

DRAFT SCIENTIFIC OPINION

Draft Guidance for submission for food additive evaluations¹

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This guidance document refers to the applications for authorisation of a new food additive or to a modification of an already authorised food additive, combining in a single document the description of the data requirements and their context, and also a description of the risk assessment paradigm applied. The document is arranged in four main sections: chemistry and specifications, existing authorisations and evaluations, proposed uses and exposure assessment, and toxicological studies. For the toxicological studies, this guidance describes a tiered approach which balances data requirements against the risk, taking into consideration animal welfare by adopting animal testing strategies in line with the 3-Rs (replacement, refinement, reduction). This tiered approach for toxicological studies consists of 3 tiers, for which the testing requirements, key issues and triggers are comprehensively described. According to this tiered approach, a minimal dataset applicable to all compounds has been developed under Tier 1, while Tier 2 testing, generating more extensive data, will be required for compounds which are absorbed and/or demonstrate (geno)toxicity in Tier 1 tests. Tier 3 should be performed on a case-by-case basis taking into consideration all the available data, to elucidate specific endpoints needing further investigation of findings in Tier 2 tests.

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KEY WORDS

22 EFSA guidance, food additives, application, tiered approach, data requirements, risk assessment

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23 **SUMMARY**

24 The Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked by the European
25 Food Safety Authority (EFSA) to develop a guidance on the scientific data required to be submitted
26 for food additive evaluations, in order to reflect the current thinking in risk assessment.

27 The present document provides guidance on data requirements for applications supporting the
28 authorisation of a new food additive or modifications to an already authorised food additive. The
29 document is arranged in four main sections: the **Chemistry and specifications** section seeks to
30 identify the food additive, potential hazards (e.g. impurities, residuals) from its manufacture, and,
31 through the specifications, to define the material tested; the **Existing authorisations and evaluation**
32 section seeks to give an overview of previous risk assessments on the additive and their conclusions;
33 the **Proposed uses and exposure assessment** section seeks to estimate dietary exposure based on the
34 proposed uses and use levels for different EU Member States and various groups in the population; the
35 **Toxicological studies** section seeks to describe the methods which can be used to identify (in
36 conjunction with data on manufacture and composition) and characterise hazards. The document also
37 describes the risk assessment paradigm (including hazard identification, hazard characterisation,
38 exposure assessment and risk characterisation) utilised by the Panel in undertaking risk assessments.
39 Consequently, it identifies relevant data and information that should be made available to permit an
40 adequate risk assessment. The Panel stresses that applicants should base their dossier on sound science
41 and evolving principles of risk assessment.

42 For the toxicological studies, this guidance describes a tiered approach which balances data
43 requirements against the risk. The tiered approach initially uses less complex tests to obtain hazard
44 data; these are then evaluated to determine if they are sufficient for risk assessment or, if not, to design
45 studies at higher tiers. The tiered approach for toxicological studies consists of 3 tiers, for which the
46 testing requirements, key issues and triggers are comprehensively described. According to this tiered
47 approach, a minimal dataset applicable to all compounds has been developed under Tier 1, while Tier
48 2 testing will be required for compounds which are absorbed, demonstrate toxicity or genotoxicity in
49 Tier 1 tests, in order to generate more extensive data. Tier 3 testing should be performed on a case-by-
50 case basis taking into consideration all the available data, to elucidate specific endpoints needing
51 further investigation of findings in Tier 2 tests.

52 In particular, the tiered approach is designed to evaluate the following core areas: toxicokinetics,
53 genotoxicity, toxicity (encompassing subchronic toxicity, chronic toxicity and carcinogenicity), and
54 reproductive and developmental toxicity. In each of these core areas for evaluation, the general
55 considerations and tiered approach to testing are outlined. In addition to the core areas for evaluation,
56 the Panel noted that other tests may be required to allow an adequate risk assessment. Other studies
57 that may be relevant and useful for assessing the risk and establishing the safety of an additive include
58 immunotoxicity, hypersensitivity and food intolerance, studies on neurotoxicity, endocrine activity
59 and mechanisms and modes of action. A number of issues related to the design, conduct and
60 interpretation of all toxicological studies, are addressed in the document.

61 Applicants are advised to design the actual testing on a case-by-case basis taking into account
62 physicochemical data on the compound, toxicity data on structurally related compounds and any
63 available information on structure activity relationships. Inherent in the rationale of a tiered approach
64 is the concept that results of studies at higher tiers will generally supersede results at lower tiers. The
65 intention is that in developing their dossier, applicants will be able to more readily identify relevant
66 data needs, which will allow adequate assessment of risks to humans from the intended use, whilst
67 strengthening the scientific basis for the assessment. In addition, this approach takes into consideration
68 animal welfare by adopting animal testing strategies in line with the 3-Rs (replacement, refinement,
69 reduction). The Panel recommends that an integrated testing strategy, which may include alternative
70 approaches, should be used to further support the risk assessment.

71 This guidance document replaces the previous guidance document by the Scientific Committee for
72 Food (SCF) published in 2001 (SCF, 2001).

73

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136 **BACKGROUND AS PROVIDED BY EFSA**

137 Regulation (EC) No 1331/2008 of the European Parliament and Council establishing a common
138 authorisation procedure for food additives, food enzymes and food flavourings lays down a common
139 procedure for the assessment and authorisation of food additives, food enzymes and food flavourings
140 in view of updating the Community lists of permitted substances defined in the corresponding sectoral
141 food laws.

142 According to this procedure, EFSA is requested to carry out a risk assessment of the substance under
143 consideration for inclusion in the relevant Community list following an application or on the initiative
144 of the Commission.

145 Regulation (EC) No 1333/2008 of the European Parliament and Council on food additives is the
146 sectoral food law for food additives referred to in Regulation (EC) No 1331/2008.

147 In accordance with the provisions of regulation (EC) No 1331/2008 on implementing measures for the
148 sectoral food laws, the ANS Panel has adopted on 9 July 2009 a statement on data requirements, while
149 suggestions for specific scientific approaches can be found in the guidance for food additives
150 applicable at the time of the application.

151 During its second plenary meeting in September 2008, the Scientific Panel on Food Additives and
152 Nutrient Sources added to food (ANS) endorsed provisionally the guidance document for food
153 additive evaluations adopted by the Scientific Committee on Food (SCF) in 2001.

154 In the statement on data requirements for the evaluation of food additive applications, the ANS Panel
155 indicated that it would start a detailed reappraisal of the guidance document of the SCF in order to
156 reflect the current thinking in risk assessment.

157 **TERMS OF REFERENCE AS PROVIDED BY EFSA**

158 The European Food Safety Authority asks the ANS Panel to develop a guidance on submission for
159 food additives evaluation, considering especially the following aspects:

160 - Chemistry of the substance and specifications
161 - Proposed uses and exposure assessment
162 - Toxicokinetics and toxicity

163 The ANS Panel will work in close collaboration with the Scientific Committee in order to take into
164 account the ongoing developments on issues related to the guidance and to contribute to them.

165

166 **INTERPRETATION OF THE TERMS OF REFERENCE BY THE ANS PANEL**

167 The Panel considered that the guidance should not only describe scientific data essential for the risk
168 assessment but also additional information which might help in providing context for the risk
169 assessment and in decreasing uncertainties in the risk assessment⁴. The guidance document should
170 combine in a single document the description of the data requirements and their context and also a

⁴ For administrative and other requirements, readers should refer to the Scientific Statement of the Panel on Food Additives and Nutrient Sources added to Food on data requirements for the evaluation of food additives applications following a request from the European Commission (EFSA Journal 1188, 1-7, 2009) and the Practical guidance for applicants for addresses, contact points and the relevant documents for risk assessment available at the DG SANCO website: http://ec.europa.eu/food/food/fAEF/authorisation_application_en.htm

171 description of the risk assessment paradigm applied. The latter will enable stakeholders to understand
172 the use and interpretation of the data. The Panel stresses that applicants should base their dossier on
173 sound science and evolving principles of risk assessment, in order to provide a high level of public
174 health protection whilst avoiding unnecessary animal experiments. To this end, this technical guidance
175 on data requirements should also indicate possible flexibility in the data requirements compatible with
176 this aim.

177

178 **INTRODUCTION**

179 This guidance document refers to the applications for authorisation of a new food additive or to an
180 extension of the authorisation of an already authorised food additive. It describes the scientific data
181 required for the evaluation of a food additive which allow its safety in proposed uses to be evaluated
182 within the established framework for risk assessment as well as the risk assessment paradigm used by
183 the Panel. A description of the risk assessment paradigm is given, followed by guidance arranged in
184 the following four main sections:

- 185 1. The **Chemistry and specifications** section seeks to identify the food additive, potential
186 hazards (e.g. impurities, residuals) from its manufacture, and, through the specifications, to
187 define the material tested.
- 188 2. The **existing authorisations and evaluation** section seeks to give an overview of previous
189 risk assessments on the additive and their conclusions.
- 190 3. The **Proposed uses and exposure assessment** section seeks to estimate dietary exposure
191 based on the proposed uses and use levels for different EU Member States and various groups
192 in the population.
- 193 4. The **Toxicological studies** section seeks to describe the methods which can be used to identify
194 (in conjunction with data on manufacture and composition) and characterise hazards.

195 In contrast to the Scientific Committee for Food (SCF) guidance document published in 2001 (SCF,
196 2001), which describes core and supplementary toxicological studies, this guidance describes a tiered
197 approach which balances data requirements against the risk. The tiered approach initially uses less
198 complex tests to obtain hazard data; these are then evaluated to determine if they are sufficient for risk
199 assessment or, if not, to design studies at higher tiers. The intention is that in developing their dossier,
200 applicants will be able to more readily identify relevant data needs which will allow adequate
201 assessment of risks to humans from the intended use whilst strengthening the scientific basis for the
202 assessment. In addition, this approach takes into consideration animal welfare by adopting animal
203 testing strategies in line with the 3 Rs (replacement, refinement, reduction). The Panel recommends
204 that an integrated testing strategy, which may include alternative approaches, should be used to further
205 support the risk assessment.

206 The Panel has sought to provide an overall concept with clear information on a tiered approach for risk
207 assessment. Using this tiered approach, a minimal dataset applicable to all compounds has been
208 developed under Tier 1. Compounds which are absorbed or for which toxic or genotoxic effects are
209 found in Tier 1, will require Tier 2 testing to generate more extensive data. Tier 3 defines detailed
210 testing for specific endpoints, for which Tier 2 testing results raised concerns. A diagram of the tiered
211 approach is presented in Appendix C.

212 Applicants are advised to design the actual testing on a case-by-case basis taking into account
213 physicochemical data on the compound, toxicity data on structurally related compounds and any
214 available information on structure activity relationships. Inherent in the rationale of a tiered approach
215 is the concept that results of studies at higher tiers will generally supersede results at lower tiers.

216 The guidance document includes the following three appendices: a diagram outlining the tiered
217 toxicity testing for food additives (Appendix A), the general data requirements⁵ as published before
218 (Appendix B), and the Specifications as required by the Commission (Appendix C).

⁵ EFSA, 2009. Data requirements for the evaluation of food additive applications.

219 **RISK ASSESSMENT PARADIGM**

220 The risk assessment process comprises four steps; hazard identification, hazard characterisation,
221 exposure assessment and risk characterisation. In carrying out its risk assessments, the Panel seeks to
222 define a health based guidance value e.g. an Acceptable Daily Intake (ADI)⁶ (IPCS, 2004) applicable
223 to the general population.

224 The ADI is established for compounds for which a threshold mechanism of toxicity can either be
225 demonstrated or reasonably expected based on the available data. The ADI does not apply to infants
226 below 12 weeks (JECFA, 1978; SCF, 1998) and the use of food additives for infant formula represents
227 a special case for which recommendations were given by the Joint FAO/WHO Expert Committee on
228 Food Additives (JECFA) (JECFA, 1972; 1978) and by the SCF (SCF, 1996; 1998). The Panel
229 endorses these recommendations.

230 In cases when an ADI cannot be derived, for example in the case of an additive where the available
231 data are considered to have certain deficiencies which nonetheless do not prevent the Panel reaching a
232 conclusion regarding safety, the Panel will consider a Margin of Safety (MOS) approach to conclude
233 whether or not there would be a risk at the proposed use and use levels. For compounds for which no
234 safe level of exposure can be anticipated, for example genotoxic carcinogens, an ADI would not be
235 established. In assessing the risk from levels of unavoidable contaminants or residuals in the additive
236 which are genotoxic and carcinogenic, the Panel generally uses the Margin of Exposure (MOE)
237 approach described in the European Food Safety Authority (EFSA) Scientific Committee opinion
238 (EFSA, 2005).

239 *Hazard identification and characterisation*

240 The chemical and technological assessment identifies the hazards of an additive, which are then
241 further characterised via their biological and toxicological dose-response relationships. Traditionally,
242 the Panel has sought to identify the most sensitive endpoint from a range of toxicological hazards and
243 their dose-response relationships, for identification of a so-called “Point of Departure” (POD). This
244 POD is used to establish an ADI, by application of uncertainty factors to account for toxicokinetic and
245 toxicodynamic differences between individuals and species. Typical PODs include the No Observed
246 Adverse Effect Level (NOAEL) or a BMDL value (the lower confidence bound of the benchmark dose
247 (BMD)). The EFSA Scientific Committee has recently endorsed the benchmark dose procedure and
248 the use of the BMDL₀₅ for continuous data or the BMDL₁₀ for quantal data as a preferred approach to
249 the NOAEL, to define the POD for deriving health-based guidance values (EFSA, 2005; 2009a). The
250 Panel expects to increasingly use BMDL values rather than the NOAEL for deriving an ADI, and this
251 should be considered when designing toxicology studies.

252 The default uncertainty factors used by the Panel are a factor 10 for toxicokinetic and toxicodynamic
253 differences between individuals, and an additional factor 10 for toxicokinetic and toxicodynamic
254 differences between species. Additional uncertainty factors may be applied depending on the adequacy
255 of the entire database. Furthermore, where human and animal kinetic data are available, they can
256 potentially be used in risk assessment to derive chemical-specific adjustment factors (CSAFs) for
257 interspecies extrapolation (Meek et al., 2003). CSAFs may be higher or (more usually) lower than
258 default uncertainty factors.

259 The International Programme on Chemical Safety (IPCS) has published guidance on the use of
260 quantitative toxicokinetic and toxicodynamic data for the derivation of CSAFs as part of its project on
261 the Harmonisation of Approaches to the Assessment of Risk from Exposure to Chemicals (IPCS,
262 2005). Toxicokinetic data can also be of value in developing adjustment factors for groups of related
263 chemicals that share common physical or chemical characteristics or toxicokinetic or toxicodynamic
264 pathways (Bokkers and Slob, 2007; Dorne and Renwick, 2005; IPCS, 2005; Naumann et al., 2001).

⁶ The ADI is the estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk.

265 *Exposure Assessment*

266 Assessment of the exposure to food additives is the process of the qualitative and/or quantitative
267 evaluation of their likely intake by the European population, taking into account their dietary and non-
268 dietary sources as appropriate. Exposure assessment is an essential component for quantifying risk and
269 for determining whether a food additive poses an unacceptable risk to the European population.
270 Typically, data on actual food consumption from national or international surveys in Europe are
271 combined with the intended use levels of the food additive to estimate the exposure to a food additive.
272 This exposure assessment is intended to cover the population of all European Member States taking
273 into account the variation of exposure due to differences in food consumption across the Member
274 States and between various groups of the population, in particular toddlers, children, adolescents,
275 adults and elderly.

276 Dietary exposure to a food additive is determined by summing the contribution made by each food in
277 which the food additive is intended to be used. This in turn is achieved by multiplying the
278 concentration of the food additive in a given food or food category by the food consumption of this
279 food or food category. The concentration of the food additive may be derived from maximum
280 permitted levels laid down in legislation, or from normal use levels as determined analytically or as
281 indicated by industry. Finally, the result is divided by the corresponding body weight of the
282 population affected to give the exposure on a kg body weight and day basis.

283 *Exposure assessment and outcome of the risk assessment*

284 The overall evaluation of the additive for potential human risk should be made in the context of the
285 known or likely human exposure in comparison with the ADI derived from the POD, with application
286 of an appropriate uncertainty factor. In the first instance the ADI is compared with the human
287 exposure estimate resulting from use of the additive at the proposed uses and use levels, and in the
288 second instance the comparison also includes exposure from other sources, where relevant. When
289 using the MOS approach, the Panel would generally consider a MOS of 100 between a NOAEL or
290 BMDL and the anticipated exposure, sufficient.

291 *Unavoidable genotoxic and carcinogenic residuals*

292 The Panel considered that it would be possible to use a MOE approach for unavoidable genotoxic and
293 carcinogenic residuals. The Scientific Committee described that for contaminants a MOE of 10,000 or
294 higher, if it is based on the BMDL₁₀ from an animal study, and taking into account overall
295 uncertainties in the interpretation, would be of low concern from a public health point of view and
296 might be reasonably considered as a low priority for risk management actions (EFSA, 2005). .
297 However the Panel considered that for unavoidable residuals, the MOE should be at least 10,000 and
298 preferably as large as possible, and that this should be reflected in the specifications. Whenever
299 possible, it would be prudent to establish levels of this type of residuals in the specifications as low as
300 reasonably practicable.

301

302 **1. CHEMISTRY AND SPECIFICATIONS**

303 The chemistry and specifications of a substance (or mixture of substances), in terms of chemical
304 structure(s) and physico-chemical properties, is critical information required for risk assessment and
305 subsequent risk management. The purity of a single substance needs to be defined by specifications,
306 and adequate chemical characterisation of simple mixtures needs to be performed. It may not always
307 be possible to fully characterize more complex mixtures, but as much information as possible is
308 required to understand the extent to which variability in composition is controlled during manufacture.
309 The information required with respect to identity is set out in detail in subsections 1.1.1 to 1.1.7 and
310 the complementary information on Specifications in Section 1.2. Section 1.3 describes information
311 requirements for the manufacturing process. Information on the manufacturing process is used in the
312 risk assessment to identify impurities, residuals, reaction intermediates, precursors and reagents that
313 could have an influence in the toxicological evaluation. Hazards that might need to be controlled in the
314 material of commerce need to be identified and specified (e.g. genotoxic compounds, heavy metals).
315 Section 1.4 describes the information requirements for analytical methods to detect and measure the
316 additive in food. Section 1.5 describes information requirements for evaluating the stability of the
317 additive during storage and over time, when used in different food types. The identification of
318 degradation products might trigger toxicological evaluation of one or more degradation products to
319 characterise any additional hazards and risks.

320 **1.1. Identity of the substance**

321 **1.1.1. *Single substances (e.g. sorbic acid, sodium ascorbate, propyl gallate, glycerol, etc)***

- 322 • Chemical name, when appropriate, according to IUPAC nomenclature rules.
- 323 • CAS number (if this has been attributed) from the ChemIDplus database, E number
324 (where appropriate), EINECS number (where appropriate), and other identification
325 numbers.
- 326 • Synonyms, trade names, abbreviations.
- 327 • Molecular and structural formulae.
- 328 • Molecular weight (g/mol) or atomic weight (for elements).
- 329 • Spectroscopic data (printout) such as NMR or MS spectra or other data.
- 330 • Description of physical and chemical properties: appearance, melting point, boiling
331 point, specific gravity, stereochemistry (if any).
- 332 • Solubility (reference e.g. JECFA, 2006 - general method for solubility) in water and
333 other common solvents.
- 334 • Influence of pH on solubility - ionisation constant(s).
- 335 • Octanol: water partition ratio.
- 336 • Particle size, shape and distribution, if applicable.
- 337 • Other data that the applicant considers may be useful to support the identity of the
338 substance.

339 **1.1.2. *Simple mixtures (e.g. sorbitol syrup, lecithins, etc)***

340 These are mixtures whose components can be fully chemically characterised.

- 341 • Chemical name, when appropriate, according to IUPAC nomenclature rules.

342 • Chemical composition-identity of the components of the mixture as required in point
343 1.1.1.

344 • CAS number (if this has been attributed) from the ChemIDplus database, E number
345 (where appropriate), EINECS number (where appropriate), and other identification
346 numbers.

347 • Synonyms, trade names, abbreviations.

348 • Proportion of each component of the mixture.

349 • Molecular and structural formulae of each component of the mixture.

350 • Molecular weight (g/mol) of each component of the mixture.

351 • Spectroscopic and chromatographic data (printout of spectra/chromatogram) which
352 allow the identification of the components of the mixture.

353 • Description of physical and chemical properties: appearance, stereochemistry of each
354 component (unless not applicable).

355 • Solubility (reference e.g. JECFA general method for solubility (JECFA, 2006)) in
356 water and other common solvents.

357 • Particle size, shape and distribution, if applicable.

358 • Other data that the applicant considers may be useful to identify the mixture and its
359 components.

360 **1.1.3. Complex mixtures others than those derived from botanical sources (e.g. mineral
361 hydrocarbons, caramels, etc)**

362 These are mixtures whose components cannot be always fully chemically characterised. The level of
363 chemical characterisation required depends on the proposed use and use levels.

364 • Starting materials or source materials

365 • Chemical name, when appropriate, according to IUPAC nomenclature rules.

366 • CAS number (if this has been attributed) from the ChemIDplus database, E number
367 (where appropriate) EINECS number (where appropriate) and other identification
368 numbers.

369 • Synonyms, trade names, abbreviations.

370 • Chemical description, the level of principal components in so far as these are known
371 and level of unidentified components.

372 • Description of physical and chemical properties.

373 • Solubility (reference e.g. JECFA general method for solubility (JECFA, 2006)) in
374 water and other common solvents.

375 • Particle size, shape and distribution, if applicable.

376 • Other data that the applicant considers may be useful to identify the mixture and its
377 components.

378 **1.1.4. Polymers (e.g. anionic methacrylate, agar, alginate and xanthan gums, pectins, modified
379 starches, celluloses, polyvinylpyrrolidone, etc)**

380 • Chemical name, when appropriate, according to IUPAC nomenclature rules.

- CAS number (if this has been attributed) from the ChemIDplus database, E number (where appropriate), EINECS number (where appropriate), and other identification numbers.
- Synonyms, trade names, abbreviations.
- Chemical and structural formula and molecular weight or number average molecular weight and weight average molecular weight (if feasible).
- Structural formulae of monomers and starting materials, other agents involved in the polymerisation.
- Degree of substitution, percentages of substituted groups (where appropriate).
- Description of physical and chemical properties.
- Solubility (reference e.g. JECFA general method for solubility (JECFA, 2006)) in water and other common solvents.
- Particle size, shape and distribution, if applicable.
- Other data that the applicant considers may be useful to identify the polymer and its constituents.

1.1.5. Additives derived from botanical sources (such as steviol glycosides from Stevia, or rosemary extracts)

399 In agreement with the EFSA Guidance on Safety assessment of botanicals and botanical preparations
400 intended for use as ingredients in food supplements (EFSA, 2009b), the following information for
401 plant-derived additives is required in addition to the chemical information listed in sections 1.1.1 –
402 1.1.4.

403 Concerning the plant being the source of the additive, this includes:

- The scientific (Latin) name (botanical family, genus, species, subspecies, variety with author's name, chemotype, if applicable).
- Synonyms (botanical name) that may be used interchangeably with the preferred scientific name.
- Common names (if a trivial or a common name is used extensively in the monograph, it should be firmly linked to the scientific name and part used).
- The part used (e.g. root, leaf, seed, etc.).
- The geographical origin (continent, country, region).
- Growth and harvesting conditions (wild or cultivated; cultivation practices, time of harvest in relation to both season and stage of the plant growth).

414 Furthermore data on the chemical composition of the plant-derived food additive should be provided
415 with emphasis on the concentrations of constituents of relevance; this includes the concentrations of
416 the following:

- Compounds classified according to their chemical structure (e.g. flavonoids, terpenoids, alkaloids, etc.).
- Constituents being characteristic for the food additive (chemical fingerprint, markers).

420 • Constituents that provide reasons for concern due to their chemical, pharmacological or
 421 toxicological properties.

422 In addition, since processing (e.g. extraction solvent, temperature) may influence the composition of
 423 the plant-derived food additive, the composition should be characterized for each proposed production
 424 process to facilitate read across.

425 Information on maximum levels for microorganisms and possible contaminants, including e.g. heavy
 426 metals, mycotoxins, pesticide residues and polycyclic aromatic hydrocarbon (PAH) residues, should
 427 be provided (EFSA, 2009b).

428 **1.1.6. Nanomaterials**

429 The following information for nanomaterials, taken from Table 1 of the EFSA Guidance on
 430 engineered nanomaterials (ENMs) (EFSA, 2011a), is required in addition to the chemical information
 431 listed in sections 1.1.1 – 1.1.4.

432 **Table 1:** Parameters for characterisation and identification of ENMs (EFSA, 2011a)

Parameter	Requirements	Description
Chemical composition/identity	Essential	Information on chemical composition of the ENM – including purity, nature of any impurities, coatings or surface moieties, encapsulating materials, processing chemicals, dispersing agents and/or other formulants e.g. stabilisers.
Particle size (Primary/ Secondary)	Essential (two methods, one being electron microscopy)	Information on primary particle size, size range and number size distribution (indicating batch to batch variation – if any). The same information would be needed for secondary particles (e.g. agglomerates and aggregates), if present.
Physical form and morphology	Essential	Information on the physical form and crystalline phase/shape. The information should indicate whether the ENM is present in a particle-, tube-, rod-/shape, crystal or amorphous form, and whether it is in free particulate form or in an agglomerated/ aggregated state, as well as whether the preparation is in the form of a powder, solution, suspension or dispersion.
Particle and mass concentration	Essential for dispersions and dry powders	Information on concentration in terms of particle number and mass per volume when in dispersion, and per mass when as dry powder.
Specific surface area	Essential for dry powders	Information on specific surface area of the ENM.
Surface chemistry	Essential (for ENM with surface modifications)	Information on ENM surface – including any chemical/biochemical modifications that could modify the surface reactivity, or add a new functionality.
Surface charge	Essential	Information on zeta potential of the ENM.
Redox potential	Essential for inorganic ENMs	Information on redox potential. Conditions under which redox potential was measured need to be documented.
Solubility and partition properties ^a	Essential	Information on solubility of the ENM in relevant solvents and their partitioning between aqueous and organic phase (e.g. as log K _{ow} if appropriate).
pH	Essential for liquid dispersions	pH of aqueous suspension.
Viscosity	Essential for liquid dispersions	Information on viscosity of liquid dispersions.
Density and pour density	Essential for granular materials	Information on density/porosity of unformulated ENM and pour density.
Dustiness	Essential for dry powders	Information on dustiness of powder products – such as spices, creamers and soup powders.

Chemical reactivity/catalytic activity ^b	Essential	Information on relevant chemical reactivity or catalytic activity of the ENM and of any surface coating of the ENM.
Photocatalytic activity	Essential for photocatalytic materials	Information on photocatalytic activity of relevant materials used in food packaging, coatings, and printing inks and internal reactions.

433 a) Dispersion, solution, dissolved: An insoluble ENM introduced to a liquid form a ‘dispersion’ where the liquid and the
 434 ENM coexist. In a true solution the ENM is dissolved (and thus not present) (see OECD ENV/JM/MONO(2010)25).
 435 b) If an ENM has catalytic properties, it may catalyse a redox or other reaction which may perpetuate resulting in a much
 436 larger biological response even with small amounts of the catalytically active ENM. Thus, compared to a conventional
 437 biochemical reaction which uses up the substrate, ENM reaction centres may perpetuate catalytic reactions.
 438

439 The Panel considers that for natural nanomaterials, similar characterisation to that required for ENMs
 440 should be carried out and provided.

441 **1.1.7. Substances containing microorganisms or derived from microorganisms**

442 The following information is required for additives of microbial origin.

443 • The microbial origin of food additives produced by fermentation or cultivation, including:

444 - Name of the microorganism

445 - Taxonomic classification of the microorganism

446 - History of modification of the production organism

447 • Whether the microorganism fulfils the requirements for a Qualified Presumption of Safety
 448 (QPS) (EFSA, 2007). In such cases no further data on the microorganism itself are required.

449 • Information on residual levels of toxins.

450 • Information on the production process.

451 • Information on the identity of residual intermediates or microbial metabolites in the final
 452 product.

453 • In the special case of food additives consisting of, containing, or produced from genetically
 454 modified microorganisms (GMMs), these have to be authorised in accordance with Regulation
 455 (EC) No 1333/2008⁷ and Regulation (EC) No 1829/2003⁸, and the Guidance of the GMO
 456 Panel on the risk assessment of products GMMs should be followed (EFSA, 2011b).

457 **1.2. Specifications**

458 The specifications of an additive define the requirements concerning the identity, the purity and the
 459 limits of any impurity present in the additive, indicating also the appropriate methods of analysis.
 460 Analytical information on at least 5 batches of the proposed additive, produced according to the

⁷ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food additives. Official Journal of the European Union L354/16, EN, 31.12.2008. Available at: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0016:01:EN:HTML>

⁸ Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Union L268/1, EN, 18.10.2003. Available at: http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_268/l_26820031018en00010023.pdf

461 method of manufacture and using the analytical methods described, should be provided in order to
462 show that the additive can be consistently manufactured within its proposed specifications. A rationale
463 for the proposed specifications should be provided.

464 The following information is required about the specifications of an additive.

- 465 • The definition of the article of commerce.
- 466 • The proposed specifications should include the purity in percentage and the method of
467 determination to allow the identification of the substance (chromatograms, spectra,
468 etc.).
- 469 • The proposed specifications should include the impurities: nature, limits (including for
470 individual heavy metals, and where appropriate, for microorganisms and mycotoxins)
471 and methods of determination and their validation.
- 472 • The proposed specifications should be submitted in a format modelled on recent EU
473 (see Appendix B) or other internationally accepted specifications.
- 474 • Where the proposed specifications differ from any already existing EU, JECFA or
475 other internationally recognised specifications, these specifications should be set out
476 alongside the proposed new specification, and any differences pointed out.
- 477 • The specifications for additives derived from **botanical sources** may be based on
478 nutritional or biologically active components or, when these are not known, on
479 selected chemical markers. In agreement with the EFSA Guidance on Botanicals
480 (EFSA, 2009b), specifications for botanical sources should indicate:
 - 481 a) The identity of the article of commerce.
 - 482 b) The purity of the article of commerce in percentage; concentrations of major
483 groups of constituents present in the botanical preparation (e.g. amino acids,
484 lipids, polysaccharides, volatile oil, inorganic ions, polyphenols, alkaloids,
485 terpenes, alkenylbenzenes, lignin, saponins, etc.) as well as the major
486 constituents within these classes. Methods of determination (chromatograms,
487 spectra, etc).
 - 488 c) Limits for specific undesirable/toxic substances known to be present in the
489 plant. Validated methods should be provided for the analysis.
 - 490 d) Information on maximum levels for microorganisms and possible
491 contaminants including e.g. heavy metals. Validated methods should be
492 provided for the analysis of substances considered in the specifications.
 - 493 e) Compliance with recent EU or other internationally accepted specifications
494 (e.g. pharmacopoeia) where appropriate.
 - 495 f) Where the proposed specifications differ from internationally recognised
496 specifications, the latter specifications should be set out alongside the
497 proposed new specifications, and any differences pointed out.
- 498 • The specifications should describe the material in full (100%) and state the percentage
499 of the material that is unidentified.

500 **1.3. Manufacturing process**

501 The information on the manufacturing process is used in the risk assessment to identify impurities,
502 reaction intermediates, precursors and reagents that could present a hazard. Where hazards are
503 identified, they might need to be controlled in the material of commerce (e.g. genotoxic compounds,
504 heavy metals).

505 Two descriptions of the manufacturing process should be provided: a generic (non-confidential)
506 description of the process and another (which may be confidential) with more detailed information
507 including the following:

- 508 • Method of manufacture (e.g. raw materials, the process by which the raw materials are
509 converted to the finished product), production controls and quality assurance.
- 510 • For substances synthesised chemically: i) factors such as reaction sequence, side
511 reactions, purification and preparation of the product to be commercialised, which
512 may assist in determining likely impurities and their influence on the toxicological
513 evaluation; ii) information on substances entering the manufacturing process, e.g.
514 identity of the extraction solvent, reagents, special precautions (light and temperature)
515 should be provided.
- 516 • For substances derived **from botanical sources**: i) information on the method(s) of
517 manufacture should include the process by which the raw material is converted into a
518 preparation, such as extraction or other procedure(s), and plant extract:ratio; ii)
519 information on substances entering the manufacturing process, e.g. identity of the
520 extraction solvent, reagents, special precautions (light and temperature); iii)
521 standardisation criteria (e.g. see European Pharmacopoeia) (EFSA, 2009b).

522
523 In submissions requesting approval of a currently permitted EU additive that is to be manufactured by
524 a new method involving significant change in its production methods or starting materials used, or in
525 which there is a change in form from conventional bulk material to nanoscale dimensions, the main
526 differences between the existing manufacturing method and the new manufacturing method should be
527 highlighted, including information on, or prediction of, any new impurities that may be present as a
528 result.

529 **1.4. Methods of analysis in food**

530 A minimum of a single laboratory validated analytical method should be provided for the
531 determination of the substance and its degradation and reaction products in the foodstuffs to which the
532 substance is intended to be added. The method(s) provided should be specific and fit-for-purpose.
533 They should be applicable to all the types of foodstuffs to which the substance may be added.
534 Method(s) should be given in full except where the analytical methods used are well established and
535 may be given by reference only.

536 In the case of additives made from nanomaterials, the Panel refers to the EFSA opinion on the
537 potential risks arising from nanoscience and nanotechnologies on food and feed safety (EFSA, 2009c;
538 2011a), which states that "*in the absence of exposure data, and where it is not possible to determine
539 the nanoform in the food/feed matrix, it should be assumed that all added ENM is present, ingested
540 and absorbed in the nanoform*". The Panel noted that in such cases conventional chemical methods
541 may be used to measure the total amount of the additive present.

542 **1.5. Stability of the substance, and reaction and fate in food**

543 The stability of the additive during storage, as produced and in food, should be evaluated and
544 described. This information requirement for establishing the stability of the additive during storage
545 conditions in different food types and over time in foodstuffs is to identify hazards which might arise
546 from degradation products to characterise any additional hazards and risks. Appropriate information
547 should be provided on:

- 548 • The chemical/physico-chemical stability of the food additive in its food additive
549 preparation and under the conditions of storage and effect of storage temperature,

550 environment [light, oxygen, moisture, relative humidity (water activity)] or any other
551 factor that might influence the stability of the food additive preparation.

552

- 553 The chemical/physico-chemical stability of the additive during storage of the
554 processed food: e.g. effect of the nature of the food to which the substance is added,
555 processing temperature, pH, water activity or any other factor. If possible, the nature
556 and reactivity of degradation products and nature of interaction/reaction of any
degradation product with food components.
- 557 Technologically intended reactions with food constituents and the resulting products
558 in food.

559

560 **2. INFORMATION ON EXISTING AUTHORISATIONS AND EVALUATIONS**

561 Information on existing authorisations and evaluations should be provided. This should include details
562 of the following:

563

- 564 the body which carried out the evaluation;
- 565 when the evaluation was undertaken;
- 566 details of the evaluation identifying the critical studies and their NOAELs/LOAELs
and BMDL values, and
- 567 any uncertainties described, health-based guidance values (e.g. ADIs) and the
568 uncertainty factors used in this evaluation.

569

570 **3. PROPOSED USES AND EXPOSURE ASSESSMENT**

571 *Introduction*

572 Historically, exposure assessment of food additives followed a tiered approach from crude estimates
573 (Tier 1) to more refined estimates (Tiers 2 and 3), as outlined in the report from the Commission on
574 dietary food additive intake in the EU (EC, 2001). Tier 1 started with crude estimates (Budget
575 method), based on theoretical food consumption data and the maximum intended use levels of the food
576 additive (SCOOP report) (EC, 1997). Tier 2 estimates were calculated by using data on actual food
577 consumption and the maximum intended use level of the food additive, thus representing a refined
578 estimate of potential exposure compared to Tier 1. For the re-evaluation of already authorised food
579 additives, Tier 3 estimates (further refinement of exposure estimates at Tier 2) were calculated by
580 using data on actual food consumption and normal use levels of the food additive. Data on the normal
581 use level are available from the food industry or post marketing surveillance by food enforcement
582 authorities in the Member States. The highest normal use levels reported by industry were used for
583 exposure estimation at Tier 3.

584 Since the concept of the Tier 1 (Budget method) was developed for post marketing surveillance, Tier 1
585 calculations are not required for new authorisation of a food additive or a modification of an existing
586 authorisation. Tier 3 estimates are only relevant for already authorised food additives, as no normal
587 use level would exist for applications for the authorisation of a new food additive. Overall, the Panel
588 considered that this historical tiered approach was no longer appropriate.

589 *Data required for the estimation of exposure in accordance with this guidance document*

590 As already indicated in the introductory section on the risk assessment paradigm, assessment of the
591 exposure to food additives is the process of the qualitative and/or quantitative evaluation of their likely
592 intake by the European population, taking into account their dietary and non-dietary sources as
593 appropriate. To enable this assessment, information should be provided on known or anticipated
594 human exposure to the proposed additive from food (including natural dietary sources) and any other
595 potential sources (e.g. from drinking water, consumer products (cosmetics), pharmaceuticals etc.).
596 When a modification of the conditions of use of an already authorised food additive is requested, the
597 exposure estimates should also take into account all existing authorisations. Exposure estimates are
598 also to be provided on any potential exposure to relevant residues or contaminants present due to the
599 use of the additive.

600 For the purpose of carrying out an exposure estimation in accordance with this guidance document,
601 data are required for the relevant one of the two different scenarios:

602 i. **Scenario 1** refers to applications for the authorisation of a new food additive;

603 ii. **Scenario 2** refers to a modification of the proposed uses or use levels of an already
604 authorised food additive.

605 The exposure estimation is calculated for the applicable scenario by entering the data requested in the
606 section on Proposed Uses and Use Levels in a template which will be available to the applicant. This
607 template will provide a first simplified calculation of exposure estimates by combining the data on the
608 proposed uses and use levels for a new authorisation (**Scenario 1**) with summary statistics data
609 calculated from the EU Comprehensive Food Consumption Database (EFSA, 2011c). In the same
610 way, this template will provide the basis of refined estimates for **Scenario 2** by combining the data on
611 the proposed new uses and use levels for a modification of an existing authorisation and the normal
612 use levels of the existing authorisation with the EU Comprehensive Food Consumption Database
613 (EFSA, 2011c).

614 Anticipated exposure is calculated for each individual food group in which the additive is proposed.
615 Mean and 95th percentile anticipated exposures are calculated for each food category for the following
616 age groups: European toddlers (12 months up to 35 months), children (36 months to 9 years),
617 adolescents (10-17 years), adults (18-64 years) and elderly (over 65 years). The main food groups
618 contributing (more than 5%) to the total exposure of the additive should be indicated either as a single
619 value or as several values when the exposure estimates are similar or highly variable between Member
620 States. In addition, the range of the contribution to the total exposure (minimum to maximum across
621 Member States) should be indicated for all contributors to the total exposure, regardless their relative
622 contribution (i.e. including food groups that are contributing less than 5 %). The template will
623 automatically calculate and identify the main food groups contributing to the exposure of the additive.
624 These main contributors should be described in the main text of the exposure section of the
625 application.

626 **3.1. Proposed uses in food and corresponding use levels**

627
628 The data requested for an authorisation of a new additive should indicate in which foods this additive
629 is proposed to be added/used, and the intended use level of the food additive (Scenario 1). The data
630 requested for a modification of the proposed uses or use levels include the new proposed use levels,
631 and both the maximum permitted levels and the normal use levels of the already authorised uses
632 (Scenario 2). The normal use level is considered to be the concentration of a food additive used in a
633 given food in order to provide the intended technological effect. In most cases, normal use levels are
634 expected to be lower than the maximum permitted use level in a food category. The Panel will not be

635 able to conclude on the safety of a food additive if only *quantum satis* use is proposed since exposure
636 estimates cannot be calculated in this case.

637 Data on the normal use level are available from the food industry or from post-marketing surveillance
638 by food enforcement authorities in Member States. In principle, a normal use level is the average level
639 of the food additive determined in a number of samples being representative for the food in a given
640 European Member State. It is likely that within the European Member States different levels of food
641 additives are typically found for the same food category. If so, the highest reported normal use levels
642 within the European Member States should be used for exposure estimation.

643 In order to support the calculation of the most refined possible exposure estimations, each food or food
644 category in which the food additive is used or proposed to be used should be defined at the highest
645 level of detail possible for the two following food classification systems:

646 - FoodEx classification system (used for the EFSA comprehensive database)⁹
647 - Food classification system defined in the Annex II of Regulation 1333/2008¹⁰.

648 **3.1.1. Authorisation of a new food additive (Scenario 1)**

649
650 Data required for a new authorisation should be as follows:

651 • Proposed use and use level of the food additive in the final food product for each food
652 item or food category. For food additives prepared by extraction from natural sources
653 (e.g. beetroot red, rosemary extracts, etc), the use levels provided should be related to
654 i) the additive itself, and ii) the corresponding concentration of the compound and
655 other components in the mixture.

656 • If the intended use can be achieved by different chemical forms of the food additive
657 (e.g. potassium nitrate/sodium nitrate, lutein/lutein esters), data are required on the
658 proposed use level of each of the chemical forms of the additive and whether they are
659 proposed to be used in combination or replacing each other in the final product.

661 **3.1.2. Modification of an existing authorisation (Scenario 2)**

662
663 Data required for a modification of an existing authorisation should be as follows:

664
665 • If applicable, proposed use level of the food additive in the final food product for each
666 food or food category for the newly proposed uses. For food additives prepared by
667 extraction from natural sources (e.g. beetroot red, rosemary extracts, etc), the use
668 levels provided should be related to i) the additive itself, and ii) the corresponding
669 concentration of the compound and other components in the mixture.

670 • If applicable, use level of the food additive in the final food product proposed to
671 replace the existing maximum permitted level for each food or food category for

⁹<http://www.efsa.europa.eu/en/datex/datexfooddb.htm>,

<http://www.efsa.europa.eu/en/datexfooddb/docs/datexfooddbchronicgday.xls>

¹⁰ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food additives.

Official Journal of the European Union L354/16, EN, 31.12.2008. Available at: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0016:01:EN:HTML>

672 already authorised uses. For food additives prepared by extraction from natural
673 sources (e.g. beetroot red, rosemary extracts, etc), the use levels provided should be
674 related to i) the additive itself, and ii) the corresponding concentration of the
675 compound and other components in the mixture.

676 • The normal use levels of the food additive in the final food product for the already
677 authorised uses of the food additive.

678 • The maximum permitted levels of the food additive as laid down in the relevant
679 regulation for the already authorised uses of the food additive in the final food
680 product.

681 • If the intended use can be achieved by different chemical forms of the food additive
682 (e.g. potassium nitrate/sodium nitrate, lutein/lutein esters), data are required on the
683 proposed use level of each of the chemical forms of the additive, and whether they are
684 proposed to be used in combination or replacing each other in the final product.

685 If carry over of the food additive itself or any other toxicological relevant residue may occur, for
686 example where a food additive is not intended to be part of the final food product, during manufacture
687 (e.g. coating material, additives for the stabilisation of vitamin preparations, etc.), data are requested
688 on the carry over of the food additive and its resulting concentration in the final food product.

689 **3.2. Exposure data**

690 **3.2.1. Assessment of exposure to the food additive from other uses**

691 The evaluation of the safety of a food additive is based on the combined exposure from all sources.
692 Other potential sources of exposure to the additive or toxicologically relevant components of the
693 additive should therefore be taken into account (e.g. natural occurrence in food, non-additive use in
694 food supplements, use as flavouring, use as food contact material, use in pharmaceutical or cosmetic
695 products).

696 For these sources, the average anticipated exposure and exposure at the 95th percentile are requested
697 for the population groups as indicated above. Subsequently, the Panel may decide to request further
698 information (including quantitative data) regarding the exposure resulting from these additional
699 sources, depending on their relevance.

701 **3.2.1.1. Assessment of aggregate exposure to the same compound from different sources**

702 For the estimation of total exposure to the food additive, data are requested on aggregated exposure to
703 the food additive from all sources, as outlined above. Aggregated exposure is the sum of:

- 704 - average exposure to the food additive from its use as food additive at the proposed use and the
705 corresponding use levels,
- 706 - average exposure from its natural sources as appropriate,
- 707 - average exposure from food fortification and supplements as appropriate, and
- 708 - average exposure from other uses, from those listed above.

709 Since high percentiles of overall exposure should only be calculated from individual data, in order to
710 avoid gross overestimations, high percentile estimates for each food category or other source should
711 be provided but may not be used for that calculation.

712 3.2.1.2. *Estimate of exposure to residues or contaminants*

713 Finally, exposure to any toxicologically relevant components coming into foods from the use of the
714 food additive (e.g. potential residues of degradation products, reaction products, or contaminants
715 arising from the use of the additive) should be provided taking into account specific legislative purity
716 criteria as applicable. It is recommended that the same template is used as for the food additive itself,
717 in order to describe the anticipated exposure for average and 95th percentile consumers to this
718 compound for the population groups, as indicated above.

719 3.2.2. *Submission of data*

720 The applicant will be requested to provide these data in the form of a template that will be made
721 available.

722

723 **4. TOXICOLOGICAL STUDIES (TOXICOKINETICS AND TOXICITY)**

724 The tiered approach, described below, is designed to evaluate the following core areas:

725 - Toxicokinetics
726 - Genotoxicity
727 - Toxicity encompassing subchronic, chronic toxicity and carcinogenicity
728 - Reproductive and Developmental toxicity

729 These are normally assessed on the basis of toxicological studies performed *in vitro*, and *in vivo* using
730 laboratory animals. Further details of these core areas are given below. Experimental studies (e.g.
731 toxicokinetics data, SARs, data from other toxicity and neurotoxicity studies) and human data
732 (epidemiological studies and case reports, if available) should be included in the evaluation. A number
733 of issues related to the design and conduct of all toxicological studies are addressed in the next section.

734 **ISSUES TO BE CONSIDERED IN THE DESIGN AND PERFORMANCE OF TOXICOLOGICAL STUDIES**

735 The following aspects should be considered in the design, conduct and interpretation of toxicological
736 studies on food additives.

737 • Toxicological studies should be carried out with the additive as manufactured according to the
738 proposed specifications, unless there are scientific reasons why this is not appropriate. In such
739 cases the scientific reasons should be clearly and adequately described.

740 • Ethical approval and welfare standards for animal and human studies should comply with
741 relevant EU standards and regulations on the protection of humans and animals used for
742 scientific purposes.

743 • Applicants are reminded that Council Directive 2010/63/EU¹¹, on the protection of animals
744 used for experimental and other scientific purposes, requires that care is taken to avoid
745 unnecessary use of animals. Studies carried out should be those necessary to demonstrate the
746 safety of an additive and planned in accordance with the principles of replacement, reduction,
747 and refinement. Since adequate human data are unlikely to be available, *in vivo* studies using
748 experimental animals from species relevant to humans are still needed in order to assess
749 possible risks to humans from the ingestion of food additives. There are some exceptions to
750 this (e.g. initial assessment of genotoxic potential by *in vitro* studies), and alternative validated
751 methods for other endpoints in toxicity, involving fewer or no animals, are being developed.
752 Studies submitted using alternative methods will be considered by the Panel on a case-by-case
753 basis.

754 • Studies on toxicokinetics and toxicity of food additives should be conducted using
755 internationally agreed test guidelines. Test methods described in OECD test guidelines
756 (OECD TG) or in Council Regulation (EC) No 440/2008¹² laying down test methods pursuant
757 to Regulation (EC) No 1907/2006¹³ of the European Parliament and of the Council on the

¹¹ Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

¹² Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

¹³ Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

758 Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) are
759 recommended. The most up-to-date edition of any test guideline should be followed. However
760 it should be noted that these guidelines provide minimum criteria for acceptance of studies and
761 a specific protocol should be derived for each study which may need additional requirements
762 above these minimum criteria. These may serve as screens for more specialised endpoints and
763 their results may point to the need for additional specialised studies (e.g. neurotoxicity and
764 immunotoxicity). Use of any methods differing from internationally agreed test guidelines
765 including protocols for special studies should be justified and their acceptance will be assessed
766 on a case-by-case basis.

- 767 • Studies should be carried out according to the principles of Good Laboratory Practice (GLP)
768 described in Directive 2004/10/EC¹⁴. Applicants need to be aware that studies that fail to meet
769 the minimum requirements of internationally agreed test guidelines, or which are conducted
770 post 1987 and are not GLP compliant, can be rejected on this basis. The Panel does not
771 generally apply this to historical studies being re-evaluated or mechanistic studies used in
772 support of mode of action analyses.
- 773 • Substances should normally be administered via the oral route. Consideration should be given
774 to the choice of mode of administration, bearing in mind the form in which humans are likely
775 to consume the substance and the influence this will have on rate of absorption and subsequent
776 systemic exposure. For substances that are to be added to solid foods, or added to both solid
777 foods and beverages, administration should normally be via the diet. In the event of
778 palatability problems following incorporation of high concentrations into the diet,
779 administration by oral gavage or use of additional pair feeding control groups should be
780 considered. For substances that are only to be used in beverages, administration via drinking
781 water may appear to be the mode of choice, but for practical reasons this may limit the
782 maximum amount that can be administered and may not adequately reflect the fact that
783 humans can consume beverages such as soft drinks in significant quantities over a short time
784 period. Thus, alternative modes of bolus administration, such as gavage, could be used for
785 such substances. For other substances that may be consumed by humans as a bolus, such as an
786 additive for use in food supplements marketed in the form of capsules or tablets,
787 administration by oral gavage (or in the case of non-rodents, by capsule) should be
788 considered. The effect of method of administration on toxicokinetics and local effects should
789 be assessed.
- 790 • For ENMs, as described in the corresponding 2011 EFSA Guidance document, toxicological
791 testing methods may require modifications (e.g. range of organs studied) based on
792 toxicokinetic studies on the ENMs and characterisation of the ENMs tested (EFSA, 2011a).
793 For nanomaterials which exist as a permitted non-nanoform food additive, the limited
794 additional testing on the nanoform establishes whether read-across from the non-nanoform is
795 feasible for more complex testing. For novel nanomaterials, all toxicological tests need to
796 incorporate the nanospecific characterisation and additional endpoints described in the EFSA
797 Guidance.
- 798 • As a special case, botanical food additives derived from conventional food sources with a long
799 term history of food use, may benefit from a “presumption of safety” under certain
800 circumstances when an adequate body of knowledge exists. This has to be evaluated on a case-
801 by-case basis. In agreement with the EFSA “Guidance on Safety assessment of botanicals and
802 botanical preparations intended for use as ingredients in food supplements” (EFSA, 2009b), a
803 “presumption of safety” could be applied to botanicals and botanical preparations used as food

¹⁴ Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004, on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances

804 additives when data would allow the conclusion that exposure to known levels of the botanical
805 ingredient has occurred in large population groups for many years without reported adverse
806 effects. The Panel noted that the Guidance on botanicals states that “*an important requirement*
807 *is that the technical data, the data on exposure and the available toxicological data are*
808 *provided, and that no significant increase of intake compared to historical levels is to be*
809 *expected due to the intended levels of use*”. However, the Panel considered that the definition
810 of what is considered a significant increase, compared to historical levels, will be judged on a
811 case-by-case basis. This implies that not only use levels but also chemotypes of botanicals and
812 the chemical composition of the botanical preparations should be in line with historically used
813 ones. Methods of extraction of the botanical preparation used as food additive should be
814 considered, since processes differing from the traditional methods of food preparing may lead
815 to compositional differences and concentrate undesirable components. For botanical
816 preparations with a potential to contain toxic, addictive, psychotropic or other substances that
817 may be of concern, presumption of safety can only be applied if there is convincing evidence
818 that these undesirable substances in the specific plant parts or preparations are either absent in
819 the source material, or significantly reduced if not excluded, or inactivated during processing.
820 Furthermore, the presumption of safety approach can only be applied when intakes due to the
821 intended levels of use are within the range of intake levels derived from the European Member
822 States’ mean diets or from studies on specific subgroups. It is recognized that the acceptability
823 of presumption of safety approach relies mainly on the objective of not significantly
824 increasing exposures beyond the levels linked to the history of use.

825 **CORE AREAS FOR EVALUATION**

826 **4.1. Toxicokinetics (ADME)**

827 **4.1.1. General considerations**

828 Toxicokinetics (ADME) is an important tool in human health risk assessment and greater
829 application of toxicokinetics as part of an improved assessment could offer more efficiency, use
830 fewer animals and provide better data for risk assessment purposes. Toxicokinetic data provide
831 valuable information for selection of appropriate species and doses for toxicity testing, and also for
832 risk assessment through the comparison of internal dose in experimental animals and humans.
833 Administration of a chemical does not automatically mean that all of the dose will be bioavailable.
834 Therefore, data on systemic exposures to the chemical and its metabolites, as well as an
835 understanding of the major processes involved in its absorption, distribution, metabolism and
836 excretion (ADME), can assist in the interpretation of toxicity studies and the prediction of
837 differences or similarities across animal species or from animal to man (Creton et al., 2009).
838 Toxicokinetic processes and metabolism may become saturated at doses higher than those expected
839 to be relevant to human exposure, which can result in toxicity that would not be relevant to the
840 intended use and usage level (Bus and Reitz, 1992; Counts and Goodman, 1995; Slikker et al.,
841 2004b).

842 • Toxicokinetic data can be derived from a suite of studies covering ADME, including *in vitro*
843 and *in vivo* studies, single and repeated dose kinetics. Whole animal studies using single or
844 repeated dosing may be needed to define toxicokinetic parameters. However, the design of
845 toxicokinetic studies should be flexible based on the particular substance being tested.

846 • Systemic exposure to the parent compound or metabolites is assessed by measuring plasma (or
847 whole blood or serum) concentrations, although tissue concentrations may be measured in
848 some cases. Commonly measured parameters include the area under the curve (AUC) of
849 plasma concentration of the compound against time after oral administration, maximum
850 concentration (Cmax), time to reach maximum concentration (Tmax), elimination half life

851 (T%) and bioavailability. Estimates of bioavailability require comparison of results following
852 oral administration with those obtained from intravenous administration. In particular,
853 assessment of systemic exposure greatly aids the interpretation of dose-response relationships,
854 which can be nonlinear due to induction, alteration or saturation of processes involved in the
855 ADME of the compound. Furthermore, toxicokinetic information may be used to determine
856 that a lack of toxicological response is not due to a lack of systemic exposure.

857 • *In vitro* studies, employing proteins, carrier proteins, enzymes, subcellular organelles, cell
858 cultures, and perfused organs, can also provide useful information for the investigation of
859 absorption, distribution and metabolism, mechanisms of toxicity, effects on enzymes and other
860 specific aspects. Such *in vitro* studies can be especially useful in defining possible species
861 differences.

862 • Studies in humans should only be performed if there are adequate data from animal and other
863 related studies to demonstrate the likely safety in humans at the proposed level of exposure.
864 Toxicokinetic information in humans can not only provide confirmation of the validity of the
865 animal models used in terms of metabolism, but also whether toxicokinetic parameters
866 estimated from animal data are applicable for humans. This information can be used to define
867 chemical specific adjustment factors.

868 • For substances with limited bioavailability, studies on the distribution and metabolic fate of
869 the additive may require use of compounds labelled with radioactive or stable isotopes.

870 • For some food additives such as complex mixtures, conventional metabolism and
871 toxicokinetic studies may not be feasible for all components in the mixture, but should be
872 provided for the relevant constituents.

873 • In some cases where a matrix effect is thought to impact on the safety of specific levels of
874 substances by affecting their toxicokinetic parameters, appropriate testing and/or other data
875 should be provided to demonstrate the occurrence of the matrix effect with the preparation and
876 its effect on toxicokinetics. A matrix effect should be judged on a case-by-case basis.

877 **4.1.2. Tiered approach to toxicokinetic testing**

878

879 **Tier 1 Absorption studies and *in vitro* gastrointestinal metabolism**

880 • The aim of Tier 1 toxicokinetic testing is to establish whether the compound or breakdown
881 products are absorbed from the gastrointestinal tract. There are a number of established
882 models for absorption studies (including *in vitro*, *in vivo* and *ex vivo* models-absorption and
883 bioavailability models). Physicochemical factors which affect absorption are molecular
884 weight, ionisation constant, hydro- and lipophilicity. Demonstration of negligible absorption,
885 either through experimental studies or from theoretical considerations, may provide a
886 scientific justification for not undertaking higher tiered toxicological studies on an additive.
887 The required sensitivity to determine negligible absorption levels will generally necessitate *in*
888 *vivo* studies using labelled compounds. In general, there is a need for case-by-case evaluation
889 when determining negligible absorption.

890 • The stability of the compound in the gastrointestinal tract needs to be investigated to ascertain
891 that it neither breaks down nor is metabolised to components that may be absorbed. The use of
892 *in vitro* gastrointestinal metabolism models, including gut flora, may assist in this evaluation.
893 The use of absorption and bioavailability models such as the Ussing chamber (Ussing *et al.*,
894 1951; Grass *et al.*, 1988 and Gotoh *et al.*, 2005) and the inverted sac model (Wilson *et al.*,
895 1954; Kato *et al.*, 2004) could provide information about the differences in absorption along

896 the gastrointestinal tract and provide quantitative absorption information (Bohets *et al.*, 2001;
897 Versantvoort *et al.*, 2000).

898 If negligible absorption of the additive, its residuals and its intestinal (e.g. microflora or chemical)
899 breakdown products is demonstrated, a limited number of toxicity studies would be accepted. Further
900 details on toxicity studies required at Tier 1 are given in the respective sections below. In case of
901 absorption of the compound, its metabolites or breakdown products (e.g. microflora or chemical) from
902 the gastrointestinal tract, then Tier 2 toxicokinetic testing should be carried out.

903

904 ***Tier 2 Studies to define distribution, metabolism and excretion and other basic toxicokinetic
905 parameters following a single dose***

906 For some additives (e.g. high molecular weight polymers and mixtures) when there is absorption of
907 low molecular weight components, Tier 2 studies (both in toxicokinetics and in other endpoints) of
908 these components may be more relevant than studies on the additive itself.

909

- 910 • Tier 2 toxicokinetic studies (OECD TG 417) should provide data on systemic exposure to the
911 compound and definition of basic single dose toxicokinetic parameters ($T_{1/2}$, AUC,
912 bioavailability, Cmax and Tmax) together with *in vivo* assessment of its absorption,
913 distribution, metabolism and excretion including identification and quantification of
914 metabolites. It is often desirable to have parameters determined at a range of dose levels to
915 examine the linearity of kinetic parameters and possible saturation of these parameters.
- 916 • The assessment of the validity of the chosen animal model might require comparative *in vitro*
917 metabolism studies using animal and human enzymes, subcellular fractions and/or cells.

918

919 ***Tier 3 Studies to define toxicokinetic parameters following repeated administration.***

920 The trigger for requesting Tier 3 studies will be limited or slow excretion or any other mechanism that
921 may underlie possible bioaccumulation. In these cases the following data should be considered to
922 expand the available database. Further details are found under the section Additional Tier 3 Studies.

923

- 924 • Tier 3 toxicokinetic studies with repeated doses in experimental animals, normally this would
925 involve studies to steady-state which would be approximately five terminal half lives.
- 926 • Data to support physiologically-based kinetic (PBK) modelling and to help predict the
927 absorption, distribution, metabolism and excretion of a compound in humans.
- 928 • Human kinetic data from volunteer studies. It should be done on a case-by-case basis.

929 Evidence of differences in toxicokinetics due to age, disease state, etc may require consideration of
930 specific toxicokinetic studies that will refine the risk assessment.

930 **4.2. Genotoxicity**

931 ***4.2.1. General considerations***

932 As outlined in the EFSA Scientific Committee (SC) opinion on genotoxicity testing strategies (EFSA,
933 2011d), genetic alterations in somatic and germ cells are associated with serious health effects, which
934 in principle may occur even at low exposure levels. Mutations in somatic cells may cause cancer if
935 mutations occur in proto-oncogenes, tumour suppressor genes and/or DNA damage response genes,

936 and are responsible for a variety of genetic diseases (Erickson, 2010). Accumulation of DNA damage
937 in somatic cells has also been proposed to play a role in degenerative conditions such as accelerated
938 aging, immune dysfunction, cardiovascular and neurodegenerative diseases (Hoeijmakers, 2009;
939 Slatter and Gennery, 2010; De Flora & Izzotti, 2007; Frank, 2010). Mutations in germ cells can lead to
940 spontaneous abortions, infertility or heritable damage to the offspring and possibly to the subsequent
941 generations.

942 In view of the adverse consequences of genetic damage to human health, the assessment of mutagenic
943 potential is a basic component of chemical risk assessment. To this aim, both the results of studies on
944 mutation induction ("mutagenicity") and tests conducted to investigate other effects on genetic
945 material are taken into consideration. For definitions of the terms "mutagenicity" and "genotoxicity",
946 the EFSA SC opinion on genotoxicity testing strategies (EFSA, 2011d) or the REACH "Guidance on
947 information requirements and chemical safety assessment" (ECHA, 2008) may be consulted.

948 Genotoxicity testing is performed with the following aims:

949 - to identify substances which could cause heritable damage in humans,
950 - to predict potential genotoxic carcinogens in cases where carcinogenicity data are not available, and
951 - to contribute to understanding of the mechanism of action of chemical carcinogens.

952 For an adequate evaluation of the genotoxic potential of a chemical substance, different end-points
953 (i.e. induction of gene mutations, structural and numerical chromosomal alterations) have to be
954 assessed, as each of these events has been implicated in carcinogenesis and heritable diseases.

955 The genotoxic potential of any new additive has to be assessed as part of the evaluation process. The
956 recommendations concerning genotoxicity testing in this technical guidance are based on the scientific
957 opinion on genotoxicity testing strategies (EFSA, 2011d).

958
959 The Scientific Committee recommended a step-wise (tiered) approach for the generation and
960 evaluation of data on genotoxic potential, comprising:

961 • a basic battery of *in vitro* tests aimed to evaluate the genotoxic potential of the substance
962 assessing induction of gene mutation, structural (clastogenicity) and numerical (aneuploidy)
963 chromosomal alteration,
964 • consideration of whether specific features of the test substance might require substitution of
965 one or more of the recommended *in vitro* tests by other *in vitro* or *in vivo* tests in the basic
966 battery,
967 • in the event of positive results from the basic battery, review of all the available genotoxicity
968 data on the test substance, and
969 • where necessary, conduct of an appropriate *in vivo* study (or studies) to assess whether the
970 genotoxic potential observed *in vitro* is expressed *in vivo*.

971
972 Indicator tests, which detect primary DNA damage, are not part of the basic battery; however, such
973 tests could be useful in the follow-up of *in vitro* positive results.

974 Before embarking on any testing, it is important for the appropriate conduct of the tests to consider
975 other relevant knowledge on the substance. Supporting information may also be available from
976 Structure Activity Relationship (SAR) data, and 'read-across' of data between structurally-related
977 substances. This information can also be important for interpretation of genotoxicity testing results and
978 particularly relevant for the choice of any *in vivo* study.

979
980 In rare cases there may be scientific grounds (e.g. insufficient metabolic activation *in vitro*, the
981 involvement of specific conditions such as reactions in the gastrointestinal tract or structural similarity

982 with known mutagens/carcinogens) for requiring *in vivo* testing even in case of negative results *in*
983 *vitro*.

984 The opinion on genotoxicity testing strategies of the Scientific Committee (EFSA, 2011d) may be
985 consulted for further general aspects such as scope of genotoxicity testing, definition of terms, data
986 interpretation and follow up of e.g. equivocal or inconclusive results.

987 The Panel noted that the SC in its recent opinion also considered whether genotoxicity data would
988 always be necessary for substances in food and feed for which human exposures are very low and
989 whether, instead, the TTC approach might be helpful in assessing the likelihood of carcinogenic or
990 transmissible genotoxic effects (EFSA, 2011e). Such low-exposure substances include impurities,
991 metabolites and degradation products of deliberately added substances, for which genotoxicity data
992 may be unavailable. It is anticipated that the Scientific Committee will adopt an opinion on the use of
993 the TTC approach by the end of 2011.

994 **4.2.2. Tiered approach to genotoxicity testing**

995 The principle of tiered testing to examine genotoxic potential *in vitro* and whether this is expressed *in*
996 *vivo* is well established in genotoxicity testing strategies. There is a recommended battery of *in vitro*
997 tests that determine possible genotoxicity hazards (EFSA, 2011d). Tier 1 testing is mandatory for all
998 food additives. A positive result in Tier 1 requires follow-up in Tier 2. This Tier 2 testing determines
999 whether the hazard is expressed *in vivo*. There are a number of reasons why the genotoxic potential
1000 may not be observed *in vivo* and in case of negative results it is crucial to demonstrate exposure of the
1001 tissue either through direct toxicity or using kinetic data. A valid negative Tier 2 outcome is regarded
1002 as showing an absence of genotoxicity *in vivo*. If Tier 2 is positive it is usually assumed that the
1003 compound is a somatic cell genotoxin and will be potentially carcinogenic and mutagenic in germ
1004 cells. Such compounds are usually not considered acceptable as food additives.

1005

1006 **Tier 1 Basic test battery**

1007 In line with the recommendations of the Scientific Committee (EFSA, 2011d), the following two *in*
1008 *vitro* tests are required as the first step in genotoxicity testing:

1009

- a bacterial reverse mutation assay (OECD TG 471), and
- an *in vitro* mammalian cell micronucleus test (OECD TG 487).

1011 This combination of tests fulfils the basic requirements to cover the three genetic endpoints with the
1012 minimum number of tests; the bacterial reverse mutation assay covers gene mutations and the *in vitro*
1013 micronucleus test covers both structural and numerical chromosome aberrations. The addition of any
1014 further *in vitro* mammalian cell tests in a basic battery would significantly reduce specificity with no
1015 substantial gain in sensitivity (EFSA, 2011d). There may be circumstances under which deviation
1016 from the above-mentioned tests may be justified. In such cases a scientific justification should be
1017 provided and additional types of considerations or mechanistic studies may be needed. If there are
1018 indications for the substance of interest that specific metabolic pathways would be lacking in the
1019 standard *in vitro* systems, or it is known that the *in vitro* test system is inappropriate for that substance
1020 or for its mode of action, testing may require either appropriate modification of the *in vitro* tests or use
1021 of an *in vivo* test at an early stage of testing. It may be advantageous to include *in vivo* assessment of
1022 genotoxicity at an early stage and incorporate such testing within other repeated-dose toxicity studies
1023 that will be conducted anyway, especially when the test substance can be dosed up to the limit dose
1024 which would be applicable in a separate *in vivo* genotoxicity study. Some practical aspects that need to

1025 be considered when combining genotoxicity testing with repeated-dose toxicity testing are described
1026 in the SC opinion on genotoxicity testing strategies (EFSA, 2011d).

1027 In the case of positive results from the basic battery of tests, it may be that further testing *in vitro* is
1028 appropriate to optimise any subsequent *in vivo* testing, or to provide additional useful mechanistic
1029 information.

1030 In cases where all *in vitro* endpoints are clearly negative in adequately conducted tests, it can be
1031 concluded with reasonable certainty that the substance is not a genotoxic hazard.

1032 In the case of inconclusive, contradictory or equivocal results from *in vitro* testing, it may be
1033 appropriate to conduct further testing *in vitro*, either by repetition of a test already conducted, perhaps
1034 under different conditions, or by conduct of a different *in vitro* test, to try to resolve the situation.

1035
1036 ***Tier 2 Follow-up of results from the basic test battery***

1037 Before embarking on any necessary follow-up of positive *in vitro* results by *in vivo* testing, not only
1038 the results from the *in vitro* testing should be reviewed, but also other relevant data on the substance,
1039 such as information about chemical reactivity of the substance (which might predispose to site of
1040 contact effects), bioavailability, metabolism, toxicokinetics, and any target organ specificity.
1041 Additional useful information may come from structural alerts and 'read-across' from structurally
1042 related substances. It may be possible after this to reach a conclusion to treat the substance as an *in*
1043 *vivo* genotoxin. If, after such a review, a decision is taken that *in vivo* testing is necessary, tests should
1044 be selected on a case-by-case basis using expert judgement, with flexibility in the choice of test,
1045 guided by the full data set available for the substance.

1046 *In vivo* tests should relate to the genotoxic endpoint(s) identified as positive *in vitro* and to appropriate
1047 target organs or tissues. Evidence, either from the test itself or from other toxicokinetic or repeated
1048 dose toxicity studies, that the target tissue(s) have been exposed to the test substance and/or its
1049 metabolites is essential for interpretation of negative results.

1050 The approach to *in vivo* testing should be step-wise. If the first test is positive, no further test is needed
1051 and the substance would be considered as an *in vivo* genotoxin. If the test is negative, it may be
1052 possible to conclude that the substance is not an *in vivo* genotoxin. However, in some cases, a second
1053 *in vivo* test may be necessary as there are situations where more than one endpoint in the *in vitro* tests
1054 is positive and an *in vivo* test on a second endpoint may then be necessary if the first test is negative. It
1055 may also be necessary to conduct a further *in vivo* test on an alternative tissue if, for example, it
1056 becomes apparent that the substance did not reach the target tissue in the first test. The combination of
1057 assessing different endpoints in different tissues in the same animal *in vivo* should be considered.

1058 In line with the recommendation of the SC, the Panel considers the following tests as suitable *in vivo*
1059 tests:

- 1060 • an *in vivo* micronucleus test (OECD TG 474),
- 1061 • an *in vivo* Comet assay (no OECD TG at present; internationally agreed protocols available,
1062 e.g. see <http://cometassay.com>), and
- 1063 • a transgenic rodent assay (draft OECD TG; OECD, 2010).

1064
1065 The *in vivo* micronucleus test covers the endpoints of structural and numerical chromosomal
1066 aberrations and is an appropriate follow up for *in vitro* clastogens and aneugens. The current OECD
1067 TG only considers peripheral blood and bone marrow as target tissues. There may be circumstances in
1068 which an *in vivo* mammalian bone marrow chromosome aberration test (OECD TG 475) may be an
1069 alternative follow up test. The Panel noted that local genotoxic effects (e.g. in the upper
1070 gastrointestinal tract) cannot be ruled out solely on the basis of inactivity in bone marrow, especially

1071 for directly acting, electrophilic molecules. The *in vivo* Comet assay is considered a useful indicator
1072 test in terms of its sensitivity to substances which cause gene mutations and/or structural chromosomal
1073 aberrations *in vitro* and can be performed with many tissues. Transgenic rodent assays can detect point
1074 mutations and small deletions and are without tissue restrictions.

1075 When the *in vivo* and *in vitro* results are not consistent, then the differences should be clarified on a
1076 case-by-case basis. For example, in the *in vivo* micronucleus test, certain substances may not reach the
1077 bone marrow due to low bioavailability or specific tissue/organ distribution. In certain cases, for
1078 example when it is known that the test substance is metabolised in the liver and the reactive
1079 metabolites formed are too short-lived to reach the bone marrow, even demonstration of the
1080 bioavailability of the parent substance in the bone marrow does not indicate that bone marrow is an
1081 appropriate target. A negative result of the *in vivo* micronucleus assay can be considered as
1082 meaningful only if there is definitive evidence from toxicokinetic data that the tested substance as well
1083 as the relevant reactive metabolite(s) can reach the bone marrow in significant amounts.

1084 More detailed advice on strategies for *in vivo* follow up is given in the opinion on genotoxicity testing
1085 strategies (EFSA, 2011d).

1086 Normally, if the results of appropriate and adequately conducted *in vivo* tests are negative, then it can
1087 be concluded that the substance is not an *in vivo* genotoxin. If the results of the *in vivo* test(s) are
1088 positive, then it can be concluded that the substance is an *in vivo* genotoxin.

1089

1090 ***Follow-up of results from Tier 2 by carcinogenicity studies and germ cell assays***

1091 The Panel considered that an adequately conducted and powered carcinogenicity study may
1092 demonstrate that an *in vivo* genotoxin does not give rise to carcinogenicity. However, mutations in
1093 somatic cells are also known to be responsible for a variety of genetic diseases (Erickson, 2010).
1094 Furthermore, such an *in vivo* genotoxin may be a germ cell mutagen and it is recognised that standard
1095 reproductive toxicity studies do not cover all germ cell effects. The Panel noted that the Scientific
1096 Committee concluded that a substance that is positive in tests in somatic tissues *in vivo* would
1097 normally be assumed to reach the germ cells and to be a germ cell mutagen, and therefore potentially
1098 hazardous to future generations. In the contrary situation, a substance that is negative in tests in
1099 somatic tissues *in vivo* would be assumed to be negative in germ cells, also because no germ cell
1100 specific mutagen is known. Accordingly, the Scientific Committee concluded that routine testing for
1101 genotoxicity in germ cells is not necessary. The Scientific Committee further concluded that clear
1102 evidence of genotoxicity in somatic cells *in vivo* has to be considered an adverse effect *per se*, even if
1103 the results of cancer bioassays are negative, since genotoxicity is also implicated in diseases other than
1104 cancer (EFSA, 2011d). Hence, careful consideration should be given to animal welfare issues such as
1105 suffering and numbers before conducting any further *in vivo* studies.

1106 There is no Tier 3 for genotoxicity testing.

1107 **4.3. Toxicity testing (subchronic, chronic and carcinogenicity)**

1108 ***4.3.1. General considerations***

1109 The major objective of a toxicity study on a food additive is to provide information on treatment-
1110 related changes in blood, urine and clinical biochemistry parameters, gross and histopathological
1111 changes in organs and tissues following prolonged exposure to the additive via an appropriate oral
1112 route. The clinical observations may also provide information on neurofunctional and neurobehavioral
1113 effects of the additive under investigation.

1114 Data from a subchronic toxicity study should normally be submitted. Such studies often establish the
1115 main toxicological profile of the substance, providing information on the target organs and tissues
1116 affected (hazard identification), on the nature and severity of any effects, and on the dose-response
1117 relationships (hazard characterisation). They should allow determination of the BMDL₁₀ using a BMD
1118 analysis or of the dose at which adverse effects found at higher dose levels are no longer observed, i.e.
1119 the NOAEL. The subchronic toxicity study is used for estimating the appropriate dose levels for
1120 chronic toxicity studies and it can provide indications for the need for additional studies on particular
1121 effects, such as neurotoxic or immunological effects.

1122 Subchronic toxicity will usually be investigated in one species only, normally the rat, although other
1123 species may have to be used, either alternatively or additionally. A scientific justification, e.g.
1124 metabolic differences, needs to be provided for the choice of species. However, if there is evidence
1125 that there are significant toxicokinetic differences between the chosen rodent and humans, then testing
1126 should be performed in a different, adequate species.

1127 For subchronic and chronic toxicity studies and for carcinogenicity studies, the highest dose level
1128 should normally be chosen to identify the principal target organs and toxic effects while avoiding
1129 suffering, severe toxicity, morbidity, or death. For food additives, which may be relatively non-toxic,
1130 it may be impossible for animal welfare reasons to identify such a dose level in a meaningful way.

1131 The highest dose level in chronic or carcinogenicity studies should be chosen to elicit some evidence
1132 of toxicity, as evidenced by, for example, depression of body weight gain (approximately 10%), and
1133 has previously been referred to as the Maximum Tolerated Dose (MTD). In the case of food additives
1134 given via the diet, the highest dose should normally not exceed 5% of the diet, in order to avoid
1135 nutritional imbalances. This upper dose is acceptable even if no toxicity is produced. The OECD
1136 Guidance Document 116 provides additional guidance on dose selection for chronic toxicity and
1137 carcinogenicity studies.

1138 *Subchronic toxicity*

1139 Within Tier 1, a subchronic toxicity study should normally be conducted for a period of at least 90
1140 days (OECD TG 408) in rodents, modified to include assessment of some additional parameters
1141 described in the more recent guideline on repeated-dose 28-day oral toxicity study in rodents (OECD
1142 TG 407). The additional parameters place more emphasis on endocrine-related endpoints, (e.g.
1143 determination of thyroid hormones, gross necropsy and histopathology of tissues that are indicators of
1144 endocrine-related effects), and (as an option) assessment of oestrous cycles. The modified 90-day
1145 study should allow for the identification of chemicals with the potential to cause neurotoxic,
1146 immunological, reproductive organ effects or endocrine-mediated effects, which may warrant further
1147 in-depth investigation. Preceding range-finding studies conducted for shorter periods can provide an
1148 indication of target organs and help in selection of appropriate doses for 90-day studies. When range-
1149 finding studies have been conducted, the results should be submitted. Studies of shorter duration than
1150 90-days are generally not sufficient, by themselves, for evaluation of potential subchronic toxicity.

1151

1152 *Chronic toxicity and carcinogenicity*

1153 In Tier 2, a chronic toxicity study may reveal effects not evident in subchronic studies, or it may
1154 confirm effects observed in subchronic studies at the same or perhaps lower doses. The chronic
1155 toxicity of a food additive may be evaluated in a stand-alone study, using the relevant OECD TG 452.
1156 Alternatively, the use of a combined protocol to study chronic toxicity and carcinogenicity in the same
1157 experiment will often be appropriate in the testing of food additives, in accordance with OECD TG
1158 453. The combined test provides greater efficiency in terms of time and cost compared to conducting
1159 two separate studies, without compromising the quality of the data in either the chronic phase or the
1160 carcinogenicity phase. Careful consideration should however be given to the principles of dose
1161 selection when undertaking a combined chronic toxicity and carcinogenicity study (OECD TG 453).
1162 In carrying out such a combined study, sufficient satellite animals will normally be included in the

1163 design of the study to enable the chronic toxicity aspects of the study to be assessed, without
1164 compromising the carcinogenicity part of the study. An OECD Guidance Document (No.116) on the
1165 design and conduct of chronic toxicity and carcinogenicity studies, supporting OECD TGs 451, 452
1166 and 453 is currently under development, providing useful additional information on dose selection and
1167 the conduct of such studies (OECD GD 116, 2010).

1168 In rats, chronic toxicity studies will normally be carried out for a 12-month period. Carcinogenicity
1169 studies should cover the majority of the lifespan of the animals, generally 24 months in the rat and 18
1170 or 24 months in the mouse, in accordance with OECD TG 453. *In utero* exposure is not required in
1171 carcinogenicity studies unless specific considerations suggest otherwise.

1172 Information to be derived from these studies should include histopathological investigations and
1173 clinical observations including ophthalmology, measurements of body weight, food/water
1174 consumption and food efficiency, made at appropriate intervals as specified in the OECD Test
1175 Guidelines. For additives where previous subacute or subchronic toxicity tests indicated the potential
1176 to cause neurofunctional or neurobehavioral effects, further investigations of such effects should be
1177 carried out using appropriate methodology (referred to under Additional Studies, Tier 3). Microscopic
1178 examination should cover all organs and tissues in the body. It is however acceptable to examine
1179 control and top dose animals only for microscopic changes, provided no significant treatment-related
1180 pathological changes are observed in the top dose group. Tissues from lower dose groups should
1181 always be retained in case further examination is required.

1182 Carcinogenicity and chronic toxicity studies will usually be investigated only in one species, the rat.
1183 Traditionally, carcinogenicity testing for food additives has been conducted in two species, the rat and
1184 the mouse, as recommended in the 2001 SCF Guidelines. In recent years there has been considerable
1185 debate about the value of the two rodent species approach to carcinogenicity and about the continued
1186 use of the mouse as a second species, particularly within the ICH (ICH, Proceedings of the Third
1187 International Conference, 1995). A number of studies have assessed the relative individual
1188 contribution of rat and mouse carcinogenicity studies and whether the use of rats or mice alone would
1189 result in a significant loss of information on carcinogenicity relevant to human risk assessment. This
1190 debate has led to the suggestion that there may be no need for routine conduct of two long-term rodent
1191 carcinogenicity studies, with the rat being the preferred species for testing. Overall, the Panel
1192 considers that it is appropriate to perform the carcinogenicity studies in the rat.

1193

1194 **Strategies for carcinogenicity testing**

1195 OECD TG 451 indicates that before commissioning carcinogenicity studies, all available data should
1196 be evaluated. These data include the identity, chemical structure, and physico-chemical properties of
1197 the additive; results of any *in vitro* or *in vivo* toxicity tests including genotoxicity tests; anticipated
1198 use(s) and potential for human exposure; available (Q)SAR data, mutagenicity/genotoxicity,
1199 carcinogenicity and other toxicological data on structurally-related substances; available toxicokinetic
1200 data (single dose and also repeated dose kinetics where available) and data derived from other repeated
1201 exposure studies. Assessment of carcinogenicity should only be carried out after initial information on
1202 toxicity has been obtained from 90-day toxicity and/or longer term toxicity tests. In the event of a
1203 carcinogenic response being demonstrated in the study, additional mechanistic information together
1204 with good data on toxicokinetics are usually essential for risk assessment, both with respect to
1205 extrapolation to humans and possible determination of a threshold for non-genotoxic carcinogens.

1206 **4.3.2. Tiered approach to toxicity testing**

1207 The **Tier 1** for toxicity testing consists of a modified 90-day toxicity test (OECD TG 408 with
1208 extended parameters from the OECD 407) that should allow for the identification of chemicals with

1209 the potential to cause neurotoxic, immunological or reproductive organ effects or endocrine-mediated
1210 effects, which may warrant further in-depth investigation at higher tiers. The results from the repeated
1211 dose 90-day oral toxicity can be used to identify a BMDL or a NOAEL.

1212
1213 In the case of food additives for which Tier 1 toxicokinetics testing indicates a lack of systemic
1214 exposure, the Tier 1 studies should look for both pathological and physiological effects in the
1215 gastrointestinal tract. The effects of unabsorbed materials on gastrointestinal function and tolerance
1216 also need to be investigated.

1217
1218 **Tier 2** Studies on chronic toxicity (12 months) and carcinogenicity in a single species, generally the
1219 rat. Either separate studies (OECD TGs 452 and 451, respectively) or the combined study (OECD TG
1220 453).

1221 **Tier 3** Carcinogenicity study in a second species triggered by results of Tier 2 (equivocal results or
1222 species specific findings) or specialised studies to investigate the mode of action or mechanism of
1223 toxicity or carcinogenicity observed in Tier 1 or Tier 2 tests. In the last decades, several alternative
1224 models including short-term tests with transgenic mouse models (p53⁺⁻, rasH2, Tg.AC, Xpa⁻/ and
1225 Xpa⁻/p53⁺⁻) have been developed to add to or refine the classical carcinogenicity bioassay, and may
1226 provide appropriate information at Tier 3. Although not a complete replacement to the rodent 2-year
1227 cancer bioassay, transgenic mouse models are a refinement and may result in a significant reduction in
1228 the use of experimental animals.

1229 Tier 3 may also include specialised testing for neurotoxicity, immunotoxicity or endocrine-mediated
1230 effects. The purpose of investigations into mechanisms and modes of action is to determine the
1231 relevance for man of effects observed in the test species as part of their mode of action framework.

1232 **4.4. Reproductive and developmental toxicity**

1233 **4.4.1. General considerations**

1234 Food additives showing systemic bioavailability should be tested in reproductive toxicity and
1235 developmental toxicity studies. The objective of a reproductive toxicity study is to provide
1236 information about effects and potency of food additives on male and female libido, fertility, on the
1237 female's ability to carry pregnancy to term, on maternal lactation and care of the young, on the
1238 prenatal and postnatal survival, growth, functional and behavioural development of the offspring, on
1239 the reproductive capacity of the offspring and to identify histologically any major target organs for
1240 toxicity (including reproductive organs) in the parents and offspring. The major objective of a prenatal
1241 developmental toxicity study is to identify the potential of a substance to cause lethal, teratogenic or
1242 other toxic effects on the embryo and foetus, by examination for embryonic and foetal resorptions or
1243 deaths, foetal weight, sex ratio, and external, visceral and skeletal morphology. Exposure to an
1244 additive, prenatally via the mother and postnatally via maternal milk, may also impair postnatal
1245 development and function, including neurological function and behaviour, immunological function
1246 and endocrine activity.

1247 Decisions on whether tests are necessary for reproductive and developmental toxicity will need to be
1248 considered in the light of the toxicity data and toxicokinetics information available. For a decision on
1249 whether a developmental toxicity study will be necessary, consideration also needs to be given as to
1250 whether the substance may cross the placenta. Such information may not be readily available, since
1251 ADME studies do not routinely include pregnant animals.

1252

1253 **4.4.2. Tiered approach to reproductive and developmental toxicity testing**

1254

1255 **Tier 1**

1256 The data from Tier 1 testing are relevant when considering the need for reproductive and
1257 developmental testing in Tier 2.

1258 • Tier 1 testing for reproductive and developmental toxicity studies need not be performed
1259 where absorption is negligible. The Panel suggested to use the value of 1.5 µg/kg bw/day
1260 (TTC for Cramer class III; Munro et al., 2008) as the limit to required developmental toxicity
1261 studies. In the case of intakes below this value, the Panel considered that no developmental
1262 toxicity studies are required.

1263 • The repeated dose 90-day oral toxicity study (OECD TG 408) offers only limited information
1264 on reproductive toxicity and no information on developmental toxicity; it can inform about
1265 effects on the reproductive organs and, if assessed, the oestrous cycle, but it does not assess
1266 fertility and the whole reproductive cycle from *in utero* exposure onwards, through sexual
1267 maturity to conception, gestation, prenatal and postnatal development.

1268 Decisions on whether tests are necessary for reproductive and developmental toxicity need to
1269 be considered in the light of the toxicity data and toxicokinetics information available. If the
1270 Tier 1 toxicokinetic study shows that the test substance is bioavailable in the test species
1271 (normally rodents) or suspected to be bioavailable in humans, Tier 2 testing for reproductive
1272 and developmental toxicity is required. Indications of effects on reproductive organs or
1273 parameters in the 90-day oral toxicity will also trigger Tier 2 testing for reproductive and
1274 developmental toxicity.

1275 **Tier 2**

1276 • Tier 2 testing for reproductive and developmental toxicity comprises a prenatal developmental
1277 toxicity study (OECD TG 414) in the rabbit and an Extended One-Generation Reproduction
1278 Toxicity Study (EOGRTS) (OECD TG 443). Cohorts for the preliminary assessment of
1279 additional more specific endpoints should be routinely incorporated in the EOGRTS for
1280 studies on food additives (see details below). When evaluating existing additives, the Panel
1281 could consider a multi-generation study, instead of a EOGRTS, acceptable, provided that
1282 sufficient information on possible neurotoxicity and immunotoxicity is available (for example
1283 from an extended 90-day study, OECD TG 408).

1284 • In the EOGRTS, administration of the test substance should normally be via the diet or by oral
1285 gavage to both sexually mature male and female animals covering a defined pre-mating period
1286 (minimum of 2 weeks) and a 2-week mating period, with parental males being treated until at
1287 least the weaning of the F1, for a minimum of 10 weeks, and parental females during
1288 pregnancy and lactation until weaning of the F1. Dosing of the F1 offspring should begin at
1289 weaning and continue until scheduled necropsy in adulthood. The testing will be conducted in
1290 one laboratory species only, primarily rodents, with the rat being the preferred species of
1291 choice provided that careful consideration has been taken in relation to all the other available
1292 information. However, based on other information available, alternative species can be used
1293 provided that a rationale is outlined by the applicant.

1294 • The EOGRTS (OECD TG 443) in the rat will provide information evaluating specific life
1295 stages not covered by the other toxicity studies; fertility and reproductive function, and short-
1296 to long-term developmental effects from exposure during pregnancy, lactation and prepubertal
1297 phases as well as effects on juveniles and adult offspring will be assessed, by efficiently
1298 integrating several endpoints that cover the whole reproductive cycle (from gametogenesis
1299 through to maturation of the following generation) as well as preliminary assessment of
1300 additional more specific endpoints (i.e. developmental neurotoxicity and developmental
1301 immunotoxicity). According to the OECD guideline (TG 443), the selected parameters to be
1302 measured fall into the following categories:

1303 - reproductive endpoints
1304 - developmental (pre- and postnatal) endpoints
1305 - specific endpoints (developmental neurotoxicity & immunotoxicity)

1306 and focus on physical, functional and behavioural development in animals exposed from the
1307 beginning of embryogenesis through to adulthood. Relevant observations generally include
1308 pup body weight, pre-weaning physical and functional developmental landmarks including
1309 reflex development, the onset of sexual maturity as measured by vaginal opening in females
1310 and cleavage of the balanopreputial gland in males, sensory and locomotor function, and some
1311 indication of cognitive ability (learning and memory).

1312 • The EOGRTS protocol includes endpoints, termed 'triggers' (e.g. P fertility, F1 oestrous cycle
1313 evaluation, F1 litter parameters and developmental landmarks, F1 pup survival postnatally
1314 and malformations, and F1 live birth index and body weight) which can be used for
1315 determining whether assessment of a second generation (F2) is required. Where these triggers
1316 are positive, the EOGRTS may be extended to include the F2 generation which may help
1317 clarify any equivocal findings or provide further characterisation on fertility in the F1 mating.
1318 It is expected that with the additional parameters evaluated in the F1 generation in the
1319 EOGRTS, the F2 with their limited parameter assessments would seldom affect the hazard
1320 characterisation for risk assessment (Piersma et al., 2011). However, when predicted human
1321 exposures are considered adequately characterised, MOE considerations may be factored into
1322 the decision to require the assessment of a F2 generation. Consideration should also be taken
1323 on all the other information available.

1324 **Tier 3**

1325 In devising appropriate Tier 3 testing, a case-by-case approach should be adopted with careful
1326 consideration given to animal welfare issues and on all available data. Tier 3 testing comprises
1327 additional studies for endocrine, developmental neurotoxicity and developmental immunotoxicity,
1328 mode of action studies, and is triggered by results in Tier 2 studies. These Tier 3 Studies are described
1329 in the next section.

1330 **4.5. Additional Tier 3 studies**

1331 In addition to the core areas for evaluation, the Panel noted that other tests may be required to allow an
1332 adequate risk assessment. These studies generally examine specific biological processes which may
1333 not be fully considered in the core areas for evaluation, for example effects on gastrointestinal
1334 physiology and function. Other studies that may be relevant and useful for assessing the risk and
1335 establishing the safety of an additive include immunotoxicity, hypersensitivity and food intolerance,
1336 studies on neurotoxicity, endocrine activity and mechanisms and modes of action.

1337 **4.5.1. Human studies**

1338

1339 *Introduction*

1340 Useful information could be gained from human studies conducted before or after the marketing of a
1341 food additive. Similarly, experience gained from the investigation of the safety of human therapeutic
1342 agents may be applicable in some circumstances to human studies with food additives.

1343

1344 *Indications for human volunteer studies*

1345 Studies of food additives in humans should only be performed if there are adequate data from animal
1346 and other related studies to demonstrate the likely safety in humans at the proposed level of exposure.
1347 Any proposed studies should have clear scientific objectives and adequate protocols, include
1348 provisions for review in the event of occurrence of unexpected results, and comply with the relevant
1349 ethical and legal standards. These include approval by an appropriately constituted review or ethical
1350 body, adherence to the principles of informed consent by volunteers, and the maintenance of records
1351 that are open to inspection.

1352
1353 *Types of human volunteer studies*

1354 Human volunteer studies are generally of two types: absorption, metabolism, distribution and
1355 elimination studies, and tolerance studies. Other special studies e.g. on allergy, behaviour or cognitive
1356 function may sometimes be appropriate. Human volunteer studies may also be indicated when
1357 knowledge is required about special subgroups of the general population who may be genetically
1358 predisposed to low tolerance or particularly exposed to certain additives. Studies of the absorption,
1359 metabolism, distribution and elimination of additives in humans would greatly enhance the predictive
1360 value of the traditional chemical, biochemical and toxicological investigations in laboratory animals
1361 used to demonstrate safety. Comparison of the results of such human studies with those obtained in
1362 laboratory animals enables validation of the database acquired in animal experiments and the detection
1363 of any significant differences between animals and humans, which can be of importance for the
1364 interpretation of unusual or adverse findings.

1365 Gastrointestinal absorption may be followed by determination of blood levels at intervals after
1366 administration, giving some indication of bioavailability. Information on kinetics and metabolism
1367 following absorption can be obtained from blood and urine measurements. Human studies are
1368 particularly appropriate for investigating tolerance of a substance or a food. They may be appropriate,
1369 for example, for investigating symptoms which cannot be studied in animals (e.g. headaches,
1370 gastrointestinal discomfort). They may include physical examination, blood chemistry, haematology,
1371 urine analysis and organ function tests. At the same time monitoring for any adverse reactions, and
1372 recording their nature, frequency, intensity and dose relationship should be carried out. A number of
1373 publications contain useful information on the conduct of clinical studies (EMA, 2002).

1374

1375 **4.5.2. Immunotoxicity, Hypersensitivity/allergy and Food Intolerance**

1376 In exposed individuals, food additives may interact with the immune system in several ways and
1377 induce changes in the immune response resulting in either immunosuppression or immunostimulation.
1378 Immunostimulation may lead to hypersensitivity reactions, including autoimmunity and allergy. An
1379 allergic response to an additive can be induced by the presence of allergenic components or residues,
1380 in particular proteins, or alternatively because the additive itself is an allergen (e.g. a protein or a
1381 peptide) or capable of acting as a hapten.

1382 Preliminary experimental data indicative of an effect on the immune system may be obtained from the
1383 Tier 1 and Tier 2 testing strategies for (sub)chronic toxicity testing, and these may trigger further Tier
1384 3 studies investigating immunotoxicity.

1385 **Immunotoxicity**

1386 The tiered approach to testing outlined in this guidance includes, at **Tier 1**, a 90-day study in rats
1387 (OECD TG 408). This study involves investigation of the effect of the food additive on a number of
1388 parameters that may be indicative of an immunotoxic or immunomodulatory effect. These include:
1389 changes in spleen and thymus weights relative to body weight in the absence of overt toxicity,
1390 histopathological changes in these and other organs of the immune system (e.g. bone marrow, lymph
1391 nodes, Peyer's patches), as well as changes in total serum protein, albumin:globulin ratio and in the

1392 haematological profile of the animals, notably in lymphocyte numbers and in the total and differential
1393 blood cell counts.

1394 The effects may be confirmed or, alternatively, seen for the first time in **Tier 2** studies, notably the
1395 EOGRTS (OECD TG 443), but also in chronic toxicity/carcinogenicity studies conducted according to
1396 OECD TGs 452, 451 or 453. In the EOGRTS, a cohort of animals is specifically dedicated to assess
1397 the potential impact of exposure on the developing immune system. In subchronic and chronic studies,
1398 haematological and clinical chemistry data are generally provided, together with phenotypic analysis
1399 of spleen cells (T-, B-, NK-cells) and bone marrow cellularity. The EOGRTS provides additional
1400 information on the primary IgM antibody response to a T cell dependent antigen, such as sheep red
1401 blood cells (SRBC), or keyhole limpet hemocyanin (KLH).

1402
1403 The evaluation of the potential of a food additive to adversely affect the immune system may be based
1404 on an integrated assessment of the results obtained from these toxicity studies (Tiers 1 and 2). If, these
1405 results indicate that the food additive has such a potential, additional Tier 3 studies should be
1406 considered, on a case-by-case basis. These will normally be designed to investigate the underlying
1407 mechanisms of the effects seen, and/or their biological significance.

1408 **Tier 3** studies may include specialised functional, mechanistic, and disease model studies (Draft
1409 Guidance for Immunotoxicity risk assessment for chemicals-WHO/IPCS, 2011). The Panel noted that
1410 there are no OECD guidelines for these extended specialised studies, but based on IPCS, such studies
1411 may include the following:

- 1412 • mitogen stimulation assays for B and T cells
- 1413 • natural killer cell functional analysis, macrophage quantification and functional analysis,
1414 interleukin-2 functional analysis, cytokines production by lymphocytes
- 1415 • complement assays: total serum haemolytic activity or individual components (C3a, C5a,...)
- 1416 • kinetic evaluation of humoral response to a T-cell-dependent antigen (primary and secondary
1417 responses to SRBC, tetanus toxoid or other), or to a T-independent antigen such as
1418 pneumococcal polysaccharides, trinitrophenyl-lipopolysaccharide, or other
- 1419 • delayed-type hypersensitivity response to a known sensitizer of T effector cells, or
1420 reversibility evaluation
- 1421 • infectivity challenge (Trichinella, Candida or other in rat, Listeria or other in mouse), or
1422 tumour challenge (MADB106 or other in rat, or PYB6 sarcoma in mouse).
- 1423 • Alternative methods using human cells from umbilical cord such as hematopoietic progenitor
1424 clonogenic assays.

1426 **Allergy**

1427 At present there are no validated studies in laboratory animals which would allow assessment of the
1428 potential of a substance to cause allergic reactions in susceptible individuals following oral exposure.
1429 Studies on dermal or inhalation sensitisation may provide relevant information for possible hazards
1430 from occupational exposure to additives and could be helpful in assessing consumer safety even if
1431 their relevance to oral allergenicity remains unclear. It is also possible to use double-blind placebo-
1432 controlled oral food challenges, or prick testing in humans.

1433 Where the additive is a potential allergen (e.g. a protein or a peptide) or contains residues of proteins
1434 or other known potential allergenic molecules, the principles discussed in the EFSA Guidance on the
1435 Allergenicity of GMOs should be followed in evaluating allergenic components. These principles for
1436 the determination of allergenicity include the investigation of structural aspects of the protein or
1437 peptide, *in silico* (or bioinformatics) approaches, IgE binding and cell-based methods, analytical
1438 profiling techniques and animal models (EFSA, 2010).

1439 Since no single experimental method yields decisive evidence for allergenicity and allergic responses,
1440 a weight of evidence approach taking into account all the information obtained from various test
1441 methods is recommended.

1442 Where allergenicity of a food additive has been identified, it has generally been accepted to date that
1443 defining a threshold/NOAEL for such effect is difficult since different thresholds exist for induction
1444 and elicitation of the allergenic response together with idiosyncratic reactions. Therefore, the Panel
1445 will take such an adverse effect into account on a case-by-case basis.

1446 **Intolerance reactions**

1447 Intolerance reactions to food additives are not immune-mediated. They result from
1448 pharmacological effects such as release of bioactive amines, e.g. histamine or tyramine, and
1449 other still undefined mechanisms. Such reactions are difficult to predict and mostly rely on
1450 human studies reporting observations of adverse effects.

1451 At present, no validated experimental *in vitro* and *in vivo* methods are available which would allow
1452 assessment of a substance's potential to cause intolerance reactions in susceptible individuals
1453 following oral exposure. Moreover, it is not feasible to undertake clinical studies of sufficient power
1454 prior to marketing. Therefore, post-marketing surveillance is essential in order to identify possible
1455 sensitive populations.

1456

1457 **4.5.3. Neurotoxicity**

1458 Initial indications of potential neurotoxic effects of a test substance will be obtained through the 90-
1459 day toxicity study (Tier 1). Other information, such as screening results, SARs or physicochemical
1460 properties indicative of any neurotoxic potential should also be considered.

1461 Where initial indication of potential neurotoxicity is seen at Tier 1, further neurotoxicity testing
1462 (OECD TG 424) should be considered. Such testing is aiming to confirm or further characterise (and
1463 quantify) the potential neurotoxic response induced by the test substance and should be carried out on
1464 a case-by-case basis. Information from the other studies should also be considered to improve the
1465 design with respect to dose selection in order to address confounding effects by general toxicity.
1466 Further specialised studies can also be performed to elucidate mechanisms in order to extrapolate from
1467 animals to humans and to further characterise and complete the risk assessment.

1468 The tiered approach to testing outlined in this guidance includes, at **Tier 1**, a 90-day study in rats
1469 (OECD TG 408). This study involves investigation of the effect of the food additive on a number of
1470 parameters that may be indicative of a neurotoxic effect. These include: changes in clinical signs,
1471 functional observational battery, motor activity and brain weight relative to body weight in the
1472 absence of overt toxicity, histopathological changes in this organ.

1473 The effects may be confirmed or, alternatively, seen for the first time in **Tier 2** studies, notably the
1474 EOGRTS (OECD TG 443), but also in chronic toxicity/carcinogenicity studies conducted according to
1475 OECD TGs 452, 451 or 453. In the EOGRTS, a cohort of animals is specifically dedicated to assess
1476 the potential impact of exposure on the developing nervous system. In the studies, data will be derived
1477 from detailed clinical observations, auditory startle, a functional battery, motor activity and
1478 neuropathology assessments of the F1-pups and adult animals.

1479
1480 The evaluation of the potential of a food additive to adversely affect the nervous system may be based
1481 on an integrated assessment of the results obtained from these toxicity studies (Tiers 1 and 2). If these
1482 results indicate that the food additive has such a potential, additional Tier 3 studies should be
1483 considered on a case-by-case basis. These will normally be designed to investigate the underlying
1484 mechanisms of the effects seen, and/or their biological significance.

1485 **Tier 3** studies may include more extensive behavioural and morphological tests in a developmental
1486 neurotoxicity study. Guidance for these tests can be found in OECD TG 426.

1487

1488

1489 **5. SUPPLEMENTARY REQUIREMENTS**

1490 **5.1. Integrated (alternative) testing strategies**

1491 The Panel noted the continuing development of integrated testing strategies (ITS) and welcomed the
1492 use to complement the data required in these guidance. Alternative methods may be used aiming to
1493 fulfil the goals as determined by the concept of the 3Rs. ITS are anticipated to improve or replace
1494 current traditional toxicological approaches. ITS approaches comprise methods that can efficiently
1495 generate toxicological data for both hazard identification and risk assessment, hereby aiming to reduce
1496 costs and minimize the need for experimental animals.

1497 The most recent overview on the alternative methods available and the time frame to further develop
1498 the methods for a full replacement of in vivo testing was published by a group of authors with respect
1499 to the requirements of the 7th amendment to the European Union's Cosmetics Directive
1500 (76/768/EEC¹⁵) (Adler et al.,2011). The review is also applicable in other fields.

1501 **5.2. Mechanisms and Modes of action**

1502 Studies on the mode of action may be used to investigate the relevance of findings in animals for
1503 humans. These studies can examine the mode of action for carcinogenic effects or other endpoints
1504 such as endocrine disruption, and should use the appropriate MOA (mode of action) frameworks when
1505 assessing the data (IPCS, 2006; Boobis, 2006; Boobis, 2008).

1506 **5.3. Review of published literature**

1507 Applicants should review the published literature for relevant references. This should be based on the
1508 principles underpinning systematic reviews. The methods used to identify relevant data and other
1509 information, including the scope and criteria of literature searches, should be described.

1510 **5.4. Reporting and referencing of studies**

1511 *Overview and evaluation of toxicological data*

1512 In compiling the data in the submission, applicants should also seek to interpret the data and draw
1513 conclusions. The significant findings of each study (both commissioned and published) should be
1514 highlighted, together with identification of the POD, the BMDL₅ value for continuous data, the
1515 BMDL₁₀ for quantal data value or the NOAEL, if one has been determined, and any other relevant
1516 information. There should also be an evaluation of the whole dossier clearly describing the POD from
1517 individual studies and identifying the critical one. The reasons for disregarding any findings should be
1518 carefully explained. Where necessary, the conclusions should include an interpretation of the
1519 significance of the findings in terms of possible mechanisms underlying any effects observed, a
1520 discussion of whether these are relevant to humans and, if so, the possible significance of the
1521 extrapolation of such findings to humans.

1522

¹⁵ Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1976L0768:20100301:en:PDF>

1523 *References and study reports*

1524 1. List of references

1525 References should be quoted as follows:-

1526 i. Published data

- 1527 • Journals: Author(s) (full list including all names and initials), date, title of article,
1528 journal, volume number, page numbers.
- 1529 • Books: Author(s), date, title of chapter/book, editor(s) (if relevant), publisher,
1530 location, page numbers (if relevant).

1531 ii. Unpublished data

- 1532 • Name of petitioner, date, title of report, report reference, name of investigator(s) (if
1533 any), name of laboratory, address of laboratory.

1534 2. Appended papers and study reports

- 1535 • Copies of key papers from the references cited which might be needed for an
1536 independent safety evaluation should be submitted with the dossier.
- 1537 • Copies of all unpublished study reports should be submitted in full. Summaries of
1538 unpublished studies are not acceptable.

1540 **REFERENCES**

1541 Adler S, Baskett D, Creton S, Pelkonen O, van Benthem J, Zuang V, Andersen KE, Angers-Loustau
 1542 A, Aptula A, Bal-Price A, Benfenati E, Bernauer U, Bessems J, Bois FY, Boobis A, Brandon E,
 1543 Bremer S, Broschard T, Casati S, Coecke S, Corvi R, Cronin M, Daston G, Dekant W, Felter S,
 1544 Grignard E, Gundert-Remy U, Heinonen T, Kimber I, Kleinjans J, Komulainen H, Kreiling R,
 1545 Kreysa J, Leite SB, Loizou G, Maxwell G, Mazzatorta P, Munn S, Pfuhler S, Phrakonkham P,
 1546 Piersma A, Poth A, Prieto P, Repetto G, Rogiers V, Schoeters G, Schwarz M, Serafimova R, Tähti
 1547 H, Testai E, van Delft J, van Loveren H, Vinken M, Worth A, Zaldivar JM., 2011. Alternative
 1548 (non-animal) methods for cosmetics testing: current status and future prospects. *Arch Toxicol.*
 1549 85(5):367-485. doi: 10.1007/s00204-011-0693-2.

1550 Bohets H, Annaert P, Mannens G, 2001. Strategies for absorption screening in drug discovery and
 1551 development. *Current Topics in Medicinal Chemistry* 1, 367-383.

1552 Bokkers, BGH, Slob W, 2007. Deriving a data-based interspecies assessment factor using the NOAEL
 1553 and the benchmark dose approach. *Critical Reviews in Toxicology* 37, 355-373.

1554 Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W,
 1555 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev
 1556 Toxicol.* 36:781-92.

1557 Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J,
 1558 Vickers C, 2008. IPCS framework for analyzing the relevance of a noncancer mode of action for
 1559 humans. *Crit Rev Toxicol.* 38:87-96.

1560 Bus JS, Reitz RH, 1992. Dose-dependent metabolism and dose setting in chronic studies. *Toxicology
 1561 Letters* 64-65, 669-676.

1562 Counts JL, Goodman JI, 1995. Principles underlying dose selection for, and extrapolation from, the
 1563 carcinogen bioassay – dose influences mechanism. *Regulatory Toxicology and Pharmacology* 21,
 1564 418-421.

1565 Creton S, Billington R, Davies W, Dent MP, Hawksworth GM, Parry S, Travis KZ, 2009. Application
 1566 of toxicokinetics to improve chemical risk assessment: implications for the use of animals.
 1567 *Regulatory Toxicology and Pharmacology* 55, 291-9.

1568 De Flora S, Izzotti A, 2007. [Mutagenesis and cardiovascular diseases Molecular mechanisms, risk
 1569 factors, and protective factors](#). *Mutat Res.* 621(1-2):5-17.

1570 Dorne JMC, Renwick AG, 2005. The refinement of uncertainty/safety factors in risk assessment by
 1571 the incorporation of data on toxicokinetic variability in humans. *Toxicological Sciences* 86, 20-26.

1572 EC (European Commission), 1997. Reports on Tasks for scientific co-operation. Task 4.2. Report on
 1573 Methodologies for the Monitoring of Food Additive Intake across the European Union. DG
 1574 Industry.

1575 EC (Commission of the European Communities), 2001. Report from the Commission on dietary food
 1576 additive intake in the European Union (542 final).

1577 ECHA, 2008. Guidance for the Implementation of REACH. Guidance on information requirements
 1578 and chemical safety assessment. Chapter R.7.a: Endpoint specific guidance. Section R.7.7
 1579 Mutagenicity and carcinogenicity, p377. European Chemicals Agency, Helsinki. Available at:
 1580 http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7a_en.pdf?vers=02_02_10.

1582
 1583 EFSA, 2005. Opinion of the Scientific Committee on a request from EFSA related to a harmonised
 1584 approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA
 1585 Journal* 282, 1-31.

1586 EFSA, 2007. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of
 1587 selected microorganisms referred to EFSA - Opinion of the Scientific Committee. *EFSA Journal*
 1588 587, 1-16.

1589 EFSA, 2009a. Guidance of the Scientific Committee on a request from EFSA on the use of the
 1590 benchmark dose approach in risk assessment. *EFSA Journal* 1150, 1-72.

1591 EFSA, 2009b. Guidance on Safety assessment of botanicals and botanical preparations intended for
 1592 use as ingredients in food supplements. *EFSA Journal* 1249, 1-19.

1593 EFSA, 2009c. The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed
 1594 Safety. *EFSA Journal* 958, 1-39.

1595 EFSA, 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms
 1596 and derived food and feed. *EFSA Journal* 2011;9(5):2140 [36 pp].

1597 EFSA, 2011a. Guidance on the risk assessment of the application of nanoscience and
 1598 nanotechnologies in the food and feed chain. *EFSA Journal* 2010; 8(7):1700

1599 EFSA, 2011b. Scientific Opinion of the Scientific Panel on Genetically Modified Organisms (GMO)
 1600 on the Guidance on the risk assessment of genetically modified microorganisms and their food and
 1601 feed products or EFSA guidance on the submission of applications for authorisation of genetically
 1602 modified food and feed and genetically modified plants for food or feed uses under Regulation
 1603 (EC) No 1829/2003. *EFSA Journal* 2011;9(7):2311 [165 pp].

1604 EFSA, 2011c. Use of the EFSA Comprehensive European Food Consumption Database in exposure
 1605 assessment. *EFSA Journal* 2097, 1-34.

1606 EFSA, 2011d. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety
 1607 assessment. *EFSA Journal* 2011;9(9):2379

1608 EFSA, 2011e. Exploring options for providing preliminary advice about possible human health risks
 1609 based on the concept of threshold of toxicological concern (TTC). (pending adoption)

1610 EMEA, 2002. Guideline for Good Clinical Practice (CPMP/ICH/135/95). Available at:
 1611 http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002874.pdf

1613 Erickson RP, 2010. Somatic gene mutation and human disease other than cancer: an update. *Mutat
 1614 Res.* 705(2):96-106.

1615 Frank SA, 2010. Evolution in health and medicine Sackler colloquium: Somatic evolutionary
 1616 genomics: mutations during development cause highly variable genetic mosaicism with risk of
 1617 cancer and neurodegeneration. *Proc Natl Acad Sci U S A.* 107 Suppl 1:1725-30. Gotoh Y, Kamada
 1618 N, Momose D, 2005. The advantages of the Ussing chamber in drug absorption studies. *Journal of
 1619 Biomolecular Screening* 10, 517-523.

1620 Grass GM, Sweetana SA, 1988. In vitro measurement of gastrointestinal tissue permeability using a
 1621 new diffusion cell. *Pharma Research* 5, 372-376.

1622 Gotoh Y, Kamada N, Momose D, 2005. The advantages of the Ussing chamber in drug absorption
 1623 studies. *J Biomol Screen.* 10(5):517-23.

1624 Hoeijmakers JH, 2009. DNA damage, aging, and cancer. *N Engl J Med.* 361(15):1475-85. IPCS, 2004
 1625 IPCS Risk Assessment Terminology. Part 1:IPCS/OECD Key Generic Terms used in Chemical
 1626 Hazard/Risk Assessment. International Programme on Chemical Safety. World Health
 1627 Organization, Geneva.

1628

1629 JECFA, 1972. WHO Technical Report Series, Evaluation of certain food additives and the
 1630 contaminants mercury, lead, and cadmium. Sixteenth report of the Joint FAO/WHO Expert
 1631 Committee on Food Additives. No. 505. World Health Organization, Geneva.

1632 JECFA, 1978. Evaluation of certain food additives. Twenty-first report of the Joint FAO/WHO Expert
1633 Committee on Food Additives. No. 617. World Health Organization, Geneva.

1634 JECFA, 2006. Combined Compendium of food additive specifications. Volume 4 of the Joint
1635 FAO/WHO Expert Committee on Food Additives. World Health Organization, Geneva.
1636 (ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e00a.pdf).

1637 ICH, 1995. <http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html>

1638 IPCS, 2005. Chemical-specific adjustment factors for interspecies differences and human variability:
1639 Guidance document for use of data in dose/concentration response assessment (Harmonization
1640 Project Document No. 2). (http://whqlibdoc.who.int/publications/2005/9241546786_eng.pdf)

1641 IPCS, 2006. Framework for analysing the relevance of a cancer and a non-cancer mode of action for
1642 humans. (Harmonisation project document No. 4).
1643 (http://www.who.int/ipcs/methods/harmonization/areas/cancer_mode.pdf)

1644 Kato T, Hayashi Y, Inoue K, Yuasa H, 2004. Functional characterization of the carrier-mediated
1645 transport system for glycerol in everted sacs of the rat small intestine. *Biological & Pharmaceutical*
1646 *Bulletin* 27, 1826-30.

1647 Meek B, Renwick A, Sunich-Mullin C, 2003. Practical application of kinetic data in risk assessment -
1648 an IPCS initiative. *Toxicology Letters* 138, 151-160.

1649 Munro IC, Renwick AG, Danielewska-Nikiel B, 2008. The threshold of toxicological concern (TTC)
1650 in risk assessment. *Toxicology Letters* 180, 151-6.

1651 Naumann BD, Silverman KC, Dixit R, Faria EC, Sargent EV, 2001. Case studies of categorical data-
1652 derived adjustment factors. *Human and Ecological Risk Assessment* 7, 61-105.

1653 OECD, 1997. OECD Guidelines for the Testing of Chemicals – Test Guideline 424, Neurotoxicity
1654 Study in Rodents (adopted 21 July, 1997). Organisation for Economic Cooperation and
1655 Development, Paris. Available at:
1656 <http://oberon.sourceoecd.org/vl=53690256/cl=12/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s24/p1.idx>

1657 OECD, 1997. OECD Guidelines for the Testing of Chemicals – Test Guideline 471, Bacterial Reverse
1658 Mutation test (adopted 21 July, 1997). Organisation for Economic Cooperation and Development,
1659 Paris. Available at: <http://oberon.sourceoecd.org/vl=45301079/cl=15/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s37/p1.idx>

1660 OECD, 1997. OECD Guidelines for the Testing of Chemicals – Test Guideline 474, Mammalian
1661 Erythrocyte Micronucleus Test (adopted 21 July, 1997). Organisation for Economic Cooperation
1662 and Development, Paris. Available at:
1663 <http://lysander.sourceoecd.org/vl=66362086/cl=26/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s39/p1.idx>

1664 OECD, 1997. OECD Guidelines for the Testing of Chemicals – Test Guideline 475, Mammalian Bone
1665 Marrow Chromosome Aberration Test (adopted 21 July, 1997). Organisation for Economic
1666 Cooperation and Development, Paris. Available at:
1667 <http://lysander.sourceoecd.org/vl=65973088/cl=15/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s40/p1.idx>

1668 OECD, 1998. OECD Guidelines for the Testing of Chemicals – Test Guideline 408, Repeated Dose
1669 90-day Oral Toxicity Study in Rodents (adopted 21 September, 1998). Organisation for Economic
1670 Cooperation and Development, Paris. Available at: <http://www.oecd-ilibrary.org/docserver/download/fulltext/9740801e.pdf?Expires=1301310917&id=0000&accname=freeContent&checksum=2EDAE00F6D3BF1EC02B516E2C31AC345>

1671 OECD, 2001. OECD Guidelines for the Testing of Chemicals – Test Guideline 414, Prenatal
1672 Developmental Toxicity Study (adopted 22 January, 2001). Organisation for Economic
1673 Cooperation and Development, Paris. Available at:
1674 <http://www.oecd-ilibrary.org/docserver/download/fulltext/9740801e.pdf?Expires=1301310917&id=0000&accname=freeContent&checksum=2EDAE00F6D3BF1EC02B516E2C31AC345>

1680 http://titania.sourceoecd.org/vl=11846807/cl=16/nw=1/rpsv/cgi-
1681 bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s14/p1.idx

1682 OECD, 2007. OECD Guidelines for the Testing of Chemicals – Test Guideline 426, Developmental
1683 Neurotoxicity Study (adopted 16 October, 2007). Organisation for Economic Cooperation and
1684 Development, Paris. Available at: <http://www.oecd-ilibrary.org/docserver/download/fulltext/9742601e.pdf?expires=1301311587&id=0000&accname=freeContent&checksum=CFE3FCB1C200CF6FEE11F5E84211BCB6>

1687 OECD, 2008. OECD Guidelines for the Testing of Chemicals – Test Guideline 407, Repeated Dose
1688 28-Day Oral Toxicity Study in Rodents (adopted 3 October, 2008). Organisation for Economic
1689 Cooperation and Development, Paris. Available at: <http://www.oecd-ilibrary.org/docserver/download/fulltext/9740701e.pdf?expires=1301311242&id=0000&accname=freeContent&checksum=7D5879166B44415747D7055280A7AE8C>

1692 OECD, 2009. OECD Guidelines for the Testing of Chemicals – Test Guideline 452, Chronic Toxicity
1693 Studies (adopted 7 September, 2009). Organisation for Economic Cooperation and Development,
1694 Paris. Available at: <http://www.oecd-ilibrary.org/docserver/download/fulltext/9745201e.pdf?expires=1301311343&id=0000&accname=freeContent&checksum=7C65EE89AA25AB5BF5418117B43B4AE8>

1697 OECD, 2009. OECD Guidelines for the Testing of Chemicals – Test Guideline 453, Combined
1698 Chronic Toxicity\Carcinogenicity Studies (adopted 7 September, 2009). Organisation for Economic
1699 Cooperation and Development, Paris. Available at: <http://www.oecd-ilibrary.org/docserver/download/fulltext/9745301e.pdf?expires=1301311105&id=0000&accname=freeContent&checksum=F37C5B8A0C8EECDFE4EEE276233C186>

1702 OECD, 2009. OECD Guidelines for the Testing of Chemicals – Test Guideline 451, Carcinogenicity
1703 Study (adopted 7 September, 2009). Organisation for Economic Cooperation and Development,
1704 Paris. Available at: <http://www.oecd-ilibrary.org/docserver/download/fulltext/9745101e.pdf?expires=1301311482&id=0000&accname=freeContent&checksum=7E9D56C36F4D041F751FD9DD92E75AB2>

1707 OECD, 2010. OECD Draft Guidance document N° 116, Design and Conduct of Chronic Toxicity and
1708 Carcinogenicity Studies, supporting TG 451, 452, 453 (April, 2010). Organisation for Economic
1709 Cooperation and Development, Paris. Available at:
1710 <http://www.oecd.org/dataoecd/58/18/44960015.pdf>

1711 OECD, 2010. OECD Guidelines for the Testing of Chemicals – Test Guideline 487, In vitro
1712 Mammalian cell Micronucleus Test (adopted 22 July, 2010). Organisation for Economic
1713 Cooperation and Development, Paris. Available at:
1714 <http://lysander.sourceoecd.org/vl=65973088/cl=15/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s62/p1.idx>

1716 OECD, 2010. OECD Guidelines for the Testing of Chemicals – Test Guideline 417, Toxicokinetics
1717 (adopted 22 July, 2010). Organisation for Economic Cooperation and Development, Paris.
1718 Available at: <http://www.oecd-ilibrary.org/docserver/download/fulltext/9741701e.pdf?expires=1301311739&id=0000&accname=freeContent&checksum=4FCF1ABEDFB378AFE5BAE80E766F9467>

1721 OECD, 2011. OECD Guidelines for the Testing of Chemicals – Test Guideline 443, Extended One-
1722 Generation Reproductive Toxicity Study (adopted 28 July, 2011). Organisation for Economic
1723 Cooperation and Development, Paris. Available at: http://www.oecd-ilibrary.org/environment/test-no-443-extended-one-generation-reproductive-toxicity-study_9789264122550-en

1725 Piersma AH, Rorije E, Beekhuijzen ME, Cooper R, Dix DJ, Heinrich-Hirsch B, Martin MT, Mendez
1726 E, Muller A, Paparella M, Ramsingh D, Reaves E, Ridgway P, Schenck E, Stachiw L, Ulbrich B,
1727 Hakkert BC. Combined retrospective analysis of 498 rat multi-generation reproductive toxicity
1728 studies: on the impact of parameters related to F1 mating and F2 offspring. *Reprod Toxicol*.
1729 31(4):392-401.

1730 SCF, 1996. Opinion on additives in nutrient preparations for use in infant formulae, follow-on
1731 formulae and weaning foods. 7 June 1996.
1732 http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_40.pdf

1733 SCF, 1998. Opinion of the Scientific Committee of Food on the applicability of the ADI (Acceptable
1734 Daily Intake) for food additives to infants. 17 September 1998.
1735 http://ec.europa.eu/food/fs/sc/scf/out13_en.html

1736 SCF, 2001. Guidance on submissions for food additive evaluations by the Scientific Committee on
1737 Food. 12 July 2001 http://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

1738 Slatter MA, Gennery AR, 2010. Primary immunodeficiencies associated with DNA-repair disorders.
1739 Expert Rev. Mol. Med 12: e9.

1740 Slikker WJr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG,
1741 Dorman DC, Gaylor DW, Hattis D, Rogers JM, Woodrow Setzer R, Swenberg JA, Wallace K,
1742 2004. Dose-dependent transitions in mechanisms of toxicity: case studies. *Toxicology and Applied
1743 Pharmacology* 201, 226–294.

1744 Ussing HH, Zerah K, 1951. Active transport of sodium as the source of electric current in the short-
1745 circuited isolated frog skin. *Acta Physiologica Scandinavica* 23, 110-127.

1746 Versantvoort CHM, Rompelberg CJM, Sips AJAM, 2000. Methodologies to study human intestinal
1747 absorption. *RIVM Report 63003001*.

1748 WHO/IPCS, 2011. Draft Guidance for Immunotoxicity Risk Assessment for Chemicals.
1749 (<http://www.who.int/ipcs/methods/harmonization/areas/immunotoxicity/en/index.html>)

1750 Wilson TH, Wiseman G, 1954. The use of sacs of everted small intestine for the study of the
1751 transference of substances from the mucosal to the serosal surface. *Journal of Physiology* 123, 116-
1752 125.

1753

1754 **APPENDICES**

A. TIERED TOXICITY TESTING FOR FOOD ADDITIVES

TIER 1

- **Absorption**
- **Genotoxicity**
 - in vitro testing
- **Toxicity**
 - Extended 90-day toxicity study



Triggers for Tier 2

- *Systemic exposure*
- *Toxicity in the 90-day study*
- *+ve in vitro genotoxicity*

TIER 2

- **ADME**
- **Genotoxicity**
 - in vivo testing
- **Toxicity (stand-alone or combined)**
 - Chronic toxicity
 - Carcinogenicity
- **Reproductive & Developmental toxicity**



Triggers for Tier 3

- *Bioaccumulation*
- *+ve in vivo genotoxicity*
- *Chronic toxicity/carcinogenicity*
- *Reproductive & Developmental toxicity*

TIER 3

- **ADME**
 - PBK modelling, volunteer studies
- **Carcinogenicity**
 - mode of action
- **Reproductive & Developmental toxicity**
 - F2 generation
 - developmental immunotoxicity & neurotoxicity
- **Specialised studies**
 - e.g. immunotoxicity, neurotoxicity, mode of action

B. DATA REQUIREMENTS FOR THE EVALUATION OF FOOD ADDITIVE APPLICATIONS,

USE A STATEMENT FROM THE PANEL MADE AT THE TIME

Scientific Statement of the Panel on Food Additives and Nutrient Sources added to Food (Question No EFSA-Q-2007-188)

1. Introduction

The present statement defines the general data requirements, while specific scientific approaches are suggested in the guidance for food additives applicable at the time of the application. During its second plenary meeting in September 2008, the Panel endorsed provisionally the guidance document for food additive evaluations adopted by the Scientific Committee on Food (SCF) in 2001. In order to reflect current thinking in risk assessment, the Panel will commence a detailed reappraisal of the guidance in September 2009. It is anticipated that, following a period of public consultation, this new guidance will be finalised in July 2011. Applicants should also take into consideration the opinions adopted in 2009 by the Scientific Committee of EFSA on Nanoscience and Nanotechnologies, on the use of the benchmark dose approach in risk assessment and on the replacement and reduction of animal testing, as well as the guidance on transparency in the scientific aspects of risk assessments adopted in 2009 and the guidance on the safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements adopted in 2008.

2. Data requirements

A dossier submitted in support of an application for the evaluation of a food additive should enable an assessment to be made of the additive based on the current state of knowledge and permit verification that the additive does not, on the basis of the scientific evidence available, pose a safety concern to the health of the consumer at the level of use proposed, as laid down in Article 6 of Regulation (EC) No 1333/2008 (EC, 2008).

The application dossier should include all the available data relevant for the purpose of the risk assessment (i.e. full published papers of all references cited, full copies of the original report of unpublished studies and corresponding individual raw data). When these papers and reports are not originally in English, the original language version and a complete English translation should be provided.

The documentation on the gathering of the data used in the dossier should also be provided. This documentation should specify the data gathering conducted and especially the literature search strategies (assumptions made, key words used, databases used, limitation criteria, etc.).

The comprehensive outcome of the literature search should also be provided. The individual raw data of the unpublished studies should be available on request from EFSA, preferably in a computer-readable format. The individual results of examinations and raw data, including microscopic slides, should also be available on request from EFSA. The safety evaluation strategy and the corresponding testing strategy should be described and justified with rationales for inclusion and exclusion of specific studies. Information should be provided on:

- the applicant and the application dossier (administrative data)
- the identity and characterisation of the additive (including the proposed specifications and analytical method)
- the manufacturing process
- the stability, reaction and fate in foods to which the additive is added
- the case of need and proposed uses

- the existing authorisations and evaluations
- the exposure assessment
- the biological and toxicological data.

Regarding the biological and toxicological data, the following core areas should normally be covered:

- Toxicokinetics
- Subchronic toxicity
- Genotoxicity
- Chronic toxicity/carcinogenicity
- Reproductive and developmental toxicity

Applicants are reminded that for each study performed it should be stated whether the test material conforms to the proposed or existing specification. Where the test material differs from this specification, the applicant should demonstrate the relevance of these data to the food additive under consideration.

Overall conclusions should be proposed by the applicant on the safety of the proposed uses of the additive. The overall evaluation of potential human risk should be made in the context of known or likely human exposure, including that from other sources. A summary of the information given in the dossier should also be provided. The dossier should be presented in a standard way. For this purpose, EFSA will establish standard templates for the different sections of the application dossiers and for the reporting of the toxicological studies. Once established, these templates should be used. Details of any applications made to other evaluation bodies or regulatory agencies together with their status and outcome should be disclosed. During the evaluation process, EFSA may request any additional data that is considered necessary for the safety assessment.

3. Administrative requirements

In order to enable EFSA to process adequately the application dossier and contact the applicant as necessary for the purpose of the evaluation of the application, the following information should be provided.

1. Applicant's contact details: name of the applicant or company, address (street, number, postcode, city, country), telephone, fax, e-mail (if available).
2. Manufacturer's contact details: name of the manufacturer(s) of the substance (if different from above), address (street, number, postcode, city, country), telephone, fax, e-mail (if available).
3. Contact person's details (for all correspondence with EFSA): name of the contact person, position, address (street, number, postcode, city, country), telephone, fax, email (if available).
4. Type of application (i.e. new food additive, new use of a permitted food additive)
5. Proposed (or existing) common name of the additive
6. Chemical name of the additive according to the IUPAC nomenclature
7. CAS number of the additive (if defined)
8. E number of the additive as defined in the European legislation on food additives (if applicable)
9. ELINCS and/or EINECS number of the additive (if attributed)
10. Date of submission of the dossier

11. Table of contents of the dossier
12. List of documents and other particulars. The applicant must identify the number and titles of volumes of documentation submitted in support of the application. A detailed index with reference to volumes and pages shall be added
13. List of parts of the dossiers requested to be treated as confidential, where necessary. The list shall make reference to the relevant volumes and pages of the dossier.

4. Additional Technical Information

Petitioners are also advised to provide reviews of the scientific literature for their additive and to report these with their criteria search strategies and search terms. These reviews should also summarise any existing authorisations for the additive including pending or unsuccessful submissions for any uses together with the basis used by the relevant authorities in making these decisions. The Panel will make its own evaluation on the specifics of the application and is not bound by these evaluations. Whilst other evaluations might inform the decision, petitioners are reminded that there can (and will) be differences in the scientific interpretation of the significance of findings and that the acceptability of individual findings is judged within the basis of the risk management context which determines the acceptability of both risk and uncertainty. Systematic reviews provide a tool for undertaking these literature reviews. The Panel encourages the application of the key elements of the systematic review process. The minimum requirements for a literature review are the search strategies applied, the definition of inclusion and exclusion criteria, documentation of how these were applied to the searches and a review of those papers meeting the inclusion criteria. Copies of these references should also be supplied. After the initial premarketing evaluation these searches and reviews need to be kept up to date to facilitate future re-evaluations.

Summary document

- A document summarising the data submitted in support of the proposed use of a food additive should also be provided.
- This summary should describe elements considered by the petitioner essential for the safety evaluation of the additive.
- The summary document shall be a standalone document and include a summary of the relevant information in any references
- The petitioner should highlight the crucial parameters related to the safety assessment of the proposed additive.
- The summary document should not contain any confidential information as it will be made available to the public on request.
- The summary document should essentially contain following elements.

Technical information

1. The chemical/physico-chemical identity and characteristics of the proposed additive.
2. Description of the source materials and the manufacturing process including information whether the additive is from plant, microbial, GMO or nano-material origin.
3. Information on the stability of the proposed additive and its reaction and fate in food.
4. Information on proposed use levels of the additive.
5. Information on previous evaluations and authorisations of the proposed additive.
6. Information on the estimated exposure of the proposed additive.

Toxicological information

1. Information on the toxicological evaluation of the food additive

2. Description of the toxicological data including descriptions of the results of individual studies
3. Review of results and conclusions.

Petitioners are invited to present their own conclusions as to the likely safety-in-use of the substance, drawing attention to any unusual features in the data presented.

5. Procedure

In assessing a food additive application the initial step is an administrative check by the Panel Secretariat that the required data are present or that there is a rationale for its absence. Dossiers failing to comply with these requirements will be rejected and their status updated to reflect this. In some cases it may be necessary to consult the Panel or one of its Working Groups on the merits of the rationales prior to making this decision. Following the initial screening, the dossier will be placed on the work programme of a Working Group and a rapporteur(s) assigned to carry out an initial evaluation of the scientific data. The rapporteur will develop drafts for discussion by the Working Group and subsequently the Panel. At any stage during this process additional data or clarification (including supporting evidence for rationales or interpretations of results) may be requested.

C. SPECIFICATIONS AS REQUIRED BY THE COMMISSION

E number	
Synonyms	
Definition	
EINECS	XX-XX-X
Colour Index No	
Chemical names	
Chemical formula	
Molecular/ Atomic weight /Weight average molecular weight	
Particle size of powder	
Assay ¹⁶	
Description	
Appearance of a solution	
Identification	
Spectrophotometry, spectrometry, chromatography, Infra Red, X-ray diffraction	
Density/specific gravity	XX (20°C) (25/25°C)
Refractive Index	
Specific rotation	
pH	XX-XX (XX% aqueous solution)
Degree of hydrolysis/ decomposition/ properties during burning	
Precipitation reaction	
Colour reaction	
Melting range or point	XX to XX °C
Viscosity	
Solubility	
Boiling point	
Specific identification tests and parameters	
Congealing range	
Distillation range	
Drop point	
Isoelectric point	
Solidification point	
Sublimation point	
Vapour pressure	
Microscopic observation/ examination	
Purity	
Loss on drying	
Loss on ignition	
Water or HCl insoluble matter	
Water content	
Conductivity	
Acid/Hydroxyl value	
Acidity/ alkalinity	
Oil content	
Fat	
Protein	
Total sugars	
Starch	

¹⁶ In accordance with Directive 2008/84/EC on specifications of food additives others than colours and sweeteners, the following definition of assay taken from the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) ([ftp://ftp.fao.org/docrep/fao/009/a0691e/a0691e00a.pdf](http://ftp.fao.org/docrep/fao/009/a0691e/a0691e00a.pdf)) should be considered: A quantitative assay requirement is provided here, where applicable, to indicate the minimum acceptable content, or maximum acceptable content range, of the principal functional component(s) of the additive.

Sodium chloride	
Ash	Not more than XX% (XXX°C)
Viscosity	Not less/more than XXX mPa.s
Wax	
Residual Solvents	Not more than XXmg/Kg
Residue on ignition	
Non-volatile residue	
Organic Volatile impurities	
Aldehydes	
Unsaponifiable matter	
Saponification value	
Ester value	
Iodine value	
Peroxide value / peroxides	
Oxidising/reducing substances	
Readily carbonisable substances	
Specific parameters for impurities	
Other specific parameters indicating the degree of purity	
Chlorinated compounds	
3-Monochloropropane-1,2-diol (3-MCPD)	
Polycyclic Aromatic Hydrocarbons	
Organic compounds other than colouring matters	
Pentachlorophenol	
Epoxides	
Mercury	Not more than XX mg/Kg
Cadmium	Not more than XX mg/Kg
Arsenic	Not more than XX mg/Kg
Lead	Not more than XX mg/Kg
Aluminium/ aluminium oxides	Not more than XX mg/Kg (expressed as Al)
Copper	
Nickel	
Antimony	
Chromium	
Selenium	
Fluorides	
Microbiological criteria	
<i>Salmonella spp</i>	
<i>Escherichia Coli</i> (coliforms)	
<i>Staphylococcus aureus</i>	
Yeast and moulds	
Total bacterial count	
Total plate count	
Other safety or purity related microbiological criteria	

ABBREVIATIONS

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ANS	Scientific Panel on Food Additives and Nutrient Sources added to food
AUC	Area under the curve
BMD	Benchmark dose
BMDL	Benchmark dose Lower
CAS	Chemical Abstracts Service
CSAF	Chemical-Specific Adjustment Factor
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of Notified Chemical Substances
EMA	European Medicines Agency
ENM	Engineered Nanomaterials
EOGRTS	Extended One-Generation Reproduction Toxicity study
EU	European Union
GD	Guidance document (OECD)
GLP	Good Laboratory Practice
GMM	Genetically Modified Microorganism
GMO	Genetically Modified Organism
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPCS	International Programme on Chemical Safety
ITS	Integrated Testing Strategies
IUPAC	International Union of Pure and Applied Chemistry

JECFA	Joint FAO/WHO Expert Committee on Food Additives
KLH	Keyhole Limpet Hemocyanin
LOAEL	Low Observed Adverse Effect Level
MOA	Mode of action
MOE	Margin of Exposure
MOS	Margin of Safety
MS	Mass Spectroscopy
MTD	Maximum Tolerated Dose
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
OECD	The Organisation for Economic Co-operation and Development
PAH	Polycyclic Aromatic Hydrocarbon
PBK	Physiologically-based Kinetic
POD	Point of Departure
QPS	Qualified Presumption of Safety
(Q)SARs	(Quantitative) structure-activity relationships
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
3-Rs	Replacement, refinement, reduction
SC	EFSA Scientific Committee
SCF	Scientific Committee on Food
SCOOP	Scientific Cooperation
SRBC	Sheep Red Blood Cells
TG	Testing guideline (OECD)
TTC	Threshold of Toxicological Concern
WG	Working Group