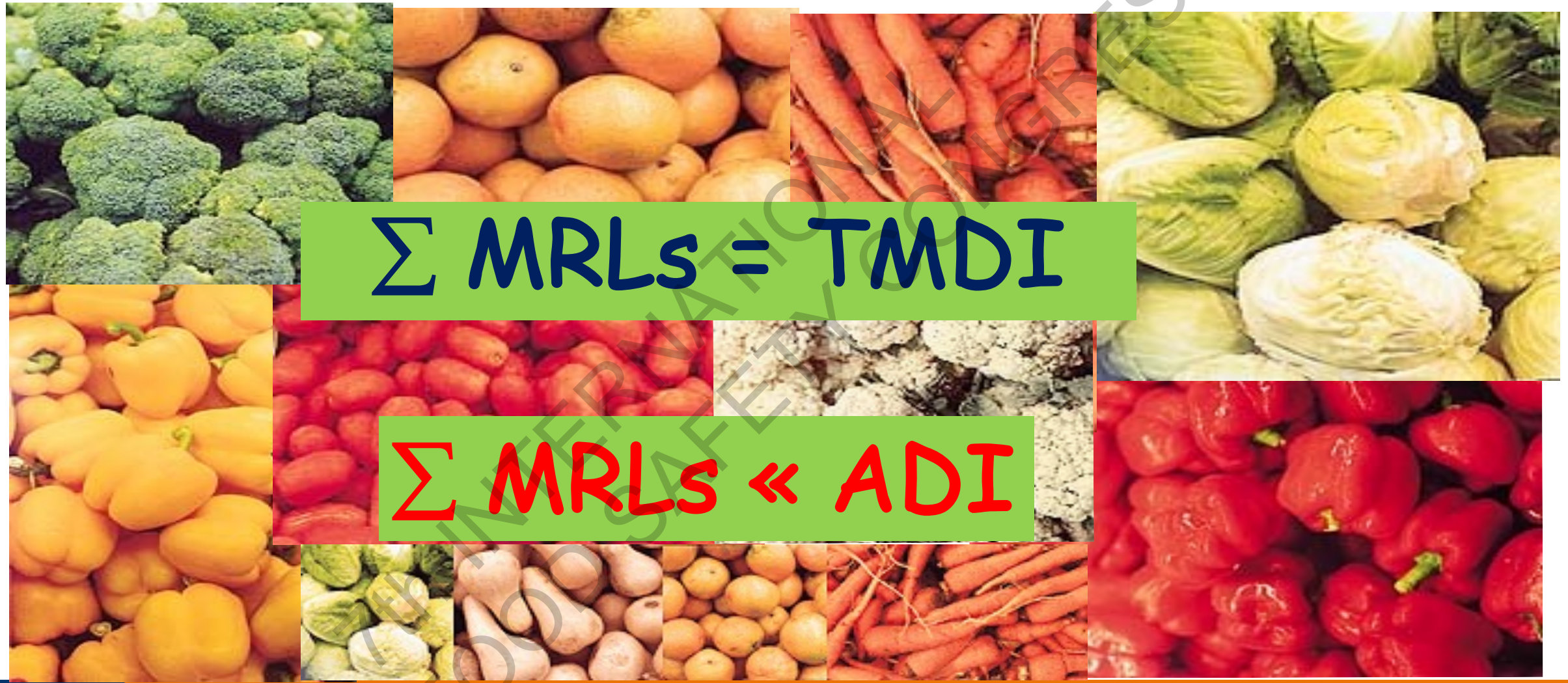
ACTIVE PRINCIPLE/METABOLITE

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

PESTICIDES RISK ASSESSMENT



CONSUMERS RISK ASSESSMENT



$\sum$ MRLs = TMDI

$\sum$ MRLs « ADI

EMERGING FOOD TOXICOLOGICAL ISSUES

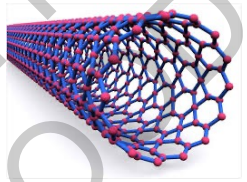
- **Food Additives** substances added to **food** to preserve flavor or enhance taste, appearance, or other sensory qualities.
- **Novel Food** substances without a history of "significant" consumption as of May 15, 1997 in the EU
- **Contaminants** substances that have not been intentionally added to food. (natural, bioaccumulating)
- **Nanomaterials** materials of which a single unit is sized (in at least one dimension) between 1 and 100 nm (the usual definition of nanoscale
- **Botanicals** preparations, based on plants, algae, fungi or lichens, are widely present on the European market in the form of food supplements.
- **Endocrine Disruptors** exogenous substance or a mixture, that alters function(s) of the endocrine system, causing adverse health effects in an intact organism, or its progeny, or (sub)populations
- **Mixture**
- **……insect proteins, microbiota**

NANOMATERIAL

Nanomaterial: a natural, incidental or manufactured material containing:

- particles in an unbound state, means a minute piece of matter with defined physical boundaries,
- particles as an aggregate. a collection of strongly bound particles with a defined external surface area.
- particles as an agglomerate, a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual aggregate components

Definition: 50% or more of the particles in the number-size distribution, one or more external dimensions is in the size range 1–100 nm.



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 of the particles** 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

FRAMEWORK FOR STEP-WISE HAZARD IDENTIFICATION AND RISK CHARACTERISATION

Step 0 In vitro digestion	
In vitro gastrointestinal digestion*	
Does the nanomaterial degrade quickly and fully under gastrointestinal tract conditions?	
yes	**not**
Expected not to show nanorelated behaviors	Step 1a and Step 1b

FRAMEWORK FOR STEP-WISE HAZARD IDENTIFICATION AND RISK CHARACTERISATION

STEP 1a Review existing information	STEP 1b Generate new in vitro data
❖ Review all existing physicochemical and toxicological information as well as information relevant to grouping/read-across.	❖ Dissolution under lysosomal conditions, ❖ *In vitro* genotoxicity and ❖ *In vitro* cell toxicity.

FRAMEWORK FOR STEP-WISE HAZARD IDENTIFICATION AND RISK CHARACTERISATION

In vitro tests for induction of gene mutations:

- AMES test (OECD TG 471 is not a recommended)

- Hypoxanthine-guanine phosphoribosyl transferase gene (Hprt) (OECD TG 476)
- Xanthine-guanine phosphoribosyl transferase gene (xprt) (OECD TG 476)

- Mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490)

In vitro tests for structural and numerical chromosome damage:

- Mammalian cell micronucleus test (OECD TG 487)

- Comet assay

The interpretation of the results from the in vitro genotoxicity studies would be supported by an assessment of cellular uptake (and nuclear uptake, if feasible) of nanoparticles.

FRAMEWORK FOR STEP-WISE HAZARD IDENTIFICATION AND RISK CHARACTERISATION

In vivo tests for induction of genotoxicity:

- Micronucleus test (OECD 474)
- Mammalian alkaline Comet assay (OECD 489)
- Transgenic rodent somatic and germ cell gene mutation assay (OECD 488)

FRAMEWORK FOR STEP-WISE HAZARD IDENTIFICATION AND RISK CHARACTERISATION

In vitro toxicity testing:

Co-culture as they more closely mimic conditions in vivo;

- Human colorectal epithelial cells (CaCo-2) combined with immune cells and mucus-secreting cells
- Primary human oesophageal epithelial cells, either in monoculture or (better) in co-culture, may be used to represent the gastrointestinal tract.
- Primary human monocyte-derived macrophages or human monocytic cell line THP-1 for immunotoxicity evaluation.

FRAMEWORK FOR STEP-WISE HAZARD IDENTIFICATION AND RISK CHARACTERISATION

Where in vitro methods indicate lack of toxic effects, and in vitro dissolution of the nanomaterial in lysosomal and gastrointestinal conditions is fast

an argument can be put forward for waiving in vivo studies on a case-by-case basis.

FRAMEWORK FOR STEP-WISE HAZARD IDENTIFICATION AND RISK CHARACTERISATION

STEP 2a
Pilot in vivo study

- A pilot study for dose finding and assessment of absorption, tissue distribution and accumulation and elimination phases (≈ 14d) is recommendable.

STEP 2b
In vivo studies

- *(In vivo genotoxicity)*
- Modified 90-day oral toxicity study

The results from this study can be used to identify a point of departure (such as BMDL or a NOAEL).

STEP 3
Targeted in-depth investigations

Additional toxicokinetic study (optionally in human studies),
reproductive and developmental toxicity,
immunotoxicity,
neurotoxicity,
carcinogenicity,
endocrine effects,
gut microbiome

SIMPLE QUESTIONS versus DIFFICULT ANSWERS

What material(s)

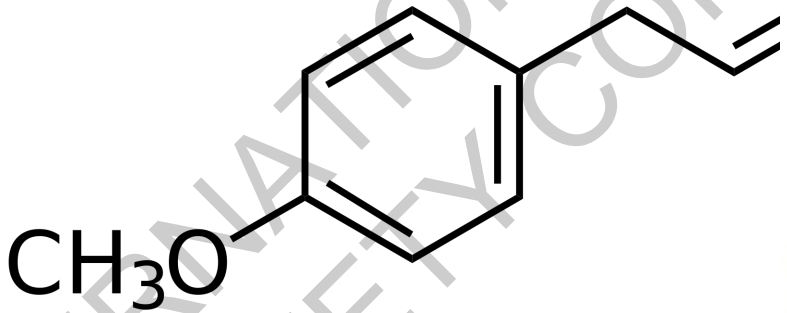
are we **exposed to**?

Single component ??

or

Complex mixture ??

?? MATRIX MATTERS ??



BASIL

ESTRAGOLE

PESTO

BASIL

The chemical composition of the essential oil of Basil oil varies according to the season.

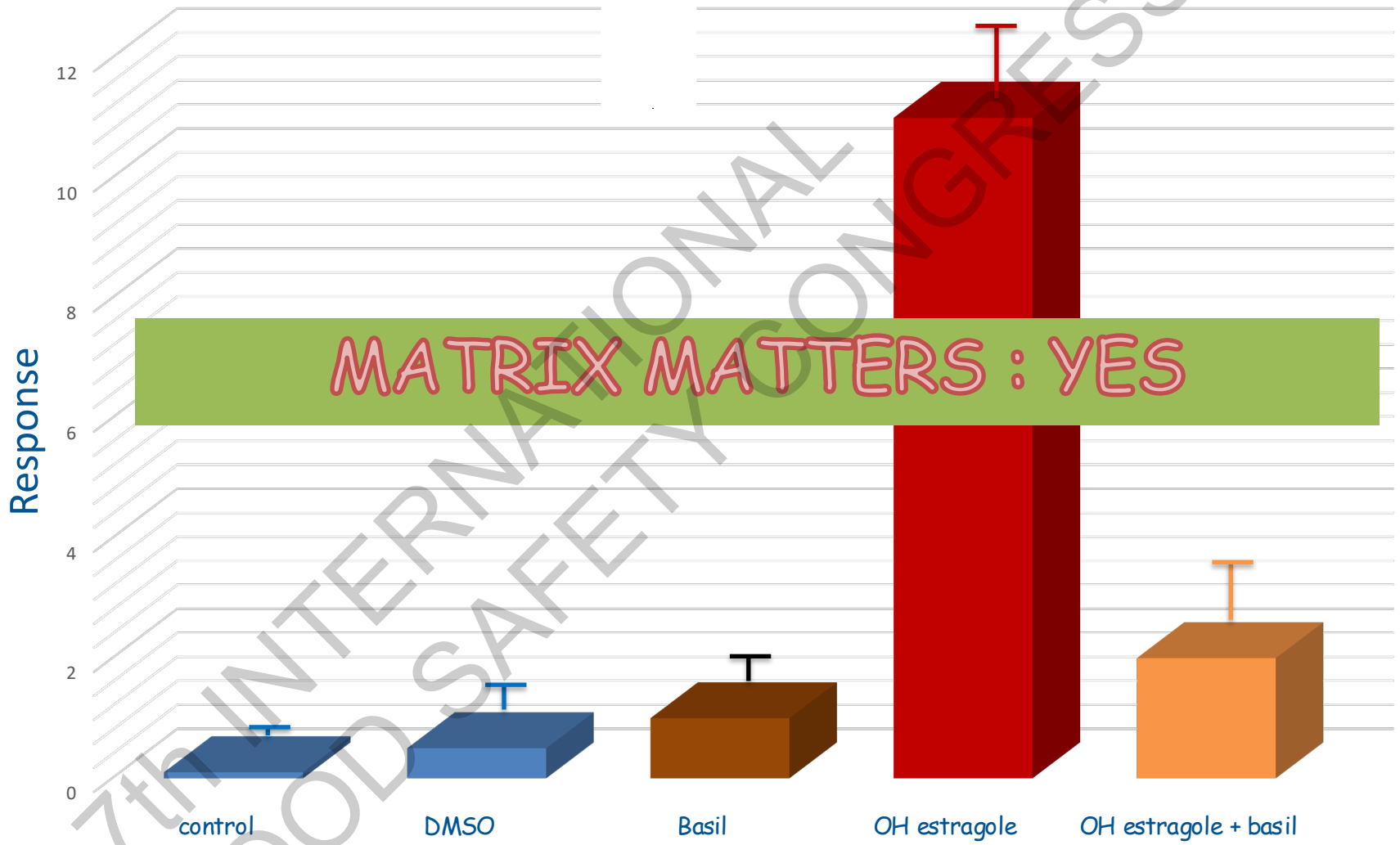
- Oxygenated monoterpenes (60.7-68.9%),
- Sesquiterpene hydrocarbons (16.0-24.3%)
- Oxygenated hexquiterpenes (12.0-14.4%).

- 29 compounds representing 98.0-99.7% of the oily composition

- Linalool the main constituent of essential oils (56.7–60.6%):
- epi-α-cadinol (8.6-1.4%),
- α-bergamotene (7.4-9.2%),
- γ-cadinene (3.3– 5.4%),
- germacrene D (1.1-3.3%) e
- camphor (1.1-3.1%).

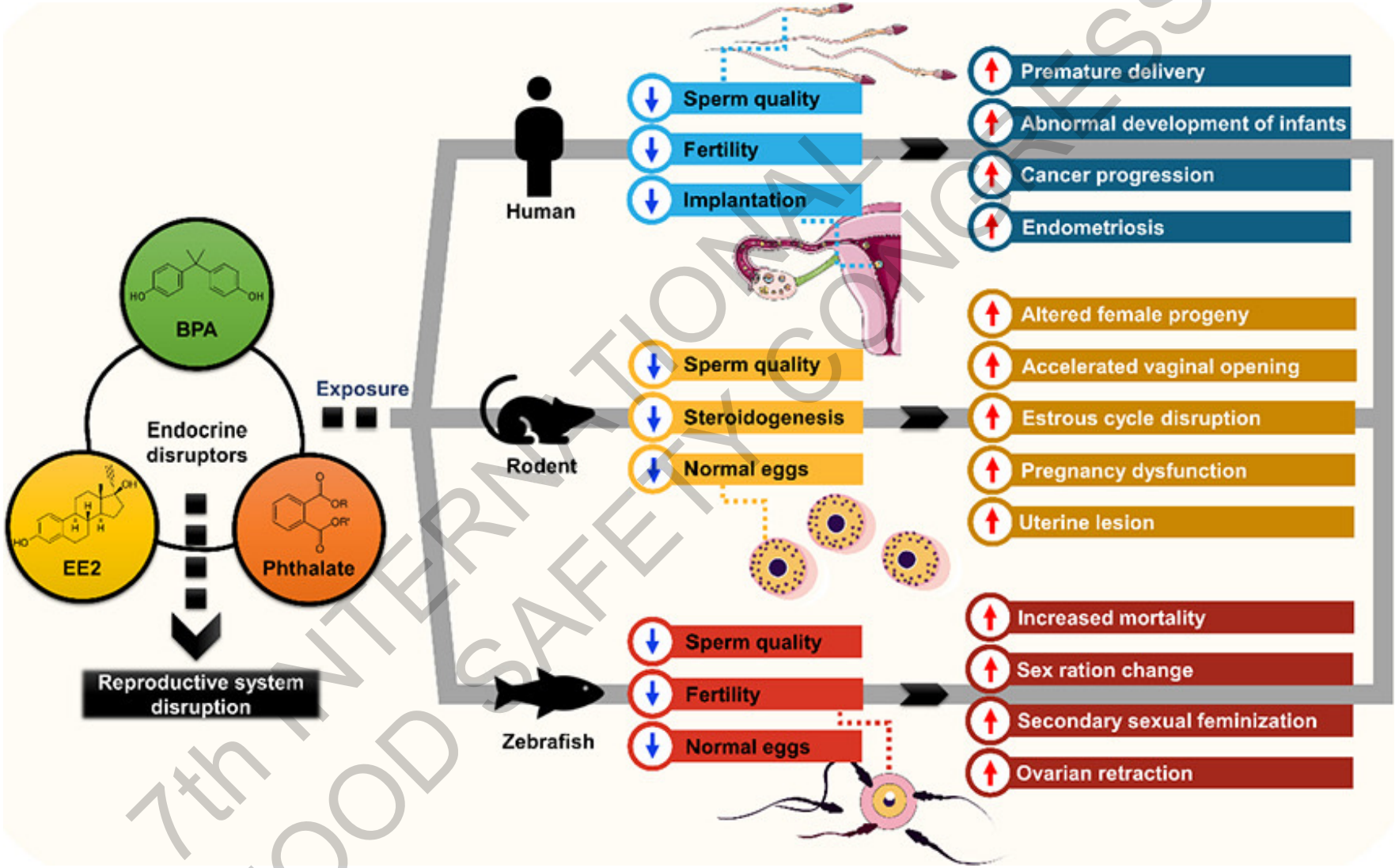
In addition, components such as methylchavikol, methylcinnamat, **estragole (< 0.5%)** , linolen, eugenol, cis-geraniol, 1,8-cineol, β-caryophyllene, and viridiflorol reported as important components

GENOTOXICITY



MATRIX MATTERS : YES

ENDOCRINE DISRUPTORS



CRITERIA FOR ENDOCRINE DISRUPTION

COMMISSION REGULATION (EU) 2018/605 OF 19TH APRIL 2018

An **active substance** **shall be considered as having endocrine disruption properties** that may cause adverse effect in humans if, it is a substance that meets all of the following criteria, unless there is evidence demonstrating that the adverse effect identified are **not relevant to humans**:

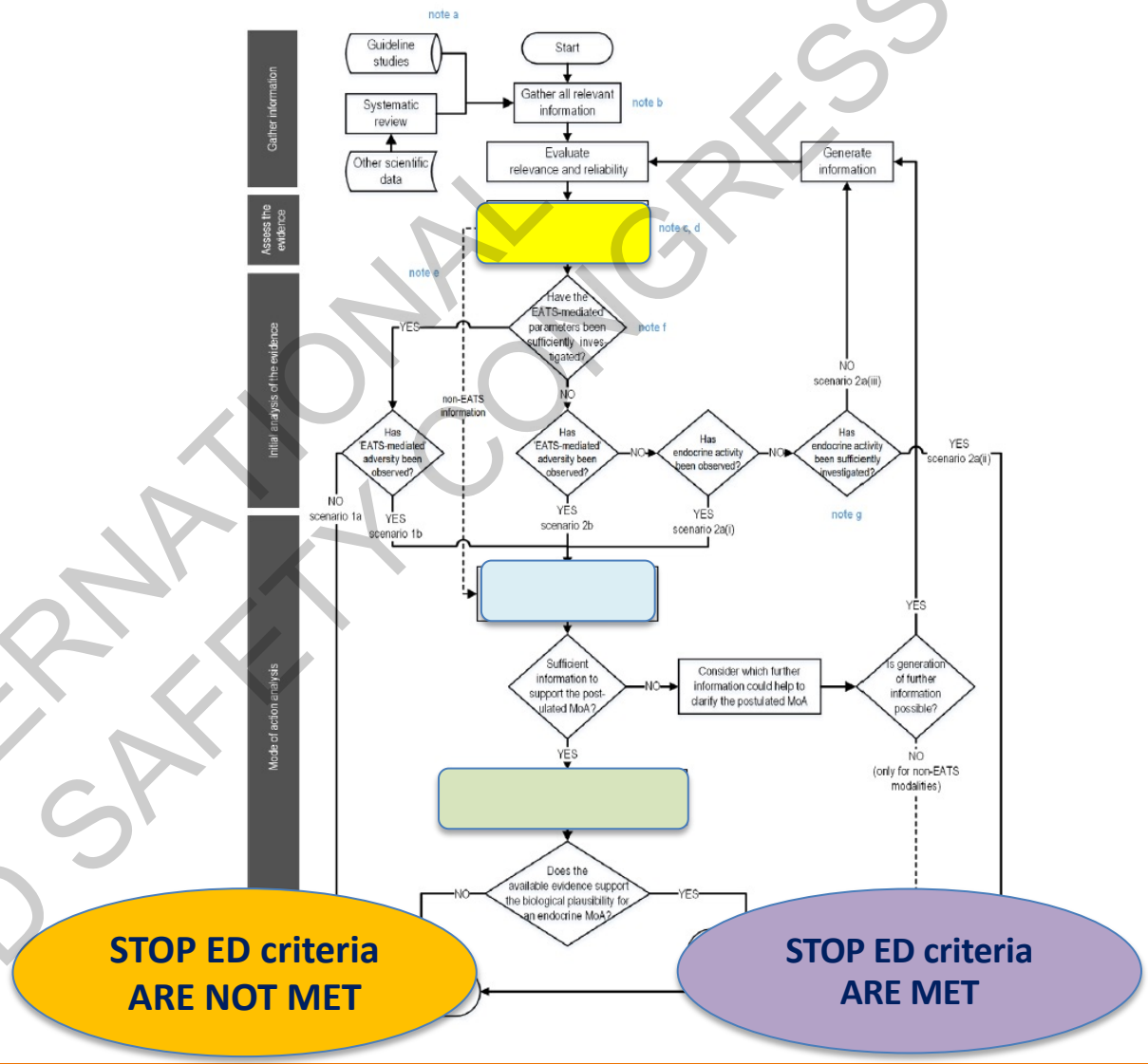
1. It shows an adverse effect in **an intact organism** or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub) population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
2. It has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;
3. The adverse effect is a consequence of the endocrine mode of action

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

Assemble, assess, intergrate and report the **lines of evidence for endocrine activity and adversity**

postulate MoA(s) considering the adversity and/or endocrine activity identified above

Establish the **biological plausibility** for the link between the adverse effect(s) and endocrine activity fcr the postulated MoA(s)



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Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

Level 1 — Existing data
- Existing or new non-test information

Level 2 — In vitro assays
- providing data about selected endocrine mechanism(s)/ pathways(s) (Mammalian and non mammalian methods)

Level 3 — In vivo assays
- providing data about selected **endocrine mechanism(s)/pathway**(s)(a)

Level 4 — In vivo assays
- providing data on **adverse effects** on endocrine relevant endpoints

Level 5 — In vivo assays
- providing more comprehensive data on **adverse effects on endocrine relevant endpoints** over more extensive parts of the life cycle of the organism(b)

ILGINIZ IÇIN TEŞEKKÜRLER.

SORULAR?