Guidance on Novel Foods

From Animal Toxicity Studies to Safe Levels of Intake in Humans

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efsa
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OUTLINE

- **The EFSA Guidance document**
- **Aspects from previous applications**
- **Comments raised in the public consultation**
- **Requests for additional information**
  Examples from the past and considerations of the NDA Panel.

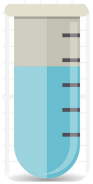
Section 2.10

Toxicological Information

2.10.1 GENERAL CONSIDERATIONS (1)

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.



2.10.1 GENERAL CONSIDERATIONS (2)

All **relevant knowledge on the NF** should be considered for the toxicological testing including:

- the 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 kinetic and toxicological data
- 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') should be considered.

2.10.1 GENERAL CONSIDERATIONS (3)

- Consider **tiered toxicity testing** approach proposed for food additives as the default approach:

  - It 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

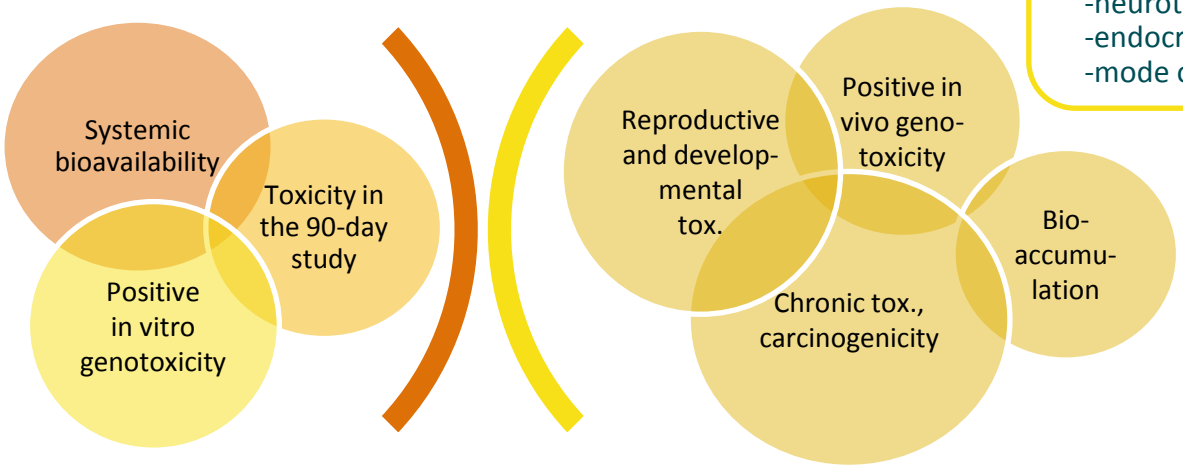
- Absorption
- Genotoxicity in vitro
- Extended 90-day toxicity study

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

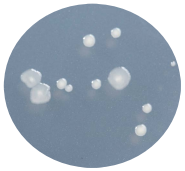
Flow-chart provided by ANS Unit

2.10.2 GENOTOXICITY

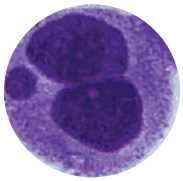
- Basic component of chemical risk assessment to identify substances which could cause **heritable damage** in humans;
- **To predict potential genotoxic carcinogens** in cases where carcinogenicity data are not available.
- For some complex mixtures and whole foods, it may be necessary to **focus on specific constituents** of the NF.
- Deviations can be argued on a case-by-case basis.
- 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.**

2.10.2 GENOTOXICITY (EFSA SC 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 test in vitro 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.

GENOTOXICITY: EXAMPLE HYDROXYTYROSOL

- Bacterial reverse mutatation test **negative**
- *In vitro* chromosome aberration test **positive**
- *In vivo* chromosome aberration with rats **negative**

Clastogenicity *in vitro* is not expressed *in vivo*

➔ ***No concerns regarding genotoxicity***

GENOTOXICITY: PUBLIC CONSULTATION

- *Further guidance was required* ***on the tests that are specifically recommended to assess the genotoxicity of NF*** *instead of referring to horizontal guidance documents.*

The tiered **approach and the type of genotoxicity tests** needed to evaluate the genotoxicity of a **NF does not differ** from other substances, e.g. additives.

Genotoxicity testing **may not be applicable** for Novel Foods, if neither their **source**, their well characterised **composition** nor the **production process** give raise for such concerns:

- some whole foods such as chia seeds, baobab fruit, insects or products derived therefrom.
- examples from the past: krill oil or rapeseed protein.

2.10.3 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.

2.10.3 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.

2.10.3 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 EXAMPLES – 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?

SUBCHRONIC STUDIES: SELECTING A REFERENCE POINT (RP)

Parameters often determining the RP (BMDL)

- Reduced body weight (gain)
- Changes in organ weight
- Clinical chemical parameters
- Effects in haematology
- Urinalysis

Criteria are:

- (Statistically) different from concurrent control
- Dose-Response
- Pattern of effects

SUBCHRONIC TOXICITY: NON-ADVERSITY

Possible criteria:

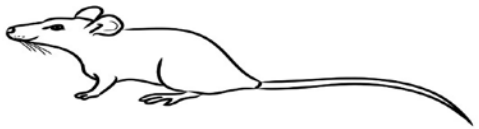
- No dose-response
- Absence of findings in histopathology. However, changes in organ weights are often first signs that organs are affected, other findings may provide pattern
- Within range of historical controls
  - ideally provided with the study report
  - should be from same lab, same rat strain, relevant years
  - allow the assessment of study control value
  - alone not sufficient to invalidate statistically significant findings
- Underlying mechanism (e.g. demonstrated low palability)

See also Draft Guidance on Biological Relevance →public consultation

SUBCHRONIC TOXICITY: BENEFICIAL OR ADVERSE ?

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

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



Despite the consideration of the above criteria, expert judgement is needed.

SCIENTIFIC COMMITTEE ON DEFAULT VALUES

in the absence of actual measured data (EFSA, 2012)

Applicable default uncertainty factor:

- Animal → Humans 10
  - inter-species toxicokinetics 4
  - inter-species toxidynamics 2.5
- Interindividual differences in humans 10
- Subchronic → chronic exposure 2

Rationale for deviation should be provided.

2.10.4 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.

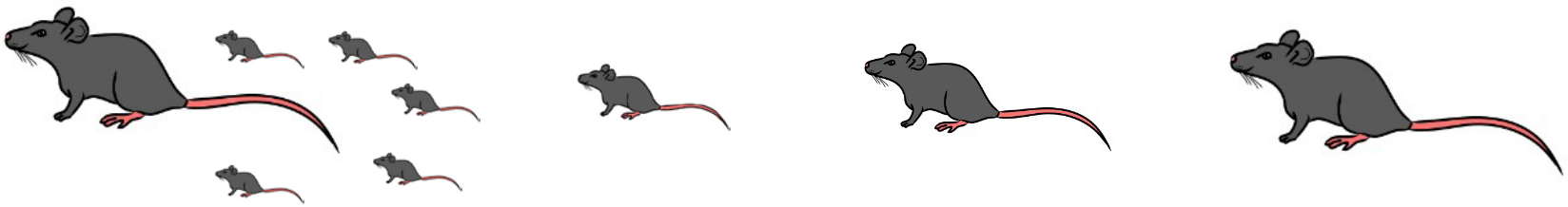
- **Further guidance** on the triggers for these studies and their implementation are outlined in the Guidance on food additives (EFSA ANS Panel, 2012) and respective OECD Guidelines (OECD TG 451, 452 or 453).

2.10.5 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.



2.10.7 SPECIFIC CASES (1)

2.10.7.1 Insects

- Present guidance applicable also for insects
- Consider Opinion of the EFSA Scientific Committee on potential hazards related to the use of farmed insects as food (EFSA Scientific Committee, 2015).
- Important factors to consider are, among others:
  - Species and substrate to be used
  - Methods for farming and processing,
  - Additional biological and chemical hazards when collecting from the wild.

2.10.7 SPECIFIC CASES (2)

2.10.7.2. Microorganisms

Wide variety of microorganisms (MO) and fungi are used in food, often in high concentrations and viable.

Qualified presumption of safety (QPS)

Some MO have been assigned the QPS status constituting a preliminary safety assessment (EFSA BIOHAZ Panel, 2015). This QPS list includes **taxonomic groups** that (1) have not raised safety concerns so far, and (2) others for which some safety concerns exist, but could be defined and addressed with "qualification".

MO with QPS status would be freed from the need for an exhaustive safety assessment (other than satisfying the criteria and qualifications specified previously and assessing the risk of antimicrobial resistance).

2.10.7 SPECIFIC CASES (3)

For those MO for which safety properties are less well understood, a safety assessment should be provided.

- **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 (e.g. toxins, D-lactate, etc.).

- **Phenotypic characterisation of potential antimicrobial resistances** following EFSA recommendations (EFSA FEEDAP Panel, 2012)
- **Characterisation of other potentially adverse phenotypic features** e.g. potential toxin production, haemolytic activity, infectivity, adverse immune effects, etc.
- Numbers of **viable MO in the final product** and stability.

2.10.7 SPECIFIC CASES (4)

2.10.7.3. Engineered Nanomaterials

- If the NF contains or consists of "engineered nanomaterials", the applicant should consider the **Guidance on the risk assessment of the application of nanoscience and nanotechnologies** in the food and feed chain from EFSA's Scientific Committee (EFSA Scientific Committee, 2011) which is currently under review by EFSA (EFSA-Q-2016-00281).

REQUESTS FOR ADDITIONAL INFORMATION (1)

Genotoxicity

- Absence of genotoxicity/mutagenicity studies
- Only Ames test provided, test for potential clastogenic activity is lacking (in vitro micronucleus test or in vitro chromosome aberration test)
- Not in compliance with OECD Guidance documents
- Invalid dose used
- Poor and incomplete description of the studies, only publications provided, unclear if representative of the NF
- No study report provided

REQUESTS FOR ADDITIONAL INFORMATION (2)

Subchronic toxicity

- Absence of sub-chronic toxicity studies
- Poor studies not in compliance with OECD and GLP
- Full study reports missing
- Published article provided (full study report missing, unclear whether all endpoints were studied, unclear whether test material was representative of the NF)
- Study in accordance with OECD and GLP, but unclear whether test material corresponds to the NF
- Inadequate considerations of statistically significant findings.

REQUESTS FOR ADDITIONAL INFORMATION (3)

Developmental / reproductive toxicity

- Insufficient description of the study (unclear method of administration, beginning of treatment not clearly described)
- Inappropriate methodology of the study (no control, only one dose tested)
- Clarification regarding missing animals or animals which died during the study
- Lacking explanation on the clinical findings reported
- Provide full study reports

REQUESTS FOR ADDITIONAL INFORMATION (4)

Other issues related to toxicological data

- No identification and review of relevant studies reported in the literature
- Studies mentioned in the application have not been made available by the applicant
- Potential endocrine function not explored

Thank you

for your attention !