

Annex to: Scientific opinion on the assessment of the potential BSE risk posed by ruminant collagen and gelatine other than derived from hides and skins. doi:10.2903/j.efsa.2025.9435

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## **Annex A – Protocol for the effect of incineration, co-incineration and combustion on TSE hazards in Category 1 animal by-products**

### **A.1 Introduction**

#### **A.1.1 Scope of this protocol**

This document outlines the protocol for the Request for a scientific opinion on the presence of biological and chemical hazards in ash from Category 1 material after incineration, co-incineration, and combustion. The protocol will be used as input for the scientific opinion of the EFSA Panel on Biological Hazards (BIOHAZ) on the assessment of the presence of biological and chemical hazards in ash from Category 1 material after incineration, co-incineration, and combustion.

This protocol was developed with the aim of defining the methods for collecting data, appraising the relevant evidence, and analysing and integrating the evidence in light of the identified uncertainties. It was developed following the principles and process defined in a project that aimed to further improve EFSA's scientific assessment processes (EFSA, 2015) and based on the recommendations for protocol development described in the Guidance on protocol development for EFSA generic scientific assessments (EFSA, 2023).

The protocol was drafted by the WG members and was approved by the BIOHAZ Panel at their 174<sup>th</sup> plenary meeting (4-5 December 2024).

#### **A.1.2 Background and Terms of Reference (ToR) as provided by the requestor**

Background: see scientific opinion.

##### **ToR1**

To assess the effect of incineration, co-incineration, and combustion of Category 1 material referred to in Article 8 Regulation (EC) No 1069/2009 on the BSE/TSE hazards in the ash resulting from these treatments.

**If the outcome of ToR1 is that there is residual TSE/BSE infectivity, then there is NO need to proceed to ToR2.**

##### **ToR2**

To assess the effect of incineration, co-incineration, and combustion of Category 1 material referred to in Article 8 Regulation (EC) No 1069/2009 on the biological hazards other than the BSE/TSE and on the chemical hazards in the ash resulting from these treatments.

#### **A.1.3 Interpretation of the ToRs of the mandate**

- The effect of incineration, co-incineration and combustion on TSE is assessed by considering the available evidence on the effect of heat treatment on prions.
- ToR1 will be addressed by defining the effect as the absence of residual BSE/TSE hazards (prions) with high level of certainty (>99%).
- The presence of prions will be assessed by detection of TSE infectivity or seeding activity.
  - TSE infectivity is mainly determined by bioassay in laboratory rodents. Endpoint titration studies are the most reliable for quantification of infectivity, but survival time estimates or other bioassay approaches could be also considered if deemed reliable by the WG.
  - Seeding activity determined by protein misfolding cyclic amplification (PMCA) is considered a reliable proxy for infectivity because it is conceptually similar to infectivity, usually as sensitive as bioassays, and the available evidence suggests that it provides reliable estimates when measuring reduction in infectivity after heat treatment and other prion inactivation treatments. Data on seeding activity ( $SA_{50}$ ) will therefore be considered along with  $ID_{50}$  infectivity.
- Other proxies that could in principle be used, but will not be considered, are:
  - Direct measurement of  $PrP^{Sc}$ : it is not sensitive enough to detect different  $\log_{10}$  reductions or trace amounts of prions.
  - Protein content before and after heat treatment cannot be considered a good proxy because of limited sensitivity and differences between different proteins, especially when insoluble protein aggregates such as  $PrP^{Sc}$  are considered.
  - Lack of detection of peptides larger/smaller than the minimum  $PrP^{Sc}$  size after the treatment cannot be considered a good proxy because of the potential lack of sensitivity and indirect evidence of discrepancies with direct measurements of infectivity by bioassay.
- The opinion will assess the residual infectivity for the most thermoresistant animal TSE field strain as a worst-case scenario (WCS).

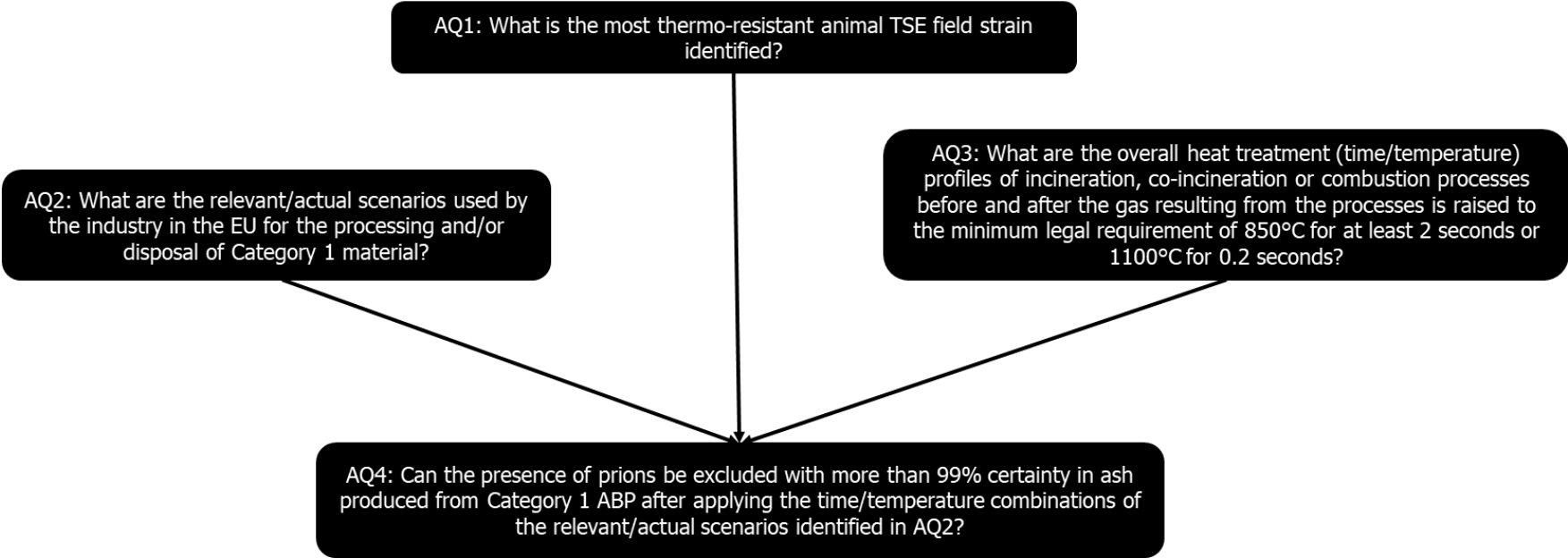
## A.2 Problem formulation

The ToR1 of the mandate was translated into four assessment question(s) (AQ). Their relationship is shown through the conceptual model in Figure A.1.

- AQ1: What is the most thermo-resistant animal TSE field strain identified?
- AQ2: What are the relevant/actual scenarios used by the industry in the EU for the processing and/or disposal of Category 1 material?
- AQ3: What are the overall heat treatment (time/temperature) profiles of incineration, co-incineration or combustion processes before and after the gas resulting from the processes is raised to the minimum legal requirement of 850°C for at least 2 seconds or 1100°C for 0.2 seconds?
- AQ4: Can the presence of prions be excluded with more than 99% certainty in ash produced from Category 1 ABP after applying the time/temperature combinations of the relevant/actual scenarios identified in AQ2?

The approach for each AQ, i.e., whether to apply a quantitative, qualitative or semi-quantitative approach, has been specified in Table A.1. There was no need to prioritise AQ.

**ToR1:** To assess the effect of incineration, co-incineration, and combustion of Category 1 material referred to in Article 8 Regulation (EC) No 1069/2009 on the BSE/TSE hazards in the ash resulting from these treatments



**Figure A.1:** The relationship between the assessment questions (AQ) for the assessment on the presence of biological and chemical hazards in ash from Category 1 material after incineration, co-incineration, and combustion

### **A.3. Methods that will be applied for conducting the assessment.**

The overall approach, the evidence needs and the methods, for answering each AQ (i.e. the use of a literature review, data from databases, expert judgement or primary data collection) are described in Table A.1

The methods that will be used for evidence integration across AQ and for accounting for the remaining uncertainty are provided in Table A1 based on the conceptual model.

**Table A.1:** Assessment questions and sub-questions for the assessment of the potential BSE risk posed by ruminant collagen and gelatine derived from ovine, caprine or bovine material other than hides and skins

Step 1.1	Step 1.2	Step 1.3	Step 2.1	Step 2.1
Assessment questions (reflecting clarification of ToRs)	Sub-assessment questions (if needed)	Overall approach	Evidence needs	Description of method to be used
AQ1: What is the most thermo-resistant animal TSE field strain identified?		Qualitative	<ul style="list-style-type: none"> <li>Data from scientific literature</li> <li>Previous EFSA opinions</li> </ul>	<ul style="list-style-type: none"> <li>Expert knowledge and literature selected by WG members.</li> <li>Very few papers using field strains are available. Key papers are limited and are on the radar of the experts making it very unlikely that any relevant paper will be missed.</li> <li>Animal TSE field strains are those characterised from cases confirmed by surveillance in the natural host populations.</li> <li>Laboratory strains will be considered in the narrative of the answer acknowledging that they are more thermo-sensitive.</li> </ul>
AQ2: What are the relevant/actual scenarios used by the industry in the EU for the processing and/or disposal of Category 1 material?		Qualitative	<ul style="list-style-type: none"> <li>Industry data (hearing experts)</li> </ul>	<ul style="list-style-type: none"> <li>Questionnaire to EFRA on industry practicalities relating to the processing and disposal of Category 1 animal by-products.</li> <li>Invite hearing experts from industry to WG meetings to ascertain how the different types of incinerators, co-incinerators and combustion plants operate.</li> </ul>
AQ3: What are the overall heat treatment (time/temperature) profiles of incineration, co-incineration or combustion processes before and after the gas resulting from the processes is raised to the minimum legal requirement of 850°C for at least 2 seconds or 1100°C for 0.2 seconds?		Qualitative	<ul style="list-style-type: none"> <li>Data from scientific literature</li> <li>Previous EFSA and EU Commission former Scientific Steering Committee (SSC) opinions,</li> <li>Industry data (hearing experts)</li> </ul>	<ul style="list-style-type: none"> <li>Invite hearing experts from industry to working groups meetings to provide specific details, for example, residence time and temperature profiles.</li> <li>This AQ3 allows the WG members to ascertain the actual time/temperature profiles that the ABP material is subjected to before and after the gas resulting from the processes is raised to the minimum legal requirement of 850°C for at least 2 seconds or 1100°C for at least 0.2 seconds. Thus, the actual exposure times to temperatures contributing to the reduction of infectivity can be considered.</li> </ul>
AQ4: Can the presence of prions be excluded with more than 99% certainty in ash produced from Category 1 ABP after applying the time/temperature combinations of the relevant/actual scenarios identified in AQ2?		Qualitative	<ul style="list-style-type: none"> <li>Data from scientific literature</li> <li>Previous EFSA opinions</li> <li>RA conducted by other bodies and not peer reviewed.</li> </ul>	<ul style="list-style-type: none"> <li>For the purpose of this AQ, the term 'presence of prions' encompasses either the presence of TSE infectivity measured by bioassays or the presence of seeding activity, which is considered a reliable and sensitive proxy.</li> <li>TSE strains other than those identified in AQ1 and laboratory strains will be considered in the narrative of the answer acknowledging that they may experience a higher level of reduction without concluding on the residual infectivity.</li> <li>The answer to AQ4 will be provided by consensus judgement with the working group, considering the impact on the most thermos-resistant animal TSE field strains (AQ1) of the actual/total exposure times to temperatures contributing to the reduction of infectivity (AQ3) in the relevant/actual scenarios used by the industry in the EU (AQ2).</li> </ul>
<b>Step 2.2: Integration of evidence across assessment questions and remaining overall uncertainty</b>				
<b>ToRs as clarified</b>	<b>Integration of evidence between AQ and uncertainty analysis</b>			
ToR 1	<ul style="list-style-type: none"> <li>AQ3 will help ascertain the actual temperature/time profiles in the incinerator, etc., allowing a better understanding of the exposure time to temperature that could contribute to the reduction of infectivity.</li> </ul>			

- AQ4 requires the ascertainment of the impact of the time/temperature combinations of the selected relevant/actual scenarios (AQ2) on the most thermo-resistant animal TSE field strains (AQ1).
- For this purpose, evidence on inactivation dynamics of animal TSE field strains, processing parameters, and the specific characteristics of the standard processes applied will be collected.
- If not available at the temperature ranges of the relevant scenarios, all data extracted from the relevant literature on the effect of heat on prions at temperature and time combinations the closest to the minimum requirement of the legislation will be assessed.
- From all scenarios considered, the one with the most stringent conditions will be evaluated first. If the answer to AQ4 for that scenario is NO, then it can be assumed the same answer for all other scenarios. Then the assessment will stop.
- AQ4 has been designed as a binary question that only allows two answers: YES/NO. Since the question requires to assess the exclusion of prions in the final material with high certainty, it was not considered to assign any degree of uncertainty associated to the answer, hence there will not be any quantification of the level of uncertainty associated to the answer.
- The sources of uncertainty will be identified while describing the data from relevant studies on heat inactivation of prions at low and high temperatures using the most thermo-resistant animal TSE field strain and other field and laboratory strains.
- The available data will be reviewed together with the uncertainty, and whenever it is concluded that there are not data available on the conditions under assessment and no conclusions will be produced at conditions other than those reported in the studies, then the answer would be NO to AQ4, even if the reduction of the prions in the final material can be ascertained and acknowledged.
- The answer to AQ4 would be YES only if there is more than 99% certainty that the ashes produced under the conditions of a selected scenario do not contain any prions. The final answer for each scenario will be produced by consensus judgement with the working group.

## References

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