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## Guidance on protocol development for EFSA generic scientific assessments

More, Simon ; Bampidis, Vasileios ; Benford, Diane ; Bragard, Claude ; Hernández-Jerez, Antonio ; Bennekou, Susanne Hougaard ; Koutsoumanis, Konstantinos Panagiotis ; Lambré, Claude ; Machera, Kyriaki ; Mullins, Ewen ; Nielsen, Søren Saxmose ; Schlatter, Josef ; Schrenk, Dieter ; Turck, Dominique ; Younes, Maged ; Kraft, Andrew ; Naegeli, Hanspeter ; Tsaioun, Katya ; Aiassa, Elisa ; Arcella, Davide ; Barizzzone, Fulvio ; Cushen, Maeve ; Georgiadis, Marios ; Gervelmeyer, Andrea ; Lanzoni, Anna ; Lenzi, Paolo ; Lodi, Federica ; Martino, Laura ; Messens, Winy ; et al

**Abstract:** EFSA Strategy 2027 outlines the need for fit-for-purpose protocols for EFSA generic scientific assessments to aid in delivering trustworthy scientific advice. This EFSA Scientific Committee guidance document helps address this need by providing a harmonised and flexible framework for developing protocols for EFSA generic assessments. The guidance replaces the 'Draft framework for protocol development for EFSA's scientific assessments' published in 2020. The two main steps in protocol development are described. The first is problem formulation, which illustrates the objectives of the assessment. Here a new approach to translating the mandated Terms of Reference into scientifically answerable assessment questions and sub-questions is proposed: the 'APRIO' paradigm (Agent, Pathway, Receptor, Intervention and Output). Owing to its cross-cutting nature, this paradigm is considered adaptable and broadly applicable within and across the various EFSA domains and, if applied using the definitions given in this guidance, is expected to help harmonise the problem formulation process and outputs and foster consistency in protocol development. APRIO may also overcome the difficulty of implementing some existing frameworks across the multiple EFSA disciplines, e.g. the PICO/PECO approach (Population, Intervention/Exposure, Comparator, Outcome). Therefore, although not mandatory, APRIO is recommended. The second step in protocol development is the specification of the evidence needs and the methods that will be applied for answering the assessment questions and sub-questions, including uncertainty analysis. Five possible approaches to answering individual (sub-)questions are outlined: using evidence from scientific literature and study reports; using data from databases other than bibliographic; using expert judgement informally collected or elicited via semi-formal or formal expert knowledge elicitation processes; using mathematical/statistical models; and - not covered in this guidance - generating empirical evidence ex novo. The guidance is complemented by a standalone 'template' for EFSA protocols that guides the users step by step through the process of planning an EFSA scientific assessment.

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## Guidance on protocol development for EFSA generic scientific assessments

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### Abstract

EFSA Strategy 2027 outlines the need for fit-for-purpose protocols for EFSA generic scientific assessments to aid in delivering trustworthy scientific advice. This EFSA Scientific Committee guidance document helps address this need by providing a harmonised and flexible framework for developing protocols for EFSA generic assessments. The guidance replaces the 'Draft framework for protocol development for EFSA's scientific assessments' published in 2020. The two main steps in protocol development are described. The first is problem formulation, which illustrates the objectives of the assessment. Here a new approach to translating the mandated Terms of Reference into scientifically answerable assessment questions and sub-questions is proposed: the 'APRIO' paradigm (Agent, Pathway, Receptor, Intervention and Output). Owing to its cross-cutting nature, this paradigm is considered adaptable and broadly applicable within and across the various EFSA domains and, if applied using the definitions given in this guidance, is expected to help harmonise the problem formulation process and outputs and foster consistency in protocol development. APRIO may also overcome the difficulty of implementing some existing frameworks across the multiple EFSA disciplines, e.g. the PICO/PECO approach (Population, Intervention/Exposure, Comparator, Outcome). Therefore, although not mandatory, APRIO is recommended. The second step in protocol development is the specification of the evidence needs and the methods that will be applied for answering the assessment questions and sub-questions, including uncertainty analysis. Five possible approaches to answering individual (sub-)questions are outlined: using evidence from scientific literature and study reports; using data from databases other than bibliographic; using expert judgement informally collected or elicited via semi-formal or formal expert knowledge elicitation processes; using mathematical/statistical models; and – not covered in this guidance – generating empirical evidence *ex novo*. The guidance is complemented by a standalone 'template' for EFSA protocols that guides the users step by step through the process of planning an EFSA scientific assessment.

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\* The author is a US EPA employee. The views expressed in this document are those of the author and do not necessarily reflect the views or policies of the US EPA.

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## Summary

In a 2014–2018 project, the European Food Safety Authority (EFSA) defined the principles for the scientific assessment process (impartiality, methodological rigour, transparency and engagement) and developed a four-step approach (plan/do/verify/report) to aid in their fulfilment (EFSA, 2015, 2016, 2018). One key component of this approach was to plan the methods for the scientific assessment upfront in a protocol. In 2020, EFSA published a 'Draft framework for protocol development for EFSA's scientific assessments' (EFSA, 2020a) to aid in the gradual implementation of protocols in all EFSA generic mandates. The draft framework was tested on 21 EFSA mandates. In 2021, EFSA also outsourced a project to develop a framework for problem formulation, that is, the first step in protocol development (Risk Sciences International, 2022). More recently, EFSA Strategy 2027<sup>1</sup> outlined the need for fit-for-purpose protocols for EFSA generic scientific assessments to aid in delivering trustworthy scientific advice.

The scope of this EFSA Scientific Committee guidance, which updates the draft framework published in 2020 by integrating the improvements identified via the testing phase and the framework for problem formulation developed by the external contractor, is to provide all EFSA scientific panels and units/teams with a harmonised but flexible framework for developing protocols for 'generic mandates'. These are the assessments defined by Art. 29 and Art. 31 of Regulation (EC) 178/2002, and Art. 43 of Regulation (EC) 396/2005<sup>2</sup> that are not related to processes of regulated products. Generic mandates are relevant to all EFSA scientific panels and units.

This guidance helps fulfil the requirement to develop protocols especially for the cases when domain-specific guidance laying out the methods for conducting the assessment does not already exist. For regulated products, the protocol is predominantly covered by the relevant regulatory and scientific framework (EFSA, 2018). However, there can be cases when the regulatory framework is not fully detailed and for which this guidance will be useful. The guidance can also be applied by external contractors working on scientific assessments outsourced by EFSA.

The process for protocol development described in this guidance starts *after the clarification of the mandate requirements and the acceptance of the mandate*.

The guidance is complemented by a separate, supporting document that acts as a 'template' for EFSA protocols (Annex A) and guides the users step by step through the process of planning an EFSA scientific assessment.

By providing the plan for the scientific assessment upfront, protocols safeguard against methodological flaws like hypothesising at the end, when the results are already known and help protect from cognitive biases by requiring assessors to articulate scientific approaches before acquiring knowledge about the available results. Protocols also help streamline the implementation of the scientific assessment and contribute to improving the integrity, transparency and defensibility of the results of an assessment.

To ensure the delivery of fit-for-purpose and efficient scientific advice, EFSA protocols must be adapted to the mandate requirements. This implies tailoring (i) the level of detail of the protocol and (ii) the approach to publishing and disseminating the protocol to each mandate. Therefore, EFSA protocols can range from being brief summaries of the assessment questions and sub-questions and of the approach to perform the assessment, to highly detailed documents that illustrate, for example, the final search strategies, the tools for appraising evidence or the statistical analysis plan. Annex A to this guidance provides generic examples designed to demonstrate the approach and assist in developing EFSA protocols. For mandates addressing recurrent scientific questions, standard protocols can be developed and reused over time upon minor, ad hoc adaptations, when needed, depending on the specific question. As for publishing and disseminating the protocols, depending on the mandate requirements, external parties may or may not be consulted on a draft version of the protocol.

In addition to the mandate requirements, several reasons can reduce the possibility of developing highly detailed protocols. These include, for instance, the impossibility to fully formulate the problem upfront that is inherent in some types of scientific assessments; the limited knowledge of the available

<sup>1</sup> EFSA strategy 2027 (EFSA, 2021): science, safe food, sustainability, Publications Office. <https://data.europa.eu/doi/10.2805/886006>

<sup>2</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Text with EEA relevance.

evidence at the time of protocol development; the unavailability of or lack of expertise in applying certain methods; or time constraints.

The two main steps in protocol development are problem formulation (Step 1), which describes what the assessment aims to achieve, and the specification of the evidence needs and the methods that will be applied to address the problem (Step 2), which is concerned with defining how the assessment will be conducted. The overall process for protocol development is iterative and may require various extents of literature scoping and consultations within the expert group responsible for the assessment as well as the mandate requestor. In some exceptional situations when there is the need for a highly detailed protocol and it is not possible to plan everything upfront, the protocol can be developed in phases.

Problem formulation includes: (1) translating the mandate into assessment question(s) and making them *operational*; (2) breaking down each assessment question into sub-questions, making them *operational* and defining the conceptual model (i.e. the relationship among assessment question(s) and sub-questions); (3) determining the relative priority of the assessment question(s) and sub-questions; (4) selecting the overall approach for the assessment; and (5) documenting the process and the methods applied for formulating the problem. While steps 2–5 are not always needed, making assessment (sub-)questions *operational* is an essential part of problem formulation, necessary to define the evidence needs and the methods for identifying, analysing, synthesising and integrating evidence, or for eliciting expert judgement. The new 'APRIO' paradigm (Agent, Pathway, Receptor, Intervention and Output) helps this process by providing a formal, structured yet adaptable tool that is considered broadly applicable within and across the various EFSA domains. Owing to its cross-cutting nature, APRIO is expected to help harmonise problem formulation and foster consistency in protocol development. APRIO may also overcome the difficulty of implementing some traditional approaches for question formulation in systematic reviews across the multiple, diverse EFSA disciplines (e.g. the PICO/PECO approach: Population, Intervention/Exposure, Comparator, Outcome). Therefore, even if not mandatory, the use of APRIO is recommended. Different examples of assessment questions and sub-questions formulated using APRIO are given in Appendix A of this guidance.

Following problem formulation, the second part of the protocol specifies the evidence needs and the methods that will be applied for dealing with such evidence and carrying out the assessment. The guidance outlines five possible approaches to answering individual (sub-)questions that can also be used in combination: (a) using evidence from scientific literature and study reports; (b) using data from non-bibliographic databases; (c) using expert judgement; (d) using mathematical/statistical models; and (e) generating empirical evidence *ex novo* by carrying out primary research studies. Depending on the mandate requirements, each approach can be used with flexibility (e.g. narrative vs. systematic review or informal collection of expert judgement vs. formal expert knowledge elicitation). As for the process for answering assessment question(s), this can be by integrating evidence across sub-questions or by answering sub-questions in a logical sequence. The template for EFSA protocols (Annex A to this guidance) provides examples of how to plan these aspects step by step.

The guidance emphasises that uncertainty is inherent in each step of the scientific assessment process (from problem formulation to drawing conclusions). Therefore, the approach to addressing uncertainty should be planned *for each step of the scientific assessment*. The extent to which uncertainty can be defined at the protocol phase will vary between assessments based on a variety of factors including data availability, established practices in the field and/or the level of detail required by the protocol at hand.

According to EFSA procedures, a protocol – or, when developed in phases, a phase of the protocol – must be finalised before the start of the scientific assessment. Amendments to the final version of the protocol are possible if they are reported and the reasons for the change explained. The latter can also be related to organisational issues, i.e. it is not possible to retain the original plan due to time and/or resources constraints. Ideally, an attempt should be made to assess the impact of the protocol amendments on the final output. In some cases, the amendments to the protocol may require an agreement with the mandate requestor.

The approach proposed in this guidance document is expected to be applied with flexibility to accommodate the requirements of each specific EFSA mandate and support a fit-for-purpose and efficient risk assessment process.

The guidance ends with two recommendations for future developments to facilitate and harmonise protocol development at EFSA: (i) to develop an interactive repository of EFSA protocols, complemented by a writing interface; and (ii) to finalise the harmonised classification of EFSA assessment questions and sub-questions started in the 'Draft framework for protocol development for EFSA's scientific assessments' (EFSA, 2020a).



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## Disclaimer

This guidance consists of two main documents:

- 1) The present document, which illustrates the background, rationale and theoretical and practical aspects related to (EFSA) protocols.
- 2) Template for protocols (Annex A), i.e. a practical tool that helps implement the guidance by guiding the users step by step through the process of developing EFSA protocols and provides suggestions, examples, and links to relevant documents.

## 1. Introduction

The European Food Safety Authority (EFSA) contributes to the safety of the food chain from farm to fork, protecting human life and health, taking account of animal health and welfare, plant health and the environment as mandated in Regulation 178/2002<sup>2</sup>. It does so by delivering independent and transparent scientific advice to policymakers, through cooperation with EFSA partners, and in an open dialogue with society.

In 2019, EFSA responsibilities were increased by the publication of Regulation (EU) 2019/1381 (i.e. the 'Transparency Regulation', entered into application in 2021),<sup>3</sup> aimed to build stakeholders' and citizens' trust in EFSA through increased transparency and improved communication, while making its risk assessments more reliable and its operational model more sustainable. In 2021, the various requirements placed on the Authority by the Transparency Regulation were captured, as implementation actions, in EFSA 2027 Strategy.<sup>1</sup>

### 1.1. Background and Terms of Reference as provided by the requestor

#### 1.1.1. Background

In a 2014–2018 project, EFSA defined the four principles for the scientific assessment process (i.e. impartiality, methodological rigour, transparency, and engagement) and developed and tested a 4-step approach (plan/do/verify/report) to ease their fulfilment (EFSA, 2015, 2016, 2018). One fundamental aspect of this approach was to plan the methods for the scientific assessment upfront in a protocol, to reduce data-driven decisions and increase transparency.<sup>4</sup>

In 2019 EFSA decided to gradually implement the 4-step approach in all generic (i.e. 'non-application') mandates received as of 2020.<sup>5</sup> To this end, an EFSA Scientific Committee (SC) working group including external experts and one representative from each EFSA scientific unit was established to develop recommendations for protocol development. In April 2020 a first set of recommendations was endorsed by the SC and published in draft (EFSA, 2020a), to allow a testing phase that lasted for over 1 year and involved all generic assessments started after April 2020. Feedback and proposals for improvement were collected. Meanwhile EFSA also started a contract aimed to deliver a standardised approach for problem formulation, to be incorporated as appropriate in the fine-tuned recommendations for protocol development.

In 2021, the need to regularly implement protocols for generic mandates was outlined by the Management Board (MB) in EFSA 2027 Strategy.<sup>1</sup> In particular, to achieve the first Strategic Objective to 'deliver trustworthy scientific advice and communication of risks from farm to fork', and the operational result to deliver 'generic scientific advice with quality and efficiency', the Strategy requires, as key action, to *implement fit-for-purpose protocol development and publication, including problem formulation and the use of appraisal tools*. The obligation to prepare protocols for generic mandates was also operationalised by EFSA through its Quality Management System.

#### 1.1.2. Terms of Reference

In view of the above, EFSA Scientific Committee proposed to EFSA to fine tune the draft recommendations published in 2020 and translate them into a guidance document for protocol

<sup>3</sup> Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC.

<sup>4</sup> See Section 3 of this guidance for further clarifications on the advantages of protocols.

<sup>5</sup> See Section 1.2 for the definition of EFSA generic mandates.



development for EFSA generic scientific assessments (see next section). The Committee indicated that the guidance should consider the feedback and proposals for improvement collected during the testing phase of the draft recommendations and incorporate as appropriate the framework for problem formulation delivered by the external contractor.

The Committee also indicated that the guidance should aim to provide indications for the content of protocols for EFSA generic mandates, ensuring flexibility (i.e. different extents of planning depending on the situation) and enhancing harmonisation across EFSA's areas.

## 1.2. Scope, degree of obligation and applicability of this guidance

The scope of this guidance, which updates and replaces EFSA 'Draft framework for protocol development [...] (EFSA, 2020a), is to provide all EFSA scientific panels and units/teams with a harmonised but flexible framework for developing or updating protocols for 'generic mandates' (also called 'generic scientific assessments').

These are the assessments defined by Art. 29 and Art. 31 of Regulation (EC) 178/2002, and Art. 43 of Regulation (EC) 396/2005<sup>2</sup> that are *not* related to processes of regulated products. Generic mandates can be received by all EFSA Panels and units, including those mostly dealing with applications.<sup>6</sup>

EFSA's Quality Management System requires protocols to be developed for all EFSA generic mandates, and this guidance helps fulfil this requirement, especially for the cases when domain-specific guidance does not already exist. When domain-specific guidance exists that lays out the methods for conducting the assessment, those methods are referenced and, if needed, summarised in the protocol. In those cases, Panels should integrate into their protocols any additional and relevant aspects illustrated in the present guidance document.

The process for protocol development illustrated in this guidance starts after the negotiation and acceptance of the mandate, when, according to EFSA Standard Operating procedures, the Terms of Reference and the requirements of the mandate have already been clarified with the mandate requestor.

The term 'scientific assessment' used in this document covers not only risk assessments but also other types of evaluations that occur at EFSA, for example benefit and efficacy assessments.

For the development of dossiers in support of applications of regulated products, the protocol is typically already covered by the relevant regulatory and scientific framework (i.e. EU legislation and complementary EFSA and non-EFSA guidance documents), which describes the data requirements for the application and the methods for collecting, analysing and synthesising those data (EFSA, 2018). However, there can be cases when some of these aspects are not fully detailed in the regulatory framework and for which this guidance can be useful.

This guidance can also be applied by external contractors working on scientific assessments outsourced by EFSA.

In addition, although this document is not meant for developing methodological guidance, some of the principles and recommendations illustrated here can also be valuable for that purpose.

## 2. Process for developing this guidance

This guidance was developed over multiple phases by an ad hoc working group of EFSA experts and staff as illustrated in the following sections and subsequently revised and adopted by the EFSA Scientific Committee.

### 2.1. Publication of a draft framework for protocol development (EFSA, 2020a)

The first phase of the process for developing this guidance consisted of the definition of a 'Draft framework for protocol development for EFSA's scientific assessments', which was published in 2020 as a technical report (EFSA, 2020a) (hereafter referred to as 'EFSA's 2020 draft framework').

The framework was defined by a Scientific Committee working group composed of EFSA experts and staff who used, as starting point, the outcomes of a previous EFSA project aimed at defining and testing the use of protocols in EFSA assessments (the so-called 'PROMETHEUS' project (EFSA, 2015,

<sup>6</sup> For EFSA operational management see: <https://www.efsa.europa.eu/en/people/operationalmanagement#risk-assessment-production-department>. For EFSA panels and Scientific Committee see: <https://www.efsa.europa.eu/en/science/scientific-committee-and-panels>.

2016, 2018))<sup>7</sup> and considered relevant information reported in the scientific literature. Membership from all EFSA units ensured a reflection of all EFSA scientific domains.

The technical report was divided into two parts: the first, describing the rationale for protocols and their advantages, and the second, illustrating the steps and recommendations for developing EFSA protocols. One important outcome of this project was a preliminary, harmonised classification of EFSA assessment questions and sub-questions,<sup>8</sup> which showed multiple similarities across EFSA domains and set the scene for defining a harmonised approach for problem formulation (i.e. the first step in protocol development).

In 2020 the framework was endorsed by the EFSA Scientific Committee and published as a draft to allow its testing and fine tuning.

## 2.2. Test of the draft framework for protocol development within EFSA

A further phase of the project focused on pilot testing EFSA's (2020a) draft framework to fine tune it based on the needs of the users.

The testing ran from June 2020 to June 2021 and included all EFSA generic mandates that started in approximately June 2020. At the end of the test period, 21 generic mandates were included: 10 from the former department on Risk Assessment and Scientific Assistance and 11 from the former one on Scientific Evaluation of Regulated Products.

Feedback and suggestions for improvement were collected from EFSA staff responsible for the mandates through a questionnaire administered using EUSurvey.<sup>9</sup> The questionnaire focused on five main aspects:

- 1) General feedback on the mandate on which the draft framework for protocol development was tested (including any plan for disseminating the protocol).
- 2) Description of the sub-questions embodied in the mandate and approach(es) applied to answering each of them.
- 3) Feedback on specific sections of EFSA's (2020a) draft framework.
- 4) General feedback on the specificity, structure, clarity, fit-for-purpose, user friendliness, appropriateness of case examples and length of EFSA's (2020a) draft framework.
- 5) Further feedback on any general difficulties encountered when drafting the protocol.

In addition to the feedback provided via the survey, some EFSA units/teams shared more extensive considerations and suggestions via e-mail.

Out of the 21 mandates used to test EFSA's (2020a) draft framework, 19 were registered in the survey. Of those 19, the questionnaire was completely answered for 13 mandates only, for various reasons, including the fact that some mandates were still in a preliminary stage.

The results of the survey showed the need for a series of changes to the EFSA's (2020a) draft framework for protocol development which included clarifying some methodological aspects (e.g. how to translate mandates into scientifically answerable questions and sub-questions, how to determine the relative priority of the sub-questions within a mandate or how to plan evidence synthesis/integration and uncertainty analysis in a protocol), and better defining several concepts (e.g. what it is meant by 'different extent of planning' in a protocol, what is a deviation from a protocol or when to consider a protocol 'final').

In addition, a scoring system based on stars was used to rate seven general features of the draft document (Table 1). This highlighted the need to make the framework more fit-for-purpose, user friendly and supported by illustrative examples. Although achieving sufficiency, the score for specificity, structure, clarity and length also suggested the possibility for further improvement.

<sup>7</sup> PROMoting METHods for Evidence Use in Scientific assessments. <https://www.efsa.europa.eu/en/methodology/evidence>

<sup>8</sup> See Section 6.1 of this guidance for clarifications on these concepts.

<sup>9</sup> <https://ec.europa.eu/eusurvey/home/welcome>

**Table 1:** Mean rating from 1 to 5 (1 star: not satisfactory at all; 2 stars: not satisfactory; 3 stars: sufficient; 4 stars: good/more than average; 5 stars: very good/exemplary) of the seven general criteria (referring to 13 out of 21 mandates)

Seven criteria	Mean
Structure of the report	3.4
Length of the document (maximum score when not too short, not too long)	3.4
Specificity of the recommendations	3.2
Clarity of the recommendations	3.0
Fit-for-purpose	2.7
User friendliness	2.3
Inclusion of case example (i.e. whether the report includes examples that clarify the recommendations)	2.3

### 2.3. Outsourced project on problem formulation

In EFSA's (2020a) draft framework for protocol development, the section on problem formulation was only briefly touched on, as it had been planned to develop such part based on the outcomes of an outsourced project.

The project, launched in 2021 and awarded to the contractor Risk Sciences International (RSI),<sup>10</sup> included the following tasks:

- Carrying out a review of available frameworks for problem formulation, to identify aspects that are key for formulating assessment questions and sub-questions and possible approaches for selecting those of 'higher priority'.<sup>11</sup>
- Screening all scientific assessments published in the EFSA Journal between 2019 and 2020, with the purpose of identifying their assessment questions and sub-questions and mapping them onto the preliminary classification available in EFSA's (2020a) draft framework, with the purpose of proposing a revised version.
- Drafting a handbook illustrating a framework and a set of recommendations for problem formulation and developing two examples to test them on real cases.

The methodology and findings of the contractor are detailed in (Risk Sciences International, 2022) and summarised in this section.

Through a narrative review of the literature focussed on methods for problem formulation, the contractor yielded 40 relevant documents, most of them dealing with genetically modified organisms and environmental risk assessments (26 out of 40). The review aimed at identifying the prevalent definitions and purposes of problem formulation, steps of the process and guidance on how to perform each step to aid in developing recommendations for the handbook.

By screening the 2019–2020 EFSA publications, the contractor identified over 700 scientific assessments (external scientific reports and guidance documents were excluded), reviewed their full texts and analysed their assessment questions and sub-questions to map them onto the draft classification reported in EFSA's (2020a) draft framework. This resulted in a revised and extended classification of EFSA Assessment Questions and Assessment Sub-questions. Through this activity, the contractor also developed a new, harmonised approach for formulating assessment questions and sub-questions, which was defined as the 'APRIO' paradigm (Agent, Pathway, Receptor, Intervention, Output), meant to be applicable to the large breadth of EFSA topics.

A handbook was also delivered, providing recommendations for a stepwise process for problem formulation for EFSA scientific assessments.

### 2.4. Revision of EFSA's (2020a) draft framework for protocol development and translation into this guidance

The final phase of the project consisted of the amendment of the draft framework for protocol development (EFSA, 2020a) and its conversion into this guidance. To do so, the working group was

<sup>10</sup> <https://risksciences.com/>

<sup>11</sup> See Section 6.4 of this guidance.

enlarged to include new experts with additional competencies in methods for evidence-based scientific assessments and to exchange views with other international organisations with remits like EFSA's.

The working group analysed the input and feedback received during the pilot phase of EFSA's (2020a) draft framework and revised and integrated the part on problem formulation and especially the APRIO paradigm delivered by the external contractor (Risk Sciences International, 2022).

To make the guidance user friendly and more fit-for-purpose, it was decided to complement it with a 'template' for EFSA protocols (Annex A), which guides users step by step through the process of planning an EFSA scientific assessment by providing suggestions, examples, and links to relevant documents.

A draft version of the guidance, endorsed by EFSA's Scientific Committee, was shared for public consultation between 13 March and 15 May 2023. The comments received were used for finalising the guidance. The outcomes of the public consultation are reported in Annex B.

### 3. The scientific assessment process at EFSA

The scientific assessment process at EFSA consists of two main phases (EFSA, 2015, 2016, 2018):

- 1) A planning phase, during which the protocol for the scientific assessment is developed. This includes (i) problem formulation and (ii) the specification of the evidence needs and of methods that will be applied for carrying out the assessment ('plan'). In the assessments of applications of regulated products, the protocol is covered by the relevant legislative documents and complementary guidance, which describe, in varying detail, the data requirements and the methods for dealing with these data.
- 2) An implementation phase, which implies conducting the assessment in line with the protocol ('do'); verifying the assessment process ('verify'); documenting the process (including any amendments to the protocol); reporting its results and drawing conclusions, while ensuring as much as possible accessibility of methods and data ('report').

This two-phase approach is in line with best practice in primary research and systematic review methodology (Higgins et al., 2022), but also with a growing number of initiatives that started to promote and implement the use of protocols in contexts similar to EFSA's (WHO, 2014; Woodruff and Sutton, 2014; OHAT-NTP, 2019; National Academies of Sciences, 2021; WHO, 2021).

Specifying the methods before the start of the scientific assessment process has proven to have several advantages, including limiting methodological flaws like hypothesising after the results are known (HARKing), data-contingent analysis decisions and post-hoc analysis in general by requiring assessors to articulate scientific approaches before acquiring knowledge about – and possibly be influenced by – the available results (Munafò et al., 2017). In addition, protocols safeguard against arbitrary decision-making during the assessment process and help protect from cognitive biases (Shamseer et al., 2015; Munafò et al., 2017) such as confirmation bias, i.e. the tendency to focus on evidence that is in line with expectations or favoured explanation (Kerr, 1998). Overall, all this contributes to improving the integrity, transparency and defensibility of the results of an assessment. Moreover, protocols help streamline the implementation of the scientific assessment (Kass et al., 2016) and, if shared with external parties and made open for comments, prevent duplication of effort and raise early awareness of areas of information not scoped within the protocol.

However, EFSA protocols do not need to, or cannot always, be highly detailed (next section).

### 4. Flexible approach to protocol development at EFSA

To ensure the delivery of scientific advice that is fit-for-purpose and efficient, the EFSA Quality Policy sets out the requirement for an agreement between the Authority and its 'customers' about several aspects, including the scope of the question, the deadline and the degree of 'scientific value' of the final product.<sup>12</sup>

<sup>12</sup> In EFSA Quality Policy ([https://www.efsa.europa.eu/sites/default/files/documents/TM0622127ENN\\_002-PF3.pdf](https://www.efsa.europa.eu/sites/default/files/documents/TM0622127ENN_002-PF3.pdf)) EFSA's customers are defined as the entities listed in the founding regulation that can mandate the Authority to produce advice: the European Commission, the European Parliament, the EU Member States and EFSA itself. In the Policy, 'scientific value' is defined as the extent to which four interdependent value components are satisfied beyond standard minimum requirements during the assessment process and captured in the final product: impartiality, methodological rigour, transparency and engagement. Impartiality is concerned with minimising the risk of bias and maximising objectivity, methodological rigour with minimising errors and maximising representativeness and transparency with the accessibility of inputs, intermediaries and outputs. Engagement is about the engagement of stakeholders and open dialogue.

In this context, a flexible approach to protocol development is required. This implies adapting to the characteristics of each specific mandate and, if possible, clarifying with the mandate requestor before the start of the scientific assessment process:

- the extent of planning in the protocol, i.e. level of detail in problem formulation and in the specification of the evidence needs and methods that will be applied.
- the approach to publishing and disseminating the protocol.

For mandates addressing recurrent scientific questions, a standard protocol can be developed and reused over time upon minor, ad hoc adaptations, when needed, depending on the specific question.

EFSA protocols can vary from being outlines of the assessment questions and sub-questions and of the approach to perform the assessment (along with its rationale, limitations and related sources of uncertainty), to very detailed documents providing, for example, a thorough description of the final search strategies, the tools for appraising evidence or the statistical analysis plan.

In addition, regardless of the requirements of each specific mandate, multiple aspects can reduce the possibility of developing highly detailed protocols, such as:

- Impossibility to fully formulate the problem upfront (and hence detail the methods) that is inherent in some types of scientific assessments. For instance, for assessments aimed at developing an adverse outcome pathway (AOP), the AOP itself represents the conceptual model, which in the protocol can only be preliminarily outlined.<sup>13</sup>
- Limited knowledge of the available evidence. The less information one has in advance on the type, amount and heterogeneity of the data that will be used, the more difficult it will be to develop ahead the most detailed plan.
- Unavailability of methods. If certain methods are not yet established in the scientific community (e.g. critical appraisal tools for some specific study types; or some specific methods for evidence synthesis/integration and uncertainty analysis), it can be too resource intensive to describe the specific approach upfront in a protocol.
- Limited expertise in applying certain methods. The ability to plan the methods increases as the experience in applying those methods grows. For instance, for complex scientific assessments based on extensive and heterogenous types of evidence, often there is limited experience in the methods for evidence synthesis and integration, including uncertainty analysis, so these components of the protocol may not be as fully formulated or detailed as other parts.
- Time constraints.

In a broad assessment containing multiple sub-questions, the extent of planning can vary by sub-questions, depending on their relative priority (Section 6.4), but also within the same sub-question and approach, by step of the approach. For instance, for the same sub-question, the protocol can provide an extensive plan of the methods for data collection and a generic description of the ones for evidence synthesis.

Recommendations and examples for 'low' and 'high' extent of planning in the various steps of protocol development are provided in Annex A to this guidance document.

As for the approach to publishing and disseminating protocols, this can be adapted to the requirements of the mandate as follows:

- 1) The final version of the protocol (Section 8) is stored for internal use and audit purposes and made publicly available only at the end of the scientific assessment process when it is published with the final output.
- 2) The final version of the protocol is published as the scientific assessment progresses and before its end.
- 3) A draft version of the protocol is shared with external parties through a public consultation to receive feedback and input on the methods to use for the scientific assessment and, if appropriate, refine them. The extent and method for the consultation vary depending on the engagement agreed for the assessment and the urgency for finalising it (e.g. targeted consultation of relevant stakeholders or extensive public consultation; consultation via EFSA website only or also via a workshop).

<sup>13</sup> An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that leads to an adverse health or ecotoxicological effect (OECD Environmental, Health and Safety (EHS) Programme). <https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm>



## 5. Steps in protocol development

In EFSA's assessments, protocol development starts after the negotiation and acceptance of the mandate, when the Terms of Reference have already been clarified with the requestor – including what is within and what is out of scope – and decisions have already been made on the extent of planning in the protocol and the approach to publishing and disseminating it (previous section). However, during protocol development, multiple consultations with the requestor may be needed to further clarify the mandate and ensure its Terms of Reference and requirements be properly addressed. If protocols are shared with the requestor, they help ensure, before starting the implementation phase, that the assessment will answer the question originally posed and that the plan will lead to an output that targets the requestor's needs.

The steps for developing protocols for EFSA's scientific assessments are summarised in Box 1. Step 1 (problem formulation) describes *what* the assessment aims to achieve, while Step 2 (the specification of the evidence needs and the methods that will be applied to address the problem) is concerned with specifying *how* to achieve it.

The process for protocol development is iterative and can be time- and resource-intensive, requiring various extents of literature scoping and consultations within the expert group responsible for conducting the assessment and the mandate requestors. Nevertheless, to meet the required deadline, the time dedicated to the protocol should be proportional to the overall time assigned to the mandate and, when starting an assessment, it should be decided how to split the overall time for the mandate into the planning phase and implementation phase.

The protocol should illustrate the plan for the scientific assessment from the start to the end. However, *in some exceptional situations when there is the need for a highly detailed protocol, and it is not possible to plan everything upfront* (e.g. when it is difficult to specify *a priori* the relevant endpoints, owing to the limited knowledge of the available evidence), the protocol can be developed in phases. This implies defining the plan for the first part of the assessment (e.g. up to the methods for data collection included) and carrying out the first part of the assessment accordingly. The outcomes of the first part of the assessment provide input to the planning of the second part, which is then implemented, and so on (see an example in EFSA PPR Panel, 2023). When protocols are planned in phases, each phase should be finalised and approved before moving to the next (Section 8).

**Box 1:** Steps for developing protocols for EFSA's scientific assessments and relevant sections in this guidance document. Not all steps are mandatory. ToRs: Terms of Reference.

### STEP 1 (what): Problem formulation (Section 6 and Figure 1)

**Step 1.1:** Translate the mandate ToRs into assessment question(s) and make them operational.

**Step 1.2:** Break down each assessment question into sub-questions and make them operational.

Define the relationship among assessment questions and sub-questions (**conceptual model**).

**Step 1.3:** Determine the relative priority of the assessment question(s) and sub-questions.

**Step 1.4:** Select the overall approach for the assessment.

**Step 1.5:** Document the process and the methods applied for formulating the problem.

### STEP 2 (how): Specification of the evidence needs and the methods for ...

#### Step 2.1: ... answering individual (sub-)questions (Sections 7.1 and 7.2)

- 1) Using evidence from scientific literature and study reports.
- 2) Using data from non-bibliographic databases.
- 3) Using expert judgement (including expert knowledge elicitation (EKE)).
- 4) Using mathematical/statistical models.
- 5) Generating empirical evidence *ex novo* (not covered in this guidance).

#### Step 2.2: ... answering assessment questions that consist of multiple sub-questions

- 1) Integrating evidence across sub-questions (Sections 6.3 and 7.2).
- 2) Answering sub-questions in a logical sequence (Section 6.3).

Note: Ideally the methods for **assessing uncertainty** should be **planned for each step** of the scientific assessment (Section 7.3).



As for the expertise needed, protocol development requires, especially for highly detailed plans, the involvement of multidisciplinary working groups embracing domain expertise but also expertise in, for example, information science, study design, data science, epidemiology and statistics.

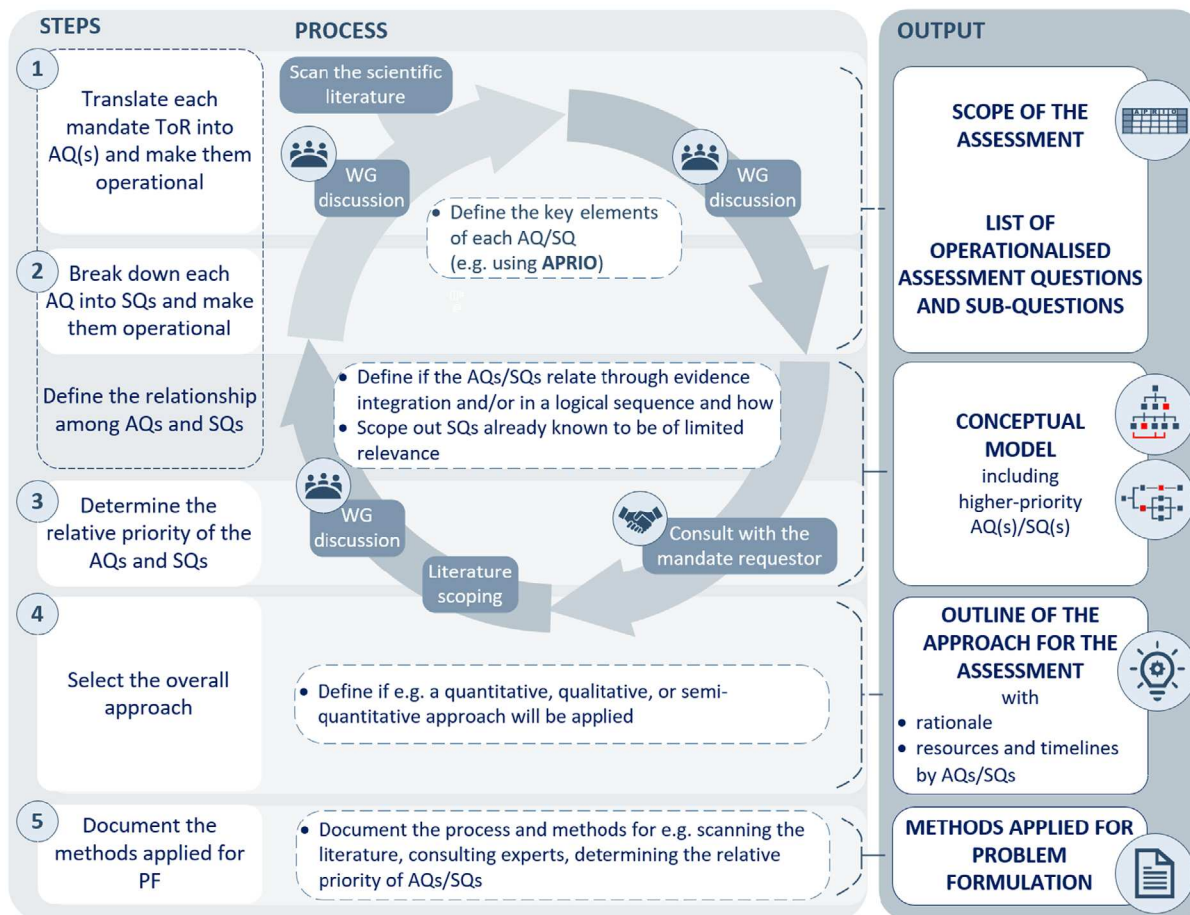
Key concepts to clarify the iterative, stepwise process for protocol development are described in the next sections, while suggestions to develop protocols step by step are given in Annex A to this guidance document, including the case of less and highly detailed protocols.

## 6. Problem formulation

Problem formulation is the first step in the planning of the scientific assessment, initiating protocol development and clarifying the objectives of the assessment.

Problem formulation starts after the mandate negotiation with the requestor and consists of the steps depicted in (Figure 1) and illustrated in this section. Examples and suggestions on how to conduct problem formulation are given in Annex A to this guidance.

### PROBLEM FORMULATION



Note: Steps 2–4 are optional. Step 2 is often needed in EFSA mandates. ToR: Term of Reference; AQ: assessment question; SQ: sub-question; PF: problem formulation; APRIO: Agent, Pathway, Receptor, Intervention, Output.

Figure 1: Steps in problem formulation

- 1) **Step 1.** The first step in problem formulation consists of translating each mandate Term of Reference into as many **assessment questions** as necessary (Section 6.1) and making them **operational** (Section 6.2). The latter can be done using the Agent, Pathway, Receptor, Intervention, Output paradigm (APRIO) or any available domain-specific approach. The APRIO paradigm is described in Section 6.2. To this scope, an initial scan of the scientific literature (including relevant assessments by national and international bodies, if any) can be conducted with different extents of rigour

depending on the situation, to identify challenging aspects for addressing the mandate and possibly capture critical elements that help formulate the questions and define the scope of the entire assessment.

- 2) **Step 2.** (Optional) The second step implies breaking down each assessment question into **sub-questions** (Section 6.1) and formulating them using APRIIO or any other specific approach, if available (Section 6.2). Often the necessary sub-questions are already broadly defined at the start of this step (e.g. 'occurrence' and 'consumption' for a question on exposure assessment). However, they are not yet **operational** (Section 6.2).

As Steps 1 and 2 progress, the **conceptual model(s)** (that is, the relationship among the assessment questions and, if present, sub-questions) is defined (Section 6.3). This can be described graphically, in tabular format or with a simple text description. In the rare situation when the mandate corresponds to one individual assessment question not broken down into sub-questions, the conceptual model is not needed. During the definition of the conceptual model, sub-questions that are already known to be of limited relevance can be identified and not included in the conceptual model (Section 6.3).

- 3) **Step 3.** (Optional) This step consists of determining the **relative priority** of the assessment questions and sub-questions, including identifying those that may require greater effort to be answered. Justifying and documenting the related choices as well as any method applied (e.g. sensitivity analysis) is also covered in this step (Section 6.4).

Overall, Steps 1–3 are typically iterative and require consulting the literature and experts. Often during these steps an interaction with the mandate requestor is needed, to ensure the mandate is properly translated, and the conceptual model(s) and all related aspects (e.g. the relative priority assigned to the assessment question(s) and sub-questions) appropriately address the Terms of Reference.

- 4) **Step 4.** (Optional). Occasionally, in problem formulation there can be the need for a description of the approach for the assessment – for the assessment overall or by individual sub-question – with fit-for-purpose rationale. A critical element would be whether a quantitative, qualitative or semi-quantitative approach will be applied. This choice will influence most of the following decisions in protocol development and during the conduct of the assessment. Another indication can be the application of a tiered approach. The latter might imply moving from crude but more conservative assumptions (e.g. the legal maximum permitted level is representative of the content of a substance in food) to a more refined, accurate approach (e.g. use of occurrence data provided by the Member States). If feasible, a description of resources and timelines implication at the level of questions and sub-questions is given.
- 5) **Step 5.** (Optional) The last step is represented by the documentation of the process and the methods applied for formulating the problem (e.g. the methods for scanning the literature and/or consulting experts). The choice to include this step (as well as its level of detail) will be case by case depending on what is decided during the negotiation phase of the mandate (Section 4).

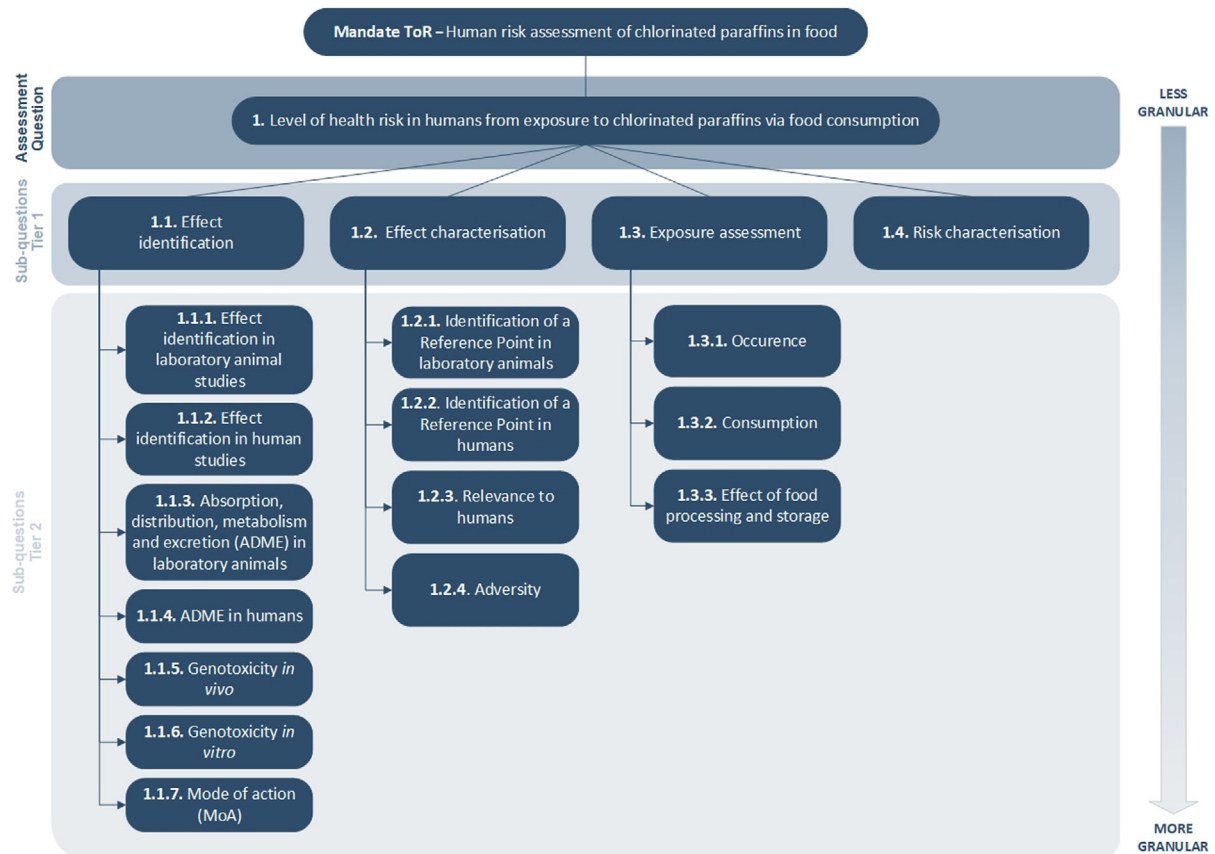
## 6.1. Translation of the mandate Term(s) of Reference into assessment question(s) and sub-questions

The number of Terms of Reference in EFSA mandates varies depending on how the mandate is written by the requestor. Problem formulation starts with the translation of each Term of Reference into as many assessment questions as deemed necessary by the assessors. For instance, in the hypothetical mandate depicted in Figure 2 on the risk to human health related to the presence of chlorinated paraffins in food, the single Term of Reference is translated into one assessment question. In the mandate – also hypothetical – on the assessment of the use of high-pressure processing (HPP) to increase microbiological food safety instead, there are two Terms of Reference divided into six sub-Terms of Reference, each of them translated into one assessment question (Figure 3).

In EFSA mandates the assessment questions are often broad and dependent on answering multiple sub-questions, which must be identified and specified during problem formulation. For instance, in the example mandate in Figure 2, the assessment question on the level of health risk in humans from exposure to chlorinated paraffins via food consumption is dependent on answering the sub-questions on effect identification, effect characterisation, exposure assessment and risk characterisation. Other examples of sub-questions are shown in Figure 3 on high-pressure processing.

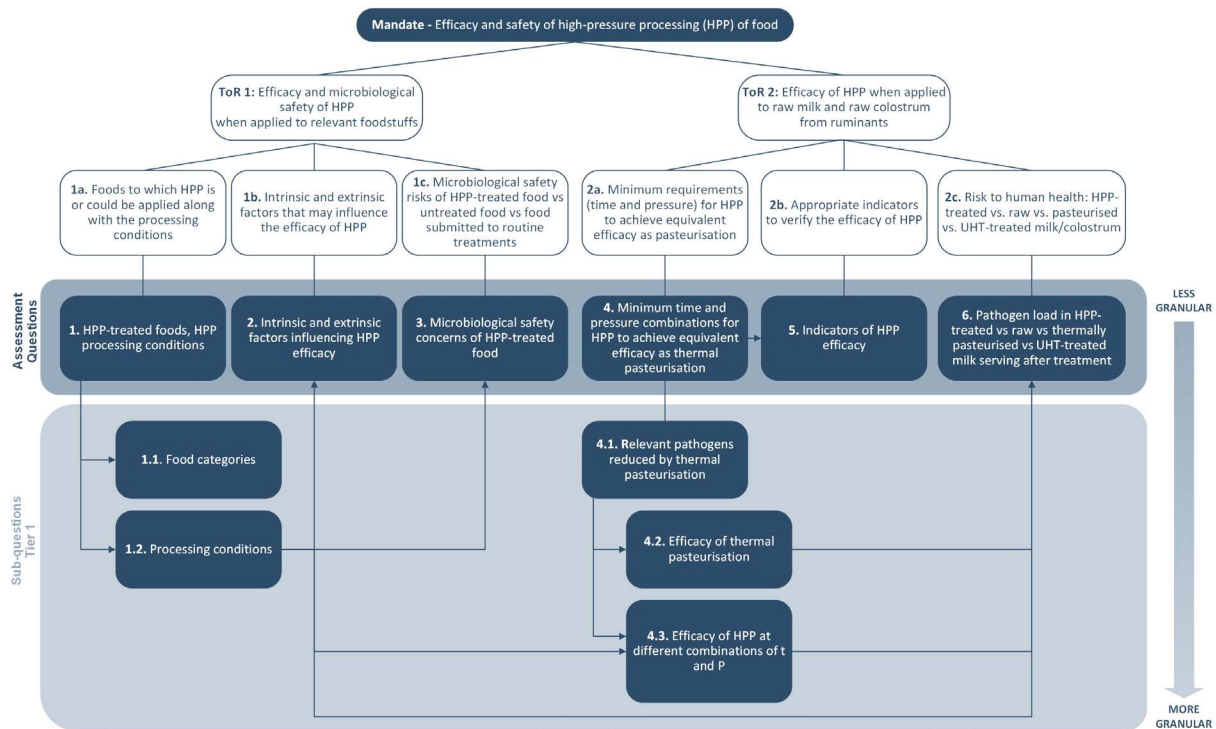
In addition, sub-questions are frequently broken down into further sub-questions, in a hierarchical structure. In the example in Figure 2 for instance, the Tier 1 sub-question on effect identification requires addressing the more granular – Tier 2 – sub-questions on effect identification in laboratory animals, effect identification in human studies or on Absorption, Distribution, Metabolism and Excretion in laboratory animals (ADME), and so on. In the same example, most sub-questions could be broken down into additional, more granular, sub-questions (e.g. Tier 3 and Tier 4).

When to stop breaking down a sub-question during problem formulation depends on the characteristics of the mandate and/or data availability. Typically, the generation of more granular sub-questions ends when the attained detail of a sub-question reflects the level at which evidence synthesis is deemed necessary and/or permitted by data availability. This is typically decided iteratively during problem formulation and can also be specified in the sectoral guidance documents, when available.



In this example, there is one assessment question corresponding to multiple Tier 1 and Tier 2 sub-questions. More granular sub-questions are shown by the arrow from top to bottom. The examples of sub-questions are illustrative and not necessarily exhaustive for the mandate. The assessment question and sub-questions are reported in the form of statements and are not yet 'formulated' (Section 6.2). ADME: Absorption, Distribution, Metabolism and Excretion; MoA: Mode of Action; ToR: Term of Reference.

**Figure 2:** Chlorinated paraffins in food (hypothetical mandate)



In this example, there are two Terms of Reference (ToRs) and six sub-ToRs. Each sub-term of reference is translated into one assessment question. The assessment questions are broken down into Tier 1 sub-questions (i.e. assessment questions 1 and 4) or not split (i.e. assessment questions 2, 3, 5 and 6). More granular sub-questions are shown by the arrow from top to bottom. The assessment questions and sub-questions are reported in the form of statements and are not yet 'formulated' (Section 6.2 and Appendix A.4). HPP: high-pressure processing; P: pressure; t: time; UHT: ultra-high temperature.

**Figure 3:** High-pressure processing technique for food (hypothetical mandate)

## 6.2. Formulation of the assessment question(s) and sub-questions: a harmonised approach based on the APRIO paradigm

Another essential element of problem formulation consists of making the assessment questions and sub-questions derived from the mandate *operational*, that is, *answerable through the scientific assessment* and mutually understandable between risk assessors and risk managers.

This process, defined as question and sub-question *formulation*, is fundamental as it aids in the definition of the conceptual model (i.e. the relationship among the assessment questions and sub-questions), the evidence needs (e.g. what studies to look for, what data to collect, what new empirical evidence to generate) and the methods for identifying, analysing, synthesising and integrating evidence or for eliciting expert judgement (Section 7).

Typically, question formulation takes place iteratively during the translation of the mandate term(s) of reference, starting at the level of the assessment question(s). Then, the operationalised assessment questions help first identify and then formulate the underpinning sub-questions.

Question and sub-question formulation can be challenging, especially when sectoral guidance does not already exist. The APRIO paradigm (Agent, Pathway, Receptor, Intervention and Output) presented in this guidance is considered a useful tool to aid in this process. APRIO was originally developed by Risk Sciences International after analysing almost 700 EFSA scientific assessments published in 2019–2020 and further refined when developing this guidance (Section 2).

The definitions of the A-P-R-I-O elements are as follows:

- **Agent** is anything that can cause an effect on a receptor.
- **Pathway** refers to any way in which an agent interacts with its receptor. It is the sequence of events leading the agent to cause an effect on the receptor. It can simply cover the route of exposure (typically dietary in EFSA assessments) or represent, for instance, the steps of introduction and spread when assessing a pathogen.



- **Receptor** refers to anything that experiences the effect of the agent. The receptor can also experience a secondary consequence to the exposure to the agent (e.g. farmers changing cropping practices as a consequence of the crops being affected by a pest).
- **Intervention** refers to any intentional measure aimed at changing directly or indirectly the exposure and/or the consequence of the exposure to the agent.
- **Output** is the form of the answer to the assessment question or sub-question, the result of an assessment process.

Table 2 shows how these elements can be interpreted in the various EFSA fields, while examples of questions and sub-questions formulated using the APRIO paradigm are given in Appendix A to this guidance.

The terms within the APRIO paradigm, adapted or extended to apply across diverse EFSA domains, may have some divergence with the use of the same or similar terminology within individual scientific disciplines. For instance, in APRIO the term 'Pathway' broadly refers to 'any way in which an agent interacts with its receptor [...] () both at micro and macro level, while in biology or toxicology it is more related to the mode or mechanism of action of an agent in biological systems. Likewise, in APRIO 'Receptor' is anything that experiences the effect of the agent [...], while again in biology or toxicology the term is usually used in relation to the molecular mechanism of action and, in toxicology, as a key concept to understand dose–response relationships. However, given the diversity and breadth of EFSA work, APRIO provides a logical, cross-cutting approach whose benefits are considered to outweigh the cost of this terminology adaptation (Section 6.2.1).

The meaning, relevance and characterisation of the APRIO elements vary depending on the question/sub-question. For instance, a treatment (e.g. a sanitiser or a physical inactivation technique) can be an Agent or an Intervention depending on the aim of the question. Notably:

- In an *efficacy question*, the aim is to assess the effect of different treatment conditions in an *experimental setting and not to implement the treatment in real conditions*. For instance, sub-question 4.3 in Figure 3 and Appendix A.4 aims at assessing the efficacy, in terms of log<sub>10</sub> units' reduction of pathogen load, of high-pressure processing at different time and pressure combinations on a pathogen in milk in an experiment. In this type of question focused on an experimental setting, the treatment is the Agent since it can cause an effect (reduction) on the Receptor (the pathogens in milk). This concept applies to all types of 'treatment' (e.g. feed additives) and receptors (e.g. aflatoxin B1 in feed). Generally, the APRIO element *Pathway is not relevant* for this type of question as the treatment is not deliberately introduced in a 'pathway to harm'.
- In an *intervention question*, the scope is to assess the impact of a treatment intentionally introduced in *real-life conditions, in a specific step of a process and under specific conditions compared with the absence of the treatment or the use of other treatments*. For instance, assessment question 6.bis in Appendix A.4 assesses the impact of high-pressure processing in milk processing at a specific step of the food chain and at a specific combination of time and pressure on the probability of listeriosis in the older human population, compared with no treatment or other treatments. For this type of question, the APRIO element *Pathway is key* as it identifies how and when the Intervention (high-pressure processing) is applied to change the consequences (probability of listeriosis per serving milk) of the exposure to the Agent (*Listeria monocytogenes*) on the Receptor (elderly EU human population).

As any other step of the scientific assessment process, problem formulation entails a level of subjectivity due to the view of the assessors, the mandate Terms of Reference and the scope of the assessment. Similarly, the APRIO elements can be characterised differently depending on the situation. However, they should be interpreted as consistently as possible within and across the various EFSA domains, using the definitions given in this guidance, to help harmonise the formulation of EFSA scientific questions.

**Table 2:** Definition of APRIO elements and examples for the different EFSA Panels/units/teams

Notes:

- The first row in the table recaps the definition of the A-P-R-I-O elements given in the text.
- The examples of APRIO elements are meant for generic mandates.
- Some examples show that an element can be either the Agent or the Receptor (or either the Agent or the Intervention), depending on the assessment question or sub-question (see explanation in previous text).

	<b>Agent (A)</b>	<b>Pathway (P)</b>	<b>Receptor (R)</b>	<b>Intervention (I)</b>	<b>Output (O)</b>
	Agent is anything that can cause an effect on a receptor	Pathway refers to any way in which an agent interacts with its receptor. It is the sequence of events leading the agent to cause an effect on the receptor. It can simply cover the route of exposure (typically dietary in EFSA assessments) or represent, for instance, the steps of introduction and spread when assessing a pathogen	Receptor refers to anything that experiences the effect of the agent. The receptor can also experience a secondary consequence to the exposure to the agent (e.g. farmers changing cropping practices as a consequence of the crops being affected by a pest)	Intervention refers to any intentional measure aimed at changing directly or indirectly the exposure and/or the consequence of the exposure to the agent	Output is the form of the answer to the assessment question or sub-question, the result of an assessment process
<b>Examples for different EFSA Panels/Units/Teams</b>					
<b>AHAW (Animal Health and Welfare)</b>	Animal housing/transport conditions Microorganism Slaughter method Invasive animal Parasite Zoonotic pathogen	Wild animals crossing EU borders Contact with infected animal excretion Import/ transport means Ingestion of contaminated feed Transmission (of the agent) among animals Inhalation	Animal (farmed, non-farmed) Diagnostic test (e.g. <i>Brucella</i> fluorescence polarisation assay)	Assessment of the impact of e.g.: <ul style="list-style-type: none"> <li>• import control measures (e.g. quarantine, sampling, testing, hold of products)</li> <li>• farm biosecurity measures</li> <li>• farm hygiene measures (e.g. washing boots before entering the premises)</li> </ul>	Prevalence of a health outcome in the target animal population Probability of spread of the agent Probability of contact with the agent Ratio of the probability of introduction (of the agent) with control measures and at baseline (i.e. without control measures) Performance of a diagnostic test (such as sensitivity or specificity)



	<b>Agent (A)</b>	<b>Pathway (P)</b>	<b>Receptor (R)</b>	<b>Intervention (I)</b>	<b>Output (O)</b>
<b>BIOHAZ (Biological Hazards)</b> <b>BIOMO</b>	Bacteria Viruses Prions Fungi Parasites Plasmid Antimicrobial-resistant bacteria or antimicrobial resistance genes Yeast Bioreactor processing Zoonotic pathogen Chemical control agent/substance/decontaminant (e.g. sanitiser/disinfectant) Biological control agent (e.g. bacteriophage) Physical inactivation technique (e.g. HPP, thermal pasteurisation, UHT)	Contamination along the food chain (e.g. primary production, processing, retail) Transcutaneous transmission (e.g. scratches) Direct contact (e.g. via broken skin or mucous membranes in the eyes, nose or mouth) with saliva or brain/nervous system tissue from an infected animal Environmental contamination	Human (consumer) Animal (farmed, non-farmed, laboratory) Bacteria Viruses Prions Food items Analytical test	Assessment of the impact of e.g.: • intensified sampling/monitoring • chemical, physical or biological interventions/control measures • pathogen growth inhibition measures	Prevalence of the microbiological contamination along the food chain Pathogen load reduction with/without control measures Estimate of the dose of a pathogen Dose–response relationship between food pathogen load and probability of an adverse effect in humans Probability of human illness per serving of a food item Cases of illness in a given population through consumption of food item Prevalence of disease caused by prions in an animal population (e.g. BSE)
<b>CEP (Food Contact Materials, Enzymes and Processing Aids)</b>	Food contact materials (e.g. phthalates, BPA)	Dietary Non-dietary (e.g. dermal, inhalatory) Environmental contamination Contamination along the food chain (e.g. primary production, processing, retail)	Human (consumer, worker) Animal (laboratory) Cell lines, organoids, etc. Analytical test Foodstuff	Assessment of the impact of e.g. a specific migration limit or restrictions in use (e.g. for plasticisers, not to be used with fatty food but aqueous food only)	Exposure/Migration level Prediction of an effect from an in silico/computational model Identification of a causal relationship between the agent and an adverse effect Description of the characteristics of the agent Dose–response relationship between the agent and an adverse effect Health-based guidance value (HBGV)
<b>CONTAM (Contaminants in the Food Chain)</b>	Natural toxins (e.g. mycotoxins) Environmental contaminants (e.g. dioxins)	Dietary Non-dietary (e.g. dermal, inhalatory) (rarely)	Human (general population, all age groups) Farm animals (ruminants, pigs, poultry, rabbits,	Generally not applicable	Identification of a causal relationship between the agent and an adverse effect Estimation of the dietary exposure

	<b>Agent (A)</b>	<b>Pathway (P)</b>	<b>Receptor (R)</b>	<b>Intervention (I)</b>	<b>Output (O)</b>
	Process contaminants (e.g. acrylamide) Metals (e.g. arsenic)		fish), horses, companion animals (cats and dogs) Relevant laboratory animals Relevant cell lines, organoids, etc.		Dose–response relationship between the agent and an adverse effect Health-based guidance value (HBGV) or Margin of exposure
<b>FAF (Food Additives and Flavourings)</b>	Food additives Flavourings	Dietary Non-dietary (e.g. dermal, inhalatory)	Human (consumer, worker) Animal (laboratory) Cell lines, organoids, etc. Analytical test	Generally not applicable	Exposure level Prediction of an effect from an in silico/computational model Identification of a causal relationship between the agent and an adverse effect Description of the characteristics of the agent Dose–response relationship between the agent and an adverse effect Health-based guidance value (HBGV) or margin of exposure
<b>FEEDAP (Additives and Products or Substances used in Animal Feed)<sup>(a)</sup></b>	Feed additives affecting the characteristics of feed: technological additives (e.g. antioxidants, preservatives, silage additives), sensory additives (e.g. colourings, flavourings) Feed additives exerting their function on the animal: nutritional additives (e.g. vitamins, trace elements, amino acids), zootechnical additives (e.g. microorganisms, enzymes) Microorganisms used as feed additives or as production organisms (for producing feed additives)	Deliberate addition during feed manufacturing Dietary (animals and humans, consumers of food of animal origin) Non-dietary (e.g. dermal, inhalatory) (workers) Environmental contamination (manure spread on land, use in freshwater and marine aquaculture)	Feed Human (consumer, worker) Animal (farmed, pets, laboratory) Cell lines, organoids, etc. Non-target species, soil (earthworms, plants), freshwater (e.g. <i>Daphnia</i> , algae, fish) and marine environment (e.g. benthic invertebrates) Analytical test	/	Identification of a causal relationship between the agent and an adverse/beneficial outcome Identification of a maximum safe/ tolerated level in feed Identification of a minimum effective level for the claimed effect on feed (e.g. stability, colour) or on animal performance (e.g. improvement in body weight of the animal) Identification/quantification of residues of toxicological relevance in food of animal origin, establishment of a safe dose and a safe intake Environmental risk assessment (non-target species) Antimicrobial resistance

	<b>Agent (A)</b>	<b>Pathway (P)</b>	<b>Receptor (R)</b>	<b>Intervention (I)</b>	<b>Output (O)</b>
	Mixtures (e.g. feed additives of botanical origin)				Safety of genetic modifications of microorganisms used as feed additives or as production strains Safety of other feed additives Combined exposure to multiple chemicals
<b>GMO (Genetically Modified Organisms) The examples are meant for generic mandates</b>	Genetically modified (GM) maize Hybrid maize (e.g. teosinte)	Environmental contamination	Genetically modified (GM) maize Hybrid maize (e.g. teosinte)	Assessment of the impact of: <ul style="list-style-type: none"> <li>• Field inspection surveys</li> <li>• Removal of wild maize (e.g. teosinte plants)</li> <li>• Clean field machinery</li> <li>• Use shallow tilling practices to control teosinte</li> <li>• Rotate crops</li> <li>• Refuge area</li> </ul>	Occurrence of teosinte in EU areas Ability of teosinte to hybridise with GM maize Exposure of NTOs to GM teosinte Exposure of TOs to GM teosinte Accumulation of GM product in GM teosinte
<b>NDA (Nutrition, Novel Foods and Food Allergens)</b>	Nutrients Substances other than vitamins and minerals added to foods Allergens	Dietary	Human (consumer) Animal (laboratory) Cell lines, organoids, cell-free systems	Assessment of the impact of the time of introduction of complementary feeding into infant diet	Identification of a causal relationship between the agent and an adverse/beneficial effect Dose–response relationship between the agent and an adverse effect Health-based guidance value (HBGV) Dietary reference values
<b>PLH (Pest risk assessment and Commodity risk assessment)</b>	Plant pest <sup>(b)</sup>	A pathway is 'any means that allows the entry or spread of a pest' <sup>(c)</sup> Movement (e.g. trade) of plants, plant parts or plant products Movement of vectors, for vector-borne diseases Movement of soil (with trade or for example with machinery)	Plant (agriculture, forest, ornamental or landscape plants) Ecosystem	Assessment of the impact of e.g: <ul style="list-style-type: none"> <li>• import control measures,</li> <li>• surveillance, eradication,</li> <li>• containment on the risk of entry, establishment and spread</li> </ul>	Pest prevalence in the target plant population in the exporting country Probability of entry of the pest in the EU territory Probability of transfer of the pest to a suitable host in the EU territory Probability of establishment (including climate suitability) of the pest in the EU territory Probability of spread of the pest in the EU territory

	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)
					Potential impact of the pest on agriculture, forestry, landscape and environment in the territory Quantitative comparison of any of the outcomes described above in different scenarios (e.g. with/without Intervention)
<b>PPR (Plant Protection Products and their Residues Pesticides)<sup>(d)</sup></b>	Pesticides (active substances, metabolites, etc.): Fungicide Herbicide Insecticide Microbial	Dermal Dietary Inhalation Environmental contamination	Human (consumer, worker, operator, etc.) Animal (farmed, non-farmed, laboratory) Cell lines, organoids, cell-free systems, computation of all approaches Food products of plant and animal origin Target species Non-target species Ecosystem/matrix e.g. groundwater Soil Analytical test	Assessment of the impact of e.g.: <ul style="list-style-type: none"> <li>personal protective equipment</li> <li>particular dose-reducing spraying technique</li> <li>conditions of use of a pesticide</li> </ul>	Identification of a causal relationship between the agent and an adverse effect Description of the characteristics of the agent Health-based guidance value (HBGV) Combined dietary exposure to multiple chemicals Assessment of dietary exposure estimations for EU consumers related to residues of a pesticide and if needed, possible measures that can adequately ensure consumer protection Environmental risk assessment (non-target species) and fate

(a): E.g. Safety of vitamin D<sub>3</sub> addition to feeding stuffs for fish (EFSA FEEDAP Panel, 2017): <https://www.efsa.europa.eu/en/efsajournal/pub/4713>, Safety for the environment of vitamin D<sub>3</sub> for salmonids (EFSA FEEDAP Panel, 2019): <https://www.efsa.europa.eu/en/efsajournal/pub/5540>, Revision of the currently authorised maximum copper content in complete feed (EFSA FEEDAP Panel, 2016): <https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2016.4563>

(b): A plant pest is defined as 'Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products' (IPPC Secretariat, 2022).

(c): Definition of pathway according to FAO IPPC ISPM 5 (IPPC Secretariat, 2022).

(d): The examples are meant for generic mandates. E.g. Scientific opinion on the toxicity of pyrethroid common metabolites (EFSA PPR Panel, 2022b): <https://www.efsa.europa.eu/en/efsajournal/pub/7582>, Scientific Opinion on the setting of health-based reference values for metabolites of the active substance terbuthylazine (EFSA PPR Panel, 2019): <https://www.efsa.europa.eu/en/efsajournal/pub/5712>, Scientific Opinion of the Scientific Panel on Plant Protection Products and their Residues (PPR Panel) on the genotoxic potential of triazine amine (EFSA PPR Panel, 2020): <https://www.efsa.europa.eu/en/efsajournal/pub/6053>, Statement on the active substance acetamiprid (EFSA PPR Panel, 2022a): <https://www.efsa.europa.eu/en/efsajournal/pub/7031>

### 6.2.1. Foreseen advantages of the APRIO paradigm

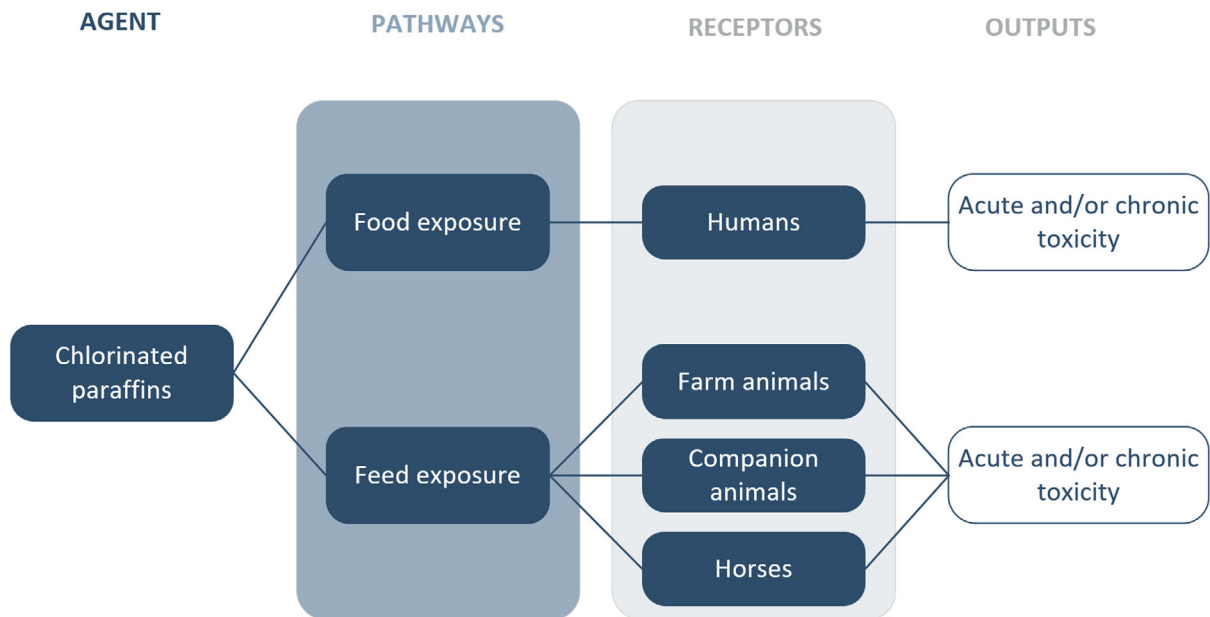
This section summarises the foreseen advantages of the APRIO paradigm for question and sub-question formulation for all types of EFSA scientific assessment and not only for those lacking sectoral guidance. To be confirmed, these advantages will require real-case applications.

- 1) First, APRIO offers a formal and structured framework for question and sub-question formulation that aids, during protocol development, in the definition of the evidence needs and the methods for dealing with such evidence and/or for eliciting expert judgement (Section 7.1).
- 2) Second, APRIO is adaptable to the specificities of the assessment. For instance, in the example of plant health risk assessment in Figure 4, the Intervention is a critical aspect of the assessment, making it appropriate to clarify in the protocol each A-P-R-I-O element. For the assessment in Figure 5 instead, the question is likely to be a causal question that would require specification of the A-P-R-O elements only (no need for the Intervention). Or, in a sub-question on the history of safe use, there may be the need to specify the Receptor, the Agent and the Output (the form of the result) (R-A-O), but not the Pathway and the Intervention, which are irrelevant to this sub-question. Or, for descriptive assessment questions and sub-questions, there is the need to specify only the Outcome. Several examples of APRIO's adaptability are provided in Appendix A.



This is a simplified example of a quantitative pest risk assessment that addresses one of the risk assessment steps, i.e. the entry.

**Figure 4:** Illustrative example of a conceptual model displaying all A-P-R-I-O elements for the various pathways linking the agent to the output



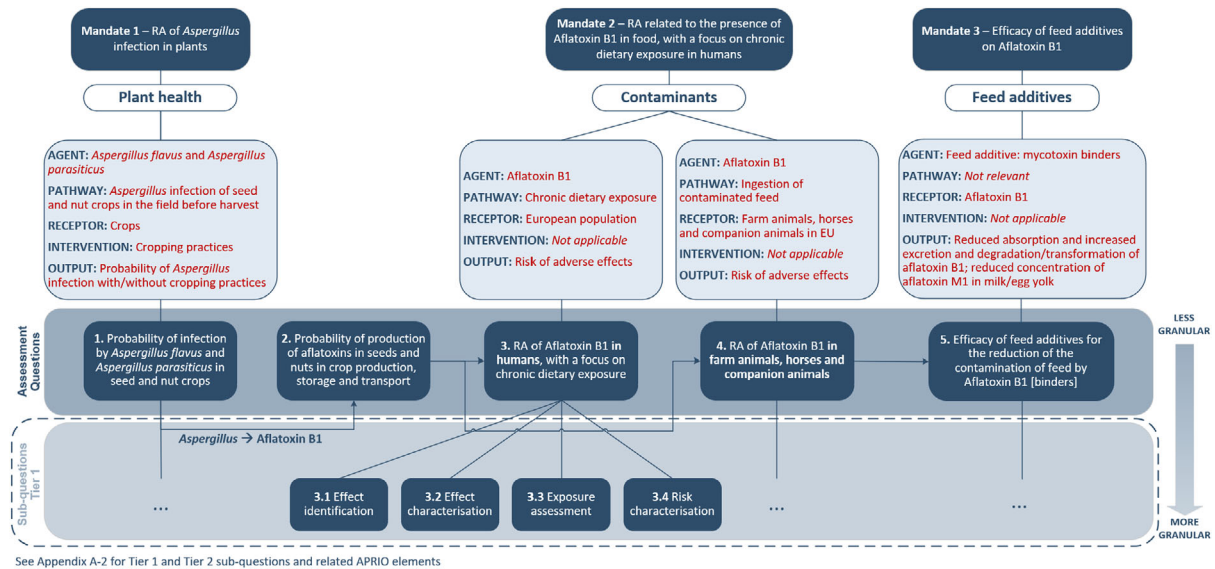
**Figure 5:** Illustrative example of a conceptual model developed using A-P-R-O. In this case, the intervention (I) is not applicable

- 3) Another advantage of APRIO is that it is broadly applicable within and across the various EFSA domains, thereby helping harmonise question and sub-question formulation and foster consistency in protocol development across EFSA scientific assessments.

This is more evident when dealing with mandates implying a pathway that encompasses several EFSA domains addressed by different panels. A hypothetical example for the areas of plant health, contaminants and feed additives is the mycotoxin aflatoxin B1 (Figure 6 and Appendix A.2).

In this case, the biological pathway starts with the fungus *Aspergillus* that can infect seed and nut crops and proliferate under specific climate conditions leading to an increased concentration of mycotoxins. The latter can then affect human health via dietary exposure and animal health through ingestion of contaminated feed. Some feed additives, e.g. clay minerals like bentonite, can act as mycotoxin binders to suppress or reduce the absorption of mycotoxins, promote their excretion or modify their mode of action. These additives exert their effect after ingestion by the animal by reducing the bioavailability of aflatoxin B1 from contaminated feed, subsequently resulting in reduced concentrations of aflatoxin M1 in milk and dairy products. In such a complex biological pathway, APRIO helps read across domains and harmonise question and sub-question formulation.

- 4) Last, owing to its broad applicability, APRIO may overcome the difficulty of implementing the traditional PECO/PICO/PO/PIT acronym for question formulation in systematic review across the multiple, diverse EFSA disciplines (Box 2).



See Appendix A-2 for Tier 1 and Tier 2 sub-questions and related APRIO elements

The three mandates are hypothetical. Further details are given in Appendix A.2. More granular sub-questions are shown by the arrow from top to bottom. RA: Risk Assessment.

**Figure 6:** Example of APRIO as a tool to harmonise question and sub-question formulation across different domains



**Box 2:** The APRIO paradigm in relation to the PICO/PECO/PO/PIT framework

The APRIO paradigm mirrors and extends the scope of the 'PICO' acronym introduced in evidence-based medicine in the 1990s to increase question clarity when reviewing evidence from clinical trials. The acronym, standing for population, intervention(s), comparator(s) and outcome(s) became a standard for systematic review in the healthcare field (Higgins et al., 2022). The efforts to apply these concepts to the environmental and occupational medical research and toxicology fields led to the addition of the 'PECO' framework, with E standing for exposure (EFSA, 2010; Morgan et al., 2018). For PECO (which is more relevant to most EFSA mandates) broader groupings of Outcomes compared with traditional PICO statements have typically been considered. The EFSA guidance on systematic reviews (EFSA, 2010) further elaborated the paradigm by including 'PO' for descriptive questions of populations or systems (such as the ones about prevalence, occurrence, consumption or incidence, for which the population (P) and the outcome of interest (O) need to be specified) and 'PIT' for test accuracy questions, where the acronym stands for population (P), index test (s) (I) and target condition (T). However, EFSA mandates include a broader spectrum of assessments, especially as regards as the variety of the agents considered (e.g. chemical contaminants, food additives, GMOs, food packaging, food ingredients, microorganisms, invasive insects, plant growing conditions) and the nature of the organism that ultimately incurs the harm or benefit of the agent under assessment (e.g. humans, farmed and non-farmed animals, crops, ecosystem services, environment).

Some reasons for a more widely applicable approach than the PICO/PECO/PO/PIT framework at EFSA are noted below:

- The assessments of biological hazards, plant and animal health typically require the explicit reference to the pathway(s) to harm/benefit that identifies possible route(s) leading a receptor to incur the harm/benefit. This aspect, covered by the element P(athway) in the APRIO approach, is missing in the PICO/PECO/PO/PIT framework.
- The 'A' (Agent) and 'R' (Receptor) elements of APRIO have a broader meaning compared with the 'I'/'E' (Intervention/Exposure) and 'P' (population) in the PICO/PECO framework.
- In the PICO framework, the term 'I' (Intervention) is used to address the efficacy of therapies expected to produce a benefit (as in clinical trials), whereas in APRIO it refers to any intentional measure aimed at changing directly or indirectly the exposure and/or the consequence of the exposure to the agent. Notably, in APRIO a question on efficacy (e.g. efficacy of a high-pressure processing technique applied to foods) will not imply any 'I' element, since the agent tested for efficacy would not be a measure introduced in the pathway to harm to prevent (or reduce the consequence of) an exposure. It will rather be an agent able to modify the level of exposure once it has already occurred.
- The output of the assessment question/sub-question (the 'O' element) is not explicitly covered by PICO/PECO/PO framework, leaving the type of answer to the question and/or sub-question unspecified, whereas in APRIO the 'O' makes it explicit the way the answer will be expressed (e.g. probability of introduction of highly pathogenic avian influenza H5N8 virus or reduction in the occurrence of aflatoxin B1 after processing), aiding the clarification of the evidence needs.

**6.3. Definition of the conceptual model**

Within and across the term(s) of reference of a mandate, assessment questions can be related to varying degrees or be completely independent of one another. The same applies to sub-questions.

Clarifying the relationship between assessment questions and sub-questions in the so-called conceptual model is an essential part of problem formulation. The conceptual model illustrates all assessment questions and sub-questions derived from translating the mandate term(s) of reference and provides a description (logical or mathematical) of their relationships. A conceptual model can vary from purely descriptive to mathematical, with all sub-questions expressed as parameters/variables. A pathway to harm represents an example of a conceptual model. The conceptual model can be depicted graphically, in tabular format or via a simple text description.

In the rare situation when a mandate corresponds to one individual assessment question that is not broken down into sub-questions, the conceptual model is not needed.

In most cases, though, a well defined conceptual model is fundamental to plan, in the protocol, the process for answering assessment question(s), i.e.:

- 1) By integrating evidence across sub-questions (Section 7.2).

In this case, the evidence is integrated across the same tier (e.g. Tier 2) sub-questions to address the sub-question they all pertain to, up to the level of the assessment question. In the hypothetical mandate on chlorinated paraffins in food in Figure 2, for instance, the conceptual model depicts

multiple related Tier 2 sub-questions, whose underpinning evidence must be integrated across to address Tier 1 sub-questions up to the human health risk assessment question. For example, the evidence underpinning the two Tier 2 sub-questions on consumption and occurrence is combined to address the Tier 1 sub-question on exposure assessment, and so on.

2) By answering the sub-questions in a logical sequence.

In some cases, evidence integration across sub-questions is not needed as the sub-questions are organised in a logical sequence that requires the answer to the first sub-question to feed into the next, until the assessment question is answered. For instance, in the example in Figure 3, the answer to sub-question 4.1 on the list of relevant pathogens to reduce by thermal pasteurisation feeds into the next two sub-questions aimed at assessing the efficacy of thermal pasteurisation and high-pressure processing for those pathogens (sub-questions 4.2 and 4.3). Then the combination of the answers to those two sub-questions helps answer assessment question 4 on 'Minimum time and pressure combinations to achieve an equivalent efficacy as thermal pasteurisation'. This example also illustrates that the answer to some (sub-)questions can feed into sub-questions that are part of another assessment question or into other assessment questions. For instance, the processing conditions for the treatment of raw milk defined under sub-question 1.2 give input to the evaluation of the minimum high-pressure processing requirements to treat raw milk assessed in assessment question 4.

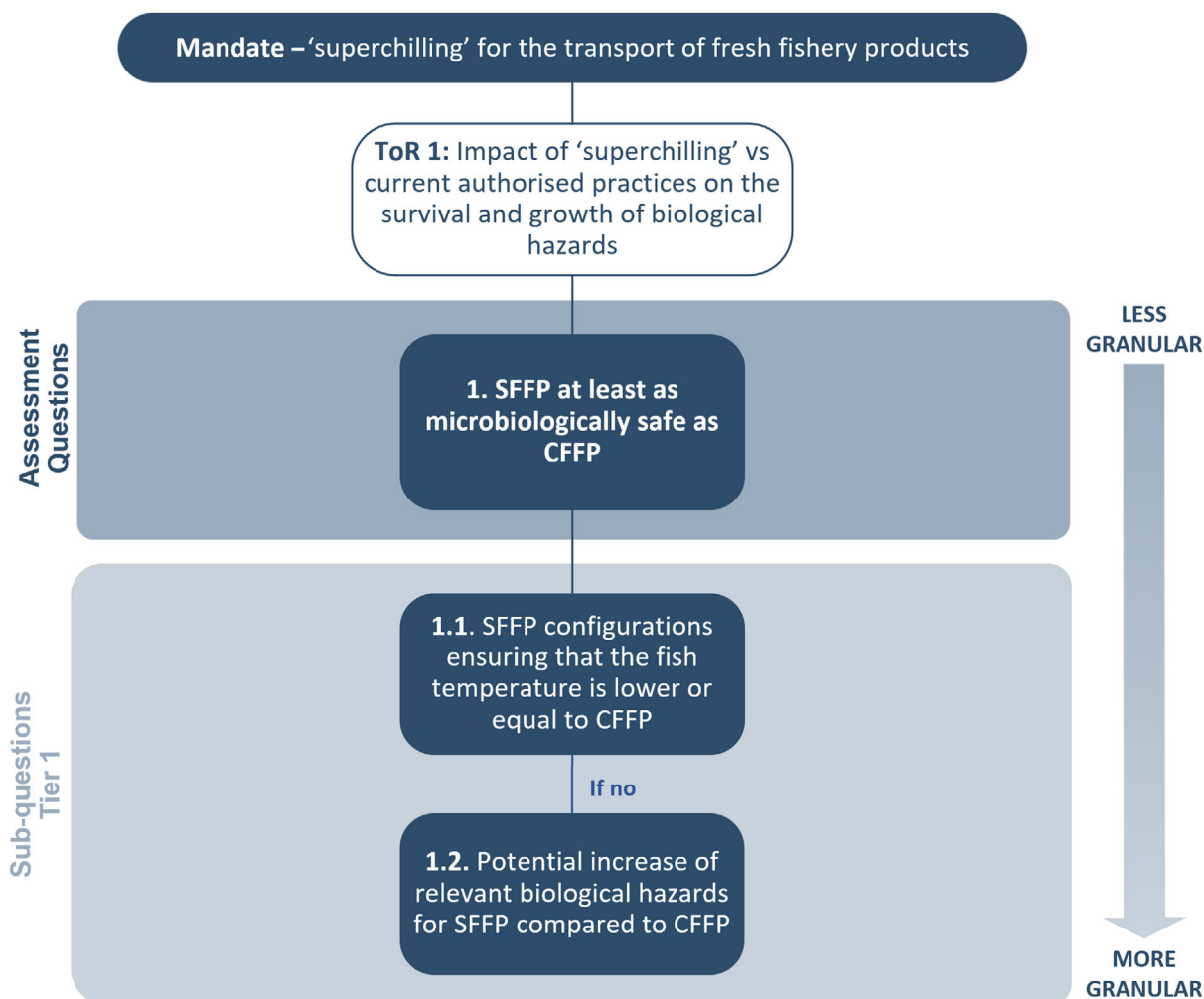
The definition of the conceptual model normally takes place iteratively as the assessment questions and sub-questions are progressively identified and formulated. In some cases (e.g. for 'pathway to harm' types of assessment), the conceptual model can be defined already at the start of problem formulation, as it represents the necessary condition to identify the assessment question(s) and sub-questions(s).

It is recommended to follow the approach of constructing the questions and sub-questions and the conceptual model together before proceeding with the subsequent steps of the protocol and addressing issues related to, for example, data sources and methods for dealing with data. In fact, given that the overall conceptual model informs about how the responses to the questions and sub-questions will be used or combined to address the Terms of Reference, the conceptual model may reveal gaps in the thinking applied to break down the problem. For instance, it may reveal that additional questions/sub-questions may be necessary or that their targeting and definition may need to be changed.

In addition, as the definition of the conceptual model progresses, this may help identify sub-questions that are already known to be of limited relevance and thus not to be included in the assessment. Examples could be:

- A sub-question focusing on a pathway (e.g. of exposure) that is either plausible but considered very unlikely to contribute significantly compared with other known and substantial pathways, or highly implausible.
- A sub-question focusing on an intervention that, while potentially effective, based on already available knowledge is expected to be considerably less effective than other known interventions.
- A sub-question that aims at providing information which is customarily collected in similar assessments, however, it is not relevant for the assessment and it does not contribute to addressing the Term(s) of reference or mandate.

In some cases, the conceptual model can also depict a sort of decision tree where the answer to a sub-question leads to scoping out subsequent alternative sub-questions. An example is the conceptual model represented in Figure 7, where the sub-questions are answered in a logical sequence and the need for sub-question 1.2 is conditional to the answer to sub-question 1.1.



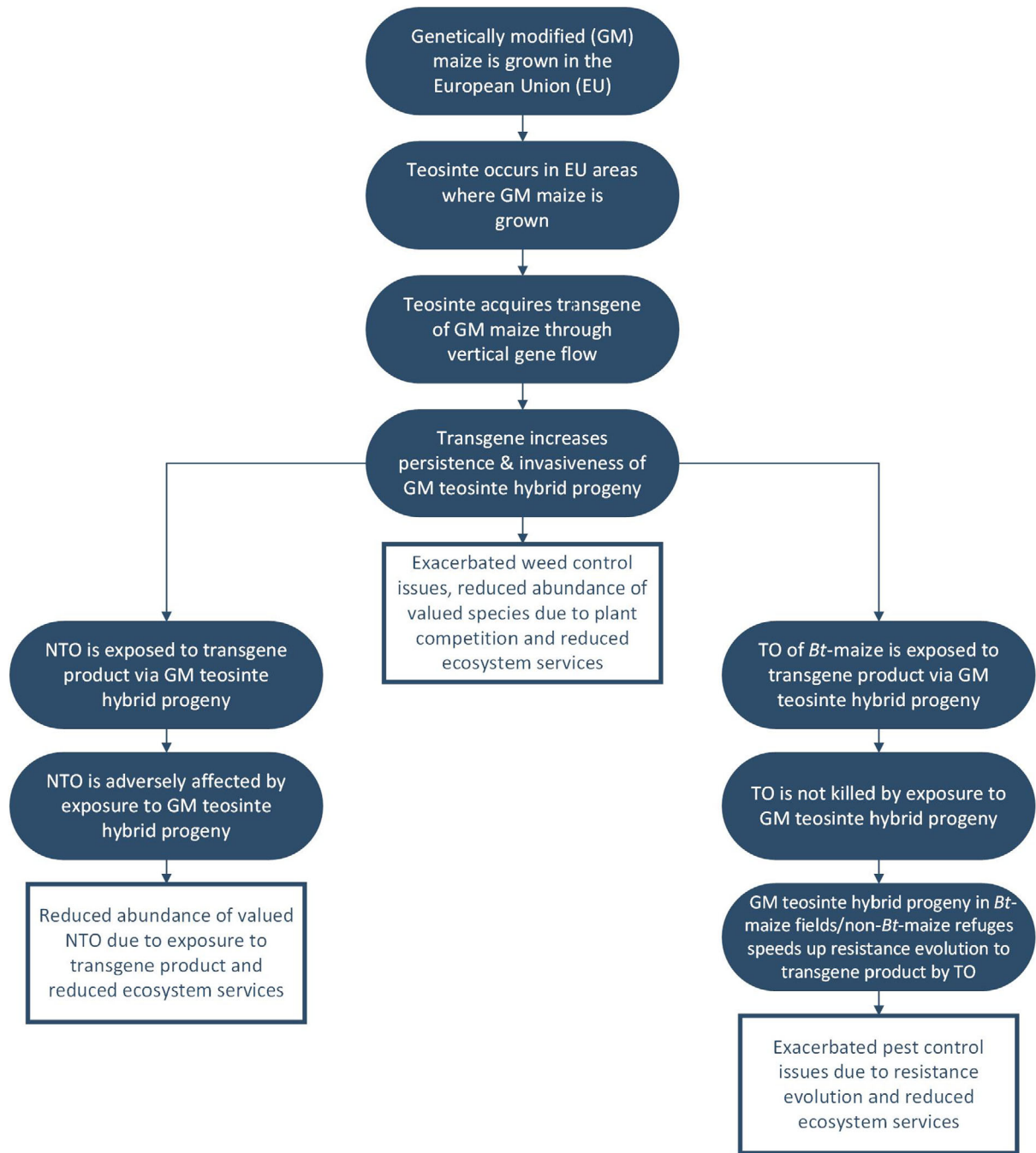
To answer the assessment question, the sub-questions are answered in a logical sequence. More granular sub-questions are shown by the arrow from top to bottom. ToR: Terms of Reference.

**Figure 7:** Superchilled fresh fishery products (SFFP) (illustrative, simplified example) where the aim is to evaluate if the superchilled fresh fishery products stored/transported in boxes without ice are at least as safe (from a microbiological food safety perspective) as the currently authorised practice in boxes with ice (conventional fresh fishery products, CFFP)

Notably, the decision to scope out sub-questions can also happen after the definition of the conceptual model, during the assessment process, when the evidence becomes clearer and this may be anticipated in the protocol by setting some ‘decision moments’ throughout the assessment. For instance, a sub-question on occupational and environmental exposure could be deemed out of scope in a mandate on chlorinated paraffins in food, if not specifically included in the Terms of Reference.

The rationale for excluding a sub-question should be carefully documented and reviewed while planning and/or conducting the scientific assessment. The reasons for scoping something out may be more important than the rationale for scoping something ‘in’.

Identifying the assessment questions and sub-questions embodied in an EFSA mandate and defining the conceptual model can be straightforward (e.g. because the mandate covers a well-established risk assessment paradigm), whereas other times it can be more challenging, resulting in a time-intensive and resource-intensive process. In all cases, the nature of the conceptual model (e.g. graphic, table or text only) and the effort to construct it should be fit-for-purpose and proportionate to the complexity of the specific needs of the term(s) of reference. For example, a conceptual model can simply depict the assessment questions and sub-questions and their hierarchical organisation (Figure 2), or describe a complex pathway that interlinks the assessment questions, sub-questions and relevant events of the pathway (see a simplified example in Figure 8).



Bt-maize: genetically engineered maize that expresses insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt); NTO: non-target organism; TO: target organism.

**Figure 8:** Example of a conceptual model based on the pathway to harm approach for the cultivation of genetically modified (GM) maize in EU areas infested with teosinte, which interlinks the assessment questions, sub-questions and relevant events of the pathway (simplified from EFSA GMO Panel, (EFSA, 2022))

## 6.4. Definition of the relative priority of the assessment question(s) and sub-questions

Within a mandate, some assessment questions and sub-questions can be deemed as 'higher priority' compared with others based on some critical considerations including:

- 1) The possible impact of the assessment question or sub-question on the overall conclusions, i.e. if it ultimately 'drives' the outcomes (e.g. a dominant pathway, critical position of a parameter in a model or a highly variable parameter).
- 2) When the answers to individual assessment questions or sub-questions might be useful for answering multiple Terms of Reference or assessment questions.
- 3) The level of novelty of some of the APRIO elements characterising the assessment and/or the entire question or sub-question (e.g. a newly emerged pathogen and/or pathway).
- 4) The level of novelty of the type of evidence that is likely to be considered.
- 5) The scientific controversy (sustained disagreements) on answers to the assessment question or sub-question, which requires explicitly estimating the level of associated uncertainty.

Often the assessment questions and/or sub-questions identified as higher priority require a greater effort to be answered. This includes not only the burden of the process for data collection, appraisal/validation, synthesis, integration and computation and analysing uncertainty, but also the extent to which the results can be expected to require detailed communications to and/or consultations with various stakeholders.

The justification for defining one or more assessment question(s) and sub-question(s) as a higher priority (including any method applied for identifying them, e.g. sensitivity analysis)<sup>14</sup> should be documented in the protocol (see Annex A to this guidance). Doing so provides transparency in decision-making by the working group/panel that carries out the scientific assessment. The documentation may be used to justify changes in resource requirements (e.g. the need to include additional specific expertise) or time if the scientific assessment is more complex than expected during the negotiation of the mandate.

## 7. Methods' specification

The second step in protocol development consists of specifying the evidence needs and the methods for conducting the assessment (Box 1 in Section 5).

The evidence needs are concerned with, for example, what studies to look for, what data to collect or what new empirical evidence to generate. Notably, defining the evidence needs does not necessarily imply the availability of the best possible data, and this must be considered when planning data collection and analysis.

As for the specification of the methods, this implies defining (i) the methods for answering individual (sub-)questions and, if needed as indicated in the conceptual model (see Section 6.3), (ii) the methods for integrating evidence across (sub-)questions.

### 7.1. Approaches to answering individual (sub-)questions

In EFSA mandates, there are five possible approaches that can be applied to answering individual sub-questions or assessment questions, when the latter are not split into sub-questions. All these approaches can be undertaken by EFSA or outsourced to external parties.

This section provides an overall description of these five approaches and the steps within each of them that shall be carefully considered when developing a protocol. Examples and suggestions on how to plan each step – with low and high extent of planning – are given in Annex A to this guidance (i.e. template for protocols).

The five approaches for answering (sub-)questions are:

- 1) Using evidence from scientific literature and study reports.
- 2) Using data from databases other than bibliographic databases.

<sup>14</sup> See e.g. the protocol of EFSA's 'Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)' (Annex A to the revised guidance, available here: <https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2023.EN-7982>).



Overall, the first and the second approach represent the case when a (sub-)question is answered *using already existing data* as opposed to generating empirical evidence *ex novo* (fifth approach). In this guidance they are described separately to emphasise the fact that they follow two different stepwise processes that require specific planning in the protocol (see next sections and Annex A).

- 3) Using expert judgement informally collected or, especially for quantitative assessments, elicited via (semi-formal or formal) expert knowledge elicitation (EKE) processes.
- 4) Using mathematical/statistical models. These are simplified representations of reality, constructed to gain insights into selected attributes of a physical or biological system and their relationships. This relationship is usually stated via a mathematical construct, for example equations.
- 5) Generating empirical evidence *ex novo* by carrying out primary research studies. When applied, in most cases this approach is commissioned by EFSA to external parties.

These approaches can be combined to answer the same (sub-)question. For instance, a sub-question can be addressed using evidence from scientific literature and evidence from non-bibliographic databases. Or, within the same EFSA mandate, some sub-questions can be answered using one approach and others using another approach. Hence EFSA protocols can contain 'nested' protocols for specific sub-questions. For instance, there can be a nested protocol for a systematic review of a sub-question or for a formal EKE process for some others. The extent of planning of the methods can also vary by step of the approach. For example, there can be an extensive plan of the methods for data collection and a generic description of the ones for evidence synthesis.

Depending on mandate requirements clarified before starting protocol development, each approach can be conducted with a different extent of rigour or extensiveness (e.g. narrative vs systematic review, or informal collection of expert judgement vs formal EKE). Regardless of the degree of rigour or extensiveness, common and best practice in all these approaches implies: (i) a clear formulation of the assessment (sub-)question, (ii) the use of the appropriate, multidisciplinary expertise for making all decisions for planning and implementing each approach and related steps, and (iii) the documentation of the approach applied.

In addition, every approach entails a level of uncertainty that stems from: (i) the limitations in the evidence and (ii) the methods applied (Section 7.3). The most appropriate way to deal with uncertainty is to characterise/document it and try and minimise all individual sources of it in every step of these approaches and then, if feasible, assess their combined impact on the outcomes (next sections and Section 7.3).

### 7.1.1. Using evidence from scientific literature and study reports

An approach to answering individual (sub-)questions consists of using evidence from scientific literature and study reports submitted to EFSA by stakeholders.

Scientific literature is typically represented by primary research studies available, in the form of bibliographic records, in bibliographic databases or other sources.<sup>15</sup> In most cases, this type of literature is retrieved by EFSA via literature searches. However, there can be situations when scientific literature is retrieved and submitted to EFSA by stakeholders like Member States, contractors, non-

<sup>15</sup> Relevant definitions (also reported in the Glossary):

- Primary research study: the original study in which data were produced. The term is sometimes used to distinguish such studies from secondary research studies (i.e. reviews) that re-examine previously collected data.
- Secondary research study: a review (e.g. a systematic review) that re-examines existing data from one or more primary research studies.
- Bibliographic record: the information used to identify a full-text document. Typically, this includes the author's name(s), publication date, the title of the document and publication details of the document (e.g. the name, volume and page numbers of a scientific journal, or the URL and publisher of a website).
- Full-text document: a document (e.g. journal article, dissertation) in which details of one or more studies are reported.
- Bibliographic database: a searchable database which contains summary records (often with abstracts and sometimes linking to full-text documents) of scientific literature and, in some cases, provide indexing terms (e.g. subject headings) to assist in searching
- Sources of evidence from scientific literature other than bibliographic databases: any repository of information other than a bibliographic database that contains (primary and secondary) research studies in the form of bibliographic records (which include bibliographic citations and, in many cases, abstracts and full-text documents). Examples include internet search engines which access information in a variety of formats, internet pages, online journals and their tables of contents, and reference lists within full-text documents.



governmental organisations (NGOs) or industries. For instance, scientific literature can be included in a dossier in support of an application and be used by EFSA to address a generic mandate, if relevant. Another example is when it is yielded by EFSA through calls for data.

Evidence from scientific literature and study reports often comes in the form of aggregated data for a group of subjects (e.g. treatment groups in an experiment or exposure groups in an observational study).<sup>16</sup>

Answering a (sub-)question using evidence from scientific literature and study reports can be summarised into the following stepwise process:

- 1) Formulating the (sub-)question (in this guidance, covered under 'problem formulation').
- 2) Defining the evidence needs and retrieving evidence. This implies defining the sources of evidence and, for scientific literature, the search strategy,<sup>17</sup> possibly using the search string that best balances sensitivity and precision (Higgins et al., 2022) and using multiple sources of evidence. Validation of the search strategy to understand the impact of missed studies can also be included.
- 3) Selecting studies for inclusion according to specific eligibility criteria, by applying an approach that helps reduce uncertainty (e.g. broad eligibility criteria for study selection and/or study selection done in parallel by two independent reviewers).
- 4) Extracting data from the included studies, possibly using a data model<sup>18</sup> and trying to reduce the uncertainty (e.g. by specifying for each variable the measurement unit used in each study, by validating data extraction done by an expert with a reviewer). This step can be swapped around with the next one.
- 5) Appraising evidence, i.e. assessing the uncertainty related to the evidence at individual study level (due to limitations in the design and conduct of each individual study), and at the level of body of evidence (e.g. due to data gaps, publication bias, unexplained inconsistencies across studies' results). Again, trying to reduce uncertainty, for example using standard critical appraisal tools and involving multiple independent reviewers. This step can be swapped around with the previous one.
- 6) Synthesising/integrating evidence accounting for uncertainty in: (i) the individual studies, (ii) at the level of the body of evidence and, when appropriate, (iii) related to the methods (see Sections 7.2 and 7.3).
- 7) Analysing any further sources of uncertainty not addressed earlier in the process (if feasible) and expressing the overall certainty in the conclusions quantitatively if possible (EFSA Scientific Committee et al., 2018b) (see Section 7.3).

### 7.1.2. Using data from non-bibliographic databases

(Sub-)questions can also be answered using data from non-bibliographic databases. These are searchable databases containing a collection of digital information or data, typically organised according to a pre-defined structure and accompanied by metadata explaining the methods used to collect and analyse the data. Examples of non-bibliographic databases are EFSA's Comprehensive food consumption database<sup>19</sup> or Eurostat's database.<sup>20</sup>

The data extracted from this type of databases can be individual or aggregated. Compared with evidence from published and unpublished scientific literature, some non-bibliographic databases can provide data representative at the EU level (e.g. Eurostat).

<sup>16</sup> Relevant definitions:

- Aggregated data: information resulting from the synthesis of individual data (e.g. mean exposure in a treatment group, standard deviation of the observations in a group, etc.).
- Individual data: information collected at the level of the finest unit on which variables are measured (e.g. exposure observed on each individual belonging to a study). They cannot be further 'disaggregated'.

<sup>17</sup> Search strategy not needed in case of unpublished study reports submitted by stakeholders to EFSA.

<sup>18</sup> A data model is a theoretical model that organises element of data and describes how they relate to one another and to the properties of real-world entities they represent. A data model explicitly determines the structure of the data and is used to store and/or exchange harmonised information. For example, EFSA Standard Sample Description 2.0 (SSD2) (EFSA, 2013) was developed to harmonise at EU level the collection of analytical data on chemical substances and microbiological agents in different matrices of non-human nature. The logical model of SSD2 includes a combination of three main groups of terms and characteristics: (1) data elements, definition and structure, (2) controlled terminologies and (3) business rules to ensure the validity of the information supplied.

<sup>19</sup> <https://www.efsa.europa.eu/it/data-report/food-consumption-data>

<sup>20</sup> <https://ec.europa.eu/eurostat/data/database>

Same as for evidence from scientific literature, these types of data are typically actively retrieved by EFSA but can also be provided through, for example, dossiers or calls for data. The latter can be ad hoc calls (e.g. on food processing conditions),<sup>21</sup> continuous data collections (e.g. EFSA's call for continuous collection of chemical contaminants occurrence data in food and feed)<sup>22</sup> or regular data collections required by the legislation (e.g. for zoonoses, antimicrobial resistance and foodborne outbreak data).<sup>23</sup> In this guidance, all these situations are deemed different from those when primary research studies are commissioned by EFSA according to a pre-defined study protocol (which falls under the fifth approach; Section 7.1.5).

When using this approach for answering a (sub-)question, the following steps are needed:

- 1) Formulating the (sub-)question (in this guidance, covered under 'problem formulation').
- 2) Defining the data needs and collecting data. This implies defining the data model and the database(s) or the data provider(s), trying to reduce uncertainty and any biases arising when data are solicited from external parties. Uncertainty is reduced, for instance, by selecting datasets that are representative of the target population or by defining upfront the variables – including their format – needed to answer the sub-questions.
- 3) Checking and validating data trying to use sound methods that better fit the context. For instance, this is achieved using plans for checks that maximise the accuracy of the results minimising the efforts, typically identifying mistakes that can be more influential.
- 4) Synthesising/integrating evidence accounting for uncertainty in the evidence and, when appropriate, in the methods (see Sections 7.2 and 7.3).
- 5) Analysing any further sources of uncertainty not addressed earlier in the process (if feasible) and expressing the overall certainty in the conclusions quantitatively if possible (EFSA Scientific Committee, 2018b) (see Section 7.3).

### 7.1.3. Using expert judgement

Another approach to answering (sub-)questions is by using expert judgement, which can be informally collected, for example, through collegial discussion within a working group or elicited via semi-formal or formal expert knowledge elicitation processes (EFSA, 2014; EFSA Scientific Committee, 2018a).

Semi-formal and formal EKE is primarily implemented for (sub-)questions aimed at estimating a quantity/rank of interest whose value is not directly substantiated by primary studies. These assessment questions/sub-questions usually relate to certainty of statements, estimations of parameters (any numeric values) and prioritisations (ranking of alternatives, as done, for example, on risk by viruses in food (Cefas, 2016)). In these cases, the elicitation process is aimed not only at providing a reliable estimate of the quantity but also at allowing for a quantification of the uncertainty in the estimate.

Semi-formal and formal EKE enables experts to use existing evidence for their judgements, to reduce as much as possible heuristic biases and the influence of group effects (e.g. groupthink) in a way that is transparent, reviewable and repeatable.

Semi-formal and formal EKE processes (EFSA Scientific Committee et al., 2018a) follow four main phases (see Annex A to this guidance for further details) (EFSA, 2014):

- 1) Problem definition (i.e. problem formulation in this guidance): identification of the assessment question(s) and/or sub-questions for which EKE is needed, definition of the risk assessment model, collation of the evidence and discussion of limitations, etc.
- 2) Pre-elicitation phase: frame of the EKE questions, selection of the experts, selection of the method to elicit the judgement (e.g. behavioural, mathematical aggregation or mixed), provision of the evidence dossier.

<sup>21</sup> E.g. in the BIOHAZ panel opinion on the high-pressure processing of food, data were collected via a questionnaire (EFSA BIOHAZ Panel, 2022, Appendix A) addressed to competent authorities, establishments using HPP and equipment providers. Questions were related to the products being treated using HPP along with their processing conditions, evaluations conducted and potential food safety problems.

<sup>22</sup> EFSA call for continuous collection of chemical contaminants occurrence data in food and feed is updated every year. The call from 2023 is available here: <https://www.efsa.europa.eu/en/call/call-continuous-collection-chemical-contaminants-occurrence-data-food-and-feed-2023>. It shows all information on the chemicals of interest and on how to submit data.

<sup>23</sup> The zoonoses, antimicrobial resistance and foodborne outbreak data are collected based on a legislative requirement that obliges Member States to report these data on an annual basis (i.e. Zoonoses Directive 2003/99/EC).

- 3) Elicitation phase: training of the experts on the selected EKE methodology, execution of the EKE according to the protocol, including collection and synthesis of judgements and reasonings, and communication of the results, including the uncertainties.
- 4) Post-elicitation phase: documentation.

In addition to the cases described, semi-formal or formal EKE can also be used for: (i) problem formulation (e.g. sensitivity analysis for selecting higher priority assessment questions and sub-questions; Section 6.4) and (ii) synthesising and integrating evidence accounting for uncertainty (e.g. the uncertainty related to the evidence; Section 7.3).

#### 7.1.4. Using mathematical/statistical models

For some EFSA assessments, particularly those requiring a pathway to harm/benefit or part of it, a mathematical/statistical model may be necessary to address individual (sub-)questions or the entire mandate/assessment question.

The use of a mathematical/statistical model is conditional to the availability of input data of appropriate validity and the understanding of the system investigated (e.g. understanding of the pathways, processing or pathogen/host specificities). Input data can come from scientific literature, study reports submitted to EFSA by stakeholders, non-bibliographic databases, expert elicitation and primary research studies.

When using mathematical/statistical models, the first thing to define, already at the level of problem formulation, is the criteria for selecting an existing model or setting a new one. All other aspects of the assessment process and related protocol are based on the structure and type of model that will be used.

This approach is particularly valuable when there is the need to evaluate/quantify the impact of some influential variables (e.g. intervention measures) or alternative conditions (e.g. storage of foods under baseline and alternative conditions) or when estimating the association between a public health outcome and the exposure to a potential hazard. When a mathematical formalisation is adopted, this allows representing conditions and/or scenarios that do not (yet) necessarily occur in real life, thereby making it possible to draw conclusions on, for example, the impact of intervention measures before they are implemented. For instance, modelling made it possible to predict the impact of prolonging the shelf life of eggs on public health (EFSA BIOHAZ Panel, 2014).

In EFSA's scientific assessments, a model taking the form of a mathematical construct can have the purpose of predicting an outcome of interest (e.g. the presence/absence of an adverse/beneficial effect or the load of pathogenic microorganisms in various stages of processing, storage and distribution). A model can even be used to estimate the surface temperature of foods under baseline and alternative conditions; the outcome of which is then used to estimate the growth of pathogens using another model.<sup>24</sup> A model can also be aimed at explaining a phenomenon (e.g. how a pest spreads in a specific environment under the influence of specific factors such as humidity, temperature).

The use of a mathematical/statistical model to answer a (sub-)question implies the steps illustrated below. Sometimes, they are implemented in a slightly different order depending on the type of model and (sub-)question to address:

- 1) Formulation of the (sub-)question(s) that will be addressed by the mathematical/statistical model (in this guidance, covered under 'problem formulation'). In many cases, mathematical/statistical models are used to address the full conceptual model and not only individual sub-question(s).
- 2) Identification/development of existing or new model(s) and related description (e.g. equations, formulas, estimators to use for the parameters).
- 3) Definition of the evidence needs to quantify the parameters/variables in the model.
- 4) Model verification and calibration (i.e. assessment that the model correctly implements the conceptual model using some evidence).
- 5) If newly developed, or adapted, validation of the model (i.e. assessment of the model's ability to represent the real phenomenon investigated).
- 6) Uncertainty and sensitivity analysis.

<sup>24</sup> E.g. BIOHAZ examples: Fish tubs opinion (EFSA BIOHAZ Panel, 2020): <https://www.efsa.europa.eu/en/efsajournal/pub/6091>. Here temperature and growth have been modelled. Superchilling opinion (EFSA BIOHAZ Panel, 2021): <https://www.efsa.europa.eu/en/efsajournal/pub/6378>. Here only temperature was modelled; no need for modelling the growth of pathogens.

As for the previous approaches to answering (sub-)questions, preference is given to methods that better fit the data and the problem to minimise the related uncertainties. Examples are represented by choosing the estimators that maximise precision or the type of mathematical formalism – e.g. deterministic vs probabilistic – that better fits the context. In some cases, the uncertainty in the methods can be addressed quantitatively. For instance, the benchmark dose (BMD) approach requires fitting several families of models to the data and averaging over those better performing, based on a pre-defined indicator (EFSA Scientific Committee, 2022).

### 7.1.5. Generating empirical evidence *ex novo*

(Sub-)questions are answerable also by generating new data via primary data collections, which can be carried out using a variety of study designs and techniques such as field observations, questionnaires and experiments.

In primary data collections, planning the methods for the study upfront in a protocol is a well-established, common practice. How to plan the methods for a study (e.g. methods for data collection, validation and analysis) can significantly vary depending on the type of primary study. Describing it goes beyond the scope of this guidance and it is recommended to refer to specific guidelines (e.g. OECD, EFSA).

For chemical risk assessments for instance, Integrated Approaches to Testing and Assessment (IATA) are the preferred methodology. The IATA should include the integrated analysis of existing information coupled with the generation of new information using testing strategies. OECD has developed a project including a variety of case studies as well as an IATA template that could facilitate the implementation of this approach.<sup>25</sup>

At EFSA, the conduct of primary research studies is usually commissioned to external parties and done according to a pre-defined study protocol.

## 7.2. Synthesising and integrating evidence within and across (sub-) questions

This section provides some general considerations on evidence synthesis and integration. Examples of how to plan them with low and high extent of planning are given in Annex A (i.e. the template for protocols).

In this guidance, *evidence synthesis* is defined as the process of summarising similar evidence. This can be, for instance, evidence from similar populations, study designs or evidence streams (e.g. human data). Defining what is 'similar' is subjective and depends on the evaluation of the assessors. Evidence synthesis can be performed on individual as well as on aggregated data from studies with a level of heterogeneity that does not affect the summary and the interpretation of the results.

*Evidence integration* is the process of combining evidence that is *diverse* (for instance, evidence from different study designs or streams of evidence). Evidence integration can happen:

- within a sub-question, for example, when combining evidence from studies of distinct designs (e.g. experimental and observational for effect identification). In this case, additional uncertainties to account for are due to e.g. the different ability to represent real-life conditions (e.g. for observational vs. experimental settings) or different ability to control for confounding factors (e.g. for randomised controlled trials vs prospective cohort studies).
- across sub-questions, for example, when aggregating evidence from sub-questions on effect identification on different species to derive conclusions on an effect for humans. Evidence integration across sub-questions can sometimes be a relatively simple process that relies on the use of mathematical equations and related calculations (e.g. when combining sub-questions on occurrence and consumption to derive an estimate of the exposure to a substance). In other cases, combining the evidence pertaining to multiple sub-questions is challenging as it can imply extrapolating conclusions from one line of evidence (e.g. *in vitro* studies) to the target population (e.g. humans). In these cases, several considerations are required beyond the assessment of the validity, precision and relevance of the individual studies. In the previous example, for instance, a model may be required for the translation of

<sup>25</sup> EFSA PPR panel opinion on Development of Integrated Approaches to Testing and Assessment (IATA) case studies on developmental neurotoxicity (DNT) risk assessment (EFSA PPR Panel, 2021): <https://www.efsa.europa.eu/en/efsajournal/pub/6599>.

the internal to the external dose. These challenges translate into uncertainties that must be accounted for and, if possible, quantified.

- across assessment questions, depending on how the mandate term(s) of reference are split (Sections 6.1 and 6.3).

A well described conceptual model is essential to define the process of evidence integration.

The methods for evidence synthesis and integration can be qualitative (e.g. narrative description of the evidence), quantitative or semi-quantitative.

In quantitative approaches mathematical/statistical models are used. An example of quantitative evidence synthesis is a meta-regression for dose-response. In this context the certainty (and consequently the weight) of each study is quantified looking at the precision of its estimates and possibly the variability across studies and the individual study validity.

Semi-quantitative approaches provide an intermediary level between qualitative and quantitative risk assessment (e.g. risk ranking based on scoring categories (EFSA BIOHAZ Panel, 2013)). Compared with qualitative approaches, they are more consistent and rigorous for assessing and comparing risks and risk management strategies and help avoid some of the ambiguities that a qualitative risk assessment may produce. They do not require the same amount of data as quantitative approaches, which means they can be applied to risks and strategies where precise data are missing (FAO and WHO, 2021).

Planning the methods for evidence synthesis and integration in a protocol can be very challenging, especially when there is very little information upfront on the amount and degree of heterogeneity of the evidence and/or on the availability of aggregated versus individual data. A stepwise approach can be indicated in the protocol, with alternative methods foreseen and 'decision moments' planned as the assessors become familiar with the available evidence.

### 7.3. Uncertainty analysis

Ideally, the approach to analysing uncertainty should be planned for each step of the scientific assessment and described in the method specification section of the protocol. However, it is acknowledged that this aspect is one of the most challenging to define upfront, which depends on several factors, including data availability, the established practices in the field and the degree of detail required by the protocol at hand (Section 4).

This section summarises some considerations on uncertainty analysis based on EFSA Scientific Committee guidance documents (EFSA Scientific Committee, 2018a,b). Annex A to this guidance provides some examples of how to plan uncertainty analysis. Further examples could be added as experience grows within EFSA (see Section 11).

Uncertainty is defined by EFSA as 'all types of limitations in the available knowledge that affect the range and probability of possible answers to an assessment question' (e.g. for an AQ or SQ). It can be related to (i) limitations in the evidence (e.g. heterogeneity, degree of relevance, degree of internal validity and/or precision) and (ii) the methods used throughout the assessment, including any assumptions made throughout the assessment process (EFSA Scientific Committee, 2018a,b).

For instance, when using evidence from scientific literature, the uncertainty related to the evidence is the one at individual study level (due to limitations in the design and conduct of the individual studies like confounding factors or inaccuracy of the methods to measure the exposure and the outcome) and at the level of the body of evidence (e.g. publication bias, unexplained inconsistency of results). For data extracted from non-bibliographic databases, the main source of uncertainty at the level of a single database is the one due to the limitations in the methods for data collection. Additional sources of uncertainty can stem from (list not exhaustive) the lack of representativeness of the data for answering a particular question, potential confounders in case of observational data, inconsistencies between data from different databases, different levels of granularity of the available information for different variables or lack of harmonisation of methods for data generation within and across different databases.

The uncertainty related to the methods is the one due to, for example, the methods applied for study selection, for data collection (including when data are collected by external parties), for the statistical analysis, or, when doing an EKE, for fitting the individual distributions elicited from the experts and aggregating them.

Uncertainty is inherent in each step of the scientific assessment process (from problem formulation to drawing conclusions) and should be considered (and, ideally, minimised) *step by step when conducting an assessment and not only at the end of the process*. For instance, by accounting for the



uncertainty in the evidence when doing evidence appraisal and synthesis/integration, and by choosing methods that are sound and minimise as much as possible inaccuracy and imprecision (e.g. the most precise/sensitive search string or the most efficient unbiased estimator). One way to account for uncertainty in the methods is sensitivity analysis (EFSA Scientific Committee, 2018b). In addition, an attempt should always be made to quantify the combined impact of identified sources of uncertainty (overall uncertainty). If the quantification of the overall uncertainty is not feasible, it should be acknowledged and the conclusions should be reported as conditional on the unquantified uncertainties.

## 8. When to consider an EFSA protocol 'final'?

Even though the process for protocol development is iterative and may require significant time, EFSA's Standard Operating Procedures clarify that such process should end *before the start of the scientific assessment*, with the approval of a final version of the protocol, which will not be necessarily highly detailed (Section 4). In the exceptional case when protocols need to be developed in phases, each phase requires finalisation and approval before being implemented.

Once finalised, the version is checked for compliance with the extent of planning defined during the negotiation phase of the mandate (conditionally to any possible constraints that may reduce the possibility of a highly detailed protocol; Section 4), formally approved, stored and published as described in the previous section.

## 9. Amendments to the protocol

An amendment to the protocol is a *change* to the *final* and approved plan (or phase of the plan, when protocols are developed in phases) that occurs *once the implementation phase has already started*.

A protocol amendment should not be confused with different versions of the protocol that can be generated when a draft protocol is published for consultation and revised before the start of the implementation phase. An amendment is also different from the process of refining a standard protocol for recurrent mandates (EFSA FAF Panel, 2023).

Amendments to the protocol must be documented and reported and the reasons for the change explained.

In some cases, the reasons for amending the protocol are pragmatic, i.e. it is not possible to maintain the original plan due to time and/or resources constraints. This can happen, for example, if the amount of evidence identified is higher than expected, or when in the protocol it was planned to do evidence appraisal in parallel by two independent reviewers and then it is done by one only. However, an amendment does not necessarily imply a simplification of the original plan. There are cases when more advanced/sophisticated methods, compared with the ones planned, are implemented.

Ideally, an attempt should also be made to assess the impact of the protocol amendments on the final output (for instance in EFSA, 2020b).

In addition, in some cases there may be the need for agreeing with the mandate requestor on the amendments to the protocol.

It is suggested that the amendments to the agreed plan are reported in the version of the protocol that is published together with the final output. Examples of the recommended format are given in Annex A to this guidance document.

## 10. Link between the protocol and the data and methodologies section of the final output

When EFSA protocols are published at the end of the scientific assessment process, they can be published as standalone documents or as appendices of the final output (e.g. a Scientific Opinion). To ensure clarity and the appropriate link between the protocol and the final output, the following approach is recommended:

- The 'data and methodologies' section of the final output cross-references the protocol for all methods that were already detailed there. This is to avoid unnecessary repetitions.
- The amendments to the protocol, their reason and, if possible, an assessment of their impact are reported in the protocol that is published with the final output and properly outlined to distinguish them from the original plan. Examples are given in Annex A to this guidance.



- For those sections in the protocol where the extent of planning was 'low', the corresponding section in the 'data and methodologies' section in the final output must be detailed (i.e. without cross-references to the protocol).

## 11. Conclusions and Recommendations

This guidance document provides a harmonised framework for developing protocols for EFSA generic scientific assessments that replaces the 'Draft framework for protocol development for EFSA's scientific assessments' published in 2020.

For problem formulation (step 1 of protocol development), the guidance applies a formal and structured paradigm for translating the mandated Terms of Reference into scientifically answerable assessment questions and sub-questions (APRIO: Agent, Pathway, Receptor, Intervention and Output). Owing to its cross-cutting nature, this paradigm is considered adaptable and broadly applicable within and across the various EFSA domains and, if applied using the definitions given in this guidance, is expected to help harmonise the problem formulation process and outputs and foster consistency in protocol development. APRIO is also considered to overcome the limitations of existing frameworks (e.g. PICO/PECO). For all these reasons, although not mandatory, the use of APRIO is recommended.

For Step 2 of protocol development – the definition of the evidence needs and the specification of the methods for the assessment – the guidance provides an overview of the approaches for answering (sub-)questions and some considerations about evidence/synthesis integration and uncertainty analysis. A 'template' for protocols (Annex A) complements the guidance by giving examples of how to implement these aspects in EFSA protocols and guiding the user step by step through the process of planning an EFSA scientific assessment.

**The approach proposed in this guidance document is expected to be applied with flexibility to accommodate the requirements of each specific EFSA mandate and support a fit-for-purpose and efficient risk assessment process.** This implies adapting to each mandate the level of detail of the protocol as well as the approach to disseminating the protocol. Depending on the needs, the latter may or may not include consulting external parties on a draft version of the protocol. All these aspects should be clarified with the mandate requestor before starting the scientific assessment process.

For mandates addressing recurrent scientific questions, a standard protocol can be developed and reused over time upon minor, ad hoc adaptations, when needed, depending on the specific question.

EFSA Panels are encouraged to incorporate the methodology proposed in this guidance in their scientific assessments in a fit-for-purpose approach and ensure alignment with possible future updates. When domain-specific guidance exists that lays out the methods for conducting the assessment, those methods are referenced and, if needed, summarised in the protocol. In those cases, Panels should integrate into their protocols any additional and relevant aspects illustrated in the present guidance document.

Continuous feedback between EFSA Panels and the Scientific Committee on the use of this guidance is encouraged. This will help identify the need for revisions.

## 12. Recommendations for future developments

### 12.1. Interactive repository of EFSA protocols

Efficiency and flexibility in protocol development at EFSA can be increased by easing the retrieval and reuse, upon adaptation, of existing protocols or their sections. This would be useful for all EFSA mandates and not only for those addressing repetitive scientific questions.

To this end, it is recommended to develop a *repository* of EFSA protocols classified according to indexing metadata and retrievable via a common search engine.

The repository could be complemented by a user-friendly *interface* that would enable loading, adapting, and reusing existing protocols. The interface could incorporate and translate into a web-based 'writing tool' the MS Word template for EFSA protocols annexed to this guidance. In such a way, the users would be able to easily select and customise only the relevant sections of the template.

The interface would also enable the automatic indexing of each section of the protocol under development and hence aid in the continuous update of the repository, as new protocols become available.

## 12.2. Harmonised classification of EFSA assessment questions and sub-questions

EFSA draft framework for protocol development (EFSA, 2020a) provided a preliminary, harmonised classification of EFSA assessment questions and sub-questions which highlighted the multiple similarities across EFSA domains despite the semantic differences and the variety of scientific topics and legislative frameworks. The subsequent revision of this classification done by Risk Sciences International by scrutinising 700 EFSA publications (Section 2.3) resulted in some changes to and an extension of the categories of sub-questions and confirmed the similarities of assessment questions and sub-questions, not only across the various EFSA scientific domains, but also between generic mandates and assessments of regulated products.

A further revision and consolidation of this classification is recommended, to provide EFSA experts and staff with a tool for reading across EFSA areas and support a common, harmonised approach for developing protocols. This is considered to help consistency and cross-fertilisation of methods within and across EFSA domains and streamline the process for protocol development.

In fact, when starting a protocol for an assessment, looking at an existing protocol on the same class of assessment questions and/or sub-questions, irrespective of whether the domain or legislative framework can help:

- formulate the questions/sub-questions more precisely.
- have a better idea of the type of evidence that may be needed.
- identify existing methods to address the questions/sub-questions and plan them in the protocol.
- adapt an existing protocol to the specific questions/sub-questions.

This can be useful also for applicants preparing dossiers in support of applications of regulated products, when the data requirements and the methods for dealing with data are not fully detailed in the legislative documents and guidance.

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## Glossary

Term	Definition
Aggregated data	Information resulting from the synthesis of individual data (e.g. mean exposure in a treatment group, standard deviation of the observations in a group, etc.). See individual data.
Agent	An agent is anything that can cause an effect on a receptor.
Bibliographic database	A searchable database which contains summary records (often with abstracts and sometimes linking to full-text documents) of scientific literature and, in some cases, provides indexing terms (e.g. subject headings) to assist in searching.
Bibliographic record	The information used to identify a full-text document. Typically, this includes the author's name(s), publication date, the title of the document and publication details of the document (e.g. the name, volume and page numbers of a scientific journal, or the URL and publisher of a website).
Data model	A data model is a theoretical model that organises elements of data and describes how they relate to one another and how they relate to the properties of real-world entities they represent. A data model explicitly determines the structure of the data and is used to store and/or exchange harmonised information. For example, the EFSA Standard Sample Description 2.0 (SSD2) (EFSA, 2013) was developed by EFSA to harmonise at the EU level the collection of analytical data on chemical substances and microbiological agents in different matrices of non-human nature. The logical model of SSD2 includes a combination of three main groups of terms and characteristics: (1) data elements, definition and structure, (2) controlled terminologies and (3) business rules to ensure the validity of the information supplied.
<i>Ex novo</i> data generation	The process of generating new data as it happens when designing and conducting an experiment or an observational study (e.g. a survey). It is also referred to as a 'primary research study' as opposed to a 'secondary research study' (see definitions). In the EFSA context, studies generating data <i>ex novo</i> are designed and conducted, for instance, by the applicants submitting a dossier to EFSA in support of an application or by EFSA, when e.g. performing surveys (e.g. baseline surveys).
Expert Knowledge Elicitation (EKE)	Expert Knowledge Elicitation is a systematic, documented and reviewable process to retrieve expert judgements from a group of experts in the form of a probability distribution (EFSA, 2014).
Extent of planning in the protocol	Level of detail in problem formulation and in the specification of the evidence needs and methods that will be applied.
Full-text document	A document (e.g. journal article, dissertation) in which details of one or more studies are reported.
Individual data	Information collected at the level of the finest unit on which variables are measured (e.g. exposure observed on each individual belonging to a study). They cannot be further 'disaggregated'.
Integration (evidence integration)	Evidence integration is the process of combining evidence that is diverse (e.g. evidence from different study designs or streams of evidence). See also synthesis (evidence synthesis).
Intervention	Intervention refers to any intentional measure aimed at changing directly or indirectly the exposure and/or the consequence of the exposure to the agent.

Non-bibliographic databases	Searchable databases containing a collection of digital information or data, typically organised according to a pre-defined structure and accompanied by metadata explaining the methods used to collect and analyse the data.
Output	Output is the form of the answer to the assessment question or sub-question, the result of an assessment process.
Overall uncertainty	The assessors' uncertainty about the question or quantity of interest at the time of reporting, taking account of the combined effect of all sources of uncertainty identified by the assessors as being relevant to the assessment (EFSA Scientific Committee, 2018b).
Parameter	Parameter is used in this document to refer to quantitative inputs to an assessment or uncertainty analysis, without specifying whether they are variable or not. In most places a non-variable quantity is implied, consistent with the use of parameters in statistics. However, in some places 'parameter' could refer to a variable quantity, as it is sometimes used in biology (e.g. glucose level is referred to as a blood parameter) (EFSA Scientific Committee, 2018b).
Pathway	Pathway refers to any way in which an agent interacts with its receptor. It is the sequence of events leading the agent to cause an effect on the receptor. It can simply cover the route of exposure (typically dietary in EFSA assessments) or represent, for instance, the steps of introduction and spread when assessing a pathogen.
Primary research study	The original study in which data were produced. The term is sometimes used to distinguish such studies from secondary research studies (i.e. reviews) that re-examine previously collected data (see secondary research study).
Prioritisation of uncertainty	The process of evaluating the relative importance of different sources of uncertainty, to guide decisions on how to treat them in uncertainty analysis or to guide decisions on gathering further data with the aim of reducing uncertainty. Prioritisation is informed by influence or sensitivity analysis (EFSA Scientific Committee, 2018b).
Receptor	Receptor refers to anything that experiences the effect of the agent. The receptor can also experience a secondary consequence to the exposure to the agent (e.g. farmers changing cropping practices as a consequence of the crops being affected by a pest).
Secondary research study	A review (e.g. a systematic review) that re-examines existing data from one or more primary research studies (see primary research study).
Semi-formal EKE	A structured and documented procedure for eliciting expert judgements that are intermediate between fully formal elicitation and informal expert judgements (EFSA Scientific Committee, 2018a).
Source of uncertainty	An individual contribution to uncertainty, defined by its location (e.g. a component of the assessment) and its type (e.g. measurement uncertainty, sampling uncertainty). A single location may be affected by multiple types of uncertainty, and a single type of uncertainty may occur in multiple locations (EFSA Scientific Committee, 2018a).
Sources of evidence from scientific literature other than bibliographic databases	Any repository of information other than a bibliographic database that contains (primary and secondary) research studies in the form of bibliographic records (which include bibliographic citations and, in many cases, abstracts and full-text documents). Examples include internet search engines which access information in a variety of formats, internet pages, online journals and their tables of contents, and reference lists within full-text documents.
Synthesis (Evidence synthesis)	Evidence synthesis is defined as the process of summarising similar evidence. This can be, for instance, evidence from similar populations, study designs or evidence streams (e.g. human data). Defining what is 'similar' is subjective and depends on the evaluation of the assessors. Evidence synthesis can be performed on individual as well as on aggregated data from studies with a level of heterogeneity that does not affect the summary and the interpretation of the results.



Uncertainty	All types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question (EFSA Scientific Committee, 2018b).
Uncertainty analysis	The process of identifying and characterising uncertainty about questions of interest and/or quantities of interest in a scientific assessment (EFSA Scientific Committee, 2018b).

## Abbreviations

ADME	Absorption Distribution Metabolism and Excretion
AOP	Adverse Outcome Pathway
APRIO	Agent (A), Pathway (P), Receptor (R), Intervention (I), Output (O)
AQ	Assessment question
BSE	Bovine Spongiform Encephalopathy
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower Limit (lower bound of benchmark dose confidence interval)
BPA	Bisphenol A
Bt-maize	Genetically engineered maize that expresses insecticidal proteins from the bacterium <i>Bacillus thuringiensis</i> (Bt)
CFFP	Conventional Fresh Fishery Products
EC	European Commission
EEA	European Environment Agency
EHS	Environmental, Health and Safety Programme
EKE	Expert Knowledge Elicitation
FAO	Food and Agriculture Organization
FCM	Food Contact Material
FPA	Fluorescence Polarisation Assay
GM	Genetically Modified
GMO	Genetically Modified Organism
HARKing	Hypothesising After the Results are Known
HBGV	Health-Based Guidance Value
HPAI	Highly Pathogenic Avian Influenza
HPP	High-Pressure Processing
IATA	Integrated Approaches to Testing and Assessment
LOAEL	Lowest-Observed-Adverse-Effect-Level
MB	Management Board
MoA	Mode of Action
MOE	Margin of Exposure
NGO	Non-Governmental Organisation
NOAEL	No-Observed-Adverse-Effect-Level
NTO	Non-Target Organism
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OHAT	Office of Health Assessment and Translation
P	Pressure
P95	95 <sup>th</sup> percentile
PECO	Population (P), Exposure (E), Comparator (C) And Outcome (O) in a question about an exposure effect
PF	Problem Formulation
PICO	Population (P), Intervention (I), Comparator (C) and Outcome (O) in a question about an intervention effect
PIT	Population (P), Index test (I) and Target condition (T) in a question about test accuracy
PO	Population (P) and Outcome of interest (O) in a descriptive question
PROMETHEUS	PROmoting (PRO) METHods (METH) for Evidence (E) Use (U) in Scientific (S) assessments

RA	Risk Assessment
RfC	Reference Concentration
SC	Scientific Committee
SFFP	Superchilled Fresh Fishery Products
SSD2	Standard Sample Description 2.0 (EFSA, 2013)
SQ	Sub-question
t	Time
TO	Target Organism
ToR	Term of Reference
UHT	Ultra-High Temperature
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

## Appendix A – Examples of APRIO-formulated assessment questions and sub-questions for some hypothetical mandates

This section provides examples of APRIO-formulated assessment questions and sub-questions for some hypothetical mandates, which all together embrace a wide breadth of EFSA domains. The examples reported can also work for other topics. For instance, the example on aflatoxin B1 illustrated in Appendix A.2 could also work for sweeteners.

The process for formulating assessment questions and sub-questions using APRIO illustrated in the tables can be summarised as follows:

- At the start of problem formulation, the assessment question is generically defined (first cell, first column).
- Then, the relevant and applicable A-P-R-I-O elements are defined (same row as above, from second to second-last column) and the assessment question is formulated (same row, first cell in last column).
- The APRIO-formulated assessment question helps define, at a high level, the relevant sub-question(s) (next cells in the first column).
- Then, the sub-question(s) are formulated by specifying their A-P-R-I-O elements moving from the second to the last column.
  - The APRIO-formulated assessment questions and sub-questions aid in the definition of the evidence needs and the methods for collecting, appraising and synthesising data as well as, if needed, eliciting expert judgement. The better the APRIO elements and, in particular, the Output are specified *a priori*, the easier the definition of the evidence needs and the higher the clarity on the related question and sub-question.
  - As the formulation of the assessment questions and sub-questions progresses, the conceptual model (i.e. the relationship among assessment questions and sub-questions) is clarified.

### Disclaimer for all the examples in this Appendix:

- The mandates are hypothetical and do not reflect any real situation.
- The examples of assessment questions and sub-questions are illustrative and not necessarily exhaustive for the mandate.
- For most APRIO elements and especially for the Output(s) there can be alternatives and those given are just examples.

## A.1. Pathway to harm on avian influenza

**Table A.1:** Examples of APRIO-formulated assessment question (AQ) and sub-questions (SQ) for a hypothetical mandate aimed at assessing the impact of possible control measures on the probability of introduction of highly pathogenic avian influenza (HPAI) H5N8 into EU poultry holdings via wild birds (see explanation and disclaimer at the beginning of Appendix A). The mandate is purely hypothetical and does not reflect any real situation. AQ: assessment question; SQ: sub-question

Examples of AQ and SQs (generally defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>AQ: Impact of control measures on the probability of introduction of highly pathogenic avian influenza (HPAI) H5N8 into EU poultry holdings</b>	Avian influenza virus HPAI H5N8	Contact with excretions of infected wild birds	Poultry flocks present in EU holdings	Control measures (i.e. washing boots before entering the premises)	Ratio of the probability of introduction with control measures and at baseline (i.e. without control measures)	What is the probability of introduction of HPAI H5N8 infection in poultry flocks present in EU holdings (via contact with wild bird excretions) applying control measures (i.e. washing boots before entering the premises) compared with baseline probability (i.e. when control measures are not applied)?
<b>Tier 1 SQ 1: Entry of HPAI H5N8 in the EU wild bird population</b>	Avian influenza virus HPAI H5N8	Wild birds migrating routes	EU wild bird population	Not applicable for this SQ	Probability of introduction	What is the probability that avian influenza virus HPAI H5N8 enters the EU via wild bird populations migrating routes?
<b>Tier 2 SQ 1.1: Prevalence of HPAI in migrating wild birds when entering the EU</b>	Avian influenza virus HPAI H5N8	Not relevant for this SQ	Migrating wild bird species entering the EU (all species)	Not applicable for this SQ	Average prevalence in a typical year	What is the average prevalence of HPAI H5N8 virus in migrating wild birds entering the EU (all species) in a typical year?
<b>Tier 2 SQ 1.2: Number of migrating wild birds entering the EU</b>	Not relevant	Not relevant for this SQ	Migrating wild bird species entering the EU (all species)	Not applicable for this SQ	Average number across all species in a typical year	What is the average number of migrating wild birds entering the EU?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 1 SQ 2: Spread of HPAI in the EU wild birds present in the EU</b>	Avian influenza virus HPAI H5N8	Transmission among wild birds present in the EU	Residential EU wild birds (all species)	Not applicable for this SQ	Prevalence at the end of the migration period	What is the prevalence of avian influenza virus HPAI H5N8 in EU residential wild birds (all species) at the end of the migration period considering the transmission of the virus among residential EU wild birds (all species)?
<b>Tier 2 SQ 2.1: Population size</b>	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	Size of the EU residential wild bird population	[already specified in the O]
<b>Tier 2 SQ 2.2: Probability of contact</b>	Infectious excretions (i.e. contaminated with avian influenza virus HPAI H5N8)	Not relevant for this SQ	EU residential wild birds	Not applicable for this SQ	Probability of contact with infectious excretions	What is the probability of contact of the EU residential wild birds with excretions contaminated with avian influenza virus HPAI H5N8)?
<b>Tier 1 SQ 3: Introduction of HPAI into EU poultry holdings</b>	Avian influenza virus HPAI H5N8	Contact with wild birds' infectious excretions introduced into the poultry holdings via several means (e.g. boots, wheels, wild birds)	EU poultry holdings	Not applicable for this SQ	Probability of introduction	What is the probability of introduction of avian HPAI H5N8 into EU poultry holdings via contact with wild birds' infectious excretions introduced into the poultry holdings via several [specified] means?
<b>Tier 2 SQ 3.1: Baseline probability</b>	Avian influenza virus HPAI H5N8	Contact with infectious excretions of wild birds introduced into the poultry holdings via several means (e.g. boots, wheels, wild birds)	EU poultry holdings	Not applicable for this SQ	Probability of introduction in the absence of control measures (baseline)	What is the probability of introduction of HPAI H5N8 into EU poultry holdings via contact with wild birds' infectious excretions introduced into the poultry holdings via several [specified] means in the absence of control measures?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 2 SQ 3.2: Probability of introduction in poultry holdings when control measures are implemented</b>	Avian influenza virus HPAI H5N8	Contact with infectious excretions of wild birds introduced into the poultry holdings via several means (e.g. boots, wheels, wild birds), various scenarios	EU poultry holdings	Control measures (e.g. washing boots before entering the premises)	Probability of introduction in the presence of control measures	What is the probability of introduction of HPAI H5N8 into EU poultry holdings via contact with wild birds' infectious excretions introduced into the poultry holdings via several [specified] means [various scenarios]) when applying control measures (e.g. washing boots before entering the premises)



## A.2. Assessment of *Aspergillus* in plants and the risk to animal and human health related to the presence of mycotoxin aflatoxin B1 in feed and food

**Table A.2:** Examples of APRIO-formulated assessment questions and sub-questions for hypothetical mandates on aspergillosis in plants (AQ1), aflatoxin B1 and feed additives (AQ3), and aflatoxin B1 in feed (AQ4) and food (AQ5 onwards). This example is also depicted in Figure 6 in Section 6.2.1. For aflatoxin B1 in food, the hypothetical mandate aims at assessing the human health risks related to the presence of aflatoxin B1 in food, with a focus on chronic exposure (see explanation and disclaimer at the beginning of Appendix A). The mandates are purely hypothetical and do not reflect any real situation. AQ: assessment question; SQ: sub-question.

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>AQ1: Probability of infection by <i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i> in seed and nut crops (Plant health)</b>	<i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i>	Infection of seed and nut crops in the field before harvest	Crops: maize, cottonseed, sorghum, nuts (e.g. hazelnut, pistachio nut, peanut, almond)	Cropping practices (e.g. early harvesting, irrigation)	Probability of <i>Aspergillus</i> infection with/without cropping practices	What is the probability of infection by <i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i> in seed and nut crops (maize, cottonseed, sorghum, nuts e.g. hazelnut, pistachio nut, peanut, almond) with and without cropping practices (e.g. early harvesting, irrigation)?
<b>AQ2: Probability of production of aflatoxins in seeds and nuts in crop production, storage and transport</b> (this is an interdisciplinary question related to different areas, that can also be addressed by modelling; e.g. see <a href="https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2012.EN-223">https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2012.EN-223</a> )	Aflatoxin	Production of aflatoxins in nuts and seeds during crop production, storage and transport	Maize seeds, cottonseed, sorghum seeds and, nuts (e.g. hazelnut, pistachio nut, peanut, almond)	Optimal storage conditions	Aflatoxins produced in seeds and nuts during crop production, storage and transport	What is the probability of producing aflatoxins during crop production, storage and transport of seed (e.g. maize, cottonseed, sorghum) and nuts (e.g. hazelnut, peanut, pistachio nut) in the EU and/or in Non-EU Countries?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>AQ3: efficacy of feed additives (binders) for the reduction of the contamination of feed by aflatoxin B1 (Feed additives)</b>	Feed additive: mycotoxin binders (clay minerals, e.g. bentonite, zeolite, etc.)	Not relevant for this AQ	Aflatoxin B1 in feed	Not applicable for this AQ	At the proposed use level of the additive in feed: <ul style="list-style-type: none"> <li>– reduced absorption of aflatoxin B1</li> <li>– increased excretion of aflatoxin B1</li> <li>– increased degradation/transformation of aflatoxin B1</li> <li>– reduced concentration of aflatoxin M1 in milk/egg yolk</li> </ul>	Is the additive efficacious, at the proposed conditions of use, to reduce the absorption of aflatoxin B1, increase the excretion of aflatoxin B1, increase the degradation/transformation of aflatoxin B1 and reduce the concentration of aflatoxin M1 in milk/egg yolk?
<b>AQ4: Risk assessment of aflatoxin B1 in farm animals, horses and companion animals (Feed contaminants)</b>	Aflatoxin B1	Ingestion of contaminated feed	Farm animals, horses and companion animals in the EU [different target species]	Not applicable for this AQ	Risk of adverse effects (if any)	What is the risk of adverse effects associated with ingestion of feed contaminated by aflatoxin B1 in farm animals, horses and companion animals [different target species] in the EU?
<b>AQ5: Risk assessment of aflatoxin B1 in humans, with a focus on chronic dietary exposure (Food contaminants)</b>	Aflatoxin B1	Chronic dietary exposure	European population	Not applicable for this AQ	Risk of adverse effects (if any)	What is the risk of adverse effects associated with chronic dietary exposure to aflatoxin B1 in the European population?
<b>Tier 1 SQ 1: EFFECT IDENTIFICATION</b>	Aflatoxin B1	Chronic dietary exposure	Humans	Not applicable for this SQ	Effects associated with chronic dietary exposure and considerations on their adversity and on causality	What [adverse] effects are [causally] associated to chronic dietary exposure to aflatoxin B1 in humans?
<b>Tier 2 SQ 1.1: Effect identification in laboratory animal studies</b>	Aflatoxin B1	Sub-chronic and chronic oral exposure	Relevant laboratory animals [species specified and strain]	Not applicable for this SQ	Effects associated to sub-chronic and chronic oral exposure	What adverse effects are associated to chronic oral exposure to aflatoxin B1 in laboratory animals [species and strain specified]?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 2 SQ 1.2: Effect identification in human studies</b>	Aflatoxin B1	Chronic dietary exposure	Humans	Not applicable for this SQ	Effects associated with chronic dietary exposure	What adverse effects are associated to chronic dietary exposure to aflatoxin B1 in humans in epidemiological studies?
<b>Tier 2 SQ 1.3: Absorption, distribution, metabolism and excretion (ADME) in laboratory animals</b>	Aflatoxin B1	Chronic oral exposure	Relevant laboratory animals [species specified]	Not applicable for this SQ	Characterisation of toxicokinetic parameters (i.e. ADME)	What is the ADME of aflatoxin B1 in laboratory animals [species specified] following chronic oral exposure?
<b>Tier 2 SQ 1.4: ADME in humans</b>	Aflatoxin B1	Chronic dietary exposure	Humans	Not applicable for this SQ	Characterisation of toxicokinetic parameters (i.e. ADME)	What is the ADME of aflatoxin B1 in humans following chronic dietary exposure?
<b>Tier 2 SQ 1.5: Genotoxicity <i>in vivo</i></b>	Aflatoxin B1	Not relevant for this SQ	For example, mammalian erythrocyte micronucleus test, transgenic rodent somatic and germ cell gene mutation assays, <i>in vivo</i> Comet assay	Not applicable for this SQ	Genotoxic potential	Is aflatoxin B1 showing genotoxic potential in <i>in vivo</i> tests?
<b>Tier 2 SQ 1.6: Genotoxicity <i>in vitro</i></b>	Aflatoxin B1	Not relevant for this SQ	For example, bacterial reverse mutation test, <i>in vitro</i> mammalian cell micronucleus test	Not applicable for this SQ	Genotoxic potential	Is aflatoxin B1 showing genotoxic potential in <i>in vitro</i> tests?
<b>Tier 2 SQ 1.7: Mode of action (MoA)</b>	Aflatoxin B1	Oral and other relevant exposures	Relevant laboratory animals [species specified], <i>in vitro</i> assays with relevant human cell lines, organoids,	Not applicable for this SQ	Mode of Action	What is the MoA that can explain the effect(s) associated to exposure to aflatoxin B1 observed in laboratory animals and

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
			cell-free systems [specified], computational approaches			humans [assessed in previous SQs]?
<b>Tier 1 SQ 2: EFFECT CHARACTERISATION</b>	Aflatoxin B1	Chronic dietary exposure	Humans	Not applicable for this SQ	Establishment of a health-based guidance value (HBGV) or Margin of Exposure (MOE) based on a Reference Point (e.g. NOAEL, LOAEL or BMDL) and related uncertainty <sup>(a)</sup>	What is the HBGV or margin of exposure based on a reference point at which the adverse effect(s) associated to chronic dietary exposure to aflatoxin B1 occur(s) in humans?
<b>Tier 2 SQ 2.1: Identification of a reference point in laboratory animals</b>	Aflatoxin B1	Chronic oral exposure	Laboratory animals [relevant species specified]	Not applicable for this SQ	Identification of a Reference Point (e.g. NOAEL, LOAEL or BMDL) from experimental animal studies	What is the Reference Point (e.g. NOAEL/LOAEL/BMDL) at which the adverse effect (s) associated to chronic oral exposure to aflatoxin B1 occur(s) in laboratory animals [species specified]?
<b>Tier 2 SQ 2.2: Identification of a reference point in humans</b>	Aflatoxin B1	Chronic dietary exposure	Humans	Not applicable for this SQ	Identification of a Reference Point (e.g. NOAEL, LOAEL or BMDL) from human epidemiological studies	What is the Reference Point (e.g. NOAEL/LOAEL/BMDL) at which the adverse effect (s) associated to chronic oral exposure to aflatoxin B1 occur(s) in human epidemiological studies?
<b>Tier 2 SQ 2.3: Relevance to humans</b>	Aflatoxin B1	Chronic dietary exposure	Humans	Not applicable for this SQ	Relevance of the effects observed in laboratory animals	What is the relevance to humans of the effects associated with chronic oral exposure to aflatoxin B1 observed in laboratory animals, also considering the MoA [assessed in previous SQs]?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 2 SQ 2.4: Adversity</b>	Aflatoxin B1	Chronic dietary exposure	Humans	Not applicable for this SQ	Adversity to humans of the effects observed in laboratory animals	What is the adversity to humans of the effects associated with chronic oral exposure to aflatoxin B1 observed in laboratory animals, also considering the MoA [assessed in previous SQs]?
<b>Tier 1 SQ 3: EXPOSURE ASSESSMENT</b>	Aflatoxin B1	Chronic dietary ingestion of contaminated food	European population (all age groups and countries)	Not applicable for this SQ	Mean and P95 estimates of the chronic dietary exposure (per kg body weight per day)	What is the estimate of the mean and P95 chronic dietary exposure per kg body weight per day to aflatoxin B1 in the European population (all age groups and countries)?
<b>Tier 2 SQ 3.2: Occurrence</b>	Aflatoxin B1	Contamination along the food chain	Food items	Not applicable for this SQ	Mean occurrence in each food item [codified according to FoodEx2 <sup>(b)</sup> in the EU market and as submitted to EFSA via the 'Call for continuous collection of chemical contaminants occurrence data in food and feed']	What is the mean occurrence of aflatoxin B1 in each food item as codified according to FoodEx2 in the EU market as submitted to EFSA?
<b>Tier 2 SQ 3.3: Consumption</b>	Food items potentially contaminated with Aflatoxin B1	Chronic dietary ingestion	European population (all age groups and countries)	Not applicable for this SQ	Individual chronic consumption (adjusted by body weight) [from the surveys included in the Comprehensive Food Consumption database]	What is the individual chronic consumption* (adjusted by body weight) of each food item (potentially contaminated with aflatoxin B1) of the different population groups and age classes in the EU population?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
						*Note: the individual chronic consumption is the target which is estimated with the individual average consumption over the survey period
<b>Tier 2 SQ 3.4: Effect of food processing and storage</b>	Industrial and household food processing, e.g. milling, sorting, cleaning, heat treatment, fermentation and storage	Not relevant for this SQ	Food items contaminated by aflatoxin B1	Not applicable for this SQ	Percentage of reduction or increase in the occurrence after processing and storage for each item and food beverage	Does the application of industrial and household food processing techniques [specified] and storage affect (reduce or increase) the occurrence of aflatoxin B1 in contaminated food items?
<b>Tier 1 SQ 4: RISK CHARACTERISATION</b>	Aflatoxin B1	Chronic dietary exposure	European population (all age groups)	Not applicable for this SQ	Comparison of the estimated exposure to the HBGV, or calculation of the Margin of exposure between the estimated exposure and the reference point	What is the risk of adverse health effects for the European population (all age groups) exposed to aflatoxin B1 via the diet when considering the HBGV or Margin of exposure?

(a): Note on dose–response assessment: in most cases (and especially if it is a first-time assessment), at the time of drafting a protocol, it’s not yet known whether a no-observed-adverse-effect-level (NOAEL), lowest-observed-adverse-effect-level (LOAEL) or a Benchmark dose lower limit (BMDL) will be identified for a particular incidence of effect from epidemiological studies and experimental animal studies to derive health-based guidance value (HBGV) or calculate a margin of exposure (MOE).

(b): <https://www.efsa.europa.eu/en/data/data-standardisation>



### A.3. Diagnostic test accuracy *Brucella* Fluorescence Polarisation Assay

**Table A.3:** Example of an APRIO-formulated assessment question for a hypothetical mandate on the suitability of the *Brucella* fluorescence polarisation assay (FPA) (diagnostic test accuracy) (see explanation and disclaimer at the beginning of Appendix A). The mandate is purely hypothetical and does not reflect any real situation. AQ: assessment question. AQ: assessment question

Example of AQ (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQ formulated using APRIO
<b>AQ: sensitivity and specificity of FPA for <i>Brucella melitensis</i></b>	<i>Brucella melitensis</i> in various sample media	Not relevant for this SQ	Multiple [specified] diagnostic tests <sup>(a)</sup> including <i>Brucella</i> FPA	Not applicable for this SQ	Estimated comparative performance (i.e. sensitivity (Se) and specificity (Sp))	What is the performance (i.e. Se and Sp) of <i>Brucella</i> FPA compared with standard tests for diagnosis of brucellosis using various sample media?

(a): The diagnostic assays are considered the 'receptor' as they experience the effect of the agent that is added to the test system and causes a reaction of the test reagents.

## A.4. High-pressure processing of food

**Table A.4:** Examples of APRIO-formulated assessment questions (AQs) and sub-questions (SQs) for a hypothetical mandate on the efficacy and safety of high-pressure processing of food (see explanation and disclaimer at the beginning of Appendix A). This example is also shown in Figure 3, Section 6.1. The mandate is purely hypothetical and does not reflect any real situation. AQ: assessment question; SQ: sub-question

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<i>Examples also depicted in Figure 3, Section 6.1:</i>						
<b>AQ1: HPP-treated foods, HPP processing conditions</b>	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	List of (broad) food categories to which HPP is or could be applied (to increase microbiological food safety) with processing conditions (e.g. P, t, T), focusing on commercially processed foods	[already specified in the output]
<b>Tier 1 SQ 1.1: Food categories</b>	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	List of (broad) food categories to which HPP is or could be applied (to increase microbiological food safety), focusing on commercially processed foods	[already specified in the O]
<b>Tier 1 SQ 1.2: Processing conditions</b>	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	List of HPP processing conditions (e.g. P, t, T) of commercially processed foods	[already specified in the O]
<b>AQ2: Intrinsic and extrinsic factors influencing HPP efficacy</b>	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	List of intrinsic (i.e. food related) and extrinsic (i.e. process related) factors that may influence the efficacy of HPP on vegetative microorganisms (log <sub>10</sub> reduction) when applied to foods	[already specified in the output]
<b>AQ3: Microbiological safety concerns of HPP-treated food</b>	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	List of potential microbiological food safety concerns in HPP-treated food vs untreated food or food subject to routine treatments aiming to increase microbiological food safety	[already specified in the output]
<b>AQ4: Minimum time and pressure</b>	HPP with varying t and P (and any	Not relevant for this AQ	Pathogens present in ruminants' raw	Not applicable for this AQ	Minimum requirements (t and P combinations) of HPP to achieve	What are the minimum requirements (t and P

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>combinations for HPP to achieve equivalent efficacy as thermal pasteurisation</b>	other relevant factor)		milk and raw colostrum		an equivalent efficacy on relevant pathogens (log10 reduction) as with thermal pasteurisation	combinations) of HPP applied to ruminants' raw milk and raw colostrum to achieve an equivalent efficacy on relevant pathogens (log10 reduction) as with thermal pasteurisation?
<b>Tier 1 SQ 4.1: Relevant pathogens reduced by thermal pasteurisation</b>	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	List of relevant pathogens reduced by thermal pasteurisation of raw milk and raw colostrum from ruminants	[already specified in the O]
<b>Tier 1 SQ 4.2: Efficacy of thermal pasteurisation</b>	Thermal pasteurisation applied according to EU legal requirements (i.e. at least 72°C for 15 s, at least 63°C for 30 min or equivalent)	Not relevant for this SQ	Pathogens present in ruminants' raw milk and raw colostrum [specified in SQ 4.1]	Not applicable for this SQ	Log10 unit reduction of pathogens [specified in SQ 4.1]	What log10 reduction of the pathogens [specified in SQ 4.1] is achieved by thermal pasteurisation of ruminants' raw milk and raw colostrum applied according to EU legal requirements [specified]?
<b>Tier 1 SQ 4.3: Efficacy of HPP at different combinations of t and P</b>	HPP with varying t and P (and any other relevant factor)	Not relevant for this SQ	Pathogens present in ruminants' raw milk and raw colostrum [specified in SQ 4.1]	Not applicable for this SQ	Log10 units' reduction of pathogen load [specified in SQ 4.1]	What log10 reduction of the pathogens [specified in SQ 4.1] is achieved by HPP with varying t and P (and any other relevant factor) on ruminants' raw milk and raw colostrum?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>AQ5: Indicators of HPP efficacy</b>	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	No need to specify for this AQ	List of inherent components of milk or colostrum appropriate as indicators of HPP efficacy on ruminants' raw milk and raw colostrum, considering the minimum requirements as defined in AQ4	[already specified in the O]
<b>AQ6: Pathogen load in HPP-treated vs raw vs thermally pasteurised vs UHT-treated milk serving after treatment</b>	Relevant pathogens (from SQ4.1)	During milk processing	Contaminated milk batch	Untreated, HPP (at a specific level of time and pressure), thermal pasteurisation or UHT treatment (at a specific time-temperature)	Probability of residual contamination and pathogen load after treatment in a milk serving	What is the probability of residual contamination and pathogen load in a milk serving when the milk batch has been treated during milk processing using HPP or thermal pasteurisation or UHT or left untreated?

Alternative examples not in Figure 3, Section 6.1:

<b>AQ6bis: Probability of listeriosis in the older human population after consumption of ruminants' milk treated in different ways (or untreated)</b>	<i>Listeria monocytogenes</i>	Intake of ruminants' milk immediately after one of the treatments (and without treatment for raw milk)	Elderly EU human population	None or HPP (specific time and pressure) or thermal pasteurisation (specific time and temperature) vs UHT treatment	Probability of listeriosis per serving of milk	What is the probability of listeriosis for the older EU human population per serving of HPP-treated vs raw vs thermally pasteurised vs UHT-treated ruminants' milk immediately after one of the treatments (and without treatment for raw milk)?
<b>Tier 1 SQ 6bis.1: Consumption</b>	Milk from ruminants	Dietary ingestion	Older EU human population	Not applicable for this SQ	Individual milk consumption (serving size)	What is the individual consumption (serving size) of ruminants' milk by the older EU population?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 1 SQ 6bis.2:</b> <i>Listeria monocytogenes</i> contamination of raw milk	<i>L. monocytogenes</i>	Not relevant for this SQ	Ruminants' raw milk	Not applicable for this SQ	Contamination load in terms of log10 units	What is the <i>L. monocytogenes</i> contamination load of ruminants' raw milk (log10 units)?
<b>Tier 1 SQ 6bis.3:</b> Efficacy of 'conventional treatments' applied to ruminants' raw milk	Conventional treatments	Not relevant for this SQ	<i>L. monocytogenes</i> in ruminants' raw milk contaminated with	Not applicable for this SQ	Reduction of contamination load in terms of log10 units	What is the efficacy of 'conventional treatments' in terms of log10 unit reduction of <i>L. monocytogenes</i> when applied to ruminants' raw milk from ruminants contaminated with <i>L. monocytogenes</i> ?
<b>Tier 1 SQ 6bis.4:</b> Efficacy of HPP applied to ruminants' raw milk	HPP treatment	Not relevant for this SQ	<i>L. monocytogenes</i> in ruminants' raw milk	Not applicable for this SQ	Reduction of contamination load in terms of log10 units	What is the efficacy of HPP treatment in terms of log10 unit reduction of <i>L. monocytogenes</i> when applied to ruminants' raw milk contaminated with <i>L. monocytogenes</i> ?
<b>Tier 1 SQ 6bis.5:</b> Dose-response relationship	<i>L. monocytogenes</i>	Oral exposure via ingestion of contaminated ruminants' raw milk	Elderly human population	Not applicable for this SQ	Probability of listeriosis at different pathogen loads (expressed as log10 units)	What is the probability of human listeriosis for the older population following oral exposure to different <i>L. monocytogenes</i> loads (expressed in log10 units) via ingestion of ruminants' raw milk?

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 1 SQ 6bis.6:</b> Probability of listeriosis for the older human population following consumption of ruminants' conventionally treated milk and raw milk	<i>L. monocytogenes</i>	Oral exposure immediately after one of the treatments (and without treatment for raw milk) until the moment of consumption	Elderly EU human population	None or thermal pasteurisation vs UHT treatment	Probability of human listeriosis per serving of ruminants' conventionally treated and raw milk	What is the probability of human listeriosis for the older population per serving of ruminants' raw vs thermally pasteurised vs UHT-treated milk immediately after one of the treatments (and without treatment for raw milk) until the moment of consumption?
<b>Tier 1 SQ 6bis.7:</b> Probability of listeriosis for the older human population following consumption of HPP-treated milk from ruminants	<i>L. monocytogenes</i>	Oral exposure immediately after HPP (and without treatment for raw milk) until the moment of consumption	Elderly human population	HPP treatment	Probability of human listeriosis per serving of HPP-treated milk from ruminants	What is the probability of human listeriosis for the older population per serving of HPP-treated ruminants' milk immediately after treatment (and without treatment for raw milk) until the moment of consumption?



### A.5. Exposure assessment of plasticisers

**Table A.5:** Examples of APRIO-formulated assessment questions (AQs) and sub-questions (SQs) for a hypothetical mandate on the exposure assessment of substances potentially used as plasticisers in food contact materials (FCMs). The scope is to assess the dietary exposure assessment of such substances, with the aim of addressing the relative contribution to dietary exposure of EU consumers (see explanation and disclaimer at the beginning of Appendix A). The mandate is purely hypothetical and does not reflect any real situation. AQ: assessment question; SQ: sub-question

Examples AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>AQ1: assessment of total dietary exposure (due to food being subject to environmental contamination and/or contact along the food chain, e.g. migration from FCMs)</b>	[Prioritised] phthalates, structurally similar substances and replacement substances potentially used as plasticisers in materials and articles intended to come into contact with food (e.g. certain phthalates, terephthalates, citrates, benzoates)	Chronic and acute dietary ingestion of contaminated food	EU population (different population groups and age classes)	Not applicable for this AQ	Population mean and 95th centile of total [see def] dietary exposure to each [prioritised] substance	What is the population mean and 95th centile of total [see def] dietary exposure to each prioritised substance in the different population groups and age classes in the EU population, via chronic and acute dietary ingestion of food?
<b>Tier 1 SQ 1.1: Concentration in food</b>	Prioritised substances [same as above]	Environmental contamination or contamination along the food chain (e.g. migration from food contact materials into food)	All food consumed by the EU population	Not applicable for this SQ	Distribution of concentration for the different foods	What is the distribution of the concentration of the prioritised substances in food items [specific list] contaminated from the environment or along the food chain (e.g. migrated from FCM) consumed by the EU population?
<b>Tier 2 SQ 1.1.1: List of relevant analytical tests</b>	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	List of relevant analytical tests for assessing concentration in food	[already specified in the O]
<b>Tier 2 SQ 1.1.2: Accuracy of the analytical results</b>	[Prioritised] substances [same as above]	Not relevant for this SQ	Relevant analytical tests [specified in previous SQ]	Not applicable for this SQ	Accuracy of the results measured using, for example, recovery, standard deviation, limit of detection	What is the accuracy of the results of the relevant analytical tests [specified in previous SQ] measured using e.g. recovery, standard deviation, limit of detection?

Examples AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 1 SQ 1.2: Food consumption</b>	Food items potentially contaminated with the prioritised substances	Chronic and acute dietary ingestion	EU population (different population groups and age classes)	Not applicable for this SQ	Individual consumption (adjusted by body weight) for each food item and beverage	What is the individual acute and chronic consumption (adjusted by body weight) of each food item and beverage (potentially contaminated with the prioritised substances) of the different population groups and age classes in the EU population?
<b>AQ2: Assessment of dietary exposure from FCMs only (considering migration levels from plastic and rubber FCMs as well as other possibly relevant materials, e.g. (printed) paper and board including articles throughout the whole food chain, including food manufacturing and processing equipment, as well as packaging, kitchenware and tableware)</b>	[Prioritised] substances [same as above]	Chronic and acute dietary ingestion of food contaminated via FCMs	EU population (different population groups and age classes)	Not applicable for this SQ	Population mean and 95th centile of dietary exposure from FCMs only for each [prioritised] substance	What is the population mean and 95th centile of dietary exposure from FCMs only for each [prioritised] substance in the different population groups and age classes in the EU population, via chronic and acute dietary ingestion of food contaminated via FCM?
<b>Tier 1 SQ 2.1: List of relevant FCMs</b>	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	FCMs in which the [prioritised] substances [same as above] occur	[already specified in the O]

Examples AQ and SQs (generally defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 1 SQ 2.2: Use of relevant FCMs</b>	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	No need to specify for this SQ	Qualitative description of the use of the FCM in the food chain: steps of the food chain when the FCM is used; how often and under what conditions of use; frequency of use (market share)	[already specified in the O]
<b>Tier 1 SQ 2.3: Migration from relevant FCMs into food</b>	[Prioritised] substances [same as above]	Migration from FCMs [listed in previous SQ] into food	All food consumed by the EU population potentially contaminated from FCMs	Not applicable for this SQ	Distribution of migration levels for the different foods [mg/kg food]	Which is the distribution of migration levels for the different foods (mg/kg food) of the prioritised substances for food consumed by the EU population for each identified FCM [listed in previous SQ], according to the relevant use [defined in previous SQ]?
<b>Tier 1 SQ 2.4: Consumption of food contaminated via FCMs</b>	Food items for which there is evidence [assessed in previous SQ] on migration of the prioritised substances from relevant FCMs [listed in previous SQ]	Chronic and acute dietary ingestion	EU population (different population groups and age classes)	Not applicable for this SQ	Individual consumption (adjusted by body weight) for each food item and beverage	What is the individual acute and chronic consumption (adjusted by body weight) of each food item and beverage in which migration of the prioritised substances from relevant FCMs [listed in previous SQ] is plausible [assessed in previous SQ], of the different population groups and age classes in the EU population?

## A.6. Non-cancer hazard assessment of formaldehyde in air

**Table A.6:** Examples of APRIO-formulated assessment questions (AQs) and sub-questions (SQs) for a hypothetical mandate on the level of lifetime inhalation exposure to formaldehyde expected to be without adverse non-cancer health effects (see explanation and disclaimer at the beginning of Appendix A). AQ: assessment question; SQ: sub-question.

**Disclaimer:**

- This example shows how APRIO can work in a hazard assessment framework that is (slightly) different from EFSA's.<sup>26</sup>
- In this example, the way the APRIO elements (mainly the O) are defined is different from e.g. the aflatoxin B1 example, as well as the degree of granularity of the SQs, showing how these can change (and still be valid) depending on the view of those formulating the problem.

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>AQ: Level of lifetime inhalation exposure to formaldehyde expected to be without adverse non-cancer health effects in the general population</b>	Formaldehyde	Lifetime inhalation exposure	General population, including susceptible individuals	Not applicable for this AQ	Airborne formaldehyde concentration below which adverse non-cancer effects are not expected to occur in any individual	What is the airborne formaldehyde concentration below which adverse non-cancer health effects are not expected to occur in any person, including susceptible individuals, exposed for a lifetime?
<b>Tier 1 SQ 1: HAZARD IDENTIFICATION</b>	Formaldehyde	Lifetime inhalation exposure	General population, including susceptible individuals	Not applicable for this SQ	Identification of non-cancer human health effects expected to result from inhalation exposure to formaldehyde	What are the non-cancer adverse health effects that are expected to result from inhalation exposure to formaldehyde in the general population, including susceptible individuals, exposed for a lifetime?
<b>Tier 2 SQ 1.1: Hazard Identification – endpoint sensory irritation</b>	Formaldehyde	Lifetime inhalation exposure	General population, including susceptible individuals	Not applicable for this SQ	Determination of (increased) sensory irritation as a potential adverse health effect of exposure	Does (increased) sensory irritation represent a potential adverse health effect of lifetime formaldehyde inhalation exposure in the general population, including susceptible individuals?

<sup>26</sup> This hypothetical example is modelled after a publicly available draft hazard identification and dose–response assessment developed by the US EPA. However, this example is for illustrative purposes only and is in no way related to the US EPA assessment. This content does not represent the views or policies of the US EPA.

<b>Examples of AQ and SQs (generically defined)</b>	<b>Agent (A)</b>	<b>Pathway (P)</b>	<b>Receptor (R)</b>	<b>Intervention (I)</b>	<b>Output (O)</b>	<b>AQs and SQs formulated using APRIO</b>
<b>Tier 3 SQ 1.1.1: Hazard Identification – endpoint sensory irritation (human data)</b>	Formaldehyde	Inhalation exposure (air concentrations are reported, can be estimated or can be inferred)	Exposed humans (i.e. worker, residential and controlled exposure studies)	Not applicable for this SQ	Determination of sensory irritation as a potential adverse health effect from human studies (i.e. epidemiological and controlled exposure)	Do the available human studies (i.e. epidemiological and controlled exposure) support the determination of sensory irritation as a possible adverse health effect of lifetime inhalation exposure to formaldehyde?
<b>Tier 3 SQ 1.1.2: Hazard Identification – endpoint sensory irritation (animal data)</b>	Formaldehyde	Inhalation exposure	Laboratory animals [species specified]	Not applicable for this SQ	Determination of sensory irritation as a potential adverse health effect from animal studies	Do the available animal toxicological studies [species specified] on sensory irritation support determination of a possible adverse health effect of lifetime inhalation exposure to formaldehyde?
<b>Tier 3 SQ 1.1.3: Hazard Identification – sensory irritation (mechanistic data) (this row in the table summarises multiple SQs on different mechanistic data)</b>	Formaldehyde	Routes and durations of exposure relevant to understanding MoA for inhalation exposure (e.g. inhalation and non-inhalation exposure) considering ADME understanding	Humans Human and animal cell systems Laboratory animals [species specified]	Not applicable for this SQ	Determination of MoA for sensory irritation; identification of additional potential effects on endpoints not listed in previous SQs; assessing human relevance of animal data; identifying mechanisms of increased susceptibility	Do the available mechanistic data support: determination of a MoA for sensory irritation; potential for effects on endpoints not studied in the above evidence; assessing the human relevance of the animal data; and identifying mechanisms of increased susceptibility for lifetime inhalation exposure to formaldehyde?
<b>Tier 2 SQ 1.2: Hazard identification – endpoint pulmonary function</b>	Formaldehyde	Similar to SQ 1.1	Similar to SQ 1.1	Not applicable for this SQ	Similar to SQ 1.1	
<b>Tier 2 SQ 1.3: Hazard identification – endpoint immune effects (e.g. allergic conditions; asthma)</b>	Formaldehyde	Similar to SQ 1.1	Similar to SQ 1.1	Not applicable for this SQ	Similar to SQ 1.1	

Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<b>Tier 2 SQ 1.4: Hazard identification – endpoint respiratory tract pathology</b>	Formaldehyde	Similar to SQ 1.1	Similar to SQ 1.1	Not applicable for this SQ	Similar to SQ 1.1	
<b>Tier 2 SQ 1.5: Hazard identification – endpoint female reproductive or developmental effects (e.g. spontaneous abortion; fertility)</b>	Formaldehyde	Similar to SQ 1.1	Similar to SQ 1.1	Not applicable for this SQ	Similar to SQ 1.1	
<b>Tier 2 SQ 1.6: Hazard identification – endpoint male reproductive effects (e.g. fertility; organ damage)</b>	Formaldehyde	Similar to SQ 1.1	Similar to SQ 1.1	Not applicable for this SQ	Similar to SQ 1.1	
<b>Tier 2 SQ 1.7: Hazard identification – endpoint nervous system effects (e.g. ALS incidence; neurobehavioral effects)</b>	Formaldehyde	Similar to SQ 1.1	Similar to SQ 1.1	Not applicable for this SQ	Similar to SQ 1.1	
<b>Tier 1 SQ 2: Non-cancer DOSE–RESPONSE (SQ answered if any human adverse health effect is determined with sufficient certainty)</b>	Formaldehyde	Lifetime inhalation exposure	General population, including susceptible individuals	Not applicable for this SQ	Reference concentration (RfC; in mg/m <sup>3</sup> ) expected to be without non-cancer effects in any person and characterisation of certainty in the RfC	What is the reference concentration (RfC in mg/m <sup>3</sup> ) for lifetime inhalation exposure to formaldehyde below which adverse non-cancer human health effects are not expected to occur in any person including susceptible individuals and what is the certainty in the RfC?
<b>Tier 2 SQ 2.1: Dose–response: endpoint sensory irritation (selected value) (SQ addressed only if increased sensory irritation is determined, with</b>	Formaldehyde	Lifetime inhalation exposure	General population, including susceptible individuals	Not applicable for this SQ	Toxicity value (mg/m <sup>3</sup> ) expected to be without sensory irritant effects and characterisation of certainty in that value	What is the toxicity value (mg/m <sup>3</sup> ) for lifetime inhalation exposure to formaldehyde below which sensory irritant effects are not expected to occur in any



Examples of AQ and SQs (generically defined)	Agent (A)	Pathway (P)	Receptor (R)	Intervention (I)	Output (O)	AQs and SQs formulated using APRIO
<p><b>sufficient certainty, to represent a human health adverse effect)</b>  <b>(This and the SQs below would be repeated for all other endpoints mentioned under hazard identification)</b></p>						<p>person, including susceptible individuals? What is the certainty in that sensory irritation toxicity value?</p>
<p><b>Tier 3 SQ 2.1.1: Dose–response: endpoint sensory irritation (candidate values)</b></p>	Formaldehyde	Lifetime inhalation exposure	General population, including susceptible individuals	Not applicable for this SQ	Candidate toxicity values (in mg/m <sup>3</sup> ) based on one or more PODs and related uncertainties, that are expected to be without sensory irritant effects	<p>What are the candidate toxicity value(s) (mg/m<sup>3</sup>) for lifetime inhalation exposure to formaldehyde, based on one or more PODs and considering uncertainties that exist, below which sensory irritant effects are not expected to occur in any person, including susceptible individuals?</p>
<p><b>Tier 4 SQ 2.1.1.1: Dose–response: endpoint sensory irritation (points of departure, PODs)</b></p>	Formaldehyde	POD study-specific exposures	POD study-specific populations or models	Not applicable for this SQ	Identification of points of departure (in mg/m <sup>3</sup> ) based on one or more studies informative to quantifying sensory irritant responses and characterisation of the related uncertainties	<p>What are the best supported and most appropriate points of departure (PODs) across studies, based on a list of [specified] criteria given the available evidence on sensory irritation?</p>

## **Annex A – Template for EFSA protocols**

## **Annex B – Outcomes of the public consultation on the draft EFSA Scientific Committee guidance on protocol development for EFSA generic scientific assessments**

Annex A and B can be found in the online version of this output (in the 'Supporting information' section): <https://doi.org/10.2903/j.efsa.2023.8312>