UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACOLOGICHE E BIOMOLECOLARI

SIMPOSIO
BOTANICALS E PROBLEMATICHE DI SICUREZZA: UN BINOMIO INSCINDIBILE

SICUREZZA E EFFICACIA A CONFRONTO: IL CASO ALOE

CORRADO LODOVICO GALLI – ERT

20° Congresso Nazionale
Società Italiana di Tossicologia – SITOX
Sostanze di origine naturale: farmaci, veleni o entrambi
Bologna, 25-27 ottobre 2021 – Savoia Regency Hotel

POINT of DEPARTURE

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 22 November 2017 doi:

10.2903/j.efsa.2018.5090

Safety of hydroxyanthracene derivatives for use in food

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Maged Younes, Peter Aggett, Fernando Aguilar, Riccardo Crebelli, Metka Filipic, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Gunter Georg Kuhnle, Claude Lambre, Jean-Charles Leblanc, Inger Therese Lillegaard, Peter Moldeus, Alicja Mortensen, Agneta Oskarsson, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Raul J Andrade, Cristina Fortes, Pasquale Mosesso, Patrizia Restani, Fabiola Pizzo, Camilla Smeraldi, Adamantia Papaioannou and Matthew Wright

MEDICINAL PRODUCTS

According to the European Medicines Agency monographs, the maximum dosage should not exceed **30 mg/day** of hydroxyanthracene derivatives in medicinal products used as a laxative for adults, elderly and adolescents over 12 years

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WORLD HEALTH ORGANIZATION

It is recommended that products containing anthraquinone glycosides should not be used for longer than 1–2 weeks, due to possible incidence of electrolyte imbalance

World Health Organization, 1999. WHO Monographs On Selected Medicinal Plants, Vol. 1. World Health Organization, Geneva.

EFSA Scientific Opinion

Considering the possible presence of aloe-emodin and emodin in extracts,

the Panel concluded that

hydroxyanthracene derivatives should be considered as genotoxic and carcinogeni

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unless there are specific data to the contrary……..

……..although uncertainty persists.

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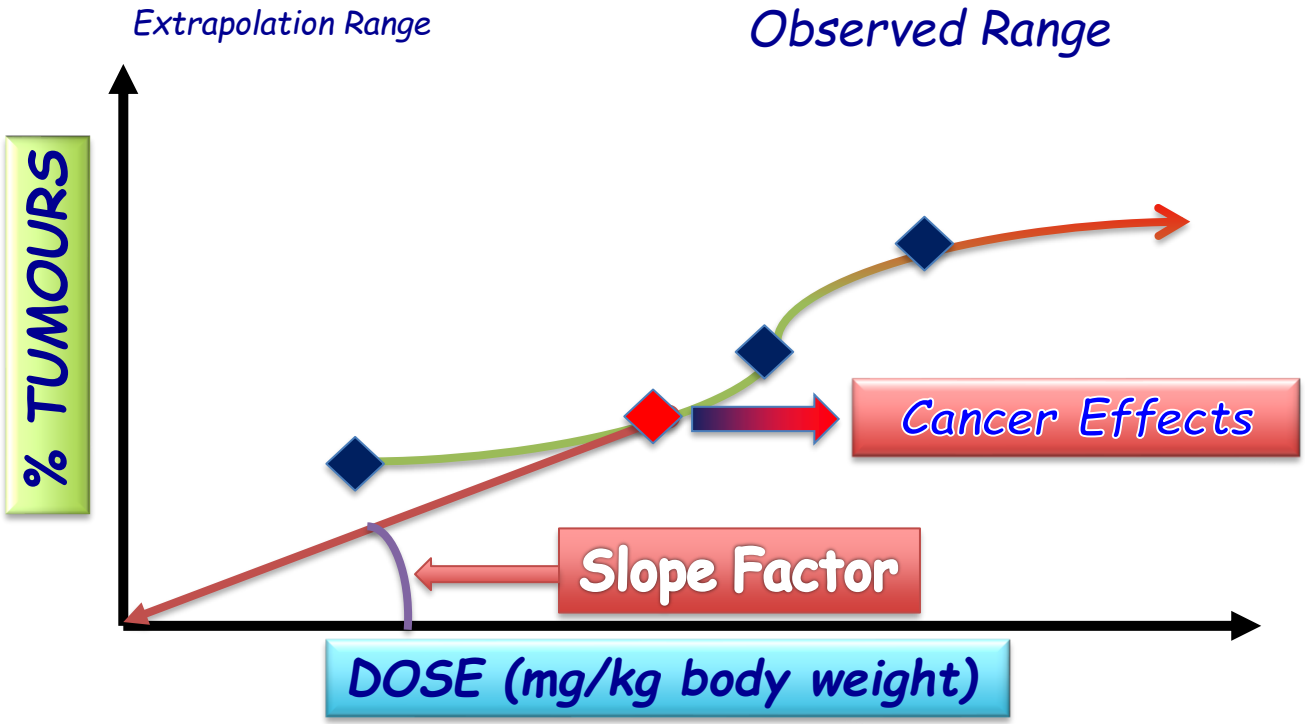
GENOTOXIC and CARCINOGENS



SIMPLE QUESTIONS versus DIFFICULT ANSWERS

What material(s)

are we **exposed to**?

Single component ??

or

Complex mixture ??

Simposio:

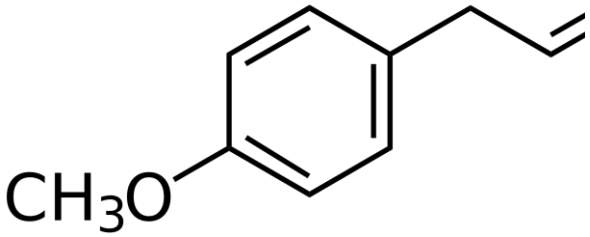
BOTANICALS: CARATTERIZZAZIONE E VALUTAZIONE DELLA SICUREZZA

MATRICE VS SINGOLO COMPONENTE

Corrado Lodovico Galli - ERT

Wednesday February 12, 2020

?? 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

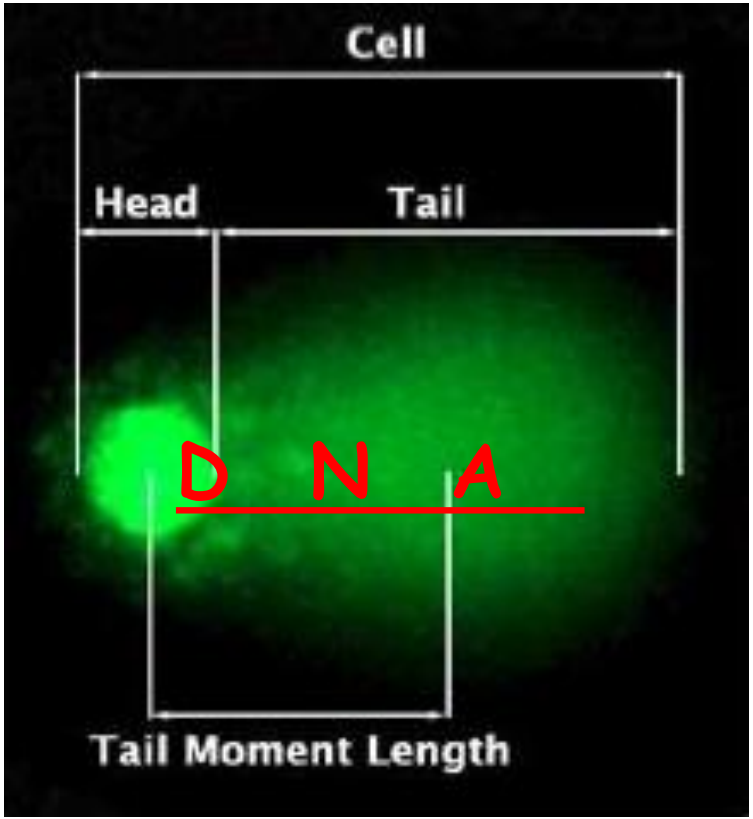
- Linalool the main constituent of essential oils (56.7–60.6%):
- epi-a-cadinol (8.6-1.4%),
- a-bergamotene (7.4-9.2%),
- γ-cadinene (3.3– 5.4%),
- germacrene D (1.1-3.3%) e
- camphor (1.1-3.1%).

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

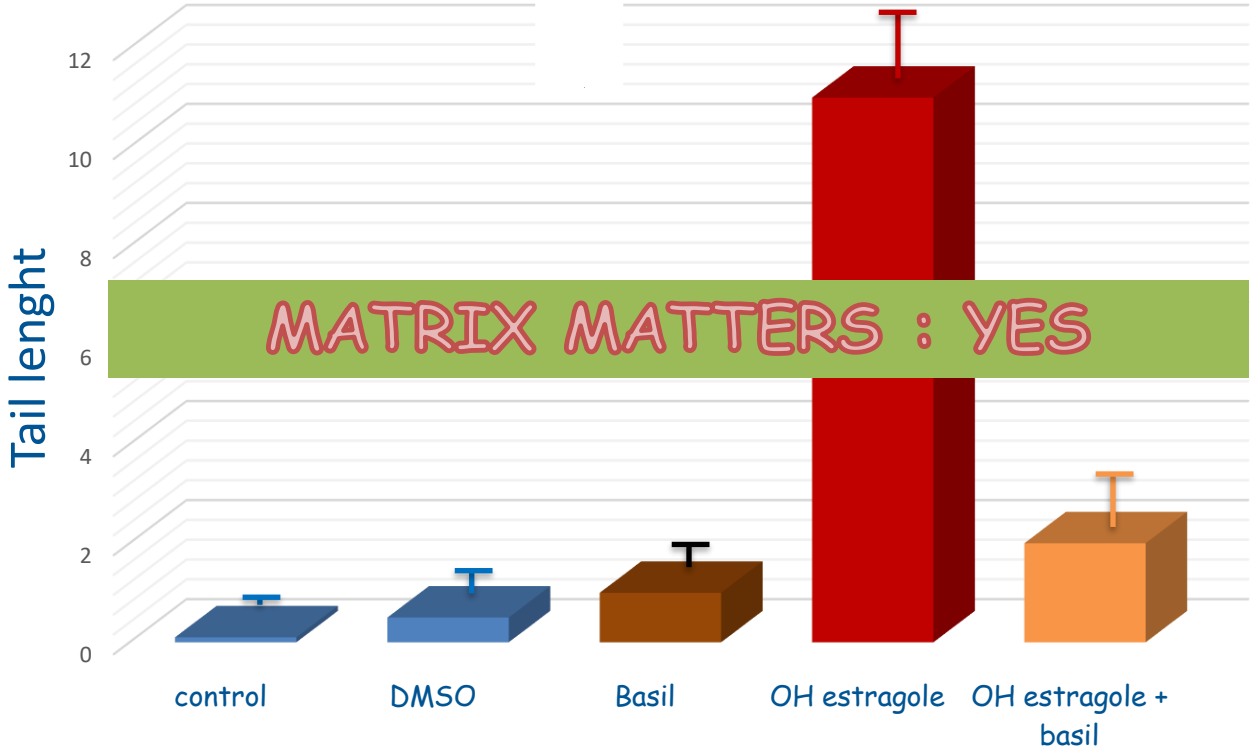
DNA FRAGMENTATION IN THE MOUSE IN VIVO COMET ASSAY

The comet assay is a gel electrophoresis method used to visualize and **measure DNA strand breaks** in individual cells, using microscopy. ...

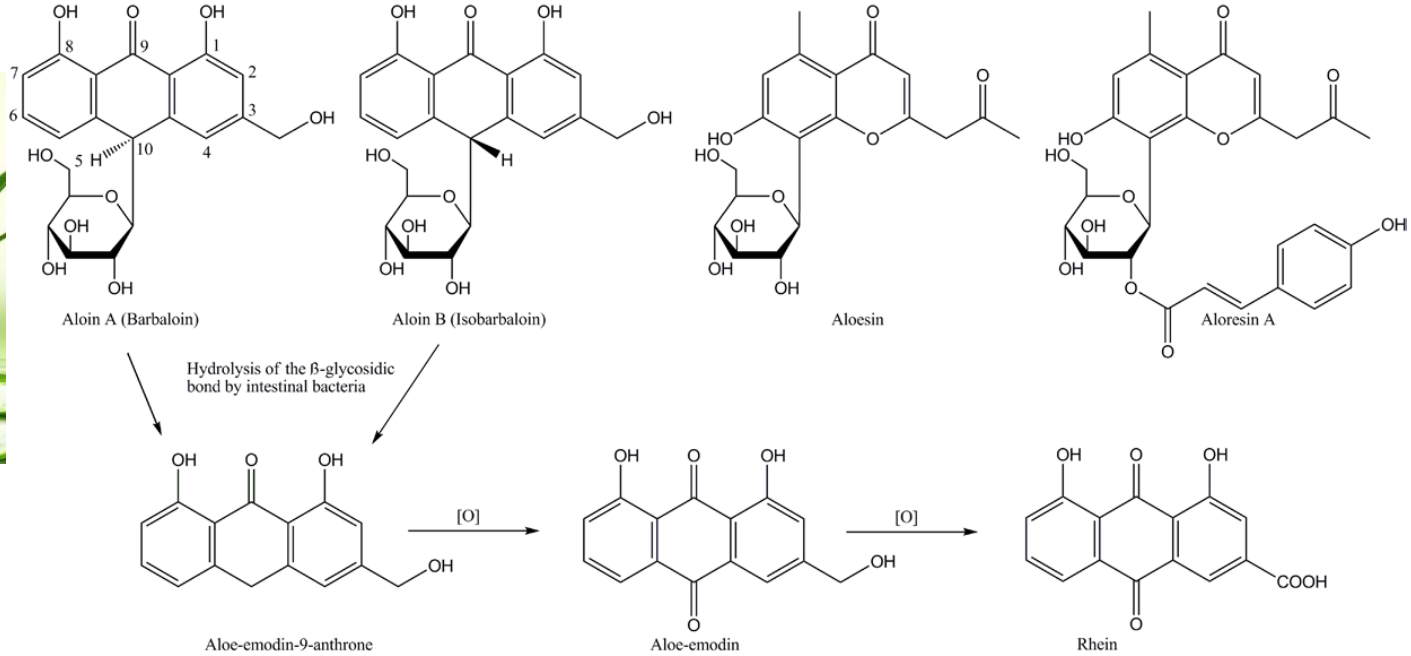
The relative size of the tail is an index of DNA damage.



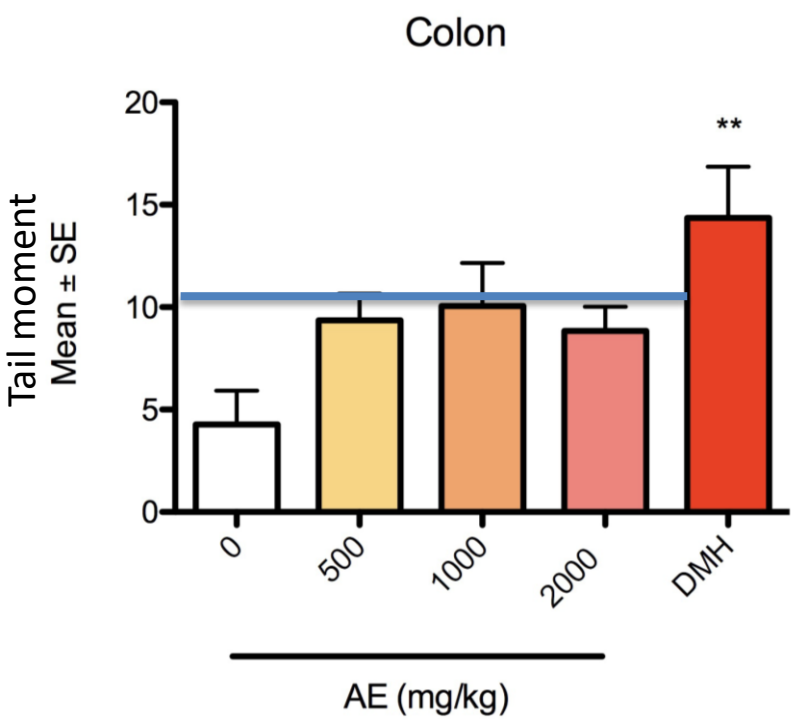
GENOTOXICITY

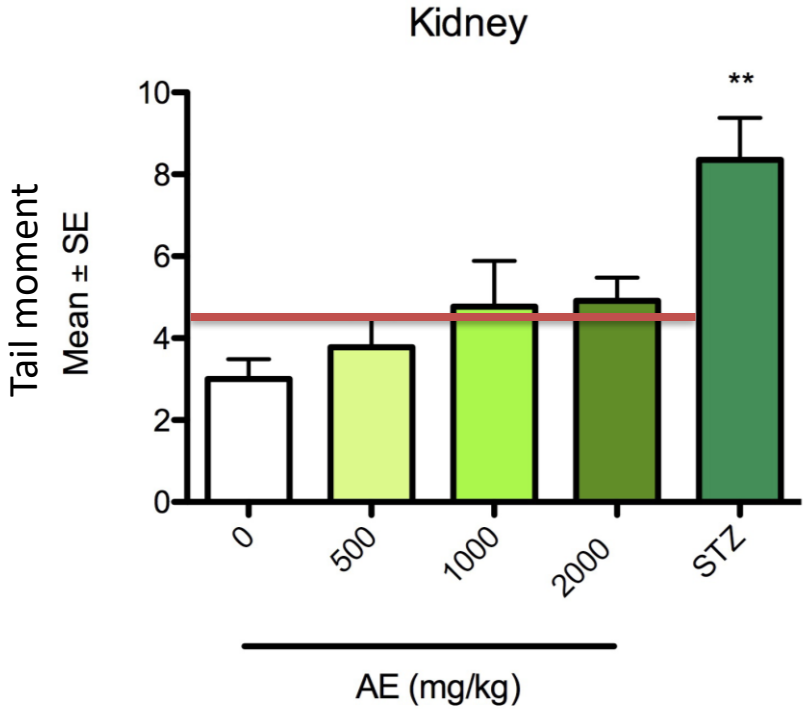


HYDROXYANTHRACENE DERIVATIVES



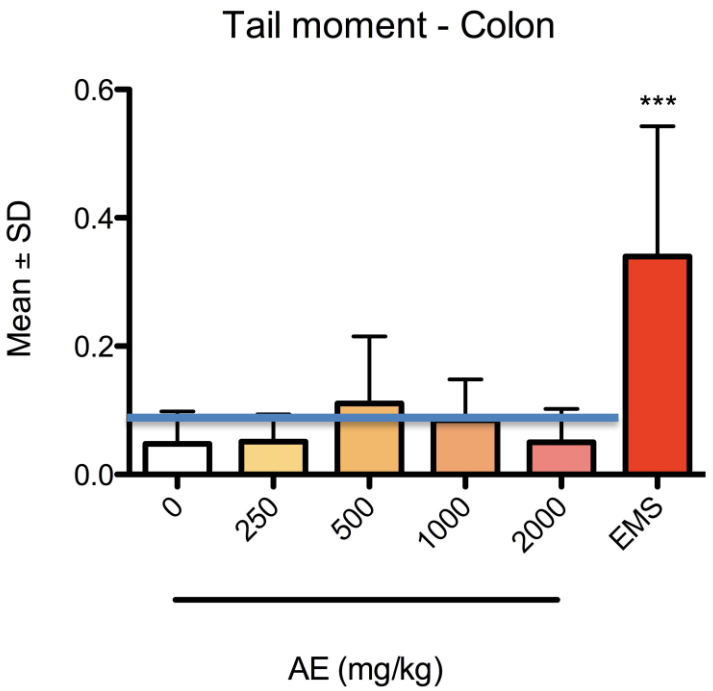
ALOE-EMODIN-INDUCED DNA FRAGMENTATION IN THE MOUSE IN VIVO COMET ASSAY

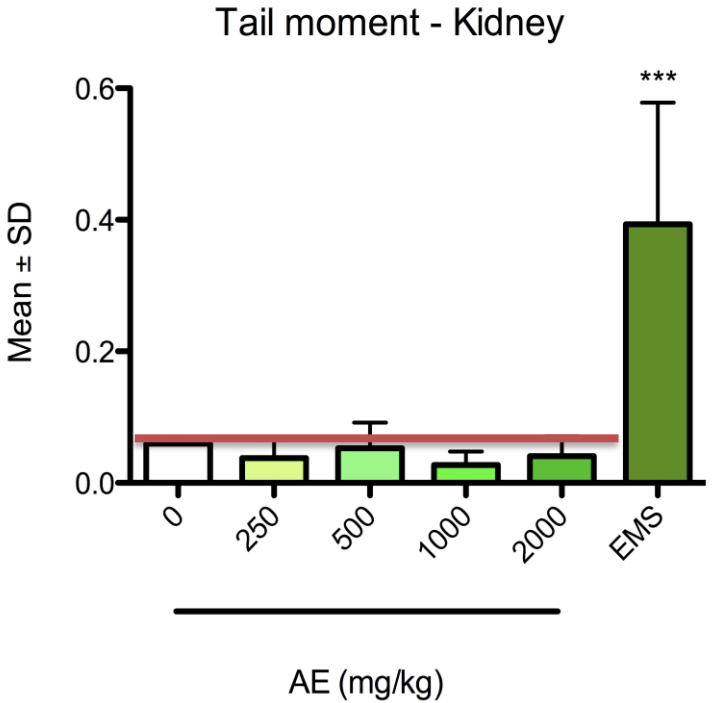




Statistic: two-sample t-test after log-transformation

ALOE-EMODIN - ENZYME-MODIFIED IN VIVO ALKALINE COMET ASSAY IN MICE





Statistic: 1-way ANOVA

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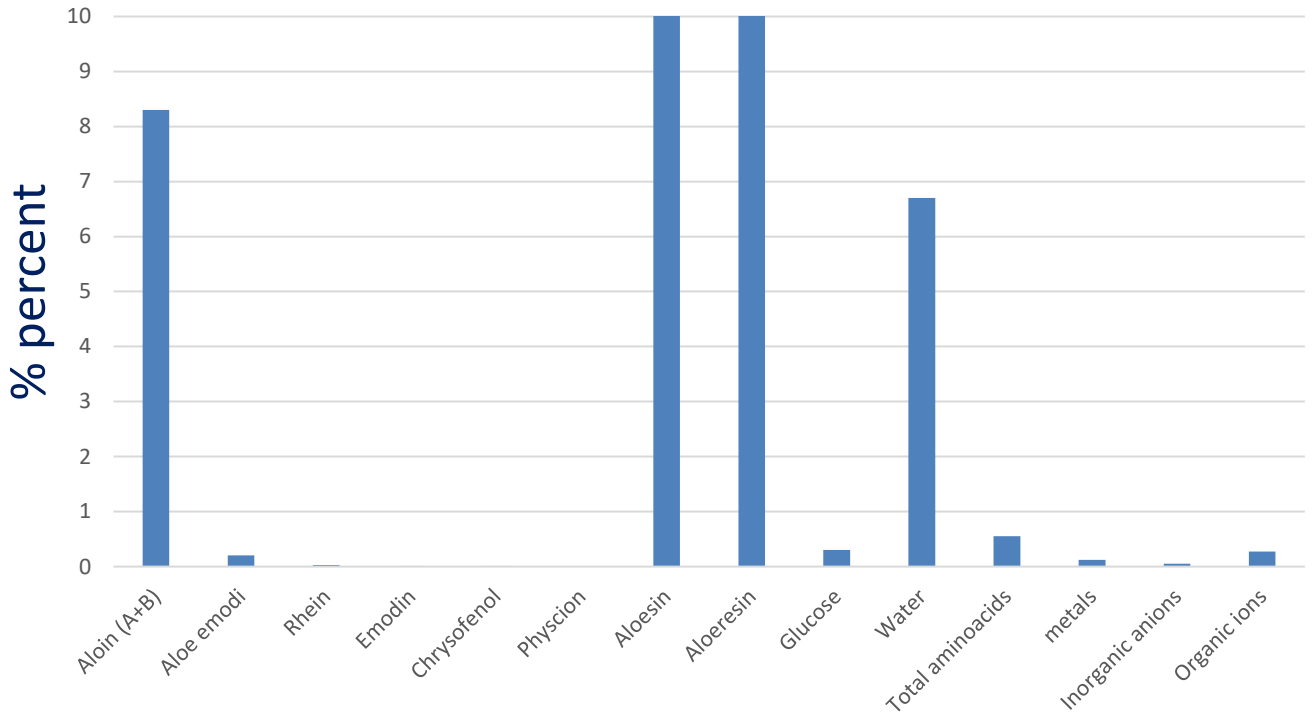
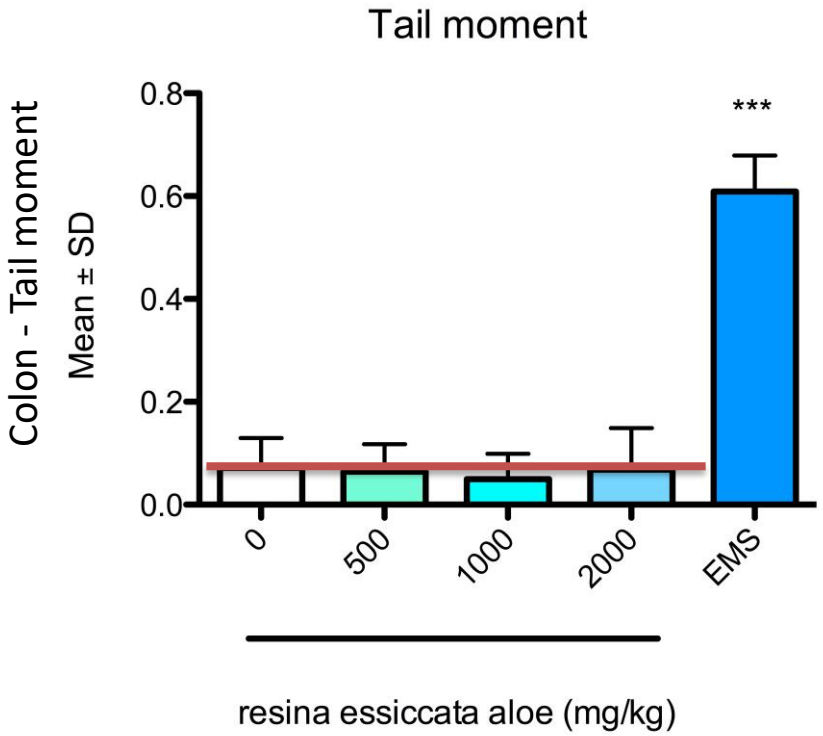
COMPOSITION OF DRIED ALOE FEROX JUICE



ALOE-FEROX - IN VIVO ALKALINE COMET ASSAY IN MICE



EVALUATION OF GENOTOXIC DAMAGE BY AMES TEST OF A RHUBARB DRY EXTRACT

WITHOUT METABOLIC ACTIVATION

Dose level (µg/plate)	TA1535 Rev/pl.	TA1537 Rev/pl.	TA98 Rev/pl.	TA100 Rev/pl.	WP2 *uvrA* Rev/pl.
Untreated	16	19	36	125	29
0.00	15	20	34	130	34
50.0	13	28	38	150	26
158	18	26	35	157	39
500	19	25	37	154	31
1580	11	24	39	171	37
5000	19	30	40	177	43

EVALUATION OF GENOTOXIC DAMAGE BY AMES TEST OF A RHUBARB DRY EXTRACT WITH METABOLIC ACTIVATION.

Dose level (µg/plate)	TA1535 Rev/pl.	TA1537 Rev/pl.	TA98 Rev/pl.	TA100 Rev/pl.	WP2 *uvrA* Rev/pl.
Untreated	14	18	31	114	26
0.00	19	20	29	126	26
50.0	13	14	33	155	22
158	18	19	31	148	27
500	12	17	32	149	30
1580	11	21	34	135	30
5000	12	33	34	132	34

EVALUATION OF GENOTOXIC DAMAGE IN VITRO MICRONUCLEUS TEST IN HUMAN LYMPHOCYTES OF A RHUBARB DRY EXTRACT.

Main Assay	S9	Treatment time (hours)	Harvest time (hours)	Dose level (µg/mL)	Incidence of micronucleated cells (%)	Statistical significance
1	–	3	32.5	0.0	0.50	-
				5000	0.65	NS
				3330	0.55	NS
				2220	0.85	NS
				Linear trend		NS
				Historical control data (95% confidence limits)		0.00-0.75
	+	3	32.5	0.0	0.90	-
				5000	0.55	NS
				3330	0.60	NS
				2220	0.85	NS
				Linear trend		NS
				Historical control data (95% confidence limits)		0.00-0.85
2	–	31	31	0.0	0.55	-
				3000	0.30	NS
				2500	0.40	NS
				2080	0.35	NS
				Linear trend		NS
				Historical control data (95% confidence limits)		0.00-0.96

NS = Not significant

??? UNCERTAINTIES ????

Considering the possible presence of aloe-emodin and emodin in extracts,

the Panel concluded that

hydroxyanthracene derivatives should be considered as genotoxic and carcinogenic

unless there are specific data to the contrary……..

……..although uncertainty persists.

❖ *In its scientific opinion, the European Food Safety Authority (EFSA) concluded that the hydroxyanthracene derivatives (HAD) aloe-emodin, emodin and danthron as well as all Aloe extracts containing them are genotoxic and can cause cancer in the intestine.*

❖ if a harmful effect on health has been identified, the substance and/or the ingredient containing the substance shall:

- (i) be placed in Annex III, **Part A**, and its addition to foods or its use in the manufacture of foods shall be prohibited;

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❖ *The EFSA Panel further considered that there is a safety concern for certain extracts containing HADs (such as Rheum, Cassia and Rhamnus extracts) although scientific uncertainty persists.*

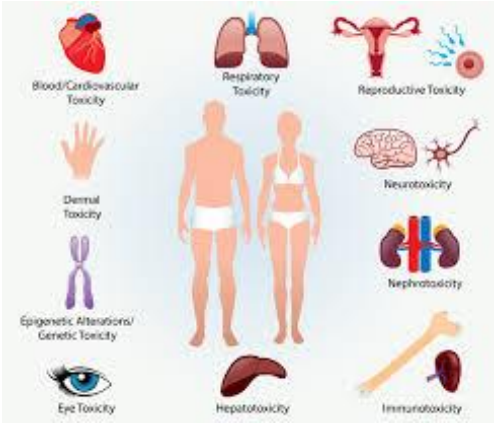
❖ if the possibility of harmful effects on health is identified but ***scientific uncertainty persists***, the substance shall be placed in Annex III, Part C.

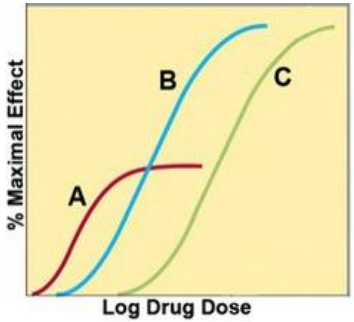
- *These conclusions, seem to be NOT in line with previous assessments on the botanical sources of these substances by other European and international bodies, including*
  - *European Medicines Agency,*
  - *World Health Organization*
- ***cannot be neglected or invalidated.***

SIMPLE QUESTIONS versus DIFFICULT ANSWERS

What material(s)

are we **exposed to**?

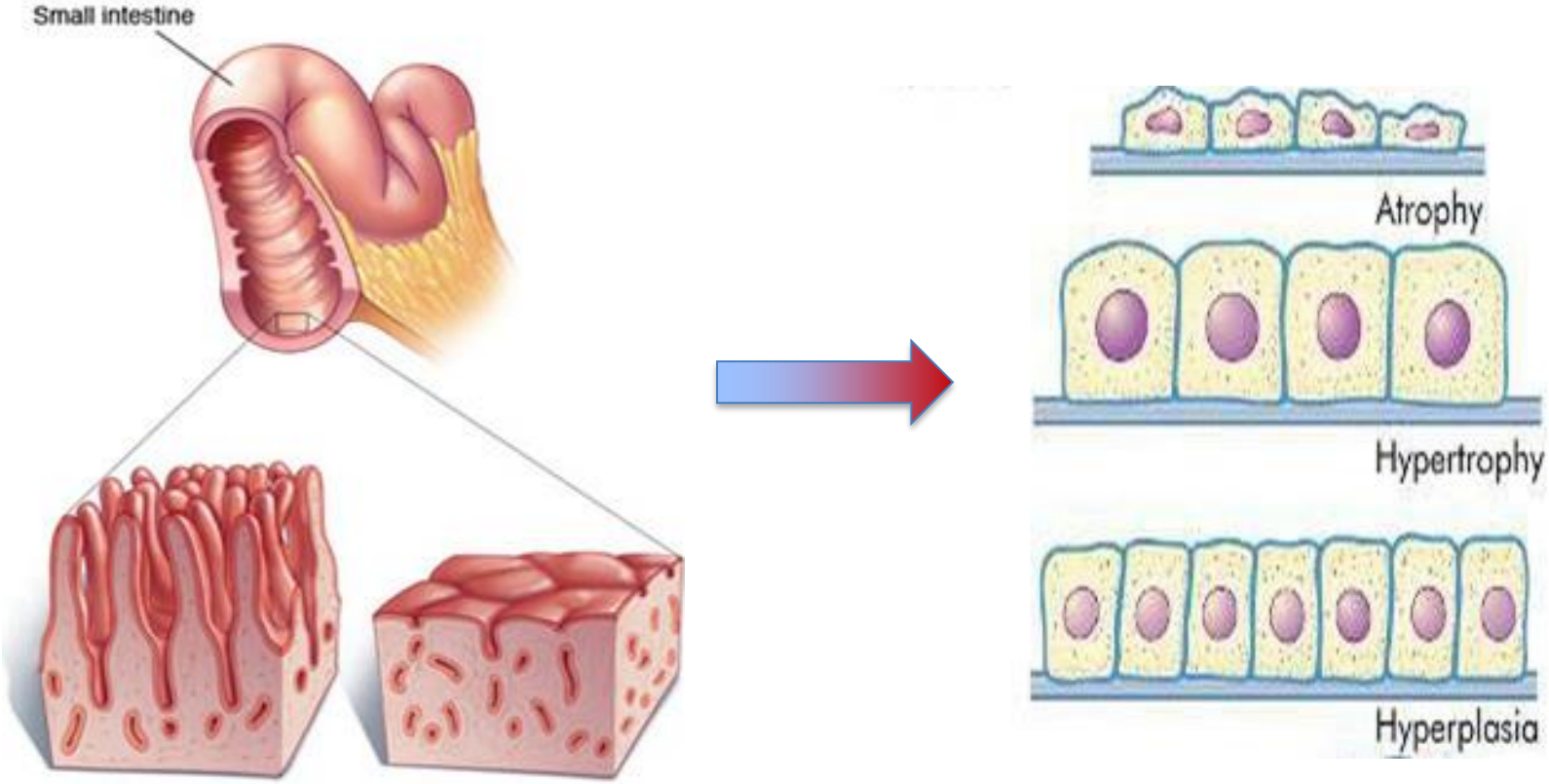




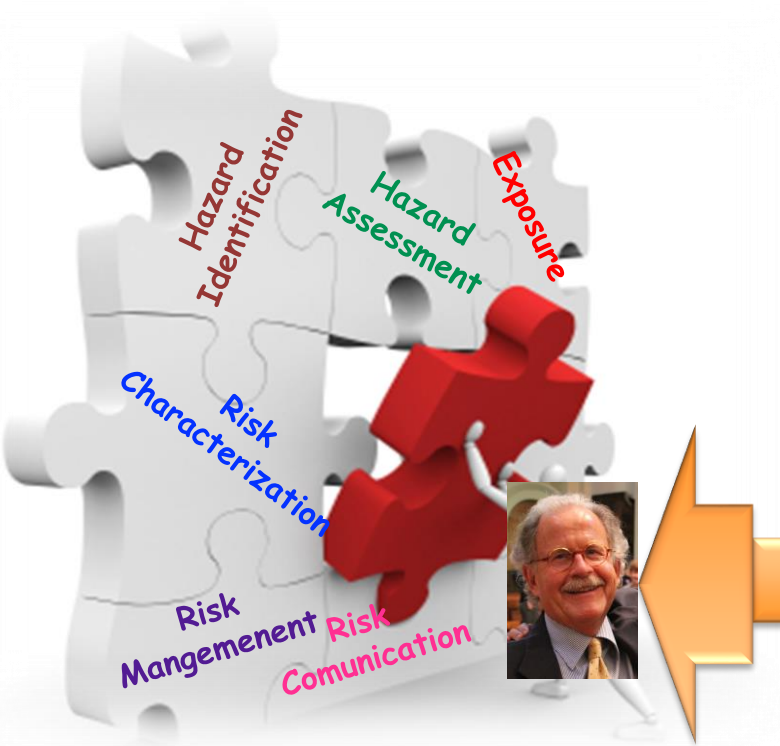
At what *dose* is toxicity observed

- Where do they cause toxicity?
- What are the mechanism of toxicity?
- Who is susceptible?

SIMPLE QUESTIONS versus DIFFICULT ANSWERS



TAKE HOME MESSAGE



Weight of Evidence

Expert Judgement