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BSE risk posed by ruminant collagen and gelatine derived from bones

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Abstract

The European Commission requested an estimation of the BSE risk (C-, L- and H-BSE) from gelatine and collagen derived from ovine, caprine or bovine bones, and produced in accordance with Regulation (EC) No 853/2004, or Regulation (EC) No 1069/2009 and its implementing Regulation (EU) No 142/2011. A quantitative risk assessment was developed to estimate the BSE infectivity, measured in cattle oral infectious dose 50 ($CoID_{50}$), in a small size batch of gelatine including one BSE-infected bovine or ovine animal at the clinical stage. The model was built on a scenario where all ruminant bones could be used for the production of gelatine and high-infectivity tissues remained attached to the skull (brain) and vertebral column (spinal cord). The risk and exposure pathways defined for humans and animals, respectively, were identified. Exposure routes other than oral via food and feed were considered and discussed but not assessed quantitatively. Other aspects were also considered as integrating evidence, like the epidemiological situation of the disease, the species barrier, the susceptibility of species to BSE and the assumption of an exponential dose–response relationship to determine the probability of BSE infection in ruminants. Exposure to infectivity in humans cannot be directly translated to risk of disease because the transmission barrier has not yet been quantified, although it is considered to be substantial, i.e. much greater amounts of infectivity would be needed to successfully infect a human and greater in the oral than in the parenteral route of exposure. The probability that no new case of BSE in the cattle or small ruminant population would be generated through oral exposure to gelatine made of ruminant bones is 99%–100% (almost certain) This conclusion is based on the current state of knowledge, the epidemiological situation of the disease and the current practices, and is also valid for collagen.

KEYWORDS

BSE, cattle, collagen, exposure, feed, food, gelatine, goats, humans, risk, sheep

CONTENTS

SUMMARY

The European Food Safety Authority (EFSA) was asked by the European Commission to deliver a scientific opinion by 31 October 2024 on the estimation of the BSE risk of gelatine and collagen derived from ovine or caprine (Term of reference 1)/bovine (Term of reference 2) material other than hides and skins, i.e. from bones, and produced only in accordance with: (a) all of the requirements laid down in Sections XIV and XV of Annex III to Regulation (EC) No 853/2004, excluding the provisions by which bones defined as specified risk material in Article 3(1)(g) of the TSE Regulation are prohibited, as well as point 1. (b) in Chapter III of both Sections; (b) or the relevant provisions of Regulation (EC) No 1069/2009 and its implementing Regulation (EU) No 142/2011. A number of clarifications were agreed with the requestor on the interpretation of the ToR: classical BSE and atypical L and H BSE should be all included when considering the BSE risk; other TSE agents (classical and atypical scrapie) should not be considered; the BSE risk should be assessed for all susceptible species populations, i.e. cattle, small ruminants and humans; the BSE risk should be considered from ruminant collagen and gelatine (C&G) produced from bones of bovine, ovine and caprine animals born and raised in the EU and also sourced from third countries; the risk assessment should be conducted for a scenario where all bones could be used for the production of gelatine and collagen. Exposure routes other than oral via food and feed were considered in the assessment but not quantified.

A probabilistic model was developed to estimate the BSE infectivity load, measured in cattle oral infectious dose 50 ($CoID_{50}$), contained in the gelatine produced from all the bones of one adult bovine/ovine animal infected with any of the three BSE strains (C, H and L) and at the clinical stage of the disease. The data used to parameterise the model were sourced from the industry via personal communications, previous EFSA opinions, scientific literature and expert knowledge.

The exposure pathways for humans from the use of ruminant C&G were described and included: food (oral), cosmetics (topical and parenteral), medicinal products and medical devices (oral and parenteral) and industrial purposes (different possible routes of exposure). Based on the outputs of the model and the data from the EFSA's food consumption database, the maximum amount of infectivity that the consumer would be exposed to through food consumption, if all the gelatine consumed was sourced from an infected batch, was estimated. The risk pathways for ruminants from the use of ruminant C&G were also described and included: the addition of collagen or gelatine, or of former foodstuffs containing collagen or gelatine, directly into compound feed for livestock or in pet food, and the feeding to livestock of technological additives or nutritional supplements containing collagen or gelatine. A dose–response equation was used to determine the probability of BSE infection in cattle or sheep occurring as a result of exposure to gelatine added to compound feed for ruminants, applying the exponential dose–response relation for the single-hit model with fixed probability of infection *r* (infectivity), assuming that the total daily requirement of crude protein is provided using gelatine.

When integrating the evidence with the quantitative outputs of the model, further considerations were taken on board. These included the epidemiological situation of BSE in Europe and the rest of the world, the use of multiple worst-case scenarios in the probabilistic models, the susceptibility of different species to BSE and the extrapolation of the gelatine model to collagen.

The results of the probabilistic model showed that the median number of $CoID_{50}$ in the gelatine produced with the bones of one BSE-infected bovine/ovine animal (C, H or L), at the clinical stage, is 0.26 and 4 $CoID_{50}$, respectively. The median number of $CoID_{50}$ per kg of a small size batch of gelatine (containing 755 kg of bovine or ovine bones) including the bones of one BSE-infected bovine/ovine animal is 3.4×10^{-4} and 5.3×10^{-3} $CoID_{50}$/kg, respectively. Given that worst-case scenarios were considered in some assumptions of the models and that several input variables were approximated to worst-case estimates, the infectivity will have been overestimated by the models.

For oral exposure via food, the median amount of infectivity that a consumer could be exposed to in one single exposure event (daily consumption) if all the gelatine consumed was sourced from an infected batch, based on median values of infectivity, was estimated to be between 7.1×10^{-6} $CoID_{50}$ and 0.26 (bovine BSE), and 1.1×10^{-4} and 4.06 $CoID_{50}$ (ovine BSE) depending on whether it was assumed that the infectivity was uniformly distributed in gelatine or aggregated in a single exposure event, respectively. This is an overestimation of the exposure since it is based on worst-case model outputs and median consumption figures of the highest consumption group. Taking into consideration all factors related to the worst-case scenarios, in case of oral exposure, the median dose in a single exposure event would be well below 1 $CoID_{50}$.

Potential parenteral exposure routes to BSE-infected gelatine and collagen in humans (through cosmetic, medical and surgical products) were identified but not quantified in this assessment. For humans, exposure to infectivity cannot be directly translated to risk of disease because the transmission barrier has not yet been quantified. This transmission barrier is considered to be substantial, i.e. much greater amounts of infectivity would be needed to successfully infect a human than a bovine, or small ruminant and greater in the oral route than in the parenteral route of exposure.

In this risk assessment, the BSE risk for ruminants has been quantified as the number of potential new BSE cases generated due to oral exposure to bovine/ovine-derived BSE infectivity via feed containing contaminated gelatine. The dose–response equation applied to the outputs of the model showed that in up to 87% and 96% of the iterations, the number of new BSE cases potentially generated in bovine and small ruminants, respectively, due to exposure to an infected batch containing all the bones of one BSE-infected bovine animal is below 1. In the hypothetical scenario where all the bones from one adult BSE-infected ovine animal are included in one small batch of gelatine, the results of the dose–response equation applied to the outputs of the model showed that in up to 7.9% and 25% of the iterations, the number of new BSE cases potentially generated in bovine and small ruminants, respectively, is below 1.

Taking into account the multiple unlikely events that may lead to the potential exposure of cattle and small ruminants to BSE infectivity through consumption of gelatine in feed, the current lack of production of gelatine with small ruminant

bones, the results of the model, the impact of the worst-case scenarios and the lack of evidence that the C-BSE agent might be circulating in the small ruminant population, it is concluded that the probability that no new case of BSE in the cattle or small ruminant population would be generated through oral exposure to gelatine made of ruminant bones is 99%–100% (almost certain).

The conclusions are based on the current state of knowledge of the epidemiological situation of the disease in the EU and third countries, and the current practices related to the production of collagen and gelatine. If any of these factors change, the conclusions of this assessment should be reviewed. Since collagen is not currently produced using ruminant bones, it was not possible to estimate the residual infectivity in collagen produced with infected bovine/ovine animals. However, as for gelatine production, the collagen extraction process also involves pretreatment and hydrolysis processes which have a similar capacity to result in a reduction in BSE infectivity. Therefore, the BIOHAZ Panel assumed in this assessment that the conclusions for gelatine are also valid for collagen.

It is recommended to assess the transmission risk associated with the non-oral exposure to collagen and gelatine; to periodically reassess the risks addressed in this opinion, should the relative plausibility and likelihood of the risk and exposure pathways change; to maintain the current EU-wide surveillance system; to evaluate the impact of the specific industrial processes for the production of C&G on the infectivity of naturally occurring BSE agents; to assess the BSE risk associated with the uses of the waste generated through the degreasing process; to undertake research activities aimed at the production of new data regarding the susceptibility of cattle to infection with H-BSE or L-BSE via the oral route.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

The current international standard for BSE, Chapter 11.4 of the terrestrial animal health code of the World Animal Health Organisation (hereafter referred to as 'the Code chapter') lays down the following provisions for gelatine and collagen derived from cattle:

- gelatine and collagen prepared exclusively from hides and skins are considered safe commodities, meaning they are exempt of any BSE-related conditions – article 11.4.1, point d).
- gelatine and collagen prepared from bones from healthy cattle are subject to certain BSE-related conditions, including as regards the industrial process, when originating from a country with a controlled or undetermined BSE risk – article 11.4.15.

This distinction is generally reflected in the EU rules laid down primarily in Regulation (EC) No 999/2001[1] (hereafter referred to as 'the TSE Regulation'), though these rules cover gelatine and collagen derived from ovine and caprine animals in addition to that from cattle:

- gelatine and collagen derived from hides and skins from healthy ruminants shall not be subject to restrictions on placing on the market – article 16, point 1.b).
- gelatine and collagen derived from healthy bovine, ovine and caprine animals, other than from hides and skins, are subject to certain BSE-related conditions when originating from a country with a controlled or undetermined BSE risk – article 16, points 2 and 3. Additional conditions apply for importation into the Union, depending on the risk status of the country of origin – Annex IX, Chapter C. These conditions are however significantly more detailed than those in the Code Chapter.

In addition to the TSE Regulation, Regulation (EC) No 853/2004[2] (2) establishes in Section XIV and XV of Annex III the specific requirements for the production of gelatine and collagen for food, including as regards the industrial process to which ruminant bones from ruminants originating from a country with a controlled or undetermined BSE risk must be subject.

Finally, Regulation (EC) No 1069/2009[3] and its implementing Regulation (EU) No 142/2011[4] establish the conditions for the production of gelatine and collagen for its use in feed.

These provisions have been initially established and may have evolved over time based on several past EFSA opinions, in particular the following:

- Quantitative assessment of the human BSE risk posed by gelatine with respect to residual BSE (EFSA BIOHAZ Panel, 2006a);
- Potential BSE risk posed by the use of ruminant collagen and gelatine in feed for non-ruminant farmed animals (2006);
- BSE risk from cohort animals: bovine hides and skins for technical purposes (2006);
- Potential BSE risk posed by the use of ruminant collagen and gelatine produced in accordance with Section XIV and XV of Annex III to Regulation (EC) No 853/2004 or classified as Category 3 as referred to in Article 10 of Regulation (EC) No 1069/2009 and produced in accordance with Regulation (EU) No 142/2011, in feed for non-ruminant farmed animals (2020).

When it comes to use, gelatine and collagen derived from ruminant bones are allowed for human consumption (provided their production is in accordance with Regulation (EC) No 853/2004) and for the feeding of any animal species other than ruminants but remain prohibited for ruminants.

Taking note of the quasi-eradication of classical BSE in the world, including in the Member States of the European Union, the World Animal Health Organisation proposed in 2017 to launch an in-depth review of the current international standards for BSE, which has eventually led the Code commission to circulate, after its meeting of September 2022, of a significantly modified draft Code chapter.[5]

Among many other evolutions, the proposed Code chapter recognises any gelatine and collagen derived from bovines as safe commodities, regardless of the tissues used as raw material (i.e. including bones) and of the BSE risk category of the country of origin of the bovines from which the tissues originate (i.e. including countries with a controlled or undetermined BSE risk). It is likely that the proposed Code chapter will be adopted in May 2023 by the World Animal Health Organisation.

[1]Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 147, 31.5.2001, p. 1–40.

[2]Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

[3]Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). OJ L 300, 14.11.2009, p. 1–33.

[4]Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. OJ L 54, 26.2.2011, p. 1–254.

[5]https://www.woah.org/app/uploads/2022/11/a-tahsc-sept-2022-report-1.pdf (Annex 10, page 118).

In this context, the question to which the Commission is confronted with is whether it would be appropriate to propose aligning the TSE Regulation with the likely future international standards, according to which no BSE-related restriction will apply to gelatine and collagen derived from any ruminant material.

1.1.2 | Terms of reference

In accordance with Article 29 of Regulation (EC) No 178/2002,[6] the Commission requests EFSA:

1. To estimate the BSE risk (C-, L- and H-BSE) of gelatine and collagen derived from ovine or caprine material other than hides and skins, i.e. from bones, and produced only in accordance with:

   – all of the requirements laid down in Sections XIV and XV of Annex III to Regulation (EC) No 853/2004, excluding the provisions by which bones defined as specified risk material in Article 3(1)(g) of the TSE Regulation are prohibited, as well as point 1. (b) in Chapter III of both Sections.
   – or the relevant provisions of Regulation (EC) No 1069/2009 and its implementing Regulation (EU) No 142/2011.

2. To estimate the BSE risk (C-, L- and H-BSE) of gelatine and collagen derived from bovine material other than hides and skins, i.e. from bones, and produced only in accordance with:

   – all of the requirements laid down in Sections XIV and XV of Annex III to Regulation (EC) No 853/2004, excluding the provisions by which bones defined as specified risk material in Article 3(1)(g) of the TSE Regulation are prohibited, as well as point 1. (b) in Chapter III of both Sections.
   – or the relevant provisions of Regulation (EC) No 1069/2009 and its implementing Regulation (EU) No 142/2011.

1.2 | Interpretation of the Terms of Reference (if appropriate)

During the discussion about the ToR, clarification was sought from the requestor, and agreement reached, on the following points:

1. Classical BSE and Atypical L and H-BSE should be all included when considering the BSE risk.
2. Other TSE agents present in the relevant species, such as classical and atypical scrapie, should not be considered.
3. The BSE risk should be assessed for all susceptible species populations, i.e. cattle, small ruminants and humans. It was acknowledged by the requestor that if humans are to be considered, only the human exposure to the BSE agent will be estimated, but not the risk.
4. Possible future alignment with international standards requires that the BSE risk should be considered from ruminant collagen and gelatine (C&G) produced from bones of bovine, ovine and caprine animals born and raised in the EU and also sourced from third countries. Given the lack of knowledge about the BSE status of many countries, an extra level of uncertainty would have to be added to the conclusions.
5. Regarding the type of material, the first indent of ToR 1 and ToR 2 specifies that the provisions set out in Regulation (EC) No 853/2004, by which bones defined as specified risk material in Article 3(1)(g) of the TSE Regulation are prohibited, should be excluded from consideration in the risk assessment. This implies that the BSE risk of ruminant C&G derived from bones declared specified risk material (SRM) under Regulation No 853/2004 should be estimated in the Opinion. The second indent of ToR 1 and ToR 2 does not specify that the provisions by which bones defined as specified risk material in Article 3(1)(g) of the TSE Regulation are prohibited for the production of collagen and gelatine should be excluded from consideration in the risk assessment. The ABP regulations state that only Category 3 material can be used to produce C&G. This implies that the BSE risk of C&G derived from bones produced under Regulation (EC) No 1069/2009 and its implementing Regulation (EU) No 142/2011 should not be estimated in the Opinion. This distinction is only valid in the EU, as in other parts of the world, and particularly in countries with a negligible BSE risk, SRM is not defined, and therefore, no categorisation of raw materials for the production of gelatine and collagen is made even for feed purposes. Considering the possible future alignment with the international standards, the risk assessment should be conducted for a scenario where all bones could be used for the production of gelatine and collagen. This is a sort of worst-case scenario, where any connotations related to TSE are ignored.
6. In relation to exposure assessment, one of the potential uses of ruminant collagen and gelatine could be the reauthorisation of its inclusion in ruminant feed, which would be the first time since the total feed ban in which some form of cannibalism is authorised in the EU. The requestor confirmed that the direct exposure of cattle/other ruminant species to collagen and gelatine via ruminant feed should be considered in the risk assessment.
7. The ToR 1 and ToR 2 state that the provisions of point 1 (b) of Chapter 3 of Sections XIV and XV of Annex III of Regulation (EC) No 853/2004 should be excluded from consideration. This point covers the production process from bones in negligible risk countries. It was not clear whether the production process set out in point 1 (a) of Chapter 3 of Sections XIV and

[6]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

XV of Annex III from bones from ruminants from controlled or undetermined risk countries should also be applicable to ruminant bone material derived from ruminants from negligible risk countries, by exclusion of the point 1(b).

Extra clarification was requested from the European Commission on the assumption that the scenario to be assessed is the use of all potential raw material (i.e. all bones of all animals, with no regard for current TSE restrictions) subject to the minimum treatments required for the production of collagen and gelatine, i.e. without any regard for any additional measures that might be taken with the purpose of reducing TSE infectivity in the product. The requestor confirmed that the assessment should be conducted for a scenario where all ruminant bones could be used for the production of gelatine and collagen, i.e. without any regard for any additional measures that might be taken with the purpose of reducing TSE infectivity in the product.

NON-FOOD RISK PATHWAYS

The quantitative estimations of exposure to BSE agents in humans or of risks of a new BSE case in cattle or small ruminants will be calculated considering exclusively the consumption of food or feed containing contaminated gelatine. Other exposure pathways, e.g. through cosmetics or medicinal products containing potentially contaminated gelatine, are only described for a more complete overview of the general use of gelatine.

2 | DATA AND METHODOLOGIES

2.1 | Data

2.1.1 | Data related to production practices

The previous C&G opinion (EFSA BIOHAZ Panel, 2020) requested information from various producer and industry associations to gain insight into the prevailing production practices for C&G and the use of these products in feed in the European Union. Topics including the source and type of raw materials and the various processing methods were included in the communications, although a structured survey was not conducted, and differing levels of engagement were achieved with stakeholders. The information and data provided were deemed to be confidential, and no specific references to any company or association were included in the opinion, but the information reviewed was used to describe generic practices for production and use of C&G in the EU at that time.

The same organisations were approached to support the preparation of this opinion and the development of a probabilistic model. Text from the 2020 opinion was sent to each organisation and information was requested on any changes. All those who responded confirmed that their processes and practices were unchanged. Full referenced descriptions of the production of collagen and gelatine were provided in the 2020 opinion, and readers are directed to that opinion for further details. The text in Section 3.3 provides a summary for context, and ease of reference.

2.1.2 | Data for the model

A probabilistic model was developed to estimate the BSE infectivity in the gelatine produced from the bones of one adult infected animal as detailed further in Section 2.2.2, with two versions: one for a bovine animal and a second for an ovine animal. Once the parameters of the model were identified and defined, data to populate the model were retrieved, using the following data sources:

- Data on adult tissue weights and tissue infectivity in bovine and ovine animals were obtained primarily from previous EFSA opinions (EFSA BIOHAZ Panel, 2005b, 2005c, 2006a) in which quantitative risk assessments (QRA) of the residual BSE risk in the production of C&G had been conducted. A search for any updated literature was also conducted. Given the lack of any data available in the public domain for ovine, unpublished data being prepared for publication, provided by a working group (WG) member, were also used.
- Data on the reduction of TSE infectivity by the application of alkaline and acidic methods, as used in the production of C&G, were sourced by reviewing previous Scientific Steering Committee (SSC) and EFSA Opinions (EFSA BIOHAZ Panel, 2005b, 2005c; SSC, 2000a, 2000b, 2001, 2002, 2003) and key scientific publications, as described in EFSA BIOHAZ Panel (2020). No relevant recent publications were identified that could add to this body of knowledge.
- The parameters specifically addressing industrial processes were defined using information sourced directly via personal communication with industry stakeholders. References have been added as personal communications.
- For a small number of parameters, no data were available. In these cases, the parameter estimates were agreed based on the expert knowledge of the WG members.

The papers considered in this assessment were selected by the WG experts, based on the topic and their relevance. A systematic literature search was not performed.

Where there were multiple data sources with different estimates for the same parameter, the WG discussed and, where appropriate, agreed on a probability distribution to be included in the model, representing associated uncertainty around

the true average value, based on the robustness of the data sources and their representativeness of the natural phenomena and processes under assessment.

2.1.3 | Information and data on the use of C&G in humans and animals

Information on the use and consumption of collagen and gelatine was obtained from the following sources:

- A non-systematic literature review.
- Information obtained from producer and industry associations for the 2020 opinion, and updated for this assessment, as described in Section 2.1.1.
- Previous EFSA Opinions, particularly the 2020 Opinion on collagen and gelatine (EFSA BIOHAZ Panel, 2020).
- Opinion of the Scientific Committee on Consumer Products of the European Commission (SCCP, 2005).
- Guidelines produced by the Scientific Committee of Consumer Safety (SCCS) of the European Commission, like for example the SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 12th revision (SCCS, 2023).
- Note for guidance of the European Commission on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products.[7]
- A review of the grey literature and relevant websites.
- Specific summary statistics of consumption data (for chronic and acute consumption) of gelatine, obtained from the EFSA data consumption database,[8] extracted in January 2024. Dietary surveys and food consumption data for each country are divided by category: age (from infants to adults aged 75 years or older); food group (over 2500) and type of consumption, covering both regular and high consumption. These statistics contain data for the total population (all subjects/all days) and for consumers only/consumption days only, expressed in grams per day or grams per day per kilogram of body weight (g/kg body weight (bw) per day) codified under the first version of the FoodEx food classification system.

2.2 | Methodologies

2.2.1 | Approach to address the ToR

The following assessment questions (AQ) were formulated to address the ToR:

- AQ1: What is the BSE infectivity (cattle oral infectious dose 50, $CoID_{50}$) in the gelatine hypothetically produced by all the bones from one adult BSE-infected ovine animal?
- AQ2: What is the total amount of BSE infectivity ($CoID_{50}$) per kg of gelatine in a hypothetical production batch that includes one BSE-infected ovine animal?
- AQ3: What is the BSE infectivity ($CoID_{50}$) in the gelatine produced by all the bones from one adult BSE-infected bovine animal?
- AQ4: What is the total amount of BSE infectivity ($CoID_{50}$) per kg of gelatine produced in a batch that includes one BSE-infected bovine animal?
- AQ5: What is the maximum amount of BSE infectivity, expressed in $CoID_{50}$, that a human could be exposed to in a hypothetical worst-case scenario due to gelatine from a batch that includes one BSE-infected animal?
- AQ6: What is the probability of a new case of BSE in cattle, sheep or goats, due to exposure to gelatine from a batch that includes one BSE-infected animal?

The final wording of AQ2 and AQ4 is different to that proposed in the original protocol (see Annex A). Following the discussions in the BIOHAZ Panel, it was agreed to remove the subquestions related to infectivity estimation at population level. The conceptual framework showing the relationship between the AQs is displayed in Figure 1.

[7] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52011XC0305%2804%29.
[8] https://www.efsa.europa.eu/en/data-report/food-consumption-data.

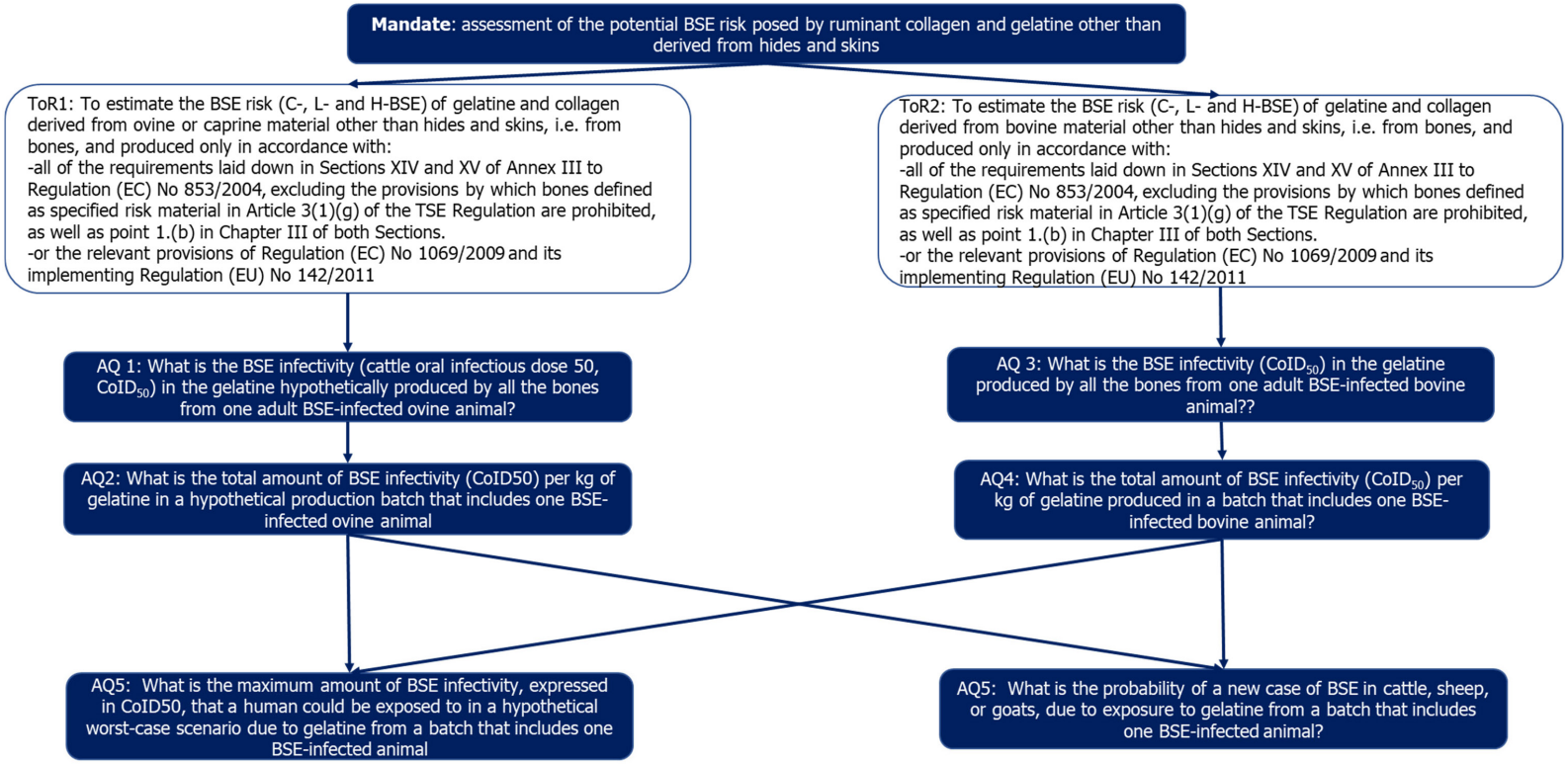

FIGURE 1 The relationship between the assessment questions (AQ) for the assessment of the potential BSE risk posed by ruminant collagen and gelatine derived from ovine, caprine or bovine material other than hides and skins.

The ToR of this mandate focus on two specific scenarios, (a) the use of small ruminant bones as a substrate, and (b) the use of bovine bones, including (in both cases) the skull and vertebral column. At present, the industry does not use small ruminant (SR) bones for the production of gelatine, and therefore, the processes cannot be assessed from real data. It is therefore assumed that any process using SR bones would be equivalent to that using bovine bones as the substrate. Since the bovine model is built with actual infectivity data of infected cattle as opposed to the ovine model built on experimental infectivity data, it was decided to describe first the bovine model and provide answers to AQ2 and AQ4, and then the ovine model with the answers to AQ1 and AQ3. It is also assumed that all the steps involved in the production of gelatine will be the same, regardless of substrate once the bones are crushed and degreased. The potential reductions in infectivity relating to each step, as inferred from the experimental data, and presented in detail in the previous opinion (EFSA BIOHAZ Panel, 2020) are therefore unchanged (See Section 3.6.1).

2.2.2 | The quantitative risk assessment: Probabilistic model

A probabilistic model was developed to estimate the BSE infectivity load, measured in $CoID_{50}$, contained in the gelatine produced from the bones of one adult animal infected with any of the three BSE strains (C, H and L) (subsequently referred to as 'BSE') and at the clinical stage of the disease. The model structure and framework was similar to that developed for the previous EFSA risk assessment on the same topic (EFSA BIOHAZ Panel, 2020). The structure, assumptions and parameters of the model are described in Sections 3.6.1 and 3.6.2, and the limitations are included in the sections dealing with the uncertainty analysis and further considerations (Sections 3.10 and 4).

When possible, any known uncertainty associated with the parameter values has been described by appropriate probability distributions. Depending on the situation and the data available, either the Pert, log-normal or the uniform distributions have been selected. In this way, uncertainty is considered in the model and represented by 5th and 95th percentiles, which indicate the range within which 90% of the results lie. The model was run for 50,000 iterations using Latin Hypercube sampling to ensure convergence. Not all uncertainty has been estimated in the calculations, as not all can be quantified. Therefore, the 5th and 95th percentiles describe the amount of quantified uncertainty included in the model. Mean values of the distribution are also reported given the skewness of some of the output distributions of the model. As far as possible, ranges representing the variability within a population were excluded from the model with all parameter values representing the average (mean) of the population. This was achieved by different methods depending on the parameter: disaggregating the data into categories (e.g. a separate bovine and ovine model and inclusion of different tissue types), using maximum estimates and worst case scenarios (e.g. an adult animal at clinical stage). While the ideal is to separate variability and uncertainty in the model, it was decided to use fixed values representing average values for certain parameters for which no further data were available. The impact on model outputs was assessed for these and all other parameters. Overall, it is considered that the model overestimates the average BSE infectivity in the final product.

A sensitivity analysis was conducted for each parameter represented by a probability distribution. This method is preferred for large numbers of input parameters, as all values that provide an insignificant contribution are removed from the analysis. Further methodology for the model assumptions and parameterisation are described in Section 3.6.

2.2.3 | Uncertainty analysis

The assessment of uncertainty was undertaken following the EFSA 'Guidance on Uncertainty Analysis in Scientific Assessments' (EFSA Scientific Committee, 2018a), the EFSA scientific opinion on 'The principles and methods behind EFSA's Guidance on Uncertainty Analysis in Scientific Assessments' (EFSA Scientific Committee, 2018b) and the Guidance on Communication of Uncertainty in Scientific Assessments (EFSA, 2019). Special attention was given to: (i) the interpretation of the ToR, i.e. framing of the mandate, (ii) the identification of sources of uncertainty, which were listed, together with their expected impact on the outcome of the assessment and (iii) the uncertainties in the answer to the ToR. The QRA probabilistic model described in Section 2.2.2 models uncertainty on the BSE infectivity contained in the gelatine produced from the bones of one infected adult animal. Other sources of uncertainty not accounted for by the model were identified and considered when assessing the overall uncertainty in the answer to the ToR via consensus judgement within the working group. The uncertainty analysis was conducted following the recommendations of the guidance, describing probabilities that express uncertainties using the numeric expression of the approximate subjective probability scale followed by the words (e.g. extremely unlikely, almost certain, etc.).

3 | ASSESSMENT

3.1 | Summary of previous EFSA RA on C&G

The potential risk from BSE in bovine derived products such as gelatine was first assessed by the EU Scientific Veterinary Committee (SVC) in 1992 and 1994, as reported Scientific Steering Committee (SSC) of the European Commission (SSC, 2000a), when it was concluded that *'whatever the tissue source, there is a negligible risk from trading in gelatine for*

technical use, for consumption or in cosmetics. Additional guarantees are therefore not necessary'. Following the identification of the zoonotic potential of BSE in 1996, several further assessments of the potential risk associated with BSE in collagen and gelatine were conducted by the SVC, the SSC, EFSA and other EU agencies. The human and animal BSE exposure risk associated with collagen and gelatine has been kept under periodic review. The risk assessments conducted immediately after the announcement of the zoonotic potential of BSE are documented in an SSC Opinion on the safety of gelatine produced in March 1998 (SSC, 1998) and in the updated Opinion produced in January 2000 (SSC, 2000a). These risk assessments supported the principle of sourcing of raw materials from geographical areas that were uninvolved in the BSE epidemic, or at least excluding tissues identified as a risk. In 1997, the use of skull, brain, eye, spinal cord and tonsils for any purpose was prohibited by Commission Decision 97/534/EC.[9]

Further updates on the safety of gelatine were produced by the SSC in June 2001 (SSC, 2001), September 2002 (SSC, 2002), December 2002 (SSC, 2002b) and March 2003 (SSC, 2003). All of these continued to support the principle of safe sourcing. The 2003 SSC Opinion concluded that the production processes for gelatine derived from bone had a TSE inactivation capacity of 4.5 logs. In addition to the recommendations in previous opinions, the 2003 SSC Opinion recommended that bones used for the production of gelatine should come from animals that passed *ante* and *post-mortem* inspection. It further recommended higher standards, and perhaps *'special grade gelatine'* for parenteral or ophthalmic administration.

In 2000, the SSC produced an Opinion on a quantitative risk assessment on the use of vertebral column for the production of gelatine and tallow (SSC, 2000a). The SSC concluded that 'the additional safety gained from the removal of vertebral column for the production of tallow and gelatine is limited in countries with a lower BSE risk but should be considered as sufficiently important to exclude it in higher risk countries.'

The SSC produced an Opinion on the safety with respect to the TSE risks of collagen produced from ruminant hides in 2001. The SSC concluded that 'on the basis of current knowledge it can be considered that the parts of ruminant hides used for the production of collagen do not present a risk with regard to TSE, provided contamination with potentially infected materials is avoided.' Based on the SSC opinion, the European Commission introduced Decision 2003/721/EC[10] laying down the specific public health conditions for the manufacture of collagen intended for human consumption. In 2005 (EFSA BIOHAZ Panel 2005), an opinion on the safety of an alternative process for the production of collagen concluded that '*the production process proposed by industry ensures equivalent or higher safety of collagen intended for human consumption compared to the safety achieved by applying the standards of Part V(1) of Decision 2003/721/EC'*.

In 2006, the EFSA Scientific Panel on Biological Hazards was asked to assess the *validity of the outcome of a 2004 quantitative risk assessment of the residual BSE risk in a variety of bovine derived products, including gelatine extracted from bovine bones* (EFSA BIOHAZ Panel, 2006a). The conclusions reached by the Panel are described below in Section 3.7.1.

More recently a further QRA was requested (EFSA BIOHAZ Panel, 2020), to examine the risk posed to cattle by ruminant collagen and gelatine, from raw material fit for human consumption or from Category 3 animal by-products, used in feed intended for non-ruminant animals, including aquaculture animals. This opinion concluded that '*the probability that no new case of BSE in the cattle population would be generated through any of the three identified risk pathways was 99–100% (almost certain)'*.

The current risk assessment follows on from these previous risk assessments and considers the implications for both the human and animal exposure that would result from the removal of the precautionary measures (i.e. restrictions on the geographical source, or tissue types of source materials) put in place at the start of the BSE epidemic.

3.2 | Current legal framework for the production of C&G

Currently, collagen and gelatine production in the EU is tightly regulated. Regulation (EC) No 142/2011 for ABP and Regulation (EC) No 853/2004 for foodstuff both define collagen as a protein-based product derived from hides, skins, bones and tendons of animals.

Specifications for the production of collagen intended to be used in food are laid down in Section XV, Annex III, Regulation (EC) No 853/2004. Under Point 3, Chapter 1 of that Section, authorised raw materials must be derived from animals which have been slaughtered in a slaughterhouse, and whose carcasses have been found fit for human consumption following ante- and post-mortem inspection.

Gelatine is defined in the above-mentioned EU legislation as a natural, soluble protein, gelling or non-gelling, obtained by the partial hydrolysis of collagen produced from bones, hides and skins, tendons and sinews of animals. Gelatine production processes are specified by Section XIV, Annex III, Regulation (EC) No 853/2004. According to Pont 3, Chapter 1 of Section XIV, the same raw materials can be used as for collagen production. In addition, collagen and gelatine production from ABP is approved subject to certain conditions and requirements. In EU MS, ABP are regulated by Regulation (EC) No 1069/2009 and its Implementing Regulation (Regulation (EU) No 142/2011) (referred to as the ABP Regulations). Regulation (EC) No 1069/2009 lays down health rules as regards ABP and derived products not intended for human consumption. Regulation (EU) No 142/2011 lays down implementing rules for Regulation (EC) No 1069/2009, including processing standards, hygiene conditions and the format for documentary evidence that has to accompany consignments of ABP and

[9]Commission Decision of 30 July 1997 on the prohibition of the use of material presenting risks as regards transmissible spongiform encephalopathies (Text with EEA relevance). OJ L 216, 8.8.1997, p. 95–97.

[10]Commission Decision of 29 September 2003 amending Council Directive 92/118/EEC as regards requirements for collagen intended for human consumption and repealing Decision 2003/42/EC. OJ L 260, 11.10.2003, p. 21–31.

derived products for the purposes of traceability. More detailed information on the legal framework for the production of collagen and gelatine can be found in the 2020 EFSA Opinion (EFSA BIOHAZ Panel, 2020).

Third countries from which the import of (fresh and treated) raw materials for gelatine and collagen production is permitted are listed in Regulation (EU) 2021/405.[11] Regulation (EU) 2020/2235[12] lays down model official certificates to be used for the entry of collagen or gelatine intended for human consumption to the EU. Regulation (EU) 2022/2292[13] sets out other requirements for the entry in the EU of collagen and gelatine intended for human consumption. Under that Regulation, imported raw materials intended for the production of gelatine or collagen must come from approved premises.

New legislation following the EFSA opinion of 2020

Regulation (EC) 999/2001 lays down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Prior to 2021, under Annex IV of Regulation (EC) No 999/2001, collagen and gelatine derived from non-ruminant animals could be fed to farmed animals, including ruminant animals but there was a prohibition on the feeding of collagen and gelatine derived from ruminant animals to farmed animals. Collagen and gelatine derived from ruminant and non-ruminant animals could be fed to pets and fur animals. In 2021, following a positive vote from the Standing Committee on Plants, Animals, Food and Feed and the approval of the European Parliament and the Council, the European Commission adopted Commission Regulation (EU) 2021/1372[14] amending Annex IV. This regulation permitted the feeding of collagen and gelatine derived from ruminant animals to farmed animals other than ruminants. The scientific basis for this legislation was the opinion adopted by EFSA on 22 September 2020 on the potential cattle BSE risk posed by ruminant collagen and gelatine in feed for non-ruminant farmed animals. As already described in Section 3.1, EFSA concluded that the probability that no new case of BSE in the cattle population would be generated through any of the three risk pathways identified in that opinion was greater than 99% (almost certain).

Despite the current regulatory framework, it is important to note that the current risk assessment is conducted under a hypothetical scenario of complete deregulation of the source material and of the production and trade of collagen and gelatine, without any regard for any additional measures that might be taken with the purpose of reducing TSE infectivity in the product.

3.3 | Production and trade of collagen and gelatine

3.3.1 | Production of collagen and gelatine

The industrial processes for the production of collagen and gelatine are described in the 2020 EFSA Opinion (EFSA BIOHAZ Panel, 2020), and summarised here for ease of reference.

Pigs, cows and fish are the principal species currently used for collagen and gelatine production (Alipal et al., 2021). According to the feedback received from the industry, collagen can be derived from the hides, skins, bones, tendons and sinews of animals, but only hides and skins are used for the production of collagen by the members of the Collagen Casing Trade Association (Bierwagen, 2023 by email on 20 October 2023). In Europe, gelatine and collagen peptides (obtained through further hydrolysation of gelatine) are produced mostly from pig skins, pig bones, cattle hides, cattle bones as well as a small amount of fish skin, with ~21% of the gelatine produced in Europe being made from bovine raw materials, two-thirds of which is produced from bovine bones specifically (Vermeulen, 2023b by email on 31 October 2023). In regions, such as South America, bovine hide is the main raw material for gelatine production, as opposed to Europe, in which porcine sources are still preferred for gelatine manufacturing, probably due to the BSE crisis and to a low percentage of the population being Muslim (Dille et al., 2021). Materials of small ruminant origin are not cited in the literature as sources of collagen or gelatine, and this is supported by information from industry confirming that they are not currently used for commercial collagen and gelatine production. There is an increasing exploration and development of plant-based sources of collagen and gelatine, such as agar, carrageenan, pectin, xanthan gum, modified corn starch and celluloid (Alipal et al., 2021) to circumvent religious restrictions.

When gelatine is produced from bones, it is usually produced from bone chips, a dry material that is stripped of any soft tissues that were adhered to the bones. The current commercial practices for producing bone chips through degreasing

[11]Commission Implementing Regulation (EU) 2021/405 of 24 March 2021 laying down the lists of third countries or regions thereof authorised for the entry into the Union of certain animals and goods intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council (Text with EEA relevance) C/2021/1810. OJ L 114, 31.3.2021, p. 118–150.

[12]Commission Implementing Regulation (EU) 2020/2235 of 16 December 2020 laying down rules for the application of Regulations (EU) 2016/429 and (EU) 2017/625 of the European Parliament and of the Council as regards model animal health certificates, model official certificates and model animal health/official certificates, for the entry into the Union and movements within the Union of consignments of certain categories of animals and goods, official certification regarding such certificates and repealing Regulation (EC) No 599/2004, Implementing Regulations (EU) No 636/2014 and (EU) 2019/628, Directive 98/68/EC and Decisions 2000/572/EC, 2003/779/EC and 2007/240/EC. OJ L 442, 30.12.2020, p. 1–409.

[13]Commission Delegated Regulation (EU) 2022/2292 of 6 September 2022 supplementing Regulation (EU) 2017/625 of the European Parliament and of the Council with regard to requirements for the entry into the Union of consignments of food-producing animals and certain goods intended for human consumption C/2022/6496. OJ L 304, 24.11.2022, p. 1–30.

[14]Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals C/2021/6012. OJ L 295, 18.8.2021, p. 1–17.

follow a continuous process with a number of steps, described below, as provided by Gelatine Manufacturers of Europe (GME) (Vermeulen, 2023b by email on 31 October 2023):

- After the crushing of the bones, they are treated with hot water to separate the chipped bone from an emulsion of fat and small particles. The turbulent mixing of the bones with hot water and the sliding and rubbing of the crushed bone loosens meat and other soft tissues from the bones. All these impurities are removed from the bones by gravity or centrifugation. The wet bone chips are then dried with hot air in a rotating drier. The sliding and rubbing of the chips in the drier help the removal of any remaining meat or other soft tissue. The smaller particles are separated from the dried degreased bone chips by sieving and the light particles are separated by gravity.
- The conditions for the degreasing depend on the size and design of the equipment and the production rate. The temperature of the hot water can range from 75°C to 90°C and the degreasing time from 15 to 30 min, depending on the input flow and the bone/water ratios. The temperature of the chips will not normally exceed 85°C, although the air entering the dryer can be injected at over 400°C. The drying time can range from 20 to 60 min. The amount of dried degreased bone chips obtained from 1 kg of crushed fresh bone is usually about 200 g (20%). The water content of dried degreased bone chips is ~8%, the fat content is <3% and the ash content ~63%.

The purpose of the degreasing process is to remove any soft tissues (including bone marrow, brain, spinal cord and dorsal root ganglia (DRG), that may contain infectivity) that could be adhered to or contained within the bones. It should be noted that this step would lower the total amount of any infectivity present in the substrate through removal of the infective tissues, not by reducing prion infectivity per se.

The fat obtained as a by-product of this degreasing process can be used in several applications. Some of the fat, if the equipment allows it (stainless steel pipes, etc.), can be used in food. If the equipment is not suited for food production, then the fat is either sold to the feed industry, oleochemical companies who will produce glycerine and fatty acids, or to the biofuel industry (biodiesel) (Vermeulen, 2023b by email on 31 October 2023).

The flesh removed during the degreasing becomes PAP (Processed Animal Protein), once it meets the requirements set out in Annex X of Commission Regulation (EU) No 142/2011. Most of the obtained PAP is sold to the pet food industry (Vermeulen, 2023b by email on 31 October 2023).

After degreasing, the bone chips are demineralised using dilute hydrochloric acid (at a minimum concentration of 4% and pH < 1.5) over a period of at least 2 days. Bone chips are then pretreated with acid or alkali before the gelatine is extracted using water, then filtered and sterilised. The ossein obtained from this process can additionally be immersed for 2 h in a solution of 0.3 M sodium hydroxide (NaOH) at pH 13 at room temperature (SSC, 2002).

The acid pretreatment involves the use of acidic solutions (sulfuric or phosphoric acid, pH 2–3.5, 12–24 h), with subsequent washing steps to remove the acid (EFSA BIOHAZ Panel, 2006b; Grobben et al., 2004). The alkaline pretreatment commonly uses a saturated lime solution (pH > 12, 20–60 days) (EFSA BIOHAZ Panel, 2006b; Grobben et al., 2004) or, alternatively, NaOH (pH 13.5, 5–7 days) (SSC, 2002). The solution is replaced regularly and, from time to time, air is pumped into the mixture to prevent the growth of anaerobic microorganisms. At the end, the solution is first treated with sulfuric acid (H_2SO_4) to remove the lime and further washed with water to remove the acid (EFSA BIOHAZ Panel, 2006b; Grobben et al., 2004).

To extract the gelatine from the material pretreated as described above, the material is mixed with hot water in a multistage process. The temperature of the water rises with each extraction step until the residue is boiled to obtain the remaining gelatine. The final extract contains about 5% gelatine. Coarse particles are removed from the gelatine solution via filtration through diatomaceous earth or special filters. Salts are removed by deionisation (ion exchange with a cation and anion exchanger). To concentrate the gelatine, water is extracted by means of vacuum evaporation. The gelatine extract is then sterilised at ultra-high temperature (138–140°C) for 4 s, and quickly cooled to obtain a gel that is finally dried in a stream of warm air. Depending on the manufacturing process, there are different types of gelatine (type A, manufactured through acid processing; type B, manufactured through alkaline processing).

3.3.2 | Trade of gelatine

3.3.2.1 | *World production and trade*

In 2007, the worldwide production of gelatine was about 326,000 tons. The worldwide demand for gelatine doubled to 620,000 tons in 2019 and the scale of the gelatine trade keeps growing year by year. About 46% of raw materials for the production of gelatine come from porcine skin, 29.4% and 23.1% come from bovine hides and bones, respectively, while about 1.5% come from other sources as fish, poultry, etc. (Karim & Bhat, 2009; Sultana et al., 2018). The world production of gelatine is distributed between Europe and America, with 78% of the total production, and the remaining 22% produced mainly by China, India, Russia and Pakistan (Alipal et al., 2021).

3.3.2.2 | *European production*

There are 120,000–125,000 tons of gelatine and collagen peptides produced in Europe (Vermeulen, 2023b by email on 31 October 2023), with the volumes varying from year to year due to the availability of raw materials. Only hides, skins and

bones from carcasses that have been found fit for human consumption, complying with the Regulation (EC) No 853/2004 for the production of gelatine for food, are used. Pig skin and bovine hides, as well as pig and bovine bones, are processed separately. To ensure full traceability, gelatine is produced in batches.

There is limited availability of raw materials in the EU for the production of C&G and, currently, not all collagen and gelatine sold in the EU is produced with raw materials from animals slaughtered in the EU. Gelatine producers in Europe use dried bone chips as raw material, produced by intermediate degreasing units and these are not available in every MS. A total of 31,250 tonnes of raw material for the production of gelatine were imported into the EU in 2022 from third countries, mainly from Brazil, Turkey, Switzerland, United Kingdom, Argentina, China and India (TRACES), with an internal trade within the EU of 87,592 tonnes.

Additionally, gelatine and collagen are imported into the EU. For example, approximately 22,030 tons of gelatine produced with mammalian material was imported into the EU in 2019 (Juschus, 2020 by email on 16 January 2020). As mentioned in Section 3.2, there are specific legal requirements in place in the EU to ensure that collagen and gelatine raw materials and final products from third countries are safe.

3.4 | BSE infectivity

3.4.1 | The concept of prion infectivity and the use of $CoID_{50}$ in the context of RA

It is well accepted that prion infectious particles consist of aggregated misfolded PrP (PrP^{Sc}). However, the molecular mechanisms of prion replication and the ultrastructure of infectious particles are still not fully understood.

In vivo, prion infectivity has been associated with PrP^{Sc} aggregates of variable size. Low-resolution structural studies such as sedimentation velocity (SV), size exclusion chromatography and asymmetric fast-flow-field fractionation have been carried out to characterise the nature of infectious particles. They revealed the existence of a broad size spectrum of infectious PrP^{Sc} aggregates, whose size varies from a few to hundreds PrP^{Sc} molecules, in the brain of terminally sick animals solubilised in specific conditions (Silveira et al., 2005; Tixador et al., 2010). These PrP^{Sc} assemblies showed structural heterogeneity (Igel et al., 2023). Further studies showed that the size distribution of the PrP^{Sc} aggregates population varies according to the strain and host species (Cortez et al., 2021; Laferrière et al., 2013; Tixador et al., 2010). Very little is known about the structural features of BSE infectious particles. A single study reported that L-BSE prions purified from brains of transgenic mice expressing bovine prion protein are mainly composed of long fibrils with one- and two-protofilament morphologies, but also include small non-fibrillar particles, such as amorphous aggregates and two-dimensional crystals (Kamali-Jamil et al., 2021).

Infectious large PrP^{Sc} fibrils from rodent-adapted scrapie strains have now been resolved at high resolution (Caughey et al., 2022; Manka et al., 2022). However, in some prion diseases, only small oligomers with uncharacterised structure are observed in infectious purified preparations (Vanni et al., 2020), thus suggesting that infectivity may be associated with PrP^{Sc} particles of different structures.

Small particles might have greater infectious power than large size PrP^{Sc} multimers (Silveira et al., 2005; Tixador et al., 2010) and, upon sonication, large particles can be broken into smaller infectious particles, resulting in increased infectivity (Terry et al., 2016). By using a protocol of dilution and solubilisation, large particles from seven different rodent-adapted prion strains were disaggregated into small size PrP^{Sc} oligomers (two to four PrP^{Sc} monomers) that retained strain-specific infectious properties (Bohl et al., 2023).

The current gaps in the understanding of the nature of prion infectious particles and the molecular mechanisms of prion replication affect some important aspects of this risk assessment, as: (i) it is not possible to correlate the risk of disease transmission with exposure to a defined number of individual 'infectious particles'; (ii) it is not possible to incorporate into the risk assessment the size distribution of the population of PrP^{Sc} aggregates, as well as their modification during the production of collagen and gelatine (breakage of large aggregates, polymerisation/depolymerisation). In the present risk assessment, no change in the infectious properties of PrP^{Sc} particles was assumed as a realistic scenario.

Due to the uncertainties mentioned above, it is not possible to directly measure the amount of prions in a tissue or product (e.g. particle count or similar). Operationally, the reference method to quantify prion infectivity is approached by titration, i.e. its capacity to transmit the disease (generally by end-point titration) in animal models. A range of inoculation routes can be used (intracerebral, intravenous, oral, intraperitoneal), with by far the most common approach being the bioassay of a sample $\log_{10}$ dilution series by the intracerebral (ic) route, given that it is generally the most efficient and fastest. The attack rates in the different challenge groups allow extrapolation to calculate the dose that causes disease in 50% of recipient animals (the ID_{50}) and, thus, to estimate the amount of infectivity in the tested tissue in terms of the ID_{50}.

In a TSE context, the ID_{50} concept is an operational definition meaning the dose that will establish infection with disease in 50% of the challenged animals. Infection, meaning evidence of replication of the agent, can be indirectly identified by clinical, pathological or bioassay methods (SSC, 2000b). The response to a particular agent strain depends not only on the dose but also on the species, the route of infection and, where relevant, other factors such as the breed or strain of the host (assumed to be related to the PRNP gene sequence of the donor, recipient species and other unknown host factors) (SSC, 2000b).

The ID_{50} is relative to the reported model and inoculation route, i.e. it varies with the recipient species and route of administration (e.g. a BSE brain homogenate will have a different ID_{50} if tested in mice or cattle, or if inoculated by the oral

or intracerebral route). This is important because, for practical reasons, most studies to determine infectivity in tissues from animals with BSE have been carried out by intracerebral inoculation in mice, and the IC ID_{50} values thus determined cannot be used directly to estimate risk in other species. Experiments with the same infectious material inoculated into different species and by different routes of administration allow comparative relationships to be established. A comparison of the titration of BSE infectivity in RIII mice and in transgenic mice overexpressing bovine PrP (Tgbov XV) showed that Tgbov XV mice are approximately $10^{4.4}$ times more sensitive to BSE infection than conventional RIII mice (Buschmann & Groschup, 2005). A comparison has also been made between cattle and RIII mice showing that 1 ID_{50} ic/ip (intraperitoneal) in RIII mice is equivalent to $10^{2.7}$ ic ID_{50} in cattle (Hawkins et al., 2000). An end-point titration of the same inoculum was performed in cattle by the oral route using serial dilutions of brain homogenate (ranging from 100 g to 1 mg) (Konold et al., 2012; Wells et al., 2007). The study showed that one cattle oral ID_{50} ($CoID_{50}$), the dose estimate at which 50% of orally inoculated cattle would be clinically affected, was equal to 150 mg of that brain homogenate, and that BSE transmission in cattle could be produced by oral exposure to as little as 1 mg of the same brain homogenate (≤ 100.4 RIII mouse i.c./i.p. ID_{50} units). It has also been shown that, under the experimental conditions used, one bovine oral ID_{50} ($CoID_{50}$) was approximatively equivalent to $10^{5.4}$ bovine IC ID_{50} or $10^{2.7}$ RIII mouse ic/ip ID_{50}.

These correlations allow the conversion of the IC_{50} measured in rodent models into $CoID_{50}$ for the purposes of this RA. It is important to take into consideration that these assumptions are based on a limited number of experiments and measurements and that bioassay titrations have an intrinsically limited accuracy.

3.4.2 | Tissue distribution and calculation of infectious titre in bovine animals

The parameters for TSE infectivity in bovine material used in this RA are based on the same data and assumptions used in the most recent opinion on collagen and gelatine (EFSA BIOHAZ Panel, 2020) and are summarised here for ease of reference.

The limited data on tissue positivity are generally based on the presence/absence of detectable PrP^{Sc} rather than the direct demonstration of infectivity. When infectivity has been directly measured, this too tends to be a single assay to establish the presence/absence of infectivity rather than a quantification of infectivity using an end-point titration, although an estimation of titre can be calculated from such data (for full discussion of such methods, see EFSA BIOHAZ Panel, 2014a). Where infectivity titres have been established/estimated, these titres are not absolute, because they are specific for the model used (e.g. conventional mice, cattle or bovinised transgenic mice), but some parallel titration studies have enabled conversion factors to be estimated (see Section 3.4.3). Failure to detect infectivity in a bioassay means that the tissue being tested is either negative, or that infectivity is below the limit of detection of the model. Agent distribution and detectable levels of infectivity vary substantially depending on the BSE strain, the tissue and the stage of disease incubation, but for the purposes of this RA all estimates are based on end-stage disease in which tissue distribution and infectivity levels are maximal.

The known BSE agents consistently replicate and accumulate in the central nervous system (CNS) and can be detected in the nerves of the peripheral (autonomic and motor) nervous system, including muscle spindles and autonomic ganglia.

In C-BSE, in addition to the nervous system involvement, accumulation of disease-associated PrP is detectable in structures associated with the lymphatic system, notably the gut-associated lymphoid tissue (GALT) of the digestive tract. These tissues are currently listed as specified risk materials, and closely regulated (the specified risk materials (SRM) in Regulation (EC) No 999/2001).

The current lack of information on the possible presence or distribution of infectivity in tissues of H- or L-BSE-infected cattle does not allow judgement of whether the current list of bovine SRM is also appropriate for these cases. Where data exist from both field cases and experimental animals (i.e. for L-BSE only), there is good agreement of the data on abnormal PrP distribution (see EFSA BIOHAZ Panel, 2014a, for review), but there are no quantitative data for infectivity. There are no data for field cases of H-BSE. In both H-BSE and L-BSE, disease-related PrP accumulation has been reported consistently in CNS tissues, peripheral ganglia and nerves, muscles (predominantly the muscle spindles), adrenal glands and retina. In contrast to C-BSE, no lymphoid tissues or gastrointestinal tissues from H-BSE- or L-BSE-affected animals have tested positive for the presence of disease-specific PrP or infectivity. However, as the available data are limited, an assumption has been made for the purposes of this risk assessment that tissue infectivity is the same for all three known strains of BSE.

The number of studies providing data on potential tissue infectivity in bovine bones is very small, all related to C-BSE specifically, and pre-date transgenic mouse assays. Most data come from a small number of natural cases and oral challenge pathogenesis studies (Wells et al., 1998, 1999), in which tissues harvested at various time points post challenge were pooled for bioassay in conventional mice (Arnold et al., 2009; Wells et al., 1998, 1999). Infectivity was reported in sternal bone marrow from animals near the clinical end-point. Subsequent bioassays in cattle of tissue from these same experimentally challenged animals did not identify infectivity in bone marrow (Sohn et al., 2009).

In a different pathogenesis study (Hoffmann et al., 2007), bioassay was not used to test for infectivity, but immunohistochemistry and western blotting were applied to look for PrP^{Sc} accumulation as a marker of disease. Bone marrow was negative. In natural disease, no infectivity has been detected in bone marrow (SSC, 2002). Compact bone has never been tested. There are no data available for infectivity in bones for either H- or L-BSE.

These largely negative results were interpreted as representing maximum hypothetical levels of infectivity of less than 0.1 cattle intracerebral (ic) ID_{50}/g, which is beyond the end point of detection of the assays used. Due to the small group size of four animals in the study that yielded the single positive result, this estimate must be regarded as an upper limit that

equates to 10^{-6} $CoID_{50}$/g (Sohn et al., 2009). In the context of this risk assessment, the amount of infectivity in bone marrow included in the probabilistic model has been fixed at $10^{-11.3}$ $CoID_{50}$/g, after the correction of the transformation of ID_{50} ic into ID_{50} oral assuming that these calculated maximum infectivity levels represent a hypothetical worst-case scenario.

3.4.3 | Tissue distribution and calculation of infectious titre in small ruminants

A total of only two field cases of classical BSE in goats have been reported through EU surveillance programmes (Eloit et al., 2005; Spiropoulos et al., 2011), and there are no reported cases of ovine BSE. However, experimental challenges of sheep with C-BSE and L-BSE have resulted in disease, confirming that ovine BSE is biologically plausible, and presents with the same tissue distribution as scrapie, with disease-associated PrP detected in the central nervous system (CNS), and peripheral lymphoreticular tissues (LRT) (Bellworthy, Hawkins, et al., 2005; Simmons et al., 2016). However, there is almost no field or experimental data that allows the quantification of prion infectivity in the central nervous system (CNS) of small ruminants affected with BSE. There are no data available for C-BSE infected goats, and only one study carried out in orally challenged sheep (with C-BSE adapted to ovine), described by Zerr (2022), and raw unpublished data available (Andreoletti, 2023, 2024 by email, 25 November 2023, and Andreoletti (2024) 3 February 2024 [See Section 3.4.3.1 and Appendix B]). These data were considered relevant for the estimation of the BSE infectivity in sheep tissues used in the probabilistic model (parameter P8 of the hypothetical ovine model described below in Section 3.6.2).

3.4.3.1 | *C-BSE agent quantification in the central nervous system of affected sheep*

In the above yet unpublished study (See Appendix B for details), 24-h-old lambs of susceptible genotypes were exposed to ovine adapted C-BSE infected brain through natural suckling, using an established dosing protocol (Ryder et al., 2009; Simmons et al., 2011). Some challenged animals were killed and sampled at predetermined preclinical time points, while others ($n = 5$) were allowed to develop clinical signs before being killed. It is data from these clinical end-stage animals that are considered relevant for this opinion.

At necropsy, the brain and spinal cord, and a panel of lymphoid tissues, were collected, and ovine BSE confirmed by western blot (WB) demonstrating the presence of PK-resistant abnormal prion protein (PrP^{Sc}) with a banding profile typical of experimental ovine BSE in the brainstem of each animal.

The replicative capacity of prions can be measured by in vitro amplification techniques and the values obtained can be converted in infectivity values by parallel in vitro and in vivo titration of reference material.

Protein misfolding cyclic amplification (PMCA) is a methodology that mimics prion replication in vitro, but in an accelerated form, allowing amplification of minute amounts of PrP^{Sc} and prion infectivity (Saborio et al., 2001). A PMCA protocol that was specifically optimised for the detection of C-BSE agent in sheep (Huor et al., 2019) was used to measure the levels of prion seeding activity in different CNS areas (obex, cervical spinal cord, thoracic spinal cord and lumbar spinal cord), collected from these C-BSE orally challenged animals at the clinical stage of the disease. Ten-fold dilution series of 10% homogenates from the collected samples were prepared and subjected to two rounds of PMCA. Six individual replicates of each sample and each dilution were tested. The presence of PrP^{res} in each amplification product was established by western blot. These results were then used to estimate the seeding activity (SA_{50}/g of tissue) in the original sample using the application of the Spearman–Karber approach for the optimal design of an LD_{50} bioassay (Markus et al., 1995).

The seeding activity titres as measured by PMCA (as SA_{50}/g of tissue) can then be converted to Cattle Oral ID_{50} ($CoID_{50}$) by comparing parallel end-point titration data of a reference ovine BSE inoculum with bioassay in TgBov mice (See Appendix B for details). This comparison indicated that the PMCA protocol used was ~1500 times more sensitive (3.18 log_{10}) than bioassay in TgBov mice for the detection of ovine C-BSE prions.

The conversion of ID_{50} ic in TgBov into ID_{50} ic in Cattle (i.e. hosts with homologous PrP) can then be undertaken using the same principle of parallel titrations of reference inoculum. Several such studies (See Appendix B) in a range of species result in a conversion factor of 1, so for this study 1 ID_{50} ic as measured in bovine PrP over-expressing mice (TgBov) is considered equivalent to 1 ID_{50} ic as measured in cattle. The final conversion of $CicID_{50}$ to $CoID_{50}$ is described elsewhere (see Section 3.4.1).

When considered together these elements indicate that 1 $CoID_{50}$ can be considered to be equivalent to $10^{8,68}$ SA_{50} as measured by PMCA.

3.4.3.2 | *Estimation of infectious titres ($CoID_{50}$) in CNS of C-BSE affected sheep*

Based on these SA_{50} to $CoID_{50}$ conversion factors, the infectious titres in the different CNS samples collected in the C-BSE affected sheep can be estimated as the following (in $CoID_{50}$ per gram of tissue) (Table 1). These are the values used to calculate P8 in the ovine model (see Section 3.6.2.2).

TABLE 1 Estimated titre of BSE infectivity expressed as $\log_{10}$ of $CoID_{50}$/g after correction factor.

	ARQ/ARQ clinical			ARR/ARR clinical	
	Sheep 1	**Sheep 2**	**Sheep 3**	**Sheep 4**	**Sheep 5**
Obex	4.29	2.45	3.12	2.95	3.62
Cervical spinal cord	1.95	2.29	0.45	1.62	−0.38
Thoracic spinal cord	0.79	3.45	2.79	3.79	2.12
Lumbar spinal cord	1.79	3.62	1.29	2.45	0.45

3.5 | Inactivation of prions during the processing of raw materials for the production of C&G

TSE inactivation studies were conducted in the early 2000s and, as a result, subsequent risk assessments could use more adequate data. In an opinion produced by the SSC in 2003 on the safety with regard to TSE risks of gelatine derived from ruminant bones or hides, it is stated that '*the production processes (steps) reported on in the attached report of the TSE/BSE ad hoc Group have a TSE infectivity inactivation capacity exceeding 4.5 logs. This is considered to be sufficient for the production of safe gelatine, provided they are applied in combination with appropriate sourcing of animals and raw materials*'. It is further stated that *'The SSC considers that the filtering, ion-exchange and UHT sterilisation (at least 138°C during 4 seconds) steps at the end of the production chain do have a TSE infectivity reduction capacity. However, at this moment it is impossible to quantify the additional TSE infectivity reduction within the overall production process, even though it has been calculated to be higher than 10^2 for the acid, manufacturing process and 10^1 for alkaline process' (*SSC, 2003*)*. Likewise, the Food and Drug Administration (FDA) of the USA, in 2003, commented on the safety aspects of collagen and gelatine that *'the data obtained from these new studies show that the reduction in BSE infectivity is sufficient to protect human health'* (Schrieber & Gareis, 2007).

At the peak of the C-BSE crisis, several laboratory studies were conducted to estimate the prion infectivity reduction factors (RF) attained by specific treatments used in the industrial production of gelatine and collagen. Together, the results of these studies (described in full in EFSA BIOHAZ Panel, 2020, and summarised briefly below for ease of reference) supported the general contention that the global industrial processes used to produce collagen/gelatine have the capacity to reduce prion infectivity that could be present in the raw materials. It should be noted that these studies looked at the prion reduction achievable by the commercial production steps and did not propose any additional methodology specific for this purpose. The only steps in the commercial production of collagen and gelatine with the specific aim of reducing prion infectivity are the restrictions placed on the raw material used (e.g. the exclusion of bovine skull and spinal column from raw material) as specified in EC regulations Nos 853/2004 and 142/2011.

The data available on the effects on BSE titre of acidic or alkaline treatments, and other methods that may be used in the production of C&G (such as degreasing, and heat treatment), are presented in Table 2 (which has been copied directly from the 2020 opinion for ease of reference).

TABLE 2 TSE inactivation during treatment processes for the production of gelatine.

Acidic treatments					
Process	**TSE strain**	**Matrix**	**Bioassay model**	**$\log_{10}$ reduction**	**Source**
Acid treatment	ME7	Brain	Not reported	1.17	SSC (2000a) [Inveresk Research International 1998b[a]]
Acid treatment	301V	Bone and spinal cord	Not reported	3.7	SSC (2001) [Grobben, 2001[a]]
Acid process after extraction (including degreasing and demineralisation)	301V	Bone	Not reported	2.6	Grobben et al. (2004)
Acid process (including Filtration, Ion-Exchange and UHT sterilisation)	301V	Bone	Not reported	≥ 4.8[b]	Grobben et al. (2004)

TABLE 2 (Continued)

Alkaline treatments					
Process	**TSE strain**	**Matrix**	**Bioassay model**	**log_{10} reduction**	**Source**
Lime Treatment 20d	ME7	Brain	Not reported	2.33	SSC (2000a) [Inveresk Research International 1998b[a]]
Lime Treatment 45d	ME7	Brain	Not reported	2.23	SSC (2000a) [Inveresk Research International 1998b[a]]
Lime Treatment 60d	ME7	Brain	Not reported	2.1	SSC (2000a) [Inveresk Research International 1998b[a]]
Soda alkali treatment (0.25M, 5d, 15°C)	ME7	Brain	Not reported	4.82	SSC (2000a, 2001) [Shepherd, 1999[a]]
Soda alkali treatment (0.3M, 7d, 15°C)	ME7	Brain	Not reported	5.25	SSC (2000a, 2001) [Shepherd, 1999[a]]
Alkaline Treatment	301V	Bone and spinal cord	Not reported	4.2	SSC (2001) [Grobben, 2001[a]]
Alkaline process after extraction (including degreasing and demineralisation)	301V	Bone	Not reported	3.7	Grobben et al. (2004)
Alkaline process (including Filtration, Ion-Exchange and UHT sterilisation)	301V	Bone	Not reported	≥4.9	Grobben et al. (2004)

Acidic and alkaline combinations					
Process	**TSE strain**	**Matrix**	**Bioassay model**	**log_{10} reduction**	**Source**
Combined acid and lime treatment	ME7	Brain	Not reported	2.84	SSC (2000a) [Inveresk Research International 1998c[a]]
Short NaOH treatment after demineralisation in the acid process (0.3 M, 2 h, pH 13)	301V	Bone	Not reported	≥5.4	SSC (2002) [Grobben, 2002[a]]

Heat/pressure treatment					
Process	**TSE strain**	**Matrix**	**Bioassay model**	**log_{10} reduction**	**Source**
133°C/20′/3bar	301V	Bone	Not reported	≥6.6	SSC (2002) [Grobben, 2002[a]]
Heat and pressure process and degreasing	301V	Bone and spinal cord	Not reported	≥6.5	Grobben et al. (2005)
Autoclaving (133°C/20 min′/3bar) and degreasing	301V	Bone	Not reported	4	Grobben et al. (2006)
Degreasing, then heat and pressure	301V	Bone	VM	6.5	Grobben et al. (2005)

Single steps					
Process	**TSE strain**	**Matrix**	**Bioassay model**	**log_{10} reduction**	**Source**
Degreasing	NA[c]	NA	NA	98%–99% removal of infectivity – Indirect assumption based on removal of protein	EFSA BIOHAZ Panel (2005a) [Manzke et al., 1997[a]]
Degreasing	NA	NA	NA	1.5–2 (extrapolated from assumption above)	Grobben et al. (2004)
Degreasing	NA	NA	NA	1.7–2 (extrapolated from assumption above)	Grobben et al. (2006)
Filtering	NA	NA	NA	0.5–1	EFSA BIOHAZ Panel (2005a) [Grobben, 2004 and unpublished results[a]]
Filtration, Ion-Exchange (individual step in gelatine production)	263K	Gelatine extracts (spiked at different points in the process)	Hamster	1.5	SSC (2002, 2003) [Rohwer, 2001[a]]

(Continues)

TABLE 2 (Continued)

Single steps					
Process	**TSE strain**	**Matrix**	**Bioassay model**	**log_{10} reduction**	**Source**
UHT sterilisation (138–140°C, 4 s) (Individual step in gelatine production)	263K	Gelatine extracts (spiked after acid/alkali steps)	Hamster	4	SSC (2002, 2003) [Rohwer, 2001[a]]

[a]References in squared brackets that have been used for the assessments undertaken in the respective opinions were not available for review because they were either provided to the SSC WG confidentially and not meant for public release, or never published.

[b]≥ in this column indicates the limits of detection for the assay that were reported.

[c]NA, not applicable.

These studies all used laboratory-adapted rodent prion strains, derived from naturally occurring scrapie or C-BSE rather than material derived directly from animals with naturally occurring disease. They involved the comparative titration of infectivity, by bioassay, in samples before and after the application of a bench-scale, downsized, protocol of the industrial step being assessed. Some studies were sponsored by the industry and have never been published in peer-reviewed journals, and therefore, detailed methodologies are not consistently available, reducing the opportunities for comparison and scrutiny. Although the principles highlighted below are expected to be valid for bovine C-BSE, the actual effect that the acid/alkaline/autoclaving conditions may have on the reduction of the infectious titre of H- and L-BSE, as compared with C-BSE and ovine-passaged H-, L- and C-BSE, could differ. Indeed, the strains involved in naturally occurring scrapie or BSE can display very different resistance/sensitivity to decontaminating treatments (e.g. autoclaving or chemical inactivation), so the resistance/sensitivity of C-BSE, H-BSE and L-BSE prion strains in cattle to the inactivation treatments used for C&G production may vary substantially from that observed using rodent adapted strains.

The gelatine production process steps that can provide a potential reduction in prion infectivity in the final product are:

- Washing with detergents, degreasing and demineralisation of raw bones;
- Strong alkaline/acidic treatment;
- Extraction by mixing with hot water in a multistage process;
- Purification and concentration via filtration of raw gelatine using diatomaceous earth, or other special filters (e.g. perlite), and salt removal by anion/cation exchange resins.
- Ultra-high temperature (UHT) sterilisation of the final product.

The details of the results presented in the SSC update opinion (Grobben, Taylor, & Steele, 2001 in SSC, 2001b) showed that UHT sterilisation inactivates approximately 4 log_{10} ID_{50} of scrapie infectivity in 4 s exposure to 138–140°C; filtration and ion-exchange remove approximately 1.5 log_{10} ID_{50} infectivity, both removals being achieved by mechanical trapping. There are no data on the additive effect of the reduction capacity of the gelatine purification and sterilisation steps. Nevertheless, it is acknowledged that the inactivation would be more than 5 log_{10} ID_{50} of TSE infectivity.

It cannot be assumed that the log_{10} reductions achieved by each step individually can be directly added together to estimate a total reduction factor (RF). Changes to the physicochemical composition of the infectious agent might occur during individual steps and could impact positively or negatively on the effectiveness of subsequent processes.

The preliminary step in bone preparation – the crushing and degreasing – becomes key to any change in the amount of infectivity potentially present in a batch of commercial gelatine made from bovine or small ruminant bones which have not been subject to any selection based on TSE status or regulations. The capacity of this step to remove the prion infectivity that might be associated with raw crushed bones has never been directly assessed. However, degreasing reduced the amount of several brain-specific proteins by 98%–99% (Manzke et al., 1997, as cited by Grobben et al., 2004), and the final dried bone chips are required to have a fat content of no more than 1%, which is equivalent to a 2 log_{10} reduction. Therefore, degreasing is likely to reduce the level of infectivity that could be associated with infective central nervous system (CNS) or DRG (dorsal root ganglia) associated with raw bone material by up to 2 log_{10}. However, this infectivity is not destroyed, it is merely displaced into the 'waste' streams, which are out of the scope of this opinion.

3.6 | QRA of the residual BSE infectivity in gelatine

3.6.1 | Bovine model

3.6.1.1 | *Model structure*

A probabilistic model was developed to estimate the BSE infectivity load, measured in $CoID_{50}$, contained in the gelatine produced with the bones of one adult bovine animal older than 30 months of age infected with any of the three BSE strains (C, H and L) (subsequently referred to as 'BSE') and at the clinical stage of the disease.

The objective of the model was to assess a worst-case scenario (WCS) in which one BSE-infected animal at the clinical stage bypasses controls with bones used to produce gelatine. The assumption is made that all the bones from the BSE-infected bovine animal are used to produce gelatine in the same batch, including the skull with full brain and vertebral column with associated tissues.

Collagen is not currently made from bones, at least in Europe, so there is no accurate corresponding data on the production method or yield of collagen from bones or impact on any infectivity present. Therefore, it was decided to develop the model focused only on the production of gelatine from bones.

To address the terms of reference for this opinion, the input raw materials were not limited to food-grade material, or those materials classified as Category 3 Animal By-Products. Instead, two different outputs are estimated, based on the residual infectivity measured in $CoID_{50}$, at the end point of gelatine production using all the bones from an infected bovine animal. In the case of the bovine model:

- P23 (AQ2): Total number of $CoID_{50}$ in the gelatine produced with the bones of one BSE-infected bovine animal after acidic treatment.
- P24 (AQ4): Number of $CoID_{50}$/kg of gelatine in a small size batch that contains the bones of one BSE-infected bovine animal.

3.6.1.2 | *Model assumptions and parameters*

The description of the parameters in the bovine model, including the units, the distributions used, the references from which the estimates were calculated and explanations on the sources of data and equations, are given in Table 3. The model not only represents the worst-case scenario in that all the bones, including the skull with full brain and vertebral column with associated tissues, from the BSE-infected bovine animal, are used to produce gelatine, but also some maximum values have been applied when parameterising the model. The model assumptions are as follows:

- The starting point of the model is one adult animal (> 30 months of age) infected with any of the three BSE strains (C, H and L) and at clinical stage.
- All the bones (including those bones of highest risk classified as SRM) are used to produce one batch of gelatine.
- The very limited data available suggest that the infectivity load and tissue distribution in H-BSE and L-BSE may not be as widespread as in C-BSE. In the absence of any data on tissue infectivity in atypical BSE, the WCS applied is that infectivity in the tissues would be the same as in classical BSE and with the same tissue distribution, even though there is no evidence of lymphoid involvement in these disease variants. This assumption might additionally mask possible differences in resistance/sensitivity to inactivation treatments of the BSE strains.
- The infectivity contained in raw skeletal bones comes from the intrinsic infectivity contained in the target tissues. Due to the very limited presence of nervous or lymphatic tissues in compact bone, it has been assumed that any intrinsic infectivity related to skeletal material would be in the bone marrow.
- The infectivity contained in the skull is restricted to the brain. Other lymphoid tissues in the head: tonsil, retropharyngeal lymph nodes, sub-maxillary lymph nodes, parotid lymph node, etc., are assumed to be removed during the harvesting of the head, tongue, etc. at slaughter or during further processing. Nevertheless, the lymphoreticular system (LRS) of BSE-affected cattle was found to be generally free of detectable PrP^{Sc} and infectivity, with the exception of the tonsils, the Peyer's patches of the gut and the mesenteric lymph node (Balkema-Buschmann et al., 2019).
- The entire target tissues are assumed to remain attached to the bones (WCS), not being reduced by any cross-contamination to adjacent carcasses during dressing at slaughter.
- Despite the lack of infectivity detected in bone marrow, it is assumed that bone marrow contains a maximum level of homogeneously distributed BSE infectivity corresponding to the limits of detection of the bioassays at the time these tissues were investigated.
- All the BSE infectivity in contaminating tissues, i.e. in the brain, the spinal cord and the dorsal root ganglia (DRG) and trigeminal ganglia (TG), is homogeneously distributed and included in the total amount of bone material used to produce gelatine.
- The lowest standard batch size, as provided by the industry, is used to parameterise the model resulting with the highest concentration of infectivity per kg of gelatine of the standard batch.
- The processing of bones to produce gelatine follows an initial pretreatment which comprises the crushing, degreasing, washing and drying of the raw bones to remove fat and other soft tissues, water, etc., to constitute dry bone chips, which are then subject to demineralisation followed by acidic and/or alkaline pretreatment. Because the acidic pretreatment is reported to be more common and results in a lower reduction factor compared to the alkaline treatment, it is assumed that only the acidic treatment for the bones reduces infectivity as a WCS.
- The removal of fat during degreasing is a necessary step in the production of dry bone chips. As stated in EFSA BIOHAZ Panel (2020), '*the capacity of this step to remove the prion infectivity that might be associated with raw crushed bones has never been directly assessed. However, degreasing reduced the amount of several brain-specific proteins by 98–99% (*Manzke et al., 1997*), and the final dried bone chips are required to have a fat content of no more than 1%*'. Since the source material under consideration in the model in EFSA BIOHAZ Panel (2020) did not include the skull or vertebral column or their contents in some of the scenarios modelled, it did not consider any reduction of infectivity during degreasing, although

it was stated that *'degreasing is likely to reduce the level of infectivity that could be associated with CNS or DRG contamination of raw bone material by up to 2 log_{10}'*. In this case, it was decided to model the reduction, as a reduction of the fat content, containing proteins from infectious tissues, and not as a reduction of the BSE infectivity measured in log_{10}.

- For all the other processes in the production of gelatine, such as washing, demineralisation, purification and concentration via filtration, salt removal or UHT sterilisation, it was considered they do not have an impact on BSE infectivity in the model, as another WCS. However, it is acknowledged that other pretreatments of the raw material or purification steps of the gelatine production process can contribute to further reduction of the TSE infectivity. For example, as stated in the 2020 EFSA BIOHAZ Panel opinion, the UHT treatment applied for the sterilisation of the gelatine extract was estimated to provide a higher than 2.2 log_{10} reduction. The ultrafiltration and nanofiltration were shown to provide reduction of TSE infectivity, variable according to the type of filter used.
- For the calculation of the number of $CoID_{50}$/kg of gelatine in a small batch of gelatine that contains the bones of one BSE-infected bovine animal, it was assumed that all the residual BSE infectivity in gelatine is homogeneously distributed in the batch. According to the industry, the size of a batch can range between 755 and 8048 kg of bovine bones, depending on the equipment, corresponding to the minimum and maximum number of bovine animals that could be included in a single batch of gelatine, respectively.
- The entire batch of gelatine is diverted to cattle feed and is consumed by cattle to the level that the gelatine provides the entire protein requirement in a single day, which represents another WCS.
- Certain parameters have been assigned probability distributions due to the uncertainty around the true average value. With the limited data available, some ranges might also represent variability in all the possible values that the parameter describes. Uncertainty is considered in the model and represented by 5th and 95th percentiles, which indicate the range within which 90% of the results lie. The model was run for 50,000 iterations using Latin Hypercube sampling. Not all uncertainty has been estimated in the calculations, as not all can be quantified. Therefore, the 5th and 95th percentiles describe the amount of quantified uncertainty included in the model. Mean values of the distribution are also reported given the skewness of some of the output distributions of the model.

The framework of the assessment with the principles of the QRA and the link to the assessment questions are visually displayed in Figure 2.

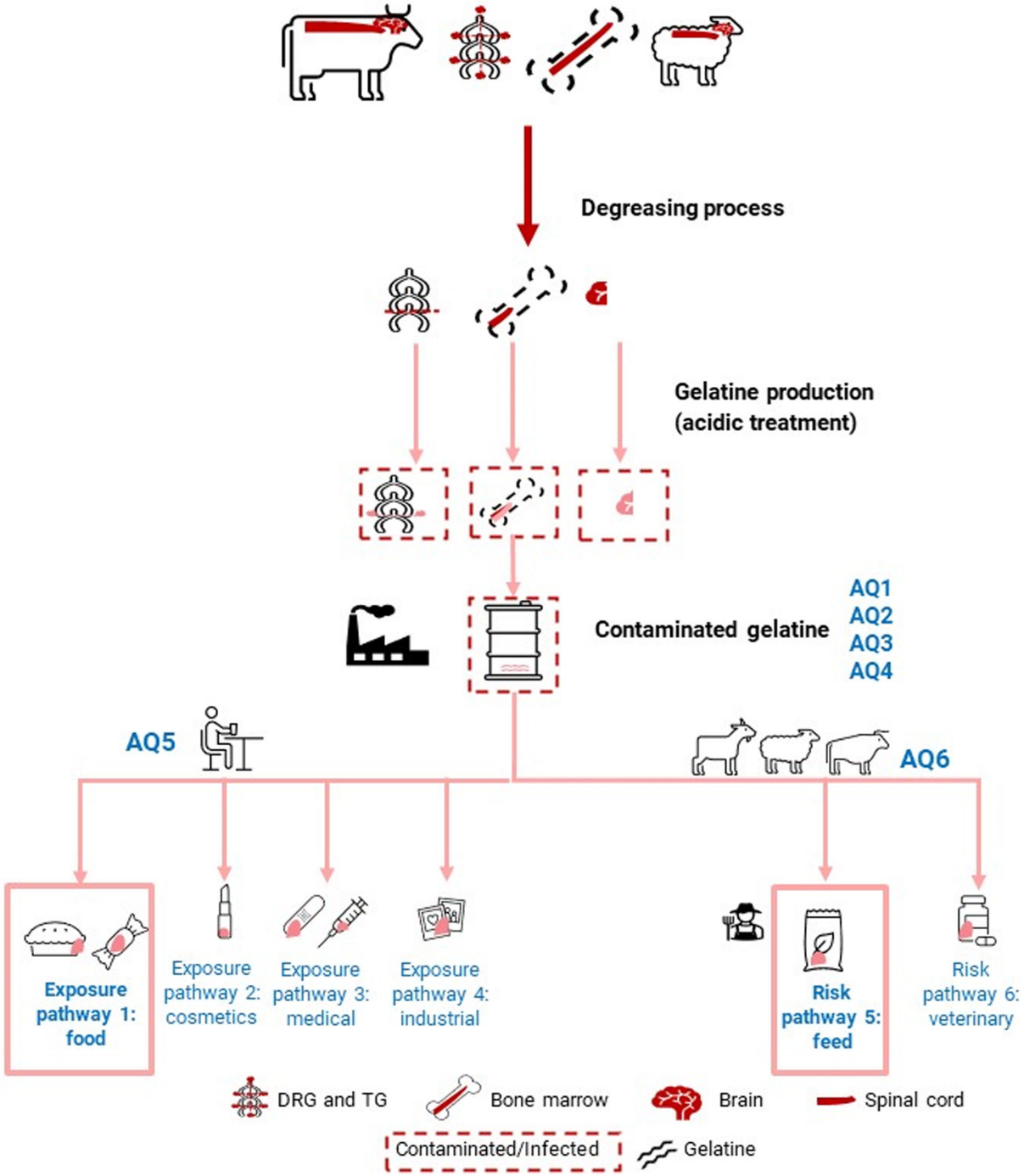

FIGURE 2 Framework of the assessment.

TABLE 3 Summary of all input and output variables of the bovine model, including model notation, the distributions used, the reference(s) from which estimates were calculated and any assumptions that were made.

Parameter	Description	Unit (per individual unless stated otherwise)	Value	Assumptions	References
P1	Weight of the bones of an adult cow	kg	58	58 kg, if no specified risk materials (skull, vertebral column) are removed	EFSA BIOHAZ Panel (2006b)
P2	Weight of the brain of an adult cow	g	Normal (480.54, 7.79)		Ballarin et al. (2016)
P3	Weight of the spinal cord of an adult cow	g	Uniform (200, 306.9)		EFSA BIOHAZ Panel (2005a), Vařechová (2015)
P4	Weight of the bone marrow of an adult cow	g	0.325 × P1 × 1000		EFSA BIOHAZ Panel (2005a)
P5	Weight of CNS-associated nerves and other high-infectivity tissues (trigeminal nerve ganglia (TRG) and dorsal root ganglia (DRG)) of an adult cow	g	50		EFSA BIOHAZ Panel (2005a)
P6	BSE-infectivity in brain, spinal cord and CNS-associated nerves	$CoID_{50}$/g	Lognormal (2.5%, 1.25, 50%, 6.66, 97.5%, 33.3)		EFSA BIOHAZ Panel (2020), Konold et al. (2012)
P7	BSE-infectivity in bone marrow	$CoID_{50}$/g	10^(−11.3)	Limit of detection of the test. 10^(−6) ic route. Transformation into $CoID_{50}$: 1 ID_{50} IC cattle = 10^(−5.3) ID_{50} oral	EFSA BIOHAZ Panel (2020) (Andreoletti, 2023
P8	Reduction in weight of fatty tissues during degreasing	%	Uniform (0.975, 0.99)		EFSA BIOHAZ Panel (2006b, 2020)
P9	Reduction of infectivity due to the acidic processing of bones	Log_{10}	Pert (1, 2.6, 3.7)		EFSA BIOHAZ Panel (2020)
P10	Yield of gelatine from bones	%	Uniform (0.041, 0.045)		EFSA BIOHAZ Panel (2020)
P11	Number of animals whose bones are included in a small size batch of gelatine	No animals	303	Small batch (303 bovine animals)	Vermeulen 2023b, by email on 31 October 2023
P12	Weight of protein replaced by gelatine per animal and meal	Protein kg/meal	0.3	WCS: all crude protein in feed compound via gelatine. See Section 3.9.2.1	
P13	Weight of brain in raw bones of one bovine animal after reduction due to degreasing	g	P2-(P2 × P8)		
P14	Weight of spinal cord in raw bones of one bovine animal after reduction due to degreasing	g	P3-(P3 × P8)		
P15	Weight of bone marrow in raw bones of one bovine animal after reduction due to degreasing	g	P4-(P4 × P8)		
P16	Weight of CNS-associated nerves and other high-infectivity tissues in raw bones of one bovine animal after reduction due to degreasing	g	P5-(P5 × P8)		
P17	Total amount of infectivity in brain	$CoID_{50}$	P6 × P13		

TABLE 3 (Continued)

Parameter	Description	Unit (per individual unless stated otherwise)	Value	Assumptions	References
P18	Total amount of infectivity in spinal cord	$CoID_{50}$	P6×P14		
P19	Total amount of infectivity in bone marrow	$CoID_{50}$	P7×P15		
P20	Total amount of infectivity in CNS-associated nerves and other high-infectivity tissues	$CoID_{50}$	P6×P16		
P21	Total amount of BSE-infectivity before acidic treatment	$CoID_{50}$	P17 + P18 + P19 + P20		
P22	Total amount of BSE-infectivity before acidic treatment (log_{10})	log_{10} of $CoID_{50}$	log_{10} (P21)		
P23	Total number of $CoID_{50}$ in the gelatine produced with the bones of one BSE-infected bovine animal after acidic treatment	$CoID_{50}$	10^(P22-P9)		
P24	Number of $CoID_{50}$/ kg of gelatine in a small size batch that contains the bones of one BSE-infected bovine animal	$CoID_{50}$/kg	P23/(P1×P10×P11)		

3.6.1.3 | *Results and sensitivity analysis*

The results of the bovine model for P23 and P24 with mean, median and 5th and 95th percentiles are displayed in Table 4.

TABLE 4 Mean, median and 5th and 95th percentiles of P23 and P24 of the bovine model.

Parameter	5%	50%	Mean	95%
P23: Total number of $CoID_{50}$ in the gelatine produced with the bones of one BSE-infected bovine animal after acidic treatment	0.025	0.26	0.771	3.075
P24: Number of $CoID_{50}$/kg of gelatine in a small size batch that contains the bones of one BSE-infected bovine animal	3.3×10^{-5}	3.4×10^{-4}	1×10^{-3}	4×10^{-3}

The results of the sensitivity analysis showed that outputs P23 and P24 were strongly affected by the uncertainty associated with, in decreasing order, P9: Reduction of infectivity due to the acidic processing of bones; P6: BSE infectivity in brain, spinal cord, DRG and TRG and CNS-associated nerves; and P8: Reduction in weight of fatty tissues during degreasing. The results of the sensitivity analysis were consistent when applying different methods for the sensitivity analysis: inputs ranked by the effect on the output mean, regression coefficients and correlation coefficients.

The model can be run using the Monte Carlo tool (risk assessment using Monte Carlo) of the EFSA's Shiny proxy open-source platform, at the following link (it requires registration): https://efsab2c.b2clogin.com/efsab2c.onmicrosoft.com/b2c_1_signin/oauth2/v2.0/authorize?response_type=code&client_id=be616b14-895a-4257-9e38-b503da802ba3&scope=be616b14-895a-4257-9e38-b503da802ba3%20openid%20email&state=lNFXOR16Kks2PYYz-rM0QmNFhjqtsgUwSdTXjX3L6MA%3D&redirect_uri=https://r4eu.efsa.europa.eu/login/oauth2/code/shinyproxy&nonce=nnD0hbZQzJ_sYF_7wsWpYBCpwNwIRLlEFFPIH3x2a5A

The two files required to reproduce P23 and P24 of the bovine model can be accessed in the following link: https://doi.org/10.5281/zenodo.12188658

3.6.2 | Hypothetical ovine model

3.6.2.1 | *Model structure and limitations*

As described in Section 3.4.3, data on tissue infectivity do not exist for caprine BSE and are very limited for ovine BSE, so it was not possible to develop a fully parameterised species-specific model for BSE infectivity in gelatine produced with ovine and/or caprine bones.

Nevertheless, a hypothetical model was developed for sheep, in parallel with the bovine model, using the same framework and scenarios, with real ovine data where it was available and cattle data when it was not. In the absence of any species-specific data, this hypothetical model is also used as a proxy for the estimation of the BSE infectivity in goats.

3.6.2.2 | *Model assumptions and parameters*

The model was developed to estimate the BSE infectivity load, measured in $CoID_{50}$, contained in the gelatine produced with the bones of one adult ovine animal infected with any of the three BSE strains (C, H and L) (subsequently referred to as 'BSE') and at the clinical stage of the disease.

The raw input materials were not limited to food grade material or Category 3 ABP, and instead, there are two different estimates based on the residual infectivity measured in $CoID_{50}$, at the end point of gelatine production using all the bones from an infected ovine animal. In the case of the ovine model:

- P25 (AQ1): Total number of $CoID_{50}$ in the gelatine produced with the bones of one BSE-infected ovine animal after acidic treatment.
- P26 (AQ3): Number of $CoID_{50}$/kg of gelatine in a small size batch that contains the bones of one BSE-infected ovine animal.

The description of the parameters in the ovine model, including model notation, the distributions used, the references from which the estimates were calculated and explanations on the sources of data and equations, are given in Table 5.

In addition to the assumptions described for the bovine model, that also apply to the ovine model, there are specific assumptions for the ovine model:

- Since it has been acknowledged that gelatine is not produced with the bones of small ruminants, it is assumed that all of the steps involved in the production of gelatine would be the same for small ruminants that for bovines, regardless of substrate, once the bones are crushed and degreased.

- Regarding the average weight of the bones of an adult sheep, little information is available in the published literature. None of the studies on which the values are based contain direct measurements of the live weight of adult sheep at slaughter and the results are highly dependent on the assumption of a dressing percentage of 40%. Some of the information comes from papers published in the 1980s and 1990s for Australian Merino sheep. This may not provide an accurate estimate of the current bone weights of an adult sheep taking into account the between breed variability and farm management practices. This may lead to an overestimation or an underestimation of the BSE risk from gelatine and collagen derived from ovine or caprine material.
- There are no actual data on the inactivation of BSE strains in sheep tissues during gelatine production. When needed, the parameterisation of the bovine model was applied to the ovine model.
- In relation to BSE infectivity in brain, spinal cord, DRG and TG (parameter P8): the estimates of infectivity using experimental data of C-BSE infectivity in obex, cervical spinal cord, thoracic spinal cord and lumbar spinal cord of ARQ/ARQ and ARR/ARR inoculated sheep at clinical stages (50–54 mpi), as in Table 1 have been used. To estimate the average infectivity in whole brain, with associated uncertainty, a distribution was parameterised by bootstrapping the normal distribution for each individual experimental result available: a combination of animal and tissue (3×4) with units of $\log_{10}$ $CoID_{50}$. The minimum, most likely and maximum values of the bootstrapped normal distribution were used to fit a Pert distribution and transformed into $CoID_{50}$. In brief, five normal distributions were fitted to the available obex readings and 15 for the available spinal cord sample results (five for each cervical, thoracic and lumbar spinal cord). In most animal prion diseases, including C-BSE, the brain stem and particularly the obex region, accumulate the highest levels of infectivity while the spinal cord may have much lower levels of infectivity. When pooling the data, in order to sample all the available CNS values, we included data from the obex and three different spinal cord regions (cervical, thoracic and lumbar). However, to obtain an infectivity value that represents the whole brain rather than just from the obex section, as a direct comparison for the cattle model parameterisation, and to avoid giving extra weight to the higher number of spinal cord sample results, five of the 15 normal distributions were selected randomly to bootstrap together with the five distributions of obex readings. Due to the low number of experimental animals and field cases, it was assumed that despite the variability of genotypes, all five sheep samples can be used as one representative population.
- Since there are no data on the size of a batch of gelatine using small ruminant material, it was assumed the size would be the same as if it was produced with bovine material. For that amount of gelatine, approximately 10 times more ovine animals would be required.

TABLE 5 Summary of all input and output variables of the ovine model, including model notation, the distributions used, the reference(s) from which estimates were calculated and any assumptions that were made.

Code	Description	Unit (per individual unless stated otherwise)	Value	Assumptions	References
P1	Live weight of an adult sheep	kg	Uniform (54.5, 71.8)		AHDB (2019), Matthews et al. (2019)
P2	% of live weight of the bones of an adult sheep	%	Uniform (0.055, 0.063)		Butterfield et al. (1983), Perry et al. (1992)
P3	Weight of the bones of an adult sheep	kg	(P1 × P2) + 1	The bones of the head were not included in P2. It was assumed by the WG that the bones of the head would weigh 1 kg	EFSA WG
P4	Weight of the brain	g	Pert (100, 150, 200)		Adkin et al. (2018)
P5	Weight of the spinal cord	g	Uniform (50, 64)		Adkin et al. (2018)
P6	Weight of bone marrow	g	0.325 × P1 × 1000	Same as per bovine	
P7	Weight of CNS-associated nerves and other high-infectivity tissues	g	Uniform (40, 80)		EFSA BIOHAZ Panel (2010)
P8	BSE infectivity in brain, spinal cord, DRG and TG	$CoID_{50}$/g	10^(Pert (1.47,2.45,3.65))		Huor et al. (2022), Andreoletti, 2023 by email, 25 November 2023, and Andreoletti (2024) 3 February 2024 See section 3.4.3
P9	BSE infectivity in bone marrow	$CoID_{50}$/g	10^(−11.3)	Same as per bovine	
P10	Reduction in weight of fatty tissues during degreasing	%	Uniform (0.975, 0.99)	Same as per bovine	
P11	Reduction of infectivity due to the processing of bones	log_{10}	Pert (1, 2.6, 3.7)	Same as per bovine	
P12	Yield of gelatine from bones	%	Uniform (0.041, 0.045)	Same as per bovine	
P13	Weight of a small size batch of gelatine	kg	755.68	Weight of a small size batch using bovine bones	
P14	Weight of protein replaced by gelatine per animal meal	Protein kg/meal	0.03		
P15	Weight of brain in raw bones of one ovine animal after reduction due to degreasing	g	P4-(P4 × P10)		
P16	Weight of spinal cord in raw bones of one ovine animal after reduction due to degreasing	g	P5-(P5 × P10)		
P17	Weight of bone marrow in raw bones of one ovine animal after reduction due to degreasing	g	P6-(P6 × P10)		
P18	Weight of CNS-associated nerves and other high-infectivity tissues in raw bones of one ovine animal after reduction due to degreasing	g	P7-(P7 × P10)		
P19	Total amount of BSE-infectivity in brain	$CoID_{50}$	P15 × P8		
P20	Total amount of BSE-infectivity spinal cord	$CoID_{50}$	P16 × P8		

TABLE 5 (Continued)

Code	Description	Unit (per individual unless stated otherwise)	Value	Assumptions	References
P21	Total amount of BSE-infectivity in bone marrow	$CoID_{50}$	P17 × P9		
P22	Total amount of BSE-infectivity in NS-associated nerves and other high-infectivity tissues	$CoID_{50}$	P18 × P8		
P23	Total amount of BSE-infectivity before acidic treatment	$CoID_{50}$	P19 + P20 + P21 + P22		
P24	Total amount of BSE-infectivity before acidic treatment (log_{10})	Log_{10} of $CoID_{50}$	Log_{10}(P23)		
P25	Total number of $CoID_{50}$ in the gelatine produced with the bones of one BSE-infected ovine animal after acidic treatment	$CoID_{50}$	10^(P24-P11)		
P26	Number of $CoID_{50}$/kg of gelatine in a small size batch that contains the bones of one BSE-infected ovine animal	$CoID_{50}$/kg	P25/P13		

3.6.2.3 | *Results and sensitivity analysis*

The results of the ovine model for P25 and P26 with mean, median and 5th and 95th percentiles are displayed in Table 6.

TABLE 6 Mean, median and 5th and 95th percentiles of P25 and P26 of the ovine model.

Code and description	5%	50%	Mean	95%
P25: Total number of $CoID_{50}$ in the gelatine produced with the bones of one BSE-infected ovine animal after acidic treatment	0.35	4.06	13.31	55.3
P26: Number of $CoID_{50}$ per kg of a small size batch of gelatine in which the bones of one BSE-infected ovine animal, older than 30 months of age, at the clinical stage of BSE, have been included	4.7×10^{-4}	5.3×10^{-3}	1.7×10^{-2}	7.3×10^{-2}

The results of the sensitivity analysis showed that outputs P25 and P26 were strongly affected by the uncertainty associated with, in decreasing order, P11: Reduction of infectivity due to the processing of bones; followed by P8: BSE infectivity in brain, spinal cord, DRG and TG and P10: Reduction in weight of fatty tissues during degreasing. The results of the sensitivity analysis were consistent applying different methods for the sensitivity analysis: inputs ranked by the effect on the output mean, regression coefficients and correlation coefficients.

The two files required to reproduce P25 and P26 of the ovine model in the Monte Carlo tool (risk assessment using Monte Carlo) of the EFSA's Shiny proxy open source platform can be accessed in the following link: https://doi.org/10.5281/zenodo.12188658

3.7 | Risk characterisation

3.7.1 | The relationship between $CoID_{50}$ and risk

The relationship between BSE infectivity and risk characterisation in different host species remains a challenge in TSE risk assessment today. Various approaches and assumptions have been adopted over the years, despite the lack of a full understanding of the quantitative relationship between PrP^{Sc} particles and infectivity (see Section 3.4), as well as between the amount of infectivity and the probability of infection (see Section 3.7.3). The risk that low dose prion exposure might present to a consumer would then be a combination of the probability of the consumer receiving the aliquot containing the infectivity, and the complex and poorly understood impact of the parameters governing the susceptibility of the individual consumer/exposed host, like the species barrier, age at exposure, maturity of the immune system, *PRNP* genotype, etc. (see Section 3.7.4) (EFSA BIOHAZ Panel, 2015, 2017).

The EFSA opinion on 'quantitative risk assessment of the animal BSE risk posed by meat and bone meal' (EFSA BIOHAZ Panel, 2005c) adopted the approach of the SSC opinion 'Oral exposure of humans to the BSE agent: infective dose and species' (SSC, 2000b), in which, it was recognised that the minimum infectious dose of the BSE agent for humans was not known and that risks from exposure to amounts of infection below the minimal infectious dose could not be determined with the state of the scientific knowledge at the time of the opinion. The SSC assumed a linear dose response curve down to the low dose range, as a 'conservative assumption' because a worst-case assumption would be that there is no threshold dose for prions. So, if a population as a whole is exposed, the SSC suggested using the probability scenario in the BSE context, assuming a linear dose–response curve down to the low dose range. For example, a product containing an evenly distributed residual infectivity of 10^{-3} ID_{50}/g and given to each of 1 million individuals, may result in 500 individuals being infected.

Following this rationale, in EFSA BIOHAZ Panel (2005b), the number of animals being infected, given the above assumptions, could be estimated by multiplying the mean residual exposure level, expressed in $CoID_{50}$ per animal per period of time by the population size exposed, dividing the number obtained by 2 (because the $CoID_{50}$ infects only half of the animals exposed). If this calculation was applied to the EU adult (over 2 years of age) cattle population (45 million individuals), this means that there might be on average 1 infected bovine case of BSE in the EU per period of time when the residual risk is around 10^{-7} $CoID_{50}$/animal/period of time. For this reason, any residual risk equal or higher than 10^{-7} $CoID_{50}$/animal/period of time can be considered to represent a risk of infection in cattle. The calculations assumed a homogeneous distribution of infectivity within a batch, while acknowledging that *'In practice clumping or inadvertent concentration of infectivity might pose a higher risk to an individual although the average risk to a large population would remain the same'*.

A quantitative assessment of the human BSE risk posed by gelatine with respect to residual BSE risk (EFSA BIOHAZ Panel, 2006a) explored 'how can the output of the QRA be interpreted in terms of potential human infections?' Following the same assumption of a linear dose–response curve of infectivity at low doses, the estimated exposure via gelatine was compared to the exposure of the UK population during the period 1980–1996. This opinion concluded that the previous recommendation of not using the skull and vertebrae from bovine animals older than 12 months in the production of gelatine was not supported by the results of the QRA as the relevant exposures are regarded as very small compared to the historical exposure due to meat and meat products in the diet of the UK population. This was based on a worst-case mean

estimate for the human exposure due to gelatine made by the acid or alkali methods from a mixture of bones, including skull (containing brain) and vertebral column (including spinal cord), sourced from cattle from a GBR IV country with unreliable surveillance, of 1.71×10^{-6} $CoID_{50}$ units per person per week. This is equivalent to an annual exposure of 8.9×10^{-5} $CoID_{50}$ units per person but assumes all gelatine in a person's diet is extracted from bovine bones; a more realistic proportion was considered to be 2%–5% of the total intake.

In 2020, EFSA published an opinion on 'potential BSE risk posed by the use of ruminant collagen and gelatine in feed for non-ruminant farmed animals ' (EFSA BIOHAZ Panel, 2020), in which a probabilistic model was developed to estimate the BSE infectivity load, measured in cattle oral ID_{50} ($CoID_{50}$)/kg, in the gelatine produced from the bones and hide of one infected animal older than 30 months with clinical BSE (worst-case scenario). The 'individual risk' was estimated by calculating the 'total risk/ number of individuals exposed', i.e. by dividing the total BSE infectivity contained in the gelatine produced in the entire EU in a single year by the total cattle population (approximately 42 million individuals), and assuming a homogeneous distribution of infectivity. If all the undetected BSE cases in the EU in a single year contributed raw material to the production of gelatine, the 50th percentile of the total infectivity in the gelatine obtained would be 6.3 $CoID_{50}$ and 2.6×10^{-2} $CoID_{50}$, if the animals were slaughtered in negligible risk or controlled risk countries, respectively. Applying a linear dose response at low-dose levels, the average amount of infectivity to which every individual in the 42 million cattle in the EU would be exposed to in one single year would be below a previously estimated threshold of 10^{-7} $CoID_{50}$/animal per year, required to generate a new case of BSE.

3.7.2 | Aggregation versus dilution

In the context of this risk assessment, $CoID_{50}$ is used as the common unit to quantify the amount of infectivity in the raw material, and in the gelatine produced from it. One $CoID_{50}$ contains multiple PrP^{Sc} infectious particles, possibly distributed as a population of PrP^{Sc} multimers of different sizes, which might individually be capable of resulting in successful infection. For example, in one experimental study, a dose equivalent to a 150-fold dilution of 1 $CoID_{50}$, i.e. –2.2 $\log_{10}$ $CoID_{50}$, was able to cause disease in 1 out of 15 orally exposed cattle (Wells et al., 2007).

The recent EFSA BIOHAZ Panel risk assessment (2020) was reviewed by ANSES (Schmidely et al., 2022). The review commented/pointed out the assumption of the homogeneous distribution of infectivity in such a matrix, since it is not documented, resulting in a possible bias in the estimation of the infectious titre in the results of cattle exposure.

Prion particles may tend to agglomerate in some matrices, and it is not known whether or not they are uniformly distributed in gelatine and collagen. Their capacity to be dispersed or aggregate under conditions associated with the gelatine and collagen production process is unknown. Two opposing scenarios can be characterised:

- Prion particles spread evenly within a batch of final product, i.e. the approach that has been reported in previous C&G opinions and is once again a model output parameter in this opinion. A hypothetical estimate of ID_{50} per unit (kg) of product can then be calculated (model outputs P24 and P26 of the bovine and ovine model, respectively), acknowledging that this is biologically simplistic.
- Prion particles aggregate into clusters. As we are dealing with very low doses, approximately 1 $CoID_{50}$ per batch of gelatine, the worst-case scenario would be that all prion particles in a batch aggregate into a single cluster. In this scenario, the maximum exposure would be represented by assuming the entire infectious content of a batch of gelatine in a single event (model outputs P23 and P25 of the bovine and ovine model, respectively). This is biologically implausible.

In practice, the distribution of infectivity in a batch of gelatine could potentially be characterised by any intermediate scenario between these two hypotheses. Of note, while concentration or clumping of infectivity may pose a higher risk to an individual, the risk to a large population would remain the same (EFSA BIOHAZ Panel, 2005c 'Quantitative risk assessment of the animal BSE risk posed by meat and bone meal with respect to the residual BSE risk'). As previously mentioned (EFSA BIOHAZ Panel, 2005c, 2020), the uncertainty arising from the assumption of a homogenous distribution of BSE infectivity in gelatine does not affect risk estimation at the population level if a linear dose response is assumed, as in the case of the risk assessment in this opinion of the possibility of a new case for bovines and small ruminants (AQ6). However, it can have a significant impact when assessing maximum exposure from a single event, as in AQ5 for evaluating human exposure. Therefore, the two extreme scenarios are considered in this scientific opinion to assess the maximum human exposure. This approach provides boundaries that represent minimum and maximum values of potential maximum exposures from a batch of gelatine contaminated with BSE.

3.7.3 | Dose–response relationship

3.7.3.1 | *General concepts*

In experimental TSE transmissions undertaken by the intracerebral route (Fryer and McLean (2011) – see below), with higher doses, the incubation time increases as the dose decreases, but at low doses (i.e. below the $CoID_{50}$), the probability of infection decreases as the dose decreases. However, for cattle challenged experimentally with C-BSE by the oral route, the variation in incubation time was less clear, and the rate at which the probability of infection decreased with dose appeared

to be much slower than for experiments in mice infected by the parenteral route (Wells et al., 2007). The attack rate in these cattle orally exposed to different doses of BSE followed a logistic regression curve, which allowed the data to be fitted to a logit dose–response model and resulted in maximum likelihood estimates of the amount of brain containing 1 $CoID_{50}$ and the probability of infection as a function of dose. These experiments provide us with direct experimental estimates in cattle, taking into account the limitations described above for the use of experimental data, but are based on doses relatively close to 1 $CoID_{50}$.

In the context of developing risk assessments for residual BSE infectivity, it is necessary to estimate the risk of disease at doses well below 1 $CoID_{50}$. Two conceptual approaches can be considered:

- in the case where the particles act independently and even a single PrP^{Sc} particle has a small but finite probability of causing infection (no threshold dose, worst case scenario), the probability of infection versus dose can be modelled using logistic regression. This concept is sometimes referred to as the infectious unit being divisible.
- if one assumes a cooperative effect of the particles and thus the existence of a threshold dose, then probit analysis (Hahn & Soyer, 2005) could be used (see below).

3.7.3.2 | *Experimental data*

Gale (1998) used negative exponential and log-probit dose–response curves to fit titration data for BSE-infected bovine brain in mice that were intracerebrally inoculated with doses ranging from 0.02 to 20 ID_{50}. The two curves were similar over the experimental range of doses but differed significantly in their probability of infection for exposures of less than 0.01 ID_{50}. Indeed, the negative exponential curve decreased linearly with dose, as expected if the prions were acting independently and with no threshold dose. In contrast, the risk predicted by the log-probit curve decreased much more rapidly at lower doses, which would be consistent with the prions acting cooperatively and with a threshold dose (i.e. an individual would need to ingest a minimum number of prions to be infected).

Fryer and McLean (2011) analysed data from 127 intracerebral titration experiments in mice, with doses ranging over 10 orders of magnitude. They confirmed that the probability of infection decreases as the dose decreases, with evidence of infection at very low doses, three orders of magnitude below the $ID_{50,}$ challenging the view that a threshold dose exists below which the probability of infection is zero (or reflecting the possibility that there may be some aggregation of PrP^{Sc} leading to heterogeneity of inoculum aliquots). The authors developed a within-host model that assumed a stochastic birth–death prion accumulation and no threshold dose for infection, which closely matched the experimental data and demonstrated a sigmoidal change in the probability of infection with relative dose. These data supported the assertion that the probability of infection is approximately linear at low doses. Therefore, available data support the assumption that there is no threshold dose for prions and that the probability of infection declines linearly at low doses. This allows for the application of the simplest model to explain the phenomenon and represents the worst-case scenario in the absence of data showing the need for cooperativity of infectious prions. In agreement with previous opinions, it was thus considered that a linear dose response curve at the low dose range is pertinent when estimating the potential for infection in an individual exposed to low prion doses (< 1 ID_{50}).

As a result, and in agreement with EFSA BIOHAZ Panel (2005b), *from a practical point of view, this implies that one can derive an estimate of the number of animals being infected, given the assumptions introduced in the QRA, by considering both the individual exposure level, expressed in $CoID_{50}$ per animal, and the population size exposed* (see Section 3.9.2).

3.7.3.3 | *Single versus repeated/cumulative exposure*

In this opinion, it has been assumed that there is a finite probability of infection resulting from a given low dose (below 1 $CoID_{50}$) and that accumulation of infectivity over a period is not a significant factor in achieving a dose that will result in disease.

3.7.4 | Transmission barrier

The minimum $CoID_{50}$ that would be required to consistently cause disease in any exposed individual is unknown. Many factors can influence the ability of a TSE agent to infect a host, such as the amount of infectivity, the age/developmental maturity of the animal challenged, host genetics, exposure route and the possible potentiating effects of intercurrent disease or injury. While these and other factors have been considered to potentially affect the success of infection following exposure, the precise roles and interdependence (if any) of these factors have not been unequivocally established. This has been discussed in detail in a previous EFSA opinion (EFSA BIOHAZ Panel, 2015) and summarised briefly below for ease of reference.

In the absence of an objective dose–response relationship based on a measurable amount of isolated/purified infectious agent, TSE infectivity is usually expressed as ID_{50} (as described in Section 3.4.1), but it has been also shown that infection is possible at the very low dose of a 1000-fold dilution of the ID_{50} (Fryer & McLean, 2011) (Section 3.7.3.2). For disease to occur, the infecting prion particles must be able to enter the central nervous system of the host and 'convert' the cellular prion protein (PrP^{C}) to PrP^{Sc} at a rate which enables accumulation of sufficient PrP^{Sc} to cause disease within the lifespan of the host. Host genetics substantially influence this, and in small ruminants, this has even been exploited to breed for

disease resistance (EFSA BIOHAZ Panel, 2014b, 2017). There is also evidence to suggest that the *PRNP* gene has a significant effect on the likelihood of clinical disease developing in humans that show evidence of infection (Gill et al., 2013, 2020; Ironside et al., 2006).

For abnormal PrP to reach the nervous system and for disease to develop, many events have to occur:

- There must be exposure to a sufficient dose of the agent. There are no data on the dose–response relationship for human TSE, or whether different doses would be required for an effective infection to occur depending on different host variables.
- The agent must be taken up from the gastrointestinal tract (unless it is introduced parenterally via injection or surgical applications). For sheep, cattle and humans alike there is a modelled association between the development of the gut-associated lymphoid tissues of Peyer's Patches (PP) and susceptibility to natural TSE infection. This association may explain the observed changes in susceptibility with host age (St Rose et al., 2006). It has been speculated that entry might be facilitated by other 'co-factors' such as mechanical loss of mucosal integrity within the gastrointestinal tract, and/or co-infection with some unrelated pathogen, but no conclusive evidence has ever been found to support any specific contributing factors. Differences in normal host physiology may also influence the outcome of exposure.
- The agent must enter the nervous system and be successfully transported to the neuronal cell bodies in the central nervous system (CNS).

All of these stages are multifactorial. There is also no data available to establish whether or not repeated exposure might increase the probability of successful infection.

Whether successful infection ultimately results in a case of clinical disease will also depend on the incubation period (which can be influenced by dose, host genetics and possibly other unidentified factors) relative to the remaining biological or commercial lifespan of the individual host at the time of infection.

The presence/absence of a transmission barrier does not in itself control the success of infection, or the resulting incubation period. For example, BSE caused variant Creutzfeldt–Jakob disease (vCJD) through oral transmission across a species barrier, with a wide exposure of the population, but only a few human cases have been identified, and in general with a young age at onset (i.e. with an incubation period substantially shorter than average lifespan). In contrast, another TSE, kuru, is an example of transmission through ingestion of infectious material without a species barrier (human to human), and with a recorded incubation period of 5–50 years (Liberski, 2013). It is, therefore, impossible to define an experimental model that encompasses all this potential variability and that directly measures the likely transmission across a species barrier (i.e. all the components required to achieve successful infection).

There is also data from experimental cross-species challenges that show that the species of the donor can influence the ability of a TSE strain to infect a recipient host of a different species on subsequent passage. In some cases, this is absolute (e.g. experimental transmissions have demonstrated that some strains can obtain or lose their ability to infect a host following passage through an intermediate host of another species (Torres et al., 2014)). In the case of BSE isolates specifically, it has been shown that BSE passaged through ovinised transgenic mice has an increased 'virulence' on subsequent passage into a different species (Padilla et al., 2011).

When it is considered that none of the factors listed above have a substantial impact on transmission (in general when transmission is within species, particularly within host genotype), then the working assumption is that there is no species barrier. This is the assumption made within this opinion with regard to bovine to bovine transmission and bovine to ovine transmissions. It is also the working assumption with regard to ovine to ovine transmission and to ovine to bovine transmissions, although this may not be the worst-case scenario, as discussed above (Padilla et al., 2011).

Based on the observed situation with real data (human vCJD cases and their relationship to the BSE epidemic in cattle), it is assumed that there is a significant (but not absolute) transmission barrier for BSE into humans, but there is no way in which to quantitatively estimate the size of this barrier, or determine if it may be different based on different species of origin (in this case bovine vs. ovine or caprine).

3.7.5 | Efficiency of different exposure routes

Experimental studies have demonstrated that the route of exposure has a crucial impact on the efficiency of prion transmission, i.e. on the amount of infectivity that is required to transmit a prion strain to a specific host species.

This phenomenon has been extensively characterised by Kimberlin and Walker using scrapie adapted strains in rodent models. Data obtained using the mouse-adapted scrapie strain 139A were collated by Kimberlin (1996) to provide comprehensive estimates of the relative efficacy of various peripheral exposure routes using the intracerebral route (the most efficient route for prion disease transmission) as a benchmark:

- intravenous (iv)/intracerebral (ic): 1/10
- intraperitoneal (ip)/intracerebral (ic): 1/100
- subcutaneous (sc)/intracerebral (ic): 1/10,000
- intramuscular (im)/intracerebral (ic):1/10,000
- oral or intragastric (ig)/intracerebral (ic): 1/100,000

These values have recurrently been used as a 'guideline' (generally with appropriate disclaimers) to conduct prion disease risk assessments. Despite their interest, these ratios must be used with caution, since they only remain valid for the model (139A in Compton-White (CW) mice) in which they were established.

Indeed, the final capacity of an inoculum to transmit the disease by a given route of exposure depends on both the nature of the TSE agent (strain) and on the donor and recipient hosts (species/genetic background). As supporting evidence to this statement:

- about 40,000 LD_{50} ic units of scrapie strain 263K are equivalent to 1 LD_{50} unit by the ip route in hamsters (Kimberlin & Walker, 1977) while for the 139A scrapie strain in CW mice 1 LD_{50} IP was on average equivalent to 430 LD_{50} ic. units (Kimberlin et al., 1983).
- In 139A scrapie /CW mice (Kimberlin & Walker, 1989) and BSE in cattle (Wells, 2007) 1 ID_{50} by the oral route was approximately equivalent to $10^{5.5}$–$10^{5.6}$ ID_{50} ic units. This contrasts with data reported in natural scrapie in VRQ/VRQ sheep, where 1 ID_{50} oral unit was equivalent to fewer than 102.7 ID_{50} ic units (Douet et al., 2014).

For obvious reasons, data related to the efficacy of prion disease transmission by peripheral routes in humans are relatively limited. The iatrogenic CJD cases that resulted from the use of pituitary extract-derived growth hormone provide an estimation of what could be the minimal amount of infectivity that would be sufficient to transmit a prion disease to a human host by non-oral routes, illustrating the very limited exposure that is necessary for infection to occur via parenteral routes in the absence of a species barrier.

Historically, transmissions to non-human primates have been considered as the most relevant models for characterising the pathogenesis of C-BSE/vCJD in humans. In a recent paper, Mortberg et al. (2022) systematically reviewed all the published data related to prion disease transmission in non-human primate models. They identified a total of 76 articles in which original transmission of prion disease in primates had been reported, accounting for a total of 883 individual animals, among which 190 were inoculated with C-BSE (from cattle or after passage into non-human primates) or vCJD (human origin or adapted to non-human primates).

When considering C-BSE originating from cattle origin, disease transmission was reported in animals exposed by ic, ic/ip, oral and iv routes.

Results of a European Union–funded non-human primate risk assessment study, designed to determine the dose of C-BSE at which 50% of macaques will be infected, show that a single 5-g dose of brain administered orally was able to transmit disease to 12 out of 12 macaques (Holznagel et al., 2013). This study was originally designed based on a previous oral C-BSE transmission study in cynomolgus macaques, which reported that only one out of two individuals orally challenged with 5 g of C-BSE cattle brain material was infected (Lasmézas et al., 2005).

In another study, Cynomolgus macaques have been inoculated by the oral route with 500 mg brain material (cattle C-BSE). However, according to currently available results, no positive transmission had been observed after more than 70 months of incubation (Comoy et al., 2015).

In summary, converging evidence indicates that various peripheral exposure routes (iv, ip, sc, im) should be considered efficient for transmitting cattle C-BSE to humans. However, their relative efficacy remains unknown.

Data obtained from TSE animal models (principally rodent scrapie models) provided quantitative estimates for the relative efficacy of transmission of particular prion strains in specific hosts.

These quantitative estimates cannot be directly transposed to the risk of C-BSE transmission by peripheral routes to humans. However, in the absence of any other relevant source of information, these values represent the only means by which to approach the estimation of the relative risk of C-BSE transmission following exposure by peripheral routes in humans.

All the evidence collected in TSE experimental models (including non-human primates) strongly support the contention that, in an individual exposed to C-BSE by the oral route, the efficacy of disease transmission will be significantly lower (several log_{10}) than in an individual exposed by a parenteral route.

3.8 | Exposure of humans to BSE infectivity through the use of C&G (AQ5)

3.8.1 | Exposure pathways for humans from the use of ruminant C&G

3.8.1.1 | *Introduction*

Following several statements of the Scientific Veterinary Committee in early 1990s, the Scientific Steering Committee published a scientific report and opinion on the safety of gelatine (SSC, 2000a) in which it classified its end use for humans (human consumption and cosmetic products, oral or topological, parenteral, ophthalmic, implantable product, as component in manufacture and industrial use) in three risk categories: BSE free or negligible risk, lower risk and high risk. At that time, due to the available data on infectivity in tissues and on the inactivation of the BSE agent by the different industrial processes of gelatine, the SSC recommended that ruminant bones from animals certified fit for human consumption and sourced from BSE-free or BSE-negligible risk countries be used for the production of gelatine. It also proposed that data should be produced on the equivalency in TSE infectivity inactivation/elimination with other already documented and validated processes.

Depending on the final application and the desired properties, gelatine derived from porcine material can be mixed with gelatine of bovine origin after production (Vermeulen, 2023b by email on 31 October 2023).

The uses for bovine-derived gelatine/collagen can be broadly split into three categories: food 50%, pharma 39% and other 12%. There is a greater use of gelatine/collagen derived from non-bovines in food. There is currently no inclusion of bovine gelatine/collagen into animal feed.

Collagen and gelatine are widely used in foods, food additives and nutritional supplements for human consumption. They are also used in medicinal products, medical devices, cosmetics and for industrial purposes. Taking these uses into account, the exposure pathways can be broadly broken down as follows:

- Exposure pathway 1: Collagen and gelatine used for human consumption (oral);
- Exposure pathway 2: Collagen and gelatine used in cosmetics (topical and parenteral);
- Exposure pathway 3: Collagen and gelatine used in medicinal products and medical devices (oral and parenteral);
- Exposure pathway 4: Collagen and gelatine used for an industrial purpose (different possible routes of exposure).

3.8.1.2 | *Exposure pathway 1: Collagen and gelatine used for human consumption*

This exposure pathway encompasses the exposure of humans to collagen or gelatine containing the BSE agent through the consumption of food, food supplements or food additives.

Collagen and gelatine have multiple applications in the food industry due to their properties: stabilising, thickening, emulsifying, binding, gelling, clarifying and foaming (Usman et al., 2023). A wide variety of foods contain gelatine including biscuits, bread, breakfast cereals, chocolate bars, pasta, savoury snacks and sweets. Gelatine is included in dairy products such as ice cream, candies, bakery products and desserts for texture, gelling, foaming, stabilising and melt-in-mouth properties (Shirsath & Henchion, 2021; Usman et al., 2023). In meat products, gelatine helps retain juices and to provide a good heat transfer medium during cooking. It is also used as a clearing agent in the brewing of wine and beer (Alipal et al., 2021). Collagen and gelatine are also used to produce frozen dough, since they allow the control of the quality of certain frozen foods like conventional baked food, jellies or functional candies (Cao et al., 2022).

Gelatine may be used as a flavour enhancer, functional ingredient or as an additive to improve the profile of products (Shirsath & Henchion, 2021). Multiple gelatine hydrolysates and gelatine-derived peptides are used as nutritional and functional foods due to their potential antioxidant and cryoprotective properties (Liu et al., 2015). Their antioxidant properties are used to prevent deterioration of products' quality and to maintain their nutritional value (Shirsath & Henchion, 2021).

Gelatine is used as a capsule material in food supplements. Examples include gelatine capsules filled with fish oil, vitamin D3, probiotics, enzymes, amino acids or herbal extracts. Gelatine is used as a gelling agent for the production of food supplements such as gummies or jellybeans with added vitamins, minerals or other nutrients. The antioxidant and antihypertensive properties of gelatine, together with its positive effects on bone & joint health and skin health (promoting cartilage regeneration in osteoarthritis) and its postulated ability to prevent a variety of health conditions has resulted in its use in a wide range of food supplements (Shirsath & Henchion, 2021).

The precise amount of gelatine used annually in the EU and the species of origin is not known. According to the feedback received from stakeholders, the gelatine content in any final food does not exceed 2% on average, with the exception of certain types of confectionery (up to 8% gelatine) (van den Brink, 2020 by email on 21 February 2020).

The EFSA (EFSA BIOHAZ Panel, 2006a) Opinion of the Scientific Panel on Biological Hazards on the 'Quantitative assessment of the human BSE risk posed by gelatine with respect to residual BSE risk' states: '*When considering their estimates for the daily consumption levels of gelatine the SSC QRA recognised that it was prudent to consider a worst case scenario where all of the daily ration of gelatine was assumed to be derived from bovine bones* (EFSA QRA Report 2004; see below). '*The average daily consumption of gelatine by humans is estimated to range between 1 and 5 grams. In practice, the average daily exposure of the EU consumer to EU bovine bone-derived gelatine ranges between 0,05 and 0,1 grams only, as gelatine is also produced from pig skin and pig bones (no TSE risk) and from bovine hide splits that can be regarded as presenting a negligible TSE risk. This would result in an average societal risk reduction by a factor of 20 to 50, but the fact that entire batches may be produced from EU bovine bones may not be ignored. To assess whether or not, for example, the removal of the vertebral column results in a significant decrease of the human exposure risk, it is reasonable to assume that all gelatine in a given batch was produced from bovine bones. This is the worst case scenario. However, to calculate the overall societal risk, the fact that only a fraction of the human food gelatine is obtained from cattle bones, should also be taken into account.*'

The average daily consumption of gelatine by humans is thus estimated to range between 1 and 5 g. In practice, the average daily exposure of the EU consumer to EU bovine bone-derived gelatine ranges between 0.05 and 0.1 g only, as gelatine is also produced from pig skin and pig bones (no TSE risk) and from bovine hide splits that can be regarded as presenting a negligible TSE risk (EFSA BIOHAZ Panel, 2005a, 2020).

Collagen is mostly used as a nutritional supplement. It is also used in the food industry. For example, its antioxidant properties make it ideal to improve the product quality or extend the shelf life of meat products such as ham, sausage and canned foods. Collagen, used as an additive in fermented milk and beverages, is a developing trend in the beverage industry (Cao et al., 2022). One of the most frequent uses of collagen is in the production of collagen casings (films), natural casings, artificial collagen casings, cellulose casings, synthetic polymer casings and new collagen-co-extrusion casings and edible collagen coatings (Cao et al., 2022).

All collagen and gelatine produced for human consumption must comply with the requirements set out in Regulation (EC) No 853/2004.

3.8.1.3 | *Exposure pathway 2: Collagen and gelatine used in cosmetics*

This risk pathway encompasses the exposure of humans to collagen or gelatine containing the BSE agent through the use of cosmetics. Gelatine in cosmetics may be administered by the topical or parenteral route (intradermal).

Collagen and gelatine are used in many cosmetic and health care products. They are an ingredient in face creams, body lotions, shampoos, hair sprays, sunscreens and bath salts and bubbles so they are likely to be used by a very wide spectrum of the human population. Very little information is available in the published literature or elsewhere in relation to the specific cosmetic products that contain pure collagen and/or gelatine, the quantity of pure collagen and/or gelatine in those products, the total quantity of cosmetics containing collagen and/or gelatine and the dose of collagen and gelatine used by individuals. There are multiple cosmetic products using multiple exposure routes (topical, intradermal, intramuscular, etc.) with different composition and concentration of collagen and/or gelatine.

The legal requirements for the production of cosmetics in the EU are set out in Regulation (EC) No 1223/2009.[15] A specific process for the production of collagen and gelatine for use in cosmetics is not set out in this legislation. Under Annex II of the Regulation, Category 1 and Category 2 materials, as defined in the ABP Regulations, are prohibited in cosmetic products. This prohibition is based on an Opinion of the Scientific Committee on consumer products of the European Commission (SCCP, 2005). It was re-stated in notes of guidelines produced by the Scientific Committee of Consumer Safety of the European Commission (SCCS, 2023). These guidelines set out detailed procedures to ensure the safety of cosmetic products.

Category 3 animal by-products can be used in cosmetic products. Collagen and gelatine from an animal source must comply with the requirements set out in ABP Regulations with a view to ensuring that conditions for controlling potential risks to public and animal health are in place.

3.8.1.4 | *Exposure pathway 3: Collagen and gelatine used in medicinal products and medical devices*

This risk pathway encompasses the exposure of humans to collagen or gelatine containing the BSE agent through the use of medicinal products or medical devices. Gelatine in medicinal products or medical devices may be administered by the parenteral route.

Collagen and gelatine are used in a wide variety of medicinal products and medical devices, including implantable medical devices. As above for cosmetic products, there are multiple medicinal products leading to multiple exposure routes (with different composition and concentration of collagen and/or gelatine).

In the production of medicines, gelatine is used for a variety of purposes including use as a binding and compounding agent in the manufacture of medicated tablets and capsules. Its ability to entrap functional components in a carrier and provide protection against oxidation or degradation during storage makes it ideal as a substrate (Gómez-Guillén et al., 2011; Shirsath & Henchion, 2021). In vaccine production, it can be used in culture media (EMA, 2018). Gelatine in medicinal products may be administered by the oral, topical or parenteral route. Blood plasma substitutes based on gelatine are widely used for the temporary replacement of blood in the circulatory system after surgery or accidents (Schrieber & Gareis, 2007).

The legal requirements for the production and use of medicinal products in the EU are primarily laid down in Directive 2001/83/EC[16] and Regulation (EC) No 726/2004.[17] Very stringent procedures are in place for the manufacture and use of medicinal products taking the route of administration and other factors into account. Guidelines and recommendations to minimise the risk from medicinal products were produced by the European Medicines Agency (EMA) (EMA, 2018). These contain the following information in relation to minimising the risk of BSE transmission.

'All medicinal products, including vaccines, have been thoroughly evaluated before they are authorised to be marketed. To receive this authorisation to market their product, a pharmaceutical company has to describe in detail (in a dossier) the results of all the studies demonstrating the quality, safety and efficacy of the medicinal product. The dossier also documents the method of production and control of each component of the medicinal product and all factors concerning the risk of BSE transmission are presented. The dossier is evaluated by the relevant National Authorities or the EMA, taking into account all existing guidelines and legal texts. It is only when a dossier is complete and fully satisfactory that a marketing authorisation for a medicinal product is granted'.

For all bovine materials used in the manufacture of vaccines (and all other medicinal products), an assessment is made of the risk of BSE contamination. This is carried out in accordance with the European *Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents* via *human and veterinary medicinal products*. This legally mandatory guideline was first applied in 1991 and has been regularly updated since. Factors taken into special account are:

[15]Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast) (Text with EEA relevance). OJ L 342, 22.12.2009, p. 59–209.

[16]Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28.11.2001, p. 67–128.

[17]Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. OJ L 136, 30.4.2004, p. 1–33.

- The country of origin of the animals used,
- The nature of the tissue used (e.g. brain is considered the highest risk, serum and muscle tissue are of the lowest risk),
- Information on traceability (origin and follow-up of herds, type of feed, etc.),
- Whether the manufacturing processes of both the materials of bovine origin and the vaccine could reduce or destroy any BSE, if it were to be present.

Therefore, safety related to the risk of transmitting BSE is assessed by taking into account not only the geographical origin of animals but also their feeding, their age at slaughter, technique of slaughter and deboning, nature of tissues used, as well as manufacturing processes which must comply with European guidelines, directives and recommendations. It is the assessment of all these criteria which ensures the freedom of a medicinal product from contamination with the agent that causes BSE before it is authorised and marketed.

According to the EMA guidelines, '*Manufacturers of vaccines strictly control the quality of the materials derived from animals by obtaining them only from known, well controlled sources with systems in place to ensure the materials do not pose a risk of contamination with BSE, and by only sourcing the materials from animals which are fit for human consumption*'.

In 2001, the European Medicines Agency (EMA, 2001) was advised by a panel of international experts that the risk of BSE contamination of vaccines used in the EU is 'extremely low'. The Committee of Proprietary Medicinal products (CPMP) concluded in line with the Center of Biologics Evaluation and Research of the US Food and Drug Administration (US FDA) that the risk of transmission of BSE through the use of bovine material during vaccine manufacture is 'very remote and theoretical'.

In surgery, the main uses of gelatine are as pharmaceutical grade sponges for use in surgical operations (Shirsath & Henchion, 2021), and swelling gelatine particles that have haemostatic properties by limiting blood flow and forming a mechanically stable matrix around a bleeding site (Liu et al., 2015). Injectable gelatine micro-cryogels (GM) have also been developed to improve cell therapy in the treatment of deep layer tissues of a wound (Alipal et al., 2021).

Gelatine has been developed as a main ingredient in products for treating skin burns due to its adhesion properties that can also provide a lubricating effect (Said, 2020). Gelatine is also used as hydrogel, in nano-microsphere containers, nanofibers, pharmaceutical additives and cell transplantation carriers (Alipal et al., 2021). Finally, gelatine is used as a matrix for intravenous infusions, injection drug delivery microspheres and implants (Shirsath & Henchion, 2021).

The legal requirements for the production of medical devices in the EU are set out in Directive 93/42/EEC.[18] Directive 90/385/EEC[19] is a specific directive for implantable medical devices. All medicinal products and medical devices derived from animals, including gelatine, must also comply with the ABP Regulations.

3.8.1.5 | *Exposure pathway 4: Collagen and gelatine used for an industrial purpose*

This risk pathway encompasses the exposure of humans to collagen or gelatine containing the BSE agent through the use of industrial products. There is a considerable lack of information in relation to the use of those products, similar to that described above for cosmetic and medicinal products. Gelatine in industrial products may result in exposure of humans by the parenteral route or direct contact.

Gelatine has many applications in microbiology as a substrate for the measurement of proteolytic activity and gelatinase production and is used in culture media as a gelling agent. (Liu et al., 2015). In forensic science, gelatine lifters are used to remove impressions found at crime scenes (Liu et al., 2015). Gelatine can be used for air filters that are capable of retaining and detecting airborne microorganisms causing diseases and allergic reactions (Alipal et al., 2021; Liu et al., 2015).

In the food industry, gelatine is not only included in the formulation of the food itself but has been applied also in the formulation of refined material for packaging films and coatings to preserve foods and extend their shelf-life, and as a microencapsulation agent (Said, 2020).

In photography, the transparency, adhesion, firmness and the hydrophilic nature of gelatine and its ability to create a clear film of gel upon cooling in cold water have contributed to its use in photographic emulsion making since the 19th century (von Waldthausen, 2015).

As set out in Article 36, Regulation (EC) No 1069/2009, business operators may place derived products, including collagen and gelatine, on the market provided that they ensure the control of risks to public and animal health by:

(i) Safe sourcing in accordance with Article 37 of the Regulation;
(ii) Safe treatment in accordance with Article 38, where safe sourcing does not ensure sufficient control; or
(iii) verifying that the products are only used for safe end uses in accordance with Article 39 where safe treatment does not ensure sufficient control.

Certain types of Category 1, Category 2 and Category 3 raw materials can be used provided that they meet these requirements.

[18]Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. OJ L 169, 12.7.1993, p. 1–43.

[19]Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices. OJ L 189, 20.7.1990, p. 17–36.

Specific requirements are set out in EU Regulation 142/2011 in relation to photo-gelatine and, particularly, in relation to the import of photo-gelatine. Currently, photo-gelatine can only be imported from the United States and Japan. Strict conditions for these imports are set out in the Regulation.

3.8.2 | Estimation of maximum human exposure through the use of C&G

3.8.2.1 | *Food exposure pathway*

The values of consumption of gelatine were extracted from the EFSA's food consumption database, for acute consumption (a one-off or very short-term exposure to a substance, usually less than 24 h) as a worst-case scenario. The French national dietary survey (INCA3, 2014–2015) in 2014 reported the highest consumption levels with a median consumption of gelatine of 21.07 g per day in very elderly people (5th–95th percentiles: 6.12–94.87) and a 99th percentile of 105.08 g consumed in a day across 4 days. EFSA recommends as the highest reliable percentile that can be estimated when applying the following minimum sample size for each percentile: five samples for the P50, 11 samples for the P75, 29 samples for the P90 and 59 samples for the P95. In this case there are only four measurements, and it was agreed to report the median consumption among the group with the highest consumption, as in the French survey. The median consumption value in the highest consumption group (21.07 g) was selected to estimate the exposure to potentially infected gelatine. In the EFSA QRA report (2004), it was reported that the average daily consumption of gelatine by humans was estimated to range between 1 and 5 g, and only between 0.05 and 0.1 g of EU bovine bone-derived gelatine.

The bovine model estimated a median concentration of infectivity in a small size batch of gelatine of 3.4×10^{-4} $CoID_{50}$/kg. For a median consumption of 21.07 g, the median amount of infectivity that the consumer in the highest consumption group would be exposed to is 7.1×10^{-6} $CoID_{50}$ of bovine BSE if all the gelatine consumed was sourced from an infected batch.

The ovine model estimated a median concentration of infectivity in a small batch of gelatine of 5.3×10^{-3} $CoID_{50}$/kg. For a median consumption of 21.07 g, the amount of infectivity that the consumer in the highest consumption group would be exposed to is 1.1×10^{-4} $CoID_{50}$ of ovine BSE if all the gelatine consumed was sourced from an infected batch. Similar calculations have been done for age categories with maximum consumption above 10 g of gelatine and presented in Table 7.

If all infectivity was clumped or aggregated in an amount of gelatine smaller than that of a single meal (exposure event), the median amount of infectivity that a single consumer would be exposed to in a single exposure event would be 0.26 $CoID_{50}$ (bovine BSE) or 4.06 $CoID_{50}$ (ovine BSE).

TABLE 7 Median acute consumption per day of gelatine in different age classes and EU countries from the EFSA Comprehensive European Food Consumption Database, and corresponding amount of BSE infectivity in a single exposure event using the outputs of the probabilistic model.

Country	Population group	50th percentile consumption per day (gr)	Number of consumption days (number of days available)*	Infectivity bovine BSE	Infectivity ovine BSE
France	Other children (from 36 months up to and including 9 years of age)	6.53	7	2.2×10^{-6}	3.4×10^{-5}
France	Adolescents (from 10 up to and including 17 years of age)	5.23	14	1.8×10^{-6}	2.7×10^{-5}
France	Adults (from 18 up to and including 64 years of age)	7.48	39	2.5×10^{-6}	3.9×10^{-5}
Hungary	Elderly (from 65 up to and including 74 years of age)	10	7	3.4×10^{-6}	5.3×10^{-5}
France	Very elderly (from 75 years of age and older)	21.07	4*	7.1×10^{-6}	1.1×10^{-4}

*These consumption values refer to 'consumption days only' and must be carefully interpreted because of the low number of consumers available.

3.8.2.2 | *Non-food exposure pathways*

In this risk assessment, it was not feasible to evaluate the maximum level of exposure via the non-food exposure pathways mentioned above (Section 3.8.1). This was due to insufficient data and a lack of specific expertise in these areas, which fall outside the scope of EFSA's remit. The potential human exposure to BSE in C&G via these pathways will also depend significantly on the EU legal frameworks in place for these products (medicines, medical devices, cosmetic products, etc.), as outlined in Section 3.8.1. The impact of the change in C&G status will be influenced by how these specific rules and guidelines are implemented.

It is conceivable that human exposure to BSE infectivity in C&G in a single event would be quantitatively lower through non-food pathways than through food. However, the different routes of exposure must be taken into account when assessing the risk of human disease. The evidence from experimental TSE models strongly supports the contention that the efficacy of disease transmission will be significantly higher (several $\log_{10}$) in individuals exposed by the parenteral route than in those exposed by the oral route (see Section 3.7.5). As most of the non-food exposure routes will be parenteral, it is anticipated that lower exposure doses might be compensated for by more effective exposure routes. It is therefore

strongly recommended that the BSE exposure arising from these routes be assessed following any change in the status of C&G to a safe commodity.

3.9 | Characterisation of the BSE risk in cattle and small ruminants (AQ6)

3.9.1 | Risk pathways for cattle and small ruminants from the use of C&G

There are two main potential risk pathways by which ruminants could be exposed to collagen or gelatine, and a number of sub-pathways.

- Risk pathway 5: Collagen or gelatine in feed (oral)
- Risk pathway 6: Collagen or gelatine used in veterinary medical products (oral and parenteral)

3.9.1.1 | *Risk pathway 5: Collagen or gelatine in feed*

There are a number of circumstances in which ruminants could be exposed to collagen or gelatine directly through feed. These include:

- The incorporation of collagen or gelatine directly in compound feed for livestock or in pet food;
- The incorporation of former foodstuffs containing collagen or gelatine in compound feed for livestock;
- The feeding of technological additives or nutritional supplements containing collagen or gelatine to livestock;

Collagen or gelatine in compound feed for livestock or in pet food

This risk pathway would encompass the feeding to ruminants of compound feed containing food or ABP grade collagen or gelatine contaminated with the BSE agent either directly, or indirectly through cross-contamination from compound feed intended for non-ruminants or from pet food.

Under Annex IV of Regulation (EC) 999/2001, non-ruminant gelatine can be fed to all livestock species, and ruminant gelatine can be fed to all livestock species except ruminants.

Due to the high cost of production (Vermeulen, 2020 by email on 18 March 2020, 31 October 2023) and their uncertain nutritional value (Asadi Kermani et al., 2017; Khalaji et al., 2016), neither collagen nor gelatine is currently used as a protein source in compound feed for any livestock. However, collagen or gelatine in their pure state and intended for human use, may occasionally be re-categorised as ABP for commercial reasons, and used in compound feed for livestock (Vermeulen, 2019 by email 19 December 2019). This could happen, for example, if a consignment of gelatine is rejected by a food business operator. According to the manufacturers, some collagen production surplus is sold to pet food producers and it is possible to find pet food advertised with the message 'containing collagen' in the market (Vermeulen, 2019 by email 19 December 2019). The extent to which gelatine in its pure form is rejected for use in food and is instead diverted for use in compound feed is uncertain, but based on information provided by the industry, it is extremely unlikely. No collagen or gelatine is currently produced from Category 3 animal by-products in Europe (Vermeulen, 2019, 2023a by email 19 December 2019; 29 September 2023; Bierwagen, 2020 by email on 27 January 2020; CCTA, 2011). So, products used for feed, pet food or technical purposes all meet the standards of food-grade collagen or gelatine.

There are strict criteria set out in relation to the production and use of C&G in compound feed for non-ruminants, which would apply to both C&G produced under Regulation (EC) No 853/2004 and under Regulation (EC) No 142/2011. Collagen or gelatine that are being used as feedstuffs must also comply with the requirements for feed labelling as set out in Commission Regulation (EU) No 2015/1905[20] of 22 October 2015 amending Annex II to Regulation (EC) No 183/2005[21] of the European Parliament and of the Council, and the requirements of Commission Regulation (EU) No 68/2013.[22]

While it is currently not economical to use collagen or gelatine in compound feed, this could change in the future. Nevertheless, it is unclear to what extent, if any, ruminant derived collagen or gelatine produced under Regulation (EC) No 853/2004 or Regulation (EC) No 142/2011 might be used for the production of compound feed following the change to the legislation in 2021, and the change underlying this opinion whereby gelatine derived from ruminants could be used to feed ruminants.

Former foodstuffs containing collagen or gelatine in compound feed for livestock

Collagen and gelatine are commonly present in former foodstuffs, i.e. foodstuffs of animal origin which are no longer intended for human consumption for commercial or other reasons. The collagen and gelatine are produced in accordance

[20]Commission Regulation (EU) 2015/1905 of 22 October 2015 amending Annex II to Regulation (EC) No 183/2005 of the European Parliament and of the Council as regards the dioxin testing of oils, fats and products derived thereof (Text with EEA relevance). OJ L 278, 23.10.2015, p. 5–10.

[21]Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene (Text with EEA relevance). OJ L 35, 8.2.2005, p. 1–22.

[22]Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials Text with EEA relevance. OJ L 29, 30.1.2013, p. 1–64.

with Annex III to Regulation (EC) No 853/2004. These former foodstuffs could potentially be used as a 'bread meal' or 'biscuit meal' that could be added as an ingredient to compound feed or fed directly to livestock (EFSA BIOHAZ Panel, 2020). Specific requirements for using former foodstuffs as animal feed are set out in Section 10, Chapter II, Annex X of Regulation (EC) No 142/2011. Prior to 2021, collagen and gelatine derived from non-ruminant animals could be fed to farmed animals, including ruminant animals but there was a prohibition on the feeding of collagen and gelatine derived from ruminant animals to farmed animals. According to the European Former Foodstuff Processors Association (EFFPA), former foodstuffs were often not incorporated into livestock feed because of the logistical difficulties of separating foodstuffs containing collagen or gelatine of non-ruminant origin from former foodstuffs containing collagen or gelatine of ruminant origin. Consequently, it was not possible, in practice, to incorporate former foodstuffs in animal feed to any great extent. According to the feedback received from GME, food processors paid for the disposal of waste foodstuffs generally through biogas plants. EFPPA claimed that 100,000 tons of former foodstuff containing ruminant collagen and/or gelatine had to be disposed of annually through this means, rather than being used more productively as an animal feed (EFFPA, 2015).

With a view to overcoming this difficulty and following the former EFSA risk assessment (EFSA BIOHAZ Panel, 2020), Annex IV of Regulation (EC) 999/2001 was amended in 2021. As a result of this amendment, non-ruminant gelatine can now be fed to all livestock species and ruminant gelatine can be fed to all livestock species, except ruminants, thus facilitating greater use of former foodstuffs in livestock feed. Industry sources indicate that former foodstuffs are solely used in non-ruminant feed, but it is not clear to what extent former foodstuffs are now used and to what extent this has changed following the change in the legislation since 2021.

As mentioned above, the gelatine content in any final food does not exceed 2% on average, with the exception of certain types of confectionery (up to 8% gelatine) (van den Brink (EFFPA), by email on 21 February 2020). It is not envisaged that such small amounts of gelatine would be retrieved for use as a protein source, not least because of the cost of achieving this, and the unpredictability of its availability. However, some former foodstuffs (e.g. biscuits, bread, breakfast cereals, chocolate bars, pasta, savoury snacks and sweets) are typically used in feed because of their high energy content in the form of sugars, oils and starch[23] rather than the proteins these meals can contain.

However, it is unclear to what extent, if any, former foodstuffs containing ruminant derived collagen or gelatine would be used to produce compound feed if no restrictions were in place.

Collagen or gelatine as technological additives or nutritional supplements

This risk pathway would encompass the consumption of collagen or gelatine containing the BSE agent by ruminants indirectly through cross-contamination of ruminant feed by encapsulated vitamins or supplements for dogs or horses.

Currently, gelatine of non-ruminant origin (generally porcine) is produced under Regulation (EC) No 853/2004 and used in feed as technological additive to encapsulate vitamins. Gelatine and collagen peptides derived from non-ruminants are used as supplements for dogs and horses to improve joint health, nutrition and digestion, among other benefits described in marketed products. There is no evidence that ruminants themselves are fed technological additives or feed supplements containing collagen or gelatine. There are several data gaps and uncertainties identified in relation to technological additives and nutritional supplements such as: which specific feedstuffs contain encapsulated/protected vitamins or other additives produced using gelatine or supplements containing collagen; what is the average content of collagen or gelatine in those vitamins or supplements; the total production of feedstuffs containing vitamins or supplements in the EU; the proportion of all vitamins or supplements for feed used in the EU that contain collagen and gelatine; whether and, if so, to what extent the production process of the vitamins or supplements reduces the BSE infectivity. Given these very specific uses, it is likely that any collagen or gelatine contaminated with the BSE agent would be greatly diluted during the manufacturing process.

3.9.1.2 | *Risk pathway 6: Collagen or gelatine used in veterinary medical products*

This risk pathway encompasses the exposure, by various routes, of ruminants to collagen or gelatine containing the BSE agent through the use of medicinal products or medical devices.

As in the case of medicinal products for humans, as described above, collagen or gelatine may be used in a wide variety of veterinary medicinal products. For example, gelatine capsules may be used orally, vaginally or post-partum intra-uterine to livestock, including ruminants.[24] Gelatine has been used as a carrier of hormone in super-ovulation injections for cattle (Looney et al., 1981) and as protective material in cell vaccines (Song, 1997).

Precise information is not available on the types of medicinal products that contain collagen or gelatine, on the species of origin, or on the quantities of collagen or gelatine that are used for these purposes. Gelatine may also be used in medical devices that are used in livestock production including devices for delivering hormones and antibiotics, but these are prohibited in the EU.

[23] https://www.effpa.eu/animal-nutrition/.

[24] For example, Gelatin Capsules Size #7 1.5oz: 100ct | Heritage Animal Health.

Regulation (EU) 2019/6[25] and Regulation (EC) No 726/2004 constitute the European Union regulatory framework for the manufacture, authorisation and distribution of veterinary medicinal products. As for the medicinal products for human use, very stringent procedures are in place for the manufacture and use of veterinary medicinal products. Guidelines and recommendations to minimise the risk from veterinary medicinal products were produced by the European Medicines Agency (EMA) (see Section 2.1.3).

3.9.2 | Estimation of the probability of a new BSE case in cattle and small ruminants through the use of C&G

3.9.2.1 | *Calculation of the probability of infection*

A dose–response equation was used to determine the probability of BSE infection in cattle occurring as a result of exposure to gelatine added to compound feed for ruminants.

At the current time, gelatine of ruminant origin is not permitted in cattle feed and, until the price per kg of gelatine falls below that of other cheaper conventional protein sources, it is unlikely that it will be used as a routine feed ingredient. It could be envisaged as a supplement as per the use for horses but would remain a very expensive option.

To model consumption, some simple conservative assumptions were made. The worst-case scenario selected consists of compound feed being given to an adult bovine animal that is housed, taking into account that compound feed is provided at higher levels when cattle are housed than when they are on good pasture. The amount of compound feed and the protein content of that feed is dependent on a number of factors including the age of the animal or live weight (e.g. calf, pregnant cow or adult bull), breed, other feed materials used concurrently and management type e.g. dairy or beef. For cattle, on average, 0.5 kg per 100 kg live weight can be fed per day per animal (AHDB, 2024. Dairy beef production systems). Above this, care must be taken to ensure there are not digestive issues. Assuming an adult cattle live weight of 500 kg, this translates to a daily maximum intake of around 2.5 kg. Where other food is scarce or very poor quality, 1 kg per 100 kg may be required but is not usual. Rates of protein content for finishing cattle are approximately 11%–12%.[26] Using the upper percentage contribution of 12%, this gives an estimate of 0.3 kg protein per day per cow. The same rationale has been applied to estimate the amount of protein per sheep per day (input consumed).

Various approaches and mathematical assumptions have been adopted to estimate the probability of infection over the years, despite the lack of full understanding of the quantitative relationship between PrP^{Sc} particles and infectivity as well as between the amount of infectivity and the probability of infection (as further described in Sections 3.4 and 3.7). With no further information available at this time, it is assumed that the proportion of a population that becomes infected from a daily consumption, as derived in parameter P28 and P30 for the bovine and ovine models, respectively, can be estimated using the following exponential dose response equation (Teunis et al., 2004), with the following inherent assumptions applied:

- The dose is divisible and there is no threshold dose below which infection cannot occur, e.g. one unit can be distributed through 10 servings.
- There is homogenous spread of infectivity, *r*, so for 1 infectious unit distributed through 10 servings, each one contains a 0.1 probability of infection.
- One exposure is defined as one day's cattle feed ration and there is no accumulation of dose between days.

Applying the exponential dose–response relation for the single-hit model with fixed probability of infection *r*,

$$P27(\text{bov}) / P29(\text{ovi}) = 1 - e^{(-r * \text{input consumed})}, \quad (1)$$

where *r* is the pathogen infectivity constant. This equation assumes that each infectious particle's action is independent, that is, the probability of infection by each single agent is independent of the size of the dose. As $CoID_{50}$ refers to 50% of an exposed population becoming infected, and input consumed = 1, then $-r = ln(0.5)$. Substituting into Equation (1):

$$P27(\text{bov}) / P29(\text{ovi}) = 1 - e^{(Ln(0.5) * \text{input consumed})}. \quad (2)$$

Finally, the mean number of livestock infected is estimated using Equation (3).

$$P28 = P25 \times P27(\text{bovine});$$

$$P30 = P27 \times P29(\text{ovine}). \quad (3)$$

[25]Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC (Text with EEA relevance) PE/45/2018/REV/1. OJ L 4, 7.1.2019, p. 43–167.

[26]Feed options for Finishing Cattle. https://www.teagasc.ie/media/website/animals/beef/feed-options.pdf.

The equations above have been applied to calculate P27 of the bovine model and, subsequently, P28. The same was done to calculate P29 in the ovine model and, subsequently, P30. The description, units and parameterisation are displayed in Tables 8 and 9 for the bovine and ovine models, respectively.

TABLE 8 Mean, median and 5th and 95th percentiles of P25–P28 of the bovine model.

Code	Description	Unit (per individual unless stated otherwise)	Value
P25	Number of livestock meals per day available from a small batch of gelatine	No meals	ROUND(P1 × P10 × P11)/P12,0)
P26	Number of $CoID_{50}$ consumed by an adult bovine per day from an infected batch where protein is substituted with gelatine	$CoID_{50}$/day/animal	P24 × P12
P27	Probability of infection per meal per animal	Probability/cow/meal	1-EXP((LN(0.5)) × P26)
P28	Number of new animals infected from exposure to an infected batch	No animals/batch	P25 × P27

TABLE 9 Mean, median and 5th and 95th percentiles of P27–P30 of the ovine model.

Code	Description	Unit (per individual unless stated otherwise)	Value
P27	Number of livestock meals per day available from batch	No meals	ROUND(P13/P14,0)
P28	Number of $CoID_{50}$ consumed by an adult ovine per day from an infected batch where protein is substituted with gelatine	$CoID_{50}$/day/animal	P26 × P14
P29	Probability of infection per meal per animal	Probability/sheep/meal	1-EXP((LN(0.5)) × P28)
P30	Number of new animals infected from exposure to an infected batch	No animals/batch	P27 × P29

The results of the estimation of parameters P27 and P28 of the bovine model (Tables 10 and 11) and P29 and P30 of the ovine model (Tables 12 and 13) are displayed below. The bovine infectivity corresponds to P6 of the bovine model and the ovine infectivity to P8 of the ovine model.

New case in bovine × bovine infectivity

TABLE 10 Mean, median and 5th and 95th percentiles of P27 and P28 of the bovine model with BSE bovine infectivity (P6 of the bovine model)

Code and description	5%	50%	Mean	95%
P27: Probability of infection per meal per animal	1×10^{-5}	7.1×10^{-5}	2.1×10^{-4}	8.3×10^{-4}
P28: Number of animals infected from exposure to an infected batch	0.018	0.180	0.53	2.1

According to the results of P28 of the bovine model with bovine BSE infectivity, in 87% of the iterations the number of new BSE cases generated in bovines, due to exposure to an infected batch containing all the bones of one BSE-infected bovine animal, is below 1.

New case in bovine × ovine infectivity

TABLE 11 Mean, median and 5th and 95th percentiles of P27 and P28 of the bovine model with BSE ovine infectivity (P8 of the ovine model).

Code and description	5%	50%	Mean	95%
P27: Probability of infection per meal per animal	2.9×10^{-4}	3.3×10^{-3}	1×10^{-2}	4×10^{-2}
P28: Number of animals infected from exposure to the infected batch	0.72	8.36	26	107

According to the results of P28 of the bovine model with ovine BSE infectivity, in 7.9% of the iterations, the number of new BSE cases generated in bovines, due to exposure to an infected batch containing all the bones of one BSE-infected ovine animal, is below 1.

The results of the sensitivity analysis showed that outputs P27 and P28 of the bovine model were strongly affected by the uncertainty associated with, in decreasing order, P9 Reduction of infectivity due to the acidic processing of bones; P6: BSE infectivity in brain, spinal cord, DRG and TRG and CNS-associated nerves; and P8: Reduction in weight of fatty tissues during degreasing. The results were consistent when applying different methods for the sensitivity analysis: inputs ranked by the effect on the output mean, regression coefficients and correlation coefficients.

The two files required to reproduce P27 and P28 of the bovine model in the Monte Carlo tool (risk assessment using Monte Carlo) of the EFSA's Shiny proxy open source platform can be accessed in the following link: https://doi.org/10.5281/zenodo.12188658

New case in small ruminants × bovine infectivity

TABLE 12 Mean, median and 5th and 95th percentiles of P29 and P30 of the ovine model with BSE bovine infectivity (P6 of the bovine model).

Code and description	5%	50%	Mean	95%
P29: Probability of infection per meal per animal	2×10^{-7}	2×10^{-6}	1×10^{-5}	3×10^{-5}
P30: Number of animals infected from that batch	0.006	0.061	0.18	0.725

According to the results of P30 of the ovine model with bovine BSE infectivity, in 96% of the iterations, the number of new BSE cases generated in small ruminants, due to exposure to an infected batch containing all the bones of one BSE-infected bovine animal, is below 1.

New case in small ruminants × ovine infectivity

TABLE 13 Mean, median and 5th and 95th percentiles of P29 and P30 of the ovine model with BSE ovine infectivity (P8 of the ovine model).

Code and description	5%	50%	Mean	95%
P29: Probability of infection per meal per animal	1×10^{-5}	1.1×10^{-4}	3.6×10^{-4}	1.47×10^{-3}
P30: Number of animals infected from that batch	0.251	2.821	9.096	38.155

According to the results of P30 of the ovine model with ovine BSE infectivity, in 25% of the iterations, the number of new BSE cases generated in small ruminants, due to exposure to an infected batch containing all the bones of one BSE-infected ovine animal, is below 1.

The results of the sensitivity analysis showed that outputs P29 and P30 of the ovine model were strongly affected by the uncertainty associated with, in decreasing order, P11: Reduction of infectivity due to the processing of bones; followed by P8: BSE infectivity in brain, spinal cord, DRG and TG; and P10: Reduction in weight of fatty tissues during degreasing. The results were consistent applying different methods for the sensitivity analysis: inputs ranked by the effect on the output mean, regression coefficients and correlation coefficients.

The two files required to reproduce P29 and P30 of the ovine model in the Monte Carlo tool (risk assessment using Monte Carlo) of the EFSA's Shiny proxy open source platform can be accessed in the following link: https://doi.org/10.5281/zenodo.12188658

3.10 | Uncertainty analysis

Apart from the sources of uncertainty (presented in Section 3.6) related to the assumptions and factors included in the model and that can affect the response to AQ1-AQ4, other sources of uncertainty were identified, not related to the model outputs, and described in the table below.

TABLE 14 Sources and causes of uncertainty, and impact on the conclusions not described in the model.

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions
Different characteristics of C-, H- and L- BSE agents	• The total BSE infectivity present in the brain, spinal cord and dorsal root ganglia of an infected animal may vary from one animal to other, and depending on the BSE strain (C, H or L). • Almost no information is available on tissue infectivity titre for atypical (H- and L-) BSE agents. There is also no data with which to estimate the $CoID_{50}$ for H-BSE and L-BSE, or even any evidence that disease can be transmitted via this route. There is, however, experimental evidence in transgenic models that some transmission using H-BSE and L-BSE can result in a disease indistinguishable from C-BSE in the recipient animal. Therefore, for the purpose of this assessment, H-BSE and L-BSE are considered to behave in the same way as C-BSE. • There is very little information on the tissue distribution of abnormal PrP in naturally occurring cases of either classical or atypical BSE. Experimental studies suggest that tissue distribution is more restricted in atypical (H- and L-) BSE, with the infectivity being confined to the nervous system, and not detectable in tissues associated with the lymphatic system, so the assumption that tissue distribution in all cases follow the pattern of C-BSE is a 'worst-case' scenario • For this opinion, it has been assumed that C, H and L-BSE are all the same with regard to transmissibility and risk, and C-BSE used as the default because it is the only one for which there is relevant data. It is known that species susceptibility is different between different strains.	• This may lead to an overestimation or underestimation of the BSE infectivity by the model. • This may lead to an overestimation of the BSE infectivity by the model, as an infected animal with H-BSE and L-BSE may contain less infectivity, which might not be easily transmitted through food or feed. • This may lead to an over- or under-estimate of how efficiently C, H and L BSE can transmit to different hosts, relative to one another • Lack of data leads to an overestimation of tissue infectivity, particularly in the context of lymphoid tissues
BSE infectivity estimates	• $CoID_{50}$ in tissues of bovines with BSE are not measured directly by bioassay in cattle but are derived indirectly from experimental ID_{50} measured in rodents. The transformation of experimental ID_{50} values into $CoID_{50}$ values is based on few/single experiments. • $CoID_{50}$ in tissues of ovines with BSE were derived from SA_{50} measured in vitro by PMCA. The extent to which in vitro seeding activity and in vivo oral infectivity are correlated is largely unknown due to lack of experimental data in the literature. • Infectivity titres in tissues of small ruminants with BSE have been obtained from a single experiment with experimentally infected sheep, due to lack of data from natural cases. • Infectivity titres in the CNS of sheep with BSE have been estimated based on measurements available from a few specific areas rather than the whole CNS.	• This may lead to over- or under-estimation of the $CoID_{50}$ in tissues from bovines with BSE used as input in the model. • This may lead to over- or under-estimation of the $CoID_{50}$ titres in ovine CNS used as input in the model. • This may lead to over- or under-estimation of the $CoID_{50}$ titres in ovine CNS used as input in the model. • This may lead to over- or under-estimation of the $CoID_{50}$ titres in ovine CNS used as input in the model.
Inactivation of different BSE agents	The actual effect that the acid/alkaline/autoclaving conditions may have on the reduction of the infectious titre of H- and L-BSE, as compared with C-BSE and ovine-passaged H-, L- and C-BSE, could differ. Indeed, the strains involved in naturally occurring scrapie or BSE can display very different resistance/sensitivity to decontaminating treatments (e.g. autoclaving or chemical inactivation). The resistance/sensitivity of C-BSE, H-BSE and L-BSE prion strains to the inactivation treatments used for C&G production may vary substantially from that observed using rodent adapted strains. The processes involved in the manufacture of C or G might lead to alterations in the PrP^{Sc} that could result in altered biological activity. This would also apply to recycling of agents through different hosts.	This may lead to an overestimation or under-estimation of BSE infectivity in gelatine produced from bones of an infected animal

TABLE 14 (Continued)

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions
Risk pathway of collagen and gelatine used for human food consumption	Collagen and gelatine can be present in a wide variety of foods and food supplements. Very little information is available in the published literature or elsewhere in relation to the following questions: – What are the species of origin of collagen and gelatine used in foods for human consumption and food supplements? – What specific foods contain collagen and/or gelatine? – What is the amount of collagen and/or gelatine present in those foods? – What number and diversity of products might contain collagen or gelatine from a single batch? – What is the total quantity of foods containing collagen and/or gelatine on the market? – What are the consumption levels by individuals consuming products containing collagen and/or gelatine? – In relation to imports of collagen and gelatine from third countries or raw materials for producing those products, what is the true prevalence of BSE in those countries?	This may lead to an overestimation or an underestimation of human exposure to BSE through consumption of food containing contaminated gelatine derived from bovine, ovine or caprine material to humans. On the other hand, the use of values of maximum consumption of gelatine by age category in the EU represents a WCS and may lead to an overestimation of exposure.
Risk pathway for ruminants from collagen and gelatine in feed	Currently, collagen and gelatine are not routinely used in the feed of ruminants for economic reasons and because of the uncertain nutritional value of those products. Collagen and gelatine for human use are occasionally re-categorised and fed to livestock. While the GME is of the view that this practice is very unlikely, the true extent to which it occurs and the livestock species to which re-categorised collagen and gelatine are fed is not known. Also, there is a possibility that collagen and gelatine may be used as a feed for livestock to a greater extent in the future and that these materials may be produced from Category 3 animal by-products but the extent to which this might occur is unknown. Collagen is occasionally incorporated into pet food. The extent to which this happens is not clear. Very little information is available in the published literature or elsewhere in relation to species of origin of the collagen and gelatine, the specific pet food products that contain collagen and/or gelatine, the quantity of collagen and/or gelatine in those products, or the total quantity of pet food containing collagen and/or gelatine. While it seems unlikely that cross-contamination of ruminant feed with pet food containing collagen or gelatine would occur to any great extent, this is not clear. Since 2021, collagen and gelatine derived from ruminants may be fed to non-ruminants. This change in the legislation was introduced in order to allow former foodstuffs containing collagen and/or gelatine to be utilised as livestock feed. The extent to which former foodstuffs containing collagen and/or gelatine are used as feed for livestock and the levels of collagen and/or gelatine in these non-ruminant feeds are unknown. Also, the level of cross-contamination of ruminant feed with non-ruminant feed containing collagen and/or gelatine is unknown. Gelatine may be used in livestock feed as a technological additive to encapsulate vitamins and gelatine and collagen peptides are used as supplements for dogs and horses. While there is no evidence that ruminants are fed technological additives or feed supplements, there are several gaps and uncertainties as regards their use.	The uncertainties in relation to the use of collagen and gelatine for livestock feed may lead to an overestimation or an underestimation of the BSE risk for ruminants. Greater use of collagen and gelatine in ruminant feed in the future would increase the risk. On the other hand, the estimated consumption of gelatine in compound feed (0.3 kg per day per cow) represents an unrealistic WCS and leads to an overestimation of the risk to ruminants

(Continues)

TABLE 14 (Continued)

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions
Impact of other processes applied during the use of collagen & gelatine on BSE infectivity or on the structural properties of PrP^{Sc} aggregates	• It is not known if the technological processes applied during manufacture of food/feed/cosmetic/medical/other products containing collagen or gelatine reduces the level of BSE infectivity. • It is not known if the steps involved in the production of C&G or in other processes applied during manufacture of products containing collagen or gelatine could affect the native structural features of individual PrPSc aggregates, either by breaking/disassociating large aggregates into smaller ones or by merging of small aggregates into larger ones. These factors might potentially affect the number of individual PrPSc aggregates (the infectious particles) by increasing or decreasing them in the gelatine-containing products compared to their number in input tissues	• This may result in a lower level of infectivity in any BSE contaminated product and an overestimation of the risk • This may lead to over- or under-estimation of the $CoID_{50}$ in the gelatine.
Dose–response relationship of BSE infectivity expressed via $CoID_{50}$	There is uncertainty about the linear dose–response relationship of BSE infectivity expressed via $CoID_{50}$ The probability of BSE infection at very low doses is not known from experimental studies. Two conceptual approaches have been proposed: (i) the existence of a threshold dose under which no infection occurs (need for cooperativity of infectious particles); (ii) absence of a threshold dose, with risk of infection decreasing linearly even at very low doses. The few available studies indirectly support the absence of a threshold dose; therefore it was assumed that there is no threshold dose for BSE	This may lead to overestimation of the BSE risk for ruminants exposed at very low doses
Cumulative versus single exposures	There are no data on how the probability of infection could increase or not in proportion to the number of times the same individual is exposed, i.e. if maximum exposure is better represented by cumulative exposure or by a single exposure event. Due to the lack of data, in this assessment, the maximum exposure in a single meal has been estimated	This may lead to under-estimation of exposure to BSE in C&G
Individual susceptibility of small ruminants to BSE	It is known that the susceptibility of small ruminants to scrapie largely depends on the PrP genotypes and that some genotypes confer resistance to scrapie. Although different susceptibilities depending on PrP genotypes have been observed in small ruminants experimentally infected with BSE, there are not enough data to conclude about absolute resistance of small ruminants with specific PrP genotypes to BSE. In this opinion, it was therefore assumed that all small ruminants are equally susceptible to BSE	This may lead to overestimation of the probability of a new BSE case in small ruminants exposed to contaminated C&G
Incubation period of BSE	BSE is characterised by several years of incubation time before the development of disease, particularly upon low dose exposures. Therefore, animals exposed to effective doses of BSE might not develop the disease during their productive life. In this opinion, it was assumed that any effective BSE exposure would result in clinical disease within the lifespan of the exposed animals (Padilla et al., 2011)	This may lead to overestimation of the probability of a new BSE case exposed to contaminated C&G
Transmission barrier of BSE in small ruminants	It was assumed there is no species barrier when estimating the infectivity to bovine animals using ovine BSE and vice versa. It is unknown if this is accurate	Although this may not be the worst-case scenario due to the lack of data on the multiple factors associated to the species barrier, the risk would have been overestimated
Infected animals vs. generating a case	Not all animals are tested as part of national surveillance schemes. For example, in the EU only cattle showing clinical signs and three other target groups (emergency slaughter, clinical at ante-mortem inspection and fallen stock) are mandated for testing. Elsewhere surveillance may be limited to clinical animals only. The test for BSE is also not 100% sensitive with the highest probability of a test positive in the last 3 months of the incubation period	This may lead to overestimation of the number of cases of BSEs

3.11 | Further considerations and integration of evidence

3.11.1 | Epidemiological considerations

The TSE surveillance in EU member states is regulated by the Regulation (EC) No 999/2001. In the case of cattle, it is based on the compulsory testing of: (a) animals clinically suspected of being infected by BSE with no age limit; (b) emergency slaughtered animals older than 24 or 48 months of age, depending on the country; (c) animals with clinical signs at ante-mortem older than 24 or 48 months of age, depending on the country; (d) animals found dead on farm (fallen stock older than 24 or 48 months of age); (e) healthy slaughtered animals older than 30 months of age (applies only to Bulgaria and Romania). For more details, the EU summary report on TSE can be consulted. The latest edition was published in 2023 (EFSA, 2023). The EU summary report includes data from the 27 Member States (MS, EU27), the United Kingdom (in respect of Northern Ireland), plus eight non-EU reporting countries: Bosnia and Herzegovina, Iceland, Montenegro, North Macedonia, Norway, Serbia, Switzerland and Turkey. Compulsory testing of cattle in the EU began in 2001, using rapid tests carried out on dead animals where the results were known within 24 h. The level of surveillance reached a peak in 2004 when over 11 million cattle were tested. It has been reduced in recent years due to the improving epidemiological situation leading to legislative changes like the discontinuation of the testing of healthy animals slaughtered for human consumption. In the region, one million cattle have been tested annually for BSE in the EU since 2015.

In Europe, the most recent case of C-BSE was reported in Scotland in 2024. In the last 10 years, single cases of C-BSE were reported in 2014, 2015, 2018, 2021 and 2024 by the UK, in 2016 by France and in 2015 by Ireland. With regard to atypical BSE, in the last 10 years, there have been 24 cases of H-type and 20 of L-type in the EU and non-EU reporting countries according to EFSA's EU summary report on TSE (EFSA, 2023).

The declining trend of C-BSE is shown by prevalence data from active surveillance (i.e. the proportion of cases among the cattle subjected to rapid testing): an annual decrease of 36% ($p < 0.01$) has been observed over the 2013–2022 period, whereas the proportion of cases per number of tested animals of the two atypical BSE forms has remained stable (EFSA, 2023).

The total number of infected cattle with C-BSE, L-BSE and H-BSE strains combined, that may escape the surveillance monitoring in a single year was estimated to be 11.38 (2.75th–97.5th percentiles: 3.6–19.8) (EFSA BIOHAZ Panel, 2018), across the whole of the EU. This number is likely to have decreased in the last few years due to the improvement of the epidemiological situation of the disease.

In 2023, there were 45,329,020 cattle slaughtered in the EU27 + UK (adult cattle, beef and young cattle).[27] In total, about 21% of the gelatine in Europe is estimated to be produced from bovine raw material, two-thirds of which is produced from bovine bones specifically and mainly from the EU and the UK (Vermeulen, 2024 17 April 2024)). That means approximately 14% of all the gelatine and collagen peptides produced in Europe (120,000–125,000 tons per year) are derived from bovine bones coming from the EU and the UK (i.e. 16,800–17,500 tons). With a yield of 2.436 kg of gelatine per adult bovine animal according to the model (4% of all the bones of an adult bovine), the bones of approximately 7 million bovine animals would have been used to produce gelatine, i.e. 15% of the total number of cattle slaughtered in the EU27 and the UK. Considering these figures, it is plausible that the bones of an infected BSE case escaping surveillance could have been incidentally used for the production of gelatine in 2023. However, it is extremely unlikely that bones of two BSE-infected animals ended up in the same batch.

TSE surveillance in sheep and goats in the EU is based on the testing of (a) animals culled under TSE eradication measures over 18 months of age; (b) animals not slaughtered for human consumption older than 18 months of age; (c) healthy animals slaughtered for human consumption older than 18 months of age, (d) animals clinically suspected of being infected by BSE with no age limit. Minimal sample sizes for each MS, based on the sheep and goat populations of the MS, are set out in Annex III of the TSE Regulation. Since 2002, more than 10.8 million sheep and goats have been tested as part of the official EU TSE surveillance programme. According to the figures provided in the 2022 EU Summary Report (EFSA, 2023), 320,680 sheep and 109,707 goats were tested in 2022. As mentioned in Section 3.4.3, two field cases of BSE in goats have been reported retrospectively (Eloit et al., 2005; Spiropoulos et al., 2011), and there have not been reported cases of ovine BSE.

The surveillance programme in the EU to look for C-BSE in small ruminants is based on further analyses of all samples positive for TSE with a set of discriminatory methods that allow to suspect, and eventually confirm by bioassay, the possible presence of C-BSE infections in small ruminants.

There are little or no publicly available data on surveillance of TSE in cattle in third countries. In many countries, the level of surveillance has been determined by the requirements of the WOAH for the recognition and/or maintenance of the negligible or controlled risk status, as per Article 11.4.20 of the Terrestrial Code.[28] Currently, 53 members are categorised as negligible BSE risk by the WOAH, including all EU Member States, except Greece, and Argentina, Australia, Bolivia, Brazil, Canada, Chile, Colombia, Costa Rica, Iceland, India, Israel, Japan, Korea (Rep. of), Liechtenstein, Mexico, Namibia, New Zealand, Nicaragua, Norway, Panama, Paraguay, Peru, Serbia, Singapore, Switzerland, the United States of America and Uruguay. A further four countries are categorised as controlled BSE risk: Chinese Taipei, Ecuador, Greece and Russia. Two members are recognised as having zones with negligible BSE risk: the People's Republic of China with the exclusion of Hong Kong and Macau and Northern Ireland in the UK. The remaining parts of the UK are recognised as having a controlled

[27] https://ec.europa.eu/eurostat/databrowser/view/APRO_MT_PANN/default/table?lang=en.

[28] https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_bse.htm.

BSE risk. The WOAH does not mandate testing quotas of sheep and goats for TSE but the requirements for the recognition of the disease-free status from scrapie requires a formal programme of targeted surveillance and monitoring, which includes testing of sheep and goats displaying clinical signs compatible with scrapie and those over 18 months of age slaughtered, culled or found dead on farm, according to Article 14.8.3 of the Terrestrial Animal Health Code.

The WOAH surveillance requirements changed in 2023 with the approval by the general assembly of the new BSE chapter. According to the new provisions, 'the objective of BSE surveillance is to detect occurrence of BSE within the bovine population'. The surveillance groups targeted are: (a) those displaying progressive clinical signs suggestive of BSE that are refractory to treatment, and where the clinical presentation cannot be attributed to other common causes of behavioural or neurological signs (e.g. infectious, metabolic, traumatic, neoplastic or toxic causes); (b) those showing behavioural or neurological signs at ante-mortem inspection at slaughterhouses/abattoirs; (c) those unable to rise or walk without assistance, with an appropriate supporting clinical history (i.e. the clinical presentation cannot be attributed to other common causes of recumbency); (d) those found dead (fallen stock), with an appropriate supporting clinical history (i.e. the clinical presentation cannot be attributed to other common causes of death). The new system does not require a quota, or a minimum number of samples tested per year. This is unlike the previous system where 'point values' were assigned to each sample, based on the subpopulation from which it was collected and the likelihood of detecting infected cattle in that subpopulation. Depending on the status of the country, the level of surveillance aimed to detect BSE at a design prevalence of at least one case per 100,000 or 50,000 for the Type A and B, respectively, in the adult cattle population in the country, zone or compartment of concern, at a confidence level of 95%.

The surveillance data submitted to the WOAH as part of the dossier for the application for the recognition of the risk status by country members are not disclosed. Thus, the level of surveillance conducted by third countries is not publicly available or is hard to retrieve. There is a need to search at country level to ascertain the surveillance design and throughput. The website of the United States Department of Agriculture states that 'our current testing of approximately 25,000 targeted animals a year allows USDA to detect BSE at the very low level of less than 1 case per million adult cattle, assess any change in the BSE status of U.S. cattle, and identify any rise in BSE prevalence in this country'.[29] According to the Government of Canada website, the number of cattle tested for BSE in Canada in the last 10 years ranged between 16,262 in 2023 and 30,894 in 2018.[30] The website states that 'the level and design of this enhanced program continues to be in full accordance with the guidelines recommended by the WOAH'.

The following cases of BSE have been reported by third countries to the WOAH in the last 10 years:

- C-BSE: Canada (2015)
- H-BSE: Brazil (2023, 2021, 2021, 2019, 2014), Canada (2021), USA (2018), Norway (2015)
- L-BSE: USA (2017).

Unlike the EU, there is limited information in the public domain on the existence of surveillance programmes aimed to specifically identify C-BSE cases among TSE cases in small ruminant populations. This lack of evidence may be due to the absence of C-BSE in small ruminant populations. However, the absence of specific surveillance programmes makes it impossible to exclude its presence outside the EU. Therefore, it remains unknown whether C-BSE is present in small ruminants outside the EU, and if so, its prevalence and distribution.

In conclusion, there has been a very significant reduction in the number of cases of BSE in European countries with the most recent case being confirmed in 2024. There is a high level of surveillance in the EU with ~ 1 million cattle being tested annually. While some testing for BSE is being carried out in non-European countries and while 30 non-European countries have achieved negligible or controlled risk status under the WOAH requirements, there is still uncertainty in relation to the presence or absence of BSE in the cattle populations of non-European countries and, if present, what the prevalence might be.

3.11.2 | The use of multiple worst-case scenarios

A QRA was developed to practically address elements of the ToR as described in Section 2.2.2. A model framework, similar to that created for other EFSA Opinions, has been defined based on the quantitative data available, some of which for the ovine model is unpublished. Several maximum estimates and worst-case scenarios (WCS) were assumed to enable the use of the data available. As a result, model estimates are likely to be a significant overestimate of the risk with the key WCS highlighted as follows.

The main assumption of the model is that all the bones of an infected bovine/ovine animal at the clinical stage of the disease would be used to produce gelatine. The probability that a BSE-infected bovine animal is devoted to the production of gelatine has to be put into the perspective of the slaughtered cattle population in the EU, the throughput of gelatine produced from indigenous cattle and the number of cases that could trickle down the food and feed chain.

The probabilistic model applied considers the strong acidic treatment as the only processing step providing a BSE reduction factor, but as already acknowledged in the model assumptions other steps in the production of gelatine, such as washing, demineralisation, purification and concentration via filtration, salt removal or UHT sterilisation, may also

[29] www.usda.gov/topics/animals/bse-surveillance-information-center.

[30] https://inspection.canada.ca/animal-health/terrestrial-animals/diseases/reportable/bovine-spongiform-encephalopathy/enhanced-surveillance/eng/1323992647051/1323992718670.

contribute to further reduction in BSE infectivity. For example, as stated in the 2020 EFSA opinion, the UHT treatment applied for the sterilisation of the gelatine extract was estimated to provide a higher than 2.2 $\log_{10}$ reduction; and the ultrafiltration and nanofiltration were shown to provide a reduction of TSE infectivity, variable according to the type of filter used. Moreover, other technological processes applied during the manufacture of food/feed products containing gelatine may also provide some level of reduction of BSE infectivity not accounted for in this assessment. If the reduction in all these technological processes were considered, the level of residual infectivity in a batch produced including all the bones of an infected animal could be significantly reduced by several $\log_{10}$.

The fact that the concentration of BSE infectivity in the gelatine of an infected batch is calculated using a small size batch (~ 755 kg of cattle bones) is another WCS, that maximises the estimated number of $CoID_{50}$ per kg of gelatine. If a large batch (bones of 3230 cattle) was considered, the level of infectivity would be reduced by at least 1 $\log_{10}$, with all batch sizes in between giving reductions between 0 and at least 1 $\log_{10}$.

The general assumption for the exposure assessment is that all gelatine is produced from bovine bones, when in fact it would most likely be mixed in unknown proportions with gelatine obtained from other raw material, bovine or from other species. The assumption that the total daily protein requirement of an adult cow is included in feed in the form of pure gelatine is unrealistic from the economic point of view. It is also unrealistic to assume that all the protein is sourced from a batch including one BSE-infected animal. Moreover, the probability of a new case of infection in ruminants through consumption of contaminated feed was estimated considering that there is no threshold dose below which infection cannot occur. If that assumption is erroneous, the exposure to low doses of BSE infectivity would result in a lower risk of infection than the one estimated in this assessment. Equally, in terms of human exposure, the probability that all gelatine-containing food consumed by different age categories includes gelatine from an infected batch is extremely low, and if exposure did occur, the amount of infectivity would be much lower than estimated.

Even with all these worst-case scenarios, in 87% and 96% of the iterations, the number of new BSE cases generated in bovine and small ruminants, respectively, from exposure to an infected batch containing all the bones from one BSE-infected bovine animal was estimated to be lower than 1, and the maximum exposure of humans in one single event was estimated to be below 1 $CoID_{50}$. In a more realistic scenario, the amount of infectivity to which animals and humans could be exposed in a single event would be much lower.

In the case of gelatine made from bones from small ruminants, the uncertainties are higher and the data available are more limited. Thus, a hypothetical model was developed for sheep using the same framework and scenarios as for cattle, with real ovine data where it was available and cattle data when it was not. Data on tissue infectivity do not exist for caprine BSE, while for ovine BSE only one, yet unpublished, study was available, which suggests that BSE infectivity in tissues of adult infected ovine animals may be higher than in cattle tissues. Considering these higher BSE infectivity values, the exposure doses and risks estimated in the hypothetical ovine scenario were higher than for gelatine of bovine origin. In fact, in this case, in 7% and 25% of the iterations, respectively, the number of new BSE cases generated in bovine and small ruminants was estimated to be lower than 1, and the maximum amount of infectivity that a human would be exposed to in one single exposure event (daily consumption) if all the gelatine they consumed was sourced from an infected batch was estimated to be 1.1×10^{-4} $CoID_{50}$, assuming that the infectivity was uniformly dispersed in gelatine; or 4.06 $CoID_{50}$ if the BSE infectivity was clumped in a single exposure event. Nevertheless, the same considerations made above on the worst-case scenarios and the overestimation of exposure and risk in the model also apply to gelatine of ovine origin, as it must also be taken into account that bones from small ruminants are not currently used in the EU for the production of gelatine for economic/logistic reasons, and that natural cases of BSE have never been reported in sheep, and only two field cases have been reported in goats.

As described in Section 3.7.4, the model assumed there is no species barrier when estimating the infectivity to bovine animals from exposure to the ovine BSE agent and vice versa. It is unknown if this is accurate. Although this may not be the worst-case scenario due to the lack of data on the multiple factors associated with the species barrier, it is likely that the overall probability of a new BSE case has been overestimated in the modelling simulations involving bovine to ovine transmission and vice versa.

Finally, it was assumed that every animal that is successfully infected following exposure to contaminated collagen or gelatine would go on to develop clinical disease. Under that scenario, the infected animal would be included in a national surveillance scheme such that a test would be carried out and subsequently, given the high sensitivity of the test, the animal would test positive and be considered a 'case'. Given that BSE has a long incubation period in all known natural host species, counted in years rather than months, where the incubation period can be higher than the slaughter age, a proportion of infected animals may die (natural lifespan) or be killed (commercial lifespan) before developing the disease. Not all animals are part of national surveillance schemes. For example, in the EU, only cattle showing clinical signs and three other target groups (emergency slaughter, clinical at ante-mortem inspection and fallen stock) are mandated for testing. Elsewhere surveillance may be limited to clinical animals only. The test for BSE is also not 100% sensitive with the highest probability of a test positive in the last 3 months of the incubation period for an infected cow.

In conclusion, the multiple worst-case scenarios, when combined, will significantly overestimate the infectivity present in the final product. Regardless of the presentation of the abnormal PrP in the gelatine, either fragmented/diluted or clumped in one single lump of infectivity, the quantification of the infectivity in the most likely scenarios would be substantially lower.

3.11.3 | Susceptibility

The C-BSE epidemic was sustained by the recycling of C-BSE infectivity through the use of meat-and-bone meal in cattle feed, resulting in significant oral exposure of cattle. The introduction of the feed ban was a key factor in the success of control strategies. Indeed, C-BSE is not transmitted horizontally in cattle and the main route of transmission is through contaminated feed. It is therefore clear that this is an additional aspect to be considered in this risk assessment. Even the remote possibility of a case of C-BSE being caused by the consumption of gelatine would not represent an epidemic risk as long as the SRM and feed ban regulations remain in place.

Although no cases of C-BSE in sheep have been reported so far, C-BSE can be transmitted orally in this species (Bellworthy et al., 2008; McGovern et al., 2016), supporting a very low or no species barrier (Padilla et al., 2011). Additionally, there is a possibility that C-BSE is contagious once passed into sheep, as suggested by the peripheral distribution of prions in this species (Jeffrey et al., 2001; van Keulen et al., 2008) and experimental transmission studies (Bellworthy, Dexter, et al., 2005). Consequently, the occurrence of a new case of C-BSE in sheep may have the potential to result in the emergence of further cases, regardless of whether infectivity is recycled through feed. It is however uncertain if the contagiousness would be enough to sustain the persistence of the disease (Jeffrey et al., 2015). Experimental data suggest that C-BSE does not lose its zoonotic capacity after passing through small ruminants. On the contrary, it acquires higher transmissibility in 'humanised' animal models (Padilla et al., 2011). In light of the aforementioned considerations, the circulation of C-BSE in sheep populations may represent a significant risk to human health.

Although the risk posed by residual BSE infectivity in gelatine appears to be extremely low in small ruminants, it is important to emphasise that even the remote possibility of a C-BSE case in sheep could potentially result in the recycling of infectivity in sheep populations. It is therefore recommended that every effort should be made to avoid exposure of sheep to C-BSE.

3.11.4 | Future uses of C&G

Currently gelatine is not used in feed. The scenario of estimating the probability of a new case in cattle and/or small ruminants very much depends on the possibility that legislative changes and/or economic drivers may result in the diversion of gelatine to the feed industry. With the current status, the exposure and consequent generation of a new case of BSE in cattle and/or small ruminants is not possible. It is difficult to foresee what will be the future uses of C&G upon changes in legislation, but the consumption of gelatine in compound feed (0.3 kg per day per cow) used in the probabilistic model to estimate the risk of new cases surely represents an unrealistic worst-case scenario, even in foreseeable future scenarios.

3.11.5 | Extrapolation to collagen

Ruminant bones are currently not used for the production of collagen in the EU, and according to the industry, this will not change in the future. However, there is some level of uncertainty in relation to this as, according to the literature, the main sources for collagen extraction are products from the slaughter of pork and beef, with the main products studied including the Achilles tendon, pericardium, inner layer of bovine skin, bovine bones, porcine skin and porcine lung (Schmidt et al., 2016). Other alternative sources mentioned are fish by-products and poultry slaughter waste. As regards the infectivity of collagen in comparison to gelatine, the model used in the 2020 EFSA opinion indicated that the final amount of infectivity per kg of collagen was more than 15 times lower than the estimate obtained for gelatine under the worst-case assumptions that there is no reduction in the level of infectivity during the processing of collagen, and that the yield from hides is higher for collagen than for gelatine. Nevertheless, the collagen extraction process also involves pretreatment and hydrolysis processes which possibly also have a (similar) capacity to result in a reduction in BSE infectivity. In fact, collagen can be basically obtained by chemical hydrolysis and enzymatic hydrolysis. Before the collagen can be extracted, a pretreatment is performed using an acid or alkaline process, which varies according to the origin of the raw material (Schmidt et al., 2016).

4 | ANSWERS TO ToRs

- There is no evidence of the use of ruminant bones for the production of collagen. For gelatine, a probabilistic model was developed to estimate the BSE infectivity produced by all the bones from one adult BSE-infected bovine animal at the clinical stage. This model showed that the median estimate of the number of $CoID_{50}$ in the gelatine containing the bones of one BSE-infected bovine animal (C, H or L) older than 30 months of age, at the clinical stage, is 0.26 $CoID_{50}$, (5th–95th percentile: 2.5×10^{-2} – 3.075) and the median number of $CoID_{50}$ per kg of a small size batch of gelatine (containing 755 kg of bovine bones) is 3.4×10^{-4} $CoID_{50}$ per kg (5th-95th percentile: 3.3×10^{-5}–4×10^{-3}).
- There is no evidence of the use of bones from small ruminants for the production of gelatine. The probabilistic model for bovine was adapted to ovine animals, with the use of indirect experimental data and the parameterisation of the cattle model (due to lack of data) in a hypothetical scenario where all the bones from one adult BSE-infected ovine animal was used for the production of gelatine. The results of the probabilistic model showed that the median estimate of the number of $CoID_{50}$ in the gelatine containing the bones of one BSE-infected ovine animal (C, H or L), at the clinical stage, is 4.06 $CoID_{50}$ (5th-95th percentile: 0.35–55.3), and the median number of $CoID_{50}$ per kg of a small size batch of gelatine (containing 755 kg of ovine bones) is 5.3×10^{-3} $CoID_{50}$ per kg (5th–95th percentile: 4.7×10^{-4}–7.3×10^{-2}).

- Given that worst-case scenarios were considered in some assumptions and that several input variables were approximated to worst-case estimates, the infectivity as reported above will have been overestimated by the models.

For oral exposure via food, the median amount of infectivity that a consumer could be exposed to in one single exposure event (daily consumption) if all the gelatine consumed was sourced from an infected batch, based on median values of infectivity and median values of consumption in the highest consumption group, was estimated to be between 7.1×10^{-6} $CoID_{50}$ and 0.26 (bovine BSE), and 1.1×10^{-4} and 4.06$CoID_{50}$ (ovine BSE) depending on whether it was assumed that the infectivity was uniformly distributed in gelatine or aggregated in a single exposure event, respectively. This is an overestimation of the exposure since it is based on worst-case model outputs and median consumption figures of the highest consumption group. Taking into consideration all factors related to the worst-case scenarios, in case of oral exposure, the dose in a single exposure event would be well below 1 $CoID_{50}$.

- Potential parenteral exposure routes to BSE-infected gelatine and collagen in humans (through cosmetic, medical and surgical products) were identified but not quantified in this assessment.
- For humans, exposure to infectivity cannot be directly translated to risk of disease because the transmission barrier has not yet been quantified. This transmission barrier is considered to be substantial, i.e. much greater amounts of infectivity would be needed to successfully infect a human than a bovine, or small ruminant and greater in the oral route than in the parenteral route of exposure.
- In this risk assessment, the BSE risk for ruminants has been quantified as the number of new BSE cases generated due to oral exposure to bovine/ovine-derived BSE infectivity via feed containing contaminated gelatine. The dose–response equation applied to the outputs of the model showed that in up to 87% and 96% of the iterations, the number of new BSE cases potentially generated in bovine and small ruminants, respectively, due to exposure to an infected batch containing all the bones of one BSE-infected bovine animal is below 1.
- In the hypothetical scenario where all the bones from one adult BSE-infected ovine animal are included in one small batch of gelatine, the results of the dose–response equation applied to the outputs of the model showed that in up to 7.9% and 25% of the iterations, the number of new BSE cases potentially generated in bovine and small ruminants, respectively, is below 1.
- Taking into account the multiple unlikely events that may lead to the potential exposure of cattle and small ruminants to BSE infectivity through consumption of gelatine in feed, the current lack of production of gelatine with small ruminant bones, the results of the model, the impact of the worst-case scenarios and the lack of evidence that the C-BSE agent might be circulating in the small ruminant population, it is concluded that the probability that no new case of BSE in the cattle or small ruminant population would be generated through oral exposure to gelatine made of ruminant bones is 99%–100% (almost certain).
- The conclusions are based on the current state of knowledge on the epidemiological situation of the disease in the EU and third countries (no reported cases of BSE in small ruminants and very low prevalence in cattle), and the current practices related to the production of collagen and gelatine. If any of these factors change, the conclusions of this assessment should be reviewed.
- Since collagen is not currently produced using ruminant bones, it was not possible to estimate the residual infectivity in collagen produced with infected bovine/ovine animals. The collagen extraction process involves pretreatment and hydrolysis processes which also have a similar capacity to produce a reduction in BSE infectivity. Therefore, the BIOHAZ Panel assumed in this assessment that the conclusions for gelatine are also valid for collagen.

5 | RECOMMENDATIONS

- To assess the transmission risk associated with BSE exposure by the parenteral route arising from the use of collagen and gelatine in cosmetic, medical and surgical products, should the production of collagen and gelatine be de-regulated.
- To periodically reassess the risks addressed in this opinion, should the relative plausibility and likelihood of the risk and exposure pathways change. The BSE risk posed by the use of ruminant C&G in food and feed directly depends on the prevalence of the BSE agents (C-BSE, H-BSE and L-BSE) in the cattle and small ruminant populations, the detection and destruction of BSE-infected animals and the exposure to collagen and gelatine.
- It is recommended to maintain the current EU-wide surveillance system in order to:

  (1) monitor the final stages of the BSE epidemic;
  (2) detect a potential re-emergence of BSE; and
  (3) detect new BSE forms in cattle or new cases of BSE in small ruminants, should they appear.

- To evaluate the impact of the specific industrial processes for the production of C&G on the infectivity of naturally occurring BSE agents.
- To assess the BSE risk associated with the uses of the waste generated through the degreasing process, which may contain infectivity, should the skull and vertebral column of an infected carcass enter the process.
- To undertake research activities aimed at the production of new data regarding the susceptibility of cattle to infection with H-BSE or L-BSE via the oral route, and the quantitative distribution of infectivity in tissues of cattle preclinically and

clinically affected with H- and L-BSE. The current lack of such data poses a major limitation for the assessment of the risk associated with the exposure to prion disease via cattle-derived materials in food and feed. It is acknowledged that these research activities are not currently feasible due to the lack of material for challenges by the oral route.

ABBREVIATIONS

AQ	Assessment question
BIOHAZ	Biological Hazards
BSE	bovine spongiform encephalopathy
CCTA	Collagen and Casings Trade Association
C&G	Collagen and gelatine
CNS	central nervous system
$CoID_{50}$	Cattle oral infectious dose 50
CW	Compton-White
DRG	Dorsal root ganglia
EMA	European Medicines Agency
FDA	Food and Drug Administration (USA)
GALT	Gut-associated lymphoid tissue
GM	Gelatine microgel
GME	Gelatine Manufacturers of Europe
IC	intracerebral
ID	Infectious dose
IM	intramuscular
IV	intravenous
IP	intraperitoneal
LD	lethal dose
LRS	lymphoreticular system
PMCA	Protein Misfolding Cyclic Amplification
PRNP	prion protein gene
PrP	prion protein
PrP^{C}	cellular prion protein
PrP^{res}	PK- resistant prion protein
PrP^{Sc}	abnormal isoform of the cellular prion protein
RA	Risk assessment
QRA	Quantitative risk assessment
RF	Reduction factor
SA	Seeding activity
SCCP	Scientific Committee on Consumer Products of the European Commission
SCCS	Scientific Committee of Consumer Safety
SD	sedimentation velocity
SRM	Specified risk material
SSC	Steering Scientific Committee
SVC	Scientific Veterinary Committee
TG	trigeminal ganglia
ToR	terms of Reference
TSE	transmissible spongiform encephalopathy
UHT	Ultra-high temperature
USDA	United States Department of Agriculture
WB	Western blot
WCS	Worst case scenario
WG	working Group
WOAH	World Organisation for Animal Health
WR	weighted risk

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

C-BSE agent quantification in the central nervous system of affected sheep

Infectious material was derived from C-BSE affected ARQ/ARQ sheep (IC inoculated with cattle BSE agent). Twenty-four-hour-old lambs (ARQ/ARQ [$n=6$] and ARR/ARR [$n=10$]) were exposed to a dose of 0.5 g infected brain through natural suckling. A second dose of inoculum was administered by the same route at 14 days old. The results of this experiment are not published (Andreoletti (2023) by email, 25 November 2023, and Andreoletti (2024) 3 February 2024).

Some animals were killed at pre-determined time-points post-inoculation, and 5 animals (2 ARQ/ARQ; 3 ARR/ARR) were kept until the onset of clinical disease and were killed when showing locomotor difficulties (ARQ/ARQ: 20 mpi, ARR/ARR: 50-51 mpi). At necropsy the brain and spinal cord, and a panel of lymphoid tissues, were collected.

Western blot (WB) was used to confirm the presence of proteinase K resistant abnormal Prion protein (PrP^{res}) in the posterior brain stem of each animal. The PrP^{res} WB banding profile displayed typical features associated with BSE agent in sheep including a 19kDa apparent molecular weight of the non-glycosylated band, a dominant bi-glycosylated PrP^{res} band and an absence of immune-reactivity to the 12B2 monoclonal anti PrP antibody.

A Protein Misfolding Cyclic Amplification (PMCA) protocol (Saborio et al., 2001) that was specifically optimised for the detection of C-BSE agent in sheep (Huor et al., 2019) was used to measure the levels of prion seeding activity in different CNS areas (obex, cervical spinal cord, thoracic spinal cord and lumbar spinal cord), collected from the C-BSE orally challenged animals at the clinical stage of the disease. Ten-fold dilution series of the 10% homogenates from the collected samples were prepared and subjected to two rounds of PMCA. Six individual replicates of each sample and each dilution were tested. The presence of PrP^{res} in each amplification product was established by Western Blot. These results were then used to estimate the seeding activity (SA_{50}/g of tissue) in the original sample using the Spearman–Karber's approach. The amplification of a 10-fold serial dilution of the same sample (12 individual replicates per dilution point) demonstrated that two PMCA rounds (24 h per round i.e. 48 h) were sufficient to reach the maximal sensitivity level of the assay. Additional PMCA rounds improved neither the analytical sensitivity of the assay nor the number of positive replicates (Huor et al., 2019). The seeding activity (SA) of the isolate was estimated by the Karber's method to be 1010.13 SA_{50} per mL.

In order to use the titres measured in C-BSE affected sheep in the model used in this opinion it is necessary to convert seeding activity titres as measured by the PMCA (as SA_{50}/g of tissue) to Cattle Oral ID_{50} ($CoID_{50}$).

Initially The SA_{50} as measured by PMCA was converted into ID_{50} ic (TgBov) by conducting parallel end-point titrations of a reference sample (10% cerebral cortex homogenate from an ARQ/ARQ BSE affected sheep) (IC inoculation route –Table 2) by ic bioassay in bovine PrP expressing mice (TgBov) and by PMCA (Table A.1).

TABLE A.1 End point titration of BSE in sheep reference isolate by bioassay in bovine PrP expressing mice (tgBov) and Protein Misfolding Cyclic Amplification (PMCA).

	Bioassay tgBov	PMCA-positive reactions	
Sheep passaged C-BSE isolate	**Positive mice**	**Incubation period (days ± SD)**	**TgARQ substrate**
Neat	6/6	223 ± 4	ND
10^{-1}	6/6	250 ± 9	ND
10^{-2}	6/6	290 ± 12	12/12
10^{-3}	6/6	338 ± 18	12/12
10^{-4}	6/6	386 ± 38	12/12
10^{-5}	5/6	486 ± 96	12/12
10^{-6}	1/6	402*	12/12
10^{-7}	0/6	> 700	10/12
10^{-8}	0/6	> 700	5/12
w10^{-9}	ND		1/12
10^{-10}	ND		0/12
10^{-11}	ND		0/12

Incubation periods (in days) are presented as mean±SD, except for those marked (*) indicating dilutions in which less than half of the mice were scored as positive

The infectious titre was estimated to be approximatively equivalent to 106.2 ID_{50}/mL IC in tgBov by the Karber approach (Markus et al., 1995). Taking into account the 4-fold lower amount of material used to seed the PMCA reaction compared to the material used in mouse inoculations, these results indicate that the PMCA protocol used was ~ 1500 times more sensitive (3.18 $\log_{10}$) than bioassay in tgBov mice for the detection of ovine c-BSE prions.

The ID_{50} IC in TgBov then needs to be converted into ID_{50} IC in cattle (Table A.2). The conversion factor used in this study has been based on data from several published studies (carried out using different prion strains and species) which have demonstrated that the infectious titres measured by endpoint titrations of the same brain homogenate samples in natural

hosts and in PrP over-expressing mouse models (i.e. homologous for *PRNP)* were similar. For example, sheep versus mice over-expressing PrP (Douet et al., 2014), mice over-expressing PrP versus conventional mice (Thackray et al., 2002), and transgenic hamster versus conventional hamster (Peretz et al., 2006). These studies support a conclusion that it is reasonable to assume that 1 ID_{50} IC as measured in bovine PrP over-expressing mice (TgBov) is equivalent to 1 ID_{50} IC as measured in bovine animals.

TABLE A.2 Estimated titre of BSE infectivity expressed as log_{10} of $CoID_{50}$/g before correction factor (mean and standard deviation).

	ARQ/ARQ clinical		ARR/ARR clinical		
	Sheep 1	**Sheep 2**	**Sheep 3**	**Sheep 4**	**Sheep 5**
Obex	12.97 ± 0.33	11.13 ± 0.41	11.8 ± 0.46	11.63 ± 0.53	12.3 ± 0.53
Cervical spinal cord	10.63 ± 0.33	10.97 ± 0.53	9.13 ± 0.41	10.3 ± 0.44	8.3 ± 0.53
Thoracic spinal cord	9.47 ± 0.41	12.13 ± 0.41	11.47 ± 0.41	12.47 ± 0.41	10.8 ± 0.46
Lumbar spinal cord	10.47 ± 0.41	12.3 ± 0.44	9.97 ± 0.53	11.13 ± 0.41	9.13 ± 0.55

Finally, there needs to be conversion from cattle ID_{50} IC into $CoID_{50}$. This conversion factor is based on previous parallel studies in cattle and mice, which have already been described in detail in Section 3.4.1. Based on these studies 1 oral ID_{50} in cattle ($CoID_{50}$) has been considered equivalent to $10^{5,5}$ IC ID_{50} in cattle for this conversion This value has been used previously in various EFSA opinions quantifying the risk transmission in cattle orally exposed to C-BSE prions (EFSA BIOHAZ Panel, 2014a, 2020). When considered together these elements indicate that 1 $CoID_{50}$ can be considered to be equivalent to $10^{8,68}$ SA_{50} as measured by PMCA.

It is important to remember that these estimations rely on limited amounts of data which might affect the reliability that can be attributed to the computed converting factor.

Based on the SA_{50} to $CoID_{50}$ converting factor the infectious titres in the different CNS samples collected in the C-BSE affected sheep can be estimated as shown in Table 14.

ANNEX A

Protocol

Annex A is available under the Supporting Information section on the online version of the scientific output.