SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 12 July 2017

doi: 10.2903/j.efsa.2017.4970

Guidance on the assessment of the biological relevance of data in scientific assessments

EFSA Scientific Committee,
Anthony Hardy, Diane Benford, Thorhallur Halldorsson, Michael John Jeger, Helle Katrine Knutsen, Simon More, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford, Antonia Ricci, Guido Rychen, Josef R Schlatter, Vittorio Silano, Roland Solecki, Dominique Turck, Maged Younes, Jean-Louis Bresson, John Griffin, Susanne Hougaard Benekou, Henk van Loveren, Robert Luttik, Antoine Messean, André Penninks, Giuseppe Ru, Jan Arend Stegeman, Wopke van der Werf, Johannes Westendorf, Rudolf Antonius Woutersen, Fulvio Barizzone, Bernard Bottex, Anna Lanzoni, Nikolaos Georgiadis and Jan Alexander

Abstract

EFSA requested its Scientific Committee to prepare a guidance document providing generic issues and criteria to consider biological relevance, particularly when deciding on whether an observed effect is of biological relevance, i.e. is adverse (or shows a beneficial health effect) or not. The guidance document provides a general framework for establishing the biological relevance of observations at various stages of the assessment. Biological relevance is considered at three main stages related to the process of dealing with evidence: Development of the assessment strategy. In this context, specification of agents, effects, subjects and conditions in relation to the assessment question(s): Collection and extraction of data; Appraisal and integration of the relevance of the agents, subjects, effects and conditions, i.e. reviewing dimensions of biological relevance for each data set. A decision tree is developed to assist in the collection, identification and appraisal of relevant data for a given specific assessment question to be answered.

Keywords: biological relevance, adverse effect, beneficial effect, size of the effect, nature of the effect, scientific assessment

Requestor: EFSA Scientific Committee

Question number: EFSA-Q-2014-00746

Correspondence: sc.secretariat@efsa.europa.eu

Suggested citation: EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Bresson J-L, Griffin J, Hougaard Benekou S, van Loveren H, Luttik R, Messean A, Penninks A, Ru G, Stegeman JA, van der Werf W, Westendorf J, Woutersen RA, Barizzone F, Bottex B, Lanzoni A, Georgiadis N and Alexander J, 2017. Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA Journal 2017;15(8):4970, 73 pp. https://doi.org/10.2903/j.efsa.2017.4970

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

The European Food Safety Authority (EFSA) requested its Scientific Committee to prepare a guidance document providing generic issues and criteria to consider biological relevance, particularly when deciding on whether an observed effect is of biological relevance, i.e. is adverse (or shows a beneficial health effect) or not.

The guidance clarifies a number of definitions and concepts (such as, responses of a biological system to exposure, mode of action and adverse outcome pathways, thresholds, critical effect, modelling approaches, biomarkers), which are central to biological relevance, in order to achieve that these concepts are used in a consistent way across EFSA areas of activity.

The list of generic issues (e.g. nature and size of the biological changes or differences, including the relevance of the biological systems where the effects are observed) to consider when deciding on whether an observed effect is biologically relevant should be applicable to all relevant EFSA Scientific Panels and Scientific Committee.

A framework was developed in which biological relevance is considered at three main stages related to the process of dealing with evidence. Development of the assessment strategy, should specify agents, effects, subjects and conditions in relation to the assessment question(s). The collection and extraction of data should follow identification of potentially biologically relevant evidence/data as specified in the Assessment strategy. The third step should include an appraisal and an integration of the biological relevance for each data set of the agents, subjects, effects and conditions. With regard to the agent, it should be considered whether the assessment is based on the agent of concern or on a surrogate agent. It should be considered whether the study is based on the subjects of concern (e.g. for human studies in humans) or in case proxies (e.g. animal models for humans) are used whether effects occurring are biologically relevant for the subject of concern. For the effects, consideration should be given as to whether the effect is causally related to exposure to the agent. The nature of the effect should be taken into account, i.a. homeostatic response, adaptive, adverse/beneficial or directly or indirectly linked to an adverse/beneficial effect. Finally, for effects, it should be assessed whether the magnitude of the effect is sufficient to be of biological relevance (e.g. outside normal variation, and for effects directly or indirectly linked to an adverse/beneficial effect whether they are of sufficient magnitude to result in an adverse/beneficial outcome). It should be noted that the biological relevance of an effect can vary according to the assessment question. For each data set, it should be considered whether the conditions of the studies (environmental, toxicological, epidemiological, etc.) used for the assessment are relevant for the conditions under consideration.

Each step of relevance considerations may be a source of uncertainty. The assessor should address these uncertainties during the assessment process as a part of the general uncertainty analysis of the assessment. Assessing biological relevance and associated uncertainty should be addressed and reported as part of the weight of evidence assessment, and its guidance on reporting can be applied.

Several case studies covering the various EFSA areas are referred to in the guidance and annexed to the opinion to illustrate how previous assessment may fit the proposed approach.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by EFSA

As per EFSA's Founding Regulation (EC) No 178/2002 of the European Parliament and of the Council, 'the EFSA Scientific Committee shall be responsible for the general coordination necessary to ensure the consistency of the scientific opinion procedure, in particular with regard to the adoption of working procedures and harmonisation of working methods'. The EFSA Science Strategy 2012–2016 echoes this key responsibility of the Scientific Committee by setting the development and harmonisation of methodologies and approaches to assess risks associated with the food chain as one of the four strategic objectives for the European Food Safety Authority (EFSA).

The recent opinion of the Scientific Committee (SC) on 'Priority topics for the development of risk assessment guidance by EFSA's Scientific Committee' (EFSA, 2013a,b) gives recommendations for the preparation of new or revision of existing guidance documents. The criteria for prioritising guidance documents to be developed are:

- Across Panel Relevance
- Critical importance including urgency of topic to be addressed for several Panels
- Topic not being addressed by an individual Panel
- Sufficient information available to develop meaningful guidance
- International dimension.

The development of guidance on biological relevance was identified by the EFSA SC as one of the three high priority topics for 2014.

In the EFSA opinion on the hazard assessment of endocrine disruptors (EFSA Scientific Committee, 2013), the concept of biological relevance assumes that a 'normal' biological state can be defined and the definition of normality is closely linked to adversity of an effect observed during toxicity testing or in epidemiological studies. Distinguishing adverse effects from physiological adaptive effects is not only crucial in identifying a No Observed Adverse Effect Level (NOAEL) from experimental toxicity studies but also when using the benchmark dose (BMD) approach as recommended by the SC (EFSA Scientific Committee, 2017).

In its opinion on biological relevance vs statistical significance, the EFSA SC gave a wider definition of biological relevance than just a modification of a physiological system, making it more applicable to the various EFSA working areas. In that opinion, biological relevant effect is defined as an effect considered by expert judgement as important and meaningful for human, animal, plant or environmental health. It implies a change that may alter how decisions for a specific problem are taken (EFSA Scientific Committee, 2011a,b). Different EFSA guidance documents are also addressing expert judgement approaches (EFSA, 2014a, 2017).

The above definition implies that guidance is provided to the various EFSA panels on what 'harm' means, and to define a number of related concepts such as 'effect size'. When a particular risk assessment considers several effects, the overall picture, using a multivariate approach, should be considered to decide whether the available body of knowledge allows to conclude on an effect to be adverse or not. Given the broad remit of activity of EFSA, the purpose of this self-task mandate is to provide the Scientific Panels with generic issues to consider when discussing on biological relevance, i.e. being adverse (or showing a beneficial health effect) or not.

Terms of Reference

EFSA requires its SC to prepare a guidance document providing generic issues and criteria to consider when deciding on whether an observed effect is of biological relevance, i.e. is adverse (or shows a beneficial health effect) or not.

The opinion should clarify a number of definitions and concepts, such as, adverse, adaptive, harm, homeostasis, biological threshold in order to achieve that these concepts are used in a consistent way across EFSA areas of activity.

The list of criteria/generic issues (e.g. nature and size of the biological changes or differences) to consider to decide whether an observed effect is biologically relevant should be applicable to all relevant EFSA Scientific Panels and Scientific Committee.

Several case studies covering the various EFSA areas will be annexed to the opinion to illustrate the proposed approach.

Links should be established with related ongoing EFSA activities, particularly with the SC working group on weight of evidence, the activity of the Assessment and Methodological Support (AMU) Unit on promoting methods for evidence use in scientific assessments (PROMETHEUS), and the SC working group on uncertainty in risk assessment. Relevant international activities and developments in the area, such as the IPCS/WHO mode of action framework should also be considered.

In view of the horizontal aspect of this topic and the need to get a common agreement and understanding of what biological relevance means, representatives of EFSA sister agencies, European Commission non-food committees and international bodies (e.g. WHO) should be invited to participate in the working group.

1.2. Interpretation of the Terms of Reference

When addressing the mandate, the SC acknowledged that the issue of biological relevance in risk assessment has a broader meaning than biologically relevant effect as described in the Terms of Reference. In fact, it encompasses also aspects related to the definition of the problem formulation. This, in turn, guides the development of the assessment strategy, which includes the decision on which data to use for the assessment (relevance of the data).

Aspects related to the reliability of the various pieces of evidence used in the assessment are outside the scope of this mandate, as they are the subject of another SC guidance on weight of evidence (EFSA, 2017).

The purpose of this document is to discuss and provide guidance across Panels/Units of EFSA on the above-mentioned issues and how they should be addressed during the risk assessment process.

1.3. Relation to other relevant EFSA guidance documents

The guidance on the use of the weight of evidence (EFSA, 2017) builds on the conceptual approach for scientific assessments as described in PROMETHEUS (EFSA, 2015a), which describes the overall process for dealing with data and evidence. The process has four steps as shown in Figure 1 and biological relevance may need to be assessed in every step:

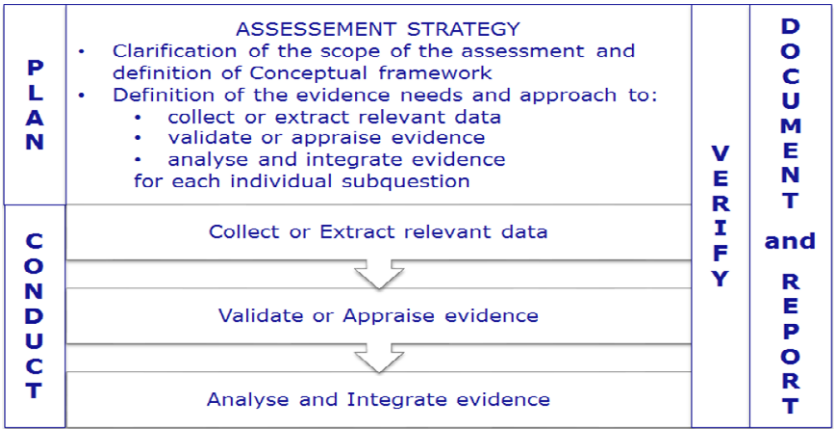

Figure 1: The process for dealing with data and evidence when conducting an assessment (EFSA, 2015a)

Transparent reporting of all assumptions and methods used, including expert judgement, is necessary to ensure that the assessment process leading to the conclusions is fully comprehensible.

'Open EFSA' aspires both to improve the overall quality of the available information and data used for its scientific outputs and to comply with normative and societal expectations of openness and transparency (EFSA, 2009a, 2014a–e). In line with this, EFSA is publishing three separate but closely related guidance documents to guide its expert Panels for use in their scientific assessments (EFSA, 2015a). These documents address three key elements of the scientific assessment: the analyses of Uncertainty, Weight of Evidence and Biological Relevance.

The first document provides guidance on how to identify, characterise, document and explain all types of uncertainty arising within an individual assessment for all areas of EFSA's remit. The Guidance does not prescribe which specific methods should be used from the toolbox but rather provides a

harmonised and flexible framework within which different described qualitative and quantitative methods may be selected according to the needs of each assessment.

The second document on weight of evidence provides a general framework for considering and documenting the approach used to evaluate and weigh the assembled evidence when answering the main question of each scientific assessment or questions that need to be answered in order to provide, in conjunction, an overall answer. This includes assessing the relevance, reliability and consistency of the evidence. The document further indicates the types of qualitative and quantitative methods that can be used to weigh and integrate evidence and points to where details of the listed individual methods can be found. The weight of evidence approach carries elements of uncertainty analysis: that part of uncertainty which is addressed by weight of evidence analysis does not need to be reanalysed in the overall uncertainty analysis, but may be added to.

This document provides a general framework to addresses the question of biological relevance at various stages of the assessment: the collection, identification, and appraisal and integration of relevant data for the specific assessment question to be answered. It identifies generic issues related to biological relevance in the appraisal of pieces of evidence, in particular, and specific criteria to consider when deciding on whether or not an observed effect is biologically relevant, i.e. adverse (or shows a beneficial health effect). A decision tree is developed to aid the collection, identification and appraisal and integration of relevant data for the specific assessment question to be answered. The reliability of the various pieces of evidence used and how they should be integrated with other pieces of evidence is considered by the weight of evidence guidance document (EFSA, 2017).

EFSA will continue to strengthen links between the three distinct but related topics to ensure the transparency and consistency of its various scientific outputs while keeping them fit for purpose (Figure 2).

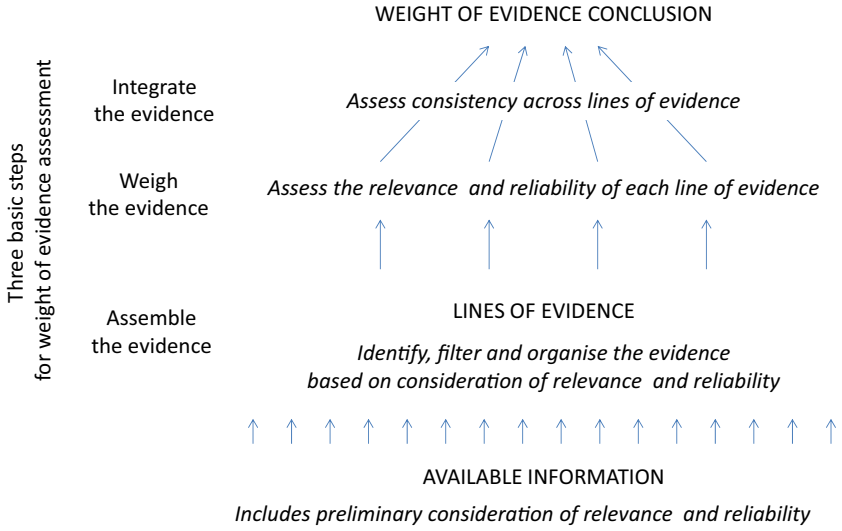

Figure 2: Relationship of relevance (including biological relevance), reliability and consistency to the three basic steps of weight of evidence assessment and to the conclusion for a weight of evidence question

1.4. Audience and degree of obligation

This Guidance is aimed at all those contributing to EFSA assessments and provides a harmonised, but flexible framework to determine biological relevance that is applicable to all areas of EFSA's work and all types of scientific assessment. In line with improving transparency (EFSA, 2006, 2009a) and reporting (EFSA 2014b, 2015a), the SC considers the application of this guidance to be unconditional for EFSA. Each assessment must clearly and unambiguously document:

- what evidence was considered;
- how the evidence was weighed and integrated in terms of relevance.

The document provides guidance on the general principles to determine the biological relevance but assessors have the flexibility to choose the degree of refinement in applying them. The SC considers that these should be fit for the purpose of the scientific assessment.

2. Data and methodologies

The process for dealing with data and evidence in an assessment as defined in the PROMETHEUS project deliverable 1 (EFSA, 2015a) was used as a framework for developing a guidance on biological relevance. A fundamental step in this process is represented by planning a strategy for the assessment including:

- the problem formulation;
- the conceptual framework;
- the definition of the evidence needs; and
- the approach for:
  - Collecting or extracting relevant data;
  - Validating or appraising evidence;
  - Analysing and integrating evidence.

In line with the 'Open EFSA' objective (i) to improve the overall quality of available information and data used for its outputs and (ii) to comply with normative and societal expectations of openness (EFSA, 2014b, 2015a), a targeted consultation of national and international scientific advisory bodies was organised on an EFSA Journal editorial presenting 'Increasing robustness, transparency and openness of scientific assessments' and a document providing the individual background and terms of reference of four related activities:

- the PROMETHEUS ('PROmoting METHods for Evidence Use in Scientific assessments') project which aims to further improve the methods for 'dealing with data and evidence' (i.e. collecting/extracting, validating/appraising, analysing and integrating data and evidence) in EFSA scientific assessments and to increase their consistency.
- Three topics for guidance developments: (i) the identification of biological relevance of adverse/beneficial health effects from experimental animal and human studies; (ii) the use of the weight of evidence in scientific assessments; (iii) the characterisation of uncertainties in scientific assessment.

A workshop was then organised on 29 and 30 June 2015 in Brussels to consult with national and international bodies including European Agencies, European Commission Scientific Committees, national agencies and international bodies with an interest in biological relevance. One objective of the workshop was to present the terms of reference of the two SC working groups on weight of evidence and biological relevance, clarify the objectives and the scope of the resulting guidance documents, and capture from the audience relevant work that should be considered by the working groups when drafting the guidance.

A Working Group composed of Panel Experts and EFSA Staff representing all EFSA areas of activity was created to address the above mandate. Members of the working group were first asked to describe in short documents how biological relevance was considered in past assessments (see Annexes). Key concepts and definitions that came out of these examples or that came out from the above consultations and that one should have in mind when considering the relevance of a data set or a piece of evidence for an assessment, have been organised into a conceptual framework and are further described in the following sections.

3. Biological Relevance – Related Concepts and Assessment Framework

The assessment strategy should specify which scientific evidence (data) would be relevant for answering the assessment question(s). However, relevance can only be determined if the question(s) for assessment is well-defined. It is therefore important to ensure a clear understanding and interpretation of the question(s) for assessment between the risk assessors and with the risk manager before developing the assessment strategy.

Having clarity on the assessment question(s) would provide guidance on how to decide which data are relevant. This is important because it identifies at an early stage what should be included or can be excluded from the assessment: which effects, which data, which model, which parameters, etc. Note that if an endpoint has relevance, then all studies testing for that particular endpoint become relevant, as both positive and negative findings may influence the answer to the assessment question.

When the relevant data have been collected, the biological relevance of the effects in each data set should then be appraised.

It is important to note that the reliability of the various pieces of evidences used and how they should be integrated with other pieces of evidence in the assessment are outside the scope of this SC guidance on relevance, as these are the subject of another SC guidance on Weight of Evidence (EFSA, 2017).

In the following text, some fundamental concepts related to biological relevance will be presented followed by a framework for consideration of relevance. This includes considerations to be done at the three steps:

- Development of the assessment strategy, in this context, specification of agents, effects, subjects and conditions.
- Collection and extraction of data, i.e. identification of potentially biologically relevant evidence/data as specified in the Assessment strategy.
- Appraisal and integration of the relevance of the agents, subjects, effects and conditions, i.e. reviewing dimensions of biological relevance for each data set.

3.1. Concepts about biological relevance

In 2011, the SC expressed an opinion addressing the concept of Statistical Significance and Biological Relevance in the context of assessing scientific evidence. The following definition of biological relevance was developed:

> *A biologically relevant effect can be defined as 'an effect considered by expert judgement as important and meaningful for human, animal, plant or environmental health. It therefore implies a change that may alter how decisions for a specific problem are taken' (EFSA, 2011).*

The guidance, in which this definition of Biological Relevance was developed, stressed that a statistically significant effect should not automatically be considered relevant for the outcome of an assessment, but that an independent evaluation of the effects as to its relevance was required. In this context, the definition implies that all effects that directly or indirectly would have the potential to influence the outcome of the assessment should be considered.

3.1.1. About responses of a biological system to exposure

A biological system usually reacts to signals from its environment, including the agent (e.g. chemical substance, nutrient, microorganism, pathogen or invasive species) under assessment. The quality of the response of the biological system, hereafter called the nature of the effect, can be either **adverse**, **adaptive** or **beneficial** and may occur at different levels, e.g. molecular, cell, organ, individual, population or ecosystem.

Adverse effects

An effect is considered 'adverse' when leading to a *change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity to compensate for additional stress or an increase in susceptibility to other influences (WHO, 2009).*

The SC acknowledges that this definition is very broad and that the relationship between the measured endpoint and the change in the functional capacity may be difficult to establish. The following text aims to provide some guidance on assessing adversity in the context of biological relevance of an effect.

An adverse effect might be primary (directly induced by the agent) or secondary (e.g. related to stress or nutritional imbalance induced by the agent) (Lewis, 2002). It is important to distinguish this for the interpretation of the effect in the context of the assessment question.

Reversibility and recovery

Adverse does not necessarily mean irreversible. An adverse change might be reversible. Whether adverse findings are reversible can be evaluated in an animal test model if animals are allowed to recover after an appropriate non-dosing period. For example, adverse changes in regenerating tissues can recover (effects on spermatogenesis can lead to the non-function of the genital system and lack of the possibility to reproduce; but recovery can happen after a withdrawal of the exposure) (Perry et al., 2013).

In environmental risk assessment, the concept of environmental harm is used, which is defined as the measurable adverse change in a natural resource or the measurable impairment of a natural resource service. It may occur directly or indirectly (European Commission, 2004), or as a measurable (or otherwise observable) loss or damage that has adverse (and significant) impact upon conservation and sustainable use of biodiversity (CBD, 2010).

The concept of 'recovery' is also used in environmental risk assessment: the return of the perturbed (ecological) endpoint (e.g. species composition, population density) to the window of natural variability as observed in the undisturbed state of the (eco)system of concern (e.g. before the stressor event took place), or to the level that is not significantly different anymore from that in control or reference systems. It should be noted that a system that has been subject to an adaptive response or to recovery might not necessarily return to the same state that it exhibited before the disturbance (EFSA, 2016a–e).

It should be noted that between reversibility on the individual level and population recovery, there is also population relevance. As an example, small effects on fecundity in density regulated systems (e.g. fish reproduction with many more eggs than can possibly develop into juvenile fish) will not translate to the population level; as there is no effect on the population level, there is no need for recovery (e.g. Hamilton et al. 2014; https://doi.org/10.1186/1741-7007-12-1).

Homeostatic capacity

When subject to a disturbance, a biological system enters in a transient state: a process variable has been changed and the system has not yet reached steady state. Some systems, including humans, have the capacity to regulate their internal environment and to maintain a stable, relatively constant condition of properties; it is called 'homeostatic capacity'. Resilience represents the amount of disturbance that can be absorbed by a system before the system changes or loses its normal function, or the time taken to return to a stable state, within the normal operation range following the disturbance (Gunderson, 2000). The homeostatic capacity varies. Reducing that capacity might be detrimental, whereas increasing the capacity could be beneficial. In order to assess homeostatic capacity, a comprehensive analysis of multiple parameters (biomarkers) will have to be undertaken (Pellis et al., 2012; Kardinaal et al., 2015).

Adaptive effects

The response to exposure to an agent can be 'adaptive', i.e. involving a process whereby a cell or organism respond to an agent so that the cell or organism will survive in the new environment that contains the agent without impairment of function (Keller et al., 2012). An adaptive response can be an active regulation of a parameter to keep it within it physiological range, i.e. be a homeostatic response (e.g. glycaemic regulation, body temperature regulation). Alternatively, an adaptive response can occur outside physiological boundaries and may eventually become adverse; therefore, it requires further considerations as to its adversity (e.g. composition of gut microbiota, liver enzyme induction). This issue is also discussed in Annex I, regarding chemicals that may affect thyroid hormone regulation).

Beneficial effects

An effect is considered 'beneficial' if it has the probability to be linked to a positive (health) effect (e.g. increase the resilience of the organism to a certain challenge) and/or the probability to be linked to a reduction of an adverse health effect in an organism, system or (sub)population, in reaction to exposure to an agent (EFSA, 2016d). The relevance of biological outcomes in terms of benefits follows similar rules as those for adverse outcomes. Yet, it should be noted that for adverse outcomes, very often data from animal test systems or in vitro studies are used. For (health) benefits, studies on the target species and population group are required, e.g. studies in humans in the case of health claims for food, whereas studies other than in the target species, are used as supportive evidence (see Annex G). In benefit assessment, EFSA is normally with the exception of certain agricultural products and processes (e.g. growth promotion of animals) not considering economic aspects. Such benefits may not necessarily be beneficial for the health of the target species (see Annex E).

3.1.2. About Mode of Action and Adverse Outcome Pathway

When an agent (e.g. chemical) causes an adverse effect in an organism, the effect is often a result of a sequence of events starting with a molecular interaction between the agent and the organism,

which might differ between species. To what extent a molecular effect should be considered biologically relevant in the target species depends on whether and how close it might be linked to an adverse outcome in an appropriate test system, either as a key event or indirectly having an impact on a key event in the in the sequence leading to an adverse outcome. Mode of Action (MoA) and Adverse Outcome Pathway (AOP) are related concepts used in this context, although the latter is still in its infancy.

The definition of MoA has evolved over time and derives from earlier works by the US EPA (1986, 2005) and the WHO. MoA analyses have been applied to a number of case studies for non-genotoxic and genotoxic chemicals (Boobis et al., 2006, 2008a). The current WHO definition for MoA is 'a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data'. MoA describes key cytological and biochemical events – that is, those that are both measurable and necessary to obtain the observed effect – in a logical framework (Boobis et al., 2008a,b; WHO, 2009; Meek et al., 2014). In many cases, also the magnitude of the effect might be critical for the determination of its biological relevance (see also Section 3.2.3).

In the US, MoA has been used as a term to reference a mechanistic understanding of the impact of a chemical on human health. Toxicologists would also refer to the same concept using the terms 'toxicity pathway, MoA, adverse outcome pathway or mechanism of action' as used by the National Research Council (NRC) report, Science and Decisions: Advancing Risk Assessment (2009) (NRC, 2009) and the Nextgen report of the US-EPA (2013). Modified Bradford Hill criteria (Meek et al., 2014) can be used to analyse the biological plausibility of key events and the weight of the related evidence. Mechanism of action is defined as the specific biochemical interaction through which a substance produces an effect on a living organism or in a biochemical system (WHO/IPCS EHC 240). MoA does not imply full understanding of mechanism of action, which refers to a detailed molecular description of individual biochemical and physiological key events leading to a toxic effect (Boobis et al., 2008a,b; WHO, 2009; EFSA, 2008).

Information from MoA can be used to establish AOPs. An AOP is defined as the information on the causal links between a molecular initiating event (MIE), intermediate key events (KEs) and an adverse outcome (AO) of regulatory concern. 'a sequence of events from the exposure of an individual or population to a chemical substance through a final adverse (toxic) effect at the individual level (from a human health perspective) or population level (from an environmental perspective)' (Ankley et al., 2010; Meek et al., 2014; OECD, 2013). Such key events should be definable and make sense from a physiological and biochemical perspective and in a toxicity pathway. Early key events including the MIE have been defined by the OECD as the 'initial point of chemical–biological interaction within the organism that starts the pathway' (OECD, 2013). An example of developing an AOP can be found at https://www.efsa.europa.eu/en/efsajournal/pub/4691.

Although not systematically developed for beneficial effects, the concepts of MoA is applicable also to beneficial effect of an agent (e.g. chemical, nutrient or additive). Examples of this are use of mechanistic and physiological information in the establishment of Dietary Reference Values (DRV) and assessments of health claims on food and food ingredients (for examples see Annex G), and in efficacy assessments of feed additives (see Annex E).

Likewise, information on MoA, i.e. pathogenic mechanism, of a microorganism, pathogen or invasive species are equally useful in the assessment of the effects of these types of agents (see e.g. Annex C).

3.1.3. About thresholds

The term 'threshold' has a variety of different meanings, depending on the context in which this term is used.

As a matter of principle, the absence of an effect can never be proven experimentally and thus the existence of a 'true' threshold in the mathematical sense is not possible. The WHO (2009) defines threshold as 'Dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur'. The WHO defines the threshold dose as 'The dose at which an effect just begins to occur—that is, at a dose immediately below the threshold dose, the effect will not occur, and immediately above the threshold dose, the effect will occur. For a given chemical, there can be multiple threshold doses, in essence one for each definable effect. For a given effect, there may be different threshold doses in different individuals. Further, the same individual may vary from time to time as to his or her threshold dose for any effect. For certain chemicals and certain toxic effects, a threshold dose may not be demonstrable. The threshold dose will fall between the experimentally

determined no-observed-(adverse-)-effect level and the lowest-observed-(adverse-)effect level, both of which have been used by different scientific groups as a surrogate for the threshold dose in the performance of risk assessments'. Note that the EFSA SC recommends the use of the BMD approach instead of the NOAEL for scientific assessment purposes (EFSA, 2017).

The WHO definition of a threshold dose indicates that there is no fixed value for a threshold. This applies both for the chemical as well as for the exposed individual. The discussion whether thresholds (experimental, mathematical, biological or 'true') exist or not and at what level (biochemical, individual or population level) does not solve the problem that when the dose decreases, the dose–response curve becomes indistinguishable from the background response at a certain point and the shape of the dose–response curve remains unknown thereafter as this dose range becomes experimentally inaccessible or non-observable. This point is largely dependent on the nature and the design of the study and its power to detect any effects. A biological threshold in this sense does not indicate a dose below which any response is zero, but a dose, below which the response may be considered to be biologically irrelevant provided sufficient power of the study.

Furthermore, thresholds may be discussed at different levels, e.g. at the molecular, cell, organ, individual, population or ecosystem level (Slob, 1999). It is important to note that chemical risk assessment in the regulatory context usually addresses risks at the population level. Even if a particular threshold would exist at a certain level (e.g. inhibition of a single enzyme molecule), it may be different or even no longer exist at higher levels of biological systems (e.g. at the cellular level with a population of enzyme molecules, at the organism level with populations of cells or at the level of population of organisms) because the resulting dose response relationship is the result of a set of dose response curves.

Thus, dose–response assessment is performed preferably in a probabilistic way (EFSA, 2017).

3.1.4. About critical effect

In toxicological risk assessment, the critical effect is defined as the toxic or adverse effect occurring at the lowest dose of an agent. The critical effect level is the dose at which the critical effect starts to occur when increasing the dose and is linked to the size or magnitude of the effect, i.e. when it can be identified. The determination of a critical effect size[1] is strictly related to its natural background variability, which might be determined using historical control data and when it becomes distinguishable from the background variability.[2]

Ecotoxicological risk assessment is also often based on the toxic effect occurring at the lowest dose or concentration. A proxy for the critical effect level in environmental risk assessment is the use of the No Observed Effect Dose (NOED) or No Observed Effect Concentration (NOEC) for these effects, but there are a number of exceptions (see Annex K).

Assuming that temporal fluctuations in physiological parameters (e.g. haematology, biochemistry) in individual healthy non-exposed animals are non-adverse, the minimal magnitude of the Critical Effect Size (CES) fold change above background for a number of continuous parameters of toxicity studies can be derived (Buist et al., 2009). If this background variability is exceeded, this can be considered as a relevant effect size for this endpoint. The background variability of a parameter may differ between individuals and between an individual and a population.

The size of an effect that would be considered biologically relevant should ideally be considered before answering the assessment question. (see also EFSA, 2017, chapter 2.5.2). Once this has been determined, a power analysis of key studies could be performed as this has implications on how the reported effects and effect estimates from those studies are interpreted. Underpowered studies may both fail to detect an effect with a relevant size and be more likely to inflate true effects or report spurious effects (Ioannidis, 2005, 2008; La Caze and Duffull, 2011). In addition, it is important to keep in mind that 'Statistically significant' does not necessarily mean 'important', 'meaningful' or 'biologically relevant' as it is sometimes misinterpreted. 'Statistically significant' just means that the observed effect size or difference is unlikely to have occurred by chance; it is a statistical statement on the property and information content of the observed data (EFSA, 2011). In other words, a statistically significant effect may exist, but may be biologically irrelevant because, although statistically significant, it is smaller than the predefined biologically relevant effect size, which can be defined based on its

[1] The term critical effect size may have different terminology, e.g. BMR or Limit of Concern (LoC), depending on the field of investigation.

[2] If it is a biomarker and not an adverse effect in itself, exceedance of background variability may not be a sufficient criterion to decide on adversity.

background variability. Conversely, lack of statistical significance should not be the sole rationale for concluding a lack of exposure related effect, just as statistical significance should not be the sole justification for concluding on the occurrence of a treatment-related effect (OECD, 2007).

An example of an experiment where a statistical significant treatment-related effect falls within the variability of the historical control data according to prior knowledge and might be considered as irrelevant for risk assessment, is given in Figure 3. The left point in Figure 3 is the mean value observed in the control group. Note that in this case the control value is at the low end of the background variability of the control group and although the middle point (value) is statistically significantly different from the control outcome, it is still within the background variability. Assuming that the natural variability among the animals used in this study (Figure 3) was by chance low, then when re-conducting the experiment, the variability would most likely be larger. The expected consequences would be that the lowest exposed group may no longer be significantly different from the controls. In that case, the right, but not the mid-point, would be the Lowest Observed Effect Concentration or Level (LOEC/LOEL).

Also, in the guidance document on toxicity endpoints from avian and mammalian reproductive toxicity studies (EFSA, 2009c), it is mentioned that although the magnitude of an endpoint in an exposed group could be statistically significantly different from that of the controls, it might not be biologically relevant. The following is a quote of this document: 'In order to determine the biological relevance of an effect it should be considered whether the effect could lead to a functional deficit later on in the study, e.g. if a reduction in the weight of pups at birth leads to a decrease in level of survival. If not, then the effect may not be biologically relevant, however if there is a carry-over of effects into the number of survivors, it can be considered biologically relevant'. That guidance document also provides more information for dealing with dose–response relationships (see chapter 2.3.1 Determining toxicity endpoints from avian and mammalian reproductive toxicity studies (EFSA, 2009c)).

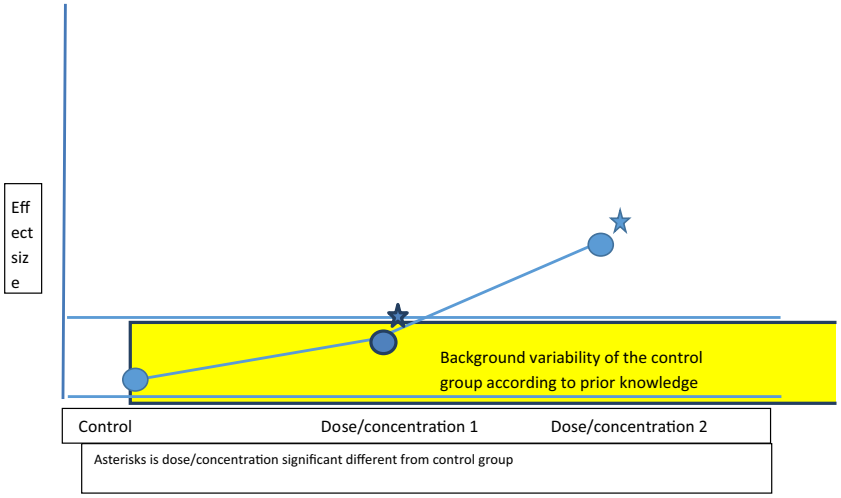

Figure 3: An example showing an experiment where a statistically significant treatment-related effect falls within the background variability for the control group according to prior knowledge and might be considered as irrelevant for risk assessment

3.1.5. About modelling approaches

In many risk assessments conducted by EFSA, modelling approaches related to biological relevance are used:

- to predict the value of endpoints, relevant to the assessment question, which cannot be measured at present time (e.g. spread of pathogens, see Annex A and Annex H);
- to estimate the value of biomarkers relevant to the assessed endpoints which cannot be measured directly, for instance as done in the example of setting DRV in Vitamin D (Annex G);
- to estimate reference points for hazard characterisation in toxicological risk assessments by BMD Modelling (EFSA, 2017)
- to assess large-scale or long-term effects on the biological system (Annex K);

- to extrapolate the outcomes of the risk assessment to various populations/receiving environments (Annex K);
- to assess the implications of uncertainties/assumptions on the outcomes of the risk assessment, for example sensitivity analysis;

Such models are quantitative or qualitative. As far as they are based on sound approaches and explicit assumptions, they can help risk assessors in understanding whether an effect size would be biologically relevant in various contexts (populations/ecosystems) and help risk managers make decisions. For further information on good modelling practice (see EFSA PPR Panel, 2014).

3.1.6. About biomarkers

Biological measurements are routinely carried out in studies on the interaction between an agent and a biological system. A wide range of such measurements are called biomarkers. The nature of these biomarkers is different and WHO identified three different classes of biomarkers: biomarker of exposure, biomarker of effect and biomarker of susceptibility (WHO, 1993).

For chemical agents, a biomarker of exposure is defined as 'an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism'. Urine, blood, faeces or nails are common media for the measurements of biomarkers of exposure.

A biomarker of effect is 'a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease'. In relation to toxicity testing, it is important to note that a biomarker of effect provides information for an effect, but does not necessarily discriminate between adverse and non-adverse effects (Blaauboer et al., 2012). Its biological relevance depends on its relation to MoA of an adverse effect or an adverse outcome pathway.

A biomarker of susceptibility is 'an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance' (WHO, 1993).

A biomarker can also be associated with a reduction in resilience capacity, or health improvement or improved resilience or maintenance of health of effect can be a single measure, but can also be a standardised and defined composite of different measures that together function as a biomarker of effect.

3.2. Framework for consideration of 'relevance'

In the framework presented below (Figure 4), biological relevance is considered at three main stages related to the process of dealing with evidence:

- Development of the assessment strategy, in this context, specification of agents, effects, subjects and conditions.
- Collection and extraction of relevant data, i.e. identification of biologically relevant evidence/data as specified in the Assessment strategy.
- Appraisal and integration of the relevance of the agents, subjects, effects and conditions, i.e. reviewing dimensions of biological relevance for each data set.

In the course of the assessment, it might become apparent that additional data would be of relevance for the assessment and the process has to be reiterated.

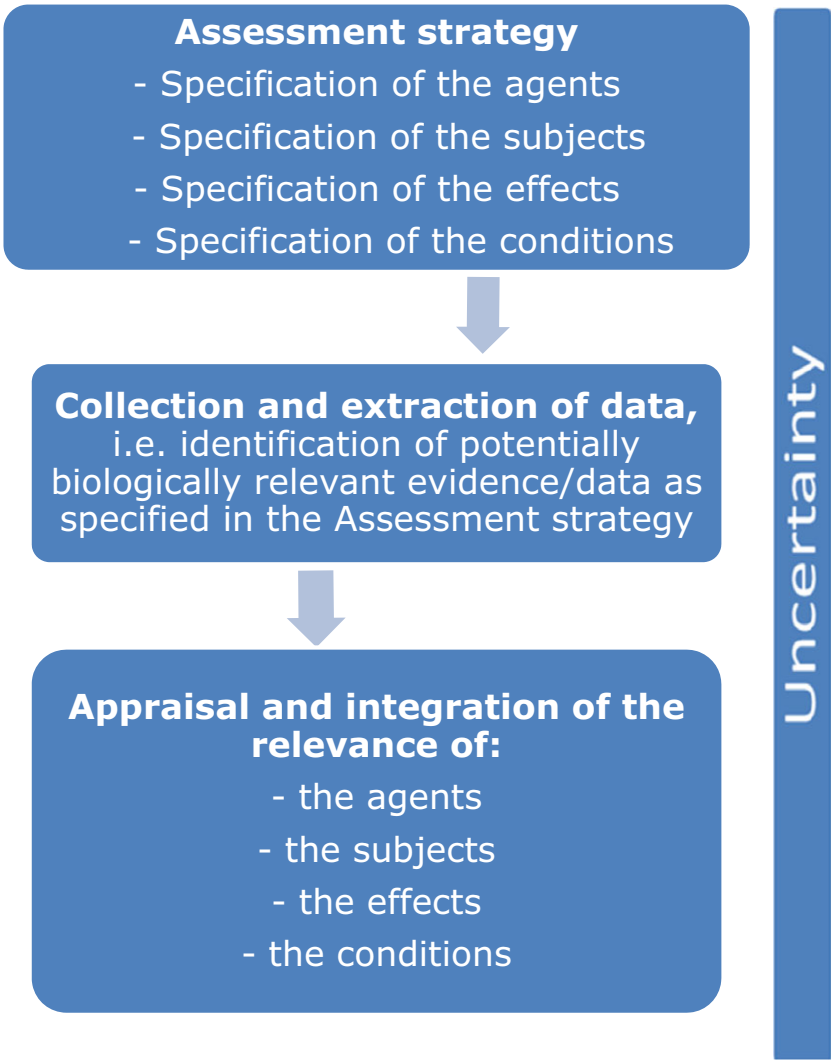

Figure 4: A framework of consideration of biological relevance at three main stages related to the process of dealing with evidence

3.2.1. Development of the Assessment strategy and relation to biological relevance

The assessment strategy should ensure that the assessment will answer the assessment question (s). When developing the assessment strategy, the scope and objectives of the risk assessment should be carefully considered, and if necessary, clarified with the requestor. Further, if any questions arise in the course of the assessment regarding the objective and scope of the assessment, they require immediately to be addressed.

A number of relevance-related considerations should take place in the course of developing the assessment strategy. One of the main considerations is to identify and specify biological relevant data, before initiating the data collection. The following considerations depend on prior knowledge:

- Which is/are the **agent(s)** of interest for the assessment or activity assessed?
- What is/are the **subject(s)**, population(s) that should be covered by the assessment, are there any subgroups or sub-population particularly relevant that the assessment should address more specifically? Are there some specific levels of protection (e.g. 95–99% of the target population) to be considered? For environmental risk assessment, this issue is translated into the concept of protection goal (see glossary and EFSA 2016b)?
- What is/are the **effect(s)** associated with the exposure to the agent(s) that is/are considered as relevant for the assessment question?

- What are the relevant **condition(s**) regarding the exposure to the agent(s): route of exposure, exposure duration, timing of exposure etc.?

In standardised assessments, i.e. assessments that strictly follow guidelines or guidance documents, the assessment questions are generally already defined in a standard form, and there is also a standard procedure for assessing them. Sometimes also regulations prescribe what kinds of data are needed for the assessment. The standardised questions and procedures are part of what Codex (2015) refers to as 'risk assessment policy', defined as 'Documented guidelines on the choice of options and associated judgements for their application at appropriate decision points in the risk assessment such that the scientific integrity of the process is maintained'. This defines what questions are relevant to a class of assessments, and what effects, data and analysis are relevant for assessing them. It would also define what kind of biological data and effects that are relevant. According to the Codex, (2015),[3] for standardised assessments, it is sufficient to confirm that the question defined by risk assessment policy is relevant to the case at hand. Where it is not, the assessor needs to interpret the terms of reference and define relevant assessment questions in consultation with the decision-maker (Codex, 2015),[3] which in effect establishes the assessment strategy specific to the case in hand. The assessor then has to decide on what kind of biological data and effects would be relevant for the outcome of the assessment.

In many areas of EFSA's work where there are not standardised procedures, current practices for conducting common types of assessments have developed, for which an assessment strategy may be predetermined and documented, e.g. in guidance documents. This may imply that specific studies and data as well as specific outcomes are considered relevant.

When using standardised procedures or current practices have developed, it is necessary to confirm that the procedure is relevant for the assessments at hand, but not necessary to reconsider the relevance of every element of the procedure. Hence, it is essential to recognise that not all the considerations of specifying biological relevant data have to be done *de novo* for every assessment.

In cases where a standard procedure is not fully relevant for answering the questions asked by the requestor, the assessment becomes case-specific and the relevance of each element will need to be considered. This is consistent with the concept of standardised and case-specific assessments in the draft guidance on uncertainty (EFSA, 2016c).

3.2.2. Collection and selection of the biologically relevant data according to specifications

The relevance-related considerations described in the previous section on the development of the assessment strategy specify which evidence is relevant or irrelevant for the assessment and needed for answering the assessment questions with the minimum possible uncertainty. The assessment strategy should also serve as a basis for defining the protocol/strategy for data collection.

Following the application of the protocol/strategy for data collection, all the data and information collected should be evaluated for their relevance for the assessment (EFSA, 2017 WoE).

Data of low quality should not be *a priori* considered irrelevant and excluded, as they may contain information important for the assessment. Instead, their implications should be considered, while taking into account the limited quality and associated uncertainty. The criteria for inclusion/exclusion of data should be explained and described within the risk assessment. If data are excluded, this should be stated in the opinion along with the rationale for their exclusion.

It should be acknowledged that in the case of 'standardised' assessments, the relevance of some evidence or data to be considered for the risk assessment is preset.

The legislation can also pre-determine the level of relevance of some of the evidence. This is the case for example for the validation of health claims where human data are considered as relevant to demonstrate and conclude on a positive effect, while other types of data (animal, *in vitro* or *in silico*) are only considered as supportive evidence. For essential nutrients, conclusions may be based on generally accepted knowledge. See also Annex G.

[3] Codex (2015) states that the risk manager should establish risk assessment policy before risk assessment, in consultation with the risk assessor and other interested parties. In current practice for EFSA assessments initiative for this tends to lie with the assessor but, in principle, the decision-maker is responsible and should at least confirm their agreement.

3.2.3. Appraisal and integration of each data set collected

Reviewing dimensions of biological relevance for each data set

To review the relevance of a particular data set, the assessor should go back to the relevance-related considerations to answer the assessment questions that have been identified during the problem formulation phase and development of the assessment strategy (see Section 3.2.1):

a) Relevance of the agent

The assessor should consider whether the data set or the study under consideration provides evidence directly on the agent subject to the assessment (e.g. nutrient, chemical substance, microorganism, pathogen or invasive species).

Studies on similar (surrogate) agents that provide indirect evidence on the agent of interest might have a certain amount of relevance to answer the assessment question; the fact that they do not address the agent of interest itself should be considered a relevance-related uncertainty and further characterised in term of impact on the assessment outcome (see Section 3.2.4).

For instance, when developing a farm-to-farm spread model in the case of the EFSA Scientific report 'Schmallenberg virus: State of the art' (EFSA, 2014c) data on the related to Bluetongue virus were used for certain parameters in case data on Schmallenberg virus were lacking.

For chemical agents, examples are the use of a structural analogue (QSARs or read-across), or a metabolite, or a precursor, or a pure compound for a formulation).

A spectrum of strains is responsible for classical scrapie in sheep, and there may be variability in properties that affect the ability to cross the human species barrier. In a study by Cassard et al. (2014), mice models were used to evaluate the zoonotic potential of classical scrapie (See Annex C). Six different isolates of classical scrapie were used. The BIOHAZ Panel concluded that the isolates used in the study were relevant for the problem under investigation. However, evidence derived from a limited number of classical isolates cannot be extrapolated to represent the whole biological variability of classical scrapie.

b) Relevance of the effect (nature and size)

For each effect, the first step is to determine whether it is causally related to the exposure or treatment (for instance according to the Bradford–Hill criteria that were developed for determining the relationship between exposure and disease) (Hill, 1965).

In the context of causality between exposure and effect, some considerations could be:

- Is the effect dose related?
- Is there potential confounding? Is the effect a result of a confounding variable (an extraneous variable that correlates with both the exposure and the effect)?
- Does the exposure precede the response according to a plausible time scale?
- Is the effect biologically plausible; is there any information on the Mode of Action?

The objective of the next step is to determine whether the observed effect, in its nature and size, is relevant for the assessment question.

A wide range of assessment questions are considered by EFSA panels relating to many different agents such as nutrient, chemicals, microorganisms, pathogens, invasive species or inserted gene elements. Also, the target biological system (e.g. human, animals, ecosystems) assessed vary widely between EFSA Panels. Hence, a large variability in the effects caused by the agent can be expected. The scheme outlined below may be relevant to the assessment of chemical substances, but should also be applicable to other agents.

Hence, as a first step considering biological relevance, the assessor has to take into account the nature of the effect caused by the agent (e.g. nutrient, substance, microorganism, pathogen or invasive species) when addressing the assessment question. In this context, the assessor may need to determine whether the effect in itself is adverse or beneficial and, if not, whether it might be related to such an outcome. Size or magnitude of the effect may be important and is the other dimension to be considered when assessing the relevance of an effect.

A number of questions can help to decide on the (non-)relevance of the effect (see Figure 5):

1) Is the effect (in itself) an adverse or a beneficial effect (see Section 3.1.1)?

- Is the nature of the effect such that it is clearly adverse according to the WHO definition or beneficial (see Section 3.1.1). For continuous data, this may also be a quantitative question related to the size of the effect, which then have to be considered in a next step.

- Does the effect represent an adaptive response (e.g. glycaemic regulation, body temperature regulation)? If so, is it within the homeostatic capacity of the organism or system? For continuous data, this may be a quantitative question related to the size of the effect (see also Section 3.1.1).
- Does the effect represent an adaptive response of a non-adverse nature? An example of such a response is the caecum enlargement, which is commonly seen in rodents as a result of a fibre rich diet. Another example is the stimulation of the immune system following exposure to microorganisms. One criterion to decide on a potential adverse effect is whether or not the effect seen occurs in isolation, e.g. without pathological changes. (see also Section 3.1.1)?
- An example of a beneficial effect is supporting defence against pathogens in the upper respiratory tract, as measured by episodes of common cold and therefore biologically relevant (see Annex G). The aim of the immune system is defence to pathogens, hence in case an agent helps to support defence to pathogens, in this case measured by reduction of the number of common cold episodes, the effect is considered beneficial. EFSA only accepts such effects when they are unequivocally demonstrated in the target species, i.e. the normal population, and only if exposure precedes the effect. If the intention is to treat an already existing disease, the agent, would be considered a drug, which is outside of the remit of EFSA.
- Any effect of statistical significance in the proper direction would be considered beneficial. As pathogens are risk factors for infections, reduction of the load of such pathogens may also be considered as beneficial; in any case it is essential to know the correlation between the load of the pathogens and the infection that they may cause,

2) *If the effect (in itself) is adverse or beneficial, is the effect size of a sufficient magnitude to be considered relevant?*

In scientific assessments, the critical effect size of adverse or beneficial effects could be considered as the effect size that would be of sufficient magnitude to be biologically relevant. As discussed above (see Section 3.1.4), the critical effect level is directly linked to the critical effects size and can be defined as the concentration or dose in the concentration/dose response relationship at which an effect occurs or at which level the function of, e.g. an organ, system or a (sub)population, is changed. In all cases, the background variability of the endpoint should be taken into account (see Section 3.1.4).

One way of taking into account normal or background variability, or more generally the natural variation of a biological system, is equivalence testing. A test of equivalence allows testing whether a value is higher (or smaller) than a given threshold specified upfront, which can be derived from historical control data gathered under comparable conditions, from estimation of the endpoint variability across populations or ecosystems or from any critical effect size deemed biologically relevant.

By comparing values with the natural variation of the biological system not exposed to the agent (i.e. responses to environmental or biological conditions other than the ones used in the assessment of the agent), equivalence testing can help to assess their biologically relevance. While statistically significant differences may point at direct biological changes caused by an agent, they may not be relevant from the safety viewpoint. By identifying those values outside normal, natural variation equivalence testing may also help concluding on the safety relevance of the effects.

Equivalence testing is used in the safety assessment of genetically modified (GM) plants. In this context, equivalence limits are not prespecified, but derived from plants varieties with an history of safe use, grown and tested under the same environmental conditions as those used for control group (see Annex GMO example for further details) (Annex F).

A critical effect size can be determined by using expert judgement (See EFSA, 2014a, Uncertainty and WoE). This was for example used in the assessment of lead where a benchmark response (BMR) of 1% for cognitive performance was chosen based on its distribution in the human population (EFSA, 2010). Another example is eggshell thinning and impact on egg cracking (Annex K) where the critical effect level, the biologically relevant percentage of egg shell thinning, starts at 18% when egg shell cracking begins to increase (EFSA, 2009c). In addition, models can be used for setting a critical effect level. For example, models of focal species could be used to determine endpoints corresponding to cut-off values set by specific protection goals (SPG). These models can be used for calculating critical effect levels for certain types of effect, for instance for egg cracking, number of surviving chicks or the size of litters, above which the population of the focal species will be negatively affected to such an extent that the population will decline over time (see Annex K extended after public consultation to include modelling).

If a consensus on a critical effect size for the adverse effect is not reached by the experts involved in the assessment, the EFSA SC recommends the use of default values. More specifically, a default critical effect size or BMR of 10% (extra risk) should be used for quantal data and 5% (change in mean response) for continuous data from animal studies. As stated in the guidance, the default BMR may be modified based on statistical and biological considerations (e.g. when endpoint-specific information is available). The rationale both for deviating from and using the default value should be described and documented (EFSA, 2017).

For beneficial effects, the same principles apply as for adverse effects to decide whether the magnitude of the effect is biologically relevant. Very often, for beneficial effects, only statistical criteria are used; see for instance the example on health claims (Annex G). However, expert judgement using a weight of evidence approach should be applied to judge the relevance of the beneficial effects observed, i.e. to decide on the magnitude to consider an effect as relevant. Cut-off values should ideally be set *a priori*, but this is usually not done (see Annex F).

Another example is assessment of efficacy of feed additives with the capacity to increase the performance of chicken for fattening, providing positive economic effect for the farmer (see Annex E). Any such effect exceeding the costs of the additive can be considered as potentially economically relevant. The magnitude of the effect is also of importance. In this example, however, the animal itself will not benefit from this positive effect.

3) *If the effect is not in itself adverse or beneficial (e.g. a biochemical parameter), is it directly or indirectly linked to a(n) adverse/beneficial outcome?*

In determining whether an effect is linked to an adverse/beneficial outcome, it could be considered, if the effect is a key event in the sequence of events leading to an adverse or beneficial outcome (see also Section 3.1.2). In this context, one of the questions resulting from the risk assessment of BPA could serve as an example (see Annex D): 'What is the biological relevance for human health of the observed proliferative and morphological changes in the mammary gland following exposure to BPA and the possible relevance for the development of breast cancer'? Ductal hyperplasia and an increase of the number of terminal end buds may be regarded as supporting evidence for tumour formation along with an increase in the proliferation of epithelial cells. However, these proliferative changes do not need to be adverse by themselves, as epithelial cell proliferation is a normal physiological process in certain life stages and per se does not lead to tumour formation and even may be reversible. However, it is generally accepted that under certain pathological conditions such as recurrent tissue damage and repair the proliferating tissue becomes more susceptible to tumour development.

Another well-known example is an alteration in circulating bioavailable thyroid hormone levels which may have a serious impact on organs or organ systems other than the thyroid itself, such as on the developing nervous system (see Annex I).

Some measured effects, such as liver enzyme induction, which may not be considered adverse in themselves, can have a modulatory influence on e.g. the toxicity of other agents. This has to be determined on a case-by-case basis.

An example of an indirect beneficial effect would be the addition of the enzyme glucanase to the feed of farm animals which has no significant nutritional value itself but which facilitates the intestinal digestion of cellulose, thereby enhancing the nutritional value of the feed (European Commission, 1996).

4) *If the effect itself is directly or indirectly linked to a(n) adverse or beneficial outcome, is the effect size of a sufficient magnitude to be considered relevant?*

To assess this, similar considerations would apply as in point 2 above.

As an example, in the risk assessment of cadmium (see Annex J) β2-microglobulin (B2M) excretion in urine was used as a biomarker for kidney damage. Renal toxicity is characterised by cadmium accumulation in convoluted proximal tubules thereby causing cell dysfunction and damage, the earliest sign of which is the decreased absorption of low molecular weight proteins from primary urine and increased excretion of B2M. To determine the relevant size of the effect, prior knowledge on the relationship between urinary excretion of B2M and renal function or damage was used. A level of 300 μg B2M/g creatinine in urine was selected since exceeding this cut-off value has been associated with accelerated decline of renal function and increased mortality. As high level criterion, a level above 1,000 μg B2M/g creatinine was selected as exceeding this level would likely be associated with irreversible damage.

Another example is the use of biomarker to determine a population reference intake for vitamin D (EFSA, 2016e). The complexity of vitamin D metabolism and the unknown contribution of its endogenous synthesis do not allow determining a reliable vitamin D Average Requirement in the

European population, hence calculating a Population Reference Intake for this population. The only possible approach relies upon the use of a biomarker (of exposure) – calcidiol or 25(OH)D – of which the serum concentration is related to bone health. Indeed, there is evidence of an increased risk of adverse musculoskeletal health outcomes below a certain threshold (50 nmol/L). Meta-regression analysis of the relationship between 25(OH)D serum concentration and total vitamin D intake allows to set an Adequate Intake of 15 μg/day for the adult European population, an intake which should ensure that most of the adult population will achieve a serum 25(OH)D concentration near or above the target of 50 nmol/L. In this case, the relationships between the altered status of 25(OH)D and adverse musculoskeletal health outcomes, on the one hand, and total vitamin D intake, on the other hand, were considered as the biologically relevant parameters.

In the re-evaluation of food additive aspartame, the critical effects were identified as reproductive effects in several animal species including humans (Annex B). The phenylalanine concentration in plasma that is not associated with damage to the offspring, from which the level obtained from a meal was subtracted, was used as a cut-off value. A bolus dose of aspartame to a normal subject reaching this value was determined based on modelling. The current aspartame intake given the current ADI was well below the dose required in PKU heterozygous individuals and it was concluded that there was no safety concern.

The figure below describes a general decision tree to decide whether a biological effect is relevant or not (Figure 5).

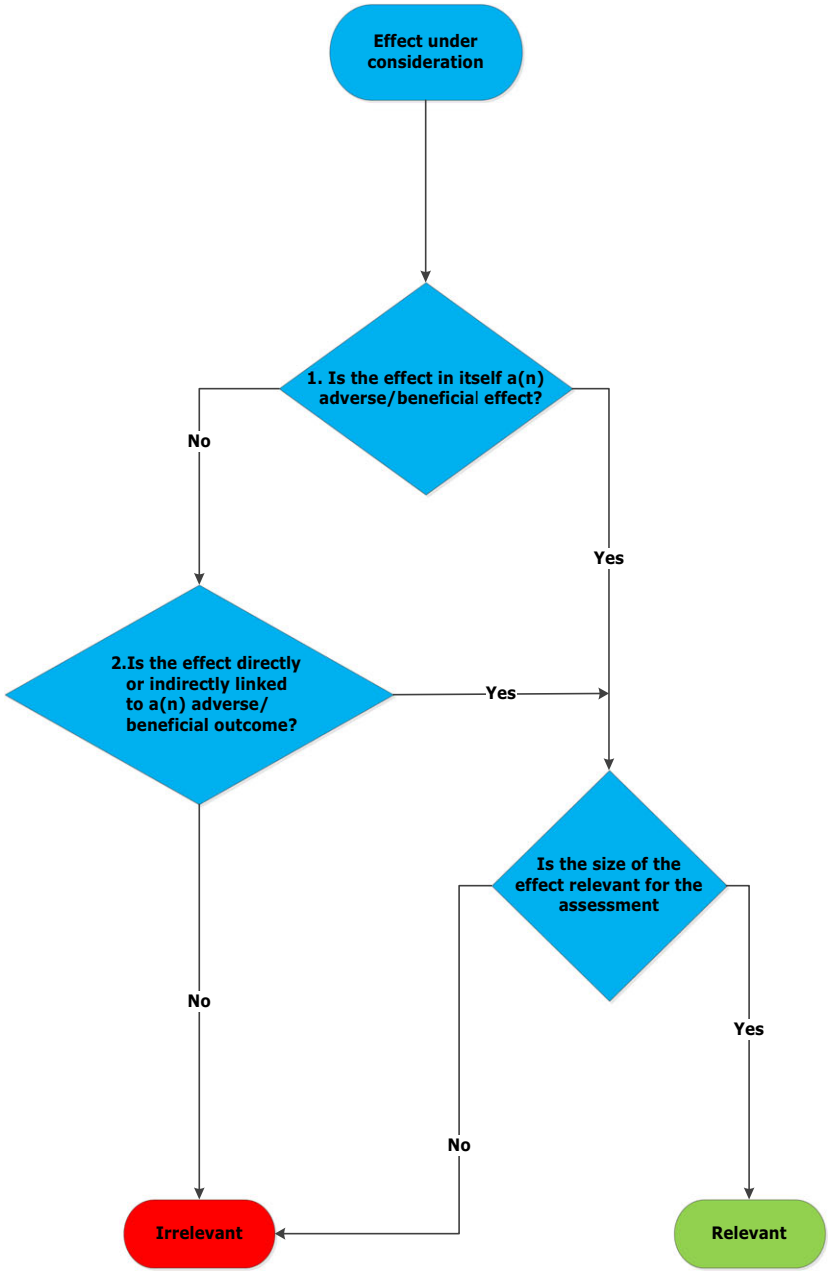

Figure 5: General decision tree to decide whether a biological effect is relevant or not

c) Relevance of the subject

In many cases, proxies for the target species are used (e.g. rats instead of man, or standard organisms to represent a group of organisms) to test the biological effects. The conclusion on whether an effect is adverse/beneficial or not, is specific to the test model under investigation.

No one to one extrapolation of the adverse or beneficial effects observed in experimental settings to humans/other species is generally possible. For example, if, following exposure to a chemical substance, a tumour occurs in one test species only or in an organ (e.g. Harderian glands; forestomach in rodents), which is not existing in humans, its relevance could be judged on the basis of the MoA. If the MoA is not known, additional information needs to be taken into consideration.

An example of a positive effect that depends on an organ only presents in certain species is utilisation of cobalt as precursor to vitamin B12 in rumen. The bacteria present in the rumen can metabolise inorganic cobalt into vitamin B12. Mammals without a rumen, as in humans, are dependent on the uptake of exogenous vitamin B12.

In order to decide on the relevance of the test species to the human situation when testing chemicals, it is important also to understand the qualitative and quantitative interspecies differences, as well as the human variability in toxicokinetics (TK) and toxicodynamics (TD) processes. For a particular agent, the level of knowledge on TK and TD processes can range from very basic (external dose and toxicity) to a full quantitative understanding (external dose to internal dose to target organ dose and metabolism (TK) to specific target organ toxicity (TD) (EFSA, 2014d).

In farm animals, (Annex E) beneficial effects should be demonstrated in Efficacy Studies performed with the target animals. Extrapolations can be made for other categories of the target animals (e.g. from chicken for fattening to hens for laying, or from piglets to pigs for fattening) or other species (from dairy cows to other animals used for milk production, or from chicken to other avian species).

The relevance of information obtained from *in vitro* or *in silico* approaches needs to be considered in conjunction with knowledge on the MoA and other available information.

In case of biological hazards; species specific pathogenicity will be considered to decide whether the effect seen in the test species is relevant for the target species.

In a study by Cassard et al. (2014), mice models were used to evaluate the zoonotic potential of classical scrapie (See Annex C). Transgenic mice over-expressing the human PrP gene and homozygous and heterozygous for methionine and valine at codon 129 were inoculated intracerebrally. The BIOHAZ Panel concluded that the mouse lines were well established and have been shown to be susceptible to different CJD and BSE strains. Although overexpression of PrP is not a natural condition in humans, and it might have impact on some biological parameters, this can be considered a scientifically appropriate approach to modelling the molecular barrier for transmission of scrapie in humans despite some limitations of these transmission models.

d) Relevance of the conditions

The conditions of the studies (environmental, toxicological, epidemiological, etc.) used for the assessment should be looked at to decide on the degree of their relevance in relation to the assessment question. Below experimental studies are used to illustrate these issues. They include:

Route of exposure

- It is evident that the exposure as applied in the toxicity test should be as close as possible to the exposure route expected in the field. This is not always feasible. For instance, in environmental risk assessment for birds and mammals the assessment of the acute risk is based on a gavage study (LD_{50}). This test does not really mimic normal exposure in the field where bolus exposure rarely occurs. Animals exposed in nature are often exposed via contaminated food over a period of time. In cases where the exposure is the result of an exposure via diet or drinking water vs bolus exposure, the outcome may be different compared with the bolus exposure (EFSA, 2005). The relevance of the exposure conditions should be taken into account in the uncertainty analysis.

  The route of exposure in a test system can sometimes be different from that in the target system. An example of this is mice models that were used in a study to examine the zoonotic potential of classical scrapie (See Annex C). The mice were inoculated intracerebrally. However, natural exposure to the classical scrapie agent in man is believed to involve the oral route through the consumption of meat from an infected animal. In this respect, the inoculation route used in the mouse model does not represent an ideal strategy for the investigation of zoonotic potential since the involvement of the digestive system, the rest of the lymphoreticular system, the enteric nervous system and peripheral nervous system have been bypassed by the direct deposition of the prions in the brain. Therefore, it was concluded that the inoculation route used by Cassard et al. (2014) cannot reproduce field conditions and does not mimic natural exposure.

Timing of exposure

- For some compounds, the timing of exposure is crucial. The test should include the most sensitive period of the animal's life cycle. For instance, some pesticides do hamper/prevent the moult of insects. When the duration of the test does not include a moulting event of the tested species the effect of the compound will not be shown.

Duration of exposure

- The duration of the test should mimic the duration of the exposure in the field. In case the duration in the field is longer or shorter than the duration of the toxicity test or the toxicity in the test did not reach a plateau (incipient toxicity), it is possible that the outcome of the standard test does not provide the answer that is needed for the risk assessment.

Formulation or the vehicle used for exposure to the agent

- By assessing the toxicity of the agent, it should be assessed whether the other compounds/ additives added to the formulation do not influence the outcome of the toxicity test (this can be in both directions: a less toxic outcome or a more toxic outcome). It is also worthwhile to assess the vehicle used to apply the toxic compound to the test organism or the test system (often the vehicle is tested on its own).

Field studies (environmental and epidemiological studies)

- For field studies, additional criteria have to be checked, for instance, whether the important animal groups are represented in the field study. These types of criteria will not be discussed in this document, but are important issues to be considered when judging whether the outcome of a test can be used in risk assessment (see for instance, the guidance document for aquatic organisms (EFSA, 2013a,b)

Other parameters, such as the number of animals per dose groups, number of doses tested, etc. are more related to reliability of the evidence (EFSA, 2017).

3.2.4. Uncertainty related to the relevance

Including evidence with less biological relevance adds to the overall uncertainty. Uncertainties arising when assessing biological relevance should be addressed and described together with other uncertainties at all stages of the assessment, including data gaps. General guidance on methods for assessing sources of uncertainty and their impact on assessment conclusions are provided by EFSA, (2016c), and can be applied to uncertainty arising from considering evidence that have limitations in their relevance as well as from other sources.

4. Reporting the assessment of biological relevance

Assessing biological relevance should be addressed and described as part of the weight of evidence assessment. General guidance on methods for reporting weight of evidence assessment conclusions is provided by EFSA, 2017, and can be applied to the assessment of biological relevance.

If the assessment of the biological relevance has been conducted following a standardised procedure previously established for use in this area of EFSA's work, the assessment of the biological relevance may be reported in the manner that is normal for that standardised procedure, provided this is transparent. The standardised procedure should be referenced and its applicability to the case in hand should be explained if it is not self-evident.

All other assessments of the biological relevance should be reported following the proposed framework according to the three basic steps of assessment of the biological relevance: (1) Development of the assessment strategy, including specification of agents, effects, subjects and conditions; (2) Collection and extraction of data, i.e. identification of potentially biologically relevant evidence/data as specified in the assessment strategy; (3) Appraisal and integration of the relevance of the agents, subjects, effects and conditions, i.e. reviewing dimensions of biological relevance for each data set. This reporting should be included in the report of the weight of evidence assessment.

Reporting should be consistent with EFSA's general principles regarding transparency (EFSA, 2006, 2009a) and reporting (EFSA, 2014a, 2015a,b). The assessment of the biological relevance should include justifying the choice of methods used, documenting all steps of the procedure in sufficient detail for them to be repeated, and making clear where and how expert judgement has been used. Where the assessment used methods that are already described in other documents, it is sufficient to refer to those. Reporting should also include referencing and, if appropriate, listing or summarising all evidence considered, identifying any evidence that was excluded; detailed reporting of the conclusions; and sufficient information on intermediate results for readers to understand how the conclusions were reached.

Guidance on reporting other parts of the wider procedure, including evidence review, problem formulation and uncertainty analysis, is provided elsewhere (e.g. EFSA, 2014b, 2015a, 2016c).

5. Conclusions and recommendations

- This guidance document is intended to guide EFSA panels and staff in the assessment of the biological relevance of scientific evidence. When addressing the mandate, the SC acknowledged that the issue of biological relevance in risk assessment has a broader meaning than the biological relevance of an effect as described in the Terms of Reference. In fact, it encompasses also aspects related to the definition of the problem formulation. This, in turn, guides the development of the assessment strategy, which includes the decision on which data to use for the assessment (relevance of the data).
- Relevance is a fundamental concept in dealing with evidence and has different implications in terms of elements to be considered at different stages of the assessment and it can only be determined when the assessment question is well defined, which forms the basis for developing an assessment strategy.
- A framework was developed in which biological relevance is considered at three main stages related to the process of dealing with evidence:
    - Development of the assessment strategy, in this context, specification of agents, effects, subjects and conditions in relation to the assessment question(s).
    - Collection and extraction of data, i.e. identification of potentially biologically relevant evidence/data as specified in the Assessment strategy.
    - Appraisal and integration of the relevance of the agents, subjects, effects and conditions, i.e. reviewing dimensions of biological relevance for each data set.
        - the agent; it should be considered whether the assessment is based on the agent of concern or on a surrogate agent (e.g. substance or biological agent behaving similarly to agent of concern).
        - the subject; it should be considered whether the assessment is based on the subjects of concern, (e.g. for human studies in humans) or in case proxies (e.g. animal models for humans) are used the relevance of effects occurring in these for the subject of concern should be considered.
        - the effect; a wide variety of effects may be considered. Consideration should be given as to whether the effect is causally related to exposure to the agent, and the nature of the effect should also be taken into account, i.a. homeostatic response, adaptive, adverse/beneficial or directly or indirectly linked to an adverse/beneficial effect. Finally, for effects where the size of the effect is critical, it should be assessed whether the magnitude of the effect is sufficient to be of biological relevance (e.g. outside normal variation, for effects directly or indirectly linked to an adverse/beneficial effect whether they are of sufficient magnitude to result in an adverse/beneficial outcome) and thereby of importance for the assessment outcome as both positive and negative findings may influence the answer to the assessment question. It should be noted that the biological relevance of an effect can vary according to the assessment question.
        - the conditions; it should be considered whether the conditions of the studies (environmental, toxicological, epidemiological, etc.) used for the assessment are relevant for the conditions under consideration.
    - It is acknowledged that in specific subject areas EFSA Panels conduct standardised assessments that strictly follow guidelines where assessment questions, biological relevance of effects and biological systems may be predefined.
- Each step of relevance considerations may be source of uncertainty. The assessor should address these uncertainties as a part of the general uncertainty analysis of the assessment. The SC Guidance on Uncertainty (EFSA, 2016c) should be followed.
- The EFSA SC acknowledges that the diversity of fields covered by the different EFSA Panels impacts how the guidance could be implemented. Depending on different types of agent (e.g. nutrient, chemical substance, microorganism, pathogen or invasive species), target subjects and different types of outcomes dealt with in the assessments, EFSA Panels may in the course of implementation identify needs for guidance documents to be developed in more specific areas.

- It is suggested that EFSA collaborate at the European and international level with relevant organisations and initiatives to harmonise developments in this area.

References

Amzal B, Julin B, Vahter M, Wolk A, Johanson G and Akesson A, 2009. Population toxicokinetic modeling of cadmium for health risk assessment. Environmental Health Perspectives 117, 1293–1301. https://doi.org/10.1289/ehp.0800317

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE and Villeneuve DL, 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry 29, 730–741. https://doi.org/10.1002/etc.34

ANSES, 2013. OPINION of the French Agency for Food, Environmental and Occupational Health & Safety on the assessment of the risks associated with bisphenol A for human health, and on toxicological data and data on the use of bisphenols S, F, M, B, AP, AF and BADGE. Available online: https://www.anses.fr/en/system/files/CHIM2009sa0331Ra-0EN.PDF

Anthony A, Caldwell J, Hutt AJ and Smith RL, 1987. Metabolism of estragole in rat and mouse and influence of dose size on excretion of the proximate carcinogen 1'hydroxyestragole. Food Chemistry and Toxicology, 25, 799–806.

Blaauboer BJ, Boekelheide K, Clewell HJ, Daneshian M, Dingemans MML, Goldberg AM, Heneweer M, Jaworska J, Kramer NI, Leist M, Seibert H, Testai E, Vandebriel RJ, Yager JD and Zurlo J, 2012. The use of biomarkers of toxicity for integrating in vitro hazard estimates into risk assessment for humans. Altex 29, 4/12.

Blus L, 2003. Handbook of Ecotoxicology: Organochlorine Pesticides. Chapter 13. 2nd Edition. CRC Press LLC, Boca Raton.

Boberg EW, Miller EC, Miller JA, Poland A and Leim A, 1983. Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfooxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. Cancer Research, 43, 5163–5173.

Boobis AR, Cohen SM, Dellarco V, McGregor D, (Bette) Meek ME, Vickers C, Willcocks D and Farland W, 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical Reviews in Toxicology, 36.

Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME (Bette), Munn S, Ruchirawat M, Schlatter J, Seed J and Vickers C, 2008a. IPCS framework for analyzing the relevance of a noncancer mode of action for humans. pp. 87–96. Published online: 10 Oct 2008. https://doi.org/10.1080/10408440701749421

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME (Bette), Vickers C, Willcocks D and Farland W, 2008b. IPCS framework for analyzing the relevance of a cancer mode of action for humans. pp. 781–792. Published online: 10 Oct 2008. https://doi.org/10.1080/10408440600977677

Buist HE, von Bölcsházy GF, Dammann M, Telman J and Rennen MA, 2009. Derivation of the minimal magnitude of the critical effect size for continuous toxicological parameters from within-animal variation in control group data. Regulatory Toxicology and Pharmacology, 55, 139–150. https://doi.org/10.1016/j.yrtph.2009.06.009

Cassard H, Torres JM, Lacroux C, Douet JY, Benestad SL, Lantier F, Lugan S, Lantier I, Costes P, Aron N, Reine F, Herzog L, Espinosa JC, Beringue V and Andréoletti O, 2014. Evidence for zoonotic potential of ovine scrapie prions. Nature Communications, 5, Article number: 5821. https://doi.org/10.1038/ncomms6821

CBD (Secretariat of the Convention on Biological Diversity), 2010. Year in review 2009. Montreal, 42 p.

Dellarco VL, McGregor D, Berry SC, Cohen SM and Boobis AR, 2006. Thiazopyr and thyroid disruption: case study within the context of the 2006 IPCS Human Relevance Framework for analysis of a cancer mode of action. Critical Reviews in Toxicology 36, 793–801.

Drinkwater NR, Miller EC, Miller JA and Pitot HC, 1976. Hepatocarcinogenicity of estragole (1-allyl-4-502 methoxybenzene) and 1'-hydroxyestragole in the mouse and mutagenicity of 1'-acetoxyestragole in bacteria. Journal of the National Cancer Institute, 57, 1323–1331.

EFSA (European Food Safety Authority), 2005. EFSA Panel on Plant Protection Products and their Residues, 2005. Opinion of the Scientific Panel on Plant protection products and their residues (PPR) on a request from EFSA related to the evaluation of pirimicarb. EFSA Journal 2005;3(8):240, 21 pp. https://doi.org/10.2903/j.efsa.2005.240

EFSA (European Food Safety Authority), 2006. Transparency in risk assessment carried out by EFSA: Guidance Document on procedural aspects. EFSA Journal 2006;4(5):353, 16 pp. https://doi.org/10.2903/j.efsa.2006.353

EFSA (European Food Safety Authority), 2008. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. https://doi.org/10.2903/j.efsa.2008.842

EFSA (European Food Safety Authority), 2009a. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009; 7(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2009b. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA Journal 2009;980, 1–139 pp. https://doi.org/10.2903/j.efsa.2009.980
EFSA (European Food Safety Authority), 2009c. European Food Safety Authority; guidance document on risk assessment for birds & mammals on request from EFSA. EFSA Journal 2009;7(12):1438. https://doi.org/10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu
EFSA (European Food Safety Authority), 2010. EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Lead in Food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
EFSA (European Food Safety Authority), 2011. EFSA Scientific Committee; statistical significance and biological relevance. EFSA Journal 2011;9(9):2372, 17 pp. https://doi.org/10.2903/j.efsa.2011.2372
EFSA (European Food Safety Authority), 2013a. Scientific opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal, 2013;11(7):3293, 131 pp. https://doi.org/10.2903/j.efsa.2013.3293
EFSA (European Food Safety Authority), 2013b. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. https://doi.org/10.2903/j.efsa.2013.3290
EFSA (European Food Safety Authority), 2014a. Discussion Paper - Transformation to an "Open EFSA". Available online: www.efsa.europa.eu
EFSA (European Food Safety Authority), 2014b. Guidance on Expert Knowledge Elicitation in Food and Feed Safety Risk Assessment. EFSA Journal 2014;12(6):3734, 278 pp. https://doi.org/10.2903/j.efsa.2014.3734
EFSA (European Food Safety Authority), 2014c. Systematic review guidance.
EFSA (European Food Safety Authority), 2014d. Schmallenberg virus: state of Art. EFSA Journal 2014;12(5):3681, 54 pp. https://doi.org/10.2903/j.efsa.2014.3681
EFSA, (European Food Safety Authority), 2014e. Modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal 2014;12(4):3638, 87 pp. https://doi.org/10.2903/j.efsa.2014.3638
EFSA (European Food Safety Authority), 2015a. Editorial: increasing robustness, transparency and openness of scientific assessments. EFSA Journal 2015;13(3):e13031, 3 pp. https://doi.org/10.2903/j.efsa.2015.e13031
EFSA (European Food Safety Authority), 2015b. Scientific report on Principles and process for dealing with data and evidence in scientific assessments. EFSA Journal 2015;13(5):4121, 35 pp. https://doi.org/10.2903/j.efsa.2015.4121
EFSA (European Food Safety Authority), 2016a. Scientific opinion on recovery in environmental risk assessments at EFSA. EFSA Journal 2016;14(2):4313, 85 pp. https://doi.org/10.2903/j.efsa.2016.4313
EFSA (European Food Safety Authority), 2016b. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal 2016;14(6):4499, 50 pp. https://doi.org/10.2903/j.efsa.2016.4499
EFSA (European Food Safety Authority), 2016c. Guidance on Uncertainty in EFSA Scientific Assessment - Draft version for internal testing. Available online: https://www.efsa.europa.eu/en/topics/topic/uncertainty
EFSA (European Food Safety Authority), 2016d. General scientific guidance for stakeholders on health claim applications. EFSA Journal 2016;14(1):4367, 38 pp. https://doi.org/10.2903/j.efsa.2016.4367
EFSA (European Food Safety Authority), 2016e. Scientific opinion on dietary reference values for vitamin D. EFSA Journal 2016;14(10):4547, 145 pp. https://doi.org/10.2903/j.efsa.2016.4547
EFSA (European Food Safety Authority), 2017. Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658
EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: executive summary. EFSA Journal 2015;13(1):3978, 23 pp. https://doi.org/10.2903/j.efsa.2015.3978
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011a. Scientific Opinion on the safety and efficacy of allylhydroxybenzenes (chemical group 18) when used as flavourings for all animal species. EFSA Journal 2011;9(12):2440, 14 pp. https://doi.org/10.2903/j.efsa.2011.2440
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011b. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. https://doi.org/10.2903/j.efsa.2011.2175
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. https://doi.org/10.2903/j.efsa.2012.2536
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. https://doi.org/10.2903/j.efsa.2012.2537
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012d. Guidance for the preparation of dossiers for sensory additives. EFSA Journal 2012;10(1):2534, 26 pp. https://doi.org/10.2903/j.efsa.2012.2534

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of BIOSTRONG® 510 (essential oil of thyme and star anise) for chickens and minor avian species for fattening and rearing to point of lay. EFSA Journal 2016;14(7):4351, 15 pp. https://doi.org/10.2903/j.efsa.2016.4351

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879, 111 pp. https://doi.org/10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms) 2011. EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150, 37 pp. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific Opinion on application EFSA-GMO-NL-2007-45 for the placing on the market of herbicide-tolerant, high-oleic acid, genetically modified soybean 305423 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer. EFSA Journal 2013;11(12):3499, 35 pp. https://doi.org/10.2903/j.efsa.2013.3499

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 2010;8(3):1461, 107 pp. https://doi.org/10.2903/j.efsa.2010.1461

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on the substantiation of a health claim related to Yestimun® and defence against pathogens in the upper respiratory tract pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA Journal 2013;11(4):3159, 12 pp. https://doi.org/10.2903/j.efsa.2013.3159

EFSA PLH Panel (EFSA Panel on Plant Health), 2010. Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options by EFSA. EFSA Journal 2010;8(2):1495, 66 pp. https://doi.org/10.2093/j.efsa.2010.1495. Available online: http://www.efsa.europa.eu/en/efsajournal.htm

EFSA PLH Panel (EFSA Panel on Plant Health), 2012. Guidance on methodology for evaluation of the effectiveness of options for reducing the risk of introduction and spread of organisms harmful to plant health in the EU territory. EFSA Journal 2012;10(6):2755, 92 pp. https://doi.org/10.2903/j.efsa.2012.2755. Available online: www.efsa.europa.eu/efsajournal

EFSA PLH Panel (EFSA Panel on Plant Health), 2016. Scientific opinion on the risk to plant health of *Ditylenchus destructor* for the EU territory. EFSA Journal 2016;14(12):4602, 124 pp. https://doi.org/10.2903/j.efsa.2016.4602

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2014. Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. EFSA Journal 2014;12(3):3589, 92 pp. https://doi.org/10.2903/j.efsa.2014.3589

EFSA Scientific Committee, 2011a. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2011b. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438, 21 pp. https://doi.org/10.2903/j.efsa.2011.2438

EFSA Scientific Committee, 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. EFSA Journal 2013;11(3):3132, 84 pp. https://doi.org/10.2903/j.efsa.2013.3132. Available online: www.efsa.europa.eu/efsajournal

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen KH, Mo re S, Mortensen A, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Aerts M, Bodin L, Davis A, Edler L, Gundert-Remy U, Sand S, Slob W, Bottex B, Abrahantes JC, Marques DC, Kass G and Schlatter JR, 2017. Update: guidance on the use of thebenchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. 10.29 03/j.efsa.2017.4658

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Benfenati E, Chaudhry QM, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C, Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A, Hempen M, Valtueña Martínez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M, 2017. Guidance on the Use of the Weight of Evidence Approach in Scientific Assessments. EFSA Journal 2017;15(8):4971, 104 pp. https://doi.org/10.2903/j.efsa.2017.4971

EMA (European Medicines Agencies), 2014. Public statement on the use of herbal medicinal products containing estragole. 24 November 2014, EMA/HMPC/137212/2005 Rev 1. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2014/12/WC500179557.pdf

European Commission, 1996. Report of the Scientific Committee on Animal Nutrition on the use of certain enzymes in animal feeding stuffs.

European Commission, 2004. Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage. Official Journal L 143, 30/04/2004 P. 0056–0075.

European Commission, 2007. Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients.

European Pharmacopoeia, 2005. Star anise oil. Monograph (MG) 2108, 5th Ednition. Council of Europe (COE)—European Directorate for the Quality of Medicines, Strasbourg, France.

Gunderson LH, 2000. Ecological resilience: in theory and application. Annual Review of Ecology and Systematics, 31, 425–439.

Hamilton PB, Nicol E, De-Bastos ESR, Williams RJ, Sumpter JP, Jobling S, Stevens JR and Tyler CR, 2014. Populations of a cyprinid fish are self-sustaining despite widespread feminization of males. BMC Biology 12, 1. https://doi.org/10.1186/1741-7007-12-1

Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295–300.

Ioannidis JP, 2005. Why most published research findings are false. PLoS Medicine, 2, e124. https://doi.org/10.1371/journal.pmed.0020124

Ioannidis JP, 2008. Why most discovered true associations are inflated. Epidemiology, 19, 640–648.

IPPC (Secretariat of the International Plant Protection Convention), 2007. International Standards for Phytosanitary Measures ISPM no. 2 Guidelines for pest risk analysis. FAO, 2007. Available online: https://www.ippc.int/largefiles/adopted_ISPMs_previousversions/en/ISPM_02_1995_En_2006-05-03.pdf

Kardinaal AF, van Erk MJ, Dutman AE, Stroeve JH, van de Steeg E, Bijlsma S, Kooistra T, van Ommen B and Wopereis S, 2015. Quantifying phenotypic flexibility as the response to a high-fat challenge test in different states of metabolic health. FASEB Journal, 29, 4600–4613. https://doi.org/10.1096/fj.14-269852

Keller DA, Juberg DR, Catlin N, Farland WH, Hess FG, Wolf DC and Doerrer NG, 2012. Identification and characterization of adverse effects in 21st century toxicology. Toxicological Sciences, 126, 297–297. https://doi.org/10.1093/toxsci/kfr350

La Caze A and Duffull S, 2011. Estimating risk from underpowered, but statistically significant, studies: was APPROVe on TARGET? The Journal of Clinical Pharmacology, 36, 637–641.

Lewis RW, Billington R, Debryune E, Gamer A, Lang B and Carpanini F, 2002. Recognition of adverse and nonadverse effects in toxicity studies. Toxicologic Pathology 30, 66–74. https://doi.org/10.1080/01926230252824725

Martins C, Cacao R, Cole KJ, Phillips DH, Laires A, Rueff J and Rodrigues AS, 2012. Estragole: a weak direct-534 acting food-borne genotoxin and potential carcinogen. Mutations Research, 747, 86–92.

Meek ME (Bette), Palermo CM, Bachman AN, North CM and Lewis RJ, 2014. Mode of action human relevance (species concordance) framework: evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. Journal of Applied Toxicology 34, 595–606. Published online 2014 Feb 10. https://doi.org/10.1002/jat.2984

Miller MD, Crofton KM, Rice DC and Zoeller RT, 2009. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. Environmental Health Perspectives, 117, 1033–1041. https://doi.org/10.1289/ehp.0800247

Minnema DJ, 1997. 90-Day subchronic dietary toxicity study of *trans*-anethole in rats. Corning Hazleton Inc., Vienna, Virginia, USA. Study no. CHV 2595-104. At finalization. Submitted to WHO by Flavor and Extract Manufacturers' Association of the United States, Washington DC, United States.

NRC (National Research Council), 2009. Science and decisions: advancing risk assessment. Washington, DC, The National Academies Press. https://doi.org/10.17226/12209

NTP-CERHR, 2008. NTP-CERHR monograph on the potential human reproductive and developmental effects of bisphenol A, 22, 1–66.

OECD, 2007. Test No. 426: developmental neurotoxicity study. OECD Publishing, Paris. https://doi.org/10.1787/9789264067394-en

OECD, 2013. Environment Directorate Joint Meeting Of The Chemicals Committee And The Working Party On Chemicals, Pesticides And Biotechnology Guidance Document On Developing And Assessing Adverse Outcome Pathways Series on Testing and Assessment No. 184.

Pellis L, van Erk MJ, van Ommen B, Bakker GCM, Hendriks HFJ, Cnubben NHP, Kleemann R, van Someren EP, Bobeldijk I, Rubingh CM and Wopereis S, 2012. Plasma metabolomics and proteomics profiling after a postprandial challenge reveal subtle diet effects on human metabolic status. Metabolomics, 8, 347–359. https://doi.org/10.1007/s11306-011-0320-5

Perry R, Farris G, Bienvenu JG, Dean CJr, Foley G, Mahrt C and Short B; Society of Toxicologic Pathology, 2013. Society of toxicologic pathology position paper on best practices on recovery studies the role of the anatomic pathologist. Toxicologic Pathology, 41, 1159–1169.

Phillips DH, Reddy MV and Randerath K, 1984. 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. II. Newborn 554 male B6C3F1 mice. Carcinogenesis, 5, 1623–1628.

Robinet C, Douma JC, Piou D and van der Werf W, 2016. Application of a wood pathway model to assess the effectiveness of options for reducing risk of entry of oak wilt into Europe. Forestry, 89, 456–472. https://doi.org/10.1093/forestry/cpw029

Slob W, 1999. Thresholds in toxicology and risk assessment. International Journal of Toxicology, 18, 259–268.

Suzuki Y, Umemura T, Hibi D, Inoue T, Jin M, Ishii Y, Sakai H, Nohmi T, Yanai T, Nishikawa A and Ogawa K, 2012. Possible involvement of genotoxic mechanisms in estragole-induced hepatocarcinogenesis in rats. Archives of Toxicology, 86, 1593–1601.

US EPA, 1986. Guidelines for Carcinogen Risk Assessment. 51 FR 33992-34003.

US EPA, 2005. Guidelines for Carcinogen Risk Assessment. 70 FR 17765-17817.

US-EPA, 2013. Next Generation Compliance: Strategic Plan 2014 – 2017. U.S. Environmental Protection Agency Office of Enforcement and Compliance Assurance Washington, DC 20460 October 2014. Available online: https://www.epa.gov/compliance/next-generation-compliance-strategic-plan-2014-2017

WHO (World Health Organisation), 2000. Evaluation of certain food additives. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. WHO, Geneva, Switzerland.

WHO (World Health Organization), 2009. Food Safety. Project to update the principles and methods for the assessment of chemicals in food. Principles and methods for the risk assessment of chemicals in food. EHC 240. ISBN 978 92 4 157240 8.

Wiseman RW, Fennell TR, Miller JA and Miller EC, 1985. Further characterization of the DNA adducts formed by electrophilic esters of the hepatocarcinogen 1′-hydroxysafrole and 1′-hydroxyestragole in vitro and in mouse liver in vivo, including new adducts at C-8 and N-7 of guanine residues. Cancer Research, 45, 3096–3105.

Abbreviations

ADI	acceptable daily intake
AMU	Assessment and Methodological Support
AO	adverse outcome
AOP	Adverse Outcome Pathway
BMD	benchmark dose
BMR	benchmark response
CES	Critical Effect Size
DRV	Dietary Reference Values
GM	genetically modified
IPCS	International Programme on Chemical Safety
KE	key event
LD_{50}	lethal dose, median
LoC	Limit of Concern
LOEC	Lowest Observed Effect Concentration
LOEL	Lowest Observed Effect Level
MIE	molecular initiating event
MoA	Mode of Action
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOED	No Observed Effect Dose
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development
PROMETHEUS	promoting methods for evidence use in scientific assessments
SC	Scientific Committee
SPG	specific protection goals
TD	toxicodynamics
TK	toxicokinetics
WHO	World Health Organization

Examples of biological relevance considerations in panel-specific scientific assessments

Annex A – AHAW

Case study of biological relevance in the area of animal health – Risk of introduction and establishment of Rift Valley Fever in the countries neighbouring the EU

Assessment strategy

Rift Valley Fever (RVF) virus is a vector-borne virus that affects ruminants. Humans can contract the infection when in close contact with infected ruminants. The causative agent is a Bunyavirus that is endemically present in Sub-Saharan Africa. The European Commission wanted to know whether the virus is moving its territory in Northern direction.

Agent	Effect	Subject	Condition
Rift Valley Fever virus	Introduction into Mediterranean countries neighbouring the EU	Ruminant Population	Prevalence of the infection in Sub-Saharan Africa Movement of ruminants into region of concern Movement of vectors into region of concern
		Humans	

Agent	Effect	Subject	Condition
RVF virus	Establishment	Ruminant Population	
			Infection dynamics model
			Geographical density of ruminants
			Temperature in the geographical regions
			Geographical density of competent vectors (literature and further extrapolated based on suitability of the habitats)
			Temperature in the geographical regions

Problem formulation: What is the risk of entry and establishment of RVF into the Mediterranean countries neighbouring the EU.

Evidence/data needed to address the question

Risk of Entry: Quantitative assessment of probability of introduction

- Prevalence of the infection in source countries in Sub-Saharan Africa (data originated from literature review and OIE outbreak information; in general data availability was limited and available data was fragmented).
- Data of movement of animals from source countries to region of concern (this is undocumented trade, so no data available; estimates were made using formal expert elicitation (Sheffield method)).
- Data on movements of competent vectors from source countries to region of concern (data were derived from literature and expert elicitation).

Risk of Establishment: Modelling infection dynamics with regard to presence and density of ruminants and competent vectors.

- Infection dynamics model (a model described in literature was used).
- Identifying competent vectors (these were derived from literature).
- Geographical density of competent vectors (derived for a small part from literature, further extrapolated based on suitability of the habitats).
- Geographical density of ruminants (data derived from FAO).
- Temperature in the geographical regions (based on temperature records of the region).

Data evaluation

Relevance of the agent

Rift Valley Fever virus is a zoonotic virus of the family Bunyaviridae that has ruminant species as reservoir hosts. Infected animals suffer from fever, young animals may die and pregnant animals may abort. The infection is primarily transmitted between animals through mosquitos. Humans can contract the infection when in close contact to animals. Most infected humans follow a subclinical or mild (fever, headache, muscle pain) course, but a small percentage of patients develops severe disease.

Relevance of the subject

For the AHAW Panel, ruminants were the relevant subjects. These species are the reservoir hosts of the virus. There is no evidence for sustainable human to human transmission.

Relevance of the effect

Two effects were examined: (1) Risk of introduction into the Mediterranean countries neighbouring the EU and (2) Risk of establishment in those countries. Both are directly related to the question asked by the requestor.

Relevance of the conditions

For both the risk of introduction and the risk of establishment, a mathematical model was used to assess the risk. This is a generally accepted assessment, because it is not possible to study these questions empirically. The introduction question included the possible routes of infection, the contact rate, prevalence of the infection in the source countries and likelihood of virus survival during the transport. Upon introduction, the infection may either fade out quickly after infecting only one or a few animals, or result in extensive transmission, which is primarily dependent on the densities of ruminants and that of competent vectors. Whether as a consequence of this spread the virus will become endemic is dependent of the host population size (in a relatively small population the influx of new susceptible animals might be insufficient for maintenance of infection) and climate (if temperatures drop in the winter to values that do not enable the vector cycle, infection will fade out in winter).

Overall conclusion

The assessment revealed that the introduction of RVF in the region of concern is highly likely, but most often takes place in regions where the combined ruminant and vector density is insufficient to result in establishment. However, according to the assessment, the region of concern has regions where RVF could become endemic.

Uncertainty

The main potential sources of uncertainties in this setting may be summarised as follows:

- mathematical models are a simplification of reality;
- uncertainty around model parameters in particular:
  - movement of animals from Sub Saharan to Northern Africa was derived using expert elicitation;
  - vector density was mostly based on the presence of suitable vector habitat only;
  - uncertainty regarding vector competence of vectors present in Northern Africa.

The uncertainty around the number of introductions was high (but also the lower limit of the estimate indicates a likely introduction). Due to sparse data, the uncertainty around the vector densities is also high and it is uncertain how the competence of vectors can vary within a certain vector species.

Case study of biological relevance in the area of animal welfare – Gas stunning and unconsciousness at slaughter

Assessment strategy

In the slaughter process, animals are killed by exsanguination. However, in order to avoid pain and suffering, they should be rendered unconscious prior to exsanguination and remain so until death occurs through loss of blood. In most cases, poultry are stunned using an electric current, but recently gas stunning has gained interest due to animal welfare advantages. It is to be expected that the industry will continue to develop new stunning methods or modify electrical or gas stunning parameters. It is therefore important to ensure that the new or modified stunning methods meet animal welfare standards. Thus, an assessment protocol has been developed to evaluate new or modified stunning methods.

Agent	Effect	Subject	Condition
Stunning method	Loss of consciousness	Poultry	Slaughter

Problem formulation: To maintain good standards of animal welfare, it is important to establish whether the new or modified stunning method (a) produces immediate loss of consciousness, (b) if loss of consciousness is not immediate, does it cause avoidable pain and suffering during the induction of unconsciousness, and (c) is the duration of unconsciousness long enough to avoid recovery of consciousness either prior to slaughter or during exsanguination.

Evidence/data needed to address the question

First, the brain mechanism associated with the induction of unconsciousness by a new or modified stunning method needs to be clearly explained. The state of consciousness can be ascertained under the controlled laboratory conditions by recording spontaneous as well as evoked activity in the brain using electroencephalograms (EEGs) before and after the application of a stunning method. The unique brain states that are incompatible with persistence of consciousness should be demonstrated using EEGs. Secondly, the correlation between EEG evidence and animal based indicators (as proxies) of unconsciousness for monitoring in slaughterhouses also need to be established. Thirdly, the duration of unconsciousness should be determined. In essence, the duration of unconsciousness should be longer than the sum time interval between the end of stunning and cutting blood vessels in the neck and the time it takes for the onset of death through exsanguination. Finally, the maximum permissible time between the end of stunning and neck cutting should be established.

Data evaluation

Relevance of the agent

Killing animals by exsanguination is a potentially painful process and the sources of pain includes, (a) cutting soft tissues, nerves and blood vessels in the neck (sawing motion or making several cuts), (b) direct activation of neurones by the blade as it transects the nerves produce intense pain and (c) the sensations produced during the injury discharge is likely to be an amalgam of all such inputs, and the overall effect is likely to be a sense of shock, comparable to an electric shock.

Relevance of the subject

Poultry is the relevant subject for the question, because it is also the target species for slaughter.

Relevance of the effect

The pain and suffering at exsanguination can be prevented by implementing pre-slaughter stunning of animals, i.e. rendering them unconscious prior to exsanguination.

Relevance of the conditions

The tests are done with the target species in a slaughterhouse setting. In this context, the brain of an animal is considered to be the seat of consciousness

Overall conclusion

Useful to test stunning methods according to the guideline.

Uncertainty

Establishing neuronal correlates of unconsciousness remains to be a challenge. For example, the magnitude of changes occurring in the EEG considered to be incompatible with persistence of consciousness varies widely. The correlation between EEG criteria and animal based indicators of unconsciousness is not widely reported, and hence, rely on expert opinion**.**

Annex B – ANS

Re-evaluation of aspartame (E 951) as a food additive

Introduction

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) of the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the re-evaluation of aspartame (E 951) as a food additive.

Aspartame (E 951) is a dipeptide of L-phenylalanine methyl ester and L-aspartic acid bearing an amino group at the α-position from the carbon of the peptide bond (α-aspartame). The major hydrolysis and degradation products of aspartame are L-phenylalanine, aspartic acid, methanol and 5-benzyl-3,6-dioxo-2-piperazine acetic acid (DKP).

For the purpose of the guidance and although the scientific assessment of Aspartame had a broader content, the example below focuses only on one effect.

Agent	Effects	Subjects	Conditions
Aspartame	Developmental effects	Rats and rabbits	Reproductive and developmental studies
			Humans heterozygous or homozygous for phenylalanine hydroxylase (PAH)
L-phenylalanine Methanol Aspartic acid		Phenylketonuria (PKU) patients	

Assessment strategy

The re-evaluation of aspartame included the assessment of the safety of its gut hydrolysis metabolites methanol (which is oxidised to formaldehyde), phenylalanine and aspartic acid. The hepatic enzyme phenylalanine hydroxylase (PAH) is necessary to metabolise the amino acid phenylalanine to the amino acid tyrosine. When PAH activity is reduced, circulating phenylalanine levels will increase. Humans heterozygous for PAH mutations, show a slightly reduced capacity to metabolise phenylalanine compared to normal individuals. Individuals homozygous for PAH mutations, phenylketonuria (PKU) patients, have a markedly reduced capacity for phenylalanine metabolism. There is long established evidence for increased severity and frequency of adverse developmental effects with high phenylalanine plasma levels in human patients with PKU. Maternal PKU syndrome refers to the teratogenic effects of PKU during pregnancy. In untreated pregnancies wherein the mother has classic PKU with a plasma, phenylalanine level greater than or equivalent to 1,200 μM (20 mg/dL), abnormalities in offspring occur at high frequencies.

The pathogenesis of this syndrome is unknown; it may be related to inhibition by phenylalanine of neutral amino acid transport across the placenta or to direct toxicity of phenylalanine and/or a phenylalanine metabolite (phenylpyruvic acid) in certain fetal organs.

Specification of the agent

Hydrolysis of aspartame in the gastrointestinal tract is essentially complete and there is no systemic exposure to aspartame but systemic exposure to aspartic acid, phenylalanine and methanol do occur.

Specification of the subject(s)

Adverse developmental effects have been reported in rats and rabbits treated with aspartame as well as with phenylalanine. Due to the very efficient hydrolysis in the gastrointestinal tract, the amount of intact aspartame that enters the bloodstream has been reported to be undetectable in several studies conducted in rats, dogs, monkeys and humans. Based on the sensitivity of the detection of radioactivity following labelling of the phenylalanine, aspartate and methyl moieties of aspartame, it can be concluded that aspartame is completely hydrolysed in the gut to yield aspartate, phenylalanine and methanol. These metabolites are then absorbed and enter normal endogenous metabolic pathways. An limit of detection (LOD) is not given for aspartame. The LOD for methanol is 3.5–4 mg/L.

Specification of the effect(s)

After birth, homozygous PKU babies show severe impairment in development and cognition if the phenylalanine intake via the diet is not strictly controlled. Adverse developmental effects were seen in children born to PKU patients and that these effects appeared to be related to maternal phenylalanine levels. It has been reported that the effects of phenylalanine in PKU mothers and their children both before and after birth had developed considerably since the initial evaluation of aspartame.

The MoA proposed for aspartame was that the toxicological effects observed in rats and rabbits during pregnancy were due to the metabolite phenylalanine. It has been postulated that phenylalanine could be responsible for some or all of the adverse effects reported for aspartame in developmental toxicity studies with rats and rabbits.

Data collection

The evaluation is based on original study reports and information submitted following public calls for data, previous evaluations, and additional literature that became available until the 15 November 2013.

A complete package of embryotoxicity, reproductive and developmental toxicity studies on aspartame in rats, mice and rabbits have been performed. Some of these studies were also conducted with the aspartame metabolite phenylalanine.

Appraisal and integration of the evidence

Relevance of the agent(s) and the subject(s)

The results of the reproductive and developmental toxicity studies in rats indicated NOAELs that ranged from 2,000 to 4,000 mg aspartame/kg body weight (bw) per day. Developmental changes in pup weight were observed at birth in studies at the dose of 4,000 mg aspartame/kg bw per day, which could be attributed to a combination of malnutrition and nutritional imbalance due to excessive exposure to phenylalanine derived from aspartame. This hypothesis was supported by the observation that administration of a dose of phenylalanine equimolar to aspartame led to a similar decrease in maternal and pup weight of rats, as observed in a concurrent aspartame group.

The data from the reproductive and developmental toxicity studies performed with rabbits were confounded both by the decrease in feed intake or the poor health of the animals, and, in many cases, by the number of deaths of pregnant rabbits in the treated groups possibly related to misdosing during gavage treatment.

Relevance of the effect(s)

The key effects observed in the reproductive studies with rats and rabbits related to a specific life stage acknowledged to be critical in both species and to humans. A spectrum of effects was observed in the rats and rabbits, particularly maternal toxicity and growth restriction of the offspring. The latter effect was recognised as an important outcome in humans because it was associated with an increased risk of perinatal mortality and morbidity. Developmental changes in pup body weight were observed at birth in studies at a dose of 4,000 mg aspartame/kg bw per day and considered to be attributed due to the excessive exposure to phenylalanine derived from aspartame. This was supported by the observation that administration of a dose of L-phenylalanine equimolar to aspartame led to a similar decrease in maternal and pup weight, as observed in a concurrent aspartame group.

Relevance of the conditions

The available reproductive and developmental toxicity studies on aspartame comprised nine studies, one in mice and eight in rats. In addition, eight embryotoxicity and teratogenicity studies were performed in rabbits, four with administration of aspartame by diet and four by gavage.

The best estimate of the critical effect level of phenylalanine exposure without damage to the offspring is 330–360 μM. In calculating a safe level of aspartame exposure (based on plasma phenylalanine concentrations), the worst-case scenario was applied, that took into account that intake of aspartame occurs in combination with a meal leading to circulating plasma phenylalanine concentrations of 120 μM.

The concentration of plasma phenylalanine derived from aspartame was, therefore, set to 240 μM (i.e. 360 – 120 μM).

Based on **modelling,** a plasma phenylalanine concentration of 240 μM would result from the administration of a bolus dose of 103 mg apartame/kg bw to a normal subject.

For a PKU heterozygous individual, the concentration of 240 μM would be reached by the administration of a bolus dose of 59 mg aspartame/kg bw.

Uncertainties

The following main assumptions were made based on the proposed MoA:

- Phenylalanine plasma level of 360 μM is the threshold for developmental effects.
- The diet results in phenylalanine plasma level not exceeding 120 μM.
- Peak plasma phenylalanine concentration can be used in the dose–response modelling as surrogate of steady-state plasma phenylalanine concentration.
- Bolus administration of aspartame can be used in the dose–concentration modelling of plasma phenylalanine to represent a more typical pattern of aspartame intake.
- The 95th percentile confidence interval of the lower bound estimate of the aspartame dose–plasma phenylalanine concentration curve provides a safe limit for plasma phenylalanine for the entire population (with the exception of homozygous PKU patients).
- The increase in plasma phenylalanine concentrations following aspartame administration will be the same in the general population as in individuals heterozygous for PKU.
- Reproductive and developmental toxicity of aspartame is solely dependent on systemic exposure to phenylalanine.
- There is no requirement for a pharmacodynamic uncertainty factor (a sensitive human population (PKU patients) was used to define the threshold).
- There is no requirement for a pharmacokinetic uncertainty factor (the aspartame plasma phenylalanine concentration was based on a more sensitive human subpopulation (PKU heterozygous)).

It was not possible to place a specific numerical value on the uncertainties related to these assumptions, but the aforementioned evaluations and considerations are more likely to overestimate than underestimate any potential developmental risk. Therefore, it is not illogical to conclude that the results of the uncertainty analysis further support the conclusion, that there is no safety concern for aspartame at the current ADI in normal and heterozygous subjects.

Annex C – BIOHAZ

Case study of biological relevance in the area of biological hazards - Zoonotic potential of classical scrapie

Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of progressive conditions that affect the brain and nervous system of many animals, including humans. Unlike other kinds of infectious disease, the infectious agent in TSEs is believed to be a protein, called the prion protein. Misshapen prion proteins are transmissible and are able to induce abnormal folding of specific normal cellular proteins that are found most abundantly in the brain: they carry the disease between individuals and cause deterioration of the brain. TSEs are unique diseases in that their aetiology may be genetic, sporadic or infectious via ingestion of infected materials and via iatrogenic means (e.g. blood transfusion). Prion diseases of humans include sporadic Creutzfeldt–Jakob disease (sCJD), new variant Creutzfeldt–Jakob disease (vCJD), Gerstmann–Straussler–Scheinker syndrome, fatal familial insomnia and kuru. Prion diseases of livestock include bovine spongiform encephalopathy (BSE) in cattle, classical scrapie in sheep and chronic wasting disease in cervids.

Host genetics substantially influence these diseases. In humans, familial prion diseases are closely associated with mutations in the prion protein gene, and the methionine/valine polymorphism at codon 129 appears to influence susceptibility, incubation period and in some respects disease phenotype.

One of the main questions in relation to the animal TSEs is their ability to infect humans. BSE is the only TSE agent identified as zoonotic. However, it has been hypothesised that other prions associated with animals such as classical scrapie can infect humans. For disease to develop in the case of exposure through foodstuffs, there must be exposure to a sufficient dose of the agent, the agent must be taken up from the gastrointestinal tract, enter the nervous system and be successfully transported to the neuronal cell bodies in the central nervous system. The infecting agent must then be able to 'convert' the cellular prion protein (PrP^{C}) to the abnormal from of the prion protein (PrP^{Sc}) at a rate which enables accumulation of sufficient PrP^{Sc} to cause disease within the life-span of the host.

Assessment strategy

In a paper, 'Evidence for zoonotic potential of ovine scrapie prions', published in Nature, Cassard et al. (2014) studied the zoonotic potential of classical scrapie by bioassay in mice, in which a range of characteristics were assessed. These included incubation periods and neuropathological characteristics. The authors concluded that the results demonstrated that scrapie prions have zoonotic potential and raise new questions about the possible link between human and animal prions. The European Commission asked the BIOHAZ Panel to scientifically appraise the paper considering the limitations, assumptions and uncertainties associated with the study design and outputs.

In line with the framework set out in Figure 1 of the main document for consideration of relevance, the agent, effect, subject and conditions can be considered as follows:

Agents/exposure	Effect/outcome	Subject/population	Conditions
Classical scrapie agent	TSE	Human	Oral exposure through the consumption of meat from an infected animal

Hence, the problem can be formulated in these terms: in humans, can the consumption of meat from classical scrapie infected sheep result in the development of a TSE?

Evidence/data needed to address the question

The evidence for the zoonootic potential of classical scrapie, as set out in the paper by Cassard et al. (2014), was evaluated by the BIOHAZ Panel using expert judgement.

Appraisal and integration of the evidence

Relevance of the agent

A spectrum of strains is responsible for classical scrapie in sheep, and there may be variability in properties that affect the ability to cross the species barrier. There is experimental evidence that some isolates may not be completely stable, and their fundamental properties may shift on transmission. There is also potential heterogeneity of geographical distribution of individual strains.

In the study by Cassard et al. (2014), six different isolates of classical scrapie were used. These had been previously studied in other animal models and showed some degree of biological variability. The BIOHAZ Panel concluded that the deliberate selection of biologically variable scrapie isolates represents an important new aspect compared to previous studies on the subject, given the known diversity within the group of TSE agents identified as classical scrapie. The Panel further concluded that no case selection will conclusively and comprehensively ever represent the total potential field exposure, but the Cassard et al. (2014) study made a good, rational and supported choice of isolates designed to be distinct from one another, to represent some of the possible range of field strains.

In conclusion, the isolates used in the study by Cassard et al. (2014) were considered relevant for the problem under investigation. However, evidence derived from a limited number of classical isolates cannot be extrapolated to represent the whole biological variability of classical scrapie.

Relevance of the subject

In the study by Cassard et al. (2014), animal models were used to evaluate the zoonotic potential of classical scrapie. Transgenic mice overexpressing the human PrP gene and homozygous and heterozyous for methionine and valine at codon 129 were inoculated intracerebrally. Historically, laboratory studies using animal models were carried out using wild-type mice. However, a substantial proportion of human and animal TSE isolates cannot be propagated into conventional mice models, which limits the usefulness of this system to characterise and compare TSE agents circulating in the field. More recently, transgenic mice with PrP derived from different species have been increasingly used in experimental transmission of TSE agents. Transgenic mice that are homozygous for methionine at codon 129 of the human PrP gene have been previously shown to be fully susceptible to human TSEs, such as sCJD and vCJD and, to a much lesser extent, cattle BSE, the only animal TSE with confirmed zoonotic potential identified so far.

Different lines of transgenic mice that express the human PrP gene were used in the study, namely the tg340, tg361 and tg650 mouse lines. The tg340 mouse line expresses methionine approximately fourfold more than normal human brain tissue at codon 129. The tg650 mouse line overexpresses methionine sixfold at the same codon. The tg361 mouse line overexpresses valine at codon129 at fourfold levels. A breeding cross between the tg340 and the tg361 provided mice that overexpress both M129 and V129 alleles at similar levels. Although PrP overexpression might circumvent the low susceptibility of gene-targeted tg mice, it is worth noting that an inevitable limitation of such transgenic mice is that only one human gene is present in the model, while disease susceptibility and incubation period are inevitably multifactorial. Additionally, if the time taken for the conversion of human PrPc to PrPSc exceeds the lifespan of the mouse, this may give a 'false negative' outcome.

The BIOHAZ Panel concluded that the mouse lines used by Cassard et al. (2014), in particular tg650 and tg340, are well established and have been shown to be susceptible to different CJD and BSE strains. Although overexpression of PrP is not a natural condition in humans, and it might have impact on some biological parameters, this can be considered a scientifically appropriate approach to modelling the molecular barrier for transmission of scrapie in humans despite some limitations of these transmission models, as mentioned above.

Relevance of the effect

In the study, serial passages were used in the transgenic mice for each of the six different strains of classical scrapie. Serial passages of bovine BSE, human sCJD isolates and human vCJD isolates were also carried out in these mouse lines for comparison with the classical scrapie isolates. Based on the attack rates observed after serial passages in transgenic mice, the potential for classical scrapie transmission is: (i) low or absent in MM129 mice; (ii) low or absent in MV129 mice; (iii) absent in

VV129 mice. The data suggest that BSE is still more efficient than scrapie in MM129 mice, while a single scrapie isolate would be more efficient than BSE in MV129 mice

Moreover, the study also showed that the serial transmission of different scrapie isolates in humanised transgenic mice led to the propagation of prions that were phenotypically identical to those that cause sCJD in humans.

In summary, the effect shown by Cassard et al. (2014) was considered relevant. The study showed that transgenic mice could be infected with classical scrapie strains but that the transmission was less efficient than for bovine BSE. It should also be noted that while serial passage maximises the chance of detecting the propagation of TSE agents, it does not mimic natural exposure in humans.

Relevance of the conditions

Intracerebral inoculation is a widely accepted and appropriate choice of inoculation in mouse models. This method can be used to assess the permeability of the transmission barrier at the molecular level, i.e. the conformational compatibility between the infecting prion strain and the PrP^{C} of the recipient species. This is an important factor which limits the propagation of prion agents among different species. Successful amplification of PrP^{Sc} would indicate that the TSE strain has the potential to convert human PrP^{C}.

Natural exposure in man is believed to involve the oral route through the consumption of meat from an infected animal. In this respect, the inoculation route used in the mouse model does not represent an ideal strategy for the investigation of zoonotic potential since the involvement of the digestive system, the rest of the lymphoreticular system, the enteric nervous system and peripheral nervous system have been bypassed by the direct deposition of the prions in the brain.

Therefore, it was concluded that the inoculation route used by Cassard et al. (2014) cannot reproduce field conditions and does not mimic natural exposure.

Overall conclusion

The BIOHAZ Panel concluded that the paper by Cassard et al. (2014), provides evidence that some classical scrapie isolates can propagate in humanised transgenic mice and produce prions that on second passage are similar to those causing one form of sCJD. However, the BIOHAZ Panel also concluded that the results from the study raise the possibility that scrapie prions have the potential to be zoonotic but do not provide evidence that transmission can or does take place under field conditions.

Uncertainty

The main potential sources of uncertainties in this experimental setting may be summarised as follows:

- Evidence derived from a limited number of classical isolates cannot be extrapolated to represent the whole biological variability of classical scrapie.
- The use of an animal model and the over-expression of PrP may not allow a direct extrapolation to human population.
- Subsequent serial passages were thought as a way to overcome the problem of allowing a longer incubation period in mice: the occurrence of a similar condition is not realistic in the field.
- The intracerebral inoculation route used by Cassard et al. (2014) cannot reproduce field conditions and does not mimic natural exposure.

Annex D – CEF

Evaluation of the toxicity of BPA for humans considering all relevant toxicological information

Assessment strategy

The EFSA scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has re-assessed in 2015 the risks to public health from BPA exposure by evaluating the toxicity of BPA for humans, including for specific (vulnerable) groups of the population (e.g. pregnant women, infants and children, etc.) and considering all relevant toxicological information available. Exposure assessment was performed for various groups in the population and finally human health risks were characterised taking into account specific groups of the population [EFSA Journal 2015; 13(1): 3978].

Although the scientific assessment of BPA had a broader content, for the purpose of this guidance the example below focuses only on one effect.

Specification of the agent

Bisphenol A (BPA) is an organic chemical used as a monomer in the manufacture of polycarbonate plastics and epoxy resins and as an additive in plastics. Polycarbonates are used in food contact materials such as reusable beverage bottles, infant feeding bottles, tableware (plates and mugs) and storage containers. Epoxy resins are used in protective linings for food and beverage cans and vats.

The scientific debate on the risks for public health of BPA is focussed on its endocrine-active properties, which might adversely impact physical, neurological and behavioural development. In addition, other perturbations of physiology, both in animals and humans, have been brought in relationship to the endocrine-active properties of BPA. Among these are, e.g. obesity, modification of insulin-dependent regulation of plasma glucose levels, perturbation of fertility, proliferative changes in the mammary gland possibly related to the development of breast cancer, immunotoxicity and adverse effects on the cardiovascular system (for an overview, see EFSA, 2006, 2008, 2010; NTP-CERHR, 2008; ANSES, 2013).

Specification of the subject

The question of interest resulting from this risk assessment of BPA was: 'What is the biological relevance for human health of the observed proliferative and morphological changes in the mammary gland following exposure to BPA and the possible relevance for the development of breast cancer'?

Specification of the effects

To update the risks to public health from BPA exposure, the complex BPA toxicity was re-evaluated by EFSA in 2015 using a Weight of Evidence (WoE) approach to identify the critical toxicological effects [EFSA Journal 2015; 13(1): 3978]. The effects on kidney weight were considered critical endpoints and taken forward to hazard characterisation to assess a reference point (BMDL10) for the derivation of a health-based guidance value (TDI). As the scientific evidence for observed reproductive- and developmental effects, neurological-, neurodevelopmental- and neuroendocrine effects, immune effects, cardiovascular effects and metabolic effects was not sufficient, they were not taken forward for risk characterisation, but these effects were included in the uncertainty evaluation.

In the WoE approach used for hazard identification, next to the general toxicity effects on kidney weight, also proliferative and morphological changes in the mammary gland were reported in several new toxicity studies and considered 'likely' (likely refers to 66–100% probability), although no reference point (BMDL10) could be calculated.

These proliferative responses and possibly enhanced sensitivity to mammary gland carcinogens seen in animal studies might be of relevance for human health and were therefore included in the risk assessment.

Collection of data relevant to the problem formulation

Earlier evidence for BPA effects on cell proliferation and differentiation and morphological changes potentially related to tumour induction in the mammary gland [EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2010] were supported by new toxicity studies

published from 2010 onwards. A number of these new laboratory animal studies in rodents (rats and mice, including a.o. transgenic mouse models and DMBA mammary tumour mouse model) and a monkey study, have reported effects on mammary tissue (mammary tumour induction, enhancement of mammary tumour growth and/or proliferative changes in mammary gland) after prenatal, perinatal and adult exposure to BPA.

Overall, using expert judgement, the CEF Panel concluded that although there were methodological weaknesses in all these studies with the exception of a US FDA/NCTR subchronic toxicity study, which was a detailed guideline study conducted in accordance with GLP, they provide further evidence that BPA may enhance mammary epithelial proliferation in animal models.

However, the proliferative changes in the mammary gland reported in these new studies, including a non-human primate study, are considered insufficient to conclude that there is a link to cancer development in later life. Nevertheless, there might be a possible role of BPA in increasing the susceptibility to mammary gland carcinogenesis.

Relevance of the agent

Although the exact MoA in respect to the reported proliferative changes in the mammary gland is not clarified, they may well fit with the conclusions of the mechanistic studies in which it is shown that BPA affects a number of receptor-dependent and independent signalling pathways, resulting in effects on hormone homeostasis and gene expression as well as cytogenetic and epigenetic effects.

It was concluded in the Panel that no single clearly defined mode of action of BPA can be identified that can contribute substantially to the understanding of the potential effects of BPA in humans. However, given that BPA appears to have multiple modes of action at the cellular level, and at least some of these MoAs involve cellular responses that are highly conserved across species (e.g. binding to oestrogen or androgen receptors), the relevance for humans of the variety of effects that have been reported for BPA in mechanistic studies cannot be totally discounted.

Relevance of the subject

Although there is no convincing evidence that BPA is carcinogenic in animals when exposed to adults or during pre- and post-natal (during lactation) development, a large number of the animal studies suggest that BPA can have a proliferative/developmental advancement effect on mammary tissue, and may also have an effect on tumour growth in animal models, particularly in sensitive transgenic models or when followed by a treatment with a complete carcinogen (DMBA).

In a large number of rodent toxicology studies, including a study with non-human primates, effects have been noted of pre- or perinatal BPA exposure on mammary gland morphology, cell proliferation and modification of gene expression. For instance, the architectural modifications induced by the BPA in mammary glands of female offspring were transient increases in the total number of epithelial structures. Moreover, time and dose-dependent modifications in gene expression profiles were observed after treatment with BPA, e.g. modulated (mainly upregulated) genes related to cell proliferation, apoptosis and differentiation, cell communication, signal transduction, immunity, protein metabolism and modification. Supportive observations also came from several studies (10) using the subcutaneous (s.c.) route of administration of BPA, which also indicated that prenatal BPA exposure results in an increased cell proliferation/apoptosis ratio in normal tissue as well as preneoplastic lesions of rat mammary gland.

In relation to possible carcinogenic effects of BPA in animals when exposed pre- and post-natally (during lactation), several studies (5) used the dimethylbenzanthracene (DMBA) mammary tumour mouse model to assess the effects of fetal or postnatal exposure to BPA on the development of mammary tumour in adults. Overall, increased susceptibility to development of mammary cancer, decreased tumour latency and increased tumour multiplicity was reported.

Also, studies were performed in transgenic mouse models, such as an adult knockout mouse model of mammary neoplasia, showing increased epithelial cell proliferation and hyperplasia in mammary glands of adult BRCA1* knockout mouse upon BPA exposure via osmotic pumps, In addition, in a female transgenic MMTV-erbB2/neu mice susceptible to develop mammary carcinoma, BPA treatment via drinking water resulted in a decreased tumour latency and increased tumour multiplicity, enhanced tumour volume and higher incidence of lung metastasis.

Also, the US FDA/NCTR subchronic (90-day) toxicity study provided some evidence of a BPA-related effect in the mammary gland of female rats. Mammary gland duct hyperplasia of minimal severity was

reported in the female groups examined at Post-natal Day (PND) 21 and in the high-dose female BPA groups examined at PND 90.

Taken together, as intraductal hyperplasia in the mammary gland is observed in humans and is considered as a precursor of ductal carcinoma both in rodents and in humans, this lesion is considered of relevance when studied in animals (e.g. rodents) to predict cancer in the human mammary gland and is considered as adverse.

Relevance of the effect

Intraductal hyperplasia in the mammary gland is observed in humans and is considered as a precursor of ductal carcinoma both in rodents and in humans. Therefore, this lesion is of high relevance to predict cancer in the human and animal mammary gland and is considered as adverse.

Ductal hyperplasia and an increase of the number of Terminal End Buds (TEBs) may be regarded as supporting evidence for tumour formation along with an increase in the proliferation of epithelial cells. However, ductal hyperplasia may not always progress to neoplastic lesions but may be reversible. Therefore, the relevance of these hyperplastic lesions, in the absence of intra-ductal hyperplasia, is questionable for humans and the level of adversity of these findings is unknown.

Increased epithelial cell proliferation in the mammary gland of rodents is linked to prolactin, which is also associated with an increased breast cancer risk in women. Thus, an increase in prolactin levels constitutes an underlying mechanism in the induction of cell proliferation, which may be indicative and therefore relevant for tumour promotion in both the human and rodent mammary gland.

In summary, based on the above indicated observations the proliferative and morphological changes in the mammary gland reported in several toxicity studies with BPA were considered relevant.

Relevance of the conditions

The experimental test species and test conditions were for most studies considered relevant, although the study reliability (e.g. data reporting, methodology) was considered low or medium for all studies on BPA-induced proliferative effects.

Although several studies were conducted with the non-relevant subcutaneous route of pre-or perinatal BPA exposure, supportive observations of increased cell proliferation/apoptosis ratio were reported in normal tissue as well as preneoplastic lesions of rat mammary gland, while in other studies with perinatal BPA exposure no such lesions were detected.

The relevance of the findings in the DMBA mammary tumour model and the sensitive transgenic models is uncertain because of limited experience with these models.

Uncertainties

The uncertainties related to the induction of proliferative changes in the mammary gland following BPA administration, i.e. intraductal hyperplasia, epithelial cell proliferation and ductal hyperplasia (including increase in the number of TEBs), were evaluated taking into account the reliability of the study results.

For the evaluation of uncertainty, the expert panel reviewed the studies considered in the WoE, and extracted key information from each study and collated that in a graphical format. The graphs summarised for each study, the life stage of the animals at treatment onset, duration of treatment and sampling time for measurements, the doses tested, whether there was a statistically significant effect at any dose, the number of strengths and weaknesses of the study, the Panel's evaluation of the reliability of the study and its relevance to the effect of interest.

Some potential sources of uncertainties:

- Several studies with subcutaneous, pre- or perinatal BPA exposure reported on intraductal hyperplasia in the mammary gland (i.e. an increase in the relative number of ducts lined by three or more layers of epithelial cells), while in other studies with perinatal BPA exposure no such lesions were detected.
- While epithelial cell proliferation is a normal physiological process in certain life stages (pre-/perinatal period, pregnancy) and per se does not lead to tumour formation, it is generally accepted that under certain pathological conditions such as recurrent tissue damage and repair the proliferating tissue becomes more susceptible to tumour development. In studies with rats

treated with BPA and, thereafter, with a well known complete carcinogen (DMBA), as well as in studies with transgenic mice, increased cell proliferation was reported along with tumour formation. In case of the study that observed cell proliferation in transgenic mice which spontaneously develop tumours, the relevance of these findings to whether proliferative changes occur at low BPA doses in normal animals was considered medium, taking into account the increased sensitivity of this mouse model to tumour development.

- Increase in the number of TEBs as well as ductal hyperplasia was reported in several studies even at very low BPA doses. However, it should be noted that these putative pre-neoplastic lesions may be reversible and will not in all cases progress to neoplasia.
- In addition, also the study reliability (e.g. data reporting, methodology) was considered low or medium for all studies on BPA-induced proliferative effects.

Conclusion

In the final assessment, the overall likelihood of the BPA-induced proliferative changes in mammary gland in animals exposed during pre-and postnatal (during lactation) development or up to 90 days (gavage) was considered 'likely', and taken forward for the risk characterisation based on the consistency of the effect in a number of studies.

The Panel concluded that the health-based guidance value should cover the lowest dose in the dose range for which the likelihood approaches 'likely' from the overall uncertainty evaluation, taking into account uncertainty of all the evaluated endpoints as well as their relevance and adversity to humans. The uncertainty evaluation approached 'likely' in the (HED) dose range of 100–1,000 μg/kg bw per day. The Panel therefore decided that the uncertainty regarding the above mentioned effects at the HED of 100 μg/kg bw per day and higher should be taken into account when establishing a health-based guidance value by including an extra factor in establishing the TDI. Thus, as the reference point was 609 μg/kg bw per day based on the mean relative kidney weight and the lower end of the dose range for which the uncertainty evaluation for other endpoints approached 'likely' is 100 μg/kg bw per day, a factor of 6 was applied.

The CEF Panel applied finally a total uncertainty factor of 150 for inter- and intraspecies differences (1 for toxicokinetics and 2.5 for toxicodynamics and 10 for intra-species differences), and the uncertainty factor of 6 (for e.g. mammary gland effects) to establish a temporary Tolerable Daily Intake (t-TDI) of 4 μg/kg bw per day.

By comparing the t-TDI with the exposure estimates, the CEF Panel concluded that there is no health concern for any age group from dietary exposure or from aggregated exposure.

Annex E – FEED

Example from FEEDAP

1. Problem formulation

The scientific evaluation of feed additives by EFSA includes:

- The safety of the additive for the target animals
- The safety of the additive for the consumer (human health)
- The safety of the additive for the user/worker
- The safety of the additive for the environment
- The efficacy of the additive

The assessment of feed additives is a standardised process, which follows legal guidelines and guidance documents. The assessment questions are already defined in a standard form in the terms of reference and the assessment follows a standardised procedure.

The evaluation of the biological relevance of an effect under consideration in the FEEDAP Panel includes adverse (unwanted) effects and beneficial (wanted) effects on potentially different species and has to address all aspects indicated above.

The feed additive considered in this example consists of two essential oils derived from steam distillation of *Thymus vulgaris* (thyme) and *Illicium verum* (star anise), quillaja bark powder, crushed herbs and spices, and other feed materials (EFSA FEEDAP Panel, 2016). Thymol and *trans*-anethole are the major components of thyme oil and star anise oil, respectively. Star anise oil may contain the genotoxic carcinogen alkenyl-benzene derivative estragole in considerable concentrations.

The additive is intended for use in chickens for fattening (the target species) at a dose of 150 mg/kg complete feed.

Problem formulation at a glance is shown in the following Table.

Agents	Effects	Subjects	Conditions
Additive/single active substances: *trans*-anethole and thymol	Short-term toxicity (mortality, clinical effects, performance parameters, haematology, blood chemistry, histopathology when needed)	Target species	Dietary exposure at recommended dose in feed (150 mg/kg feed) – Establishment of a safe dose via tolerance studies (x10 overdosing)
Single active substance: *trans*-anethole	Effects on liver enzymes and histopathology. Liver effects for *trans*-anethole in laboratory animals	Target species, Consumers	Dietary exposure via residues (data available from tolerance study, x10)
Single active substances: *trans*-anethole and thymol	Irritation, skin sensitisation	Users/workers	Exposure by inhalation, contact, systemic exposure
Single active substances: *trans*-anethole and thymol	Short-term effects (LC_{50}, EC_{50}) and long-term effects (NOEC)	Terrestrial and aquatic organisms in the environment	Exposure via manure containing residues or non consumed feed
Single active substance: estragole	Genotoxicity	All subjects	See above
Additive/single active substances: *trans*-anethole and thymol	Improving animal performances	Target species	Dietary exposure at the proposed use level
	Digestibility enhancer		

Specification of the agents

The active substances in the additive were considered to derive mainly from the thyme oil and star anise oil. The crushed herbs and spices will also contribute to the activity but to a lesser extent. Thymol and *trans*-anethole, the major components of thyme oil and star anise oil, represent about 0.2–0.4% and 4–5% of the additive, respectively. Star anise oil may contain the alkenyl-benzene derivative estragole up to 6% (European Pharmacopoeia, 2005).

Specification of the effect(s)

Adverse effects

Liver effects: Hepatotoxic effects have been reported for *trans*-anethole when administered to rats (WHO, 2000, EFSA FEEDAP Panel, 2011a). A no observed effect level (NOEL) of 300 mg/kg bw per day was derived from a 90-day study based on elevated serum activity of γ-glutamyl transferase observed at 600 and 900 mg/kg bw per day in male and female rats, respectively (Minnema 1997). The NOEL was considered as an appropriate point-of departure to derive an acceptable daily intake (ADI) of 0–2.0 mg/kg bw per day (by applying an uncertainty factor of 200 to allow for deficiency in the long-term study; WHO, 2000).

Genotoxicity: Estragole was demonstrated to be genotoxic in several in vitro and *in vivo* short-term assays and carcinogenic in mice after oral administration. As such, estragole has the ability to induce cancer in the exposed organisms through a genotoxic MoA; as a genotoxic agent, it is considered also to induce mutations in germ cells of humans and animals, with negative effects for the reproduction. Both effects are clearly defined as adverse and could be relevant for target animals, consumer, user and environment, if the conditions of the exposure to the compound allow the adverse outcome to occur.

Positive effects

An assessment of the efficacy of the feed additive is needed because the applicant claims that it increases the animal performance and digestibility of feed in chickens for fattening. Relevant performance parameters suitable for the assessment are the determination of feed intake, body weight gain and feed to gain ratio. Trials to demonstrate the efficacy of feed additives *in vivo* should be performed according to the guidance published by EFSA (EFSA FEEDAP Panel, 2011b).

Specification of the subjects

Adverse effects

The subjects of adverse effects are: (i) the target animal fed diets containing the additive (chickens for fattening), (ii) the consumer of the food products from chickens fed the additive, (iii) the workers handling the additive and (iv) terrestrial and aquatic organisms in the environment.

Positive effects

The subjects of positive effects are the target animals fed diets containing the additive (chickens for fattening).

Specification of the conditions

trans-Anethole and estragole are part of a diet for chickens. Thus, the compounds enter the body of the animals by oral uptake. Possible residues of the additive/active substances in the meat from chickens fed the additive are taken up by humans with their food. Users and workers, handling the additive or the feed with the additive may also be exposed to the compound via the skin, eye, mucosae or lung. Organisms of the environment may be exposed to the compounds or their metabolites via the manure of the chicken, which is used as a fertiliser and could contain residues of the additive.

Collection of data relevant for the problem formulation

The assessment is based on evidence/data provided by the applicant in the form of a technical dossier, prepared following the provisions of Regulation (EC) No 429/2008 and relevant Guidance documents (EFSA, 2008; EFSA FEEDAP Panel, 2011b, 2012a,b,c).

These data included the characterisation of the additive, two tolerance studies in chickens for fattening, residue data in meat and liver from chickens fed the additive at 10x the recommended dose, five long-term and six short-term efficacy studies.

Tolerance studies are designed as short-term toxicity studies to assess adverse effects of the additive in the target species at the proposed conditions of use and at x-fold (10x, 100x) the recommended dose. The endpoints considered in tolerance studies are: mortality, clinical effects,

zootechnical parameters (body weight, average daily gain, average daily feed intake, feed conversion ratio), haematological and blood chemistry parameters, gross pathology, organ weight and histopathology (if needed).

Efficacy studies are designed to demonstrate the efficacy of the additive at the lowest recommended dose. A significant effect on the performance parameters consistently observed in three long-term studies (feed intake, body weight gain and feed to gain ratio) allows the conclusion that the additive has the potential to be efficacious.

Assessment of the collected data sets for biological relevance *trans*-Anethole

Relevance of the agents

Adverse effects

trans-Anethole specified as a major component is of the additive is considered, at least in part, responsible for potential adverse effects of the additive. Literature data are available for the adverse effects of the pure compound trans-anethole (effects on liver enzymes and histopathology, liver toxicity in rat, WHO).

If adverse effects were observed in tolerance studies performed with the additive, it would not be possible to conclude which agent(s) is (are) considered responsible for the observed effects. Besides *trans*-anethole, other additive ingredients or components of the essential oils could also be responsible for potential adverse effects of the additive.

Positive effects

If a beneficial effect of a feed additive is based on economic parameters relevant for the farmer and not the animal, it can be demonstrated by a statistical significant increase of performance parameters. The efficacy of the feed additive containing thymol and trans-anethole as part of essential oils and thus its biological relevance was demonstrated by a statistically significant increase ($p < 0.05$) of the performance parameters indicated above, however, there is no direct evidence that this effect is due only to these two components.

Relevance of adverse effects of *trans*-anethole

Relevance of the methods

Possible adverse effects for target species were assessed in tolerance studies, where the additive was fed at the proposed conditions of use and at 10-fold the recommended dose. These tolerance studies included endpoints which could also detect adverse effects on the liver (liver weight, liver enzymes, histopathology.), which were observed in the rat studies. Since no adverse effects were observed at the proposed conditions of use and at up to a 10-fold of the recommended dose, it was concluded that the additive and the active substances are well tolerated by the target animals.

Relevance for the target species

Liver toxicity was observed in sub-chronic and chronic toxicity studies in rats treated with *trans*-anethole. These effects are not specific for rats and can be extrapolated to other species. They are therefore considered relevant for the target species, i.e. chicken for fattening. However, the conditions of chronic studies could be of limited relevance for target species with a short life span as is the case for the target animals of this example.

Tolerance studies performed with the additive under assessment are relevant to assess adverse effects in the target species. Liver effects were not observed in tolerance studies in chicken for fattening. The reason might be that the exposure level of *trans*-anethole as part of the feed additive was not high enough. Assuming that the additive is supplied at the proposed use level of 150 mg/kg and considering the default values of feed intake and body weight for chickens for fattening (EFSA FEEDAP Panel, 2012d), it can be calculated that this dose level would result in an exposure of 0.3 mg/kg bw of trans-anethole per day. In the experiment with the 10-fold overdose of the additive, this value would be 3 mg/kg bw per day. The NOEL derived from the 90-day rat study was

300 mg/kg bw, which provides a 1,000-fold margin of safety compared to the chicken exposed with the proposed dose level of the additive (150 mg/kg feed) and a 100-fold margin for the experiment with the 10-fold overdose. Thus, the liver toxicity of *trans*-anethole is not relevant for the target animals because the exposure level is not high enough.

Relevance for the consumer

trans-Anethole is metabolised along the same three major pathways in rat, mice and humans. Therefore, hepatotoxicity in rats was considered relevant to humans, if they are exposed to trans-anethole via residues.

The applicant provided evidence that residues of trans-anethole could not be detected in meat from chickens fed the additive at 10 times the recommended dose (limit of detection, 0.1 μg/g). Exposure of consumers to *trans*-anethole can therefore be excluded. The presence of *trans*-anethole in chickens feed at the recommended dose level will therefore not be of biological relevance for the consumer.

Relevance for the user

trans-Anethole is irritating to skin and may cause risk of serious damage to eyes after direct exposure. Handling of the compound during preparation of the additive could therefore provide adverse effects to workers. Besides *trans*-anethole, the feed additive contains a variety of other compounds, which have the potential to irritate eyes and mucous membranes and to cause allergies upon contact with skin and respiratory organs.

Relevance for the environment

trans-Anethole present in the feed of chicken for fattening will be extensively metabolised to inert compounds, excluding possible biologically relevant effects on the environment.

Relevance of positive effects for the target animals

The applicant claims that the feed additive increases the performance of chicken for fattening. This effect was proven in experimental trials with the target animals. Statistical parameters are used to confirm this claim. The effect cannot be attributed to certain compounds of the complex composition of the feed additive. The effect is of economical relevance for the farmer, because it reduces the costs for the meat production. The effect is not relevant for the animal.

Estragole

Relevance of the agent

A battery of standardised test systems is available to prove the genotoxicity and carcinogenicity of chemicals. The EFSA guidance on genotoxicity testing strategies applicable to food and feed safety recommends (EFSA Scientific Committee, 2011a,b) 'a step-wise approach for the generation and evaluation of data on genotoxic potential, beginning with a basic battery of *in vitro* tests, comprising a bacterial reverse mutation assay and an *in vitro* micronucleus assay. (…) In case of positive *in vitro* results, review of the available relevant data on the test substance and, where necessary, an appropriate *in vivo* study to assess whether the genotoxic potential observed *in vitro* is expressed *in vivo* is recommended. Suitable *in vivo* tests are the mammalian erythrocyte micronucleus test, transgenic rodent assay, and Comet assay. If the *in vivo* assay results in positive effects, the substance should be considered as an *in vivo* genotoxic agent. (…) If a two year carcinogenicity study in rodents results in a significant increase in the formation of malignant tumours compared to the control, the compound is considered as an animal carcinogen and possible human carcinogen'.

Evidence exists for the genotoxicity of estragole in V79 cells, CHO cells as well as rat and human hepatocytes *in vitro* and *ex vivo*, after oral treatment of rats with estragole (Martins et al., 2012). Estragole was also clearly genotoxic in transgenic mouse and rat strains (Suzuki et al., 2012). Clear evidence for the carcinogenicity of estragole comes from studies in mice (Drinkwater et al., 1976).

The MoA for the genotoxicity of estragole is the oxidation to 1-hydroxyestragole by CYP1A2 and conjugation with sulfate to 1-sulfooxyestragole by SULT1A1 (Wiseman et al., 1985). Spontaneous abstraction of SO_4^{2-} releases a carbocation, which form adducts with DNA and proteins. The formation of such adducts was demonstrated in the liver of mice and other mammalian species including human

liver specimens *in vitro* (Phillips et al., 1984; EMA, 2014). On the basis of this mechanism, estragole is a genotoxic hepatocarcinogen and the formation of DNA adducts is the first pre-initiation step. Although hepatocarcinogenicity of estragole has only been demonstrated in mice, the presence of the enzymes involved in the critical steps of tumour initiation is not restricted to mice and makes it likely that the same MoA takes place in other species including birds and humans (EMA, 2014). In the absence of evidence showing that estragole does not reach germ cells, it has to be assumed that estragole can exert its genotoxic effects in both somatic and germ cells.

Relevance of adverse effects of estragole

Although there is a debate about the question whether or not a threshold dose exists for genotoxic compounds, it is generally accepted that the exposure of humans and animals to carcinogenic compounds should be avoided as much as possible. With respect to the food and feed industry this means that, whenever possible, carcinogens should not be added to human food or animal feed. Therefore, the presence of the genotoxic and carcinogenic potential of estragole in the feed of farm animals, which serve as food for humans is of critical relevance for the risk assessment.

Relevance of the subjects and conditions

Relevance for the target animal

The additive is intended as a feed additive for chicken for fattening. These animals have a short life span, which makes it very unlikely that they develop cancer as a result of the exposure to the carcinogenic compound in their diet. These animals are also not used for reproduction. Thus, although genotoxicity is a strong adverse effect, the biological relevance for the target animals of this example (chicken for fattening) is limited.

Relevance for the consumer

For the assessment of the safety for the consumer, the critical question relates to whether the carcinogen (estragole) is transferred to human food obtained from chickens fed with the additive. Therefore, the ADME profile of the genotoxic compound has to be investigated and analytical data of possible residues in edible tissues of the chicken are needed, performed with methods being sensitive enough to detect very small concentrations of the critical compound and its active metabolites. If the compound is not absorbed or totally metabolised to innocuous compounds and if the absence of the genotoxic compound itself or genotoxic metabolites thereof can been proven, the use of the genotoxic compound in feeds may also be of no biological relevance for the consumer. However, it is often difficult to demonstrate the absence of the genotoxic compound or its metabolites in products derived from animals fed with the additive, for technical reasons (the sensitivity of the analytical method applied results in an 'analytical threshold'). The addition of an essential oil containing estragole is therefore of biological relevance for the consumer.

Relevance for the user

Genotoxic compounds in feed additives may create a concern for the safety of the user, if any contact with such compounds cannot be avoided. Exposure to estragole while handling the compound can occur mainly via skin contact and inhalation of the star anise oil. The presence of estragole in feed of chicken for fattening represents a biologically relevant hazard for users handling the additive, which is of biological relevance.

Relevance for the environment

Estragole is a naturally occurring compound in plants present in the European environment. Because of the relatively low concentration in the feed of chickens for fattening and the metabolism in the target animal, possible residues of estragole in the excreta of the birds will not measurably increase the concentration of this compound in the environment. For these reasons, the presence of estragole in the feed of chickens for fattening is considered without biological relevance for the environment.

Overall conclusion

During problem formulation, the presence of estragole was identified as a hazard associated with the exposure to the additive, particularly for consumers potentially exposed to residues of the additive via products of animal origin (meat) and users exposed via inhalation. Considering that the intentional addition of compounds with genotoxic-carcinogenic properties to the food chain via feed additives should be avoided (minutes of the 109th Plenary meeting of the FEEDAP Panel), the applicant reformulated the additive to remove estragole from the additive.

Uncertainties

Adverse effects

The MoA for the genotoxicity of estragole is the oxidative conversion to 1-hydroxyestragole, which is further conjugated with sulfate to the ultimate carcinogen (Boberg et al., 1983). After long-term treatment of mice, the animals developed significant increases in the incidence of hepatocarcinomas. The high sensitivity of mice to develop liver cancer limits the extrapolation of this effect to humans. However, it was demonstrated that the metabolism of estragole leading to DNA adducts in the liver is not restricted to mice and occurs also in other species including birds and humans. The carcinogenicity of estragole in mice can therefore be taken as evidence for a possible carcinogenic effect of estragole in other species including humans.

It was demonstrated that the percentage of 1-hydroxyestragole formed after application of estragole to mice and rats increases with the administered dose. At low concentrations, estragole is mainly metabolised via alternative pathways to non-genotoxic compounds (Anthony et al., 1987). Taken into consideration that the concentration of estragole residues in the tissue of chicken treated with the additive is very low, this fact increases the uncertainty that estragole residues represent a biologically relevant hazard for the consumer.

Relevant adverse effects of *trans*-anethole and other non-genotoxic irritating compounds of the feed additive are restricted to the user/worker. These effects depend on the mode and level of exposure and thus to the safety precautions which are in place at working facilities.

Positive effect

The feed additive has the capacity to increase the performance of chicken for fattening, providing positive economic effect for the farmer. Any such effect exceeding the costs of the additive can be considered as relevant. However, the animal itself will not benefit from this positive effect.

Considerable uncertainty exists about whether such effects might be attributed to certain compounds of the feed additive or they rather reflect an additive effect of the mixture.

Annex F – GMO

Scientific Opinion on application EFSA-GMO-NL-2007-45 for the placing on the market of herbicide-tolerant, high-oleic acid, genetically modified soybean 305423 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer (EFSA GMO Panel, 2013)

Assessment strategy

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean 305423 (Unique Identifier DP-305423-1) for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003,[4] i.e. to assess whether the import of soybean 305423 and/or any of its derived products in the EU would result in additional safety concerns to animal and human health or to the environment with respect to conventional soybean. Since the scope of this application excludes cultivation in the European Union the environmental risk assessment (ERA) is limited to the consequences of accidental spillage of imported soybeans and to the dissemination of faeces of animals feeding soybean 305423.

The risk assessment strategy for genetically modified (GM) plants and derived food and feed is described in EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011), the ERA of GM plants (EFSA GMO Panel, 2010), and the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2010, 2011). It is based on comparative assessment approach, that is to compare GM plants and derived food and feed with their appropriate comparators by appropriate methodologies and tools. The underlying assumption is that traditionally cultivated crops have a history of safe use for consumers and/or domesticated animals. It is designed to characterise the intended effects of the genetic modification and to identify possible unintended effects associated to it. Expected and unexpected differences observed on a series of plant characteristics between the GMO and its comparator will then be assessed as regards their biological relevance for humans and animals from the nutritional, toxicological and environmental viewpoint (EFSA GMO Panel, 2010, 2011).

Specification of the agent

1) The GM plant itself: Soybean 305423

   Soybean 305423 was transformed:

   - to express the *Glycine max-hra (gm-hra)* gene coding for a modified version of the GM-HRA protein conferring tolerance to acetolactate synthase (ALS)-inhibiting herbicides;
   - to express a fragment of the endogenous *fad2-1* gene resulting, through RNA interference, in the silencing of the endogenous *fad2-1* gene, which leads to a decreased level of the omega-6 fatty acid desaturase and a high-oleic acid phenotype.

2) The newly expressed protein as such: GM-HRA

This protein is expressed in soybean 305423 as intended trait of the genetic modification. Two forms of this protein are considered:

a) The GM-HRA protein expressed in soybean 305423.
b) The equivalent GM-HRA protein expressed in a recombinant microbial system (*E. coli*) used in toxicological studies.

Specification of the subjects

- Humans who are exposed to the agents through the consumption of soybean 305423 and derived food.
- Animal species that are exposed to the agents through the consumption of soybean 305423 and derived feed.

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1–23.

- Ecosystems, including soils, could also be exposed to the GM plant but, considering the scope of the application that excludes cultivation and the nature of the crop, the level of exposure is likely to be very low.

Specification of the effects

In soybean 305423, the genetic modification is intended to introduce two new traits (intended effects of the genetic modification): herbicide tolerance, via the expression of a endogenous enzyme modified for herbicide-resistance; and high-oleic acid phenotype, via RNAi-mediated modulation of the plant fatty acid metabolic pathway.

Intended effects are known *a priori* to occur, while the size of their change might need confirmation.

- increase in oleic acid contents in soybean 305423 compared to conventional soybean, which in turn might change the prevalence of oleic acid in the diets of animals and humans;
- tolerance to ALS-inhibiting herbicides, likely to lead to a change of weed control management of soybean 305423, which in turn might lead to changes in plant metabolism and the presence of ALS-inhibiting herbicides residues in the plant.

Unintended effects are not known *a priori* but may include:

- changes in the agronomic and phenotypic characteristics (e.g. plant height, seed weight) of soybean 305423 compared to its comparator, which may be indicative of changes in the metabolism;
- changes in the level of endogenous components of soybean 305423 compared to its comparator, which in turn may affect the nutritional balance of animal and human diets or induce toxicological effects (dependent on the specific toxic compounds whose level has changed);
- toxicological and allergenic effects of the newly expressed protein GM-HRA;
- increased allergenicity of soybean 305423 compared to conventional counterpart (*soybean is considered a common allergenic food [European Commission*, 2007]).
- the presence of open reading frames (ORFs), which might translate into peptides raising safety concern.

Indirect and/or delayed effects may also result from the changes in agricultural practices induced by the introduction of the GM plant but such effects are not relevant in the context of this application that does not cover cultivation in the EU.

The introduction of soybean 305423 on the market (through the import and processing of materials, beans and/or meals) could replace already used conventional soybeans in animal feeding and human use.

Under this scenario, the safety of soybean 305423 was assessed as regards its intended trait (newly expressed protein and modified fatty acid profile) and unintended changes observed.

Considering the modified fatty acid profile of soybean 305423, the impact of this replacement on the diet and in feedstuff formulation was assessed by an exposure assessment. For the oil derived from soybean 305423 (the main product for human consumption), a replacement dietary exposure assessment was performed to investigate whether unbalanced diet for humans might result from soybean 305423 oil consumption, including investigations on the changes in the level of fatty acids for which nutritional recommendations exist.

Possible impacts of changes in the level of endogenous toxic (antinutritional) compounds in soybean 305423 compared to conventional counterparts of relevant for food and feed safety are assessed in the application.

Specification of the condition(s)

Conditions that should be implemented to assess the effects of the agents on the subjects are described in guidance documents (EFSA, 2011).

These include:

- a set of field trials comparing the GM plant, its conventional counterpart (i.e. the genetically closest line differing from the GM plant only for the genetic transformation) and a range of commercial reference varieties (to establish natural variability); field trials should be carried out

under representative receiving environments; agronomic, phenotypic and compositional characteristics are measured and are subject to a difference and equivalence test;
- a 28-day rodent study on the *E. coli* GM-HRA protein (according to OECD TG 407);
- *in vitro* pepsin- and pancreatin-resistance tests on the on the *E. coli* GM-HRA protein;
- animal studies on the whole food/feed from soybean 305423 (a 90-day rodent study according to EFSA Scientific Committee, 2011a,b an OECD TG 408; a 12-week study in laying hens and a 77-day study in pigs, according to *ad hoc* protocols);
- allergenicity testing on whole soybean extracts (human sera);
- Dietary intake/exposure scenarios for intended changes in oleic acid (*ad hoc* protocol).

Data collection

The risk assessment strategy for GM plants and derived food and feed proposed seeks to deploy appropriate approaches to compare GM plants and derived food and feed with their respective comparators. The underlying assumption of this comparative approach is that traditionally cultivated crops have gained a history of safe use for consumers and/or domesticated animals and the risk assessment primarily focused on new proteins and/or changes in composition of the GM plant. The starting point of the data collection aims at identifying similarities and differences between the GM plant and its conventional counterpart (see above).

Data were provided by the applicant in the form of a technical dossier.

Data provision was based on requirements by EFSA GMO guidance documents (EFSA Guidance for risk assessment of food and feed from GM plants; EFSA GMO Panel, 2010, 2011).

Ad hoc additional data asked from EFSA and/or provided by the applicant. These were necessary to corroborate and to further clarify information on:

1) **Agents:** further details on RNA interference process in soybean 305423; on the structural and enzymatic activity of the newly expressed protein GM-HRA; toxicological profile and allergenicity of newly expressed protein GM-HRA.
2) **Identification of the effects:** clarification on the outcome of comparative analysis assessment (agronomic and phenotypic characteristics and particularly compositional analysis) and possible biological effects (nutritional impact) of these on consumers/animals.
3) **Identification of conditions:** comparator used in comparative assessment studies; statistical methodology used in comparative assessment studies; test conditions in toxicological and animal feeding studies; allergenicity testing of soybean extracts on human sera; dietary exposure scenarios in humans and animals.

Data included:

- a molecular characterisation, which provides information on the structure and expression of the insert(s) and on the stability of the intended trait(s);
- a comparison, under representative field conditions, of agronomic, phenotypic and compositional characteristics between the soybean 305423 and its conventional counterpart (field trials, in accordance to EFSA Guidance);
- a toxicological assessment of the newly expressed protein GM-HRA;
- An assessment of potential allergenicity, of the newly expressed protein GM-HRA as well as of the whole food derived from the GM plant by comparing the allergen repertoire with that of its appropriate conventional counterpart(s);
- a nutritional assessment to evaluate whether food and feed derived from a soybean 305423 is not nutritionally disadvantageous to humans and/or animals, in particular on fatty acid profile of soybean 305423.

Data evaluation for each data set

Relevance of the agents

GM plant: the soybean 305423 used in the assessment is relevant for the assessment, this having been substantiated by data characterising the transformation event in soybean (sequence, expression of the insert, stability of inserts).

Newly expressed protein: GM-HRA

a) The GM-HRA protein expressed in soybean 305423 was fully characterised by experimental data.
b) An equivalent GM-HRA protein expressed in a recombinant microbial system (*E. coli*): was fully characterised by experimental data, demonstrated to be equivalent to the plant protein and therefore considered adequate for toxicological studies.

Relevance of the subjects

Some limitations were identified:

Humans: The dietary intake and exposure scenario used to support the nutritional assessment for edible oil were based on UK population only.

Animals: Experimental animals were used (toxicological study on the new protein); possible extrapolation to humans/other species could constitute an uncertainty.

Relevance of the effects

To go beyond the analysis of statistical differences between the GM plant and its conventional counterpart and to put such differences into the context of the natural variation of the measured endpoints among conventional soybean varieties grown under the same conditions as the GM plant and its conventional counterpart, a test of equivalence is carried out (see box). Effects were identified and analysed as for their relevance for further assessment, based on the outcomes of both the difference test and the equivalence test in the comparative assessment and of the nutritional and toxicological studies.

1. Increase in oleic acid and MUFA in GM soybean compared to comparators

- *Is the effect in itself adverse/positive*: not adverse, might be beneficial.
- *Is the effect essentially linked to an adverse outcome*: NO, these fatty acids are normal diet constituents.
- *Is it directly or indirectly linked to a(n) adverse/beneficial outcome*? Possible (dietary perturbations).
- *Significant size of the effect*: YES (e.g. oleic acid goes up from 20% to almost 80%).

RELEVANT FOR FURTHER ASSESSMENT → Exposure assessment of European populations

2. Decrease in n-6 PUFA (linoleic acid) in GM soybean compared to comparator

- *Is the effect in itself adverse/positive*: might be adverse.
- *Is it directly or indirectly linked to a(n) adverse/beneficial outcome*? YES, deficiency in PUFA. Linoleic acid (LA) is the main dietary n-6 PUFA in the human diet. Deficiency in PUFA is associatedwith clinical symptoms (EFSA NDA Panel, 2010). EFSA has proposed an adequate intake (AI) for LA of 4 E %, based on the lowest estimated mean intakes of the various population groups from a number of European countries, where LA deficiency symptoms are not present. This AI corresponds to 9 g linoleic acid/day for an energy intake of 2,000 kcal.
- *Significant size of the effect*: YES (e.g. for C18:2, the level decreases from 50% to less than 10%).

RELEVANT FOR FURTHER ASSESSMENT → Exposure assessment of European populations

3. Changes in odd fatty acid chain in GM soybean compared to comparator

- *Is the effect in itself adverse/positive*: Not adverse, these fatty acid are a normal diet constituent.
- *Is it directly or indirectly linked to a(n) adverse/beneficial outcome?* Possible (dietary perturbations).
- *Significant size of the effect*: YES (statistically).

RELEVANT FOR FURTHER ASSESSMENT → Exposure assessment of European populations

4. Changes in levels of calcium, zinc and glycitin and related total glycitein equivalents; trypsin inhibitor

- *Is the effect in itself adverse/positive*: Not adverse, these are a normal diet constituent.
- *Is it directly or indirectly linked to a(n) adverse/beneficial outcome?* Possible.
- *Significant size of the effect:* YES from the statistical point of view (different and non-equivalence demonstrated or more likely to occur than not). These differences might be indicative of possible unintended effects of the genetic transformation but considered to need further assessment to conclude on the safety and nutritional characteristics of soybean 305423 and derived products. As regards calcium, zinc, glycitin and related total glycitein equivalents this was based on the knowledge on the biochemical roles of these endpoints in humans and animals as well as on the consideration that the differences in the levels of these endpoints observed between soybean 305423 and its conventional counterpart are of limited magnitude. These differences are therefore likely determining a negligible impact on the safety and nutritional characteristics of this GMO and derived products for humans or animals as compared to other soybean varieties. Regarding the trypsin-inhibitor activity, it is noted that the value for this antinutrient is lower than the one observed in conventional counterpart, and therefore considered not to represent a concern for safety.

Here, the assessment of biological relevance focused on implications for food and feed safety and was based on the outcomes of the difference test, of the equivalence testing as well as expert judgement.

NOT RELEVANT FOR FURTHER FOOD AND FEED ASSESSMENT

5. Allergenicity of the newly expressed protein GMHRA: NO EFFECTS: no indication that the protein is allergenic (source, bioinformatics etc.)

- *Is the effect in itself adverse/positive: the potential effect could be negative*; not applicable here.
- *Is it directly or indirectly linked to a(n) adverse/beneficial outcome?* The potential effect could be linked to an adverse outcome; not applicable here.
- *Significant size of the effect*: not applicable here.

NOT RELEVANT FOR FURTHER ASSESSMENT

6. Increased allergenicity of the whole soybean 305423 plant compared to conventional comparators: NO EFFECT: no change in 2-D immunoblot patterns; no differences at ELISA analyses

- *Is the effect in itself adverse/positive*: the potential effect could be negative; not applicable here.
- *Is it directly or indirectly linked to a(n) adverse/beneficial outcome?* The potential effect could be linked to an adverse outcome; not applicable here.
- *Significant size of the effect*: not applicable here.

NOT RELEVANT FOR FURTHER ASSESSMENT

The GMO Panel concludes that the composition of soybean 305423 differs from that of the conventional counterpart in its fatty acid profile, in the presence newly expressed protein, the levels of minerals zinc and calcium, of the isoflavone glycitin and in trypsin inhibitor activity. The variations in the fatty acid profile and the presence of newly expressed protein GM-HRA protein in soybean 305423 are consistent with the objective of the modification The safety assessment of GM-HRA identified no concerns regarding potential toxicity and allergenicity and there are no indications that the overall allergenicity of soybean 305423 has changed. Nutritional assessment on soybean 305423 oil and derived food products did not identify concerns on human health and nutrition, and there are no concerns regarding the use of feeding stuffs derived from soybean 305423. The GMO Panel considers that that the soybean 305423, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of the scope (EFSA GMO Panel, 2013).

Relevance of the conditions

Field trials: in accordance to EFSA guidance (EFSA GMO Panel, 2010, 2011).

Toxicological studies (28-day study on the newly expressed protein GM-HRA; 90-day toxicity study in rats on the whole food/feed from soybean 305423: compliant with standard OECD protocols and, as regards the 90-day study, to EFSA Scientific Committee, 2011a,b).

Animal feeding studies (broiler, laying hens, pigs): *ad hoc*, not standardised protocols were followed; these were considered adequate by the EFSA GMO panel.

Exposure assessment: *ad hoc* study protocol was used. This was considered adequate by the EFSA GMO Panel (EFSA GMO Panel, 2013).

Uncertainties

1) General assumption for comparative assessment: The underlying assumption of this comparative approach is that traditionally cultivated crops have gained a history of safe use for consumers and/or domesticated animals.
2) Use of the microbial protein as a surrogate of the plant protein: The *E. coli-de*rived GMHRA protein was fully characterised by experimental data, found to be similar to the plant protein and considered adequate for toxicological studies; however, some differences were identified between the microbial surrogate protein and the plant protein (the purification process of the microbial protein included the cleavage of the His-tag with thrombin; the resulting microbial GM-HRA protein has an additional glycine residue at the N-terminus compared with the mature GM-HRA protein expressed in soybean 305423 leaves). Not considered limitative, just noted in the scientific opinion.
3) Exposure scenarios for fatty acids: based on UK database only, not representative of the whole EU population. This was considered relevant and it was suggested, in the post-market monitoring (PMM) to focus on the collection of consumption data for the European population.

Use of statistical equivalence testing in the comparative analysis of GMOs

The comparative analysis of the characteristics of the GM plant and its comparator(s) grown under field trial conditions is a pillar of the risk assessment of GMOs, as explained in EFSA GMO Panel, 2011 and Regulation (EU) No 503/2013.

The comparative assessment requires the simultaneous application of two complementary tests on a set of compositional, phenotypic and agronomic characteristics:

- The test of difference to verify whether the biological system modified by the genetic modification (i.e. the plant containing the transformation event, or GM plant is different from its comparator(s) and has therefore the potential to cause adverse effects.
- The test of equivalence to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the range of natural variation. Such a range is defined by limit values, known as equivalence limits, which can be established either on the basis of existing knowledge, when available, or on the basis of experimental data obtained from non-GM reference varieties with a history of safe use included in the field trial (EFSA GMO Panel, 2010, 2011). GMO risk assessment experience has shown that equivalence limits have almost never been established. Therefore commercial varieties are routinely

included in the field trial to allow a direct comparison of the GMO with the commercial varieties, minimising confounding effects. Such an approach allows evaluating if the GMO's characteristic different from that of the comparator falls within the observe range of natural variability (the difference is acceptable), or if it falls outside (needing further assessment).

References

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Scientific Opinion on application EFSA-GMO-NL-2007-45 for the placing on the market of herbicide-tolerant, high-oleic acid, genetically modified soybean 305423 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer. EFSA Journal 2013;11(12):3499, 35 pp. https://doi.org/10.2903/j.efsa.2013.3499

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms) 2011. EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150, 37 pp. https://doi.org/10.2903/j.efsa.2011.2150

EFSA Scientific Committee, 2011. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438 [21 pp.] https://doi.org/10.2903/j.efsa.2011.2438

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879. 111 pp. https://doi.org/10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Scientific opinion on Statistical considerations for the safety evaluation of GMOs. EFSA Journal, 8(1):1250, 59pp.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 2010;8(3):1461, 107 pp. https://doi.org/10.2903/j.efsa.2010.1461

Annex G – NDA

Case study of biological relevance in the area of health claims: Application for substantiation of a health claim related to: Yestimun® and defence against pathogens in the upper respiratory tract (EFSA Journal 2013;11 (4):3159)

Assessment strategy

β-Glucans are dietary fibres that have been shown to have immunomodulatory activity in animals and humans after oral administration. Common colds are caused by viruses, which are pathogens that are eliminated by the body's defence mechanisms. By virtue of the effect of β-glucans on the immune system, the fibres may support defence against pathogens in the upper respiratory tract.

The food, which was the subject of the claim, that the NDA Panel was requested to evaluate, was Yestimun®, i.e. (1,3)-(1,6)-β-D-glucan produced from brewer's yeast cell wall (100% *Saccharomyces cerevisiae*), and the claim was that this food would support the defence against pathogens in the upper respiratory tract.

The NDA Panel needed to judge the claimed effect according a number of criteria:

1) Specification of the agent: is the food sufficiently characterised to evaluate the claimed effect,
2) Specification of the subjects: Which is the target group for the claimed effect,
3) Identification: Is the claimed effect in itself a relevant health effect, i.e. is it biologically relevant, and
4) Specification of the conditions: Is the in information based on human intervention studies and other information. Are the studies, on which the applicant wants to base the claim of glucans supporting defence to respiratory infection, sufficiently powered and are measures performed according accepted standards and is statistical analysis done appropriately. As long as common cold could be attributed to infection with an infectious agent, such information would be useful. Information on other respiratory conditions that are not attributable to infection is not useful. Information on immune parameters, assessed in humans, animals, or *in vitro* systems, can only provide supportive or mechanistic information, but in the absence of a substantiated effect on defence do not provide any scientific evidence for the substantiation of the claim. Does a biological effect occur after ingestion of the glucan; reduction of an already evident infection is beyond the scope of health claims on food, such effects would be considered as therapeutic.

Data collection

For the substantiation of the claim, three randomised controlled intervention studies were available. The primary endpoints were reduction in the number of common cold episodes per subject during the study periods, whereas secondary outcomes were severity of common cold episodes, duration of cold episode and the use of antibiotics and analgesics. The applicant provided information on the incidence of common cold.

Data evaluation

The NDA Panel considered that the food was sufficiently characterised for the purpose of evaluating the claimed effect.

In addition, the NDA Panel considers that supporting defence against pathogens in the upper respiratory tract, as measured by episodes of common cold, is in itself a beneficial physiological effect and therefore biologically relevant. In order to substantiate the claim, a statistically significant decrease in common cold episodes, if adequately proven with adequate confidence limits, would have been sufficient to substantiate the claim.

For the substantiation of health claims, the NDA Panel requires human data, notably intervention studies in humans. The target population is the general population and the subjects in the study, i.e. healthy individuals, represent the target population.

The evidence provided did not establish the validity of questionnaires and criteria used to assess the incidence or the severity of common cold episodes. The power of the studies were likely adequate to observe effects on the primary endpoints. However, in one of the studies post-hoc analyses were performed based on episodes that occurred in the winter months (November to March, first half of the study) to avoid the potential error that might have arisen owing to possible misdiagnosis of allergic rhinitis as common cold infections during the summer months. While this notion is understood, the post-hoc selection of the time windows for calculation of possible effects was not accepted as valid. In another study, statistical analysis did not account for the multi-centre design of the study.

Overall conclusion

In the judgement of the Panel, an effect on incidences of common cold, if appropriately shown to be statistically significant, would have been relevant for the purpose of substantiating the claim. However, even if statistically significant differences were reported, they were not judged relevant due to flaws in the statistical approach and the Panel came to the conclusion that a cause and effect relationship had not been shown.

Uncertainty

On the basis of the data presented, the NDA Panel concluded that a cause and effect relationship had not been established between the consumption of Yestimun® ((1,3)-(1,6)-β-D-glucans from brewer's yeast cell wall) and defence against pathogens in the upper respiratory tract. The opinion did not indicate that such a relation could not be there, but indicated that from the human studies provided conclusions could not be drawn.

Dietary Reference Values

Introduction

Following a request from the European Commission, the EFSA Panel on dietetic products, nutrition and allergies (NDA) was asked to deliver a scientific opinion on dietary references values (DRV) for vitamin D for the European population.

Vitamin D is a generic term for ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), which are formed from their respective provitamins, ergosterol and 7-dehydrocholesterol, following a two-step reaction involving ultraviolet B (UV-B) irradiation and subsequent thermal isomerisation. Vitamin D_2 and vitamin D_3 are present in foods and dietary supplements. Vitamin D_3 is also synthesised endogenously in the skin following exposure to UV-B. However, the properties of sunlight in Europe are not sufficient for vitamin D_3 synthesis during several months each year, resulting in the so called vitamin D winter.

Vitamin D deficiency leads to impaired mineralisation of bone due to an inefficient absorption of dietary calcium and phosphorus, and is associated with an increase in parathormone concentration. Clinical symptoms of vitamin D deficiency manifest as rickets in children and osteomalacia in adults.

Assessment strategy

A balanced diet is one that provides adequate amounts of energy and nutrients for health and well-being. DRVs comprise a complete set of nutrient reference values, such as lower threshold intake (LTI), average requirement (AR), population reference intakes (PRI), average intake (AI), and tolerable upper intake levels (UL).

PRIs can be used for instance as a basis for reference values for food labelling, or for establishing food-based dietary guidelines (FBDG). FBDG translate nutritional reference values into messages about foods and diet, which can guide consumers on to what to eat in order to fulfil their nutritional requirements.

Thus, DRVs on vitamin D should ensure that the corresponding requirements be covered in the European population, without achieving any toxic effect.

Relevance of the evidence/data

Ideally, nutritional requirements are measured in a subset of the target population, for instance using balance studies to assess the exact amount of a given nutrient, which should be consumed daily by each individual to offset losses and maintain stores at their optimal level. These data allow defining

an AR. Taking AR variance into account, it is possible to calculate a PRI, i.e. a level of intake which should cover the requirements of 97.5% of the population.

In this instance, the Panel considered that available data did not allow defining an AR, hence calculating a PRI. Instead, the Panel chose to set an AI.

In a first step, the Panel searched for biomarker of vitamin D status. Intervention and prospective observational studies were considered using endpoints related to musculoskeletal health through bone measurements (BMC, BMD) obtained via different techniques and after an appropriate study duration (e.g. at least one year), as well as the assessment of osteomalacia or bone fractures. Other health outcomes were also considered, such as adverse pregnancy-related outcomes, but the example is restricted to adult males and non-pregnant females.

Although the results were somewhat blurred by the use of different analytical methods, it was possible to conclude that there is evidence for an increased risk of adverse musculoskeletal health outcomes at serum 25(OH) concentrations below 50 nmol/l. Thus, the serum concentration of 25(OH) D can be considered as a surrogate marker of vitamin D status.

Nature and size of the effect

The next step was to assess the relationships between 25(OH)D and vitamin D intakes. In order to avoid confounding by endogenous synthesis, studies carried out during minimal sun exposure, lasting for at least 6 weeks, with oral exposure to vitamin D at least twice a week were considered. The articles which matched eligibility criteria were used to perform a quantitative analysis of the extracted data through a meta-analytic approach. Background intake was added to the supplemental vitamin D dose to generate total vitamin D estimates. When the habitual vitamin D intake was not reported, surrogates were imputed using appropriate age- and sex-specific mean vitamin D intake values (from food) from the national nutrition survey relevant to the country in which the study was performed.

Two different models of the dose-response relationship between total vitamin D intake and plasma/serum 25(OH)D concentration were explored: a linear model and a non-linear model (i.e. with the natural logarithm transformation of the total intake). Finally, the Panel decided to retain the non-linear model to better describe the dose-response shape and to be able to include results from higher dose trials (i.e. up to 50 μg/day).

A number of factors potentially influencing the dose–response relationship were investigated, in order to select factors to be included in the final model to characterise the high heterogeneity of results across individual trials. After the inclusion of the final set of covariates, the adjusted R^2 (proportion of between-study variance explained) of the final model was 85%, meaning that the fitted factors were able to characterise most of the across-trials variability in response. The models were used to predict the achieved mean serum 25(OH)D concentrations corresponding to total vitamin D intakes of 5, 10, 15, 20, 50, 100 μg/day and to estimate the total vitamin D intakes that would achieve serum 25(OH)D concentrations of 50, 40, 30, 25 nmol/l.

A number of sensitivity analyses were also carried out to evaluate whether the findings were robust to the assumptions made in the systematic review protocol and the analyses, in particular, on the background intake imputation process, on eligibility criteria (e.g. fortified food trials versus supplement trials); characteristics of participants (e.g. exclusion trials that did not explicitly exclude supplement users, persons with sun holidays, persons using sunbeds/artificial UV-B sources or going on sunny holidays). None of these sensitivity analyses raised serious concerns about the robustness of the overall analysis.

The Panel considered that the results of this meta-regression analysis could be used to set DRVs for vitamin D.

Overall relevance taking into account the exposure

The Panel used information obtained from characterising the intake-status relationship for vitamin D to derive the vitamin D intake to achieve a target serum 25(OH)D concentration of 50 nmol/l. For the purpose of deriving AIs for vitamin D, the Panel decided to focus on the *adjusted* model obtained with data mostly on adults. The estimates from that model were derived based on all covariates.

In the *adjusted model*, the total intake estimated to achieve a serum 25(OH)D concentration of 50 nmol/l, as identified by the lower limit of the 95% PI, is 16.1 μg/day. Equally, at a vitamin D intake of 15 μg/day, the predicted mean serum 25(OH)D concentration is 63 nmol/l (95% CI: 58–69 nmol/l), with a predicted value at the lower limit of the 95% PI of 49 nmol/l.

Predicted interval (PI) in the context of a meta-regression analysis illustrates the uncertainty about the true mean response predicted in a future study. Moreover, 95% PI constitutes an approximation of the interval that would include 95% of all individual responses from the populations included in previous and future studies, as it refers to the population of mean responses. The extent of this approximation could not be quantified.

The Panel therefore set an AI for vitamin D for adults at 15 μg/day, considering that, at this intake, the majority of the adult population will achieve the target serum 25(OH)D concentration near or above 50 nmol/L. The Panel decided not to set specific AIs for 'younger' or 'older' adults, because there was no evidence of a significant difference in absorption capacity between 'younger' and 'older' adults and the majority of the studies used to set the target value for 25(OH)D concentration were carried out in 'older adults'.

The unadjusted model can be also taken into account as it encompasses the whole heterogeneity across trials. In the unadjusted model, considering a vitamin D intake of 15 μg/day, the lower limit of the 95% PI is 34 nmol/L. This value is above the concentrations that have been observed in relation to overt adverse health outcomes (osteomalacia, calcium absorption). Considering a vitamin D intake of 15 μg/day, the upper limit of the 95% PI is 91 nmol/L in the unadjusted model (and 78 nmol/L in the adjusted model). These values are in the physiological range (EFSA NDA Panel, 2013).

Annex H – PLH

The EU plant health legislation aims to protect crops, fruits, vegetables, flowers, ornamentals and forests from harmful pests and diseases (harmful organisms) by preventing their introduction into the EU or their spread within the EU. This aim helps to:

- contribute to sustainable agricultural and horticultural production through plant health protection;
- contribute to the protection of public and private green spaces, forests and the natural landscape.

The Council Directive 2000/29/EC[5] provides the basis for this aim. The general principles are based upon provisions laid down in the International Plant Protection Convention (IPPC, 2007).

Directive 2000/29/EC is supported by further legislation in the form of a number of Control Directives and Emergency Measures.

In order to meet the aims of the regulation, the EU:

- regulates the introduction of plants and plant products into the EU from countries outside the EU;
- regulates the movement of plants and plant products within the EU;
- imposes eradication and containment measures in case of outbreaks, and co-finances them;
- places obligations on countries outside the EU which want to export plants or plant products to the EU.

The Panel on Plant Health (PLH) provides independent scientific advice on the risk posed by plant pests which can cause harm to plants, plant products or biodiversity in the EU. The Panel reviews and assesses those risks with regard to the safety and security of the food chain.

The EFSA plant health panel supports commission decisions on plant health by making scientifically based pest risk assessments. The risk assessment follows the structure agreed by the Secretariat of the International Plant Protection Convention (2007). The PLH panel has outlined its procedures in guidance documents on the pest risk assessment (EFSA PLH Panel, 2010) and the evaluation of risk reduction options (EFSA PLH Panel, 2012). The panel is currently working on a framework for a risk analysis that is quantitative. Aims of introducing a quantitative approach are to increase consistency, transparency and objectivity. In this new approach, the steps in the assessment are elaborated quantitatively. The steps are: (1) pest entry into the EU, (2) pest establishment in the EU, (3) pest spread within the EU, and (4) impact assessment. These steps are cumulative, as there will be no impacts without the previous steps taking place. When compared to the risk assessment for toxic or beneficial compounds, the first three steps are similar to exposure, while the impact assessment has similarity to the dose-response relationship in toxicological studies. Indeed, Robinet et al. (2016) use the term 'exposure' to describe the contact rate of native European trees with propagules of the pathogenic fungus *Ceratocystis fagacearum* that can enter Europe on wood imported from the United States.

The current example is based on an ongoing risk assessment on the potato rot nematode, *Ditylenchus destructor.* This risk assessment was elaborated following the new quantitative approach. The opinion is in preparation for possible adoption by the EFSA plant health panel in September 2016.

In the pest risk assessment on *Ditylenchus destructor*, the panel focused the assessment of entry, establishment, spread and impact on two crop species: potato (*Solanum tuberosum*) and tulip (*Tulipa* spp.). The choice of these two species was based on considerations of relevance as potato and tulip were judged, based on production areas and trade flows, to be the most relevant pathways for introduction and spread of this nematode with planting material, and also for the materialisation of impacts in crop production. Other flower bulb species could also be vectors for entry and spread, but they were not considered because the trade volumes are much smaller than those of tulips. Impacts in other bulbs species are also expected to be much smaller than those that could occur in tulips because of smaller production areas.

A modelling approach was used to estimate entry, spread and impact quantitatively. Trade data were used for assessing import volumes. Literature and expert judgement were used to estimate model parameters, taking into account uncertainty. Special attention was paid to the evaluation of risk reduction options for planting material, treatment of flower bulbs before trading, and treatment of soil prior to planting of potato.

[5] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:169:0001:0112:EN:PDF

A baseline scenario with current pest-specific phytosanitary regulations was compared with alternative scenarios without those specific regulations or with additional risk reduction options. Further information was provided on the host range of *D. destructor* and on survival of the pest in soil in the absence of hosts.

The Panel concluded that the entry of *D. destructor* with planting material from third countries is quite small compared to the yearly intra-EU spread of this nematode with planting material. Changes in pest specific regulations have little influence on entry of the pest. It was also concluded that the whole pest risk assessment area is suitable for establishment of *D. destructor*, but there is insufficient information to make a statement on the persistence of newly introduced populations. Impacts of this nematode on the quantity and quality of potato production are considered negligible. The Panel also considers the impact of this nematode on flower bulb production in the EU as very low.

Assessment strategy

The Commission asked EFSA to assess various aspects of the risks of the potato rot nematode *Ditylenchus destructor* to agriculture in the EU. The PLH panel decided to conduct a pest risk assessment according to the framework provided by Secretariat of the International Plant Protection Convention (2007), entailing an assessment of the risks of entry, establishment, spread and impact. The nematode is already present within the EU, albeit sporadically, and it is not entirely clear *a priori* whether additional entry is of relevance for the impacts of this nematode. The Panel developed a model for entry, establishment, spread and impact to assess the relative importance of entry of this nematode with trade from third countries and its spread within Europe in intra-European trade of plant products.

Evidence/data needed to address the question

To assess this question, information is needed on trade flows in plant products that can serve as a vector. Both the trade-flows from Third countries (i.c. Canada and Switzerland that have presence of this nematode but can import under certain restrictions) are considered, and those within Europe. Furthermore, information is needed on the prevalence of the nematode in the trade flows. Furthermore, information is needed on the host plants of the nematode, and the impact caused on those hosts. In addition, information is needed on the conditions for establishment. The panel focused the assessment on seed potato and flower bulbs, which constitute the most important plant product that can serve as a vector. Furthermore, ware potatoes and many flower bulb species are hosts and can suffer damage.

Data evaluation

Adequate data are available on the international trade in seed potatoes. However, no adequate data are available on the trade in flower bulbs because the data in Eurostat are not recorded at a sufficient level of resolution between species. Therefore, trade in host species and non-host species cannot be well distinguished. Data on production areas of seed potatoes across the EU are good. Data on production areas of flower bulbs are adequate.

Information on the prevalence of the nematode in the EU and in third countries is extremely sparse. Only vague descriptions are available like 'present in all parts of the area where host crops are grown', 'present, restricted distribution', 'present, few occurrences', 'present' and 'absent'. These terms were interpreted by the panel in terms of proportion of production fields infested with the nematode and proportion of planting material harvested from infested fields that carry the nematode. This interpretation is a reason for large uncertainties in the estimates of the flow of infested planting material. Furthermore, the Panel made assessments of survival of the nematode in trade flows, and of the efficacy of import and export inspection and of certification schemes to reduce or limit the levels of infestation of plant product with the nematode. Due to lack of pertinent data, these assessments were also quite uncertain.

Relevance of the agent

The potato rot nematode *D. destructor* causes rots in root crops and bulbous crops. The species is well characterised and the potential damaging effects are also well characterised. If uncontrolled, this nematode can multiply, spread and cause substantial damage. However, with current phytosanitary

measures for containing the spread with plant products, and efficient weed control in crops, reducing the number of potential hosts for the nematode, the impact of this nematode under current conditions is minor.

Nematodes can live in tubers, bulbs and rhizomes, and they can be spread with trade in such products over practically unlimited distance. Infested planting material is the main pathway for spread. Autonomous spread by nematodes is not practically relevant. Spread by farm machinery is possible but is still a short distance spread (mostly within field or farm). There is no known minimum number of nematodes that is required to cause establishment or infestation of a plant.

Relevance of the subject

The Panel made the assessment focusing on seed potatoes and tulip bulbs. Seed potatoes are the most important carrier for the nematode. Flower bulbs are also potentially important, and the trade in the host species tulip is the largest amongst flower bulbs. Both potato and tulip suffer damage if infested.

Relevance of the effect

This nematode can cause considerable damage if there are not measures in place to prevent this damage.

Relevance of the conditions

The assessment was carried out considering common practices in current trade and crop cultivation in Europe. These conditions are fully relevant.

Overall conclusion

This nematode is present in two-thirds of the EU Member States, but is currently of minor importance in Europe as current measures and agricultural practices are effective in limiting spread and impact. Lifting pest-specific measures is not expected to change this because certification of planting material of potato and flower bulbs needs to meet quality criteria that would effectively limit the presence of this agent.

Uncertainty

The key uncertainty in the assessment is the current distribution. There are no reports on structured surveys in the EU or in third countries to quantify at relevant spatial scales the prevalence of this nematode. There is thus no relevant information available on: (1) the presence in geographic areas below the level of member state, (2) proportion of infested area fields in those geographic areas in which the nematode occurs, and (3) proportion of infested planting material harvested from infested fields. The lack of quantitative information on the multi-scale presence of the organism is a great impediment to making the assessment. Instead of basing its parameter estimates for the model on data, the Panel had to resort to expert judgements. In the assessment, the Panel made use of stochastic simulations and the resulting distributions of outcomes show variability over four orders of magnitude (EFSA PLH Panel, 2016).

Annex I – PPR

PESTICIDES HAVING EFFECTS ON THE THYROID HORMONE SYSTEM

In the MRL regulation 396/2005, it was laid down that account should be taken of 'the possible presence of pesticide residues arising from sources other than current plant protection uses of active substances, and their known cumulative and synergistic effects, when the methods to assess such effects are available'.

Then, in the later pesticide regulation 1107/2009, the precautionary principle applies and therefore before placing active substances in plant protection products on the market it should be demonstrated that they do not have any harmful effect on humans.

However, not only the single active substance should not have harmful effects but should also take account of effects from mixtures of pesticides. Thus, the regulation reads 'shall have no immediate or delayed harmful effect on human health, including that of vulnerable groups, or animal health, directly or through drinking water (taking into account substances resulting from water treatment), food, feed or air, or consequences in the workplace or through other indirect effects, taking into account known cumulative and synergistic effects…'.

This would be applicable not only for dietary risk assessment but also non-dietary (operators, workers, bystanders and residents).

EFSA was in accordance with the regulation commissioned to develop the methodology for carrying out cumulative risk assessment in regard to MRL-setting and launched this work in 2006 with a scientific colloquium followed by subsequent opinions, amongst the 'Scientific opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile'. In the opinion, a methodology for grouping was developed and two cases where elaborated on; Pesticides having effects on the nervous system and pesticides having effects on the thyroid system. A total of nearly 300 pesticide dossiers were evaluated for these two cases.

Assessment strategy

In this case, two problem formulations are being answered on the basis of the same data sets.

1) For the single pesticidal active substance assessment, establish no observed *adverse* effect levels (NOAELs) in case of effects on the thyroid system is a critical effect.
2) Identification of pesticides to be included in cumulative assessment groups (CAG's) on the basis of their toxicological profile (hazard assessment) – in this case effects on the thyroid system – and establishing NO(A)EL's in this context.

The establishment of the critical effect, the NOAEL for this effect and deriving reference doses (AOEL, ADI, ARfD) is to protect the human population exposed to the pesticide when applied and to the protect the population being exposed via all routes of exposure.

The grouping of the pesticides into CAG's was developed to support the regulatory MRL-setting and as such the target population is the European Consumer.

Agents	Effects	Subjects	Conditions
Single active substance	Effects on the thyroid system	The human population	Dietary and non-dietary exposure Establishment of NOAEL in Subchronic when effect on the thyroid is a critical effect – chronic exposure
Cumulative Assessment Groups of active substances (CAGs)		The human population	Dietary exposure Establishment of NO(A)EL in subchronic – chronic exposure

Data collection and selection of the biologically relevant data

For the standard assessment of pesticidal active substances, the regulation specifies extensive data requirements in regard to mammalian toxicity (exposure usually by the oral route) on several species and exposure duration (from sub-acute to chronic).

In addition to the regulatory studies, where data from the scientific literature on pesticides and their effects on the thyroid system were available this was taken into account to support mechanistic understanding of the effect.

No specific data requirements are set in the regulations for the purpose of grouping of pesticides into CAG's.

For assessment of the histopathological findings, these are generally classified according to qualitative criteria and the data are presented as number of animals affected within a dose group. Numerical results, should according to the relevant OECD guideline, be 'evaluated by an appropriate and generally acceptable statistical method'. As the data base for grouping of pesticides for having effects on the thyroid system consists of a little less than 300 pesticide dossiers, the statistical methods applied will of course be different from study to study. But in all cases they have been assessed and peer-reviewed and NOAEL's have been established for the single substance evaluation.

The power of the studies would also be very varied going from the very low-powered dog study, with usually only four animals/sex per group, to more well-powered rodent students with 20 animals/sex per group, and in case of carcinogenic effects, there are 50 animals/sex per group. Normally, the power of a given study for the different endpoints investigated is not stated.

On studies in dog, results that are not statistically significant are also be considered for their biological significance, and individual values are been taken into account. It is considered that the statistical significance in the dog studies might not be reliable due to the high inter-individual variability.

Relevance of the effects for humans

For detailed description of the thyroid system, see Miller (2009) and the following figure[6] showing where chemicals might pertubate the thyroid system.

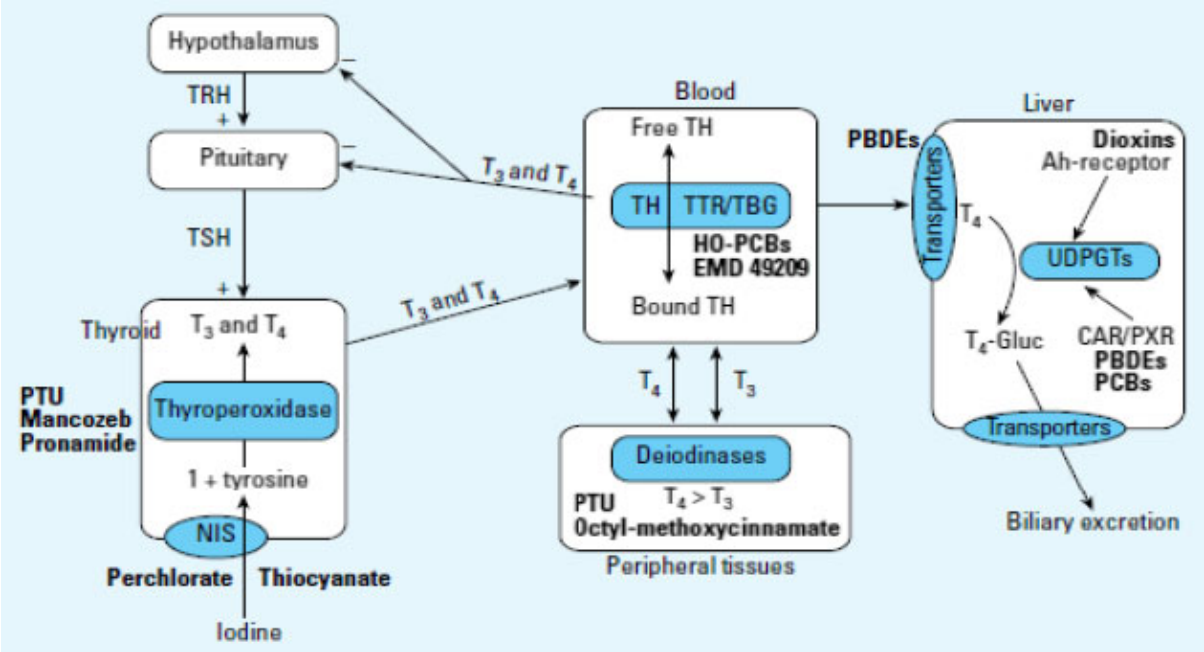

Figure I.1: Thyroid hormone system with potential targets for disruption by xenobiotics (blue). NIS, sodium/iodide symporter; TBG, thyroid hormone-binding globulin; TH, thyroid hormone (T3/T4); T3, triiodothyronine; T4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; TTR, transthyretin; UDGTs, UDP-gluuronosyl transferases (Miller et al., 2009)

When declines in circulating and tissue hormone levels occur, feedback mechanisms of the hypothalamic–pituitary–thyroid axis would result in increased secretion of thyroid stimulating hormone (TSH) and subsequently the follicular cells would increase the secretion of T3/T4 and thus levels of bioavailable T3/T4 would be re-adjusted.

Alterations in circulating bioavailable thyroid hormone levels may have serious impact on other organs or organ systems besides the thyroid itself also in humans, particularly if perturbations occur during critical windows of development' (EFSA, 2013a,b).

[6] Reproduced with permissions form Environmental Health Perspectives.

And further the PPR panel noted that 'Any degree of thyroid disruption that lowers TH levels on a population basis should be considered a biomarker of increased risk of adverse outcomes, which may have important societal outcomes' (Miller et al., 2009) (EFSA, 2013a,b). So as such adverse effects/effects on the thyroid system are considered relevant for the human population.

Is the effect in itself a(n) adverse/beneficial effect?

Single pesticide evaluation of adverse effects on the thyroid hormone system

When evaluating adverse effect on the thyroid, physiological changes preceding adverse manifestations in target organs (changes in circulating thyroid hormone levels) and indicators of perturbation of thyroid hormone homeostasis (e.g. elevation of TSH based or thyroid enlargement) would not be regarded as adverse when establishing the NOAEL for thyroid effects in a study. The assumption is that as a consequence of changes in circulating and tissue thyroid hormone levels, compensatory mechanisms including activation of the hypothalamic-pituitary-thyroid axis following a decline in peripheral thyroid hormone levels with subsequent increased production and secretion of TSH (thyroid stimulating hormone) may be expected to result in adjustment of bioavailable thyroid hormone levels. Thus, changes in circulating or tissue T3/T4 hormone levels would be transient (EFSA, 2013a,b).

Effects on the thyroid hormone system in regard to grouping for CRA

In regard to grouping of pesticides for cumulative risk assessment other considerations were also taken into account. It was noted that; 'For the evaluation of the common toxicological profile for assignment of an active substance to a CAG, different indicators may be taken into account, which could comprise downstream endpoints with obviously adverse target organ effects or upstream precursor effects e.g. a decrease in T4 levels, that may eventually lead to manifestation of an adverse organ effect.

In the context of CRA, it is therefore proposed to also consider the physiological change preceding adverse manifestations in target organs (changes in circulating thyroid hormone levels) and indicators of perturbation of thyroid hormone homeostasis (e.g. elevation of TSH or thyroid enlargement), to be of relevance for definition of cumulative assessment groups'. (EFSA, 2013a,b)

So accordingly, the following effects were considered as specific effects and indicators relevant for grouping: changes in serum T3/T4, changes in serum TSH, follicular cell hyperplasia/hypertrophy and/or increased thyroid weight and thyroid tumours and the specific NOAEL's were established.

Relevance of the conditions

It is mandatory to investigate effects on the thyroid system in pesticide active substance dossiers. The effects are always addressed after 90-day exposure in rodents – usually rats – and dogs. The following endpoints are mandatory, histopathological evaluation of the thyroid and pituitary, while estimation of hormones (T3, T4 and TSH) is optional (case by case). Also, histological evaluation of the thyroid glands and the pituitary is conducted in the mandatory carcinogenicity studies in rats and mice. All species are considered relevant for humans. The duration of exposure is considered relevant for chronic dietary exposure and non-dietary exposure.

Hazard characterisation by oral exposure is considered relevant for dietary as well as non-dietary exposure (mainly dermal). For pesticides where the inhalatory exposure is the main route – such studies might be required for repeated dose studier. However, this is rare.

Uncertainties

As discussed above, the rat is considered a very sensitive proxy in regard to effects on the thyroid system. Therefore, the PPR panel noted in regard to follicular tumours; 'concerning effects on the thyroid itself, prolonged enhanced secretion by the pituitary of TSH as a response to decreased circulating thyroid hormone levels in rat studies leads to thyroid follicular cell hypertrophy and hyperplasia, which eventually may act as a promoting factor in the development of benign and malignant follicular cell tumours. Although compensatory mechanisms based on feedback loops within the hypothalamic-pituitary thyroid axis are also operative in humans from a qualitative point of view, it appears that humans are quantitatively less susceptible to follicular cell tumour formation resulting from thyroid hormone system imbalance than rats, based on marked quantitative differences in kinetics of circulating thyroid hormones and in the extent of response to changes in thyroid hormone levels (Dellarco et al., 2006)'.

Conclusion

The same effects in regard to effect on the thyroid hormone system, namely statistical significant changes in serum T3/T4 and or TSH would be assessed differently. In the single substance evaluation, such changes, although clearly treatment related, would not be considered as adverse effects if they are not accompanied by adverse tissue manifestations. In regard to grouping based on toxicological profile for cumulative risk assessment, the effects are considered as relevant specific indicative effects on the thyroid system. Thus, different NO(A)EL's could be established based on the same data set and therefore, in different regulatory contexts, the same effect, although being regarded as biologically relevant in both settings, the impact on regulatory decision making is different.

Annex J – CONTAM Panel

Human health risk assessment of Cadmium in food:

Scientific Opinion of the Panel on Contaminants in the Food Chain; The EFSA Journal (2009) 980, 1–139

Assessment strategy

Cadmium (Cd) is a heavy metal found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources. Foodstuffs are the main source of cadmium exposure for the non-smoking general population. Cadmium absorption after dietary exposure in humans is relatively low (3–5%) but cadmium is efficiently retained in the kidney and liver in the human body, with a very long biological half-life ranging from 10 to 30 years.

For the purpose of the guidance and although the scientific assessment of cadmium had a broader content, the example below focuses only on one effect.

The kidney is the critical target organ for dietary exposure to cadmium and renal damage is characterised by cadmium accumulation in convoluted proximal tubules, thereby causing cell dysfunction and damage. The earliest signs of tubular toxicity are, respectively, decreased tubular reabsorption (increased excretion) of low molecular weight proteins (LMWP) and increased excretion of markers of cell shedding.

Problem: Characterise critical effect for the purpose of deriving a Health based guidance value

Identification of the Agents	Identification of the Effects	Identification of the Subjects	Identification of the Conditions
Cadmium	Critical effect: Kidney damage (cell dysfunction and damage of convoluted proximal tubules)	Humans	Biomarkers (decreased tubular reabsorption (increased excretion) of low molecular weight proteins (LMWP) and increased excretion of markers of cell shedding)

Data collection/data evaluation for each data set

The availability of quantitative human data for both toxicokinetics (TK) and toxicodynamics (TD) provides relevant data for hazard identification and characterisation without the need to use animal data.

Data evaluation (Biological Relevance)

Cadmium is bioaccumulating due to very slow renal excretion (TK), leading to excretion of biomarkers of kidney damage (TD).

Biological Relevance of biomarker of proximal tubular dysfunction

Is the effect in itself a(n) adverse/beneficial effect?

The CONTAM Panel based its assessment on the use of the low molecular weight protein (LMWP) β-2-microglobuline (B2M) in urine as a biomarker of Cd-induced tubular toxicity. Increased excretion of B2M is not per se associated with any objective symptom or disease. Outcome: B2M is not in itself an adverse effect.

Is the effect essentially linked to a(n) adverse/beneficial outcome?

The urinary excretion of LMWPs and the activity of some enzymes (mainly *N*-acetyl-beta-glucosaminidase (NAG)) in urine have been, respectively, used to assess tubular dysfunction and cell damage; Urinary β-2-microglobulin (B2M) has been widely used as an indicator. Outcome: B2M is essentially linked to an adverse outcome

Relevant size of the effect?

In occupational exposed subjects, adverse effects of cadmium on the kidney were observed at urinary levels of cadmium ranging from 1.1 to 15 μg/g creatinine; abnormal levels of B2M were found in the urine of workers with urinary cadmium levels greater than 1.5 μg/g creatinine.

Based on studies on the clinical relevance of urinary B2M excretion, tubular damage and renal function and damage the Contam Panel chose cut-off levels associated with renal protection and irreversible kidney damage (see page 71, EFSA Journal (2009) 980, 71–139). As an indication of abnormality, a value of 1,000 μg B2M/g creatinine was set as a high-level criterion. B2M excretion levels above this limit are likely to be irreversible kidney damage.

As a lower and more protective cut-off level, a value of 300 μg B2M/g creatinine was chosen. Exceeding the biological cut-off of *300 μg/g creatinine* for B2M has been associated with an accelerated decline of renal function associated with aging together with increased mortality.

Statistically based cut-off criteria corresponding to the 95th percentile of the B2M distribution at background urinary cadmium concentrations were also calculated. The statistically based cut-offs for the whole populations and for subjects over 50 years were 211 and 374 μg B2M/g creatinine, respectively.

BMDs and BMDLs at various cut-offs leading to extra risks of 5% in the total population, and non-occupationally exposed subjects above 50 years of age were calculated.

Calculations of BMDs and BMDLs

	Statistical cut-off [a] for U-beta-2-microglobulin (μg/g creatinine)		**U-beta-2-microglobulin > 300 μg/g creatinine**		**U-beta-2-microglobulin > 1,000 μg/g creatinine**	
	BMD5	BMDL5	BMD5	BMDL5	BMD5	BMDL5
U-Cd (μg/g creatinine)from the whole population	3.98	3.62	4.65	3.84	6.80	5.95
U-Cd (μg/g creatinine) from non-occupationally exposed subjects over 50 years	5.28	4.89	5.25	4.45	6.33	5.46

[a] 211 and 374 for whole and subjects over 50 years, respectively.

Relevance of the conditions

The relevance of the different BMDL5 calculated for risk assessment of the whole population were evaluated by the CONTAM Panel

Taking into account the slightly higher values for the subjects over 50 years and the range of the BMDL5 results for the statistical and the biological cut-off limit of 300 μg B2M/g creatinine, the CONTAM Panel selected an overall group-based BMDL5 of 4 μg cadmium/g creatinine.

The use of 300 g B2M/g creatinine as critical effect of cadmium exposure to base the risk assessment leads to a possible overestimation of the risk, but it allows protecting the most sensitive groups of the population.

To account for inter-individual variability in cadmium concentration within groups, not explicitly accounted for in the BMD modelling (i.e. when calculating the lower one-sided 95%-confidence bound for an extra risk of 5% of producing a specified change in the urinary level of the B2M, denoted BMDL5), the CONTAM Panel modified the BMDL5 value using a chemical specific adjustment factor (CSAF) for cadmium based on the estimated variance of within group cadmium concentration. After adjustment, the CONTAM Panel identified a critical cadmium concentration of 1 μg cadmium/g creatinine in urine as a modified reference point (RP) on which to base a health-based guidance value (HBGV) of cadmium dietary intake.

Converting the RP to an intake value and derive a HBGV for Cd

Subsequently, a one-compartment population TK model was fitted to 680 paired data of cadmium intake and urinary cadmium concentrations from the Swedish Mammography Cohort study

(Amzal et al., 2009). This TK model showed that a dietary intake of no greater than about 2.5 μg/kg bw cadmium per week would prevent 95% of the Caucasian population from being above the modified RP of 1 μg cadmium/g creatinine in urine after 50 years of exposure (EFSA, 2009b). In order to remain below this modified RP, it was calculated that the average daily dietary cadmium intake should not exceed 0.36 μg/kg bw, and this daily intake was used to derive the TWI of 2.5 μg/kg bw.

Annex K – Environmental Risk Assessment

In environmental risk assessment, the protection goal is normally based on protecting populations. In some cases, it is also based on individuals, for instance for all vertebrates. Sometimes the protection goal is a function, for instance nitrification, and sometimes it includes even behaviour, e.g. for bees and vertebrates.

For example, in the Avian Reproductive Test (OECD 206) the following endpoints must be assessed:

- Frequency, duration and description of signs of toxicity, along with severity, numbers affected and any remissions
- **Food consumption** and **body weight** for adults and juveniles
- Details of gross pathological examinations
- Results of residue analysis (if performed)
- **Egg production** – number of eggs laid per hen (10 weeks)
- Percentage of **cracked eggs**
- **Viability** (per cent viable embryos of eggs set)
- **Hatchability** (per cent hatching of eggs set)
- **Percentage of hatchlings** that survive to 14 days
- Number of **14 day old survivors** per hen
- **Eggshell thickness** (mm)

The test should be carried out with a minimum of three dietary concentrations of the test substance. The concentrations to be used should be based upon the results of a dietary LC_{50} test (OECD 205). The highest concentration should approximately be one half of the LC_{10}. Lower concentrations should be geometrically spaced at fractions of the highest dose (e.g. 1/6 and 1/36 of the highest dose).

As a consequence of this design, the power for each endpoint is different. For some endpoints the power will be very weak and some others strong and they will vary between compounds and over time.

All endpoints are in principle assumed to be relevant when populations are the protection goal. The hazard assessment of the bold printed endpoints above is based on a NOEC, i.e. the highest tested concentration in which the values for the observed effect are not significant different from the control. Of all other observations, the risk assessor has to consider whether these effects could influence the survival of the population.

Although an endpoint is statistically significantly different from the control it may not be biologically relevant. In order to determine the biological relevance of an effect it should be considered whether the effect could lead to a functional deficit later on in the study, e.g. if a reduction in the weight of pups at birth leads to a decrease in level of survival. If not, then the effect may not be biologically relevant, however if there is a carry-over of effects into the number of survivors, it can be considered biologically relevant.

Example involving egg shell thickness and cracked eggs

As stated above, not all outcomes of the test are biological relevant, for instance if the lowest observed effect concentrations (LOEC) for egg shell thinning is 3% than it is generally believed that the NOEC does not have a biological relevance. It is believed that the biological relevant percentage of egg shell thinning starts with 18% (Blus, 2003; EFSA, 2009a,b,c). The BMD dose equivalent to 18% effect can be calculated with an appropriate method. This BMD can be considered as the 'NOEC' for cracked eggs (see figure below).

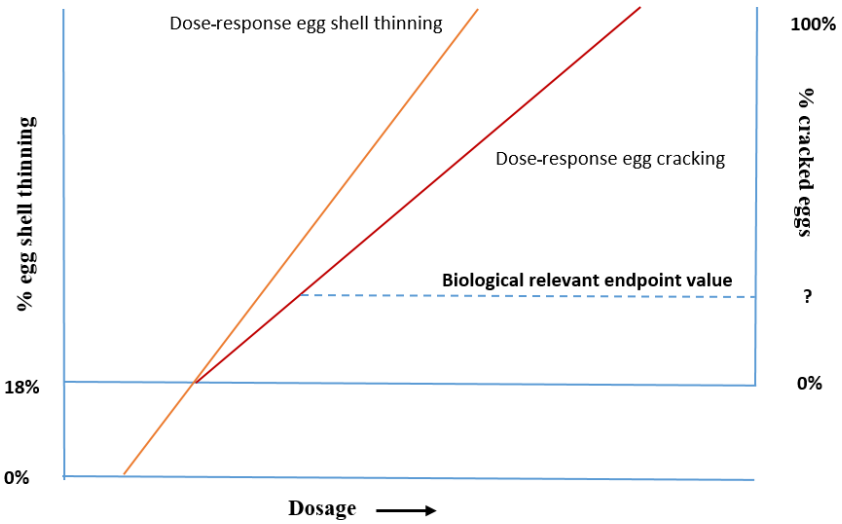

Figure K.1: Relation between egg shell thickness (orange line) and cracked eggs (red line). The dashed line is the line for effecting the reproduction of a bird species (e.g. when is the number of cracked eggs too much for maintaining a stable population)

In many cases, it will be difficult to point out what the biological relevant threshold of an endpoint will be. A tool that can be used is to run legislative acceptable models and to assess at which percentage a population will not be able to recover any more or when a population suffers to an unacceptable degree and to include in this assessment the uncertainty around the outcome.

In many cases, it will be difficult to determine what the biological relevant cut-off of an endpoint will be.

Taking a conservative approach the 'NOEC' for cracked eggs would be a good starting point for the risk assessment, but it is probably not the cut-off value at which a population will start to show signs of decreased ability to survive.

A tool that can be used is to run legislative acceptable models and to assess at which percentage a population will not be able to recover any more or when a population suffers to an unacceptable degree and to include in this assessment the uncertainty around the outcome.

In Topping and Luttik (2017), an example is provided how such a biological relevant endpoint can be assessed. For Denmark, 10 skylark scenarios were developed to cover most of the agricultural landscape of the country. According to the model simulations 10% or less cracked eggs does not affect any of the skylark populations in the long term. Based on this outcome, a BMD analysis based on a BMR of 10% cracked eggs can be determined according to the dose–response relationship between cracked eggs and egg shell thinning and this value could be used as the biological relevant endpoint size.

An alternative option would be to allow some level of population effect. In the model used for skylarks in Denmark, one could exclude the most sensitive scenario and calculate the BMR based on the next most sensitive landscape. This would result in a higher BMR and a higher BMDL. This approach would be similar to that in exposure modelling using a 90th percentile of the exposure distribution, which means that in 10% of the cases a higher exposure is expected. In this case, this would mean a BMR of 20% as the rest of the scenarios do not show at the 20% egg cracking level any long-term effects on the populations of skylarks. Although a 20% cracking rate for the most sensitive population over the simulation period would only result in small long-term population declines, this would probably be unacceptable.

The final decision on which BMR to use is a complicated one related to protection goals taking into account social and political realities and set by the risk manager, and scientific evidence available for the assessment. There is an argument for adopting the more conservative approach based on the uncertainty surrounding the effect of potential exposure to multiple pesticides, which is not currently assessed following EFSA guidance. In contrast, the need of effective pest control to support agricultural production argues for less conservatism.

Another approach is to include recovery in the risk assessment, which is for instance an option in aquatic risk assessment (not fish or amphibians) and terrestrial risk assessment for invertebrates. In

the aquatic ecosystem, a compound can be allowed on the market when recovery is seen within a period of 8 weeks but only when all important organism groups are included in the mesocosm experiment (see opinion on recovery, EFSA, 2013a,b, 2016a–e).

References

Topping CJ and Luttik R, 2017. Simulation to aid in interpreting biological relevance and setting of population-level protection goals for risk assessment of pesticides. Regulatory Toxicology and Pharmacology. 89, 40–49 pp. https://doi.org/10.1016/j.yrtph.2017.07.011