

Simposio:

BOTANICALS: CARATTERIZZAZIONE E VALUTAZIONE DELLA SICUREZZA

MATRICE VS SINGOLO COMPONENTE

Corrado Lodovico Galli - ERT

Wednesday February 12, 2020

RISK ASSESSMENT

- ① Assess the intrinsic **hazard** of a chemical and establish a level of safety;
- ② Determine the level of **exposure** to a chemical;
- ③ Compare the daily intake (*exposure*) with the *health based guidance values (HBGVs)* to ensure that the **risk is acceptable** in light of all the existing scientific evidence.



RISK ANALYSIS PARADIGM



ANIMAL-BASED TOXICOLOGICAL STUDIES

TOXICOKINETIC

- Absorption
- Distribution
- Metabolism
- Excretion

ACUTE TOXICITY

- LD₅₀ oral
- LD₅₀ dermal
- LC₅₀ inhalation
- Skin irritation
- Eye irritation
- Skin sensitization

GENOTOXICITY

- Mutagenesis
- Clastogenesis
- Aneuploidy

DEVELOPMENTAL TOXICITY

- Teratogenicity tests (Rat-Rabbit)

REPRODUCTIVE TOXICITY

- Two generation reproductive toxicity
- Extended One-Generation Reproductive Toxicity Study

SHORT-TERM TOXICITY

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

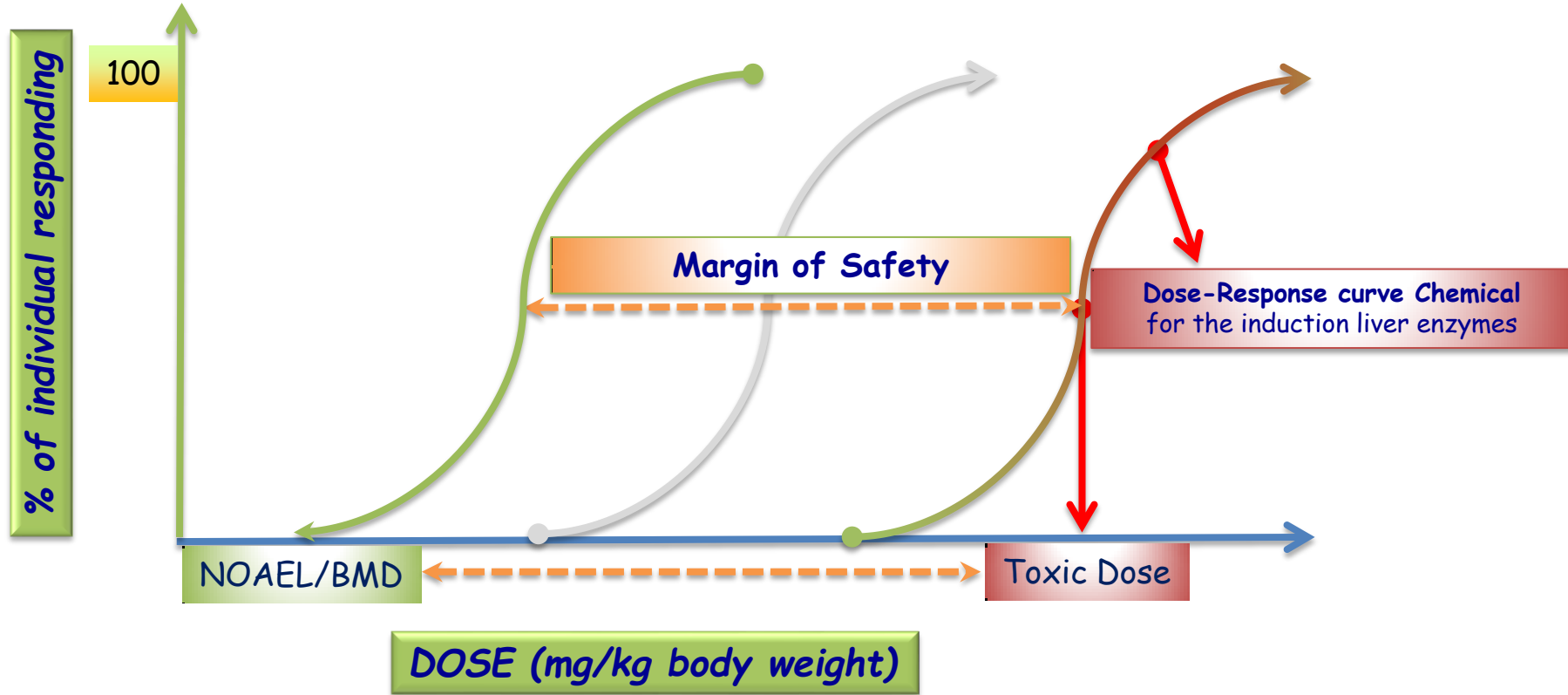
NOAEL/BMD

LONG-TERM TOXICITY AND/OR CARCINOGENICITY

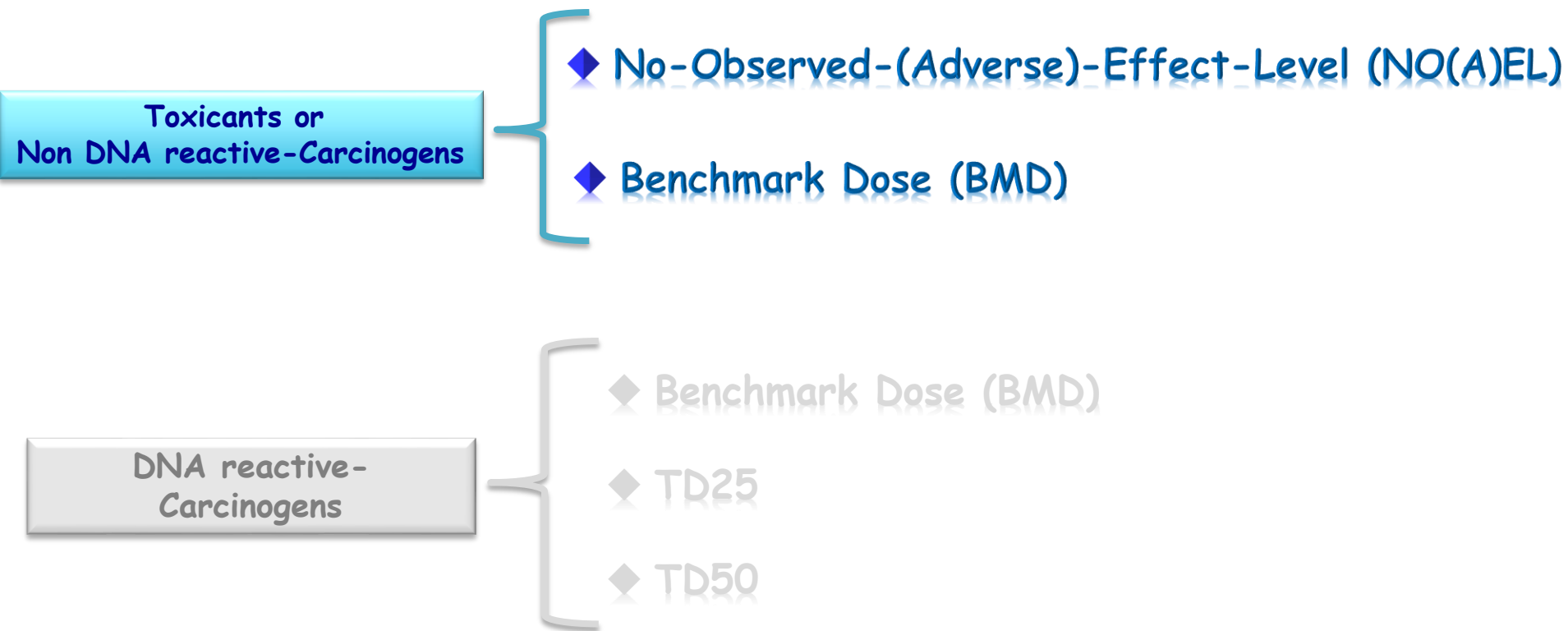
- Mouse 18 months
- Rat 104 weeks



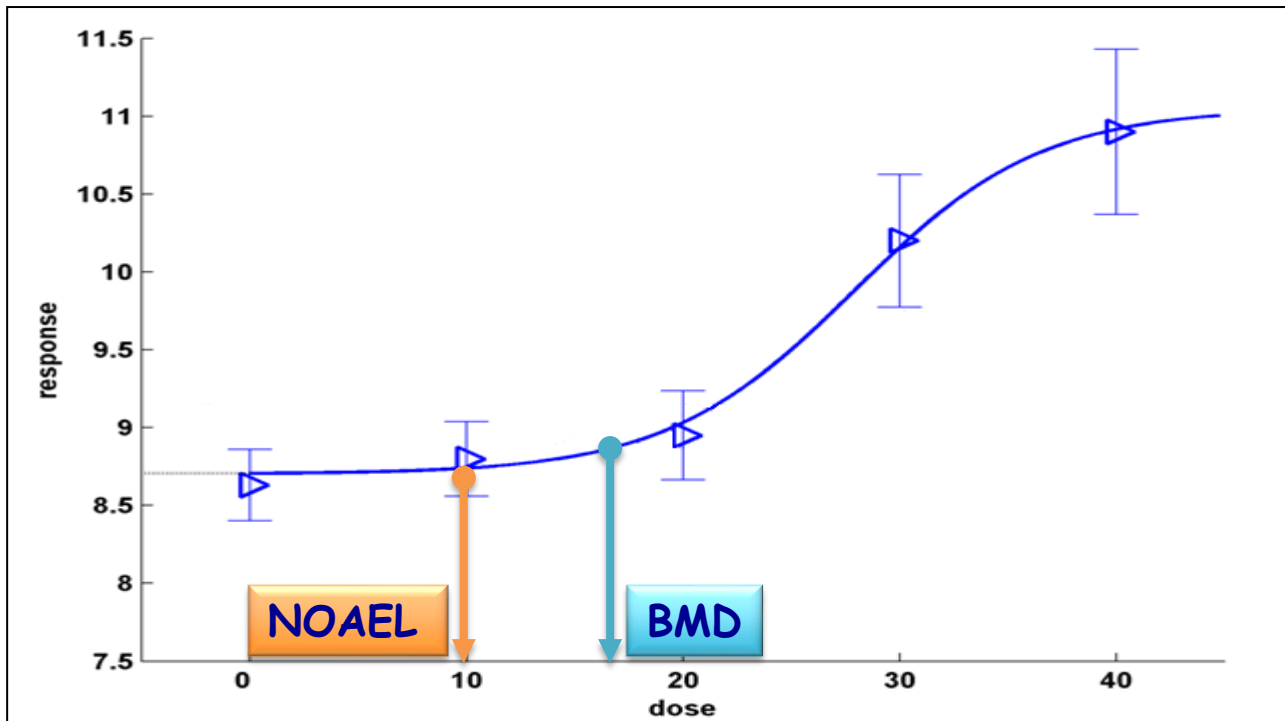
POINT of DEPARTURE (PoD): NO OBSERVED ADVERSE EFFECT (NOAEL)



REFERENCE POINTS - POINTS OF DEPARTURE

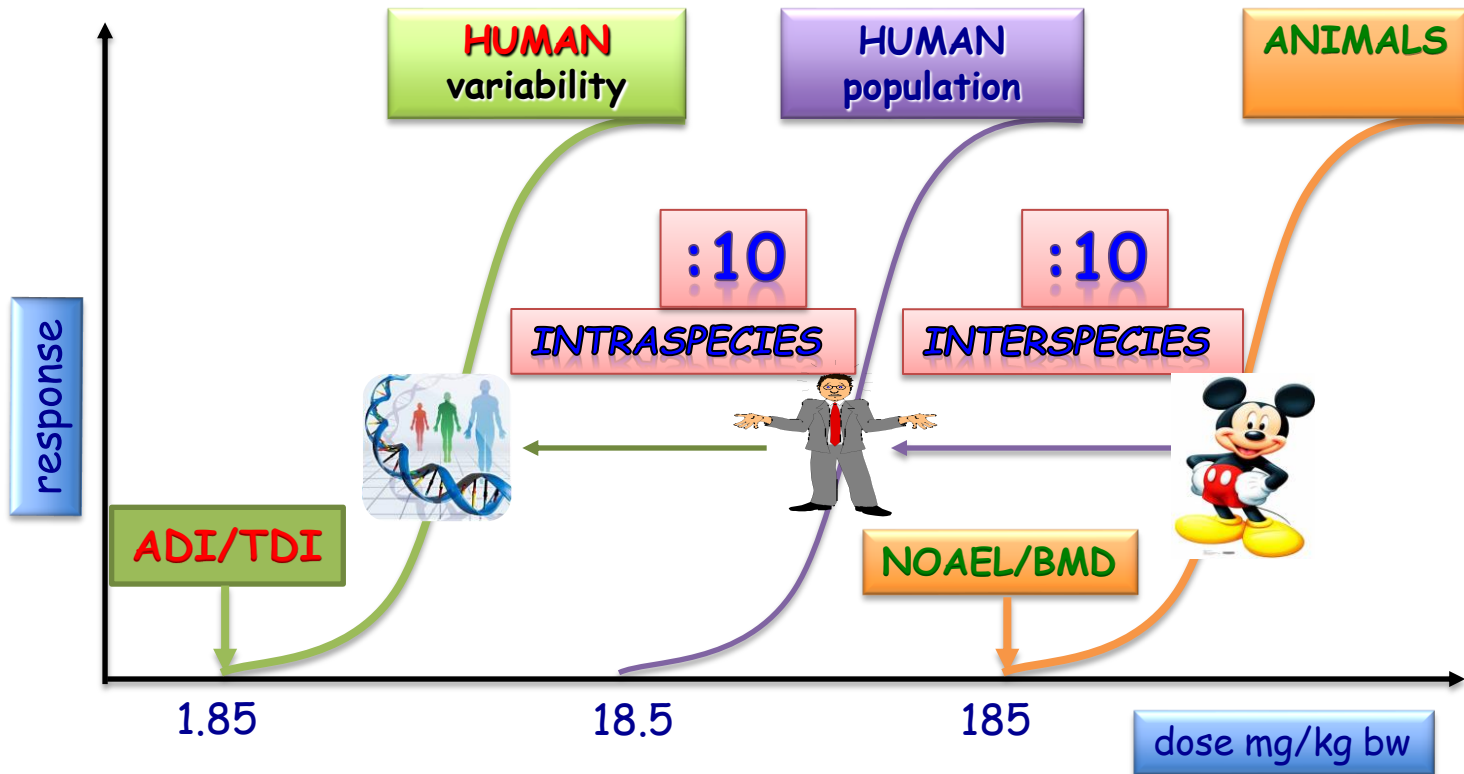


NOAEL and BMD



ANIMAL-BASED TOXICOLOGICAL STUDIES

(QUANTIFICATION OF ADVERSE HEALTH EFFECTS)



HEALTH BASED GUIDANCE VALUES (HBGVs)

NON-DNA-reactive carcinogens

ADI

(Acceptable Daily Intake)

TDI/TWI

(Tolerable Daily/Weekly Intake)

MoS

(Margin of Safety)

PDE

(Permitted Daily Exposure)

DNA-reactive carcinogens

MoE

(Margin of Exposure)

TTC

(Threshold of Toxicological Concern)



MARGIN OF EXPOSURE (MoE)

$$\text{MoE} = \frac{\text{NOAEL/BMD}}{\text{ESTIMATE DAILY INTAKE}}$$

NOAEL  25 mg/kg b.w.

EDI  0.0005 mg/kg/day

z MoE =

> 10,000



SCIENTIFIC OPINION

Guidance on Safety assessment of botanicals* and botanical preparations intended for use as ingredients in food supplements¹**

EFSA Scientific Committee²

BOTANICALS AND BOTANICAL PREPARATIONS

food supplements containing botanical ingredients

- - **botanical substances**, as whole plants, parts of them (such as roots, bark, flowers, leaves, fruits, seeds, etc), whole or crushed, but also of the juices obtained from pressing or incising the living plant (oleoresins, gums, latex, etc.) without specific treatments;
- - **botanical preparations**: obtained with treatments of the plants through extraction, distillation, pression, fraction, purification, concentration or crushing



GENERAL CONCERNS

CONTAMINATION

- both chemical and microbiological.
 - ❖ mycotoxines, pesticides, heavy metals (Cd, Pb, As, Hg), organic contaminants (IPA, PCB, Dioxins..), solvents residues,
 - ❖ as well as to the microbiological profile, mold toxins, bacterial spores, fungi.



GENERAL CONCERNS

(TEST) MATERIAL CHARACTERIZATION

Assurance that available toxicological data on related preparations are relevant to the final product as it will be marketed.

- It should be recognized that there may be significant differences in pharmacokinetic/dynamic behavior, such as the rate or extent of absorption, and pharmacological/toxic potency between a whole botanical and the equivalent amount of an isolated component.
- *i.e Stevia rebaudiana and Stevioside/Basil and Estragole*



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 the traditional intake to be expected due to the in intended levels of use as food supplement
 - if presence of otherwise toxic substances, comparison of the overall exposure with the existing safety le levels (e.g. ADI, TDI approach)
 - if presence of genotoxic and carcinogenic substances, (e.g. MoE and Threshold of Toxicological Concern tools).

HISTORY OF USE

It should be emphasised that
the absence of evidence of toxicity
is not the same as
evidence of the absence of toxicity



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

- **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...)

CASE REPORT ON WHEAT BRAN

WHEAT BRAN



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CASE REPORT ON ARTEMISIA ABSINTHIUM

ARTEMISIA ABSINTHIUM



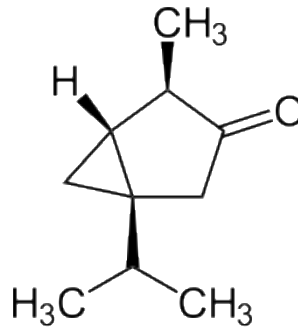
- # Guidance on Safety assessment of botanicals and botanical preparations
- # intended for use as ingredients in food supplements - EFSA Journal 2009; 7(9):1249



CASE REPORT ON ARTEMISIA ABSINTHIUM

ARTEMISIA ABSINTHIUM

THUJONE



CASE REPORT ON ARTEMISIA ABSINTHIUM

ARTEMISIA ABSINTHIUM

THUJONE

Neurotoxic

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CASE REPORT ON ARTEMISIA ABSINTHIUM

THUJONE

Neurotoxic

TDI 10 μ g/kg bw/day*

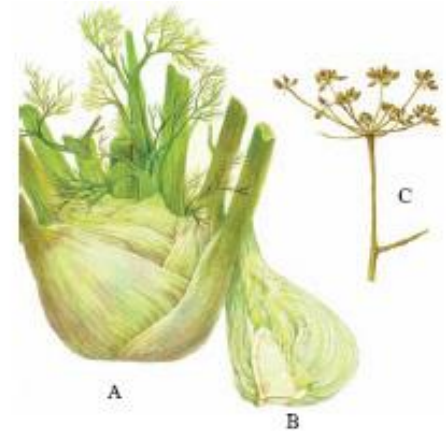
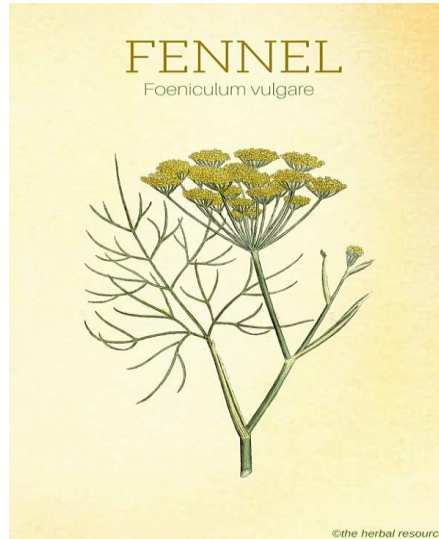
(Council of Europe 2005)

* NOAEL = 5 mg/kg bw (14 week study)

* S F = 500

CASE REPORT ON FOENICULUM VULGARE

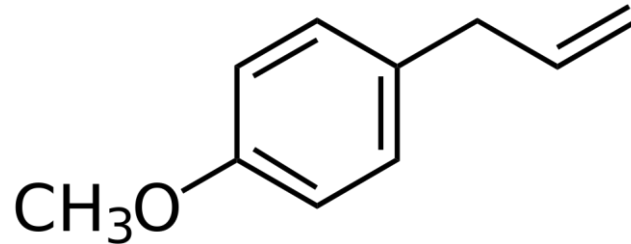
FOENICULIM VULGARE



CASE REPORT ON FOENICULUM VULGARE

FOENICULUM VULGARE

ESTRAGOLE



CASE REPORT ON FOENICULUM VULGARE

FOENICULIM VULGARE

ESTRAGOLE

both genotoxic and carcinogenic

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

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MARGIN OF EXPOSURE (MoE)

$$\text{MoE} = \frac{\text{BMDL}_{10}}{\text{ESTIMATE DAILY INTAKE}}$$

BMDL₁₀ → 9 - 33 mg/kg b.w.

EDI → 0.0033 - 0.263 mg/kg/day

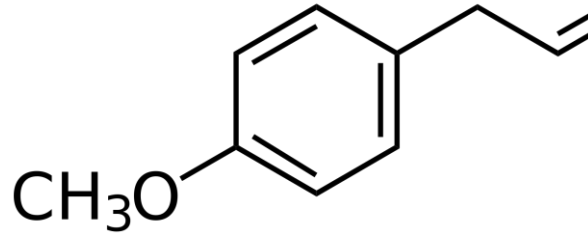
⌘ **MoE = 34 to 1000**



?? MATRIX MATTERS ??



BASILICO



ESTRAGOLE



PESTO

BASIL

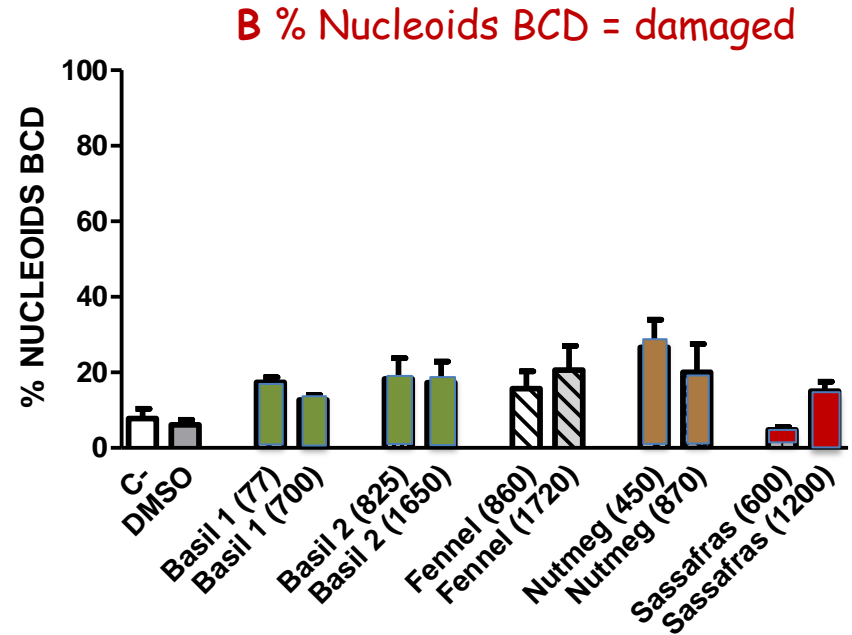
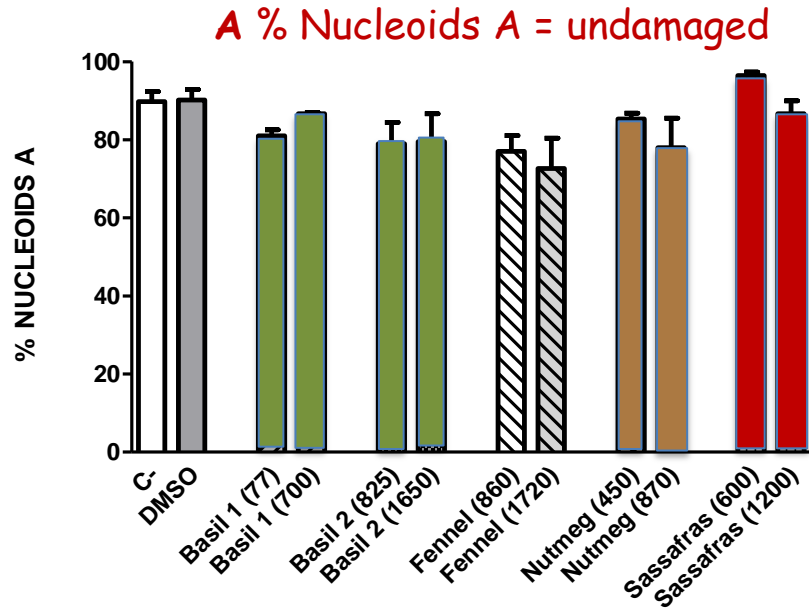
The chemical composition of the essential oil of Basil oil varies according to the season.

- 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 methylchavicol, methylcinnamat, **estragole**, linolen, eugenol, camphor, cis-geraniol, 1,8-cineol, α -bergamotene, β -caryophyllene, germacrene D, γ -cadinene, epi- α -cadinol and viridiflorol reported as important components



ALKALINE COMET TEST IN HEPG₂ CELLS - EXTRACTS (μg/mL)

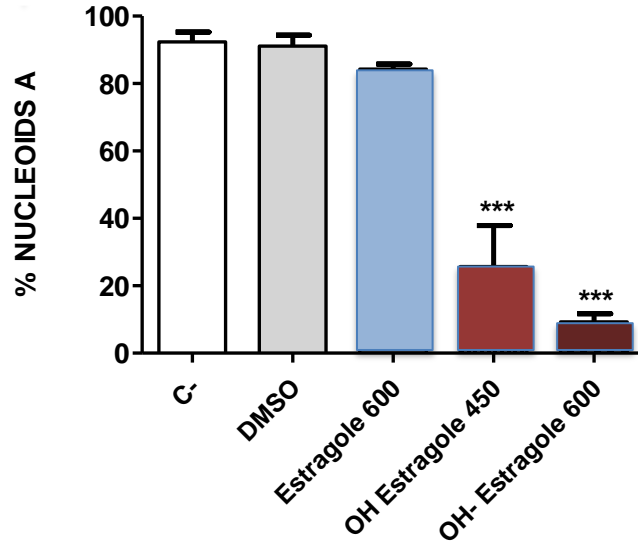


* p<0.05 **p<0.01 *** p<0.001 vs C

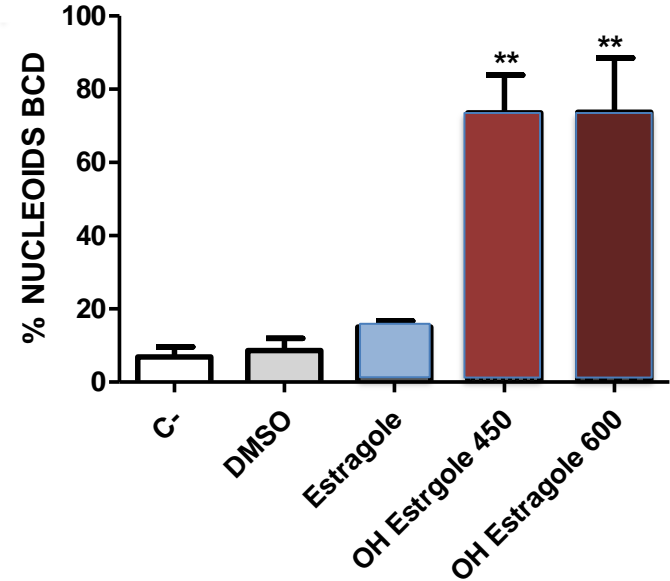


ALKALINE COMET TEST IN HEPG₂ CELLS - ESTRAGOL (μM)

A % Nucleoids A = undamaged



B % Nucleoids BCD = damaged

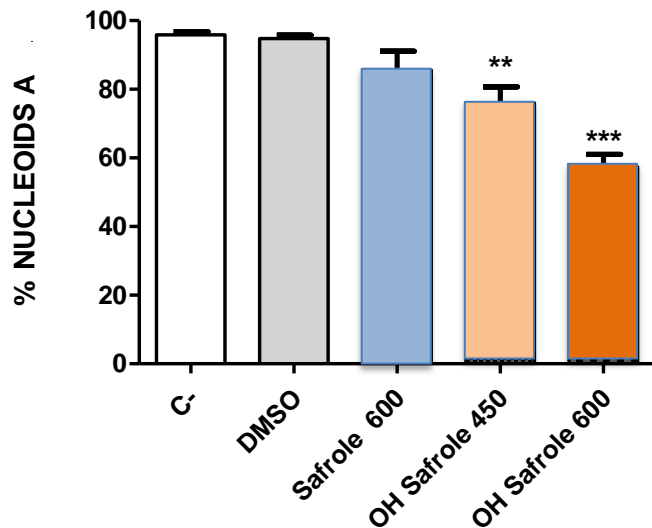


* p<0.05 **p<0.01 *** p<0.001 vs C

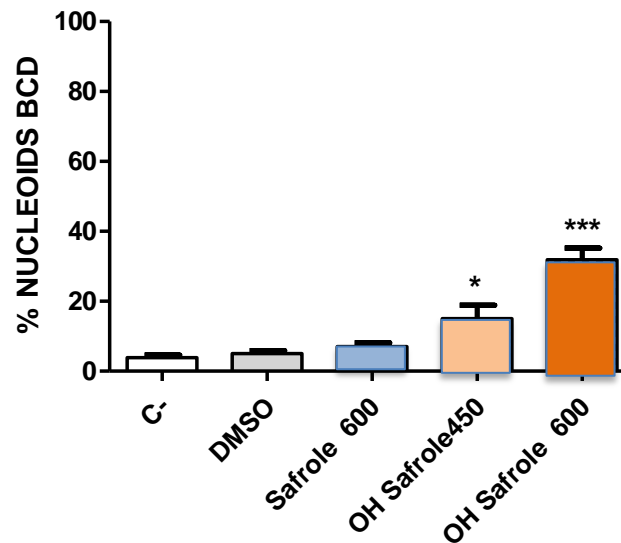


ALKALINE COMET TEST IN HEPG2 CELLS - SAFROLE (μM)

A % Nucleoids A = undamaged



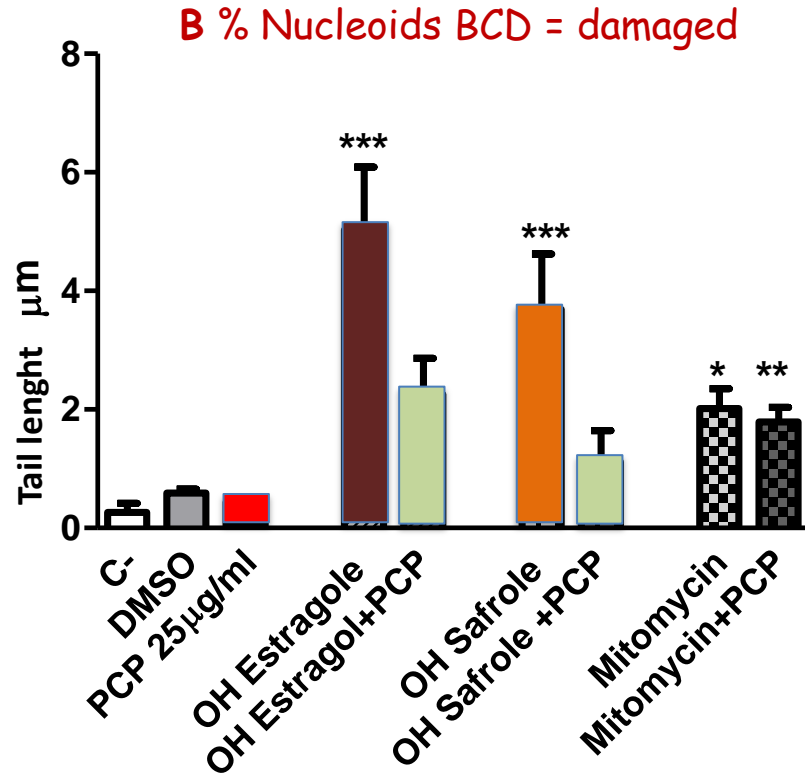
B % Nucleoids BCD = damaged



* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ vs C



ALKALINE COMET TEST IN HEPG₂ CELLS



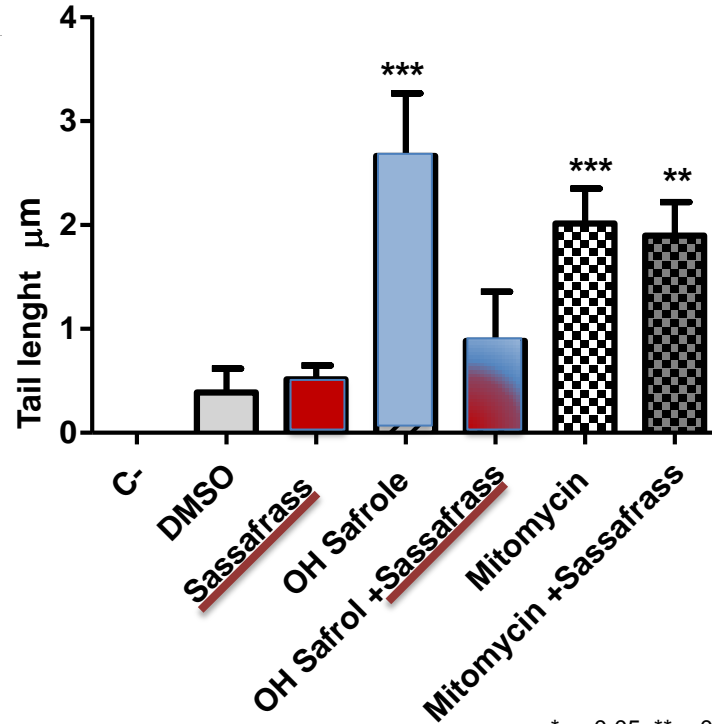
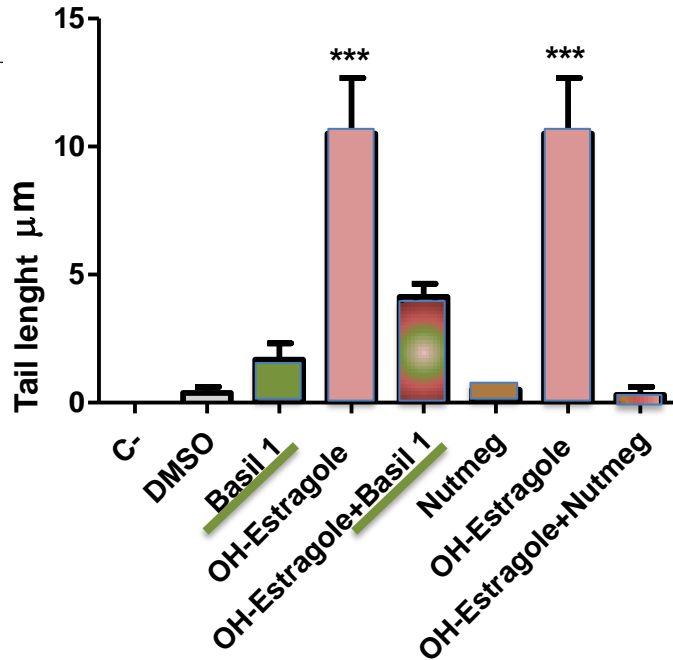
Pentachlorophenol (PCP), a substance which is able to inhibit sulfotransferase (SULTs) preventing the transformation of the 1-hydroxymetabolites to the ultimate carcinogenic 1'-sulfoxymetabolites

* p<0.05 **p<0.01 *** p<0.001 vs C



MATRIX MATTERS : YES

B % Nucleoids BCD = damaged



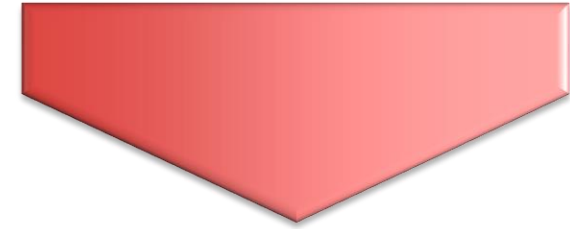
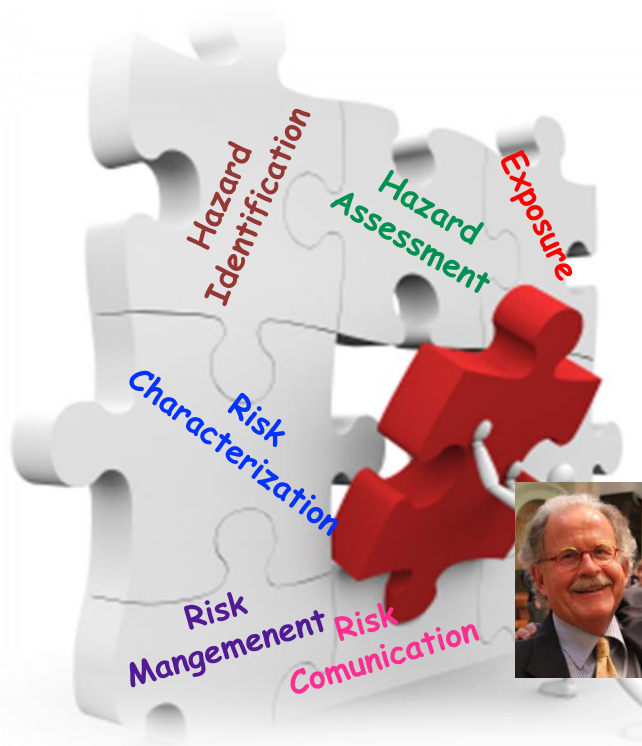
* p<0.05 **p<0.01 *** p<0.001 vs C



RISK ANALYSIS



TAKE HOME MESSAGE



Weight of Evidence

Expert Judgement

