Webinar on Scientific Aspects to consider when preparing a Novel Food Application

Speakers:
Prof. Marina Heinonen
Dr. Inge Mangelsdorf

15 June 2017
12:30 – 13:30

efsa
European Food Safety Authority

WEBINAR GUIDE TO ATTENDEES

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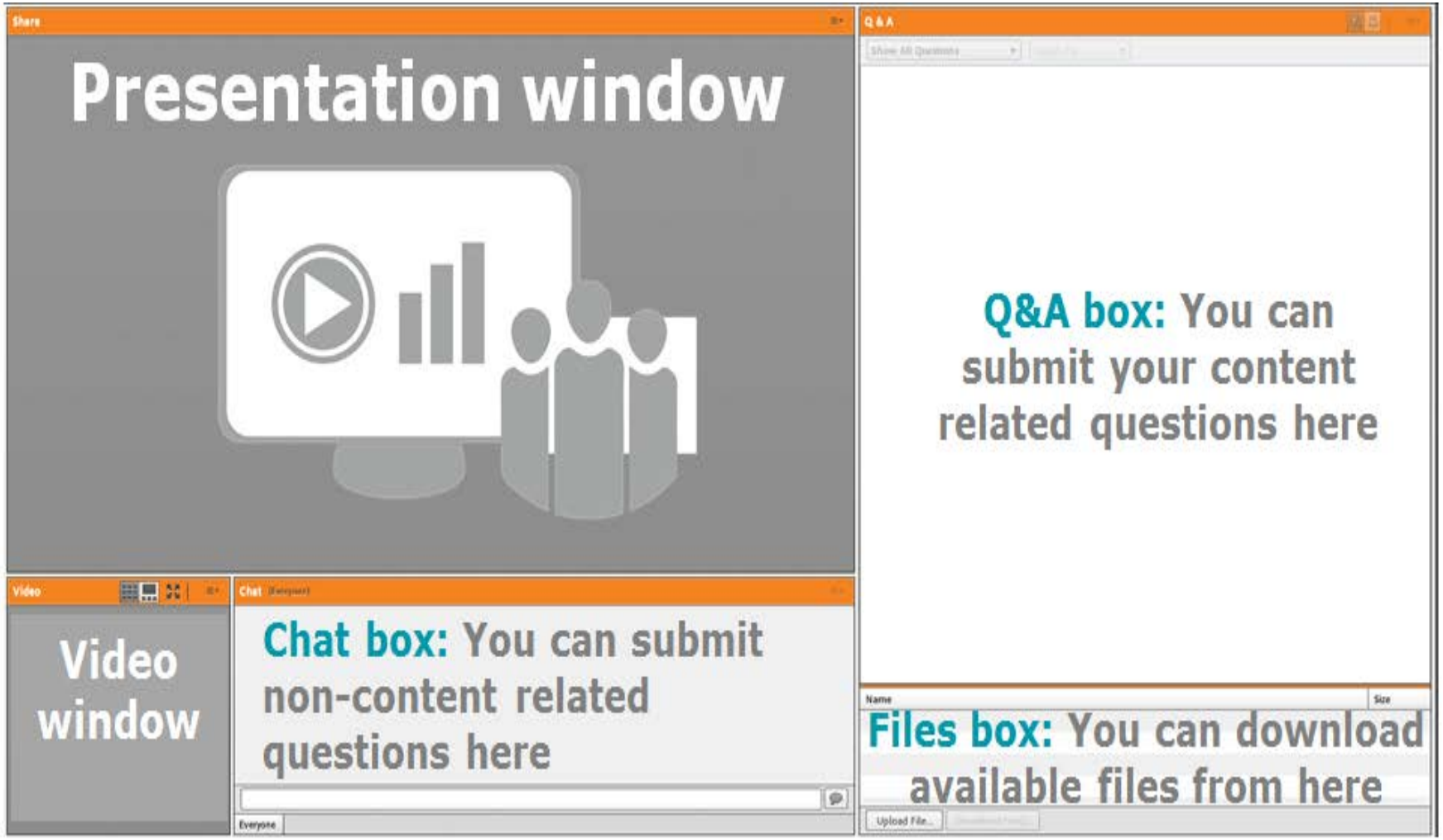
Virtual Room



Sending questions - Q&A box

- Questions should be **concise** and submitted **once**. Follow-up questions should be **self-explanatory**
- You can ask questions **until 13:30**
- You will see the **answer** right below the question row once replied by EFSA
- We will address all questions as soon as possible and until **14:00**
- If you do not receive an answer to your question, feel free to re-submit it through the **EFSA APDESK** web form later on:

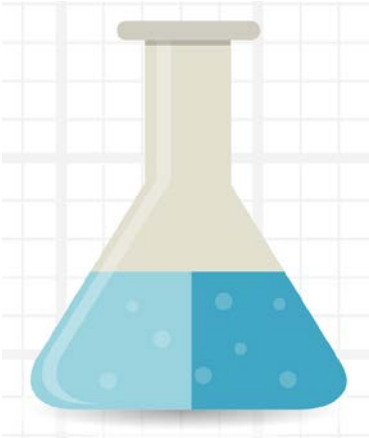
http://www.efsa.europa.eu/en/applicationshelpdesk/askaquestion

CONTENT OF THIS WEBINAR

- **Key aspects regarding compositional data and specifications**

- **Toxicological testing strategy**

Key Aspects regarding Compositional Data and Specifications



THE NOVEL FOOD GUIDANCE

- **Part 1: Administrative data**
- **Part 2: Characterisation, technical & scientific data**
  - 2.1. Introduction
  - **2.2. Identity of the NF**
  - **2.3. Production process**
  - **2.4. Compositional data**
  - **2.5. Specifications**

  also for traditional foods
  - 2.6. History of use of the NF and/or of its source
  - 2.7. Proposed uses and use levels and anticipated intake
  - 2.8. Absorption, distribution, metabolism, and excretion
  - 2.9. Nutritional information
  - 2.10. Toxicological information
  - 2.11. Allergenicity
  - 2.12. Concluding remarks
- **Part 3: Annexes to the dossier**

2.2 IDENTITY OF THE NF (1)

2.2.1. Chemical substances

2.2.2. Polymers

Foods consisting of, isolated or produced from……:

2.2.3. Microorganisms, fungi or algae

2.2.4. Material of mineral origin

2.2.5. Plants or their parts

2.2.6. Animals or their parts

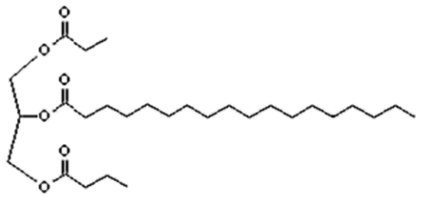
2.2.7. Cell or tissue cultures derived from animals, plants, fungi, algae

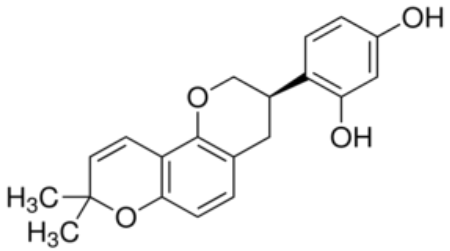
2.2.8. Foods consisting of “engineered nanomaterials” (→ guidance being updated)

2.2 IDENTITY OF THE NF (2)

2.2.1 Chemical substances

- chemical name according to IUPAC nomenclature rules
- CAS number and other identification numbers
- synonyms, trade names, abbreviations
- molecular and structural formulae; stereochemistry
- molecular mass (Da)





2.2 IDENTITY OF THE NF (3)

NF consisting of, isolated from or produced from plants (2.2.5) or from animals (2.2.6) or their parts

- Scientific (Latin) name (botanical/zoological family, genus, species, subspecies, variety with author's name/breed …)
- Synonyms that may be used interchangeably with the preferred scientific name
- For plants verification of the identity according to internationally recognised databases and methodology
- Common names
- Part(s) used
- Geographical origin (continent, country, region)

2.3. PRODUCTION PROCESS (1)

2.3.1. Detailed description …

- e.g. chemical synthesis, enzyme-catalysis, fermentation, isolation from natural source
- Potential by-products, impurities and contaminants that could raise safety concerns
- Novel aspects of the process
- Raw materials, starting substances
- Handling of sources

animals: e.g. breeding, farming, hunting conditions

algae & microorganisms: culture conditions

plants: e.g. cultivation practice, time of harvest
(→ guidance on safety assessment of botanicals and botanical preparations, EFSA, 2009)

2.3. PRODUCTION PROCESS (2)

- Operational limits and key parameters, e.g.
  - if chemical synthesis:
    - reaction sequence, side reactions
    - purification steps
    - reaction conditions

  - if plant, animal, microbial origin:
    details on the conversion of raw material into NF

- Production control and quality assurance (e.g. HACCP, GMP, ISO)
- Production flow chart

⇨ **Should allow conclusions on the impact of the process on safety and nutritional value of the NF**

FREQUENTLY MISSING INFORMATION (1)

- **Production process**
  - Steps missing
  - Insufficient information on source, raw materials, reagents used & conditions, equipment, catalysts, enzymes, extraction solvents used, pore size of filtrations
  - No flow-chart, no production yield
  - Information on quality assurance system
  - Insufficient data on the effect of the production process applied to the NF (e.g. effects of excessive heating or UV, treatment on the food)

2.4. COMPOSITIONAL DATA (1)

General requirements

- Identities and quantities of impurities, by-products or residues, chemical & microbiological contaminants
- Toxic, addictive, psychotropic, allergenic or other substances of possible concern to human health
- Type and spectrum of target analytes depending on sources and production process, e.g.:
  - chemical synthesis → residual starting materials and by-products
  - fermentation → undesirable metabolites
  - extraction → residual solvents
- Consider the source of the NF (!)

2.4. COMPOSITIONAL DATA (2)

2.4.2. Single substances and simple mixtures thereof

Single substances

- Identity tests (e.g. UV-VIS, IR, NMR, GC-MS, LC-MS)
- Physicochemical properties (e.g. appearance, melting point, boiling point)
- Solubility data in water and other common solvents
- Particle size, shape and distribution
- Minimum purity value
- Density and/or viscosity for liquid preparations

Simple mixtures (can be fully chemically characterised)

- Identities and the relative ratios of all components
- Complete mass balance

2.4. COMPOSITIONAL DATA (3)

2.4.3. Complex mixtures and whole foods

Complex mixtures (e.g. extracts, protein hydrolysates)
Whole foods (e.g. milk, meat, fruits, seeds)

⇨ **not all constituents can be fully chemically characterised and/or identified**

- Qualitative and quantitative characterisation of the main constituents (at least via sum parameters):
  - Whole foods: proximate analyses
  - Mass balance
- Micronutrients
- Components characterising the nature of the NF (e.g. peptides, phospholipids, carotenoids, phenolics, sterols)
- Amount of unidentified components ↓

BOTANICAL HAZARDS

The EFSA Compendium of Botanicals

https://www.efsa.europa.eu/en/data/compendium-botanicals

- Database of botanicals reported to contain naturally occurring substances of possible concern
- For **hazard identification**
- **Presence of a substance of concern:** note part of the plant, preparation method, dosage & conditions of use

- If adverse health effect with specific species → closely related species should also be considered **("read-across")**
- Absence of a botanical species from the database does not mean that it is devoid of hazardous compounds
- Does not include algae, cyanobacteria and fungi

2.4. COMPOSITIONAL DATA (4)

- Preferably, data on at least **five representative batches** that have been **independently produced**
- Use of validated analytical methods, preferably nationally or internationally-recognised (e.g. AOAC, ACS, EP)
- Description of methods
  - limit of detection (LOD)
  - limit of quantification (LOQ)
  - references
- Certificates of analyses and information on the accreditation of laboratories
- In-house methods: full description and validation of procedures

FIVE OR THREE BATCHES ?

Pro for five batches:

- complex mixtures
- high proportion of unidentified compounds
- novel source with no history of human consumption
- small margin of exposure (= MoE on anticipated human exposure versus adverse effect in toxicological testing)

Pro for three batches

- single chemical substances and simple mixtures
- high purity
- negligible amounts of undefined substances
- High MoE

2.4. COMPOSITIONAL DATA (5)

2.4.4. Stability

To identify hazards which might arise during storage and transport:

- Constituents and parameters
  - susceptible to changes during storage
  - having direct effect on safety
- Physicochemical, biochemical and microbiological stability of the NF under normal conditions of storage
  - effects of packaging, temperature, and environment
- Whether NF used an ingredient added to other foods

→ stability in the processed foods

FREQUENTLY MISSING INFORMATION (2)

- **Inadequate information on methods and labs**

  e.g. information (and certificates) on the accreditation of laboratories, absence of analytical reports from such laboratories, description of the applied analytical method, references to the analytical methods, inappropriate analytical method applied - inadequate LOD, missing info on LOD/LOQ.

- **Incomplete information on composition**

  e.g. information on protein content, type of polysaccharides, type of polyphenols, secondary plant metabolites..., significant amount of unidentified compounds/impurities, no information on the presence of undesirable substances even if information is available for the source of the NF or closely related species.

FREQUENTLY MISSING INFORMATION (3)

- **Batch testing**

  Missing, insufficient information (source, year, independently produced, results not complying with specifications...)

- **Stability testing**

  Missing, insufficient information (on the batches, on conditions, lacking reports), proposed conditions of use not considered and addressed

- **Specification of the NF**

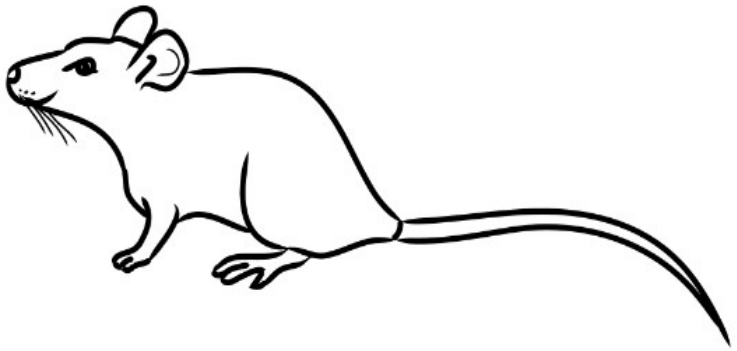
  e.g. critical components from the source or production process not added to the specification (e.g. Pd used as a catalysator, residual solvents from an extraction process..)

2.5. SPECIFICATIONS

- Key parameters → characterise and substantiate the identity of the NF
- Rationale for the selected parameters
- Minimal purity
- Limits for impurities and degradation products, in particular if toxicological or nutritional relevance
- Maximum levels of contaminants, if no legal requirements
- Methods used for analysis
- Table format

Considered and used by EC in the marketing authorisation.

Toxicological Testing Strategy



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OUTLINE

- Test substance to be tested and general considerations
- Tiered approach
  1. Toxicokinetics (Absorption, distribution, metabolism and excretion =“ADME”)
  2. Genotoxicity
  3. Subchronic toxicity
  4. Other endpoints
- Specific cases

APPROACH

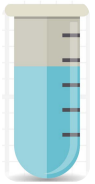
depends on the type of test material:

- Single substance, simple (well defined) mixture.
- Complex mixture (e.g. ethanolic extracts).
- Whole foods.

TEST MATERIAL

Toxicological studies should be carried out with **material**:

- **as intended to be marketed**, i.e. the test material should be manufactured according to the procedures described in the section on the production process (2.3)
- Should meet the **compositional characteristics** (2.4) and the **specifications** (2.5).
- If this is **not the case**, a **rationale** should be provided to substantiate why the material used for the toxicological studies is representative for the Novel Food (NF) and appropriate for the toxicity studies.



COMPLEX MIXTURES, WHOLE FOOD

- **Complex mixtures:** ANS Guidance: *«conventional metabolism and toxicokinetic studies may not be feasible for all components in the mixture, but should be provided for **toxicologically relevant constituents**. Toxicologically relevant constituents are generally considered to be the **major components** and those other components with known or demonstrable **biological or toxicological activity**, and should be determined on a **case-by-case basis** with a scientific justification and the rationale for their selection provided»*.

- **Whole foods** should be tested like complex mixtures.

OTHER ASPECTS TO BE CONSIDERED

- Identity, chemical structure, composition, and physico-chemical properties of the NF;
- Available information on previous **human consumption** of the NF and its source;
- Intended **uses and use levels** and the resulting intakes;
- Available **human studies** (also non-food uses if relevant);
- In case of insufficient data also
  - (quantitative) structure activity relationship **((Q)SAR**) data
  - toxicological data on structurally related substances (‘**read-across**’).

GENERAL ISSUES (2)

- **Tiered toxicity testing which integrates the core areas of kinetics, genotoxicity, repeated dose toxicity testing, and reproductive and developmental toxicity (EFSA ANS Panel, 2012).**

  - **Additional studies** may be needed to examine specific biological processes, immunotoxicity, hypersensitivity and food intolerance, studies on neurotoxicity, endocrine activity and modes of action.

- **Deviations**/non-applicability should be reasoned with sound scientific arguments

- Tests should be conducted in accordance with international guidelines (e.g. **OECD**) and according to the principles of Good Laboratory Practices (**GLP**).

TIERED APPROACH (EFSA ANS PANEL, 2012)

Tier 1

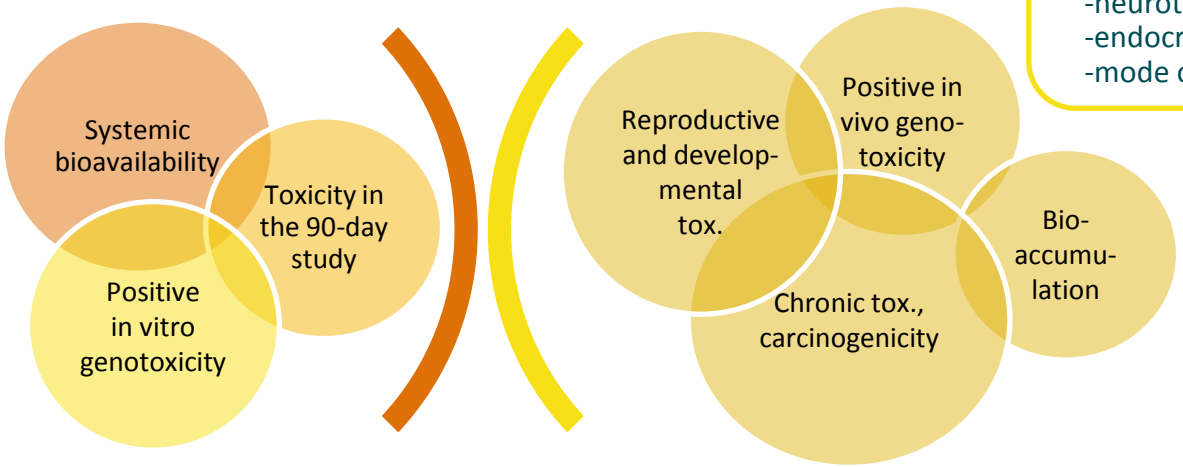
- Absorption
- Genotoxicity in vitro
- Extended 90-day toxicity study

Tier 2

- ADME: single dose
- Genotoxicity in vivo
- Chronic toxicity
- Carcinogenicity
- EOGRTS *)
- Prenatal developmental toxicity

Tier 3

- ADME: repeated dose, volunteer studies
- Carcinogenicity: Mode of Action
- Reproductive and Developmental toxicity
- Special studies
  -immunotoxicity
  -neurotoxicity
  -endocrine activity
  -mode of action



Triggers for considering Tier 2

Triggers for considering Tier 3

*) Extended One-Generation Reproductive Toxicity Study

Flow-chart provided by ANS Unit

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

Section 2.8 of the EFSA Guidance document

- Important information for the interpretation of study results.
- Without information on absorption in the animal model, conclusions cannot be drawn on the (toxicity) study.
- Relevant for the toxicological (and nutritional) aspects.
- Similarities and differences between experimental animals and humans should be considered.
- Evidence of differences in kinetics due to age, physiological state, disease state, etc. may require additional specific kinetic studies.

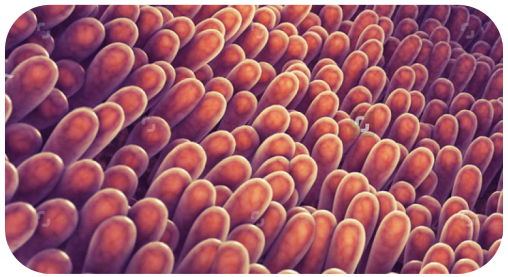
ADME - SINGLE SUBSTANCE/SIMPLE MIXTURE

NF Guidance aligns with the tiered approach suggested in the ANS Guidance for additives.

- Tier 1: Intestinal absorption no/yes ⇨
- Tier 2: Studies to define distribution, metabolism and excretion and other basic toxicokinetic parameters following a single dose.
- Tier 3: to investigate possible bio-accumulation after repeated administration.

Absorption from gastrointestinal tract

- Indirect evidence from toxic effects in 90 day study
- Measurement of test substance in plasma (AUC, Cmax, Tmax, $T_{1/2}$) after oral and iv application
- Excretion of test substance and metabolites in urine (and faeces)
- Radiolabelled test compounds may be necessary



ADME - TIER 2

Studies to define distribution, metabolism and excretion and other basic toxicokinetic parameters following a single dose

- *In vivo* data on absorption, distribution, metabolism and excretion needed.
- OECD 417 protocol to provide information on systemic exposure after a single dose (C_{max}, T_{max}, AUC, T½, bioavailability).

ADME - TIER 3

Triggered by limited or slow excretion or any other mechanism leading to possible bio-accumulation

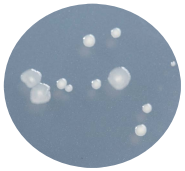
- Repeated dose toxicokinetic studies involving steady-state.
- Additional data to predict ADME in humans.
- On a case-by-case basis: human studies.

GENOTOXICITY

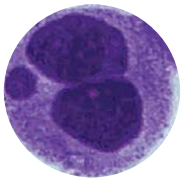
- identify substances which could cause **heritable damage** in humans;
- predict **potential genotoxic carcinogens** in cases where carcinogenicity data are not available.
- Endpoints **mutagenicity**, **clastogenicity** and **aneuploidy** shall be covered by tests
- **Deviations** can be argued on a case-by-case basis.
- For some complex mixtures and whole foods, it may be necessary to **focus on specific constituents** of the NF.
- Recommendations on test types, interpretation of results and other issues in testing the genotoxicity of substances present in food are described in **detail in the Opinion of the Scientific Committee.**

EFSA GUIDANCE ON GENOTOXICITY (2011)

Tier 1:



- Bacterial reverse mutation test (OECD TG 471)
- *In vitro* mammalian cell micronucleus test (OECD TG 487)



Tier 2: In case of positive/unclear *in vitro* test results

- *In vivo* micronucleus test (OECD TG 474)
- *In vivo* Comet assay (no OECD TG 489)
- Transgenic rodent assay (OECD TG 488)

In vivo tests may be combined, i.e. micronucleus test including comet assay of the liver.

SUBCHRONIC TOXICITY (1)

- In line with the Guidance for food additives, **a subchronic toxicity study** should normally be submitted.
- To identify adverse effects following repeated exposure via an appropriate oral route.
- Should allow the identification of a **BMDL** (or a **NOAEL)**.
- May provide indications for the need for additional studies.
- The study should normally be conducted for at least **90 d** (OECD TG 408), modified to include some **additional parameters** (as described in OECD TG 407 - 28-day oral toxicity studies in rodents) to allow the identification of substances with a potential to cause neurotoxic, immunological, reproductive organ effects or endocrine-mediated effects.

SUBCHRONIC TOXICITY (2)

- When kinetic data show a **lack of systemic availability**, studies should at least investigate pathological and physiological effects in the gastrointestinal tract.
- The effects of unabsorbed materials on gastrointestinal function and tolerance also need to be investigated.
- Additional markers of potentially **adverse nutritional and/or metabolic effects** should be considered on a case-by-case basis.
- For '**whole foods**', the testing requirements should be determined using **a case-by-case approach.** Special considerations are required with regard to dose selection and the **avoidance of possible nutritional imbalances,** (EFSA Guidance on 90d study with whole food/feed, 2011).

SUBCHRONIC TOXICITY (3) – EXAMPLES

NEEDED	NOT NEEDED
Synthetic NF ingredients:	**Well characterised source with a significant history of food use, comprehensive compositional data, no concerns from production process, knowledge on the main components; whole foods:**
lycopene, zeaxanthin, chewing gum base, resveratrol, hydroxytyrosol, 2-o-fucosyllactose, dihydrocapsiate	
Plant extracts:	
Root extract from *Glycyrrhiza glabra* L., extract of three herbal roots ("Estrog-100"); taxifolin from Sibirian Larch; Astaxanthin extracted from microalgae Noni Juice/Puree	- rooster comb extract - rapeseed protein extract - Baobab dried fruit pulp - Chia seeds - UV treated yeast, milk, bread - Milk fermented with *Bacteroides xylanisolvens*
Fementation products:	
Glucosamine from *A. niger*, Prolyl-oligopeptidase produced with a genetically modified *A. niger*, ice-structuring proteins produced with genetically modified bakers' yeast; Nattokinase extracted from soy fermented by *Bacillus subtilis*.	

SUBCHRONIC TOXICITY (4) – OVERVIEWING TABLE

Parameter	Sex	Dose (mg/kg bw per day)			
		0	100	400	800
Body weight (g)	M	559 ± 51.8	554.6 ± 56.3	525.9 ± 29.3	521.2 ± 34.2
	F	320.9 ± 26.3	324.3 ± 47.9	281.9 ± 25.7*	267.0 ± 22.3**
Food consumption (g/day)	M	29.7 ± 1.5	29.12 ± 2.2	27.92 ± 1.4	27.0 ± 1.6**
	F	21.7 ± 1.6	22.12 ± 1.3	17.21 ± 1.4**	15.3 ±1.9***
Liver W, rel.	M	27.1	27.9	28.7	29.9**
	F	26.3	27.3	28.7	30.4**
Urine volume (mL)	M	6.3 ± 1.4	7.5 ± 1.1	8.3 ± 3.0	11.4 ± 4.7*
	F	5.5 ± 2.1	5.6 ± 1.0	7.8 ±3.6	16.7 ± 8.7***
Thrombocyte counts	M	922	930	870	800**
	F	1010	1022	880	720***

Better overview of complex data: dose-response, sexes, pattern?

ADVERSE OR NOT ADVERSE ?

- Statistically different from concurrent control?
- Effect size?
- Dose-Response?
- Both sexes?
- Pattern of effects? Also histological changes?
- Underlying mechanism
  (e.g. demonstrated low palability?)
- Historical control values

See also EFSA Scientific Committee Guidance for the identification of biological relevance of adverse/positive health effects from experimental animal and human studies.

HISTORICAL CONTROL DATA

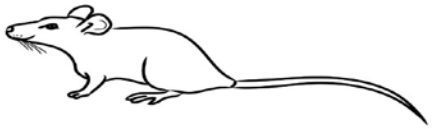
- Ideally provided with the study report
- Should be from same lab, from relevant years (about 5 years), same rat strain, ideally obtained from the same supplier, both sex, comparable age
- Mean, total range, ± 1 and 2 SD
- For assessing the value of the **control group of the study**, but not *per se* to invalidate statistically significant findings in the verum (test) group animals. OECD (ENV/JM/MONO(2002)19*: "….*it is generally not appropriate to rely on statistical comparisons with historical controls because biological parameters can vary significantly over time. Historical control data may be useful in evaluating the acceptability of the "normal" data obtained from control groups*."
- Information on the employed methods (impact on the values)

* http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2002)19

SUBCHRONIC TOXICITY: BENEFICIAL OR ADVERSE ?

Some effects may be considered beneficial for some conditions in humans, e.g. weight loss/reduced weight gain, reduced thrombocyte counts.

However, these are usually considered **adverse** in the context of toxicological studies.



Expert judgement is needed.

SELECTING A REFERENCE POINT (RP)

Parameters often determining the RP

- Reduced body weight (gain), changes in organ weight, clinical chemical parameters, effects in haematology, urinalysis

NOAEL/LOAEL *vs.* Benchmark Dose (BMD)

- BMD approach considered superior, see updated Guidance from the EFSA Scientific Committee (2016)*, EFSA Workshop on BMD**.
- EFSA's web-based BMD-Tool: https://efsa.openanalytics.eu/login (request ID and password at: amu@efsa.europa.eu) This tool is based on PROAST software developed by Dutch National Institute for Public Health and the Environment (RIVM).

* https://www.efsa.europa.eu/en/efsajournal/pub/4658
** https://www.efsa.europa.eu/en/events/event/170301-0

SCIENTIFIC COMMITTEE ON DEFAULT VALUES (2012)

Applicable default uncertainty factor:

■ Animal → Humans	**10**
o inter-species toxicokinetics (rat)	4
o inter-species toxidynamics	2.5
■ Interindividual differences in humans	**10**
■ Subchronic → chronic exposure	**2**

⇨ Default uncertainty factor (UF) of **200** as acceptable margin of exposure (MoE) between RP from animal study and the human exposure. UF can be lowered on the basis of other available data (e.g. nature, type and experience of use of the NF and its source; production process, human data on the endpoint used for the RP).

⇨ Whole foods, bulky complex NF, and NF consisting largely of macronutrients usually cannot be tested at doses 100 or 200 higher than the intended human intake. If needed (see previous slide No 18), a lower MoE may be acceptable.

CHRONIC TOXICITY AND CARCINOGENICITY

- Potential triggers for chronic toxicity or carcinogenicity studies include, among others, **critical findings in the subchronic study** as well as **results of *in vitro* or *in vivo*** toxicity tests, including **genotoxicity** tests.

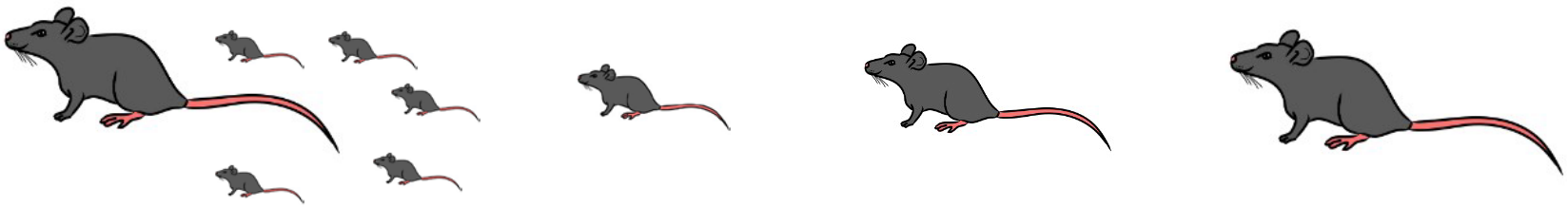
- **Further guidance** on the triggers for these studies and their implementation are outlined in the Guidance on food additives (EFSA ANS Panel, 2012) and respective OECD Guidelines (OECD TG 451, 452 or 453).

REPRODUCTIVE & DEVELOPMENTAL TOXICITY

- To be considered in the light of **kinetic** and **toxicity data**, including **read-across** data.

- Any indications of **effects on reproductive organs** or parameters, for example in the modified 90-day oral toxicity, will trigger testing for reproductive and developmental toxicity.

  Reproductive and developmental toxicity testing may not be required, if argued on a case-by-case basis.



GUIDANCE ON SPECIFIC CASES

Insects: consider Opinion of the EFSA Scientific Committee on potential hazards related to the use of farmed insects as food (EFSA Scientific Committee, 2015).

Microorganisms: without assigned QPS status: **complete strain characterisation** by fully assembled and validated whole-genome sequence analysis to enable the detection of virulence-related genes, antibiotic resistances and their potential horizontal transfer, and other potentially adverse metabolic features; **phenotypic characterisation of potential antimicrobial resistances and of other potentially adverse phenotypic features** e.g. potential toxin production, haemolytic activity, infectivity, adverse immune effects; numbers of **viable microorganisms in the final product** and stability.

Engineered nanomaterials: Guidance on the risk assessment of the application of nanoscience and nanotechnologies. EFSA's Scientific Committee, currently under review by EFSA (EFSA-Q-2016-00281).

Thank you

for your attention !