SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 7 June 2018

doi: 10.2903/j.efsa.2018.5314

Updated quantitative risk assessment (QRA) of the BSE risk posed by processed animal protein (PAP)

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Abstract

EFSA was requested: to assess the impact of a proposed quantitative real-time polymerase chain reaction (qPCR) 'technical zero' on the limit of detection of official controls for constituents of ruminant origin in feed, to review and update the 2011 QRA, and to estimate the cattle bovine spongiform encephalopathy (BSE) risk posed by the contamination of feed with BSE-infected bovine-derived processed animal protein (PAP), should pig PAP be re-authorised in poultry feed and vice versa, using both light microscopy and ruminant qPCR methods, and action limits of 100, 150, 200, 250 and 300 DNA copies. The current qPCR cannot discriminate between legitimately added bovine material and unauthorised contamination, or determine if any detected ruminant material is associated with BSE infectivity. The sensitivity of the surveillance for the detection of material of ruminant origin in feed is currently limited due to the heterogeneous distribution of the material, practicalities of sampling and test performance. A 'technical zero' will further reduce it. The updated model estimated a total BSE infectivity four times lower than that estimated in 2011, with less than one new case of BSE expected to arise each year. In the hypothetical scenario of a whole carcass of an infected cow entering the feed chain without any removal of specified risk material (SRM) or reduction of BSE infectivity via rendering, up to four new cases of BSE could be expected at the upper 95th percentile. A second model estimated that at least half of the feed containing material of ruminant origin will not be detected or removed from the feed chain, if an interpretation cut-off point of 100 DNA copies or more is applied. If the probability of a contaminated feed sample increased to 5%, with an interpretation cut-off point of 300 DNA copies, there would be a fourfold increase in the proportion of all produced feed that is contaminated but not detected.

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Keywords: BSE, cattle, PAP, risk, qPCR, technical zero

Requestor: European Commission

Question number: EFSA-Q-2017-00705

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Acknowledgements: The Panel wishes to acknowledge the European Commission, National Reference Laboratories for animal proteins and competent authorities of the selected Member States, national rendering associations, EFPRA, FEFAC and other organisations that provided data for this scientific opinion.

Amendment: An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.
Section 3.1.2. The sentence: "The rendering of other ABP (i.e. Category 3 material, over 2.4 million tonnes) (EFPRA, 2017) results in the partially segregated production of processed animal proteins (PAP) from ruminants (incorporated into the more generic 'animal PAP'), pigs and poultry" was replaced by: "The rendering of other ABP (i.e. circa 12 million tonnes of Category 3 material) (EFPRA, 2017) results in the production of 2.7 million tonnes of partially segregated processed animal proteins (PAP) from ruminants (incorporated into the more generic 'animal PAP'), pigs and poultry".
Section 3.1.4.1. The sentence: "The EU is self-sufficient in terms of PAP production, and most of the PAP used for feed in the EU is internally produced" was deleted. The sentence: "Method 1, although more demanding in terms of rendering conditions, reduces the final volume of the output and consequently the cost of transportation and disposal, being a preferred option for many producers" was deleted.
Section 3.1.6.3. The sentence: "As a result, its distribution within compound feed can be heterogeneous, a feature that might be enhanced by settling during transportation and storage" was replaced by "Despite the reduction of particle size during processing, particle size may still be variable, leading to a heterogeneous distribution of PAP within compound feed, a feature that might be enhanced by settling during transportation and storage".
Section 3.2.1.1. After "proportion of ruminant PAP produced from bovine Category 3 material (91.15%, using latest Eurostat data referred to 2016);" the following text was added: "assuming the proportion of category 3 material is proportional to the weight of meat produced by cattle, sheep and goats".

Suggested citation: EFSA Panel on Biological Hazards (BIOHAZ), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Fernández Escámez PS, Gironés R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Skandamis P, Snary E, Speybroeck N, Kuile BT, Threlfall J, Wahlström H, Adkin A, Greiner M, Marchis D, Prado M, Da Silva Felicio T, Ortiz-Pelaez A and Simmons M, 2018. Scientific opinion on an updated quantitative risk assessment (QRA) of the BSE risk posed by processed animal protein (PAP). EFSA Journal 2018;16(7):5314, 111 pp. https://doi.org/10.2903/j.efsa.2018.5314

ISSN: 1831-4732

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Summary

In October 2017, the European Food Safety Authority (EFSA) was asked by the European Commission to deliver a scientific opinion on three Terms of Reference (ToRs). ToR1: taking into account the report on a 'technical zero' produced by the European Union Reference Laboratory for Animal Proteins in feedingstuffs (EURL-AP), and taking into account EURL-AP *Standard Operating Procedure (SOP) on operational protocols for the combination of light microscopy and PCR*, to assess the impact that the implementation of the action limits envisaged in the EURL-AP report on the 'technical zero' would have on the overall limit of detection of official controls for the detection of constituents of ruminant origin in feed. ToR2: to review and update the input data, and if necessary the basis, assumptions and structure of the 2011 QRA model (i.e. model of quantitative assessment of the risk posed by processed animal proteins published in its latest version by EFSA in 2011) to estimate the risk of all types (i.e. C-, L- and H-) of cattle bovine spongiform encephalopathy (BSE) posed by the possible contamination of feed with BSE-infected bovine derived processed animal protein (PAP), taking into account new elements, in particular with regard to the improved epidemiological situation, the current regulatory framework, the laboratory methods for official feed ban controls, the data and report included in an Annex to this mandate. ToR3: based on the outcome of the updated EFSA QRA PAP model, to estimate the cattle BSE risk (C-, L- and H-BSE) posed by the possible contamination of feed with BSE-infected bovine-derived PAP, should pig PAP be re-authorised in poultry feed and poultry PAP in pig feed, taking into account the combination of light microscopy and polymerase chain reaction (PCR) method, and taking into account six different scenarios for the ruminant PCR method based on the action limits envisaged in the EURL-AP report on 'technical zero', as follows: no action limit (i.e. the PCR method applied with the current cut-off), action limit at 100, 150, 200, 250 and 300 copies.

For ToR1, information, data and scientific literature were collected and reviewed, and a qualitative assessment conducted of both the overall surveillance system for the detection of material of ruminant origin in feed (as per the legal framework in place in 2017), and the impact of the implementation of the technical zero. For ToR2, the EFSA QRA PAP model revised in 2018, the PAP model, has been updated in two ways: (1) the input data has been reviewed and updated to the most recent or most accurate data that are available and (2) other available EFSA QRA outputs have been utilised. In particular, the Cattle TSE Monitoring Model (C-TSEMM, Adkin et al., 2012) has been used to produce more accurate estimates of the number of BSE cases in the total population, and the transmissible spongiform encephalopathies infectivity model (TSE_i) in animal tissues (Adkin et al., 2014) has been used to estimate the amount of infectivity in the tissues declassified from the specified risk material (SRM) list after its last revision in 2014. All potential risk pathways were reviewed to identify those that should be quantitatively parameterised, and those that were considered to be less significant. The risk posed by infected ruminant PAP contaminating non-ruminant PAP that may then be accidentally incorporated into ruminant feed remains the only risk pathway included in the PAP model. A quantitative probabilistic model, the FEED model, was developed to answer ToR3. This model estimates the proportion of all produced feed in the European Union (EU) in a single year that is contaminated with ruminant material (using DNA as a biomarker) but is not detected by the monitoring system in place, and the impact of the implementation of the technical zero approach by modifying the interpretation cut-off point, i.e. action limit of the quantitative real-time PCR (qPCR) in terms of the DNA copy number, comparing the baseline of the current interpretation cut-off point of 10 DNA copies to 100, 150, 200, 250 and 300 DNA copies, as described in the EURL-AP technical zero report and in the ToRs. The FEED model also estimates the proportion of contaminated feed that is not detected by the monitoring system in place depending on the interpretation cut-off point applied to the qPCR.

Multiple data sets and a range of information have been used to conduct the qualitative assessment and to parameterise the models. Some of the data, such as the EC Directorate F audit reports and BSE surveillance data were already available. Others were obtained upon request, for example the real sample-based feed testing data from selected Member State (MS), data on ABP, PAP and feed production at the EU level and information about rendering and testing practices at the EU level.

Testing for the presence of ruminant DNA using the current ruminant qPCR method does not enable discrimination between bovine material that has been added legitimately, and contamination with unauthorised material. It cannot determine either if BSE infectivity is associated with any ruminant material that is detected. The actual origin of any positive signal, i.e. the type of ruminant tissue or material containing DNA, cannot be ascertained unless other methods are developed and applied. The sensitivity of the current surveillance system for the detection of material of ruminant origin in feed is limited by a number of factors such as the potentially heterogeneous distribution of

contamination, the practicalities of the actual sampling and the performance of the tests. The implementation of the action limits envisaged in the EURL-AP technical zero report will reduce the overall sensitivity by reducing the sensitivity of the test.

The updated PAP model estimated a total BSE infectivity of 0.05 cattle oral infectious dose 50% ($CoID_{50}$) (95% range: 2.4×10^{-4} to 0.33) for cattle produced in the EU28, which is four times lower than the estimate of the 2011 QRA model. This assumes a 0.1% contamination of ruminant feed with non-ruminant PAP, which may have been contaminated with up to 5% ruminant PAP and processed using the standard ABP processing method 7 (i.e. a method that will not reduce TSE infectivity). This means that, even considering the upper 95th percentile, fewer than one additional BSE-infected cow could be expected in the EU cattle population per year. The mean annual individual exposure of cattle to BSE infectivity through concentrate feed, using the same assumptions, was 5.3 times lower than that of the 2011 QRA model. A hypothetical PAP model scenario in which no controls are applied at abattoirs to remove SRM, and no reduction of BSE infectivity is achieved by rendering, results in an estimate of up to four new cases of BSE for each single infected cow arriving at the abattoir, at the upper 95th percentile.

The FEED model estimated a reduction of between 46.5% (95% range: 20.2–77.4%) and 78.6% (95% range: 48–94.9%) in the level of detection of feed containing material of ruminant origin, at the proposed interpretation cut-off points of 100 and 300 DNA copies, respectively. The proportion of total feed produced in the EU that is contaminated and removed from the feed chain, relative to the total removed if the cut-off point remains at 10 DNA copies, would be reduced by the same amount. If the probability of a feed sample being contaminated with ruminant DNA is increased from the observed level of contamination (0.5%, 0.65% and 1.9% positive feed samples in farm, feed and border samples, respectively) to 5% in all types of premises – as a scenario analysis of the increased probability of contamination due to the re-authorisation of pig PAP in poultry feed and poultry PAP in pig feed – the FEED model estimates nearly a fourfold increase in the proportion of produced feed that would be contaminated but not detected, should the interpretation cut-off point of the ruminant qPCR be increased to 300 DNA copies.

Recommendations include considering testing/speciating PAP prior to its inclusion in feedstuffs, i.e. at a point prior to the addition of any legitimate bovine-derived ingredients, thereby eliminating the need for a technical zero approach. It is also recommended that Category 1 material is clearly identified before disposal, and/or identified (together with Category 2 material) by actively monitoring the application of glyceroltriheptanoate (GTH), and to ensure physical separation of the ingredients of ruminant feed and non-ruminant feed. It is important that any technical zero action limit, if applied, is formally validated, and the production of qPCR data from dilution series of feed spiked with contaminated PAP, or legitimate bovine ingredients, is facilitated. The creation of an EU level reporting system for the monitoring of the feed ban that would inform future risk assessment exercises and allow the evaluation of the performance of the official controls.

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1. Introduction

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

The feed ban is the key animal health protection measure against TSE (Transmissible Spongiform Encephalopathies) and consists of a ban on the use of processed animal protein (PAP) in feed for farmed animals. Scientific data link the spread of classical BSE (Bovine Spongiform Encephalopathy) to the consumption of feed contaminated with infected ruminant protein in the form of PAP. Based on these findings a ban on the feeding of mammalian processed animal protein to cattle, sheep and goats was introduced in the EU in July 1994. The ban was expanded in January 2001 to the feeding of all PAP to all farmed animals, with certain limited exceptions.

In its Communication "The TSE Roadmap 2" published on 16 July 2010,[1] the Commission envisaged the possibility of re-authorising non-ruminant PAP in non-ruminant feed, subject to maintenance of the ban on cannibalism, the implementation of channelling requirements (separate production lines), and the availability of validated and operational laboratory control methods. The Commission also envisaged the possibility of introducing a tolerance level for PAP in feed for farmed animals.

On 9 December 2010, EFSA adopted a scientific opinion on the revision of the quantitative risk assessment (QRA) of the BSE risk posed by processed animal proteins (PAPs). In this scientific opinion, EFSA estimated that, based on the assumptions and data inputted into the EFSA QRA PAP model (including the 2009 EU BSE surveillance data), assuming a 0.1% contamination of feed with non-ruminant PAPs (estimated global limit of detection for PAPs in feed), the total BSE infectivity that could enter in cattle feed in the EU would be equivalent to 0.2 $CoID_{50}$. This would mean that less than one additional BSE infected cattle could be expected in the EU cattle population per year. The EFSA QRA PAP model relied on the continuation of BSE risk mitigation measures in place at the time (in particular as regards the removal of Specified Risk Material, SRM, and TSE monitoring) and the opinion recommended an update of the assessment should these measures evolve. The EFSA QRA PAP model considered both classical BSE and the two forms of atypical BSE (L and H).

Since this assessment was conducted, the EU BSE epidemiological situation has continued to improve. While 49 Classical BSE cases and 11 Atypical BSE cases were detected in the EU in 2009, only 1 Classical BSE case and 4 Atypical BSE cases were detected in the EU in 2016. In 2017, 6 Atypical BSE cases and no Classical BSE cases were detected in the EU.

In the meanwhile, BSE risk mitigation measures applied in the EU have evolved,[2] in particular the list of SRM has been revised, the BSE monitoring now focuses in most Member States on "at risk" bovine animals and the use of non-ruminant PAP in feed for aquaculture animals has been authorised.

In addition, rules for the laboratory analysis for the detection of constituents of animal origin in official controls of feed have been amended,[3] with the validation in 2012 of a PCR (Polymerase Chain Reaction) method targeting ruminant DNA, which triggered the re-authorisation in 2013 of the use of non-ruminant PAP in feed for aquaculture animals.[4] PCR methods targeting pigs DNA and poultry DNA have been validated by the EURL-AP in 2015 and 2017 respectively, however they are not in use as pig PAP is not yet authorised in poultry feed and poultry PAP is not yet authorised in pig feed.

With the introduction of the ruminant PCR method as a method for feed ban official controls, an EURL-AP *Standard Operating Procedure (SOP) on operational protocols for the combination of light microscopy and PCR*[5] has been developed and published to clarify when the light microscopy (LM) method should be used and when the PCR method should be used. According to this SOP, only the LM method may be used when testing feed or feed material intended for farmed animals (e.g. cattle) other than aquaculture animals; while the ruminant PCR method should be used in certain cases when testing feed for aquaculture animals.

[1] Communication from the Commission to the European Parliament and the Council "The TSE Road map 2: A Strategy paper on Transmissible Spongiform Encephalopathies for 2010–2015" (COM/2010/0384 final). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52010DC0384&from=EN

[2] See 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), as last amended, as well as Commission Decision 2009/719/EC of 28 September 2009 authorising certain Member States to revise their annual BSE monitoring programmes (OJ L 256 29.9.2009, p. 35), as last amended.

[3] See Annex IV to Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1), as last amended.

[4] Commission Regulation (EU) No 56/2013 of 16 January 2013 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

[5] http://eurl.craw.eu/img/page/sops/EURL-AP%20SOP%20operational%20schemes%20V3.0.pdf

However, the problems of interpretation of the PCR results highlighted in the 2010 EFSA QRA remain, i.e. authorised animal material such as dairy products can be a source of ruminant DNA and lead to qPCR positive results. While these problems remain limited in the case of feed for aquaculture animals, studies carried out by the EURL-AP suggest that these would likely become more frequent if poultry PAP would be re-authorised in pig feed and pig PAP in poultry feed. Since this issue of "false positive" results[6] occurs often due to small traces of ruminant authorised products (e.g. dairy products used as carrier for feed additives), the Commission has asked the EURL-AP to produce a report exploring the possibility of defining an action limit expressed in number of ruminant DNA copies below which the test should be considered negative ("technical zero"). The report on the "technical zero" method has been produced by the EURL-AP (hereinafter referred to as the EURL-AP technical zero report), is attached to this mandate[7] and may be used to inform the updated EFSA QRA PAP model.

Finally, in view of this mandate, the Commission has sent a questionnaire to EU Member States competent authorities on the feed ban official controls that they have carried out in 2015 and in 2016. A summary of the answers received is available in Annex II of this opinion, and may be used to inform the updated EFSA QRA PAP model. A summary of the alerts exchanges in the Rapid Alert System for Food and Feed (RASFF) related to feed ban official controls in 2015 and 2016 is also inserted in Annex III and may be used to inform the updated EFSA QRA PAP model.[8]

1.1.1. Terms of Reference

EFSA is therefore requested to:

1) Taking into account the EURL-AP report on a 'technical zero', and taking into account EURL-AP *Standard Operating Procedure (SOP) on operational protocols for the combination of light microscopy and PCR*, to assess the impact that the implementation of the action limits envisaged in the EURL-AP report on the 'technical zero' would have on the overall limit of detection of official controls for the detection of constituents of ruminant origin in feed.
2) To review and update the input data, and if necessary the basis, assumptions and structure of the current EFSA QRA PAP model to estimate the cattle BSE risk (C-, L- and H-BSE) posed by the possible contamination of feed with BSE-infected bovine derived PAP, taking into account new elements, in particular with regard to the improved epidemiological situation, the current regulatory framework, the laboratory methods for official feed ban controls, the data and report included in Annex to this mandate.
3) Based on the outcome of the updated EFSA QRA PAP model, to estimate the cattle BSE risk (C-, L- and H-BSE) posed by the possible contamination of feed with BSE-infected bovine derived PAP, should pig PAP be re-authorised in poultry feed and poultry PAP in pig feed, taking into account the combination of light microscopy and PCR method, and taking into account six different scenarios for the ruminant PCR method based on the action limits envisaged in the EURL-AP report on 'technical zero', as follows: no action limit (i.e. PCR method applied with the current cut-off), action limit at 100, 150, 200, 250 and 300 copies.

1.2. Clarification to Terms of Reference 1 and 3 as provided by the requestor

A letter of clarification to ToR1 and ToR3 of the mandate was sent by the requestor. According to it, 'by the wording "taking into account the EURL-AP SOP on operational protocols for the combination of light microscopy and PCR" in ToR1 and the wording "taking into account the combination of light microscopy and PCR methods" in ToR3, we refer not only to the combination of light microscopy and qPCR methods as it is currently in use in accordance with the EURL SOP,[5] but also to the combination of light microscopy and PCR methods as it could be, should pig PAP be re-authorised in poultry feed and poultry PAP in pig feed. The latter is described in the attached draft diagrams, which may be used for the purpose of the scientific opinion'.

[6] The expression 'false positive' results is used here to designate PCR positive results which are caused by the presence of authorised constituents of animal origin.
[7] The EURL-AP technical zero report can be accessed at: https://doi.org/10.5281/zenodo.1292462
[8] This section includes verbatim the Background section of Annex I of the mandate.

1.3. Interpretation of the Terms of Reference

It was agreed with the requestor that in the answer to ToR1, the sensitivity of the surveillance system for the detection of material of ruminant origin in feed, including the limit of detection (LOD) of the analytical methods used (LM combined with quantitative real-time PCR (qPCR)) will be considered.

It is of paramount importance for the understanding of the outputs of this risk assessment to emphasise that there are no direct associations between contamination of feed with material of ruminant origin, exposure of cattle to contaminated feed and presence of BSE infectivity in contaminated feed.

Clarification of terms:

- 'material of ruminant origin' in this opinion is defined as any physical substance containing ruminant DNA, no matter whether it is a constituent of the substrate under observation or not;
- 'contamination' is defined as the presence of material of ruminant origin in the tested substrate, regardless of whether the material of ruminant origin is authorised or not, and regardless of whether it contains BSE infectivity or not;
- 'exposure' in this opinion is defined as the access of cattle to contaminated feed (see above);
- 'risk' outputs are expressed in terms of (i) total cattle oral infectious dose 50% ($CoID_{50}$) accessed by cattle per year, (ii) additional (new) BSE-infected cows would be expected in the EU cattle population per year at the upper 95% confidence level;
- 'infectivity' relates specifically to the presence of abnormal prion protein (PrP^{Sc}) (the BSE agent), widely used as a proxy for infectivity, which can only be demonstrated unequivocally by bioassay;
- 'interpretation cut-off point' is used in this opinion as a synonym for 'action limit' and 'cut-off point' refers to the 'technical limit of detection' of the qPCR.

1.4. Additional data and information provided by the requestor

A summary of the answers received to an EC questionnaire on the feed ban official controls in the EU Member States (MS) in 2015 and 2016 was included in Annex II of the original mandate. A corrected 'Summary of answers received to a questionnaire on feed ban official controls in the EU Member States in 2015 and 2016' was submitted as Annex II of the letter of clarification to ToR1 and ToR3 of the mandate. The original questionnaire and the modified results at the EU level and by MS are displayed in Appendices E and F, respectively. This table replaces Annex II of the original mandate.

The summary is based on the results of the implementation of Reg. (EC) 882/2004 on official controls performed to ensure the verification of compliance with feed legislation. The questionnaire includes three categories of samples, collected as: (i) controls for ruminant PAP in non-ruminant PAP; (ii) controls for ruminant PAP in feed for aquaculture animals; and (iii) controls for non-authorised PAP in feed other than aquafeed. The numbers of positives indicated in the table result from different analytical methods as follows: (i) LM for the controls for ruminant PAP in non-ruminant PAP as well as for the controls for non-authorised PAP in feed other than aquafeed, and (ii) qPCR for the controls for ruminant PAP in feed for aquaculture animals.

Table G.1 in Appendix G displays a summary of the alerts exchanges in the RASFF related to feed ban official controls in 2015 and 2016, as included in Annex III of the mandate.

Figures H.1, H.2, H.3 and H.4 in Appendix H show draft diagrams of the 'Possible combinations of methods for the disclosure of prohibited processed animal proteins in feed and feed ingredients', as included in Annex I of the letter of clarification to ToR1 and ToR3 of the mandate produced by the EURL-AP.

The report on the 'technical zero' proposed for use with respect to detection of processed animal proteins in feedingstuffs has been produced by the EURL-AP (European Union reference laboratory for animal proteins in feedingstuffs) and was provided by European Commission as part of the mandate to be used to inform the update the 2011 EFSA QRA PAP model (hereinafter referred to as the '2011 QRA model') (EFSA BIOHAZ Panel, 2011a) (Annex A).

2. Data and methodologies

2.1. Data

2.1.1. Animal by-products (ABP) and feed data

2.1.1.1. Official Feed testing results

National Reference Laboratories (NRL) for animal proteins in feed and/or competent authorities (CA) from four MS, out of the six largest producers of feed in the EU, were contacted and asked to provide detailed data from the last 5 years on the official feed and PAP testing conducted by authorised laboratories in the context of the official controls with regard to the restrictions on the production and use of feed materials of animal origin, as per Regulation (EC) No 852/2004 on the hygiene of foodstuffs.

The selected MS were provided with a template to enter a number of data items at sample level. The aim was to collect data including the year of testing, sampled material, the premises sampled, the analytical test used (LM, ruminant qPCR, any other) and its result (positive, negative, inconclusive), including the DNA copy number and C_q value (see Glossary) for samples positive to ruminant qPCR. As an alternative, aggregated data were also considered fit-for-purpose. With regard to the type of material tested, and location of sampling, the following predefined categories were suggested within the template.

- Sample material: poultry PAP, pig PAP, sheep/goat PAP, ruminant PAP, fishmeal, poultry feed, pig feed, aquafeed, non-ruminant feed, bovine feed, sheep/goat feed, animal fat, milk products and products derived thereof, minerals and products derived thereof, any other (specify).
- Sampling premises: feed mill, border inspection, importer of feed materials, storage of feed materials, home mixers/mobile mixers, intermediaries of feedingstuff trade, farms keeping non-ruminants, farms keeping ruminants, farm, dealer, means of transportation, any other (specify).

Three out of the four MS were able to provide this sample level data, and these have been merged in a unique data set for further analysis.

Data retrieval from the national dedicated database management systems resulted in a list of items wider than the predefined ones, so, a few categories were added and some re-categorisation and aggregation of the data was undertaken before carrying out any data analysis.

Within the category 'sample materials', several single vegetal ingredients or preparations (e.g. barley, corn, soybean, oatmeal, ground rice, sugar beet pulp, wheat flakes, potatoes, carrots, peas, linseeds) were re-categorised into 'Vegetal raw material'; blood, blood meal, porcine blood, pig blood, poultry blood meal were all converted into 'Blood meal', and horse feed, supplementary feed for foals, any other (specify) Horses, any other (specify), birds, bird feed, any other (specify) rabbit and ostrich feed were re-categorised as 'Non-ruminant feed'.

The same applied to the list of terms that were provided to describe sampling premises. In this case, two subsequent re-categorisations were carried out: initially some categories were added (e.g. 'processing plant', 'food industry', 'drying operation') and some were re-categorised, as in the case of 'manufacturer' or 'single feed producer' that were merged into the wider 'feed mill' category. Three main sampling premises, i.e. 'farm', 'feed mill' and 'border', were generated by merging pre-existing categories. For instance, 'farms keeping non-ruminants', 'farms keeping ruminants' and 'fish farms' have been re-categorised collectively into 'farm', and the 'feed mill' category was based on the amalgamation of 'processing plant', 'drying operation', 'feed mill', 'means of transportation', 'storage of feed material' and 'dealer'.

2.1.1.2. Feed production and feed industry testing data at the EU level (FEFAC)

The European Feed Manufacturers' Federation (FEFAC) was contacted and asked to provide information on any private testing undertaken by the industry to monitor compliance with the EU legislation on feed with regard to the restrictions on the production and use of feed materials of animal origin.

FEFAC was asked to provide aggregated data at the EU level on the number of tests conducted for the last 5 years, by sample material (poultry feed, pig feed, aquafeed, bovine feed, sheep/goat feed,

any other), sample type (forages, industrial compound feed, any other), test/s applied (LM, ruminant qPCR, any other) and results (positive, negative, inconclusive). Alternatively, if these data were not available, an overall description of the industry practices in terms of feed testing could be provided.

In addition, FEFAC was also asked to provide the total amounts of compound feed produced in the EU in 2017 (or the latest year for which data were available) by species (cattle, sheep/goats, porcine, poultry, fish, other).

2.1.1.3. Information on rendering practices at the EU level (EFPRA) and selected MS

The European Fat Processors and Renderers Association (EFPRA) was contacted to retrieve the following data for 2016 (the most recent data available) at the EU level: (1) the volume of PAP poultry meal produced in the EU; (2) the volume of PAP feather meal produced in the EU; (3) the volume of PAP porcine meal produced in the EU; (4) the volume of fishmeal produced in the EU; (5) the total volume of all other PAP mixed, including ruminant and other (insect), produced in the EU; (6) the total volume of ruminant Category 3 ABP produced in the EU; (7) the yield of PAP (%), i.e. the average amount of PAP produced by tonne of Category 3 ABP processed; (8) the effective batch size of ABP for a continuous rendering process (average); and (10) the frequency of use of the standard processing methods 1–7 for ABP as defined in Regulation (EU) 142/2011.

EFPRA was also asked to provide any information on private testing conducted in the last 5 years by the industry to monitor the compliance with the EU legislation on ABPs. The aim was to retrieve any available aggregated data at the EU level on number of tests, by sample material (poultry PAP, pig PAP, sheep/goat PAP, cattle PAP, ruminant PAP, fishmeal, any other (specify)), test applied (LM, ruminant qPCR, any other (specify)), and results (positive, negative, inconclusive).

2.1.1.4. PAP production and rendering industry testing data (EFPRA)

The national rendering associations from the four MS previously selected were contacted and asked to provide data, at MS level, on: (1) the frequency of use of standard processing methods 1–7 for ABP as defined in Regulation (EU) 142/2011), and (2) any private testing conducted in the last 5 years by the industry to monitor the compliance with the EU legislation on ABPs. The aim was to retrieve any available aggregated data at the national level on the number of tests, by sample material (poultry PAP, pig PAP, sheep/goat PAP, cattle PAP, ruminant PAP, fishmeal, any other (specify)), test applied (LM, ruminant qPCR, any other (specify)), and results (positive, negative, inconclusive). These national data sets were intended to support the answers to the TORs focusing on the four MSs that are among the largest producers of feed in the EU, as representative case studies of the wider EU context.

2.1.1.5. EC Directorate F audit reports

The EC database of final reports of audits carried out by the EC Directorate F (ex-Food and Veterinary Office (FVO)) was screened to identify reports from 2009 to 2017 (hereinafter referred to as the audit reports) mentioning in their titles the following terms: 'feed sector risk based controls', 'hazards feed chain', 'animal by products', 'animal by products aquafeed', 'aquafeed' and 'feed ban, fertilisers, soil improvers'. A total of 55 audit reports have been thoroughly reviewed to identify and list:

- non-compliances with the best practices or legal requirements when processing or mixing ABP or PAP (at abattoirs and rendering plants);
- deficiencies in collection, trade, storage, transport and labelling of ABP as well as in the necessary accompanying commercial documents;
- deficiencies in the official controls of organic fertilisers/soil improvers or their usage or mixing of their components; and
- deficiencies in the general controls for ABP and feed (including aquafeed).

The identified deficiencies were summarised and used as evidence to illustrate the steps/processes of the feed chain where potential contamination can occur with the view of supporting the likelihood of contamination through the potential risk pathways that have been identified within the ABP and feed production processes. Examples of deficiencies identified in these audit reports are cited throughout the opinion where considered relevant. The deficiencies are related to single inspections conducted in one MS each, and are not necessarily indicative of widespread or systematic breaches, but are reported here to illustrate the various types of non-conformance with the regulations that have been identified in real life.

The audit reports may represent a biased reality. The criteria to identify and select the audited production lines and the premises in the selected MS could not be ascertained.

2.1.2. Model data

2.1.2.1. BSE surveillance data

Data were obtained from the 'European Union summary report on surveillance for the presence of transmissible spongiform encephalopathies (TSE) in 2016' (EFSA, 2017) which is based on data retrieved from the EU TSE database, which collects standardised surveillance data on all testing activities in all MS.

2.1.2.2. BSE prevalence

The BSE prevalence was estimated using the C-TSEMM which is a back-calculation model (Adkin et al., 2012). The model was originally developed to evaluate the performance of different BSE monitoring regimes in cattle in the EU. For full details of the model and its assumptions, see Adkin et al. (2012). Broadly, the C-TSEMM requires that, for modelling scenarios applicable to a group of countries, it must be possible to merge them together as a unique epidemiological unit, as was considered the case with the EU25 in the previously mentioned assessment, and as such to estimate the design prevalence that the surveillance regime can detect when applied to the entire cattle population of the unit. The C-TSEMM uses individual MS BSE case data, and the number of animals tested between 2002 and 2015. Following the assumptions described in Section 2.1.3 of Adkin et al. (2012), there are four surveillance components included in the model: animals clinically suspected of being infected by BSE, healthy slaughtered animals, fallen stock and emergency slaughter animals (including those with clinical signs at ante mortem inspection). The C-TSEMM requires annual historical information on the standing cattle population, the number of animals slaughtered/dying in each surveillance stream, results for those animals which have been tested, and test results classified by case type (classical BSE (C-BSE), atypical H-type BSE, atypical L-type BSE or unknown).

2.1.2.3. BSE infectivity data

The BSE infectivity was estimated using the (TSEi) model (Adkin et al., 2014; EFSA, 2014b). This model was developed to estimate the amount of infectivity associated with the intestine and the mesentery of cattle born in the EU, infected with C-BSE and that enter undetected into the food and feed chain. TSEi relies on a combination of experimental data and assumptions that might have an impact on its final accuracy. Four parameters strongly affect the model's results: (i) the variability of the infectivity titre of the ileum; (ii) the variability of the age at slaughter of the animals; (iii) the variability of the size of the ileocaecal plate weight in small intestines; and (iv) the uncertainty associated with the conversion of the infectivity titre as measured by bioassay in conventional mice and in cattle.

2.1.2.4. ABP and PAP production data

See Section 2.1.1.4.

2.1.2.5. Feed testing data

See Section 2.1.1.1.

2.2. Methodologies

Information, data and scientific literature were collected and reviewed based on the knowledge and expertise of the members of the Working Group (WG) drafting this scientific opinion and, complemented by the data listed under Section 2.1, used when answering the ToRs of the mandate.

Based on a qualitative assessment of the overall surveillance system for the detection of material of ruminant origin in feed (as per the legal framework in place in 2017), the impact of the implementation of the technical zero method has been estimated qualitatively to answer ToR1.

For ToR2, a modification and update of the 2011 QRA model (hereinafter is referred to as the PAP model) has been produced, as described in Section 3.2. This model was constructed around a single main risk pathway. The structure of the feed industry was reviewed and other risk pathways were identified and described. For some pathways, the risk in relation to animal feed was either considered too low to warrant quantification, or there were insufficient data to support their meaningful inclusion in the quantitative model. These pathways are described under Section 3.2.1.2, together with the type of data that would be required to enable their inclusion in future models.

A new probabilistic model (hereinafter referred to as the FEED model) was also developed (as described in Section 3.3) to answer ToR3. It assesses the impact of the feed testing on the removal of feed contaminated with material of ruminant origin from the feed chain, and in particular the implementation of the technical zero approach.

A major limitation for the use of the 2011 QRA model to look at the impact of any modification to testing protocols is that the qPCR method for which the technical zero approach is proposed is based on the quantification of DNA, the measurable amount of which is variable depending on tissue type, and the effects of different rendering methods. qPCR results therefore cannot be directly related to a weight of contaminating tissue. However, the level of risk to cattle in the 2011 QRA model is measured in $CoID_{50}$ which is related to weight of ABP and PAP produced. For this reason, the two models have been developed and run in parallel, together with a qualitative approach, to address the ToRs as fully as possible.

3. Assessment

3.1. Limit of detection of material of ruminant origin in feed

3.1.1. The BSE epidemiological situation

Following the emergence of BSE in cattle in the 1980s, epidemiological studies identified the inclusion of animal-derived protein (meat-and-bone meal (MBM)) as the driver for this feed-borne epidemic. The feed-borne hypothesis was supported by the fact that changes to the rendering processes for animal-derived protein had been introduced over the previous decade (Wilesmith et al., 1991) with a move from batch to continuous rendering, and a reduction in the use of hydrocarbon solvent for the extraction of tallow. These production method changes coincided with changes in feeding practices which saw the introduction of MBM into calf rations in the UK (Horn, 2001). In response to this finding, disease control measures were put in place, with the key measure being the ban on the use of ruminant protein in ruminant feed. While this measure resulted in a significant reduction in cases, disease outbreaks were still occurring which were, in some instances, argued to be caused by contamination of ruminant feed with food intended for other species, which could still legitimately contain ruminant protein. The total feed ban, imposed in the EU in 2001, prohibited the use of PAP of all species in feed for farmed animals.

Given that C-BSE has been shown to be zoonotic, further regulations are in place at slaughterhouses to ensure the removal of specified risk materials (SRM) (those tissues known to have the greatest infective load) from both the human food and animal feed chains, as per Commission Regulation 999/2001[9] (hereinafter referred to as the TSE Regulation). These measures are applied, according to age and BSE risk status of the country of origin, to the entire slaughter population. This is justified by the long incubation period of the disease and the impossibility of directly detecting prions (i.e. the TSE infectious agents) in any given raw material or by-product included in feed (EFSA BIOHAZ Panel, 2017). Methods for the identification and safe disposal of these materials are detailed in Regulation 1069/2009[10] (hereinafter referred to as the ABP Regulation).

As a result of these measures being applied, the incidence of C-BSE in the EU has decreased significantly. The total number of C-BSE cases reported in the EU went from 2174 in 2001 to one case in 2016 (Figure 1). This most recent case was born after the reinforced EU-wide feed ban imposed on 1 January 2001 (i.e. a BARB (born after the reinforced ban) case (EFSA BIOHAZ Panel, 2017). For the first time since the disease was identified, no cases of any type were reported by the United Kingdom in 2016 (EFSA BIOHAZ Panel, 2017).

[9] 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.

[10] 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. OJ L 300, 14.11.2009, p. 1–33.

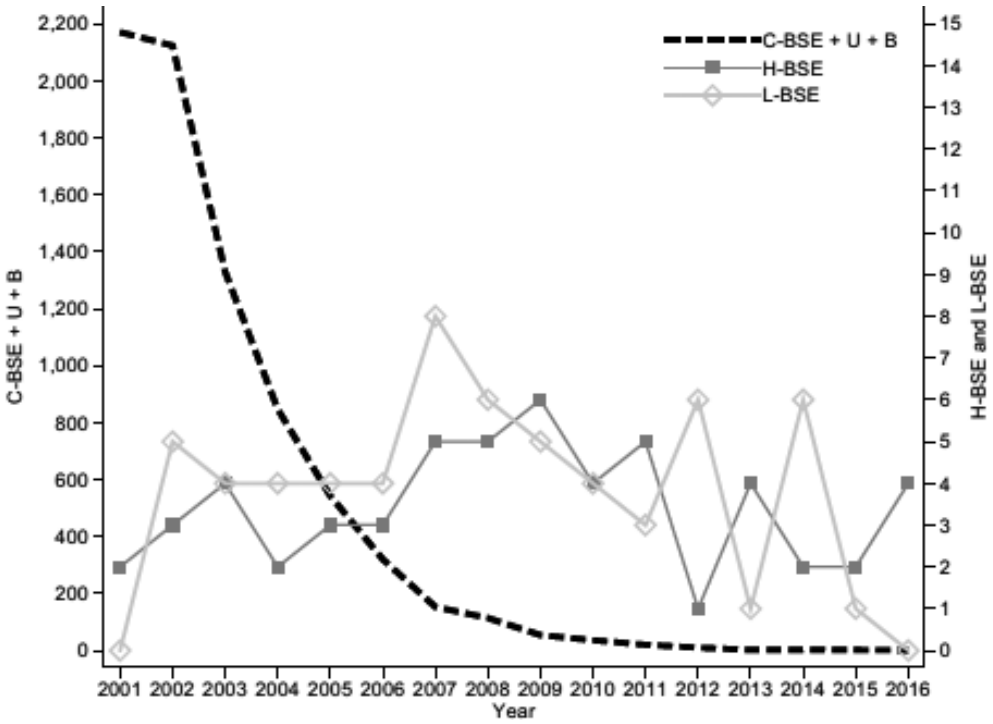


Figure 1: Number of BSE cases by type over the period 2001–2016 in the EU (EFSA, 2017)

In the early 2000s, two different forms of BSE were identified, called atypical L (low) and atypical H (high) based on their different PrP molecular masses on western blot, leading to the compulsory discrimination of any new case of BSE detected in the EU into one of the three categories, namely, C-, L- and H-BSE. It has not been determined if these atypical cases are 'new' or just 'newly identified'. Since the first case of atypical BSE was reported (in 2001), there have been a total of 115 cases, 54 atypical L and 61 atypical H, reported by MS (until the end of 2016). There is no clear pattern in the evolution of atypical BSE in its two forms, with a range of around only 0–8 cases detected per year (EFSA, 2017). The data on these forms of BSE are sparse, and their origin – as is the case for C-BSE – is unknown. It is not currently known if they share any feed-borne related risk factors with C-BSE. However, both H-BSE and L-BSE can be transmitted to a range of other species, including by the oral route, and some transmissions have led to outcomes similar to C-BSE, so an aetiological link between these atypical forms of disease and C-BSE cannot be ruled out (for summary, see EFSA BIOHAZ Panel, 2014a).

Although the agent causing C-BSE is the only prion strain that has been officially recognised as zoonotic (EFSA BIOHAZ Panel, 2011b), there is scientific evidence that there is no absolute species barrier for other BSE strains, especially in the case of L-BSE (EFSA BIOHAZ Panel, 2011b). Thus, all three types of BSE appear in the ToRs, and have been included in the estimation of the BSE prevalence by the C-TSEMM model. The relative amount of infectivity in tissues from cattle infected with the three types of BSE has been considered when estimating the residual infectivity in SRM and Category 3 material along the feed chain.

3.1.2. The overall context of PAP and feed production

A key measure to prevent the recycling of the BSE agent in the cattle population via feed was the enforcement of the ban on the use of PAP in feed for farmed animals, the so called 'total feed ban', brought into force in January 2001. The structure of the ABP and feed industry is complex and the industrial processes had to adapt as a result of the enforcement of this ban (see Section 3.1.3). Prior to the total feed ban, the production processes for ruminant and non-ruminant feed were not completely separated. During rendering processes, feed production, storage or transportation of ABP and/or feed there was ample opportunity for ingredients intended for non-ruminant feed to contaminate ruminant feed and vice-versa (TAFS, 2010).

Despite the overall effectiveness of the various feed bans (ruminant-to-ruminant feed bans or mammalian-to-ruminant feed), a total of 60 C-BSE cases in cattle born after the 1 January 2001 had

been detected in the EU by the end of 2016. The 2017 EFSA scientific opinion on the BSE cases born after the total feed ban (EFSA BIOHAZ Panel, 2017) concluded that, 'uncertainty remains high about the origin of disease in each of these animals, but when compared with other biologically plausible sources of infection (maternal, environmental, genetic, iatrogenic), feed-borne exposure is the most likely origin'. If it is assumed that there is no external source of contaminated material, there is either still ruminant feed produced in the EU that contains ruminant protein (including infectious prions) or there is a potential for the cross-feeding of ruminants with non-ruminant feed containing such ruminant protein.

There is a long chain of events between a single BSE-infected cow arriving at an abattoir, and cattle being exposed to BSE-infected ruminant material via feed or any other route. That chain encompasses the slaughtering of the infected animal within the context of the 328 million pigs, sheep, goats, beef and dairy cattle and the 6 billion chickens, turkeys and other poultry slaughtered annually in the EU (EFPRA, 2017). One part of this process is the removal of SRM (as prescribed in the TSE Regulation, as amended) which results in the production of approximately 5 million tonnes of Category 1 and Category 2 ABP in the EU annually (EFPRA, 2017), which should be destroyed. The rendering of other ABP (i.e. circa 12 million tonnes of Category 3 material) (EFPRA, 2017) results in the production of 2.7 million tonnes of partially segregated processed animal proteins (PAP) from ruminants (incorporated into the more generic 'animal PAP'), pigs and poultry. Over 155 million tonnes of compound feed are produced in the EU, of which only pet food and aquafeed are allowed to contain PAP, which in aquafeed should be non-ruminant. A flow diagram of the feed industry including authorised use and potential routes of contamination is shown in Figure 2.

The exclusion of certain bovine tissues from the SRM list in 2013 from MS with negligible risk status (by May 2017, this includes all MS except France, Ireland and Greece, and, within the UK, England and Wales) has resulted in a larger amount of ruminant material being re-categorised to low-risk Category 3, increasing the overall production of ruminant PAP, up to 625,000–750,000 tonnes, assuming that from each animal 25–30 kg is the weight of the intestine that has changed status, and 25 million cattle are slaughtered annually in the EU. It is important to remember that the 'de-classified' tissues may still contain infectivity (see Table 2), albeit at a much lower concentration than those still on the list (EFSA BIOHAZ Panel, 2014b).

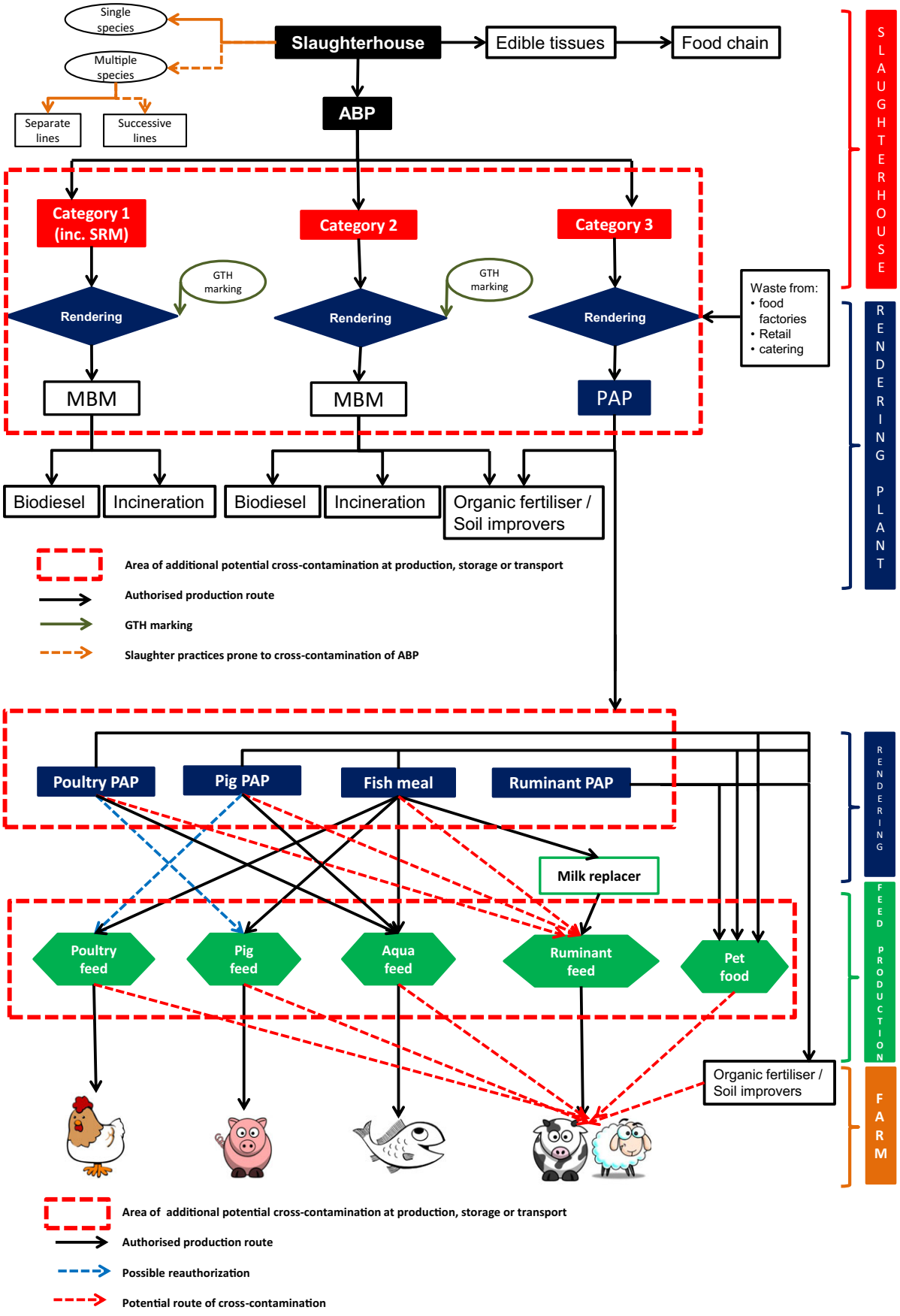


Figure 2: Flow diagram showing the interactions of the ABP industry and the feed industry and the potential routes of contamination of ruminant feed

Following the total feed ban, there has been a limited relaxation of the initial measures. In 2008, the Commission Regulation 956/2008[11] allowed the use of fishmeal in milk replacers intended for the feeding of unweaned ruminants as a supplement to, or substitute for, post-colostral milk before weaning is complete. Since June 2013 and by Commission Regulation (EC) 56/2013, PAP from non-ruminants (pig and poultry) have been allowed to be added to fish and shellfish feed. In addition, fishmeal can be used to feed pigs, poultry and farmed fish. Until July 2013, no rendered ABP were used for feed in the EU. As a result of the partial lifting of the ban in 2013, a very limited number of animal residues and derived products (only some Category 3 ABP, including former foodstuffs not containing meat and fish) can be legally recycled to livestock feed in the EU (Jędrejek et al., 2016).

Commission Regulation 893/2017[12], amending the TSE Regulation, allowed from 1 July 2017, 'the export of processed animal protein derived from ruminants, or of processed animal protein derived from both ruminants and non-ruminants, under certain conditions of storage, transport and documentation from registered suppliers so that leakage, cross-contamination and misuse are minimized'. It also allowed the export to third countries of compound feed containing PAP derived from non-ruminants produced in accordance with certain requirements. The possibility of exporting PAP offers new incentives for the industry to partially divert from the current use of PAP for pet food, organic fertilisers/soil improvers and aquafeed (which still only uses a small proportion of the Category 3 material produced), hence increasing the interest in separating Category 3 materials by species in order to produce more species-specific PAP, or at least ruminant vs. non-ruminant PAP, instead of disposing of Category 3 together with MBM due to its current low commercial value. The possible changes in feed regulations to allow the inclusion of pig PAP in poultry feed, and poultry PAP in pig feed will further increase the value and use of these products, while it will become more complicated to ensure continued segregation of both production lines and end products.

There are numerous regulations stipulating the EU controls for ABP and the food and feed chains, which require MS to devise individual risk-based protocols for testing and monitoring the feed ban. However, there is no centralised review or coordination, at the EU level, of either the plans or the data resulting from these programmes. The response to a test positive result is also discretionary, with decisions being influenced by several factors including the timescale of detection (e.g. whether the tested batch can still be located and recalled, or if it has already been distributed/consumed), and the final destination of the test positive material (e.g. aquafeed vs ruminant feed). There is an expectation that the industry will assist by self-regulation through the implementation of HACCP plans, and the regulations also allow for derogations at industry request, provided that they propose alternatives that are 'equivalent', although no indication is given of which parameters might be modified. There is no requirement to calculate the number of samples to be collected based on a 'design prevalence' when designing sampling protocols for the monitoring of the feed ban.

The only information available on how these programmes are being implemented is derived retrospectively from the testing data supplied to the EC upon request (see Appendices E and F), possible RASFF notifications (Appendix G) and the audits undertaken by the EC Directorate F. The factual and anecdotal information available from these sources can be used to inform the assessment of risks associated with each stage of the feed chain, and help to determine the impact of the implementation of action limits in the context of feed controls.

The following sections describe in more detail the different stages of the PAP and feed manufacturing chains, looking at the legal requirements, the possibility of PAP or feed contaminated with material of ruminant origin entering different pathways, and the measures in place to prevent this from happening.

3.1.3. Slaughtering and animal by-products

3.1.3.1. Classification of ABP

According to the ABP Regulation, 'animal by-products' means 'entire bodies or parts of animals, products of animal origin or other products obtained from animals, which are not intended for human consumption, including oocytes, embryos and semen'. The subsequent use/fate of these materials depends on their risk classification in three different categories (see Glossary for full definitions). Briefly:

[11] Commission Regulation (EC) No 956/2008 of 29 September 2008 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ L 260, 30.9.2008, p. 8–11.

[12] Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. OJ L 138, 25.5.2017, p. 92–116.

- Category 1 material is the highest risk, and consists principally of material that is considered a TSE risk, i.e. SRM.
- Category 2 material includes fallen stock, manure and gastrointestinal tract contents. Category 2 is also the default status of any animal by-product not defined in the ABP Regulation as either Category 1 or Category 3 material, and includes such material as slaughterhouse drain-trap waste.
- Category 3 material is considered low risk, and includes parts of animals that have been passed fit for human consumption in a slaughterhouse but which are not intended for consumption, either because they are not parts of animals that are normally consumed (hides, hair, feathers, bones) or for commercial reasons. Category 3 material also includes former foodstuffs (waste from food factories and retail premises).

3.1.3.2. Segregation/identification of ABP

Slaughterhouses vary with regard to the number of animal species they process. Larger slaughterhouses are more likely to be specialised on single species, whereas others with lower throughput might slaughter multiple species in separate, lines or in series on the same line. This may lead to the possibility of some contamination of one species' ABP with another, or indeed deliberate pooling of material to be disposed of jointly, for example for logistical reasons. Examples have been identified during inspection, and appear in the audit reports. With regard to high-risk material, the most critical procedures at abattoirs that may result in contamination are the extraction, collection, storage, identification and disposal of SRM. These procedures could lead to undesirable outcomes, for example, the mixing of SRM with Category 2 and/or Category 3 material, as reported by audit reports. Incorrect identification of bins for different categories of ABP, and/or their misuse, are two possible scenarios in a busy and complex working environment like that of a modern abattoir. The separation of blood in a multispecies abattoir can be compromised if the process is not automated and thorough cleaning and disinfection are not undertaken. Logistical constraints may lead to the possibility of wrongly identified ABP. When BSE disease surveillance required the testing of healthy slaughter animals, the time gap between the sampling of cattle brainstems and the reception of the results of TSE testing resulted in some operators disposing of bovine blood as Category 1 material that was then mixed with pig blood and identified as Category 3 in transport documents when dispatched to intermediate plants. As a result of these contingencies, ABP leaving an abattoir could either contain material from a different category or could be wrongly identified. If the former, contamination would be present at the processing plant in the next step. If the latter, inappropriate processing methods could be applied to the material, with an end use for which the material is not authorised.

The ABP Regulation considers Category 1 ABP as high risk from a TSE perspective, and all Category 1 material should be segregated, labelled and transported separately for rendering into meat and bone meal (MBM), marked indelibly to enable its identification in case of suspicion of re-use, and subsequently destroyed by incineration or conversion into biofuel. Given the nature of the tissues being segregated into these categories (e.g. heads, spinal columns), visual inspection should be sufficient to distinguish the Category 1 material but additional visual marking may be undertaken to aid identification. For example, the UK practice is to spray Category 1 material with a blue dye, but this practice is not applied in other MS. Contamination or partial mixing of different ABP streams could therefore pass undetected.

Chapter V, Annex VIII of Regulation (EU) 142/2011 states that 'Category 1 or Category 2 material derived products shall be permanently marked in processing plants with glyceroltriheptanoate (GTH)'. This is added to derived products that have undergone a preceding sanitising thermal treatment at a core temperature of at least 80°C and remain subsequently protected from re-contamination. All derived products should 'contain homogenously throughout the substance a minimum concentration of at least 250 mg GTH per kg fat'. Although the addition of GTH is a regulatory requirement, and can be used for investigating possible breaches of protocol or as part of a HACCP plan, there is no explicit regulatory requirement to use this marker to monitor for possible contamination of Category 3 material with Category 1 or Category 2 material. There is no official method for detection and quantification of GTH in fat and MBM, although there is a method validated by an interlaboratory study (Boix et al., 2010). There is no proficiency testing currently applied across MS accredited laboratories, and only isolated efforts to monitor the performance of the method are available (Marchis et al., 2013).

If ABP are not correctly labelled, contamination could occur either by incorrect classification (human error) or if storage containers and vehicles are not dedicated to single use and/or properly cleaned between loads. This could be detected by testing Category 3 ABP for the presence of GTH, but this is not done routinely, although it can be used to investigate possible breaches.

In general, Category 2 waste is treated in the same way as Category 1 despite its potential as soil improver/organic fertiliser, and transported for rendering into MBM, and marking with GTH, prior to incineration or conversion into biofuel.

The audit reports documented deficiencies in the recording of the category of the material on commercial documents, in particular absent or incorrect labelling of the unloading bays for raw ABP. Inspectors also documented the lack of enforcement of the requirements laid down by regulation concerning the marking with GTH, e.g. (i) no record-keeping of the amounts of ABP marked with GTH, (ii) Category 1 material which was not marked with GTH, (iii) no analyses for GTH concentration carried out in Category 1 processing plants, and (iv) no system in place for the systematic and/or continuous recording and monitoring of parameters which could demonstrate that the required concentration of GTH in the final products was achieved. Inspectors observed that the use and disposal of ABP from fishery plants did not started being subject to official controls immediately after the partial lift of the feed ban and that, more generally, the frequency of official controls carried out for ABP by the CA does not take into account the specific risks associated with different ABP establishments. The inspectors also reported that official controls pay little attention to Category 3 material at retailers.

3.1.3.3. Transportation/chain of custody

Point 1 of Article 21 of the ABP Regulation details the conditions for collection and identification of ABP as regards category and transport, stating 'Operators shall collect, identify and transport animal by-products without undue delay, under conditions which prevent risks arising to public and animal health'.

According to Article 17 of Commission Regulation (EU) 142/2011[13], operators consigning, transporting or receiving ABP or derived products should keep records of consignments and related commercial documents or health certificates, and should comply with the requirements for the marking of certain derived products. It also states that the processing plant must have adequate facilities for cleaning and disinfecting the containers or receptacles in which animal by-products are received, and the vehicles in which they are transported ('other than ships'). Reusable containers must be dedicated to the carriage of a particular ABP or derived product to the extent necessary to avoid contamination. However, reusable containers are allowed for different ABP or derived products provided the CA has authorised it and they are cleaned and disinfected between the different uses in a manner which prevents contamination. The method of cleaning is not specified. However, given the resistance of the BSE agent to most conventional disinfection procedures (Department of Health and Social Care, UK, 2015) and the difficulties surrounding effective cleaning and disinfection of surgical instrumentation to the extent that single-use equipment is recommended wherever possible (Department of Health and Social Care, UK 2017), it is a reasonable assumption that material of ruminant origin could remain in small amounts following routine cleaning and disinfection of containers or vehicles. Under field conditions, this cannot be seen as an absolute either in terms of what is currently done, or what could be achieved. Nonetheless thorough cleaning and disinfection substantially reduces contamination.

Registered operators transporting ABP or derived products, other than between premises of the same operator, should have information on the identification of their vehicles which allows the verification of the use of the vehicles for the transport of ABP or derived products. They should also clean and disinfect their vehicles, as appropriate, and take all other necessary measures to prevent contamination and the spreading of diseases communicable to humans or animals.

Deficiencies in commercial documentation, ABP collection, trade, storage and transportation and labelling throughout the system, from abattoirs to processing plants, have been documented in audit reports. SRM sent to a Category 3 processing plant is probably the worst-case scenario that has been reported, even if SRM might be a very small fraction of the daily throughput of a rendering plant. Another example reported by the auditors is the mixing of Category 1 material containing full bovine bodies with Category 3 material before being processed as Category 3. Storage of SRM in unidentified containers, not distinguishable from those of Category 3 material was another undesirable finding. Wrong or incomplete documentation accompanying outgoing consignments lacking the category of the derived product, information on the place of origin or place of destination, details of the transporter and/or means of transport, species, and the approval/registration number of the plant/operator of origin or destination can lead to situations similar to those described above. Structural deficiencies in

[13] 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.

processing plants could result in the collection of Category 1 material in such way that they could easily be conveyed to Category 3 containers destined for feeding fur and pet animals, for example, by not clearly identifying or labelling unloading bays with the category for which they were intended to be used. In general, audit reports highlight the low attention paid to the risk of cross-contamination during transport, undermining the overall reliability of the official controls. This weakness is mitigated by largely comprehensive 'own-control' systems put in place by the operators. The verification of the authorisation to transport ABP by registered intermediary operators not linked to the abattoir or processing plants, and the proper use of their vehicles (including the identification of the category transported and cleaning and disinfection) is a challenge for any CA, and weakens their ability to ensure that ABPs are only used, or disposed of, in accordance with the relevant EU requirements.

3.1.4. Rendering and processed animal protein

3.1.4.1. PAP production

Annex I of Commission Regulation (EU) No 142/2011 defines PAPs as 'animal protein derived entirely from Category 3 material, which have been treated in accordance with Section 1 of Chapter II of Annex X (including blood meal and fishmeal) so as to render them suitable for direct use as feed material or for any other use in feedingstuffs, including pet food, or for use in organic fertilisers or soil improvers; however, it does not include blood products, milk, milk-based products, milk-derived products, colostrum, colostrum products, centrifuge or separator sludge, gelatine, hydrolysed proteins and dicalcium phosphate, eggs and egg-products, including eggshells, tricalcium phosphate and collagen'.

The EU restrictions on the use of ABP do not need to be applied by third countries, unless required by bilateral trade agreements, and so contamination events or human error may occur undetected. If Category 3 material is imported to produce PAP, it may contain what is considered Category 1 or Category 2 in the EU. For example, in one MS, a recent import of horse PAP from a third country was found to be contaminated with material of ruminant origin (Marchis, 2018).

Chapter III, Annex IV of the Regulation mentioned above describes the standard processing methods for animal by-products. There are presently seven different rendering methods for ABP which vary with regard to the combination of temperature, pressure and time. Currently not all of them are in regular use. Unless the CA requires the application of pressure sterilisation (method 1), Category 1 and Category 2 material can be processed in accordance with processing methods 2, 3, 4 or 5 as referred to in Chapter III. Method 1 is the one with the most stringent conditions in terms of combination of temperature, time, pressure and particle size, and results in the largest reduction of TSE infectivity (Taylor and Woodgate, 2003).

According to Chapter III, Annex IV of the Commission Regulation (EU) No 142/2011, processing method 1 (pressure sterilisation) requires that 'the animal by-products with the particle size of no greater than 50 mm are heated to a core temperature of more than 133°C for at least 20 min without interruption at a pressure (absolute) of at least 3 bars… in batch or continuous systems'.

The standard methods for the processing of Category 3 ABP are variable (See Figure 3). The choice of method can vary depending on economic forces such as the price of fuel, and the market value of the end product. Apart from method 1, which requires both heat and pressure to be applied, methods 2, 3, 4, 5 and 6 require different combinations of time and temperature, making them less effective in reducing TSE infectivity.

According to Chapter III, Annex IV of the Commission Regulation (EU) No 142/2011, method 7 is defined by 'any processing method authorised by the competent authority where the following have been demonstrated by the operator to that authority:

a) the identification of relevant hazards in the starting material, in view of the origin of the material, and of the potential risks in view of the animal health status of the Member State or the area or zone where the method is to be used;
b) the capacity of the processing method to reduce those hazards to a level which does not pose any significant risks to public and animal health;
c) the sampling of the final product on a daily basis over a period of 30 production days in compliance with the following microbiological standards:

    i) Samples of material taken directly after the treatment:
    Clostridium perfringens absent in 1 g of the products

ii) Samples of material taken during or upon withdrawal from storage:
Salmonella: absence in 25 g: n = 5, c = 0, m = 0, M = 0
Enterobacteriaceae: n = 5, c = 2; m = 10; M = 300 in 1 g
where:
n = number of samples to be tested;
m = threshold value for the number of bacteria; the result is considered satisfactory if the number of bacteria in all samples does not exceed m;
M = maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more samples is M or more; and
c = number of samples the bacterial count of which may be between m and M, the samples still being considered acceptable if the bacterial count of the other samples is m or less'.

In the specifications of method 7, TSE infectivity is not mentioned or addressed, although theoretically it should not be present in Category 3 material.

The majority of the Category 1 and Category 2 materials are processed by method 1, with method 3 and method 4 occasionally applied (EFPRA, 2018). For Category 3 material, method 7 is the most frequently applied, followed by method 1 and occasionally by methods 4, 5, and 3. The main specifications of the standard methods for the processing of ABP according to Commission Regulation (EU) No 142/2011 are displayed in Figure 3.

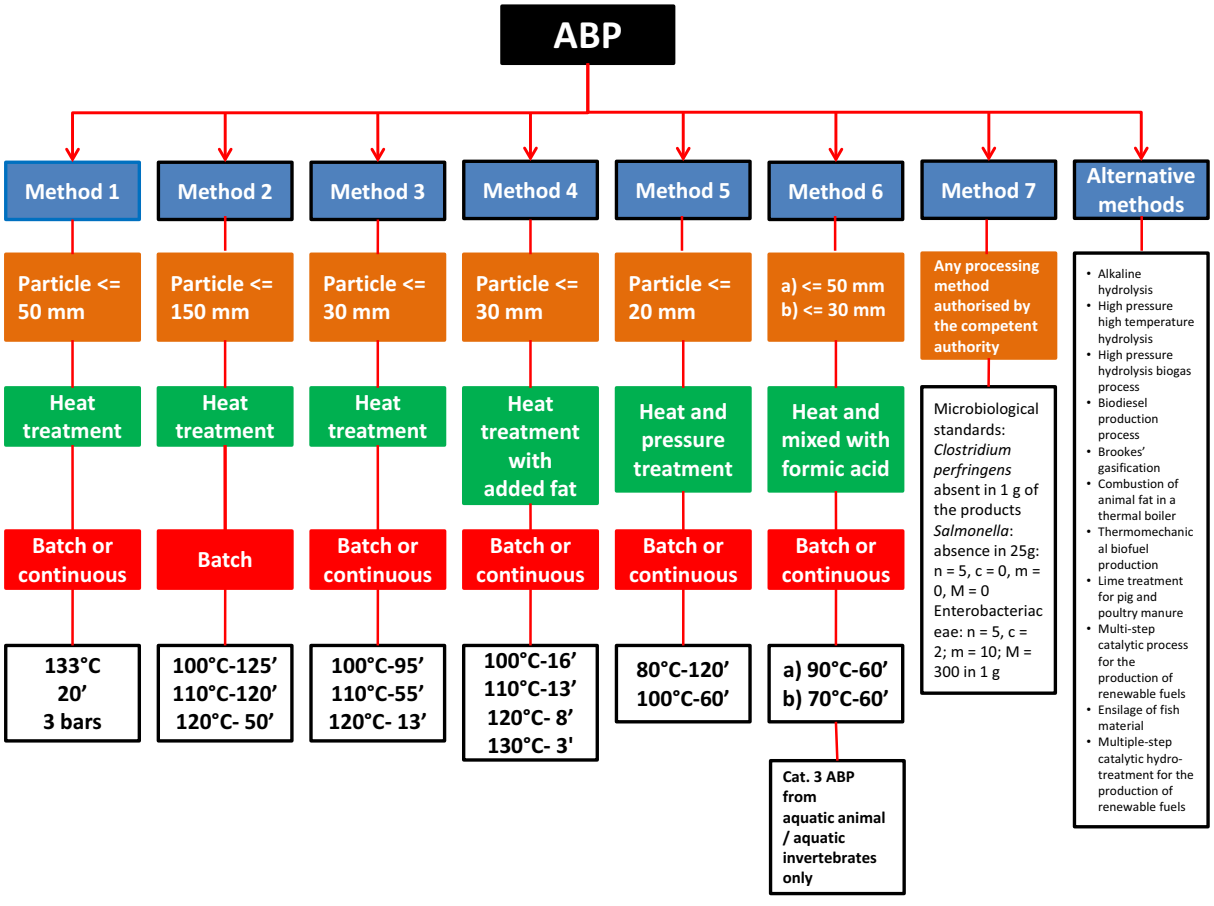


Figure 3: Specifications of the standard methods for the processing of animal by-products (ABP) according to Commission Regulation (EU) No 142/2011, as amended. Source: adapted from Spanish Renderers Association (ANAGRASA) website: http://www.anagrasa.org/es/sector/preguntas-frecuentes/index.htm

Rendered material is then transported to a range of users such as incineration plants, biofuel producers, or feed, pet food or fertiliser/soil improver manufacturers depending on which category it falls into. The potential for contamination or accidental misclassification at this stage in the process is

very similar to that raised in Section 3.1.3.2 above. The potential for such events to occur will increase when wider and more varied end use of these products is permitted.

Audit reports have also identified a number of issues/shortcomings/failures in rendering plants, related to the compliance with, and monitoring of, various aspects of HACCP procedures within plants, the production processes for MBM and PAP, its subsequent labelling and storage, staff competence and the control and monitoring of onward distribution to end users. In particular, audit reports have detailed the shortcomings identified in processing plants that questioned, for example, the monitoring of the processing parameters. There are recorded examples of

- insufficient data, or lack of recording devices to measure temperature, pressure, or retention time, to confirm that the processing methods are compliant with those authorised;
- methods being applied that had not been validated, with greater concern in the case of plants processing Category 1 material;
- records of processing parameters below the required ones, with no calibration or no regular calibration of the measuring devices, or the lack of recording devices for the processing parameters and of the particle size of the ABP processed as well as of the amounts of ABP processed.

In relation to this, the identification of deficiencies in the definition, implementation and monitoring of critical control points (CCP) of HACCP plans of processing plants has also been highlighted in some audit reports.

Correct identification of the incoming material could also be a high-risk practice if the processing method applied is not the required one for the actual category of the ABP being processed. Equally if plants are authorised to process, for example, Category 3 material only, ABP from a different category should not be present in their premises. The lack of separation of ABP into the correct categories at the abattoir due to logistical or operational constraints has resulted in the placement of MBM onto the market as PAP, for example. The inspectors also identified situations where ABP plants were in operation irrespective of whether they were approved or not, without necessarily fulfilling the relevant regulatory requirements.

3.1.4.2. PAP uses and monitoring

As a consequence of the success of the BSE control measures and the continuous decrease in the incidence of BSE, the total feed ban has been partially relaxed and PAP derived from non-ruminants have been allowed in feed for aquaculture since June 2013 (see Section 3.1.2), while the use of ruminant PAP is still forbidden in any feed for farmed animals. Table 1 shows the constituents of animal origin currently authorised in feed for different species in the EU.

At present, pig and poultry PAP can be added to aquafeed, and fishmeal can be added to pig and poultry rations and to milk replacers for unweaned calves. Future regulatory revisions identified in the TSE Roadmap 2 would allow the inclusion of pig PAP in poultry feed, and poultry PAP in pig feed.

There is currently no legal obligation to include the collection and testing of samples in early steps of the feed chain, i.e. before/during/after the production of PAP, with the exception of screening for the presence of GTH on an ad hoc basis as part of individual investigations or HACCP plans. National plans are only required to address monitoring (through testing) for unauthorised terrestrial material in feed at the feed entry/production/distribution/consumption level. It is not currently a regulatory requirement to undertake any speciation screening of PAP. However, in situations where this has been done on an ad hoc basis, there are examples of the presence of ruminant DNA in products that are not derived from bovine material, indicating contamination of material from different species either at the slaughterhouse or at the rendering facility (see Section 3.1.3).

If speciation by qPCR is performed on PAP samples and ruminant DNA is identified, this would indicate that contamination with material of ruminant origin has occurred either at the slaughterhouse, during transportation, or within the rendering plant. Field testing data (Appendix F) indicate that PAP is indeed contaminated, with variable levels of ruminant content detected.

If PAP is not tested for contamination with ruminant-derived material at the point of production, any contamination will be carried forward into any products incorporating this PAP, but diluted to a point where sampling and test sensitivity may limit its detection.

Table 1: Summary of processed animal proteins (PAP) and constituents of animal origin currently authorised in animal feed in the EU

PAP and constituents of animal origin	Ruminants	Unweaned ruminants	Non ruminants	Aquaculture	Pets and fur animals
Ruminant PAP (ruminant blood included	UA	UA	UA	UA	A
Non-ruminant PAP	UA	UA	UA	A	A
Non-ruminant blood meal	UA	UA	UA	A	A
Insect PAP	UA	UA	UA	A	
Fishmeal	UA	A	A	A	A
Ruminant collagen and gelatine	UA	UA	UA	UA	A
Non-ruminant collagen and gelatine	A	A	A	A	A
Ruminant blood products	UA	UA	UA	UA	A
Non-ruminant blood products	UA	UA	A	A	A
Hydrolysed proteins from ruminants other than those derived from hides and skins	UA	UA	UA	UA	A
Hydrolysed proteins from non-ruminants	A	A	A	A	A
Hydrolysed proteins from ruminants derived from hides and skins	A	A	A	A	A
Di and tricalcium phosphate of animal origin	UA	UA	A	A	A
Milk and milk products	A	A	A	A	A
Colostrum and derivates	A	A	A	A	A
Eggs and egg products	A	A	A	A	A

UA: unauthorised; A: authorised.

3.1.5. Feed production

According to the 2016/2017 annual report by FEFAC, the EU28 farm animals are fed with approximately 480 million tonnes of feedstuffs: 233 million are roughages produced on farm, 52 million are grains produced on farm, 40 million are purchased feed raw materials and 155.4 million tonnes (32.3%) are industrial compound feed. Direct imports of industrial compound feed from third countries are limited in the EU28 to 100,000–200,000 tonnes per year, all species combined (BIOHAZ Panel, 2017). However the import of feed materials into the EU28 reached 43 million tonnes in 2016, of which 0.3 million tonnes were fishmeal.[14]

In terms of production of compound feed, Germany (23.8%: 37 million tonnes), Spain (22.1%: 34.3 million tonnes) and France (20.4%: 31.7 million tonnes) remained the three leading EU countries in 2016, followed by UK (15.7%: 24.4 million tonnes) and the NL (14.5%: 22.5 million tonnes).

Crude protein is a key constituent of animal feed, comprising (on average) 10–18% of cattle diet (Perry and Cecava, 1995; Moran, 2005), 13–20% of pig diet (McDonald et al., 2002), 12–30% of poultry diet (McDonald et al., 2002) and 20–40% of aquafeed (Molina-Poveda, 2016). The protein component of compound feed can be derived from a range of sources, such as soybean, maize meal, cotton seed, fishmeal, synthetic proteins and PAP (if allowed). Assuming the worst–case scenario whereby the protein fraction of an average compound feed (~ 20%) was to be comprised exclusively of PAP, there would be a starting fivefold dilution of any pre-existing contamination of PAP with unauthorised material or ruminant origin, assuming the homogeneous distribution of the contamination in the PAP. This dilution effect would be less in aquafeed (where pig and poultry PAP are already permitted) or pet food, given the much higher protein content of these types of compound feed.

PAP derived from non-ruminants are allowed in aquafeed, while the use of ruminant PAP is still forbidden in any feed for farmed animals. However, milk, milk-based products, milk-derived products, colostrum, colostrum products, centrifuge or separator sludge, gelatine, hydrolysed proteins and

[14] FEFAC. The compound feed industry in the EU livestock economy. http://www.fefac.eu/files/79279.pdf

dicalcium phosphate, eggs and egg-products, including eggshells, tricalcium phosphate and collagen can be legitimately added to feed or feed ingredients. For example, bovine milk powder can be used as an anti-caking agent in mineral supplement preparations.

As indicated above, future regulatory revisions would allow the inclusion of pig PAP in poultry feed, and poultry PAP in pig feed. It will be a requirement of such arrangements that feed production lines are entirely segregated, but this increase in complexity and organisation of the feed manufacture and distribution networks (for both ingredients and finished rations) together with the need for on-farm segregation of rations on mixed species farms, will inevitably increase the likelihood of accidental contamination of ruminants with feedstuffs not authorised for that purpose. It has been previously identified (Detwiler et al., 2010), that 'while maintaining the total ban of PAPs in ruminant feed alone would in theory (e.g. under ideal, controlled conditions) be sufficient to protect cattle and sheep from exposure to potentially infected material, erroneous cross-contamination, labelling errors and fraudulent misconduct could lead to some contamination with PAPs in ruminant feed if they were to be allowed for non-ruminants. Inspections and testing can reduce, but not eliminate such a risk'.

Any overlap in the transportation, storage or handling of feed ingredients that are legitimate for some final feedstuffs, and any physical commonality in production lines, leads to the potential for contamination. Such contamination of ruminant feed with pig rations was identified as a key epidemiological consideration early in the BSE epidemic (Wilesmith, 1996; Stevenson et al., 2005) and led to the requirement that all feed production lines should be physically segregated and species specific.

3.1.5.1. Labelling of feed and feed materials

According to Articles 11 and 17 of Commission Regulation 767/2009[15], the mandatory labelling requirements for compound feed require that labels include 'a list of the feed materials of which the feed is composed, bearing the heading 'composition' and indicating the name of each feed material in accordance with Article 16(1)(a), and listing those feed materials in descending order by weight calculated on the moisture content in the compound feed; that list may include the percentage by weight. . .'.

Article 20 states that only certain feed additives have to be labelled once they are used in feed materials and compound feed. There is no labelling threshold for feed materials or feed additives incorporated into compound feed. Also, if a feed material (e.g. lactoserum) is used as a carrier in premixtures (mixtures of feed additives or mixtures of one or more feed additives with feed materials or water used as carriers, not intended for direct feeding to animals), it has to be labelled according to Regulation 767/2009. If a feed material is used in the formulation of feed additive, i.e. the feed material is specified in the description of the respective authorisation act, the labelling of the final product (the additive itself) does not have to contain an indication of that substance.

The net result of this is that feedstuffs may contain legitimate bovine-derived material, detectable by qPCR, which is not explicitly listed on the label.

3.1.6. Feed testing for the monitoring of the feed ban on the use of animal proteins

3.1.6.1. Feed sampling

There are several regulations in force to direct the monitoring of the feed chain, and compliance with the feed ban. Due to the lack of a direct test for prions or TSE infectivity, feed testing has largely relied upon the screening of feed samples by LM for the presence of any fragments that would indicate the presence of terrestrial animal tissue.

Commission Regulation 51/2013[16] reports methods to be used by the CA of MS to detect unauthorised material in feed. Feed and feed ingredient samples should be taken according to the Commission Regulation (EC) No 691/2013[17] amending Regulation (EC) No 152/2009 as regards

[15] Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. OJ L 229, 1.9.2009, p. 1–28.

[16] Commission Regulation (EU) No 51/2013 of 16 January 2013 amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed. OJ L 20, 23.1.2013, p. 33–43.

[17] Commission Regulation (EU) No 691/2013 of 19 July 2013 amending Regulation (EC) No 152/2009 as regards methods of sampling and analysis. OJ L 197, 20.7.2013, p. 1–12.

methods of sampling and analysis. However, the monitoring of the feed ban is based on the principles of self-regulation by the industry, whereby operators must have plans based on the HACCP principles in their businesses, including their own controls to ensure compliance with the legislation.

In accordance with Article 3 of Regulation (EC) No 882/2004[18] (which is being revised and updated and is due to be replaced by Regulation (EC) 625/2017[19] in 2019), MS shall ensure that official controls are carried out regularly, at 'all stages of production and all types of premises where feed is produced, handled and administered' on a risk basis and with 'appropriate frequency'. MS 'should pay special attention to the definition of criteria that can be related to a risk. The weighting given to each criterion should be proportional to the risk. The inspection frequency and the number of samples analysed in the premises should be in correlation to the sum of weightings allocated to those premises'. The frequency of official controls should be regular and proportionate to the risk, 'taking into account the results of the checks carried out by feed and food business operators under HACCP based control programmes or quality assurance programmes'.

The national control programmes 'should be based on a risk-based strategy where all stages of production and all types of premises where feed is produced, handled and administered are included'. As a result, the number of premises inspected, the frequency of inspections and the samples collected in each type of premises are different for each MS, and therefore not proportional/representative of the feed production at national level.

The audit reports provide evidence that the official feed controls are not always as risk based as they could be. Examples included the fact that there was no information exchange between the ABP inspectors and the feed inspectors as regards the registration of transporters of ABP operators, posing a challenge for the risk-based official controls for feed. Increased risk of breaches of the total feed ban on farms using soil improvers/organic fertilisers were not taken into account in the targeting of the feed samples to verify compliance with the total feed ban. In another case, no samples were collected during inspection visits to farms keeping both ruminants and non-ruminants, although such farms were considered as high priority, in terms of sampling, in the risk assessment of the regions where these were located. In a different situation, a guidance document had been prepared including instructions for inspectors about the raw materials to be prioritised during sampling. Although samples of blood meal, blood products, hydrolysed protein, collagen and gelatine and feed containing fishmeal to be taken in fish feed producing establishments, and establishments producing feed for ruminants, were considered as a priority, it was noted in one audit report that the vast majority of the samples taken so far derived from feed for aquaculture animals containing only fishmeal.

3.1.6.2. Sampling points

Some guidelines have been issued to prioritise sampling at particular steps, or premises, in the feed chain based on a risk categorisation. For example, Commission Recommendation 925/2005[20] stated that for 2006 'sampling should be targeted on batches or events where cross-contamination with prohibited processed proteins is most likely (first batch after the transport of feedingstuffs containing animal protein prohibited in this batch, technical problems or changes in production lines, changes in storage bunkers or silos for bulk material). Controls could also be extended to the analysis of dust in vehicles, manufacturing equipment and storage areas'. The Recommendation included a list of indicative premises and criteria that should be considered when drawing up a control programme. As a result, risk-based targeting of sampling, based on, for example, previous history or suspicion of non-compliance, use of derogated processed animal protein (fishmeal for non-ruminants), high throughput, multi-species processing and/or manufacturing feed mills or mobile mixers, etc., should be applied.

[18] Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. OJ L 165, 30.4.2004, p. 1–141.

[19] Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC. OJ L 95, p. 1. (Official Controls Regulation).

[20] Commission Recommendation of 14 December 2005 on the coordinated inspection programme in the field of animal nutrition for the year 2006 in accordance with Council Directive 95/53/EC. OJ L 337, 22.12.2005, p. 51–59.

If it is assumed that contamination with material of ruminant origin occurs with unprocessed ABP, then any contaminant (and associated infectivity) will be evenly distributed throughout the affected PAP. If contamination of non-ruminant PAP with ruminant PAP/MBM occurs after rendering, then the distribution of contamination throughout the batch will be much more heterogeneous. This would also be the case with any contamination that was related to transportation or storage, where 'pockets' of contamination would be the case, rather than uniform distribution. The probability that sampling methods would detect such heterogeneous contamination is low. However, regulation 691/2013 requires that 'if by a visual inspection, portions of the feed to be sampled show a difference in quality from the rest of the feed from the same lot, such portions shall be separated from the rest of the feed and treated as a separate sublot.' So, if such heterogeneous contamination was visible to the naked eye, there is provision for specifically investigating this part of the feed batch.

There are no harmonised criteria to define and identify risks with which to develop sampling strategies, precluding the possibility of having an overview of the actual monitoring of the feed ban at an EU level. Moreover, as highlighted in the EFSA opinion on the BSE cases born after the total feed ban (EFSA BIOHAZ, 2017), there is no centralised data collection with regard to feed testing, so information on the actual number of samples tested, the type of sample and the type of premises sampled, and the proportion of samples that have been found positive are not available.

The audit reports highlight the limitations of the current feed sampling strategies in some inspections, providing evidence for one situation in which, despite the fact that the national feed control programme (NFCP) foresaw that, at entry points, 112 samples should be taken on imported consignments of feed materials, only 18 were taken by the two main border inspections posts for bulk feed materials.

3.1.6.3. Sampling methods

The criteria for designing sampling plans are defined at individual MS level and are not directly based on parameters such as tonnage, for example. However, the sampling methods for the official control of feed in all EU MS should follow Commission Regulation (EC) No 691/2013 amending Regulation (EC) No 152/2009 and its guidance document[21] to ensure that they can be considered as representative of the sampled batch. This regulation defines how to take a sample, and the number of individual samples to be taken from a batch, and subsequently aggregated, based on tonnage in order to ensure that they are representative of the sampled material. It mentions that sampling tools can be used provided these are cleaned, but provides no indication of how to do this cleaning. The food business operator (FBO) can propose alternative sampling, as long as it is 'shown to be equivalent', but there is no indication of how to assess equivalence, or who should make this assessment.

The PAP in feed may vary in particle size, depending on the rendering method used to produce it (See Section 3.1.4), but can be up to 50 mm in diameter. Despite the reduction of particle size during processing, particle size may still be variable, leading to a heterogeneous distribution of PAP within compound feed, a feature that might be enhanced by settling during transportation and storage. According to Regulation (EC) 882/2004, official samples should consist of at least two final samples, each weighing 500 g, taken from a larger sample size of up to 4 kg that should be representative of the batch, and well mixed. Two further 'final samples' are prescribed: one for the producer/farmer, the other one for the legal system in the event of a result being challenged.

It is difficult to ascertain the amount of feed from which each sample has been taken (i.e. the average weight of individual feed size sampled), despite having information available regarding the type of premises in which the samples were collected. Approximately 25,000 animal feed samples are collected for testing each year, representing (w/w) in a rough estimation a very small amount (approximately 0.000008%) of the total compound feed production (ca 155 m tonnes) in the EU, assuming an average size of a feed sample 500 g. It can be assumed that if samples were collected at farms, the amount of feed from which samples are taken would be small as if they would be sourced from feed sacks (e.g. 25 kg each) or small silos, i.e. a small proportion of the overall feed production. If the samples were collected at feed mills, mobile mixers or alike, the amount of feed from which samples are taken could be considered larger, representing medium-large silos (on average, several tonnes of capacity). Finally, if they were collected at border inspection point such as a port, the amount of feed from which samples are taken would be much larger again, as in a container or a ship.

[21] Guidance Document for the implementation of Commission Regulation (EU) no 691/2013 of 19 July 2013 amending Regulation (EC) no 152/2009 as regards methods of sampling and analysis endorsed by the Standing Committee on the food chain and animal health section animal nutrition at its meeting on 16–17 June 2014). Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-guidance_documents_691_2013_en.pdf

In any case, considering the overall number of samples collected in the EU in one single year, it can be speculated that the ability of the national sampling plans to detect a batch of feed containing ruminant content is extremely low, even if assuming that the contamination is uniformly distributed.

According to the audit reports, certain weaknesses in the reporting of the sampling procedures mean that it is not always possible to prove that all the samples taken were representative of the batch being sampled. This might affect the reliability of the collected data.

Overall, it is not possible to accurately quantify the sensitivity of the surveillance system for the detection of material of ruminant origin in feed, in which the analytical and diagnostic Se of the tests is just a small component. Taking into account all the above, the overall sensitivity of the surveillance system can be considered to be extremely low.

3.1.6.4. Laboratory testing of feedstuffs for animal proteins

Microscopic examination

Method

Until recently, feed testing has been performed using LM for the identification of bone and tissue fragments of terrestrial origin in feedstuffs that should not contain them.

The LM technique enables the differentiation of fish and terrestrial material in feed. This method is predominantly focused on the presence of bone fragments, although other structures e.g. feathers, hair, scales, may provide circumstantial evidence to enable the differentiation of the respective animal types. The official method has been developed over many years, starting from Directive 98/88/EC[22]. The standardisation of the method (not the principle) was performed through an EURL-AP interlaboratory study (Veys et al., 2010). The presence of blood meal and blood products (i.e. haemoglobin) can also be shown through LM, using a specific reagent (tetramethylbenzidine/hydrogen peroxide).

At least 50 g from the 'final' sample is subsampled and ground for analysis. A portion of 10 g (accurate to 0.01 g) of the ground subsample is then used to carry out the extraction with tetrachloroethylene. The portion for extraction is 3 g in the case of fishmeal or other pure animal material, mineral ingredients or premixes which generate more than 10% sediment.

Following extraction with tetrachloroethylene the sample is divided into sediment and flotate. Microscopic slides are prepared from the sediment and, depending on the operator's choice, from either the flotate or the raw material. Test portions of fractions, spread on slides, should be representative of the whole fraction. No more than six slides per sample should be examined (total number is controlled by the protocol). For full protocol details, see Appendix D.

Interpretation

The sensitivity of this method for the detection of contamination with terrestrial animal tissues has been explored through experimental spiking studies (Veys et al., 2009, 2010), but no spiking study could be fully representative of the field situations due to variability in the size of PAP particles resulting from the rendering process. The method is able to detect the presence of material of animal origin in feed at the level of 1 g/kg (0.1%) with hardly any false negatives (Liu et al., 2011). This method does not allow discrimination between different species of terrestrial animals, as the overlapping of specific features is too high.

The result is therefore 'positive' or 'negative' over a threshold of 0.1% contamination with terrestrial PAP, with no ability to speciate the source of contamination, or to quantify it. Diagnostic sensitivity (DSe) and specificity (DSp) of the method have been estimated though a ring trial involving nine laboratories from eight MS (Lai et al., 2008). The study showed remarkable performance for the detection of both fish (DSe 100%; 95% confidence interval (CI) 90.3–100; DSp 97.1%; 95% CI 94.4–98.75) and terrestrial animal components (DSe 98.15%; 95% CI 99.95–90.1; DSp 98.1%; 95% CI 95.6–99.4) but poor ability to discriminate mammalian from poultry components.

The contamination inherent to any positive result may be attributable to commercially produced PAP of any terrestrial species, or it could be due to the accidental inclusion in compound feed of, for example, rodent carcasses.

Due to the changes described in Section 3.1.4.2, feed testing must now evolve to enable the identification of not just the presence of terrestrial tissue, but also the discrimination of the species of

[22] Commission Directive 98/88/EC of 13 November 1998 establishing guidelines for the microscopic identification and estimation of constituents of animal origin for the official control of feedingstuffs. OJ L 318, 27.11.1998, p. 45–50.

origin. qPCR methods have been developed to identify the presence of, and to speciate PAP, and they can be used independently or in conjunction with LM (See Figures H.1 and H.2 in Appendix H).

Real time Polymerase chain reaction

Method

Polymerase chain reaction methods are based on the amplification of specific DNA sequences that might be species-specific or group specific, the latter being the case in PCR methods for the detection of ruminant DNA (Prado et al., 2007). There are several available methods in the literature, for cattle and/or ruminant DNA detection. However, there is an EURL-AP SOP Detection of ruminant DNA in feed using qPCR based on a real-time PCR method developed by TNO Triskelion (Benedetto et al., 2017). This method was validated in a study organised by the EURL-AP (Fumière et al., 2013). It was also applied in a study organised by the EURL-AP to assess the implementation of the method in the NRL network as it had been validated (Fumière et al., 2012). This method allows the simultaneous detection of five ruminant species: cattle – *Bos taurus*, sheep – *Ovis aries*, goat – *Capra hircus*, red deer – *Cervus elaphus* and roe deer – *Capreolus capreolus*, and it is the one described in Regulation (EU) 51/2013, and currently used by the official laboratories.

At least 50 g of the sample is subsampled and ground for analysis and two 100 mg portions are subsampled. A full description of this method is provided in Appendix D. If results from the two tested portions are not consistent, the test should be repeated.

Several specific instructions need to be followed by laboratory staff, among them and as highlighted by EURL-APs and indicated in Commission Regulation (EU) No 51/2013, laboratories should make sure that the master mix used is fit for the purpose and does not contain bovine serum albumin (BSA) or other reagents that could lead to false positive results due to presence of bovine DNA. A list of recommended master mixes is available from the EURL-AP. If a laboratory decides to use different master mixes, a comparable performance by pretesting should be demonstrated, and a dossier describing these equivalence experiments must be submitted for approval to the EURL-AP via the NRL to demonstrate adherence to good laboratory practice.

The audit reports indicated also occasional problems with contamination of samples had occurred due to the area where the qPCR tests were performed not being adequately isolated from other areas in the laboratory, leading to uncertainties regarding the validity of some results.

Interpretation

Following the light microscopic identification of contamination of feedstuffs with terrestrial material, the qPCR method can be used to speciate the contaminant, or at least exclude the possibility that the contaminant is of ruminant origin. In the case of aquafeed, where the inclusion of pig and poultry PAP is permissible, it is used to screen for contamination with material of ruminant origin.

The qPCR has the potential to provide quantitative results, since it allows the creation of a standard curve (threshold cycle (C_q/C_t) values vs. DNA copy numbers), against which unknowns can be compared and a value can be extrapolated, allowing a quantitative estimation of the initial DNA content. Broadly speaking, the larger the C_q values (Bustin et al., 2009) the lower the number of DNA copies contained in the sample.

The qPCR method was developed with the aim of providing a high sensitivity, and 10 DNA copies is the current established cut-off point, above which a result is considered positive (Benedetto et al., 2017). However, although this test confirms the presence of species-specific DNA, it cannot distinguish tissue types, and therefore cannot identify whether the presence of ruminant DNA is a consequence of contamination, or the result of the presence of legitimate ingredients that may or may not be stated on the product label.

Assuming that the identified DNA is a consequence of contamination, the estimation of the percentage of PAP in feed through the obtained DNA copy number by qPCR is still very challenging, for several reasons:

- The composition of the compound feed: due to the presence of a variety of ingredients, the qPCR reaction can be influenced in different ways, e.g. certain compounds can inhibit the qPCR reaction which might result in late C_q values, and consequently, a lower DNA copy number estimation even in samples with the same percentage of PAP.
- The rendering/sterilisation process applied to the raw samples might affect the DNA copy number due to intensity of the thermal treatment (Pegels et al., 2011; von Holst et al., 2012).

- Despite targeting a nuclear sequence, certain variability of DNA copy numbers can be associated with the fact that the tissues in PAP may vary, e.g. be multinucleate and thus respond differently to qPCR, or the tissue may have widely spaced nuclei, or be anucleate (red blood cells).
- The use of dairy products as ingredients creates issues for test interpretation. Due to the use of DNA as the target molecule, the qPCR method also detects the presence of any other material of ruminant origin, which includes legitimate feed additives such as milk powder, which can contain a substantial number of somatic cells from which DNA can be extracted (Marchis et al., 2017). It is possible to overcome this limitation by using the official method in combination with other techniques (e.g. methods that detect dairy-specific markers) that might be able to discriminate between authorised and non-authorised material of ruminant origin. Such combination of methods has not yet been developed or validated for this purpose, although individual methods have been explored (Rasinger et al., 2016; Marchis et al., 2017; Lecrenier et al., 2018). For example, in the report prepared by the EURL-AP in which low DNA copy numbers were detected in aquafeed samples, an enzyme-linked immunosorbent assay (ELISA) test for the detection of milk proteins was performed on all samples provided, and some positive results appeared to be explained by the presence of those proteins. Nevertheless, ELISA methods targeting milk proteins are currently not validated, and there are few data with which to assess their specificity, reproducibility or robustness. There appears to be no data available, such as spiking studies that might inform on the range of DNA copies that would be expected from the legitimate inclusion of such ingredients in compound feedstuffs.

3.1.6.5. The application of these analytical methods

Directive 98/88/EC established guidelines for the microscopic identification and estimation of material of animal origin for the official control of feedingstuffs. This directive was based on the method developed by the International Association of Feedstuff analysis (IAG). The STRATFEED project harmonised the microscopic method which is the basis of Commission Directive 2003/126/EC[23] on the analytical method for the determination of material of animal origin for the official control of feedingstuffs. There are no comparative data on the analytical sensitivity of LM or qPCR methods. There are also no data on the DSe of the two analytical methods (LM and qPCR), applied either individually or in series. However, it has been accepted that the LM benchmark established in the Commission Directive 2003/126/EC on 'the Sensitivity of microscopy that, dependent on the nature of the constituents of animal origin, can detect levels of contamination lower than 0.1% of mass fraction', is also achieved by the ruminant qPCR. Beyond this threshold, the results of the LM method are not quantitative, whereas the qPCR results potentially are. Figures H.1, H.2, H.3 and H.4 in Appendix H show draft diagrams of the possible combination of methods for the detection of prohibited processed animal protein in feed and feed ingredients, produced by the EURL-AP, as submitted by the European Commission in the letter of clarification of ToR1 and ToR3 of the mandate.

The two tests, LM and qPCR, are intended to detect different material. While the LM is able to detect bone fragments and other structures (feathers, hair, scales) with limited ability to differentiate only between fish and terrestrial animals, the qPCR is aimed at detecting species-specific or group specific DNA, in this case, of ruminant origin. The idiosyncrasies of the two methods and the variability of the matrix to be tested makes it extremely difficult to estimate the detection ability of either of those tests, individually or when they are applied in combination (in series).

Due to the complexity of the technical procedures applied by the industry in order to process raw material under a range of conditions (see Section 3.1.4), and the variable nature of the components included, it is extremely difficult to replicate the full range of outputs of these technological processes for further study under laboratory conditions. Therefore, no comprehensive quantitative spiking studies have been performed.

The presence of ruminant DNA is the only available proxy for BSE infectivity in feed, although only a very small proportion of animals entering the food/feed chain are now infected. However, the currently-applied feed controls rely on a zero-risk tolerance, and the assumption that there is no legitimate inclusion of material of ruminant origin in animal feed, which is no longer the case. It is now legal for a range of ABP products to be recycled to livestock, including ruminants. Ruminant feed can contain hydrolysed proteins, collagen and gelatine from non-ruminants, and milk and milk-based products, amongst others (Jędrejek et al., 2016). Processing methods for the production of hydrolysed

[23] Commission Directive 2003/126/EC of 23 December 2003 on the analytical method for the determination of constituents of animal origin for the official control of feedingstuffs. OJ L 339, 24.12.2003, p. 78–84.

proteins, collagen and gelatine seem to affect the DNA content in such ingredients, while milk and milk products appear to retain a certain amount of DNA that can still give a positive result in qPCR.

This means that any test that identifies ruminant DNA might pick up such ingredients, but would be unable to distinguish between legitimate ruminant-derived components of the feed, and accidental contamination with ruminant PAP. This issue is further complicated by the fact that some products, for example milk powder, can be used not as primary feed ingredients, but as vehicles for, or anticaking agents in, additives such as mineral supplements and would not therefore be explicitly labelled on the final product. This could lead to the seizure and withdrawal from the market of feedstuffs which are mistakenly identified as contaminated by ruminant processed protein.

In recent years, the ways in which laboratory methods are used to test feedingstuffs for the presence of unauthorised material of animal origin have been improved and new data and information have become available.

Annex VI of Commission Regulation 152/2009 was entirely revised and amended by Commission Regulation 51/2013. The latter Regulation utilises the combination of LM and qPCR for the detection of animal proteins in feed in the EU. This Regulation also assumes their correct implementation in accordance with the SOPs produced by the EU reference laboratory for animal proteins in feeding-stuffs (EURL-AP). These SOPs complement the Regulation. The SOPs detail the operational protocols that have to be followed, depending on the type of feed being analysed. The final destination of the compound feed or feed materials determines the operational protocol which has to be followed.

According to the Feed EURL SOP 'Operational protocols for the combination of light microscopy and PCR' version 3.0 (Benedetto et al., 2015), two different operational protocols should be followed depending on the type of feed being analysed (summarised in Appendix D):

- Feed and feed material intended for farmed animals, other than aquaculture and fur animals, should be tested by LM in order to detect the presence of particles of animal origin. If particles of animal origin are detected, and if the material is intended for ruminants, the result will be considered positive. However, if the material is intended for non-ruminants (e.g. pig or poultry) or as milk replacer, samples will only be considered positive if particles specifically from terrestrial animals are found.
- For feed or feed material intended for aquaculture either the LM or the PCR method may be performed in the first instance, depending on the composition of the feed. If the composition is not known to include terrestrial PAP or blood products, LM is applied first, with PCR subsequently applied if particles from terrestrial animals are found by LM. If feed is known to contain terrestrial PAP or blood products, LM becomes irrelevant and the qPCR method is applied as the primary test.

The DSe will be different if feed is screened by LM or ruminant qPCR. Additionally, if feed is known to contain blood products, as indicated for instance from the declaration or the labelling, the qPCR method shall only be applied in the first instance at a frequency of 1 in 10 samples (1/10) chosen on a random basis (the random character of this selection must be recorded so that it could be proven). However, in case of suspicion of irregularity or positive results in previous qPCR tests, the qPCR method can be applied first on all samples of feed or feed material from the same origin.

In the overall scale of the feed chain, and considering the steps in which the detection ability of the surveillance system is already compromised/affected (heterogeneous distribution of unauthorised materials, National sampling plans, actual sampling, fractioning, testing), as it has already been concluded in a previous EFSA opinion (EFSA BIOHAZ Panel, 2017), 'the sensitivity of the control programme in place for the monitoring of the ban on feeding processed animal protein to certain animals is expected to be limited'.

3.1.6.6. Analysis of the feed testing data

The EC survey

The questionnaire used by the European Commission for the survey on the controls of the feed ban and the aggregated results by three categories (see Section 2.1.1) and by MS are shown in Appendix F.

Looking at the overall results of this questionnaire survey, a total of 1,839 samples of non-ruminant PAP were tested for the presence of ruminant PAP in 2016 by 21 MS out of the 27 that participated in the survey. The mean percentage of positive samples was 0.7%, ranging between 0% and 25%, due to one single MS that detected 10 positive samples out of 39 tested (25%).

The largest contingent of samples tested in EU MS is feed other than aquafeed, with 25,261 and 22,898 samples tested in 2015 and 2016, respectively, with only 24 and 12 samples found positive to non-authorised PAP (0.1% and 0.05%).

The proportion of positive results in the tests conducted to detect ruminant PAP in feed for aquaculture animals in the EU was 6.1% in 2015 and 2.7% in 2016, out of a total of 654 and 634 samples tested, respectively. This is a good sentinel target group for the presence of contamination in the feed productions lines affected by the partial lift of the feed ban in 2013 allowing pig and poultry PAP in aquafeed, as long as the identification of authorised material of ruminant origin has been ruled out.

Individual MS data

Analysis of detailed feed testing data provided by the four selected NRL showed that there is a very heterogeneous implementation of the official controls, based on the criteria defined in national programmes.

Only three out of the four MS (anonymously hereinafter referred to as MS1, MS2 and MS3) were able to provide sample level data over the 2013–2017 period. In total, 23,094 samples have been merged in a unique data set that has been used to obtain descriptive figures to illustrate some general patterns of the European testing activity on feed, assuming this subset of data is representative of the overall EU feed testing activity.

There are noticeable differences in the type and numbers of feed samples tested by official laboratories in the MS. For example, looking at the data from the three MS, the sample material mostly tested in MS1 and MS3 was feed (with bovine feed accounting for, respectively, 37.8% and 55.6% of the total and non-ruminant feed to terrestrial animals for respectively 21.8% and 22.2%), while in the other MS it is fishmeal (61.6%), and minerals and products derived thereof, that were the most commonly sampled matrices.

MS1 and MS2, both with a very large production of livestock feed, have very different control activities. In MS1 during the period 2013–2017 more than 11,000 samples (including different types of samples) were submitted for laboratory testing, mostly (79.9%) as a result of feed mill inspections, while MS2 only tested around 1,000 samples (including different types of samples) as part of the official controls, and mostly at border inspection points (85.6%). MS3 which has a lower feed production throughput sampled and tested 10,830 samples (including different types of samples) during the same period mostly at farms keeping either ruminants (57.9%) or non-ruminants (12.4%).

In general, feed was the most sampled target. However, vegetal raw material was the second and the fourth most tested matrix in MS1 and MS3, respectively, and fishmeal and minerals were the first and second most tested in MS2. In the three MS, a small and heterogeneous proportion of tests was applied directly to PAP for the identification of ruminant protein (1.2% in MS1, 3.7 in MS2 and 0.2% in MS3). Ruminant qPCR has been starting to be used like LM as a screening method only for the last 4 years, accounting for 2.2% (n = 506) of all tests carried out (2%, 6% and 2% in respectively MS1, MS2 and MS3). Ninety-five per cent of ruminant qPCR testing was targeted to the following six matrices: aquafeed (n = 299 i.e. 59%), non-specified PAP (12.7%), poultry PAP (9%), pig PAP (5.9%), vegetal raw materials (4.7%) and blood meal (3.4%).

In terms of analytical results, the overall proportion of positive samples identified by either test is 100-fold different (LM = 0.22%, qPCR = 21.9%). Up to 10-fold differences are also apparent between MS: MS2 found 4.2% of the samples positives while MS1 and MS3 found 0.5–0.6%. These differences may be due to different national risk ranking, or to different targeting of sample materials or sampling premises.

In addition to the general information provided in response to the EC questionnaire, specific data on C_q values and DNA copy numbers were provided by a region from MS1 (12 positive samples), MS2 (22 positive samples) and MS3 (58 positive samples). The results showed that in the last 5 years 29 out of 75 non-ruminant (i.e. from pig or poultry) PAP samples tested positive for ruminant DNA (39%). The DNA copy numbers were available for two pig PAP with 43 and 3,066 DNA copies, respectively, and five poultry PAP samples with a mean number of DNA copies of 272.8 (range: 73–819). Non-specified animal PAP (n = 15) showed high values with a mean close to 20,000 DNA copies (range: 18–106,299), whereas the mean for aquafeed positive samples (n = 64) was 118.6 (13–2,496). Nine samples from 'other' category had a mean number of DNA copies of 308.3 (range: 18–1,497). When considering the premises at which samples were collected, and after aggregation into three broad categories i.e. farm, feed mill and border, the mean numbers of DNA copies detected were 26,403 (n = 37), 7,212 (n = 43) and 157 (n = 3), respectively.

3.1.6.7. Non-compliance with the official controls

Actions following non-compliance with the official controls are covered by Regulation 882/2004. The management of information is governed by Regulation (EC) 178/2002[24], which established the RASFF system, a traceability system that allows CA to exchange information. When a non-compliant result occurs, the CA performs an evaluation of risk and identifies any possible action, which should be proportionate to the risk evaluated. When deciding which action to take, the CA shall take account of the nature of the non-compliance and of the operator's past record with regard to non-compliance. RASFF usually does not provide guidance to MS on what actions should be taken as a consequence of a TSE notification in feed. Such actions follow from the relevant legislation in place, but at present there are no harmonised criteria across MS for risk decision/actions taken on TSE notifications in feed risk evaluation. These criteria are defined at MS level, whereas the European Commission only verifies the risk evaluation before the notification is reported on the system.

According to Article X of Regulation 882/2004, when the CA identifies non-compliance, it shall take action to ensure that the operator remedies the situation. In case of suspicion of non-compliance, the competent authorities shall perform an investigation in order to confirm or to eliminate that suspicion and if non-compliance is confirmed, the CA can take a number of measures including:

- *restrict or prohibit the placing on the market, the movement, the entry into the Union or the export of animals and goods;*
- *order the recall, withdrawal, removal and destruction of goods, authorising, where appropriate, the use of the goods for purposes other than those for which they were originally intended*.

Anecdotal evidence suggests that aquafeed samples positive to ruminant DNA are not considered to be a serious problem, because the end users of these products are generally fish farms on which there are no other livestock, so the likelihood/opportunity for accidental cross-species feeding is very low. Only about 20% of positive results for non-authorised PAP in feed appear as RASFF notifications (see Appendices F and G) which are considered 'non-serious', despite triggering different responses varying from 'no action taken' through 'withdrawal from the market or from recipients', 'official detention', 'detention by the operator', 'informing the authorities', 'informing recipients' to 'seizure' and 'destruction' of the batch. It should be noted that RASFF notifications only regard products which have been distributed to other Member States or countries.

At present, there are no harmonised criteria across MS for risk decisions on TSE notifications in feed risk evaluation, although such actions follow from the relevant legislation in place. These criteria are defined at MS level, whereas the EC only verifies the risk evaluation before the notification is reported on the system. RASFF usually does not provide guidance to MS on what actions should be taken as a consequence of a TSE notification in feed.

Testing for ruminant protein in the context of official controls is carried out by the feed testing NRLs with the EURL-AP overseeing methods and laboratory performance. However, there is no centralised collection or analysis of data, or interventions, beyond individual MS level.

An intervention response to a positive test result is inappropriate if the result is attributable to a legitimate ingredient, which might not be stated on any label.

3.1.7. The technical zero approach for the ruminant qPCR

The EURL-AP technical zero report, to be used in the context of the detection of animal proteins in feeding-stuffs and submitted as part of the mandate, details the proposed application of the ruminant qPCR method based on the concept of 'DNA copy number of the ruminant target' in a sample as the result, rather than the current qualitative approach whereby a sample is declared positive/negative based on a standard cut-off point established through the calibration of the qPCR.

The concept of the technical zero method was proposed by the EURL-AP following a request from the EC to explore the possibility of defining a threshold ('action limit'), expressed in number of ruminant DNA copies, below which the test should be considered negative ('technical zero') and therefore requiring no action, with the aim of addressing the problem of a proportion of feed samples positive to the ruminant qPCR, which are argued, by the industry, to be 'false positives'. It is important to note that 'false positive' in this case is a management threshold and not a technical one. Any

[24] 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.

sample that produces a positive signal with a C_q value lower than the predefined cut-off is considered positive, regardless of the origin of the signal. Creating an 'action limit' will reduce the number of positive results that need to be acted upon, by ignoring samples with results under the threshold that could be due to contamination as well as those resulting from legitimate ingredients.

According to the EURL-AP technical zero report, 'a DNA copy number of 10 corresponds in practice to the cut-off'. The interpretation of the results as in Tables 1 and 2 of the report is consistent with the declaration of a positive sample to those with more than 10 DNA copies. The EURL-AP Standard Operating Procedure Detection of ruminant DNA in feed using real-time PCR, 2017 (Benedetto et al., 2017) in its Section 7.2.2 states that the criterion to meet for the quality control of the cut-off value corresponds 'to the cut-off value of > 9 copies'. The same SOP also states in Section 4.2 that 'the absolute LOD valid for the PCR step is 20 copies (when the cut-off is calculated at 15 copies)' and that 'in-house validation at EURL-AP and the implementation study showed also that the limit of detection of the method (thus including sample preparation and extraction) is < 0.1% in mass fraction of ruminant PAP in feed as no false negative result was recorded at this level (when the cut-off is calculated for the upper confidence interval – in terms of C_t – at 15 copies of the target)'. Acknowledging the potential variability of the cut-off point of the ruminant qPCR in terms of number of DNA copies, for modelling purposes this scientific opinion has considered the interpretation cut-off point for the declaration of a positive sample to report 10 or more DNA copies.

The application of a 'technical zero' means shifting the interpretation cut-off point, as above described, to an arbitrary level of 100, 150, 200, 250 or 300 copies. Thus, if an interpretation cut-off point of 300 ruminant DNA copies is applied, all samples containing between 10 and 300 DNA copies, which would have been declared positive in the past, would be reported as negative with the technical zero approach.

Since the LOD of contamination in a sample, established for LM, has been accepted to be 0.1%, the EURL-AP sets out the values representing the probability that the DNA copy number being produced by the qPCR when testing non-ruminant PAP that has been contaminated to a level of 0.1% with ruminant PAP for interpretation cut-off points of 300, 250, 200, 150 and 100 DNA copy numbers, respectively.

The EURL-AP technical zero report claims that, without a technical zero, the probability that there might be unauthorised material of ruminant origin in non-ruminant feed is small as it can be estimated, based on counter-analysis, that more than 95% of the aquafeed batches contain less than 0.1% of unauthorised ruminant PAP (in mass fraction). It is recognised that trying to convert the unit (DNA copy numbers) into mass fraction would require data on the distribution (in DNA copy numbers) of a given amount of ruminant PAP taken randomly in the EU. It is also stated that, considering several samples prepared at the EURL-AP for proficiency tests, the copy number of ruminant targets corresponding to a spiking level of 0.1% in mass fraction lies within a range from 424 to 798 copies, i.e. clearly beyond 300 copies. There is no information given with regard to the representativeness of the samples tested. For example, rendering processes can in principle greatly affect the DNA copy numbers in PAP, but no evidence of such a study has been provided.

Difficulties with the calculation of the percentage of ruminant PAP in feed by estimating a correlation with either DNA copy number or ng of DNA, estimated in turn through the C_q value obtained during the qPCR-based DNA amplification, have been pointed out by several research groups. As examples of these difficulties, Pegels et al. (2011) tested a qPCR method for the detection of bovine (*Bos taurus*), ovine (*Ovis aries*) and caprine (*Capra hircus*) with 126 industrial feed samples that were manufactured to reproduce rendering processes of commercial feeds destined for farmed animals. They concluded that the real quantitative capability of the assay was limited by the existing variability in terms of composition and processing treatments of the feeds, which affects the amount and quality of amplifiable DNA. In another study, the impact of different sterilisation temperatures performed under real world rendering conditions was evaluated to conclude that at high temperatures the DNA copy number was significantly reduced, and that will influence the estimation of the percentage of MBM (and consequently also PAP) when the rendering conditions are unknown. Moreover, the frequency distribution of the target analyte (DNA copy number of mass fraction MBM) showed deviations from normal distribution, especially when the variation of the C_q values exceeded a certain level (von Holst et al., 2012). Another study for the detection and quantification of fish DNA content in feedstuffs by qPCR also reported bias on fishmeal w/w concentration estimation (Benedetto et al., 2011). An estimation of the ruminant copy number of a specific sample that corresponds to 0.1% w/w is therefore uncertain, given that a different sample might result in another copy number at the same level of 0.1% w/w of contamination. Such validation of the sensitivity of the technical zero

according to internationally accepted scientific protocols, as stated in Regulation (EC) 882/2004, seems not to have been carried out. The sensitivity of the method is also dependent on the efficiency of recovery of the extraction method. Validation of the sensitivity of the technical zero using the proposed interpretation cut-off points should be carried out on feed samples containing different ruminant PAP at 0.1% w/w. Heterogeneous feed samples should be also included in the tests, i.e. aquafeed containing different ingredients (fish oil, non-ruminant PAP, non-ruminant blood products), pig feed, poultry feed, etc., following ISO 22118/2011 or other relevant protocols.

The report does not provide any specific supporting experimental evidence for samples of non-ruminant PAP, or compound feed, being spiked with a sliding scale of ruminant PAP contamination (for example, 0.001%, 0.01%, 0.1%, 1%, 10%) to enable estimation of either the mean copy number or the distribution of copy numbers detected, using a defined range of materials processed by different rendering methods (batched, continuous, atmospheric, and pressurised rendered). The Report acknowledges these points stating that 'as data to do this are lacking, a theoretical distribution based on what are reasonable assumptions is made and even if this does not fit completely to reality it seems to us it can be used for the risk assessment because we provide evidence that it is worse than reality'.

There is no evidence in the report for the selection of the normal distribution as the theoretical distribution to allocate the DNA copy numbers equating to a mean concentration of 0.1% w/w. Moreover, there is no indication of what the normal distribution represents. As mentioned by von Holst et al. (2012), 'the frequency distribution of the target analyte (DNA copy number of mass fraction in MBM) showed deviations from normal distribution, especially when the variation of the Ct values exceeded a certain level. In consequence, asymmetrical confidence intervals for the target analyte would better describe the actual distribution compared to the situation where the data follow normal distribution with symmetrical confidence intervals'. Therefore, it has been shown that in principle these data does not follow normal distribution. It could represent the variability within the process or material or uncertainty associated with an average process/material tested:

- variability associated with the rendering method used – batch, continuous, pressurised;
- variability between the copy numbers measured by different laboratories testing methods/ equipment;
- variability in the amount of contamination within a sample based on composition and input tissue types;
- uncertainty about the mean copy number from multiple laboratories testing the same sample;
- uncertainty about the mean amount of contamination across EU materials;
- The report does not provide any specific supporting experimental evidence for samples of non-ruminant PAP, or for compound feed being spiked with different levels of legitimate feed additives for comparison.

If current testing methods are to be changed by applying the technical zero approach, the number of trace back investigations initiated due to test positive results will be reduced, due to samples that are currently recorded as low positives being assigned to below the action threshold, and therefore considered negative. However, the overall sensitivity of the surveillance system for the detection of material of ruminant origin in feed will be reduced, with the extent of this reduction directly correlated to the qPCR copy number assigned as the management threshold.

The potential changes in the legislation, whereby non-ruminant PAP would be re-authorised in non-ruminant feed, subject to maintenance of the ban on cannibalism, the implementation of channelling requirements (separate production lines), and the availability of validated and operational laboratory control methods, could result in the significant reduction of the value of the microscopic analysis method following the relaxation, for example, of the pig to poultry and poultry to pig feed ban, and increased reliance on the ruminant qPCR test only. The EURL-AP report stated that 'extending the lifting of the feed ban on non-ruminant PAP in other feed than just aquafeed also increases the risk resulting from the confusion in the use of for instance pig feed or poultry feed to feed ruminants. Therefore, it seems that the main additional risk that can arise from a technical zero is linked to this additional pathway of contamination'. The implementation of the technical zero in a new legal framework of the feed ban would result in an increase in the risk that may not be accounted for in the present estimation of the PAP model (see Section 3.2) but is addressed by the FEED model (see Section 3.3).

It is important to state that the implementation of the current protocol of the ruminant qPCR for detection of ruminant content remains valid in the context of PAP testing, for which any positive result in non-ruminant PAP can only be due to the unauthorised presence of/contamination with material of

ruminant origin. The application of the technical zero would not be appropriate under these circumstances because the analysed substrate is pure PAP and would not have any other ingredients added at this point. Consequently, the technical zero is a construct that only applies to testing in the context of feed.

In the overall scale of the feed chain, and considering the steps in which the detection ability of the surveillance system is already compromised/affected (heterogeneous distribution of unauthorised material of ruminant origin, National sampling plans, actual sampling, fractioning, testing), the implementation of the technical zero, as proposed by the EURL-AP, would have a diminishing but minimal impact on the already low overall sensitivity of the surveillance system for the detection of material of ruminant origin in feed, regardless of the chosen 'action limit'. An attempt to quantify such impact is reported in Section 3.3.2 with the outputs of the FEED model.

3.1.8. Concluding remarks

- Despite the overall effectiveness of the various feed bans (ruminant-to-ruminant or mammalian-to-ruminant), a total of 60 BSE cases in cattle born after the 1 January 2001 had been detected in the EU by the end of 2017. The EFSA scientific opinion on the BSE cases born after the total feed ban (BIOHAZ Panel, 2017) concluded that feed-borne exposure is their most likely origin, either through ruminant feed contaminated with ruminant protein or cross-feeding of ruminants with contaminated non-ruminant feed.
- The feed ban is based on four major pieces of EU legislation relating to the control and eradication of TSE, feed and food law, animal health and animal welfare rules and the use of animal by-products. Other minor Regulations and Directives support the implementation in terms of specific instructions such as feed sampling or testing.
- The EU legislation requires competent authorities to monitor the feed ban for the use of the unauthorised material in feed through national plans, not validated by any EU authority. Monitoring of the feed ban relies also on the principles of industry self-regulation, whereby operators must have plans based on the HACCP principles, including their own controls, to ensure compliance with the legislation. The current legal obligation is to sample at the feed production/distribution/consumption level and not at earlier steps of the feed chain, i.e. before/during/after the production of PAP.
- As stated by the EU legislation, in the national plans, the official controls have to be applied on 'a risk basis and with appropriate frequency, taking account of identified risks', which results in the number of premises inspected, the frequency of inspections and the samples collected in each type of premises being different for each MS, and not necessarily proportional to/representative of the feed production at national level. The national control programmes 'should be based on a risk-based strategy where all stages of production and all types of premises where feed is produced, handled and administered are included'. There are no harmonised criteria to define and identify risks with which to develop sampling strategies: as a consequence, it is not possible to have an overview of the actual monitoring of the feed ban at the EU level. Also, there is no EU-level reporting system of the existing feed testing activities that would allow the collection, collation and analysis of EU-wide feed testing data.
- The production of industrial compound feed in the EU was approximately 155 million tonnes in 2016. The EU is self-sufficient in terms of PAP production. Most of the PAP used for feed in the EU is internally produced. The size of the sampling frame from which samples are to be taken is large and the heterogeneity of the distribution of cross-contamination with PAP in sampled material can also be expected to be large. Despite targeting sampling in premises indicative of high risk, the representativeness of the material properties of the samples taken with the current protocols for food and feed materials cannot be ensured.
- Two methods are currently authorised to analyse feed samples for the detection of unauthorised material of animal origin: LM and ruminant qPCR, implemented according to protocols developed by the EURL-AP. Methods are detailed in the legislation to standardise the manipulation of the collected samples in order to produce homogeneously-distributed fractions for testing.
- The two tests are intended to detect different material. Whereas the LM is able to detect bone fragments and other structures (feathers, hair, scales) with limited ability to differentiate only between fish and terrestrial animals, the qPCR is aimed at detecting group-specific DNA, in this case, of ruminant origin.

- There are no comparative data on the diagnostic sensitivity of the two analytical methods (LM and qPCR) and, together with the variability of the matrix to be tested, this makes it extremely difficult to estimate the detection ability of these tests, individually or when they are applied in combination (in series). There are no validated data either on the analytical sensitivity of the laboratory methods. It has been accepted that the benchmark established in the Commission Directive 2003/126/EC on 'the sensitivity of microscopy that, dependent on the nature of the constituents of animal origin, can detect levels of contamination lower than 0.1% of mass fraction', is also achieved by the ruminant qPCR, with its current method cut-off.
- It is not possible to accurately quantify the overall sensitivity of the surveillance system for the detection of material of ruminant origin in feed, in which the analytical and diagnostic sensitivity of the tests is just a small component. It has already been concluded in a previous EFSA opinion (EFSA BIOHAZ Panel, 2017), that 'the sensitivity of the control programme in place for the monitoring of the ban on feeding processed animal protein to certain animals is expected to be limited'.
- Official laboratories continue detecting material of ruminant origin in feed samples. The percentage of positive results in the tests conducted to detect ruminant PAP in aquafeed in the EU was 6.1% in 2015 and 2.7% in 2016. Industry representatives argue that, in some cases, this is due to the presence of authorised material of ruminant origin in aquafeed.
- The current cut-off point for a positive sample in the qPCR is, in practice, 10 DNA copies. The EURL-AP, at the request of the European Commission, has proposed the implementation of a technical zero approach in the protocol of the ruminant qPCR, to establish a higher interpretation cut-off point based on the DNA copy numbers, below which the sample would be declared negative and no legal action would follow.
- Creating an 'action limit' would reduce the number of positive results that need to be acted upon, by ignoring samples with results under the threshold although they could be due to contamination as well as resulting from legitimate ingredients.
- The technical zero is a construct that only applies to testing in the context of feed. There is no industrial process in which authorised material of ruminant origin is added while producing non-ruminant PAP, as is argued in the case of feed. The application of the technical zero would not be appropriate under these circumstances because the analysed substrate would be pure PAP and would not have any other ingredients added at this point. As a consequence, any positive signal detected at this production step would indicate the presence of unauthorised material. Currently, available data from the qPCR testing of PAP samples indicates that almost 40% of samples were contaminated with material of ruminant material prior to their inclusion in any compound feed.
- The potential changes as included in the TSE Roadmap 2, namely, pig PAP being allowed in poultry feed, and/or poultry PAP in pig feed, will pose additional risks of contamination with ruminant material. The physical separation from primary production at slaughterhouses through rendering and dedicated separate production of ingredients of ruminant and non-ruminant feed and of final compound feed production should ensure that further contamination via these routes is minimised.

3.2. The updated EFSA 2011 QRA model in 2018 (PAP model)

3.2.1. Approaches, basis, structure and parameters

To evaluate the residual BSE risk in bovine derived products, EFSA has been developing and maintaining quantitative risk assessment models since 2004. The last version of the quantitative risk assessment to estimate the specific scenario of the annual exposure risk to cattle of infectious BSE material contaminating ruminant feed was published by EFSA in 2011 (EFSA BIOHAZ Panel, 2011a).

Model results are given in terms of the oral exposure of cattle to BSE infectivity per year ($CoID_{50}$ units) for two alternative feeding regimes (Intensive and Extensive), three levels of contamination of the ruminant feed and two levels of potential inactivation (through rendering method 1 and 7 respectively).

The 2001 QRA model has always been considered a dynamic document, to be revised in light of new information and to keep up to date with the changing levels of disease. This current opinion has provided an opportunity to:

- review the 2011 QRA model for inclusion of testing controls applied by the National Feed Audit by MS,

- extend the risk assessment with a review of the basis, assumptions and structure of the 2011 QRA model to estimate BSE infection risk posed to cattle as the probability of exposure to contaminated ruminant feed containing the BSE agent, using as the units of interest the total amount of infectivity (expressed in $CoID_{50}$ accessed by cattle per year) and the number of new BSE-infected bovine cases derived from contaminated PAP;
- review and update input parameter data.

As described in Section 3.1.4.1, the standard processing method 7 for PAP is based on microbiological criteria for *Salmonella*, *Clostridium perfringens* and Enterobacteriaceae for the final product, and reduced TSE infectivity does not need to be demonstrated or, in fact, achieved. Since method 7 is the method most frequently applied to Category 3 material, the model has been run applying method 7 with no inactivation of the TSE agent.

3.2.1.1. Assumptions of the PAP model

The 2011 QRA model estimated the scenario of the BSE infection risk posed to cattle as the probability of exposure by feeding BSE material through contaminated ruminant feed, with the following cross contamination steps in the risk pathway being quantitatively modelled:

- proportion of bovine Category 1 material that would remain in the bovine by-products of an animal (bovine Category 3 material) due to incomplete removal from the carcass in the slaughterhouse (0.1–5%);
- proportion of ruminant PAP produced from bovine Category 3 material (91.15%, using latest Eurostat data referred to 2016) assuming the proportion of category 3 material is proportional to the weight of meat produced by cattle, sheep and goats;
- proportion of contamination of non-ruminant PAP with ruminant PAP (0–5%);
- proportion of contamination of ruminant feed with non-ruminant PAP (scenarios: 0.1%, 0.02% and 2%).

The 2011 QRA model did not assess the risk posed by individual batches of PAP and did not specify the exact origin, mechanism or comparative contribution of different contamination pathways and events which may occur. This was due to a lack of key information regarding the individual processes in place and the ways and frequencies at which contamination could occur. For example, contamination could occur within transport vehicles, from mislabelled products, or contaminated processing lines, among others. Each of these events may take place at a certain frequency per year and result in a range of weights of contamination to transfer into that product. In the absence of data, the 'frequency' of contamination events is not included in the 2011 QRA model, only the overall annual weight of contamination transferred to ruminant feed at the EU level is considered, therefore requiring the assumption that EU MS can be considered as a single epidemiological unit.

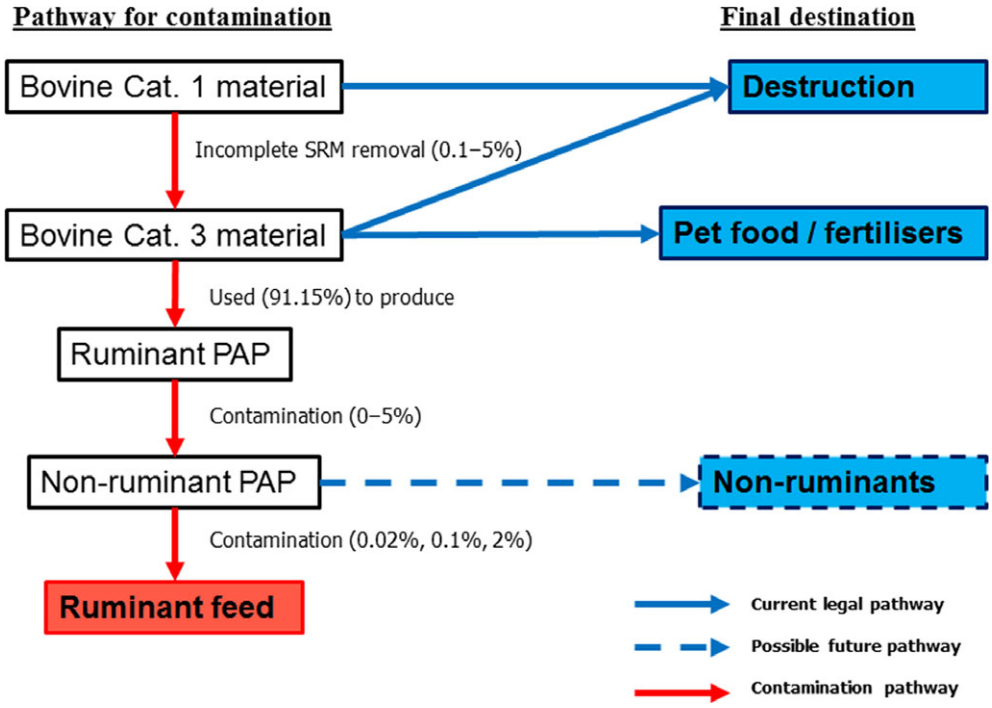
For the estimation of the range in the proportion of contamination of non-ruminant PAP with ruminant PAP (0% to 5%), 'it is assumed that if non-ruminant PAP was allowed to be used in some animal feed (e.g. porcine PAP in poultry feed), then EU regulations would require the complete separation of both rendering and handling facilities. This item reflects the conservative assumption that non-ruminant PAP may be contaminated with ruminant PAP, despite the requirements for separation of rendering facilities and handling' (EFSA BIOHAZ Panel, 2011a). However, it is not explicitly stated which separation processes (current or future) are included in this estimate and which are not.

For the estimation of the different values for the proportion of ruminant feed contaminated with non-ruminant PAP, again there was little information with which to parameterise the 2011 QRA model. The value of 0.1% was selected at that time, based on the estimation of test sensitivity of classical microscopy i.e. ruminant feed may contain up to 0.1% non-ruminant PAP without the contamination being detected. In fact, Commission Directive 2003/126/EC[23] established in its Annex that the sensitivity of microscopy, dependent on the nature of the material of animal origin, allows very small amounts (as low as 0.1%) in feedingstuffs to be detected. This LOD of 0.1% was set as a benchmark against which the suitability of other methods such as PCR was tested (Prado et al., 2007).

Detection limits of qPCR were not included in the QRA due to the fact that authorised animal material in feed can be a source of the same DNA targets as unauthorised material.

It is not known from reading the 2011 EFSA opinion why the sensitivity threshold of tested samples was used as the most likely level of homogeneous contamination. However, a plausible explanation may be that if contamination levels present at that time were significantly above the threshold, then more positive samples from surveillance would have been detected at that time. If this was the

original assumption, then the value of 0.1% represents the possible limit of contamination in 2011 and may not translate to future levels when change is applied to the PAP/feed system under consideration. For comparison, two other scenarios (0.02% and 2%) were included in the previous EFSA scientific opinion (2005). As shown in Figure 4, of the scientific opinion (EFSA BIOHAZ Panel, 2011a), the unit of interest was defined as $CoID_{50}$ per year.



Note: The yield of ruminant PAP from Bovine Category 3 material was 90% in the 2011 QRA model.

Figure 4: Flow of the annual $CoID_{50}$ through the pathway for contamination according to the 2011 QRA model (as updated for the PAP model)

A review of the structure of the 2011 QRA model was carried out to determine whether feed testing controls could be quantitatively included. To develop the QRA to include the step of a laboratory testing of individual feed samples, against a certain interpretation cut-off based on DNA copy numbers, the following needs to be considered:

1) the QRA needs to be structured so that the variability of contamination per batch can be estimated, and
2) the DNA copy number testing interpretation cut-off point (i.e. 'the technical zero') needs to be scientifically linked to a known weight of PAP.

As the structure of the 2011 QRA model was not set up to model the variability between tested samples, and the interpretation cut-off based on DNA copy numbers cannot be scientifically linked to a known weight of PAP, it was concluded that it was not possible to update the 2011 QRA model to include feed testing controls.

In order to address ToR3 of this mandate, a simpler model which permits the modelling of feed sampling has been developed (the 'FEED model') and is further detailed in Section 3.2.2.

3.2.1.2. Extension of the QRA PAP model

As mentioned, the 2011 QRA model estimated the scenario of cattle exposure to ruminant feed contaminated with BSE material. The new mandate (ToR2 and ToR3) expanded the requested estimation of the risk posed by contamination of generic feed to include the additive effect of exposure of cattle to other feed types.

As described above, there are various points in the supply and processing chain at which exposure to material of ruminant origin or contamination could occur including: at the source of ABPs (abattoirs), at processing/blending plants, at feed mills, mobile mixers or feed factories, on farm,

during storage at various stages from source premises to stores, processing plants, blending plants, compound feed mills and on farm or during transport or at the point of disposal (land).

All potential risk pathways (Figure 2) were reviewed to identify those that could be quantitatively parameterised and those that were considered to be less significant. Information from the audit reports was considered, together with an analysis of the testing results from national feed audits, to take into account where contamination was shown to have occurred, and therefore which processes were susceptible to by-pass or accidental contamination.

In particular the following pathways have been identified:

Fishmeal in milk replacer: There are two routes through which milk replacer could be contaminated with material of ruminant origin:

1) At PAP production level, as stated previously (see Section 3.2.1) Commission Regulation 956/2008 allowed the use of fishmeal in milk replacers. Commission Recommendation 925/2009 (See section 3.5.2.2) promotes risk-based targeting of sampling, based on a range of conditions including the use of derogated PAP (e.g. fishmeal for non-ruminants). A proportion of tested aquafeed samples have been shown to be positive for ruminant DNA (see Annex F), but generally deemed to be of low risk and therefore not withdrawn from use (see RASFF notifications) because they are being fed to fish. It is likely that detectable ruminant DNA is present in aquafeed following the contamination (at PAP production level) of pig or poultry PAP, which are both legitimate ingredients in aquafeed. Subsequent use of some fed fish (and their gut contents) to make fishmeal could result in the transfer of low levels of this contamination into fishmeal and hence into milk replacer.
2) At fishmeal production level, contamination could also occur if facilities or transportation were shared with the production processes for PAP of other species, with the greatest concern in the case of ruminant PAP.

There has been almost no testing of fishmeal, so data are very scarce. There are also no good data on the commercial inclusion of fishmeal in milk replacers, so this pathway cannot be quantitatively assessed at present.

Human error on mixed farms

Farms with mixed species may take delivery of compound feeds for different species (or mix their own). Possibilities are present for contamination through deliberate or accidental feeding of the wrong food by the farmer, inappropriate storage of feeds (e.g. poor segregation), or poor facilities, which could lead, for example, to animal breakouts, giving cattle access to the wrong food. The risk of contamination and the consumption of inappropriate feed under these circumstances will increase when wider use of PAP is permitted in terrestrial animal feed. There are no data readily available at the EU level on the proportion of the cattle population present on mixed farms, and there is no formal recording of actual incidents of the consumption of inappropriate feed by cattle, so this pathway cannot be quantitatively assessed at present.

Pet food

Although pet food can contain ruminant PAP (of either EU or extra-EU origin), it is not currently targeted by national monitoring programmes, as contamination with feed for farmed animals is unlikely. Generally, pet food is produced in separate production plants, and transport and storage are channelled and well separated from other lines. However pet food containing ruminant PAP may be fed to farm pets and be accidentally accessed by cattle. While feed for different farmed animals (e.g. pigs and cattle) are likely be stored close to each other on the same premises, pet food is likely to be stored and handled separately. Pets are more likely to share the same environment as the human residents, rather than those of the farmed animals, which are usually well confined. Therefore, these risk pathways could be considered low to negligible, with high uncertainty and variability between MS and have not been further explored in this scientific opinion.

Soil improvers/Organic fertilisers

Both Category 2 and Category 3 ruminant ABP may be used as soil improvers/organic fertilisers and be stored on farm. Cattle could be exposed to those materials either due to accidental access to them on farm, or by grazing where the soil improver has been spread or by being fed with fodder/grass harvested from that land (Johnson et al., 2007; De Vos and Heres, 2009).

The audit reports provide evidence for absent, or very limited, official controls for soil improvers/organic fertilisers. Inspectors stated that on occasion they could not verify whether soil improvers/organic fertilisers delivered to farms were produced in approved ABP plants with the appropriate processing method, or if transport or storage could pose a risk for contamination of feed. Situations were identified where pure PAP were being used as soil improvers/organic fertilisers, and where no system was in place for the authorisation of the components used for mixing with PAP and MBM to render them unpalatable. Similarly, there was no system to assess that these components actually rendered the soil improvers/organic fertilisers unpalatable for animals or otherwise prevented misuse of the mixture for feeding purposes. Nonetheless, the risk from this pathway has previously been assessed as negligible (SSC Opinion, 2001; Cummins and Adkin, 2007) and therefore has not been further explored.

Aquafeed

As mentioned in Section 3.1.2, aquafeed is allowed to contain PAP with the inherent possibility of it being contaminated with material of ruminant origin. However, in the EU, most of the fish feed plants are physically separated from those producing feed for ruminants, pigs and poultry. Moreover, the probability of an on-farm exposure is unlikely since cattle would not typically be kept on the same farms as aquaculture. Therefore, contamination of ruminant feed with fish feed is considered very low to negligible and this risk pathway has not been further explored in the context of the present Opinion.

Summary of risk pathways

The first two risk pathways (i.e. fishmeal in milk replacer and human error on mixed farms) were viewed as sufficiently important to consider further, however, based on the time assigned to this quantitative reassessment and the lack of available data, no extension of the 2011 QRA model for these pathways has been carried out. Therefore, the risk posed by contaminated ruminant PAP contaminating non-ruminant PAP that enters ruminant feed remains as the only risk pathway included in the updated version of the model (PAP model).

3.2.1.3. Review and update input parameter data

The 2011 QRA model has been updated in two ways: (1) the input data have been reviewed and updated to the most recent or most accurate that are currently available and (2) other available EFSA QRA outputs have been utilised, i.e. the C-TSEMM model (Adkin et al., 2012) to produce more accurate estimates of the number of BSE cases in the total population; and the TSEi (Adkin et al., 2014) has been used to estimate the amount of infectivity in the tissues declassified from the SRM list after the last revision of this list in 2014. Therefore, the update model, i.e. the PAP model, has had a number of key parameters updated.

1) Number of BSE cases in the total population: the total number of BSE cases was estimated by applying the C-TSEMM model which included 2015 surveillance data for the EU28. Since 2009, the last year of surveillance data used by the QRA (2011), BSE cases in the EU have been declining at an exponential rate, lowering the total number of infected cases contributing to infectivity loads. The C-TSEMM (2017 EFSA version updated with 2015 surveillance results) was run for C-BSE, L-BSE and H-BSE strains combined, to estimate the number of BSE cases present in the cattle population by a back calculation model taking into account diagnostic sensitivity, different MS demographics, and the 2017 testing surveillance test requirements (no healthy slaughter testing).
   The results of the C-TSEMM (2017) for EU28 showed that the estimated number of undetected infected cattle in a single year was 11.38 (2.5th percentile 3.61, 97.5th percentile 19.79).
2) Tissues included in the QRA: estimates for the amount of infectivity in bovine Category 3 material and amounts removed as Category 1 material were based on those tissues listed as SRM in 2017. Since the last update of the QRA these rules have changed, with fewer risk tissues classified as SRM and thereby slightly raising the amount of infectivity in tissues being rendered as Category 3 material. The estimated titre of infectivity of the intestines and mesenteries which can now be included in Category 3 material (from MS with negligible BSE risk status) has been calculated by the TSEi. An assumption has been made that all EU MS have achieved negligible risk status and that all intestines and mesenteries are included in Category 3 material. This may result in an overestimate of the risk posed by these materials as some may not enter Category 3 material, and not all MS have been classified as negligible

risk (currently 24 MS and two regions have negligible risk status), which would make them less likely to have contaminated material with infectivity.

The TSEi is a 'stochastic quantitative risk assessment developed to compare the level of infectivity of different TSE agents in animal tissues and to estimate the impact of amendments to the list/age for the removal of SRM on residual TSE infectivity levels for a single infected animal and at MS level per year This QRA is focused on bovine intestines and mesentery. The tissue types identified for modelling are: ileum, duodenum, jejunum, caecum, colon, mesenteric lymph nodes, mesenteric nerves and the celiac and mesenteric ganglion complex (CMGC). Processed products based on these tissues include bovine intestines (duodenum, jejunum, caecum, and colon) used to produce sausage casings, and the rendered fats from mesentery tissues. The ileum is not processed for human consumption' (Adkin et al., 2014). Using this model, the infectivity per infected animal for each tissue type (excluding the ileum) was estimated together with 2.5th and 97.5th range representing the variability per animal. In the PAP model, individual infected animals are not separately simulated. Therefore, for the number of infected individuals to be included in the model sampled, a random sample with replacement was produced for each animal to estimate the final variability range given the expected number of total infected animals, thus reducing the variability bounds.

The results of the TSEi for EU27 slaughtered cattle showed that the estimated infectivity titre from those additional tissues that would be permitted into Category 3 material was a mean of 2.8 $CoID_{50}$ per infected bovine (standard deviation (STD) of 10.7 and maximum value of 1,479).

Estimates of the total amount of BSE infectivity in the EU27 per year in the tissues included in the model are displayed in Table 2.

Table 2: Estimates of the total amount of BSE infectivity in the EU27 in selected tissues per year

Tissue type	**Infectivity per year ($CoID_{50}$/year) Mean (2.5th and 97.5th)**
Ileum	271 (130, 687)
Duodenum	0.02 (0.01, 0.04)
Jejunum	1,350 (644, 3,484)
Caecum	50 (24, 116)
Colon	0.2 (0.12, 0.4)
Mesenteric lymph nodes	3×10^{-5}
Mesentery nerves	249 (218, 284)
CMGC	64 (57, 73)
Total per year	1,985 (1,117, 4,557)

$CoID_{50}$: cattle oral infectious dose 50%.

As the PAP model is a stochastic probabilistic model, distributions are associated with key input data, where known, which represent areas of uncertainty and/or yearly variability with each input parameter. The description of the updated parameters of the PAP model, their values and data sources are displayed in Appendix B.

In addition, a scenario analysis has been run in the PAP model whereby the total amount of infectivity of the carcass of a BSE-infected animal is assumed to go through the feed chain, i.e. without any removal of SRM or reduction of BSE infectivity via rendering. In the absence of more up-to-date estimates of the total amount of infectivity in BSE-infected cattle, the value applied for this scenario was 4,160 $CoID_{50}$ (EFSA, 2005). However, other studies in the literature have reported up to 41,500 $CoID_{50}$ (Comer and Huntly, 2004). It is also important to emphasise that the difference between a fully clinical animal and a preclinically infected animal at slaughter is approximately 50 fold. For cattle infected with BSE, the mean amount of infectivity was estimated to be 9864 Bovine Oral (BO) ID_{50} per carcass (90% confidence interval: 310, 38,840) (Adkin et al., 2013). There are no further recent publications in the literature, however, a recent internal update (2018 using 2017 demographic data) to the UK BSE control model, places the mean amount of infectivity in an infected animal at clinical onset with the addition of peripheral nerves, intestines and mesenteries at 13,000 $CoID_{50}$ (Adkin, 2018). The outputs of the model have been reported as a worst-case scenario where the controls at the abattoir are lifted and there is no reduction of infectivity along the feed chain.

This model consists of four Excel worksheets. The model has been evaluated using @RISK 4.5.5, and run using Latin Hypercube sampling with 100,000 iterations. The four sheets are summarised in Appendix B. The PAP model can be accessed in the following link: https://doi.org/10.5281/zenodo.1292466.

3.2.2. Results of the PAP model

On the basis of the 2015 BSE surveillance data in the EU28, the use of processing method 7, and assuming a 0.1% contamination of ruminant feed with non-ruminant PAPs, the PAP model (Table 3) estimates that the mean of the total BSE infectivity load that could enter in cattle feed per year in the EU would be equivalent to 0.05 $CoID_{50}$ of total exposure for all cattle (95% range: 2.4×10^{-4}–0.33) being present in the 156.7 million tonnes of compound feed produced per year in the EU (FEFAC, 2017). This is four times lower than the 0.2 $CoID_{50}$ (95% range: 9×10^{-5}–1.3) estimated by the 2011 QRA model. Therefore, no additional BSE infected cows would be expected in the EU cattle population per year at the upper 95% confidence level even if a single animal was exposed orally to the total infectivity. The results of the 2018 PAP model are displayed for alternative contamination levels in Table 3.

Table 3: Annual total BSE infectivity in cattle produced in the EU by level of contamination of ruminant feed with non-ruminant PAP and using standard processing method 7

Contamination of ruminant feed with non-ruminant PAP	$CoID_{50}$ units per year			
	Mean	P2.5	P50	P97.5
a) 0.1%	0.05	2.4×10^{-4}	0.012	0.33
b) 0.02%	0.01	4.9×10^{-5}	0.0023	0.07
c) 2%	1.02	4.9×10^{-3}	0.23	6.58

BSE: bovine spongiform encephalopathy; $CoID_{50}$: cattle oral infectious dose 50%; PAP: processed animal protein.

The results in Table 4 show that the estimated individual exposure for the assumed contamination levels is extremely low indeed. The use of processing method 7 for the ruminant Category 3 material and intensive feeding of cattle assuming a 0.1% contamination of ruminant feed with non-ruminant PAP, gives a mean annual individual exposure of 2.46×10^{-9} (95% range: 0–1.6×10^{-8}). Compared to the exposure estimated by the 2011 QRA model, 1.3×10^{-8} (95% range: 6.8×10^{-12}–8.6×10^{-8}) using atmospheric processing (Method 7), the current estimate of individual exposure is 5.3 times lower. This is mainly due to the decrease in the prevalence of disease in the total population, despite the amount of residual infectivity remaining in tissues declassified from SRM and included in Category 3 material.

Table 4: Annual individual exposure of cattle to BSE Infectivity through concentrate feed, by feed system (i.e. intensive vs extensive), assuming standard processing method 7

Contamination of ruminant feed with non-ruminant PAP	$CoID_{50}$ units per animal per year			
	Mean	P2.5	P50	P97.5
Intensive feed system				
a) 0.1%	2.46×10^{-9}	0	4×10^{-10}	1.6×10^{-8}
b) 0.02%	4.91×10^{-10}	0	7.9×10^{-11}	3.3×10^{-9}
c) 2%	4.91×10^{-8}	0	7.9×10^{-9}	3.3×10^{-7}
Extensive feed system				
a) 0.1%	2.44×10^{-10}	0	2.7×10^{-11}	1.7×10^{-9}
b) 0.02%	4.88×10^{-11}	0	5.4×10^{-12}	3.4×10^{-10}
c) 2%	4.88×10^{-9}	0	5.4×10^{-10}	3.4×10^{-8}

BSE: bovine spongiform encephalopathy; $CoID_{50}$: cattle oral infectious dose 50%; PAP: processed animal protein.

Applying the worst-case scenario, i.e. the total infectivity of a whole carcass of an infected bovine going through the feed chain, and assuming a level of 0.1% contamination, the results of the model showed a mean total infectivity of 4.67 $CoID_{50}$ (95% range: 0.23–9.34) per year. That would mean up to four additional BSE-infected cows would be expected in the EU cattle population per year at the upper 95% confidence level, assuming that a maximum of nine animals were exposed evenly to the total amount of infectivity. The results of the 2018 PAP model are displayed for alternative contamination levels in Table 5.

Table 5: Annual infectivity produced by the whole carcass of a single infected animal by level of contamination of ruminant feed with non-ruminant PAP and using standard processing method 7

Contamination of ruminant feed with non-ruminant PAP	$CoID_{50}$ units per year			
	Mean	P2.5	P50	P97.5
a) 0.1%	4.67	0.23	4.65	9.34
b) 0.02%	0.93	0.05	0.93	1.87
c) 2%	93.45	4.68	93.09	186.82

CoID50: cattle oral infectious dose 50%; PAP: processed animal protein.

3.2.3. Limitations of the PAP model

- Given the time constraints and the lack of comprehensive quantitative data, the PAP model has quantified a single risk pathway as structured in the 2011 QRA model. It has been acknowledged that other two routes, namely, the use of fishmeal in milk replacers for unweaned calves and the accidental access of cattle to other species feed on mixed farms, are potential risk pathways that merit further investigation and would be an additive risk to that estimated by the PAP model.
- The modelling approach is conservative. Uncertain model input quantities have in general been defined using conservative assumptions (e.g. proportion of contamination of non-ruminant PAP with ruminant PAP, proportion of contamination of ruminant feed with non-ruminant PAP). Thus, the resulting risk estimates are expected to be biased towards higher values compared to more realistic predictions.
- The PAP model relies on the assumption that the SRM policy and TSE monitoring system in place in 2017 will continue. The model also assumes that only Category 3 material is allowed to enter in PAP produced from material of ruminant origin. In order to assess such limitation, a worst-case scenario has also been modelled in which the infectivity of an entire carcase enters Category 3 material.
- The PAP model relies on the specific scenario described, and on specific assumptions such as homogenous mixing of materials in a very large batch size, because changes to ABP manufacturing methods make it difficult to define a 'batch size' for a continuous process. This allows no estimation of the frequency of contamination events, or the variation in the concentration of contamination that may take place at an individual event level. While conservative values are used, uncertainties related to certain parameters (i.e. number of infected animals, the probability of incomplete SRM removal and the amount of infectious tissue remaining after incomplete SRM removal) were identified.
- As described earlier (Section 3.1.7), alternative test interpretation cut-off points cannot be assessed within the PAP model because of the absence of data linking test outcome with a weight of contamination that could be expressed in the $CoID_{50}$ units that is the output of this model.
- The PAP model calculations are based on the available data at present, including unofficial data about PAP production communicated to EFSA directly by the industry. Changes in PAP and feed production would require adjustment of any future model input data, for example, in the event of the lifting of the pig-poultry, poultry-pig feeding ban.
- Due to uncertainties about the dose–response relationship, and the real distribution of BSE infectivity in the feed, the estimated exposure of cattle to BSE infectivity does not necessarily reflect the individual risk for one cow to be infected with BSE.
- It has not been possible to separate the variability and uncertainty associated with some of the model parameters. However, the model accounts mostly for uncertainty. There is uncertainty associated with the magnitude and direction of the impact on the outputs of the model, should the structure have been modified in order to separate uncertainty and variability, but such revisions were beyond the remit and scope of this mandate.
- The PAP model does not account for an exogenous source of material of ruminant origin contaminated with the BSE agent and relies only on the BSE infectivity present in EU cattle presented at abattoir for slaughter.

- The assumption of considering the EU as a single epidemiological unit, also applied for the analysis of the BSE surveillance in the EU, might be questionable for the surveillance for the detection of material of ruminant origin in feed.

3.3. The FEED model

3.3.1. Approaches, basis, structure and parameters

A probabilistic model (the FEED model) was developed to estimate the specific scenario of the proportion of total feed produced in the EU in a single year that is contaminated with material of ruminant origin (using DNA as a biomarker of such a contamination) and is not detected by the monitoring system in place (see flow diagram in Figure 5). The objective of the model is to assess the impact of the application of the ruminant qPCR at different interpretation cut-off points on the identification of feed, contaminated with material of ruminant origin that could be removed from the feed chain.

The cattle risk has previously been estimated in the PAP (Section 3.2) as the BSE infectivity load that could be expected in cattle feed per year in the EU, measured in $CoID_{50}$, and the number of additional BSE infected cattle that could be expected in the EU cattle population per year as a result.

The FEED model does not estimate the cattle risk, but instead estimates the total amount of feed contaminated with material of ruminant origin that would reach the end of the feed chain undetected. It does so irrespective of the species for which the feed has been produced and irrespective of the presence, or not, of BSE infectivity associated with the material of ruminant origin causing a positive result. In this scenario, the presence of material of ruminant origin is considered a proxy for potential exposure to BSE infectivity, should cattle have accidental access to such contaminated feed.

In order to do so, the FEED model considers some specific components that need to be estimated:

- the total number of feed samples collected by all MS in a single year for the monitoring of the feed ban. This was estimated using the average of the 2-year data provided by the European Commission as part of the questionnaire survey they had previously circulated to MS for the monitoring of the feed ban;
- the distribution of feed samples collected by all MS in a single year, by type of premises. This was estimated from the actual distribution of feed samples collected by three of the four selected MS in the period 2003–2017. The types of premises from which feed samples have been collected have been grouped in four categories: farm, feed mill and border point, and other. To achieve this, a simplified re-categorisation of the types of premises was conducted as explained under Section 2.1.1.1;
- the amount of feed in the batch/lot corresponding to each of the feed samples collected. Based on standard measurements of feed production and storage facilities in the different types of premises, minimum, average and maximum values for the amount of feed represented by the samples have been applied;
- the probability that one feed sample collected by any MS in a single year is contaminated with material of ruminant origin. This was estimated using the actual prevalence of contaminated feed samples detected by three selected MS in the period 2003–2017 using a combination of LM and qPCR, split by type of premises;
- the DNA copy number that each contaminated feed sample contains. This was estimated using the actual DNA copy numbers reported from positive feed samples that had been tested by the ruminant qPCR, available in the data provided by three MS in the period 2003–2017. The estimation was done for each of the three types of premises;
- the number of feed samples declared positive/negative according to the interpretation cut-off point applied to the ruminant qPCR results;
- the estimation of the total amount of feed corresponding to the samples declared positive and/or negative according to the interpretation cut-off point applied by the ruminant qPCR;
- the estimation of the proportion of all contaminated feed that is not detected according to the interpretation cut-off point applied by the ruminant qPCR, going through the feed chain (Output 1);
- the estimation of the proportion of all produced feed in the EU in a year that is contaminated but not detected, i.e. below the interpretation cut-off point applied by the ruminant qPCR, going through the feed chain (Output 2).

The outputs of the FEED model were compared following the application of different interpretation cut-off points of the ruminant qPCR as described in the EURL-AP and in the ToR3 of the mandate: 100, 150, 200, 250 and 300 DNA copies, and using as baseline the current cut-off point of 10 DNA copies.

Model assumptions:

- All EU MS can be considered a single epidemiological unit in terms of production, distribution and usage of feed and applying the same protocols for the monitoring of the feed ban.
- The FEED model does not specify the exact origin of the contamination of a particular compound feed sample, nor does it account for the comparative contribution of different feed types. As an example, the model does not make assumptions about whether the contamination is endogenous, or could have been imported via feed ingredients from third countries.
- The distribution of feed samples collected in each type of premises is equal for each MS since the number of samples collected in a single year has been estimated at the EU level and not at MS level.
- The amount of feed, i.e. a sack, a silo or a border point production batch) from which each sample was collected is different for each of the three types of premises.
- The contamination in the form of material of ruminant origin is uniformly distributed within the amount of feed from which samples have been collected.
- The distribution of positive samples was based on the observed prevalence in each of the three premises categories.
- The detection of a positive sample in feed is based on the application of the LM and ruminant qPCR in series, or ruminant qPCR alone.
- The amount of feed from which samples have been collected and declared positive is removed from the feed chain and does not contribute to the output (exposure).

In order to parameterise the model, several data sources have been used:

- the total amount of feed produced in the EU in a single year (EFPRA, 2017). The overall weight of feed produced in the EU in 1 year at the EU level is the starting parameter of the model.
- results of the questionnaire survey administered by the European Commission on the monitoring of feed ban by MS (Appendix F): total number of tested samples for the controls for ruminant PAP in feed for aquaculture animals and for the controls for non-authorised PAP in feed other than aquafeed, and number of positives;
- feed testing data from three selected MS for the period 2013–2017, including for each sample: sample material, type of premises, tests applied, results, and the ruminant qPCR C_q values and DNA copy numbers for positive samples, when available.

The FEED model is a probabilistic model in which probability distributions are associated with key input data, where known, which represent areas of uncertainty for each input parameter. In order to assess the impact of variability of certain parameters, the model has been run using parameters with alternative scenarios:

- Fixed number of feed samples taken in the EU (average of number of feed samples collected over 2015 and 2016 in two categories: controls for ruminant PAP in feed for aquaculture animals and controls for non-authorised PAP in feed other than aquafeed) vs. the number of samples tested in 2015 and 2016 separately, in order to assess the impact of the variability of this parameter on the overall output.
- The amount of feed from which the samples have been collected has been parameterised for the three types of premises. It is difficult to ascertain the actual weight of an individual amount of feed (e.g. sack or silo or production batch) from which a sample has been taken. Such information does not accompany individual samples. Boundaries for the minimum and maximum amount of feed for the three types of premises have been assigned based on consultation within the WG, and standard sizes of storage containers and facilities. Average amounts of feed have been calculated for each of the three types of premises. In order to assess the impact of this parameter on the overall output, a scenario analysis has been conducted by running the model after fixing the amount of feed as equal for the three types of premises, using the average of the 'feed mill' category. Comparison of the main outputs with the standard settings (different averages for each of the type of premises) has been produced.

- The probability of a feed sample to be contaminated with ruminant DNA detectable by qPCR was extracted from the actual feed testing data of the three selected MS by type of premises, as follows: 0.5% for farm samples, 0.65% for feed mill samples and 1.9% for border inspection samples. As a scenario analysis of the potential impact of the partial lifting of the feed ban, should pig PAP be re-authorised in poultry feed and poultry PAP in pig feed, the probability of contamination has been set at 2%, 3%, 4% and 5% (fixed) for samples taken in the three types of premises, which are between 2.5 and 10 times larger than the observed.

The FEED model has been developed in Excel, and consists of four worksheets: 'Model', 'Input_data', 'Summary_results' and 'EU28_test'. The model has been evaluated using @RISK 7.5.2, and run using Latin Hypercube sampling with 50,000 iterations. The description of the parameters, their units, values and data sources are displayed in Appendix C. The FEED model can be accessed in the following link: https://doi.org/10.5281/zenodo.1292484.

3.3.2. Results of the FEED model

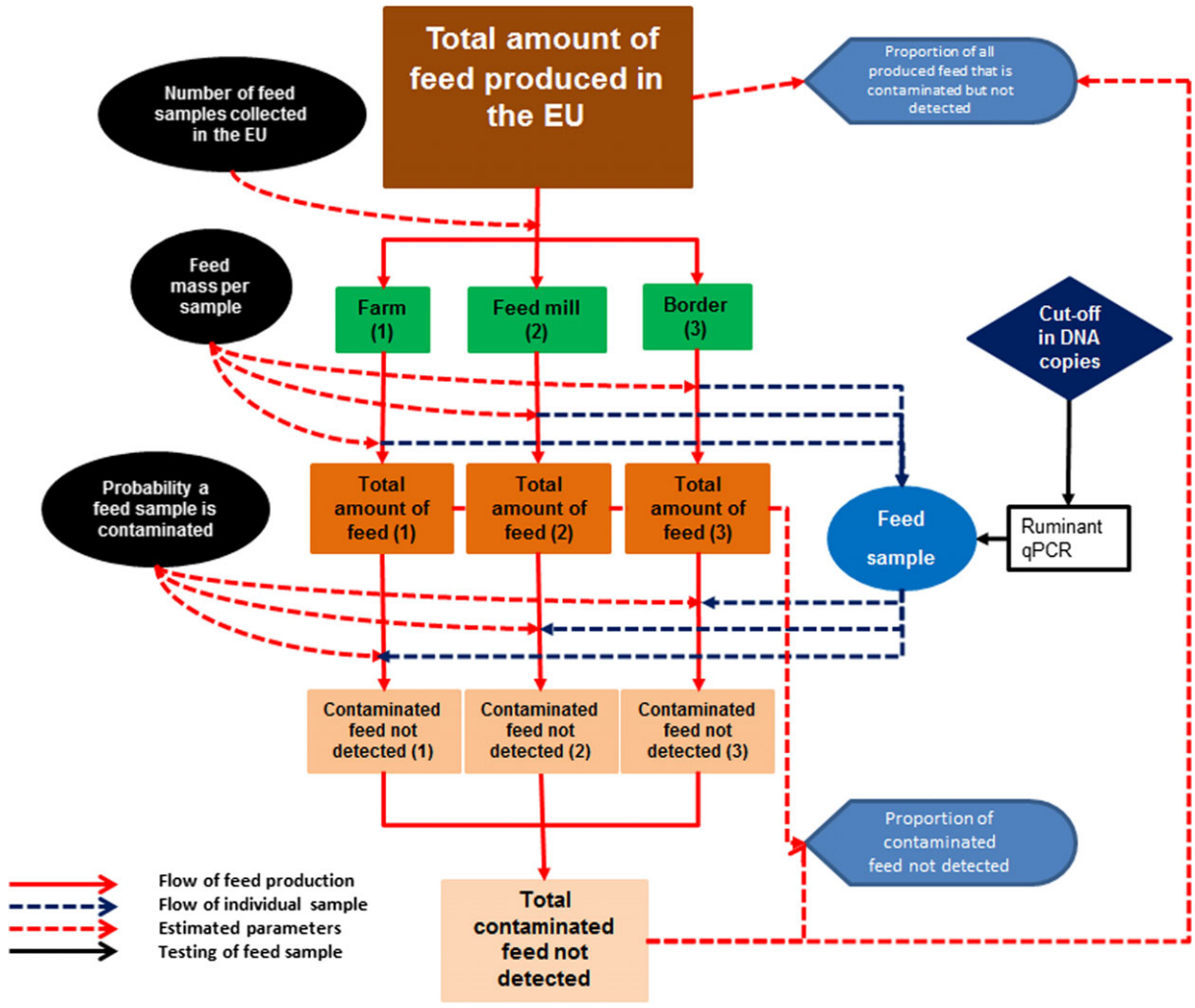


Figure 5: Flow diagram of the FEED model

The results of the model applying standard settings (24,724 samples, 7,513 kg, 16,000 kg and 265,000 kg average amount of feed per sample in farm, feed mill and border samples, respectively; and 0.5%, 0.65% and 1.9% positive feed samples in farm, feed and border samples, respectively) showed that an increase from the current cut-off point of 10 DNA copies applied to the qPCR would result in a reduction of the level of detection and removal from the feed chain of feed contaminated with material of ruminant origin, ranging from a mean of 46.5% (95% range: 20.2–77.4%) to 78.6% (95% range: 48–94.9%) between the interpretation cut-off points of 100 and 300 DNA copies,

respectively (Table 6). Based on the same limits (i.e. 100 and 300), that would also result in a mean proportion of feed contaminated and not detected, ranging from 0.0022% (95% range: 0.0009–0.004%) to 0.0037% (95% range: 0.002–0.0058%), which is equivalent to a mean of 3,604,100 kg and 5,954,600 kg annually, respectively.

Table 6: Outputs of the FEED model by DNA copy number used as interpretation cut-off point with standard settings

Cut-off point (number of DNA copies)	Proportion of contaminated feed not detected (%)				Proportion of all produced feed that is contaminated but not detected (%)			
	Mean	P2.5	P50	P97.5	Mean	P2.5	P50	P97.5
300	78.64	47.99	81.58	94.86	0.003715	0.002014	0.003655	0.005791
250	78.64	47.99	81.58	94.86	0.003715	0.002014	0.003655	0.005791
200	62.23	31.11	63.35	87.77	0.002935	0.001364	0.002852	0.004978
150	62.23	31.11	63.35	87.77	0.002935	0.001364	0.002852	0.004978
100	46.47	20.23	45.59	77.45	0.002184	0.000928	0.002067	0.00412
10	0	0	0	0	0	0	0	0

If the numbers of feed samples for 2015 (25,915) and 2016 (23,532) are applied instead of their average (24,724), there is no difference in the results regarding the mean of the proportion of contaminated feed not detected, across the proposed interpretation cut-off points (Table 7). For the proportion of all produced feed that is contaminated but not detected, the absolute magnitude of change is +/– 0.00018% across the interpretation cut-off points.

Table 7: Outputs of the FEED model by DNA copy number used as interpretation cut-off point with scenario analysis for the number of samples collected in EU in 2015 and 2016

Cut-off point (Number of DNA copies)	Proportion of contaminated feed not detected (%)				Proportion of all produced feed that is contaminated but not detected (%)			
	Mean	P2.5	P50	P97.5	Mean	P2.5	P50	P97.5
23,532 samples (2016)								
300	78.63	47.9	81.58	94.91	0.003535	0.001918	0.003474	0.005506
250	78.63	47.9	81.58	94.91	0.003535	0.001918	0.003474	0.005506
200	62.21	31.05	63.33	87.8	0.002792	0.001302	0.002716	0.004732
150	62.21	31.05	63.33	87.8	0.002792	0.001302	0.002716	0.004732
100	46.47	20.18	45.53	77.43	0.002078	0.000882	0.001966	0.003921
10	0	0	0	0	0	0	0	0
25,915 samples (2015)								
300	78.63	47.92	81.62	94.78	0.003894	0.002112	0.003831	0.006066
250	78.63	47.92	81.62	94.78	0.003894	0.002112	0.003831	0.006066
200	62.22	31.11	63.36	87.82	0.003075	0.001429	0.00299	0.005218
150	62.22	31.11	63.36	87.82	0.003075	0.001429	0.00299	0.005218
100	46.47	20.2	45.52	77.4	0.002289	0.000975	0.002162	0.00432
10	0	0	0	0	0	0	0	0

If the amount of feed from which each sample is taken is fixed for the three types of premises (Table 8), and using the average value (production batch size) for the feed mill category (16,000 kg), the absolute magnitude of change in the mean of the proportion of contaminated feed not detected is variable across the interpretation cut-off points. For the 300 and 250 DNA copy numbers, the mean proportion is 2.4% lower than the value achieved using the standard settings (Table 6), but the proportion increases for the 200 and 150 DNA copy numbers (up by 6.6% to 68.91%) and for the 100 DNA copy number (an increase of 17.4%). In terms of relative difference, using the 300 or 250 DNA copy numbers as a limit results in a 3% decrease in the proportion of contaminated feed not detected, whereas the 200 and 150 DNA copy numbers lead to a 10% increase, and the 100 DNA copy number results in an increase of 28%, giving a final output of 63.91%.

The proportion of all produced feed that is contaminated but not detected is much lower in this scenario compared with the baseline, with absolute differences in the mean proportion of 0.0024% for 300 and 250 DNA copy numbers; 0.0018% for the 200 and 150 DNA copy numbers, and 0.0011% for the 100 DNA copy number. The relative difference in the 300 and 250 DNA copy numbers was 66% reduction in the proportion of all produced feed that is contaminated but not detected, followed by the 200-150 DNA copy numbers with 61.4% reduction and the 100 DNA copy number with 52% reduction. The large reduction in the proportion of all produced feed that is contaminated but not detected is mainly due to the contribution of the amount of feed from which the samples are taken, and specifically for 'border' types of premises going from an average of 265,000 kg to 16,000 kg, despite the low proportion of samples collected in this type of premises (4.2%).

Table 8: Outputs of the FEED model by DNA copy number used as interpretation cut-off point with fixed amount of feed from which samples are taken in the three types premises

Number of DNA copies	Proportion of contaminated feed not detected (%)				Proportion of all produced feed that is contaminated but not detected (%)			
	Mean	P2.5	P50	P97.5	Mean	P2.5	P50	P97.5
300	76.26	66.13	76.54	85	0.001254	0.001001	0.001246	0.001521
250	76.26	66.13	76.54	85	0.001254	0.001001	0.001246	0.001521
200	68.91	58	69.12	78.86	0.001133	0.000888	0.001133	0.001399
150	68.91	58	69.12	78.86	0.001133	0.000888	0.001133	0.001399
100	63.91	52.94	64.02	74.32	0.001051	0.000817	0.001052	0.001307
10	0	0	0	0	0	0	0	0

The results of the scenario analysis of increasing probabilities of contamination of a feed sample with ruminant DNA showed that the proportion of contaminated feed not detected does not vary across different probabilities since this is determined mostly by the DNA copy number applied as the interpretation cut-off point. However, the proportion of all produced feed that is contaminated but not detected suffers a nearly fourfold increase from the mean of 0.0037% with current level of contamination (based on the observed 0.5%, 0.65% and 1.9% positive feed samples in farm, feed and border samples, respectively) and a qPCR interpretation cut-off of 300 DNA copy number, to a mean of 0.014% with a 5% probability of contamination in samples equal from the three types of premises, and an interpretation cut-off of 300 DNA copy number. This fourfold increase is due to the significant increase in the total amount of contaminated feed that is not detected by applying the different interpretation cut-off points in the qPCR. The impact of the probability of contamination on both output parameters of the model are displayed in Figure 6.

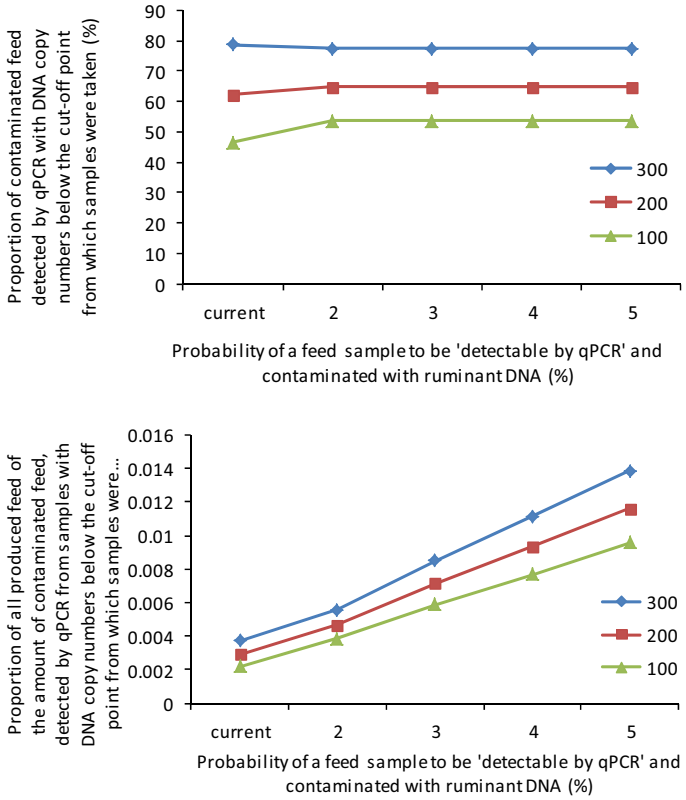


Figure 6: Results of the scenario analysis of the FEED model with four alternative probabilities of contamination of a feed samples with ruminant DNA, for three different interpretation cut-off points: 300, 200 and 100 DNA copies

3.3.3. Limitations of the FEED model

- Given the time constraints and the lack of comprehensive quantitative data, a number of assumptions have been made in order to build a model that could address the impact of the interpretation cut-off point of the ruminant qPCR. Among others, the available feed testing results from three selected MS have been extrapolated to the entire EU in terms of prevalence, and ruminant qPCR DNA copy numbers in positive samples.
- There are no direct associations between the level of contamination with material of ruminant origin, exposure of cattle to contaminated feed, and the presence of BSE infectivity in contaminated feed. The FEED model does not estimate the cattle BSE risk or the actual exposure of cattle to contaminated feed. The output of the model is considered a proxy for exposure of cattle to feed contaminated feed should cattle have access to it.
- It has not been possible to separate the variability and uncertainty associated with some of the parameters, especially for the amount of feed from which samples have been collected. However, the model accounts mostly for uncertainty. Variability has been addressed via scenario analysis. There is uncertainty associated with the magnitude and direction of the impact on the outputs of the model, should the uncertainty be reduced or eliminated altogether and the variability and uncertainty dissociated in all parameters.
- The scarcity of positive samples with available data on the DNA copy numbers means that the model is not able to detect differences in the outputs between interpretation cut-off points of 150 and 200 DNA copies, and between the 250 and 300 DNA copy numbers.
- The assumption that the amount of feed from which samples have been collected and declared positive is removed from the feed chain and does not contribute to the output (exposure) would result in an under-estimation of the absolute risk of cattle exposure to material of ruminant origin, since there is evidence of the lack of action and the difficulty of tracing back batches of feed from which the positive samples were collected (see Section 3.1.6.7).

4. Answers to the ToRs

4.1. Answer to ToR1

Taking into account the EURL-AP report on a 'technical zero', and taking into account EURL-AP Standard Operating Procedure (SOP) on operational protocols for the combination of light microscopy and PCR, to assess the impact that the implementation of the action limits envisaged in the EURL-AP report on the 'technical zero' would have on the overall limit of detection of official controls for the detection of constituents of ruminant origin in feed.

- Testing for the presence of ruminant DNA using the current ruminant qPCR method does not enable discrimination between bovine material that has been added legitimately, and contamination with unauthorised material. It cannot determine either if BSE infectivity is associated with any ruminant material that is detected. The actual origin of any positive signal, i.e. the type of tissue or material containing ruminant DNA, cannot be ascertained unless other methods are applied or developed.
- Certain bovine-derived materials, legally added to ruminant feed, may not be explicitly listed on the label and may result in a positive signal in the ruminant qPCR. The technical zero as proposed, i.e. with an interpretation cut-off point of the ruminant qPCR, arbitrarily raised from 10 to between 100 and 300 DNA copies, aims to define an action limit below which the test result should be considered negative and consequently no action would follow.
- Currently, there are no experimental data to support the assumption that samples with DNA copy numbers below the proposed interpretation cut-off points can be attributed only to the presence of authorised ingredients of ruminant origin. Creating an 'action limit' would reduce the number of positive results that would trigger the requirement for action, by ignoring samples with results under the interpretation cut-off point although they could be due either to contamination or the addition of legitimate ingredients.
- The use of a 'technical zero' is only relevant in the context of the testing of compound feed. There are no production processes in which non-ruminant PAP can be mixed legitimately with ruminant material. If PAP was the test substrate for the ruminant qPCR method, the 'technical zero' would not be appropriate or necessary, and any positive signal detected would indicate the presence of unauthorised material.
- Given the current available data, it is not possible to accurately quantify the overall sensitivity of the surveillance system for the detection of material of ruminant origin in feed, in which the analytical and diagnostic sensitivity of the tests is just a small component. The current ability of the surveillance system for the monitoring of the feed ban to detect material of ruminant origin is limited due to the heterogeneous distribution of contamination, the practicalities of the actual sampling and the performance of the tests. The implementation of the action limits envisaged in the EURL-AP report on the 'technical zero' will reduce this ability further.

4.2. Answer to ToR2

To review and update the input data, and if necessary the basis, assumptions and structure of the current EFSA QRA PAP model to estimate the cattle BSE risk (C-, L- and H-BSE) posed by the possible contamination of feed with BSE-infected bovine derived PAP, taking into account new elements, in particular with regard to the improved epidemiological situation, the current regulatory framework, the laboratory methods for official feed ban controls, the data and report included in Annex to this mandate.

- The quantitative risk assessment of the BSE risk posed by PAP previously published has been updated to take into account the improved epidemiological situation (estimation of the number of BSE cases in the cattle population), the current regulatory framework (SRM policy) and current industry data. The model cannot take into account the performance of laboratory methods for the official control of the feed ban and the data and report submitted as part of the current mandate, since there are no data with which to ascertain the correlation between DNA copy numbers and weight of contamination.
- The updated version of the 2011 QRA model, i.e. the PAP model, is based on a single risk pathway of infected ruminant PAP contaminating non-ruminant PAP, and entering ruminant feed, with a number of assumptions. Other potential risk pathways have been identified and

considered qualitatively, but were either considered to be negligible or could not be adequately parameterised for inclusion in the current model due to a lack of comprehensive quantitative data.

- The baseline results of the PAP model are based on the assumption that any BSE infectivity in ruminant material contaminating the PAP of other species will not have been reduced by the rendering methods applied to produce this material.
- The PAP model produces estimates of (1) the BSE infection risk posed to cattle as the probability of exposure to contaminated ruminant feed, using as the unit of interest the $CoID_{50}$ accessed by the cattle population per year and (2) the number of new BSE-infected cattle that might be derived from infected PAP in the EU in a single year.
- The PAP model estimates that the total BSE infectivity in cattle produced in the EU28 (using standard processing method 7, and assuming a 0.1% contamination of ruminant feed with non-ruminant PAP) is 0.05 $CoID_{50}$ (95% range: 2.4×10^{-4} – 0.33). This current estimate is four times lower than that of the 2011 QRA model (0.2 $CoID_{50}$: 95% range: 9×10^{-5} – 1.3). That would mean that fewer than one additional BSE infected cow could be expected in the EU cattle population, per year, with an upper 95th percentile. All ranges encompass both uncertainty and variability.
- The mean annual individual exposure of cattle to BSE infectivity through concentrate feed (containing PAP produced using standard processing method 7, and assuming a 0.1% contamination of ruminant feed with non-ruminant PAP) was 5.3 times lower (2.46×10^{-9} $CoID_{50}$. 95% range: 0–1.6×10^{-8}) than the estimate of the 2011 QRA model (1.3×10^{-8} $CoID_{50}$. 95% range: 6.8×10^{-12} – 8.6×10^{-8}).
- The hypothetical scenario in which the whole carcass of an infected cow enters the feed chain, i.e. without any removal of SRM or reduction of BSE infectivity via rendering, showed that for a level of 0.1% contamination the estimated mean total infectivity was 4.67 $CoID_{50}$ (95% range: 0.23–9.34) per year. That would mean that up to four additional BSE-infected cows could be expected in the EU cattle population per year, at the upper 95th percentile.

4.3. Answer to ToR3

Based on the outcome of the updated EFSA QRA PAP model, to estimate the cattle BSE risk (C-, L- and H-BSE) posed by the possible contamination of feed with BSE-infected bovine derived PAP, should pig PAP be re-authorised in poultry feed and poultry PAP in pig feed, taking into account the combination of light microscopy and PCR method, and taking into account six different scenarios for the ruminant PCR method based on the action limits envisaged in the EURL-AP report on 'technical zero', as follows: no action limit (i.e. PCR method applied with the current cut-off), action limit at 100, 150, 200, 250 and 300 copies.

- Due to the fact that the 2011 QRA model cannot be updated to take into account the impact of the application of laboratory methods, the PAP model cannot explore the impact of the proposed 'technical zero' on the risk posed to cattle.
- A probabilistic model (FEED model) was developed to quantitatively assess the impact of the implementation of different interpretation cut-off points of the ruminant qPCR on the proportion of feed containing ruminant DNA (originating from either authorised ingredients or unauthorised contamination) that would be identified and potentially removed from the feed chain, taking into account the official feed ban controls.
- The model estimates the proportion of feed contaminated with material of ruminant origin that is not detected, and the proportion of all produced feed that is contaminated with material of ruminant origin but not detected. It assumes that the batch of feed from which positive samples are taken is removed from the feed chain.
- The FEED model cannot estimate the BSE risk for cattle, but it estimates the total amount of feed contaminated with ruminant material as a proxy for exposure, should cattle have accidental access to such contaminated feed. It does so regardless of the species for which the feed has been produced, and regardless of whether or not BSE infectivity is present in the ruminant material that is causing a positive result.
- Any increase from the current ruminant qPCR cut-off point of 10 DNA copies would result in a substantial reduction of the level of detection of feed containing material of ruminant origin, and its subsequent removal from the feed chain. This potential reduction ranges from a mean of 46.5% (95% range: 20.2–77.4%) to 78.6% (95% range: 48–94.9%), for the proposed

interpretation cut-off points of 100 and 300 DNA copies, respectively. This model accounts for uncertainty only.

- When looking at the estimated proportion of the total produced feed in the EU that is contaminated but not detected, the magnitude of impact of the various proposed interpretation cut-off points of the ruminant qPCR are within the range of mean values of 0.0022% (95% range: 0.0009–0.004%) for the 100 DNA copies and 0.0037 (95% range: 0.002–0.0056%) for the 300 DNA copies, which are equivalent to approximately 3,604,100 and 5,954,600 kg of compound feed, respectively.
- There is evidence that the total amount of feed corresponding to a positive sample is not always removed from the feed chain. Thus, the model underestimates the potential exposure of cattle to material of ruminant origin.
- If the probability of a feed sample being contaminated with ruminant DNA is increased to 5% in all types of premises (from the observed 0.5%, 0.65% and 1.9% positive feed samples in farm, feed and border samples, respectively) – as a scenario analysis of the increased probability of contamination due to the re-authorisation of pig PAP in poultry feed and poultry PAP in pig feed – the FEED model estimates a four-fold increase in the proportion of produced feed that would be contaminated and not detected, if the interpretation cut-off point of the ruminant qPCR was increased to 300 DNA copies.

5. Recommendations

- To consider the testing and speciation of PAP in order to detect contamination before its inclusion in animal feed. The absence of any legitimate ruminant products at this point in feed production would negate the need for a technical zero approach, or any subsequent testing of compound feed.
- To ensure that Category 1 material is clearly identified and therefore subject to disposal as per the legislation by, for example, ensuring unequivocal visual identification of Category 1 material and/or the systematic monitoring of the application of GTH to Category 1 and Category 2 material.
- To ensure the physical segregation of the ingredients for ruminant feed and those for non-ruminant feed, at all stages, from the primary collection of ABP at slaughterhouses through rendering and feed production, in order to prevent further accidental contamination of ruminant feed with ruminant material should pig PAP be allowed in poultry feed, and/or poultry PAP in pig feed.
- To produce dilution series data from feed samples spiked with either contaminated PAP or legitimate ruminant-derived ingredients to inform the determination of a relevant 'technical zero' before the interpretation cut-off point of the qPCR is changed, and to formally validate the selected 'technical zero' action limit.
- To create an EU-level reporting system for the test results from the monitoring of the feed ban that would allow the collection, collation and analysis of MS feed testing data and subsequent dissemination throughout the EU.

Documentation provided to EFSA

1) Mandate. 23 October 2017. Annex I: Terms of Reference.
2) Mandate. 23 October 2017. Annex II: Summary of answers received to a questionnaire on feed ban official controls in the EU Member States in 2015 and 2016.
3) Mandate. 23 October 2017. Annex III: Summary of RASFF notifications of EU Member States in 2015 and in 2016 as a result of feed ban official controls.
4) Mandate. 23 October 2017. EURL-AP report on the "Technical zero" to be used with respect to detection of processed animal proteins in feedingstuffs. Authors: Gilbert Berben, Aline Marien, Olivier Fumière, Timur Kaliyev, Véronique Ninane, Viviane Planchon, Marie-Caroline Lecrenier, Pascal Veys, Vincent Baeten. July 2017. Walloon Agricultural Research Centre – CRA-W (Belgium).
5) Clarification on Terms of Reference No 1 and 3 of the request for a scientific opinion on an updated Quantitative Risk Assessment (QRA) of the BSE risk posed by Processed Animal Protein (PAP). 29 November 2017. Annex I: Possible combinations of methods for the disclosure of prohibited processed animal proteins in feed and feed ingredients.

6) Clarification on Terms of Reference No 1 and 3 of the request for a scientific opinion on an updated Quantitative Risk Assessment (QRA) of the BSE risk posed by Processed Animal Protein (PAP). 29 November 2017. Annex II: Summary of answers received to a questionnaire on feed ban official controls in the EU Member States in 2015 and 2016.

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Glossary

Animal by-products	Entire bodies or parts of animals, products of animal origin or other products obtained from animals, which are not intended for human consumption, including oocytes, embryos and semen (Article 3, Regulation (EC) No 1069/2009)
Meat-and-bone meal	Animal protein derived from the processing of Category 1 or Category 2 materials in accordance with one of the processing methods set out in Chapter III of Annex IV (Commission Regulation (EU) No 142/2011)
Processed animal protein	Animal protein derived entirely from Category 3 material, which have been treated in accordance with Section 1 of Chapter II of Annex X (including blood meal and fishmeal) so as to render them suitable for direct use as feed material or for any other use in feedingstuffs, including pet food, or for use in organic fertilisers or soil improvers; however, it does not include blood products, milk, milk-based products, milk-derived products, colostrum, colostrum products, centrifuge or separator sludge, gelatine, hydrolysed proteins and dicalcium phosphate, eggs and egg-products, including eggshells, tricalcium phosphate and collagen (Annex I, Commission Regulation (EU) No 142/2011
Category 1 material	According to Regulation (EC) No 1069/2009, Category 1 material shall comprise the following animal by-products: (a) entire bodies and all body parts, including hides and skins, of the following animals: (i) animals suspected of being infected by a TSE in accordance with Regulation (EC) No 999/2001 or in which the presence of a TSE has been officially confirmed; (ii) animals killed in the context of TSE eradication measures; (iii) animals other than farmed and wild animals, including in particular pet animals, zoo animals and circus animals; iv) animals used for experiments as defined by Article 2(d) of Directive 86/609/EEC without prejudice to Article 3(2) of Regulation (EC) No 1831/2003; (v) wild animals, when suspected of being infected with diseases communicable to humans or animals; (b) the following material: (i) specified risk material; (ii) entire bodies or parts of dead animals containing specified risk material at the time of disposal; (c) animal by-products derived from animals which have been submitted to illegal treatment as defined in Article 1(2)(d) of Directive 96/22/EC or

	Article 2(b) of Directive 96/23/EC; (d) animal by-products containing residues of other substances and environmental contaminants listed in Group B(3) of Annex I to Directive 96/23/EC, if such residues exceed the permitted level laid down by Community legislation or, in the absence thereof, by national legislation; (e) animal by-products collected during the treatment of waste water required by implementing rules adopted under point (c) of the first paragraph of Article 27: (i) from establishments or plants processing Category 1 material; or (ii) from other establishments or plants where specified risk material is being removed; (f) catering waste from means of transport operating internationally; (g) mixtures of Category 1 material with either Category 2 material or Category 3 material or both (Article 8, Regulation (EC) No 1069/2009)
Category 2 material	According to Regulation (EC) No 1069/2009, Category 2 material shall comprise the following animal by-products: (a) manure, non-mineralised guano and digestive tract content; (b) animal by-products collected during the treatment of waste water required by implementing rules adopted under point (c)of the first paragraph of Article 27: (i) from establishments or plants processing Category 2 material; or from slaughterhouses other than those covered by Article 8(e); (c) animal by-products containing residues of authorised substances or contaminants exceeding the permitted levels as referred to in Article 15(3) of Directive 96/23/EC; (d) products of animal origin which have been declared unfit for human consumption due to the presence of foreign bodies in those products; (e) products of animal origin, other than Category 1 material, that are: (i) imported or introduced from a third country and fail to comply with Community veterinary legislation for their import or introduction into the Community except where Community legislation allows their import or introduction subject to specific restrictions or their return to the third country; or (ii) dispatched to another Member State and fail to comply with requirements laid down or authorised by Community legislation except where they are returned with the authorisation of the competent authority of the Member State of origin; (f) animals and parts of animals, other than those referred to in Article 8 or Article 10, (i) that died other than by being slaughtered or killed for human consumption, including animals killed for disease control purposes; (ii) foetuses; (iii) oocytes, embryos and semen which are not destined for breeding purposes; and (iv) dead-in-shell poultry; (g) mixtures of Category 2 material with Category 3 material; (h) animal by-products other than Category 1 material or Category 3 material (Article 9, Regulation (EC) No 1069/2009)
Category 3 material	According to Regulation (EC) No 1069/2009, Category 3 material shall comprise the following animal by-products: (a) carcases and parts of animals slaughtered or, in the case of game, bodies or parts of animals killed, and which are fit for human consumption in accordance with Community legislation, but are not intended for human consumption for commercial reasons; carcases and the following parts originating either from animals that have been slaughtered in a slaughterhouse and were considered fit for slaughter for human consumption following an ante-mortem inspection or bodies and the following parts of animals from game killed for human consumption in accordance with Community legislation: (i) carcases or bodies and parts of animals which are rejected as unfit for human consumption in accordance with Community legislation, but which did not show any signs of disease communicable to humans or animals; (ii) heads of poultry; (iii) hides and skins, including trimmings and splitting thereof, horns and feet, including the phalanges and the carpus and metacarpus bones, tarsus and metatarsus bones, of: — animals, other than ruminants requiring TSE testing, and — ruminants which have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001; (iv) pig bristles; (v) feathers; (c) animal by-products from poultry and lagomorphs slaughtered on the farm as referred to in Article 1(3)(d) of Regulation (EC) No 853/2004, which did not show any signs of disease communicable to humans or animals; (d) blood of animals which did not show any signs of disease communicable through blood to humans or animals obtained from the following animals that have been slaughtered in a slaughterhouse after having been considered fit for slaughter for human consumption following an

	ante-mortem inspection in accordance with Community legislation: (i) animals other than ruminants requiring TSE testing; and (ii) ruminants which have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001; (e) animal by-products arising from the production of products intended for human consumption, including degreased bones, greaves and centrifuge or separator sludge from milk processing; (f) products of animal origin, or foodstuffs containing products of animal origin, which are no longer intended for human consumption for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arise; (g) pet food and feedingstuffs of animal origin, or feedingstuffs containing animal by-products or derived products, which are no longer intended for feeding for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arises; (h) blood, placenta, wool, feathers, hair, horns, hoof cuts and raw milk originating from live animals that did not show any signs of disease communicable through that product to humans or animals; (i) aquatic animals, and parts of such animals, except sea mammals, which did not show any signs of disease communicable to humans or animals; (j) animal by-products from aquatic animals originating from establishments or plants manufacturing products for human consumption; (k) the following material originating from animals which did not show any signs of disease communicable through that material to humans or animals:(i) shells from shellfish with soft tissue or flesh; (ii) the following originating from terrestrial animals: — hatchery by-products, — eggs, — egg by-products, including egg shells, (iii) day-old chicks killed for commercial reasons; (l) aquatic and terrestrial invertebrates other than species pathogenic to humans or animals; (m) animals and parts thereof of the zoological orders of Rodentia and Lagomorpha, except Category 1 material as referred to in Article 8(a)(iii), (iv) and (v) and Category 2 material as referred to in Article 9(a) to (g); (n) hides and skins, hooves, feathers, wool, horns, hair and fur originating from dead animals that did not show any signs of disease communicable through that product to humans or animals, other than those referred to in point (b) of this Article; (o) adipose tissue from animals which did not show any signs of disease communicable through that material to humans or animals, which were slaughtered in a slaughterhouse and which were considered fit for slaughter for human consumption following an ante-mortem inspection in accordance with Community legislation; (p) catering waste other than as referred to in Article 8(f) (Article 10, Regulation (EC) No 1069/2009)
C_t/C_q	'The nomenclature describing the fractional PCR cycle used for quantification is inconsistent, with threshold cycle (Ct), crossing point (Cp), and take-off point (TOP) currently used in the literature. These terms all refer to the same value from the real-time instrument and were coined by competing manufacturers of real-time instruments for reasons of product differentiation, not scientific accuracy or clarity. We propose the use of quantification cycle (Cq), according to the RDML (Real-Time PCR Data Markup Language) data standard (http://www.rdml.org)' (Bustin et al., 2009)
$CoID_{50}$	The oral dose which infects 50% of cattle in an experimental test
Surveillance sensitivity	'The sensitivity of a surveillance system can be considered, at the level of case reporting, the proportion of cases of a disease (or other health-related event) detected by the surveillance system' (CDC. Guidelines for evaluating surveillance systems. https://www.cdc.gov/mmwr/preview/mmwrhtml/00001769.htm)
Diagnostic sensitivity	'The probability (P) that, given the presence of disease (D), an abnormal test result (T) indicates the presence of disease; that is, P(T/D)' *(*https://medical-dictionary.thefreedictionary.com/diagnostic+sensitivity*)*

Abbreviations

ABP	animal by-products
AP	Animal proteins
BIOHAZ	EFSA Panel on Biological Hazards
BSA	bovine serum albumin
BSE	bovine spongiform encephalopathy
CA	competent authorities
CCP	critical control points
CI	confidence interval
CMGC	celiac-mesenteric ganglion complex
CNS	central nervous system
$CoID_{50}$	cattle oral infectious dose 50%
C_t/C_q	threshold cycle or quantification cycle of PCR
C-TSEMM	Cattle TSE Monitoring Model
DRG	dorsal root ganglia
DSe	diagnostic Sensitivity
DSp	diagnostic Specificity
EFPRA	European fat processors and renderers association
ELISA	enzyme-linked immunosorbent assay
EURL	European Union Reference Laboratory
FEFAC	European feed manufacturers' federation
FVO	Food and Veterinary Office
GTH	glyceroltriheptanoate
HACCP	hazard analysis and critical control points
IAG	International association of feedstuff analysis
i.c.	intracisternal
i.p.	intraperitoneal
ISO	International Organization for Standardization
LM	Light microscopy
LOD	Limit of detection
MBM	meat-and-bone meal
MS	Member state
NFCP	national feed control programme
NRL	National reference laboratory
PAP	processed animal protein
p.e.	post exposure
PrP	normal cellular prion protein
PrP^{Sc}	abnormal protease resistant isoform of prion protein
qPCR	quantitative real-time polymerase chain reaction
QRA	quantitative risk assessment
RA	risk assessment
RASFF	Rapid Alert System for Food and Feed
R_0	Basic reproduction number
Se	sensitivity
SOP	Standard operating procedure
SRM	specified risk material
ToR	terms of reference
TSE	transmissible spongiform encephalopathies
TSEi	TSE infectivity model
UK	United Kingdom
VLA	Former Veterinary Laboratory Agency, UK - current Animal and Plant Health Agency (APHA)
WG	Working Group

Appendix A – The 2011 QRA model (EFSA BIOHAZ Panel, 2011a)

The process of updating the 2011 QRA model to produce the PAP model described in this opinion resulted in some parameters being updated, but many parameters remaining the same. Where parameters have been updated or changed, details have been given in Section 3.2 and Appendix B of this Opinion.

This Appendix reproduces *verbatim* (from EFSA BIOHAZ Panel, 2011a) Sections 4.2 and 4.3 with the description of the probabilistic model for the Quantitative assessment of residual BSE Risk of processed animal protein in cattle feed, for completeness and ease of reference within this Opinion. Only the text has been reproduced here. The original opinion would need to be consulted for access to the relevant references. The layout and headings of Sections 4.2 and 4.3 have been respected.

4.2. Model basis and structure

4.2.1. Model structure

The EFSA QRA PAP model has been developed from "BSE infectivity model cattle exposure v7" the final version of the EFSA QRA model. The model now consists of four Excel worksheets. The model has been evaluated using @RISK 4.5.5, and run using Latin Hypercube sampling with 10,000 iterations. The four sheets are summarised in Table 2.

Table 2: Summary on the information provided in the 4 sheets of the "EFSA QRA PAP model" (from the 2011 EFSA opinion

Sheet	Description
1-Input data	This sheet includes all the data used for this calculation.There are 3 Run Options that the user must select: 1. Choice of BSE Prevalence Data: Default data for EU27 in 2009 is included. Or the user may specify BSE test data and related data on numbers of cattle slaughtered for any specified country or region. 2. Rendering method: Atmospheric or Pressurised steam. 3. Batch or Continuous rendering process
2-PAP to cattle	The sheet provides the calculation of exposure to individual cattle for assumed levels of contamination with PAP. Results given as Infectivity per animal per year
3-Total exposure	This sheet provides the calculation of the total exposure to BSE infectivity for all cattle in the EU due to consumption of ruminant feed. Results given as total Infectivity per year
4-Summary Results	This sheet copies the results from Sheet 2 and enables sets of runs (e.g. sensitivity cases) to be generated and compared easily

The EFSA QRA PAP model is provided as an Annex to this opinion and can be found at the following URL: www.efsa.europa.eu/en/biohazscdocs/docs/efsaqrapapmodel.xls. Moreover, detailed technical information, including information on uncertainties on the model can be found in Appendix II to this opinion.

4.2.2. Model basis

In the event that there were to be some relaxation in the use of non-ruminant PAP in animal feeds (e.g., feeding porcine PAP to poultry) there would be some increase in the risk that ruminant feeds could be contaminated with such material. Non-ruminant PAP should not represent any TSE risk to ruminants as such, but there would be a possibility that the non-ruminant PAP could itself be contaminated with ruminant PAP. The purpose of the model is to estimate the potential exposure of cattle to BSE infectivity due to the potential for contamination of cattle feed with non-ruminant PAP that could include bovine material with traces of infective material.

There are three stages in the model calculation:

1) Calculation of BSE infectivity in ruminant PAP (due to incomplete removal of SRMs);
2) Infectivity in non-ruminant PAP assuming contamination with ruminant PAP;
3) Infectivity in Cattle feed, assuming contamination with non-ruminant PAP (from step 2)

Model results are given in terms of the annual exposure of cattle to BSE infectivity ($CoID_{50}$ units) for two alternative feeding regimes (Intensive and Extensive) and for three levels of contamination of the ruminant feed.

4.2.3. BSE infectivity in Ruminant PAP

Category 1 waste, which is the material including SRM, is separated and disposed of separately in dedicated plants. This material must be completely disposed of by incineration or landfill following heat treatment.

Category 3 ABPs from ruminants may be rendered and the resulting protein material (PAP) used in products such as pet food. This assessment will assume that the starting point is the production of PAP from Category 3 waste that is made from by-products from a mixture of ruminant species that have been slaughtered for human consumption. However, it is an assumption for the opinion that this Category 3 material could be contaminated with low levels of bovine SRM.

The infectivity in ruminant PAP is calculated by combining:

- BSE prevalence in cattle population;
- Assumptions on batch size, by-products per animal, PAP yield, proportion of carcases with contaminated material present and reduction in infectivity due to rendering;
- Amount of BSE infected tissue in the mixture of by-products from contaminated carcasses;
- Infectivity of BSE infected CNS tissues.

The input data used for this model are presented in section below.

4.3. Input data

4.3.1. Prevalence

In the EFSA QRA model, the prevalence of BSE has been assessed in two steps, each one was based on rough estimates because few data and scientific results were available at that time. The first step was related to the prevalence of clinical cases per year in the cattle population, and was simply categorised in 3 categories of countries depending on their risk of BSE assessed through the Geographical BSE Risk categorisation (EFSA, 2007b; SSC, 2002). The second step was to estimate the number of sub-clinical non-detected per detected BSE positives, in order to account for the infectivity of infected animals that were dead or culled before the end of the incubation time. Based on few modelling studies, the rough estimate was 2 to 3 undetectable infected animals entering the food chain per detected cattle.

In this EFSA QRA PAP model, similar two steps are also used but since more accurate estimates, thanks to the active monitoring of BSE and additional modelling studies based on more accurate data are available the categories of previous GBR status of the MS are no longer used.

- Step 1. The prevalence of detectable cases of BSE can be obtained precisely from the comprehensive surveillance implemented since 2001 in the EU 15 and later in the other EU member states. With the surveillance system in force, almost all infected animals that reach the end of the incubation time at the time of death or slaughter are detected, given the high sensitivity of the rapid test and the comprehensive apparatus. Also, it can be postulated that in the coming years, if the control measures of BSE remain the same, the prevalence of BSE in the EU 27 should continue to decrease or at least remain constant, in light with the analysis carried out recently on 7 EU countries (Ducrot et al., 2010). So it seems reasonable to assume that the BSE prevalence in subsequent years will be less than in 2009. The EFSA QRA PAP model is based on the most recent data available on BSE prevalence in healthy and emergency slaughtered and bovine animals showing clinical signs at ante mortem inspection, at the EU-27 level, those of 2009. It was obtained on animals tested that were older than 30 months (older than 48 in some MS). In 2009, over a total number of 6,406,402 rapid tests performed on the above mentioned three surveillance streams in EU-27, 32 animals were found to be positives.[25] That leads to an overall detected prevalence in EU27 tested cattle coming from these three surveillance streams in 2009 of 5.00 positive animals per million animals tested. However, any Category 3 ABPs used to produce PAP would be derived from all slaughtered animals, and not

[25] Source: European Commission TSE monitoring database, last accessed on 16th November 2010.

just those tested. For EU-27 a total of 21,018,709 cattle were slaughtered in 2009[26] giving a detected prevalence in EU-27 slaughtered cattle of 1.52 positive animals per million slaughtered.

- Step 2. Estimates of the number of non-detected per detected BSE case in cattle have been made using different models. From Durand (1999), it was estimated (Durand, personal communication) that the percentage of infected cattle that can be detected at the time of testing (death or culling) varies from 31% to 40% if the rapid screening test detects infected animals 3 to 9 months before the end of the incubation time. In the pessimistic option (30%), it represents 2.3 non-detected per detected BSE case. Still on French data, Sala et al. (2010) carried out a simulation model of the surveillance and detection of BSE, that shows that 20% of infected animals are detected with the tests; this represents 4 non-detected per detected BSE case. Modelling studies carried out by de Koeijer (personal communication), based on a model on BSE dynamics (de Koeijer, 2007) have shown that 85% of infected animals (considering all ages) remain non-detected because they are culled or dead before the end of the incubation time, which represents 5.7 non-detected per detected case. A similar range of non-detected per detected case was also found in a study of the German BSE surveillance data (Greiner, personal communication). Finally, from back calculation models developed using UK data (Arnold and Wilesmith, 2003), it has been estimated (Arnold, personal communication) that 15.7% of the infected animals are detected, corresponding to 5.4 non detected cases per detected case.

Depending on the culling curve of cattle that can vary between countries, as well as on the age at infection and the infection dose that can modify the incubation time (higher dose, lower incubation time), the models show that the number of non-detected per detected BSE case varies, in a range of 2 to 10 in the situations seen above.

However, apart from in a few tissues such as the ileum, infectivity only develops significantly towards the end of the incubation period (see Section 3.3.6.7) of EFSA BIOHAZ Panel (2011a). This should mean that the relative infectivity in most infected but non detected animals will be much less than in those that are detected and are thus close to the end of their incubation period.

In this assessment the number of non-detected BSE infected animals per detected BSE case was assumed to follow a uniform distribution with a range from 2 to 10, the Panel considers this range as being conservative.

4.3.2. Yield of by-products per animal

As noted in the EFSA QRA Report, animals will vary in weight and the yield of by-products will vary with the animal size and according to the cutting practices in the slaughterhouse. According to data from industry provided in the context of section III.4 of the EFSA QRA report, it was estimated that when all SRMs including the vertebrae are excluded the yield of by-products would be 167 kg per bovine (EFSA, 2005a). In the current EFSA QRA PAP model this is represented as a normal distribution with a standard deviation of 10% of the mean.

4.3.3. Proportion of ruminant Cat. 3 material of bovine origin

Based on the total weight of the ruminant carcasses produced each year in the EU by species and assuming that the proportion of the weight of a carcass over the total live weight of an animal is the same for bovines, sheep and goats it has been estimated, on the basis of Eurostat data, that bovine Cat 3 material represents 90% of the material used to produce ruminant PAPs. For instance in 2007 the total weight of the bovine carcasses produced in EU-27 was 8,203,646 tonnes while for sheep and goats these weights were respectively 1,010,354 and 79,268 tonnes.

4.3.4. Batch size

In the EFSA QRA Report it was assumed that the batch sizes for production of tallow and MBM for a mixture of tissues were in a range from 150 to 1,000 tonnes based on information about the size of tallow storage tanks. The number of animals required to make up one batch of material was then calculated by dividing the batch size by the assessed average yield of by-product material per bovine slaughtered (as per 4.3.2). The probability of an infected animal being present in a batch was then determined using a Poisson distribution with the Poisson parameter given by the product of the

[26] Source: Eurostat (http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home)

number of cattle in a batch and the BSE prevalence. Most production processes are now continuous, so the batch size itself is no longer so relevant, although an effective batch size could be based on the size of product storage units. For this assessment a proxy for a continuous process is used utilising 1,000,000 tonnes as batch size for the base case, with the same range of batch sizes as used in the EFSA QRA Report included as a sensitivity case. The main effect of the batch size will be in the chance that there is an infected animal included in a batch.

4.3.5. PAP yield

In section III.6.5 of the EFSA QRA Report the yield of MBM was taken to be 40% (EFSA, 2005a); i.e., 40 kg of MBM would be produced from every 100 kg of by-products processed. This value was based on industry data for Method 1 (see 4.3.10) processing. Updated information from industry sources indicates that the yield for PAP should be 30 to 35%. This is modelled as a Uniform distribution.

4.3.6. Probability of SRM incomplete removal and quantity of SRM remaining tissue per animal

In the EU there is strict separation of SRMs and it is not considered credible that this separated material could re-enter the feed chain. For this assessment it is assumed that all bovine SRMs are removed, including the vertebral column, as per EU regulations for older cattle. However, PAP produced from bovine Category 3 by-products, which may for example be used in pet food, may still have a low level of infective material present due to incomplete removal of SRMs. In addition, recent scientific results (Buschmann and Groschup, 2005; Espinosa et al., 2007; Wells et al., 2005) have indicated that the presence of low levels of infectivity may be present in tissues that are not SRM. However, the infectivity levels found with new highly sensitive analytical techniques are 4 or more logs less than the infectivity in CNS tissue. Such low levels of infectivity would not add significantly to the overall load, and can be assumed to be included within the total amount of SRM material remaining as set out below.

The working group that defined the assumptions for the EFSA QRA Report decided that the incomplete removal of SRM in bovine animals used to produce ruminant MBM should be represented by assuming that 10% of animals slaughtered have some level of incomplete removal of SRMs. This was represented by 5% of brain (25g) and the ileum (80g CNS equivalent) giving a total of 105g of CNS equivalent per animal per animal with SRM incomplete removal. With the level of meat inspection and implementation of SRM controls in the EU this is now considered to be a highly pessimistic assumption both in terms of the likelihood and amount of contamination. This was recognised in the EFSA QRA report which stated that these assumptions represented a worst case scenario in a poorly regulated abattoir.

In the absence of data, for this revised model it is assumed that the numbers of animals with incomplete SRM removal be represented by a distribution, with the 10% value as a maximum. This was modelled as a log normal distribution with a 1st percentile value of 0.1% and a 99th percentile of 5%; this gives a mean value of 1% and a 99.9th percentile of about 10%. This was considered to be more representative of the actual situation.

Similarly in the absence of data, the amount of SRM material remaining due to incomplete removal for the purpose of the current model was assumed to be represented by a log normal distribution with mean value of 10g of CNS equivalent infected tissue and a maximum (99th percentile) of 105g.

These assumptions are combined in the EFSA QRA PAP Model with the infectivity level of CNS in an infected animal (see section 4.3.7.2) to give the total infectivity in the Cat 3 ABP from an infected but non-detected animal that is slaughtered for food of 1 Co ID50 with a 95% range of 0.002 to 8. This value can be compared to equivalent estimates from similar models discussed in section 3 (Cummins and Adkin, 2007; de Vos and Heres, 2009). The values are not reported in the same way in the published papers but have been provided directly by the authors.

Adkin reports that, for the version of the VLA model used in 2007 study, the total number of Co ID50 in Cat 3 materials per year per infected animal was a mean of 466 Co ID50 (5th 93.7, 95th 964.3). However, updates to this model in 2010 have considerably reduced this estimate. When considering the removal of vertebral column with all Dorsal Root Ganglia (DRG) for those animals greater than 30 months, thus excluding DRG from category 3 materials, Adkin reports that the mean infectivity per infected animal would be 1 Co ID_{50} (95th 0.1, 95th 4) from this model. These results are very similar to those reported above for the EFSA QRA PAP model assumptions.

De Vos also confirmed that her model does calculate the infectivity in each infected non-detected bovine both before and after SRM removal. She reports that the mean infectivity per infected non-detected bovine after SRM removal is 19.5 Co ID_{50}, but with a highly skewed distribution with a median value of

3×10^{-3} and a 95th percentile of 132. The tissue infectivity used in the De Vos model is based on that reported by Comer and Huntly (2004) which predated the results from the second stage of the VLA attack rate experiment and gives a mean infectivity about a factor of 10 greater than that used in the EFSA QRA PAP model. If the value reported by De Vos is adjusted to account for the difference in the assumed infectivity distribution the result would be in good agreement with that from the EFSA QRA PAP model.

4.3.7. Infectivity of Bovine Tissues

4.3.7.1. Estimation of the BSE oral infectious dose 50 in cattle

The Veterinary Laboratory Agency (VLA)[27] in the in the UK has carried out experiments to determine the BSE minimal oral infectious dose of BSE in cattle. In this titration experiment groups of 10 calves were each fed 300g, 100g, 10g and 1g of an homogenate made from the brain stems from clinically sick animals.

According to titration in RIII mice the used brain homogenate contained 103.5 mouse i.c./i.p.ID50 per g of tissue.

All animals inoculated with 300g and 100g came down with BSE, and 7 out of 10 in both the 10g and 1g trials. The incubation periods for both the 1g and 10g trials were comparable (between 44 – 71 months).

As it was not possible to determine an ID50 dose from this experiment, an extension of this titration experiment was carried out with doses of 1g, 100mg, 10mg and 1mg (Wells et al., 2007). The results show 3 of 5 in the 1g trial group, 7 out of 15 animals in the 100mg group, 1 out of 15 in the 10mg group, and 1 out of 15 in the 1mg group, positive for BSE. Incubation periods for the positive results in both the 1 and 10mg groups were similar to those for the 1 g trial, but two of the animals in the 100mg group had incubation periods in excess of 90 months.

In their study, Wells et al. (2007) report that the ID50 estimate from these experiments is equivalent to 0.20 g of the brain homogenate used (i.e. 5 ID50/g) with a 95% confidence interval of 0.04 – 1.00g. This value also indicates that 1 cattle oral ID50 is approximately equivalent to 102.8 mouse i.c./i.p ID50 in RIII mice.

4.3.7.2. Infectivity in the Brain and spinal cord

From titrations conducted in mice on brain from clinical or clinical suspect cases of BSE, a wide range of titres have been obtained: 102.4 –105.2 mouse i.c.or i.c./i.p. ID50/g (Fraser et al., 1992; Taylor et al., 1994). These data were used to estimate the titre at clinical onset and its variability. From this, the mean titre of brain at clinical onset was given by 103.3 mouse i.c.or i.c./i.p. ID50/g with standard deviation of 100.58 (Arnold et al., 2009). The working group preparing the EFSA QRA report considered that 'with higher titres of BSE affected brain the range could extend to 300 ID50/g' (see section III.2 of EFSA, 2005) and decided to take a precautionary view and assuming that the infectivity titre in brain of a clinically BSE infected bovine follows the following distribution:

Log normal distribution with

- Median (50th percentile): 5 Co ID50/gram
- Higher 99th percentile: 100 Co ID50/gram

For the present assessment this distribution was considered as a reasonable representation of the infectivity level in the CNS of a cattle affected with BSE.

4.3.7.3 Infectivity in the Dorsal root ganglia

In their 2009 paper Arnold et al. (2009) estimated the infectious titre in cervical and thoracic dorsal root ganglia from cattle orally inoculated with 100g brain material at different time points of their incubation. According to this study the titre in the DRG was lower than CNS, with the thoracic and cervical DRG having mean titres approximately 1 and 1.5 log10 mouse i.c./i.p. ID50/g lower than CNS respectively.

4.3.7.4. Infectivity in the Peripheral Nervous System

There have been a number of studies reporting detection of infectivity using transgenic PrP bovine mice (Buschmann and Groschup, 2005; Espinosa et al., 2007) or PrP^{Sc} (Iwata et al., 2006) in some peripheral nervous system tissues.

[27] Now known as Animal and Plant Health Agency (APHA).

According to the data reported by Buschmann and Groschup (2005) the infectivity could be detected in some but not all nerves samples from a BSE affected animal. In this study the infectivity level in the positive nerves could be estimated to be about 5-6 log10 folds lower than that in the brain from the same animal. These data are consistent with those reported by Espinosa et al., (2007) in different BSE infected animals using another bovine PrP transgenic mouse model. In this study the author report the detection of infectivity in the sciatic nerve from 30 and 33 months post cattle exposure, but its absence in animals killed at 20, 24 and 27 months post exposure (n=1 cattle per time point).

Iwata et al. (2006) reported the detection of PrPSc in some but not all nerves from 2 naturally BSE infected cases (preclinical stage of the disease). On the basis of PrP^{Sc} biochemical detection (Western Blot) it was estimated that the infectivity in the femoral and lumbar nerves of an affected cattle was 1,000 to 1,400 fold less than the PrP^{Sc} amount detected in the spinal cord.

4.3.7.5. Infectivity in non Nervous System tissues

A large range of tissues collected at various stages of the incubation were tested for the presence of BSE infectivity by mouse bioassay (conventional or bovine transgenic) (Arnold et al., 2009; Buschmann and Groschup, 2005; Espinosa et al., 2007). A more limited range of tissues was also tested by intracerebral inoculation into calves (Wells et al., 2005).

The only non-nervous tissues shown to harbour consistent infectivity in these experiments are the distal ileum and lingual tonsil.

In the distal ileum infectivity was evidenced as early as 6 months post oral exposure and seems to persist all along the incubation period. The infectious titres in the distal ileum were estimated to range between $10^{-0.06}$ and 101.94 i.c./i.p. ID50 in RIII mice per gram depending on the age of the individual (Arnold et al., 2009) (i.e. between 1 and 3 log10 fold lower than in the mean level of infectivity found in the brain from BSE affected individuals).

In lingual tonsil, infectivity was detected

- In one out of 5 calves inoculated intracerebrally with a pool of tonsil collected in orally inoculated cattle killed 10 months post exposure (Wells et al., 2005). There were no other positive results for tonsil at subsequent time points of the study (18, 22, 26, 32 and 36 months post exposure).
- In cattle killed at 20 - 24 - 27 – 30 – 33 months (n = 1 animal per time point – no younger animal tested) post inoculation in transgenic mice expressing the bovine PrP gene (1/6 mice in each case) (Espinosa et al., 2007).

On the basis of these data, it was estimated (EFSA, 2008) that the infectivity in the tonsil tissue was less than 1 bovine i.c. ID50/g or 10-6.5 Co ID50/g.

Finally, detection of minute amounts of infectivity were reported (bioassay in transgenic bovine mice) in one striated muscle sample collected in a BSE affected cattle (Buschmann and Groschup, 2005). The authors failed to detect infectivity in other muscle samples from the same animal. Using another transgenic bovine PrP mouse model other authors failed to detected infectivity in striated muscle samples (one sample per cattle) collected in cattle orally challenged with BSE (100g) and killed at 20 - 24 - 27 – 30 – 33 months (n = 1 animal per time point) (Espinosa et al., 2007). These data remain difficult to interpret. In particular, it is unclear if the detected infectivity was associated to nervous ramifications present in the muscle sample or to striated muscular cells, as reported in other TSE models (Andreoletti et al., 2004; Thomzig et al., 2004).

With the current state of knowledge it cannot be considered that striated muscles cells are harbouring BSE infectivity in cattle.

4.3.7.6. Total infectivity amount in a BSE clinical case

The total infectivity in a clinical case of BSE is summarised in Table 3. The weights of the various tissues are mainly taken from the LFRA and MLC report (LFRA and MLC, 1997) and the infectivity values are as discussed above, with the infectivity for whole brain taken to be 5 Co ID50/g. It can be seen that 90% of the infectivity is associated with central and peripheral nervous system tissues, with about 10% associated with the distal ileum.

Table 3: Infectivity in a Clinical Case of BSE (Co ID_{50})

Tissue	Weight (Kg/animal)	Infectivity		
		Co ID_{50}/g	Co ID_{50}/animal	% over the total amount of infectivity
Brain	0.5	5	2500	≈ 65%
Spinal cord	0.2	5	1000	≈ 26%
Dorsal root ganglia	0.03	0.5 – 0.1	3 – 15	< 0.4%
Trigeminal ganglia	0.02	5	100	≈ 2.5%
Lingual Tonsil	0.05[1]	0.00005	0.0025	< 0.01%
Distal ileum	0.8	0.005 – 0.5	4 – 400	< 0.01–10%
PNS	0.96[2]	0.00005	0.05	< 0.01%
TOTAL	**2.6**		**3600 – 4000**	

1: The LFRA (1997) report gives the total weight of the tonsil as 200g. 50g is an estimate of the weight of the lingual tonsil.
2: Estimate of total PNS weight from Project TS5002. 2008, Veterinary Laboratories Agency, Weybridge, Surrey, UK.

4.3.7.7. Development of the infectivity in tissues through incubation period

In the CNS, a previous analysis of the data from the VLA Pathogenesis experiment for the Over Thirty Months review risk assessment (Comer and Huntly, 2004) resulted in an estimate of a 2 month doubling time. However, a recent re-analysis of the available data (Arnold et al., 2009) indicates that the doubling time of infectivity in the brain from incubating animals may in fact be slightly less than this, with a most likely value of 1.2 and a 95% range of 1.0 to 1.9 months.

Lingual tonsil was shown to be positive in some BSE incubating animals older than 10 months post-exposure. There is no apparent modification of the infectivity level during the incubation phase.

With regard to the distal ileum, several studies indicated that after experimental oral exposure of cattle, infectivity in the distal ileum can be detected at 6, 10, 14, 18, 36, 38 and 40 months post exposure (p.e.) From 6–14 months p.e. infectivity showed a rising titre, followed in older animals by a decrease, which is likely to be highly variable between animals (Arnold et al., 2009; Wells et al., 1996; Wells et al., 1994).

4.3.8 Exposure of Cattle Feed to Ruminant PAP

Non-ruminant PAP in itself would not represent a TSE risk to ruminant animals. The risk potential is that by allowing some animal PAPs to be used in some animal feeds then there is a greater chance that ruminant feeds would be contaminated. In order for cattle feed to be contaminated with the BSE agent it would be necessary for two independent contamination events to occur.

Firstly, non-ruminant PAP is contaminated with ruminant PAP (and that the ruminant PAP had been derived from a batch including an animal with BSE). With the separation of rendering facilities and handling required within the EU this is unlikely to occur. However, at the present time the available tests are not able to differentiate species in the processed material. For this opinion it is assumed that this contamination could range from zero to 5% (modelled as a Uniform distribution). 5% contamination of non-ruminant PAP with ruminant PAP was thought to be a rather pessimistic upper estimate of the possible level of such contamination. It was thought that contamination at such a level could occur only in the most unlikely combination of conditions and so would be highly unlikely. However, it was felt that a pessimistic value was justified because of the absence of a test to differentiate species of origin in PAP.

Secondly, ruminant feed is contaminated with non-ruminant PAP. Ruminant feed should contain no animal proteins, and will be routinely tested. A base case test sensitivity of 0.1% - the present threshold of diagnostic sensitivity - will be assumed (i.e. ruminant feed may contain up to 0.1% non-ruminant PAP without being detected), but values of 0.02% and 2% will also be evaluated for comparison with the previous EFSA QRA report.

4.3.9 PAP and Ruminant Feed Production

Data received from the industry[28] indicate that the total PAP production in the EU in 2009 was 2.2 million tonnes, as shown below in Table 4, and that the total amount of Cat 3 material of ruminant origin processed in the EU in 2009 was about 3.4 million tonnes.[29]

Table 4: Total PAP production in EU

Product	Production 2009 tonnes	Proportion used in Pet Food
Poultry PAP	372,000	98%
Feather meal	215,000	50%
Porcine PAP	375,000	92%
All other PAP; mixed including ruminant	1,245,000	44%

Data on the total compound feed production in the EU is given on the website of the European Feed Manufacturers' Federation (FEFAC).[30] The total compound feed produced for cattle in the EU in 2009 is given as 38,570,000 tonnes.

4.3.10. Reduction in infectivity by processing

In order to process Category 3 materials for the production of PAPs, rendering plants must use one of seven processing methods described in Annex V to Reg. (EC) 1774/2002, where Method 1 represents the most stringent conditions. Method 1 involves strict standards for rendering the material, heating to a core temperature of more than 133°C for at least 20 min without interruption at 3 bar pressure. Method 7 does not prescribe any temperature or pressure standards on the processing of raw materials, but does include microbiological criteria based on the final product.

According to the Annex VII, Chapter II of the current Reg. (EC) 1774/2002 the following methods have to be used according to the different type of material to be processed:

- Method 1 for mammalian material (other than porcine blood and other than materials destined for incineration or petfood);
- Method 1 to 5 and 7 for porcine blood and for mammalian materials destined for incineration or petfood (this derogation implies a special channelling procedure);
- Method 1 to 5 and 7 for non-mammalian material;
- Method 1 to 7 or a microbiologically safe method for fish material.

The effects of each of the seven processing methods on BSE infectivity is not known with the exception of limited experimental data regarding Method 1. Taylor et al. (Taylor et al., 1995) found that rendering BSE contaminated tissues at 133°C for 20 min at 3 bar pressure (Method 1) eliminated BSE infectivity from a starting value of 101.7 mouse i.c./i.p ID_{50}/g, yielding a 50 fold reduction. However, this experiment was partially compromised by the relatively low starting titre of infectivity in the raw material, which made it difficult to quantify the extent of infectivity reduction at the limits of sensitivity of the mouse assay models used. In studies involving samples spiked with a ten-fold higher quantity of infectivity than that used by Taylor and co-workers, BSE infectivity remained following the rendering process, however reductions of more than 100 to 1,000 fold were measured (Schreuder et al., 1998).

Several studies have also been undertaken to investigate the effect of other rendering processes on prions with lower temperatures and/or pressures than Method 1. The studies focused on historical rendering processes in an attempt to understand the factors that may have led to the BSE epidemic.

They demonstrated that the processes historically undertaken in GB and the EC had little effect on the infectivity of the BSE or scrapie agent (Taylor and Woodgate, 1997; Taylor et al., 1995).

When Method 1 rendering is used the infectivity reduction by the saturated steam heat/pressure process (133°C and 3 bar for 20 min) has been assessed to be between 101.0 to 103.0 with 102.3 as the most likely value. These values are used with a triangular distribution when applied in the EFSA QRA PAP model.

[28] Information received from the European Fat Processors and Renderers Association (EFPRA) on 20th October 2010. The data are for the 19 EU member states that are members of EFPRA and only exclude member states with a relatively low production.

[29] Information provided by EFPRA (Stephen Woodgate, personal communication received on 1st December 2010). The data are for the 19 EU member states that are members of EFPRA and only exclude member states with a relatively low production.

[30] www.fefac.org

However, with the feed ban in place, most Category 3 material goes into pet food and is processed at atmospheric pressure. This method would have minimal effect on TSE infectivity.

In this opinion it has been assumed that the atmospheric process is used as a base case and there is no infectivity reduction, but the use of Method 1 is also assessed as a scenario analysis in Section 4.4.4 of EFSA BIOHAZ Panel (2011a).

4.3.11. Consumption of Feed Concentrate

The amounts of feed concentrate used on any farm will depend on the type of cattle and the production or rearing system. The possible range is reflected by assessing the exposure for two alternative production systems for beef cattle; i) an intensive production system in which the cattle are fed an average of 8 kg/day of feed concentrate (Normal distribution with standard deviation of 2 kg/day), and ii) an extensive production system in which the cattle are fed an average of 1.5 kg/day of feed concentrate (Normal distribution with standard deviation of 1 kg/day) (section IV.7.1 of EFSA, 2005a).

Appendix B – Input data PAP model

O2: Rendering method (to choose): Method 1/Method 7

The item numbers within the original 2011 QRA model have been retained in the updated version 7.2 of the PAP model presented in this opinion. This is to enable a direct comparison of the two versions and appraisal of the new parameter values used. Item numbers have not been reused.

Italicised text describes values and parameters that have been updated or are new in the PAP model compared to the 2011 QRA model.

Item D3: Number of cattle slaughtered per year

Name	D3
Explanation	*Total number of cattle slaughtered in EU28 in 2016*
Type	Data item
Definition	*26,599,010*
Dependent items	P2, T1
Unit	Number of cattle
Assumptions	*The current parameter has been updated from an original value of 21,018,709 to reflect current production figures for 2016*
Remark	?
References	*For 2016 bovine meat: http://ec.europa.eu/eurostat/data/database*

Item D36: Number of undetected infected animals in a single year (EU28) (new data item)

Name	*D36*
Explanation	*Number of undetected infected animals per year across the EU28. This data item is estimated by a separate model C-TSEMM, replacing the previous values of D1, D2, D3, and D4 which included expert opinion. The data refer to the parameters of a beta pert distribution fitted to the most likely, 2.5th and 97.5th percentile values*
Type	*Point estimate/data item with Monte Carlo distribution*
Definition	*2.5th percentile = 3.61; most likely = 10.86, 97.5th percentile = 19.79*
Dependent items	*P48*
Unit	*Head of cattle per year across EU28*
Assumptions	*This variable represents uncertainty associated with the point estimate*
Remark	*Estimated using surveillance and demographic data up to 2015*
References	*Main report section and Adkin* et al. *(2012)*

Item D5: BSE infectivity in bovine brain (clinical)

Name	D5
Explanation	Infectivity level in brain tissue of a BSE-infected bovine at clinical stage of disease. The data refer to the parameters of a log-normal distribution fitted to the 50th and 99th percentile values.
Type	Data item
Definition	50th percentile (median) = 5; 99th percentile = 100
Dependent items	P10
Unit	CoID50/g
Assumptions	A homogeneous distribution of infectivity in brain tissue is assumed. If this assumption is violated (i.e. if infectivity occurs in clusters), the risk outcome may show additional variability with higher risk levels occurring in rare situations. The parameters reflect a precautionary (conservative) approach.
Remark	The median value was based on published results of the VLA attack rate study (Wells et al., 2007), with the 99th percentile set by experts and used in a number of EFSA opinions, e.g. EFSA (2005). This data item captures the parameters of the lognormal distribution, which are later used to define a Monte-Carlo random distribution item.
References	EFSA BIOHAZ Panel (2011a) section 4.3.7.2 (EFSA, 2005; Wells et al., 2007)

Item D35: BSE infectivity in intestines and mesenteries (new data item)

Name	*D35*
Explanation	*Total infectivity titre in intestines and mesenteries from an average BSE infected bovine at slaughter age in the healthy slaughter and emergency slaughter exit stream in the EU28. The data refer to the parameters of a log-normal distribution fitted to the mean and standard deviation and maximum distribution value from the TSEi.*
Type	*Data item*
Definition	*Mean = 2.80. Standard deviation (STD) = 10.70. Maximum = 1478.70*
Dependent items	*P46*
Role	*This variable represents variability and uncertainty associated with the estimate*
Unit	*CoID50*
Assumptions	*Output from TSEi with associated assumptions. The model takes into account the variable age at slaughter and variable incubation period for animals destined for healthy or emergency slaughter.*
Remark	*The distribution values directly from the TSEi included the between animal variability. For use in the PAP model, the number of expected undetected infected animals (item D36) was individually sampled, and the infectivity in intestines and mesenteries was individually sampled and summed for that number of infected individuals. Therefore, the variability included in the PAP model is the variability about the average infectivity.*
References	*Main report section, and Adkin* et al. *(2014)*

Item D6: Weight of ABP removed per carcass

Name	D6
Explanation	Weight of by-products produced at slaughter after removal of all SRM per animal. Data refer to mean and standard deviation of a Normal distribution. The mean value is based on information from industry while the standard deviation was assumed by experts to be one-tenth of mean.
Type	Data item
Definition	Mean: 167. Standard deviation: 16.7
Dependent items	P5
Unit	Kg
Assumptions	Standard deviation assumed by experts to be one tenth of mean. All SRM removed, including vertebrae
Remark	Data given as a single point value with no indication of variability. Variability and uncertainty could not be differentiated.
References	EFSA BIOHAZ Panel (2011a,b) Section 4.3.2, based on data from industry as reported in the EFSA QRA report (EFSA, 2005).

Item D17: Probability of incomplete removal of SRM

Name	D17
Explanation	This parameter is an estimate of the likelihood that some SRM would remain in the by-products of an animal. The amount of such material is defined in Parameter D18
Type	Data item
Definition	Parameters to define a log-normal distribution in terms of the 1st and 99th percentiles: 1st percentile = 0.1%; 99th percentile = 5%
Dependent items	P8
Unit	Percentage. Fraction
Assumptions	In the QRA Report (EFSA, 2005), it was assumed that SRM material would remain in 10% of animals slaughtered. This was reviewed by the current working group and found to be overly pessimistic. Therefore, an updated distribution was defined with a 99th percentile of 5%. The previous 10% value would be an approximate effective maximum value (99.9th percentile) in a log-normal distribution. It is assumed that the current parameterization is more realistic.
Remark	
References	EFSA BIOHAZ Panel (2011a) Section 4.3.6

Item D18: Infected tissue remaining in Category 3 ABP per infected animal

Name	D18
Explanation	This item is related to the estimate of the total infected material (in terms of grams of central nervous system (CNS) material equivalent) that may remain in the Category 3 by-products of an infected animal due to incomplete removal of SRM.
Type	Data item
Definition	Parameters to define a log-normal distribution in terms of a mean value (10) with a 99th percentile value of 105
Dependent items	P9
Unit	Grams of CNS material/animal
Assumptions	This distribution has been defined by the experts of the working group following a review of the values used in the QRA Report (EFSA, 2005), which were regarded as being highly pessimistic given the implementation of SRM controls in the EU. The 99th percentile value (105 g) was set from the expected value defined in the QRA Report with a mean value a factor of 10 less.
Remark	
References	EFSA BIOHAZ Panel (2011a) Section 4.3.6

Item D20: Batch size equivalent to model a continuous process

Name	D20
Explanation	Effective batch size used to model a continuous process.
Type	Data item
Definition	Point values: Minimum 1,000,000. Maximum: 1,000,001
Dependent items	P4
Unit	Tonnes
Assumptions	
Remark	
References	EFSA BIOHAZ Panel (2011a) Section 4.3.8

Item D21: Yield of PAP from processing ABP

Name	D21
Explanation	Yield of PAP. This is the yield of PAP per amount of raw animal by-products processed.
Type	Monte Carlo random variable
Definition	*Parameters of a uniform distribution : Minimum = 25%; Maximum = 31%*
Dependent items	P47, P14, T2
Unit	Percentage. Fraction
Role	This variable represents variability only.
Assumptions	*The current parameter has been updated from an original value of range 30 to 35% to reflect current production procedures.*
Remark	
References	*Main report section, based on updated information received from the European Fat Processors and Renderers Association (EFPRA, 2018)*

Item D22: Proportion of ruminant PAP produced from bovine material

Name	D22
Explanation	Proportion of ruminant PAP produced from bovine material.
Type	Data item
Definition	*Value = 91.15%*
Dependent items	P6
Unit	Percentage. Fraction
Assumptions	*The current parameter has been updated from an original value of 90% to reflect current values.*
Remark	
References	EFSA BIOHAZ Panel (2011a) Section 4.3.3. *Eurostat, dataset "food_in_pagr2": Slaughtered animals for food production available at the following link: http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/. 2016 production bovine: 7798270 tonnes beef meat, 713,000 sheep and 45,000 goats. Beef meat: 91.15%* http://ec.europa.eu/eurostat/statistics-explained/index.php/File:Production_of_meat,_by_species,_2016.png

Items D23 and D24: BSE infectivity reduction by standard processing: Method 1 (D23) and Method 7 (D24)

Name	D23 and D24
Explanation	Infectivity reduction by processing. This is the expected reduction in the infectivity load in the raw material (measured in $CoID_{50}$ units) as a result of the rendering process to produce PAP. The user has the option of selecting between *Method 7 (baseline case) and Method 1 processing.*
Type	Data item
Definition	*D23: Method 1 processing*. Values to define the minimum, best estimate and maximum values for a Triangular distribution. 10; 200; 1000
D24: Method 7. Simple value of 1	
Dependent items	P13
Unit	Factor
Assumptions	For the base case, it is assumed that most Category 3 material is now processed by Method 7 and it is assumed that this would have no impact on the infectivity in the raw material. For the sensitivity case, it is assumed that pressure sterilisation (Method 1) is used as per Commission Regulation 142/2011.
Remark	The user has the option of selecting between *Method 7* (baseline case) and *Method 1* processing.
References	EFSA BIOHAZ Panel (2011a) section 4.3.10, Schreuder et al. (1998); Taylor et al. (1995)

Item D25: Proportion of contamination of non-ruminant PAP with ruminant PAP

Name	D25
Explanation	Proportion of contamination of non-ruminant PAP with ruminant PAP: The data refer to the minimum and maximum of a uniform distribution given by experts.
Type	Data item
Definition	Parameters to define the minimum and maximum values in a Uniform distribution: Min = 0%, Max = 5%
Dependent items	P15
Unit	Percentage. Fraction
Assumptions	It is assumed that if non-ruminant PAP was allowed to be used in some animal feed (e.g. porcine PAP in poultry feed), then EU regulations would require the complete separation of both rendering and handling facilities. This item reflects the conservative assumption that non-ruminant PAP may be contaminated with ruminant PAP despite the requirements for separation of rendering facilities and handling.
Remark	
References	EFSA BIOHAZ Panel (2011a) Section 4.3.8

Item D26: Proportion of contamination of ruminant feed with non-ruminant PAP

Name	D26
Explanation	Contamination of ruminant feed with non-ruminant PAP. This is the assumed level of contamination with non-ruminant PAP in cattle feed concentrate.
Type	Data item
Definition	Three set values: 0.1%, 0.02% and 2%
Dependent items	i) 0.1% – P21 & P25; ii) 0.02% – P22 & P26 iii) 2% – P23 & P27
Unit	Percentage.
Assumptions	The base case (0.1%) is set at the level of detection of animal proteins in animal feed. This assumption may be conservative since realistic contamination levels may also be below this threshold. The 2% and 0.02% values were set for the EFSA QRA report (EFSA, 2005) and have been retained for consistency.
Remark	Results for the 0.02% and 2% contamination levels have been given as alternative scenarios. However, it should be noted that 2% contamination of ruminant feed with animal proteins would be a very high level and is extremely unlikely to occur.
References	EFSA BIOHAZ Panel (2011a) Section 4.3.8. Reference (EFSA, 2005).

Items D27 and D28: Daily consumption of cattle feed concentrate: intensive system (D27) and extensive system (D28)

Name	D27 and D28
Explanation	Estimated feeding rates for animals raised for beef production. Values are given for two categories of animal husbandry Intensive and Extensive. Values are defined as the mean and standard deviation for a normal distribution.
Type	Data item
Definition	$D_{28:}$ Intensive system. Mean: 8.0. STD: 2.0 D_{29}: Extensive system. Mean: 1.5. STD: 1.0
Dependent items	i) Intensive system P20; ii) Extensive system P24
Unit	Kg/day
Assumptions	Intensive would be representative of animals fed primarily on feed concentrate. This scenario reflects the highest level of risk. Extensive system assumes some level of concentrate feeding, whilst in a truly extensive system cattle would be fed entirely on grass or conserved grass (e.g., hay/silage).
Remark	Values accepted as representative of EU by working group for the original QRA report (EFSA, 2005)
References	EFSA BIOHAZ Panel (2011a) Section 4.3.11, EFSA (2005).

Items D29, D30, D31 and D32: Total PAP produced in EU

Name	D29, D30, D31 and D32
Explanation	*Total production of PAP in EU in 2016 for Poultry (D29), Feather meal (D30), Porcine meal (D31) and all other Cat 3 PAP mixed, including ruminant (D32).*
Type	Data item
Definition	D_{29}: *505,000* D_{29}: *250,000* D_{29}: *340,000* D_{29}: *1,350,000*
Dependent items	T10, T11 and T12
Unit	Tonnes
Assumptions	*The current data values have been updated from original values from 2009 of 372,000; 215,000; 375,000; and 1,245,000 respectively.*
Remark	
References	*Data for 2016 provided by European Feed Processors and Rendering Association (EFPRA, 2018). The data are for the EU28.*

Item D33: Total ruminant Category 3 ABP

Name	D33
Explanation	Total amount of ruminant Category 3 material processed in the EU in 2009.
Type	Data Item
Definition	3,439,600
Dependent items	T2
Unit	tonnes/year
Assumptions	
Remark	The data are for the 19 EU member states that are members of EFPRA and only exclude member states with a relatively low production.
References	EFSA BIOHAZ Panel (2011a) Section 4.3.9. Data for 2009 provided by European Feed Processors and Rendering Association (EFPRA) (Stephen Woodgate, personal communication received on 1 December 2010). *Estimate for 2016 is very similar, calculated in a different way: 40% of live weight is rendered (EFPRA, EBLEX). 40% of 8,556,270 tonnes of slaughtered ruminants: 3,422,508 tonnes, which is very similar to the total ruminant Category 3 ABP in 2010: 3,439,600 tonnes.*

Item D34: Total ruminant feed produced in EU28 in 2016

Name	D34
Explanation	*Total ruminant feed produced in the EU28 in 2016.*
Type	Data item
Definition	*43,104,000*
Dependent items	T19
Unit	tonnes/year
Assumptions	*The current data value has been updated from original value from 2009 of 38,570,000.*
Remark	*Data for 2016.*
References	*Reference to http://www.fefac.org/Data for 2016 updated.*

Sheet 2: PAP to Cattle

Item P48: Number of infected animals in 2016 in EU28 (new parameter)

Name	*P48*
Explanation	*Number of undetected infected animals per year across the EU28.*
Type	*Monte Carlo random variable*
Definition	*As defined for D36*
Dependent items	*P2, T4*
Unit	*Head*
Role	*This variable represents uncertainty associated with the estimate.*
Assumptions	
Remark	*Sampled from input D36. Truncated at 0 to the left*
References	

Items P2: BSE prevalence in slaughter cattle population in 2016

Name	P2
Explanation	BSE prevalence in slaughter cattle: Probability of BSE infection in cattle slaughtered in the EU for consumption.
Type	Function item using random variables
Definition	*P48/D3*
Dependent items	P7
Unit	Percentage. Probability
Assumptions	
Remark	*P3 was the same parameter expressed as infections per million cattle and has been deleted from the QRA as not further used.*
References	

Item P4: Selected batch size

Name	P4
Explanation	*Selected batch size sampled from estimate for continuous range.*
Type	Selected Data item
Definition	*Continuous processing = D20*
Dependent items	P6, P14
Unit	tonnes
Assumptions	
Remark	
References	

Item P5: Yield of ABP per animal

Name	P5
Explanation	Yield of by-products per cattle after removal of SRM. Variability modelled as Normal distribution.
Type	Monte Carlo random variate item
Definition	Normal distribution with mean and standard deviation specified as D6
Dependent items	P6
Unit	kg/animal
Role	This variable represents variability only.
Assumptions	
Remark	
References	

Item P6: Number of cattle contributing to one batch

Name	P6
Explanation	Number of bovine animals contributing to one batch of ABP being rendered. This is the expected number of bovines that would be included in a specified batch of material. This quantity is established using the batch size in tonnes (P4), the most likely value of the fraction of bovine origin (D22) and the yield of by-product per animal (P5).
Type	Function item
Definition	P4 * 1000 * D22/P5
Dependent items	P7
Unit	Number of cattle/batch
Assumptions	
Remark	
References	

Item P7: Number of BSE-infected cattle per batch

Name	P7
Explanation	Number of infected animals in the batch. This is the number of infected but non-detected cattle in the batch of material, represented as a Poisson distribution
Type	Monte Carlo random variate item
Definition	Poisson (P6 * P2)
Dependent items	P12
Unit	Number of BSE-infected cattle/batch
Assumptions	Homogeneous mixing of BSE infectivity is assumed.
Remark	
References	

Item P8: Probability of SRM incomplete removal

Name	P8
Explanation	Probability of SRM incomplete removal. This is the probability that some SRM material would not be removed, and would therefore be left in the by-products. Sampled values from a specified log-normal distribution.
Type	Monte Carlo random variate item
Definition	As defined for D17
Dependent items	P11, T6
Unit	Percentage. Probability
Role	This variable represents both uncertainty and variability.
Assumptions	
Remark	
References	

Item P9: Amount of remaining SRM tissues per animal

Name	P9
Explanation	Quantity of remaining SRM tissue per animal. The distribution is thought to represent the actual situation (variability). The values are sampled from the distribution defined by item D18.
Type	Monte Carlo random variate item
Definition	As defined for D18
Dependent items	P11, T5
Unit	Grams of CNS equivalent tissue/animal
Assumptions	
Remark	
References	

Item P10: Sampled BSE infectivity in bovine brain

Name	P10
Explanation	Sampled BSE infectivity in bovine brain. Sampled value from a specified distribution defined by item D5.
Type	Monte Carlo random variate item
Definition	As defined for D5
Dependent items	P11, T7
Unit	CoID50/g
Role	This variable represents both uncertainty and variability.
Assumptions	
Remark	
References	

Item P46: BSE infectivity in intestines and mesenteries per infected animal

Name	*P46*
Explanation	*Infectivity per infected animal from intestines and mesenteries. log-normal distribution of the BSE infectivity in intestines and mesenteries*
Type	*Monte Carlo random variate item*
Definition	*As defined for D35*
Dependent items	*P11, T8*
Unit	*CoID50*
Role	*This variable represents both uncertainty and variability.*
Assumptions	
Remark	*Sampled from input D35. Truncated at 0 to the left*
References	*Adkin* et al. *(2014)*

Item P11: BSE Infectivity per infected animal

Name	P11
Explanation	BSE Infectivity per infected animal. This is the estimated amount of infectivity present in the raw material per infected (but not detected) animal. *Additional infectivity added from intestines and mesenteries permitted as Category 3 materials in negligible risk status MS.*
Type	Function item
Definition	(P8 * P9 * P10) + *P46*
Dependent items	P12
Unit	CoID50
Assumptions	*Assumed all member states are negligible Risk status and all this material enters Category 3 material. This is a conservative approach.*
Remark	
References	

Item P12: Total BSE infectivity per batch of PAP

Name	P12
Explanation	Total BSE infectivity per batch of PAP: Total infectivity in one batch before processing due to contamination with SRM *and infectivity in intestines and mesenteries from BSE infected cattle.*
Type	Function item using random variates
Definition	P7 * P11
Dependent items	P14
Unit	Co ID50
Assumptions	
Remark	
References	

Item P13: BSE infectivity reduction due to rendering

Name	P13
Explanation	BSE infectivity reduction during rendering. *User selects either Method 7 (Base case value) or Method 1 in Input Data sheet*
Type	Selected data item
Definition	If *Method 7* = D24If *Method 1* = D23 Triangular distribution Min: 1.00E+01. Mode: 2.00E+02 Max: 1.00E+03
Dependent items	P14, T9, T17
Unit	Factor
Assumptions	
Remark	
References	

Item P47: Yield of PAP from ABP (new parameter)

Name	*P47*
Explanation	*Yield of PAP from ABP – new parameter added to clarify distributions used in the original QRA to prevent two cells containing the same formulae. It does not alter model framework.*
Type	*Monte Carlo random variate item*
Definition	*As defined for D21*
Dependent items	*P14, T2*
Unit	*Percentage. Fraction*
Assumptions	
Remark	
References	

Item P14: BSE infectivity in ruminant PAP

Name	P14
Explanation	BSE infectivity in ruminant PAP: This is the concentration of infectivity in a batch, reduced by the rendering reduction factor, divided by the total amount of PAP produced and factored by proportion from bovine source.
Type	Function item using random variates
Definition	=(P12/P13)/(P4 * P47 * 10^6)
Dependent items	P15
Unit	CoID50/g
Assumptions	Assumes homogenous mixing of infectivity through the batch.
Remark	
References	

Item P15: BSE infectivity of non-ruminant PAP

Name	**P15**
Explanation	BSE infectivity in non-ruminant PAP.
Type	Function item using random variates
Definition	P14 * Uniform (D25(1), D25(2))
Dependent items	P28 (Baseline case), P31 and P34
Unit	CoID50/g
Assumptions	Infectivity in non-ruminant PAP assuming that this is contaminated with ruminant PAP at a level of between zero and 5% (see D25).
Remark	
References	

Item P20: Sampled daily consumption of cattle feed concentrate (intensive)

Name	P20
Explanation	Sampled daily consumption of cattle feed concentrate. Estimated consumption of cattle feed concentrate by beef animals in an intensive rearing system. Sampled value from a truncated Normal distribution; parameters defined in D27.
Type	Monte Carlo random variate item
Definition	Normal distribution with mean and STD as defined in D27
Dependent items	P21 (baseline case), P22 and P23
Unit	Kg/day
Role	This variable represents variability only.
Assumptions	
Remark	Alternate case for less intensive feeding given by P24
References	

Items P21, P22 and P23: Annual intake of non-ruminant PAP in feed (intensive)

Name	P21, P22 and P23
Explanation	Non-ruminant PAP in cattle feed. The estimated annual intake of non-ruminant PAP per animal in cattle feed at the selected contamination level. The baseline contamination is 0.1% (P21), with alternate values being given in P22 and P23
Type	Function item
Definition	P20 * D26 * 365 *1000
Dependent items	i) P28 for P21, ii) P31 for P22 and iii) P34 for P23
Unit	g/animal/year
Assumptions	
Remark	
References	

Item P24: Sampled daily consumption of cattle feed concentrate (extensive)

Name	P20
Explanation	Sampled daily consumption of cattle feed concentrate. Estimated consumption of feed concentrate by cattle in an intensive rearing system. Sampled value from a truncated Normal distribution; parameters defined in D28.
Type	Monte Carlo random variate item
Definition	Normal distribution with mean and standard deviation as defined in D28
Dependent items	P21 (Base case) and P22 & P23
Unit	Kg/day
Role	This variable represents variability only.
Assumptions	
Remark	Alternate case for less intensive feeding given by P24
References	

Items P25, P26 and P27: Annual intake of non-ruminant PAP in feed (extensive)

Name	P21, P22 and P23
Explanation	Non-ruminant PAP in cattle feed. The estimated annual intake per animal of non-ruminant PAP in cattle feed at the selected contamination level. The base case contamination is 0.1% (P25), with alternative values being given in P26 and P27
Type	Function item
Definition	P20 * D26 * 365 *1000
Dependent items	i) P37 for P24 ii) P40 for P25 and iii) P43 for P26
Unit	g/animal/year
Assumptions	
Remark	
References	

Items P28, P31 and P34: Exposure to BSE infectivity (intensive)

Name	P28, P31 and P 34
Explanation	The exposure to infectivity for one animal in an intensive rearing system, estimated as BSE infectivity in PAP per gram (P15) times the annual PAP intake in grams for an individual cow (P21). The baseline contamination is 0.1% (P28), with alternative values being given in P31 and P34.
Type	Function item
Definition	P21 * P15; P22 * P15; P23 * P15
Dependent items	Model Output
Unit	Co ID50/animal/year
Role	This item has been defined as outcome function (OF)
Assumptions	
Remark	Results for alternative contamination levels given in intensive (P28, P31 and P34), and for extensive feed system (P37, P40 and P43).Output statistics in terms of mean and percentile values at 2.5%, 50%, 97.5%, 99% and 99.9%
References	

Items P37, P40 and P43: Exposure to BSE infectivity (extensive)

Name	P28, P31 and P 34
Explanation	The exposure to infectivity for one animal in an extensive rearing system, estimated as BSE infectivity in PAP per gram (P15) times the annual PAP intake in grams for an individual cow (P24). The baseline contamination is 0.1% (P28), with alternative values being given in P40 and P43.
Type	Function item
Definition	P24 * P15; P25 * P15; P26 * P15
Dependent items	Model Output
Unit	Co ID50/animal/year
Role	This item has been defined as outcome function (OF)
Assumptions	
Remark	Results for alternative contamination levels given in intensive (P28, P31 and P34), and for extensive feed system (P37, P40 and P43).Output statistics in terms of mean and percentile values at 2.5%, 50%, 97.5%, 99% and 99.9%
References	

Sheet 3: Total Exposure

T1: Total ruminant Category 3 ABP processed

Name	T1
Explanation	Total amount of ruminant Category 3 material processed in the EU in 2009.
Type	Selected data item
Definition	D33
Dependent items	T2
Unit	Tonnes/year
Assumptions	
Remark	
References	Similar amount was calculated for 2016

Item T2: Total PAP produced from ruminant Category 3 ABP

Name	T2
Explanation	PAP produced from ruminant Category 3 ABP
Type	Function item using random variates
Definition	=T1 * P47
Dependent items	T9 and T16
Unit	Tonnes/year
Assumptions	
Remark	
References	

Item T4: Number of undetected infected animals in 2016 in EU28

Name	T4
Explanation	Number of undetected infected cattle per year
Type	Function item using random variates
Definition	P48
Dependent items	T8
Unit	Number of cattle
Assumptions	
Remark	
References	

Item T5: Infected tissue remaining in Category 3 ABP per infected animal

Name	T5
Explanation	Quantity of remaining SRM tissue per animal.
Type	Monte Carlo random variate item
Definition	P9
Dependent items	T8
Unit	Grams of CNS equivalent tissue/animal
Assumptions	
Remark	
References	

Item T6: Probability of SRM incomplete removal

Name	T6
Explanation	Probability of incomplete removal of SRM. This is the probability that some SRM material would not be removed, and therefore left in the by-products. Sampled values from a specified log-normal distribution.
Type	Monte Carlo random variate item
Definition	P8
Dependent items	T8
Unit	Percentage. Fraction
Assumptions	
Remark	
References	

Item T7: Sampled BSE infectivity in bovine brain

Name	T7
Explanation	Sampled BSE infectivity in bovine brain. Sampled value from a specified distribution.
Type	Monte Carlo random variate item
Definition	P10
Dependent items	T8
Unit	CoID50/g
Assumptions	
Remark	
References	

Item T8: Total infectivity in Category 3 ABP

Name	T8
Explanation	Total infectivity in Category 3 ABP. Infectivity in all ruminant category 3 ABP in the EU in one year prior to processing
Type	Function item using random variates
Definition	(T4 * T5 * T6 * T7) + *(T4 * P46)*
Dependent items	T9, T17
Unit	CoID50/year
Assumptions	The contribution of infectivity from intestines and mesenteries which are permitted into Category 3 materials for negligible Risk MS has been added in.
Remark	
References	

Item T9: Concentration of BSE infectivity in ruminant PAP

Name	T9
Explanation	Concentration in ruminant PAP
Type	Function item using random variates
Definition	T8/(P13 * T2 * 1000)
Dependent items	T25
Unit	CoID50/kg
Assumptions	
Remark	
References	

Items T10, T11 and T12: Non-ruminant PAP produced in EU in 2016

Name	T10, T11 and T12
Explanation	Non-ruminant PAP produced in EU in 2016
Type	Data items
Definition	D29, D30 and D31
Dependent items	T10: Poultry PAP
T11: Feather meal	
T12: Porcine PAP	
Unit	Tonnes
Assumptions	
Remark	
References	EFPRA (2018)

Item T13: Total non-ruminant PAP produced in EU in 2016

Name	T13
Explanation	Total non-ruminant PAP produced in EU in 2016
Type	Function item
Definition	∑(T10, T11 and T12)
Dependent items	T15
Unit	Tonnes
Assumptions	
Remark	
References	

Item T14: Proportion of contamination of non-ruminant PAP with ruminant PAP

Name	T14
Explanation	Proportion of contamination of non-ruminant PAP with ruminant PAP.
Type	Monte Carlo random variate item
Definition	Uniform distribution with Minimum and maximum values as per D25
Dependent items	T15
Unit	Percentage. Fraction
Assumptions	
Remark	
References	

Item T15: Amount of ruminant PAP present in non-ruminant PAP

Name	T15
Explanation	Amount of ruminant PAP present in non-ruminant PAP. The amount of ruminant PAP that would be present if all the non-ruminant PAP produced was contaminated at the level specified in T14
Type	Function item using random variates
Definition	T13 * T14
Dependent items	T16
Unit	Tonnes
Assumptions	It is assumed that all the non-ruminant PAP produced are contaminated at the level specified in T14.
Remark	
References	

Item T16: Proportion of total ruminant PAP in non-ruminant PAP

Name	T16
Explanation	Proportion of total ruminant PAP in non-ruminant PAP. This is the proportion of the total ruminant PAP produced from ruminant Category 3 ABP that is present in non-ruminant PAP
Type	Function item using random variates
Definition	T15/T2
Dependent items	T17
Unit	Percentage. Fraction
Assumptions	
Remark	
References	

Item T17: Total BSE infectivity in non-ruminant PAP

Name	T17
Explanation	Total infectivity in non-ruminant PAP. Infectivity in non-ruminant PAP due to contamination with ruminant PAP.
Type	Function item using random variates
Definition	T8 * T16/P13
Dependent items	T18
Unit	CoID50/year
Assumptions	
Remark	
References	

Item T18: Concentration of BSE infectivity in non-ruminant PAP

Name	T18
Explanation	Concentration of BSE infectivity in non-ruminant PAP, calculated by diving the total BSE infectivity in non-ruminant PAP by the total non-ruminant PAP produced in EU in 2016, expressed in $CoID_{50}$ per kg
Type	Function item using random variates
Definition	T17/(T13 * 1000)
Dependent items	
Unit	CoID50/kg
Assumptions	
Remark	
References	

Item T19: Total ruminant feed produced in EU28 in 2016

Name	T19
Explanation	Total ruminant feed produced in the EU27 in 2016.
Type	Data item
Definition	D34
Dependent items	T21
Unit	Tonnes
Assumptions	
Remark	
References	

Item T20: Contamination of ruminant feed with non-ruminant PAP

Name	T20
Explanation	Contamination of ruminant feed with non-ruminant PAP. This is the assumed level of contamination in cattle feed concentrate by non-ruminant PAP. Baseline 0.1% and alternative values at 0.02% and 2%
Type	Data item
Definition	D26
Dependent items	T21
Unit	Percentage. Fraction
Assumptions	
Remark	
References	

Item T21: Total non-ruminant PAP present in cattle feed

Name	T21
Explanation	Total non-ruminant PAP present in cattle feed, if all feed produced was contaminated at the specified level. Calculated for the three specified contamination levels.
Type	Function item
Definition	T19 * T20 = D26 * D34
Dependent items	T22
Unit	Tonnes/year
Assumptions	
Remark	Baseline 0.1% and alternative values at 0.02% and 2%
References	

Item T22: Proportion of total non-ruminant PAP present in cattle feed

Name	T22
Explanation	Proportion of total non-ruminant PAP in cattle feed, calculated by dividing the total non-ruminant PAP present in cattle feed by the total non-ruminant PAP produced in EU in 2016
Type	Function item
Definition	T21/T13
Dependent items	T23
Unit	Percentage. Fraction
Assumptions	
Remark	Note that 2% contamination would imply that 80% of the non-ruminant PAP produced would be present in the cattle feed. This is an unrealistic assumption
References	

Item T23: Total BSE infectivity in ruminant feed (0.1% contamination)

Name	T23
Explanation	Total infectivity in ruminant feed. Estimated total infectivity in all ruminant feed produced in the EU for specified contamination levels.
Type	Function item using random variates
Definition	T17 * T22
Dependent items	T24
Role	This item has been defined as outcome function (OF)
Unit	CoID50/year
Assumptions	This assumes that all the ruminant feed produced in the EU is contaminated at the same level.
Remark	
References	

Item T24: Calculation of R0 values

Name	T24
Explanation	R0. Calculated R0 value for given contamination level. R0 represents the expected number of new infections per infected animal entering the system
Type	Function item using random variables
Definition	(T23 * 0.5)/T4
Dependent items	Model Output
Unit	Number
Assumptions	
Remark	
References	

Item T25: Concentration of BSE infectivity in ruminant feed (0.1% contamination)

Name	T25
Explanation	Calculated by dividing the total BSE infectivity in ruminant feed (0.1% contamination) by the total ruminant feed produced in EU28 in 2016, expressed in CoID50/Kg
Type	Function item using random variables
Definition	T23 (0.1%)/(T19 (0.1%) * 1000)
Dependent items	Model Output
Unit	CoID50/kg
Assumptions	
Remark	
References	

Appendix C – Input Data of the FEED model

Sheet: Model (version 2.6)

Item F1: Total feed produced in the EU in a year

Name	F1
Explanation	Total feed produced in the EU in a year. It refers to compound feed. Other feedingstuffs like forages and roughages are not included
Type	Data item
Definition	156,700.000,000
Dependent items	F18
Unit	Kg
Assumptions	The overall annual weight of feed produced in the EU in 2017 has been included, assuming that EU MS can be considered a single epidemiological unit in terms of implementation of the monitoring of the feed ban
Remark & Role	
References	FEFAC. The compound feed industry in the EU livestock economy (2017) http://www.fefac.eu/files/79279.pdf

Item F2: Number of feed samples collected in the EU28 in a year

Name	F2
Explanation	Number of feed samples collected in the EU28 per year as part of the statutory monitoring of the feed ban. Average of feed samples collected in 2015 (25,915) and 2016 (23,532): 24724 samples. Data are referred to two categories: controls for ruminant PAP in feed for aquaculture animals and controls for non-authorised PAP in feed other than aquafeed
Type	Data item
Definition	24,724
Dependent items	F4
Unit	Number of samples
Assumptions	The number of feed samples reported by MS in 2015 and 2016 is representative of any year for any MS and for the entire EU
Remark & Role	Scenario analysis: between year variability. Number of samples collected in 2015 (25,915) and 2016 (23,532) have been applied in parallel to the average
References	Calculated using the data from the questionnaire survey by the EC. Appendix F of the mandate

Item $F3_i$: Distribution of feed samples by type of premises (%)

Name	$F3_i$
Explanation	Proportion of feed samples collected by types of premises. Re-categorization of the entries in the field "Type of premises" in three categories: F31: farm. F32: feed mill. F33: border point. Beta distributions (a+1, n-a+1) for the proportions for each type of premises
Type	Data item
Definition	F31: Beta distribution (10177+1,21944-10177+1) F32: Beta distribution (10841+1,21944-10841+1) F33: $1 - (F3_1 + F3_2)$
Dependent items	$F4_i$ and $F8_i$
Unit	Percentage
Assumptions	The distribution of samples collected in each type of premises is equal for each MS
Remark & Role	The re-categorization of all types of premises in three groups is a methodological approach to reduce the variability in the feed amounts from which the samples are collected and may have introduced some error in the values. 21,944 is the total number of samples with known type of premises.
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F4_i$: Annual number of feed samples collected in the EU by type of premises

Name	$F4_i$
Explanation	The distribution of the total number of feed samples collected in the EU in a single year in the three types of premises: $F4_1$: farm. $F4_2$: feed mills. $F4_3$: border point
Type	Data item
Definition	F2 * $F3_i$
Dependent items	$F9_i$ and $F10_i$
Unit	Number of samples
Assumptions	The distribution of samples by type of premises is equal for each MS. The number of samples collected in a single year has been estimated at the EU level, not at MS level. The entire EU is considered a single epidemiological unit.
Remark & Role	
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F5_i$: Probability of a feed sample to be 'detectable by qPCR' and contaminated with ruminant DNA

Name	$F5_i$
Explanation	This parameter is an estimate of the likelihood that a feed sample collected in the EU in a single year is contaminated with ruminant DNA. It has been calculated as % of the number of positive feed samples using the field 'first_analytical_test_result' to the total number of feed samples with test results in the used dataset for each type of premises. The prevalence was 51/10177 in farms, 71/10841 in feed mills and 18/926 in border inspections. Beta distributions (a+1, n-a+1) for each of the three types of premises.
Type	Data item
Definition	$F5_1$: Beta distribution (51+1, 10177-51+1) $F5_2$: Beta distribution (71+1, 10841-71+1) $F5_3$: Beta distribution (18+1, 926-18+1)
Dependent items	$F10_i$
Unit	Percentage. Probability
Assumptions	It is assumed that the observed prevalence in the dataset of the three selected MS is representative of the overall EU prevalence
Remark & Role	This parameter assumes the uncertainty around the prevalence estimate.
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item F6: Cut-off of DNA copy number applied by the ruminant qPCR

Name	F6
Explanation	The DNA copy number to be used as the cut-off to declare a feed sample positive or negative.
Type	Data item
Definition	10 (baseline and current cut-off point), 100, 150, 200, 250 and 300 (as proposed by the EURL-AP technical zero report
Dependent items	$F10_i$, $F11_i$
Unit	DNA copy number
Assumptions	It is assumed that the amount of feed from which the positive samples were taken are removed from the market, based on the interpretation cut-off applied.
Remark & Role	The interpretation cut-off point is applied to every run of model and constitutes the only parameter that determines the difference of magnitude of the outputs in combination with the two alternative scenarios for F2 and $F12_i$
References	Terms of reference of the mandate

Item $F7_i$: Probability of a contaminated feed sample with DNA copy number above the interpretation cut-off point

Name	$F7_i$
Explanation	For each type of premises, the probability of a contaminated feed sample with DNA copy number above the interpretation cut-off point determined by F6 is extracted from the data. A beta distribution is applied with the different probabilities for each cut-off point and for each type of premises
Type	Function item using random variables
Definition	$F8_1$ Beta distribution (10-29, 100-8, 150-7, 200-7, 250-5, 300-5) $F8_2$ Beta distribution (10-41, 100-14, 150-13, 200-13, 250-11, 300-11) $F8_3$ Beta distribution (10-3, 100-2, 150-2, 200-1, 250-0, 300-0)
Dependent items	$F8_i$, $F10_i$
Unit	Percentage. Probability
Assumptions	The probability of the DNA copy number above the interpretation cut-off point in the three MS is representative of the entire EU. The probabilities are taken directly from the data; P (X>=10, 100, 150 etc., then a beta distribution applied. Probability (>=10) has been fixed at 1 with no uncertainty as otherwise have a non-zero output would not have any meaning
Remark & Role	This parameter accounts for the uncertainty of the number of DNA copies of any feed sample positive to the LM + qPCR in the EU.
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F8_i$: Probability of contaminated feed samples with DNA copies below the interpretation cut-off point

Name	$F8_i$
Explanation	For each type of premises, the probability of a contaminated feed sample with DNA copy number below the cut-off point determined by F6 is extracted from the data
Type	Data item
Definition	$1 - F7_i$
Dependent items	F20
Unit	Percentage. Probability
Assumptions	The probability of the DNA copy number below the interpretation cut-off point in the three MS is representative of the entire EU
Remark & Role	This parameter accounts for the uncertainty of the number of DNA copies of any feed sample positive to the LM + qPCR in the EU
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F9_i$: Number of feed samples contaminated by type of premises

Name	$F9_i$
Explanation	It is the result of distributing the number of samples in each type of premises by the probability of a feed sample to be contaminated with ruminant DNA in each type of premises. $F10_1$: farm; $F10_2$: feed mill; $F10_3$: border point
Type	Data item
Definition	$F3_i$ * $F4_i$
Dependent items	$F11_i$ and $F15_i$
Unit	Number
Assumptions	
Remark & Role	This parameter accounts for the uncertainty of the DNA copy number of any feed sample positive to the LM + qPCR in the EU
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F10_i$: Number of feed samples contaminated with DNA copy number above the interpretation cut-off point

Name	$F10_i$
Explanation	It is the result of multiplying the annual number of feed samples collected in the EU by type of premises by the probability of a feed sample to be 'detectable by qPCR' and contaminated with ruminant DNA and by the probability of a contaminated feed sample with DNA copy number above the interpretation cut-off point
Type	Data item
Definition	$F5_i * F4_i * F7_i$
Dependent items	$F13_i$ and $F14_i$
Unit	Number of samples
Assumptions	The number of feed samples with DNA copy number above the interpretation cut-off point in the three MS is representative of the entire EU
Remark & Role	This parameter accounts for the uncertainty of the number of DNA copies of any feed sample positive to the LM + qPCR in the EU
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F11_i$: Number of feed samples contaminated with DNA copy numbers below the interpretation cut-off point

Name	$F11_i$
Explanation	It is the result of subtracting the number of feed samples contaminated with DNA copy numbers above the interpretation cut-off point to the number of feed samples contaminated, by type of premises
Type	Data item
Definition	$F9_i - F10_i$
Dependent items	$F13_i$, $F14_i$
Unit	Number of samples
Assumptions	The number of feed samples with DNA copy numbers below the interpretation cut-off point in the three MS is representative of the entire EU
Remark & Role	This parameter accounts for the uncertainty of the DNA copy number of any feed sample positive to the LM + qPCR in the EU
References	Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F12_i$: Amount of feed from which a sample is taken, by type of premises

Name	$F12_i$
Explanation	Feed sampling is conducting by extracting a number of subsamples -depending on the type of feed and premises - from a unit of feed that can range from an individual sack up to a full container ship. The model requires the parameterization of the amount of feed mass which each sample has been collected from, for the three types of premises categorized: farm, feed mill and border point. Mean values (kg) for the min and max estimated for each type of premises: farm (25, 15,000), feed mill (2,000, 30,000), border (30,000, 500,000). Scenario analysis: fixed averages for the three types using the average of feed mill (16,000)
Type	Data item
Definition	Uniform distribution: $F12_1$: Minimum = 25; Maximum =15,000. Mean: 7,513 $F12_2$: Minimum = 2000; Maximum = 30,000. Mean: 16,000 $F12_3$: Minimum = 30,000; Maximum = 500,000. Mean: 265,000
Dependent items	$F13_i$, $F14_i$ and $F15_i$
Unit	Kg
Assumptions	
Remark & Role	This parameter accounts for variability and uncertainty. This parameter is very difficult to parameterize due to the variability of the feed mass and the lack of actual data on amount of feed from which samples are taken. Proxy measurements had to be used, like the size of a sack, standard farm silo size, etc. Scenario analysis: fixed averages for the three types in order to assess the impact of this parameter on the output, using the feed mill mean for all.
References	Working group experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment

Item $F13_i$: Annual amount of contaminated feed detected by qPCR with DNA copy numbers above the interpretation cut-off point from which samples were taken, by type of premises

Name	$F13_i$
Explanation	Amount of contaminated feed detected by qPCR with DNA copy numbers above the interpretation cut-off point from which samples were taken, by type of premises. It is calculated to multiplying the number of feed samples contaminated with DNA copy numbers below the interpretation cut-off point taken in one year by the annual amount of feed from which a sample is taken, by type of premises
Type	Data item
Definition	$F10_i$ * $F12_i$
Dependent items	$F13_i$
Unit	Kg
Assumptions	The contamination in the form of ruminant DNA is uniformly distributed within the amount of feed from which the samples have been collected.
Remark & Role	
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F14_i$: Annual amount of contaminated feed detected by qPCR with DNA copy numbers below the interpretation cut-off point from which samples were taken, by type of premises

Name	$F14_i$
Explanation	Annual amount of contaminated feed detected by qPCR with DNA copy numbers below the interpretation cut-off point from which samples were taken, by type of premises. It is calculated by multiplying the number of feed samples contaminated with DNA copy numbers below the interpretation cut-off point taken in one year by the annual amount of feed from which a sample is taken, by type of premises
Type	Data item
Definition	$F11_i$ * $F12_i$
Dependent items	F18
Unit	Kg
Assumptions	The contamination in the form of ruminant DNA is uniformly distributed within the amount of feed from which the samples have been collected.
Remark & Role	
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item $F15_i$: Annual amount of contaminated feed detected by qPCR with DNA copy numbers by type of premises

Name	$F15_i$
Explanation	The annual amount of contaminated feed detected by qPCR with DNA copy numbers by type of premises is calculated by multiplying the number of feed samples contaminated by the amount of feed from which a sample is taken by type of premises
Type	Data item
Definition	$F9_i$ * $F12_i$
Dependent items	F16
Unit	Kg
Assumptions	The amount of feed for each sample collected is equal within the three types of premises, respectively. The contamination in the form of ruminant DNA is uniformly distributed within the feed fraction from which the samples have been collected.
Remark & Role	
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item F16: Annual total amount of contaminated feed detected by qPCR from which samples were taken

Name	F16
Explanation	The annual total amount of contaminated feed detected by qPCR from which samples were taken is directly calculated by adding the amounts of contaminated feed in each type of premises, as estimated by $F14_i$.
Type	Data item
Definition	Σ $F15_i$
Dependent items	F17
Unit	Kg
Assumptions	The contamination in the form of ruminant DNA is uniformly distributed within the amount of feed from which the samples have been collected.
Remark & Role	
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item F17: Annual total amount of contaminated feed detected by qPCR with DNA copy numbers above the interpretation cut-off point from which samples were taken

Name	F17
Explanation	The total amount of contaminated feed detected by qPCR with DNA copy numbers above the interpretation cut-off point from which samples were taken is directly calculated by adding the amounts of contaminated feed in each type of premises, as estimated by $F13_i$.
Type	Data item
Definition	Σ $F13_i$
Dependent items	
Unit	Kg
Assumptions	The contamination in the form of ruminant DNA is uniformly distributed within the feed fraction from which the samples have been collected.
Remark & Role	This parameter does not contribute of the output of the model.
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data (three selected MS) 2013–2017. Requested by EFSA for this mandate

Item F18: Annual total amount of contaminated feed detected by qPCR with DNA copy numbers below the interpretation cut-off point from which samples were taken

Name	F18
Explanation	The total amount of contaminated feed is directly detected by PCR with DNA copy numbers below the interpretation cut-off point from which samples were taken was calculated by adding the amounts of contaminated feed in each type of premises, as estimated by $F14_i$.
Type	Data item
Definition	Σ $F14_i$
Dependent items	F17
Unit	Kg
Assumptions	The amount of feed for each sample collected is equal within the three types of premises, respectively. The contamination in the form of ruminant DNA is uniformly distributed within the feed fraction from which the samples have been collected.
Remark & Role	
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item F19: Proportion of contaminated feed that is detected by qPCR with DNA copy below the interpretation cut-off point

Name	F19
Explanation	The proportion of contaminated feed (detected by qPCR with DNA copy numbers below the interpretation cut-off point) is directly calculated by dividing the annual total amount of contaminated feed detected by qPCR with DNA copy numbers below the interpretation cut-off point from which samples were taken (F18) by the annual total amount of contaminated feed detected by ruminant qPCR from which samples were taken, expressed in percentage (F16).
Type	Data item
Definition	(F18/F16) * 100
Dependent items	
Unit	Percentage. Fraction
Assumptions	The contamination, in the form of ruminant DNA, is uniformly distributed within the feed amount from which the samples have been collected.
Remark & Role	It is an output of the model that is reported and compared for different values of F6
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate

Item F20: Proportion of all produced feed that is contaminated and detected by qPCR with DNA copy numbers below the interpretation cut-off point

Name	F20
Explanation	The proportion of all produced feed that is contaminated and detected by qPCR with DNA copy numbers below the interpretation cut-off point, was directly calculated by dividing the annual total amount of contaminated feed detected by qPCR with DNA copy numbers below the interpretation cut-off point from which samples were taken (F18) by the total feed produced in the EU in a year (F1), and expressed in percentage
Type	Data item
Definition	(F18/F1) * 100
Dependent items	
Unit	Percentage. Fraction
Assumptions	The contamination, in the form of ruminant DNA, is uniformly distributed within the feed amount from which the samples have been collected.
Remark & Role	It is an output of the model that is reported and compared for different values of F6
References	WG experts based on consultation with field inspectors and size of standard feed deliveries and storage equipment. Feed testing data from three selected MS (period 2013–2017). Requested by EFSA for this mandate FEFAC. The compound feed industry in the EU livestock economy (2017) http://www.fefac.eu/files/79279.pdf

Appendix D – Protocols of laboratory methods for feed testing

In recent years, the laboratory methods used to test feedingstuffs for the presence of unauthorised material of animal origin have been improved and new data and information have become available. A description of feed sampling procedures including sampling and testing protocols according to National Plans is presented and discussed.

The Annex VI of Commission Regulation No 152/2009 was entirely revised and amended by Commission Regulation No 51/2013 published on the 16 of January 2013. The new regulation relies on the combination of light microscopy (LM) and qPCR for the detection of animal proteins in feed in the European Union. This regulation also relies on Standard Operating Procedures (SOPs) edited by the EU reference laboratory for animal proteins in feedingstuffs (EURL-AP) for technical implementation. SOP (s) detail the operational protocols that have to be followed, depending on the type of feed being analysed. The final destination of the compound feed or feed materials determines the operational protocol which has to be followed.

At the moment, feed and feed material for farmed animals (except aquaculture animals and fur animals) have to be processed and analysed by the LM test only, as PAP of terrestrial animals are not allowed in most of farmed animal feed, according to Regulation (EU) 56/2013. Table 1 shows PAP and material of animal origin which are allowed or forbidden in feed for different species.

According to the SOP 'Operational protocols for the combination of light microscopy and PCR' version 3.0 (Benedetto et al., 2015), two different operational protocols should be followed depending on the type of feed being analysed; one for feed or feed material intended for farmed animals others than aquaculture and fur animals, and a second for feed or feed material intended for aquaculture animals.

Protocol for feed and feed material intended for farmed animals other than aquaculture and fur animals

In this case, the LM-based method is to be followed in order to detect the presence of particles of animal origin. If particles of animal origin are detected, and if the material is not particularly intended for non-ruminants or as milk replacer, the result will be considered positive. Alternatively, if the material is intended for non-ruminants or as milk replacer, samples will only be considered positive if particles from terrestrial animals are found.

Protocol for feed or feed material intended for aquaculture animals

For this type of material, either the LM or the qPCR method may be performed in the first instance depending on the composition of the feed. If the composition is unknown, LM is the method to be applied first, with the qPCR method applied only if particles from terrestrial animals are found by LM. If the feed is known to contain PAP, the qPCR method shall be applied first.

As described in Section 3.1.6.5, if the feed is known to contain blood products, as indicated for instance from the declaration or the labelling, the qPCR method shall only be applied in the first instance at a frequency of one in 10 samples (1/10) chosen on a random basis (the random character of this selection must be recorded so that it could be proven). However, in the case of suspicion of irregularity, or positive results in previous qPCR tests, the qPCR method can be applied in the first instance on all samples of feed or feed material samples from the same origin.

Sampling for the official control of feed

Sampling of feed should follow Commission Regulation (EC) No 691/2013 amending Regulation (EC) No 152/2009 as regards methods of sampling and analysis, and the guidance document for its implementation.[31] Samples intended for the official control of feed shall be taken according to the methods described in the Regulation. Samples thus obtained shall be considered as representative of the sampled portions. The guidance document provided should be applied in all EU Member States.

[31] See footnote 21.

Light microscopy-based method

Extraction and preparation of the sediment and of the flotate.

Sample manipulation

An aggregated final sample weighs a minimum of 4 kg, or less if the feed product is expensive. This is the maximum sample size from a lot, but lot size is variable in tonnes. Lot definition is also variable, as is sampling strategy. Laboratories always receive four sub samples of 500 g each from an aggregated sample, and an aliquot of 50 g from only one of these subsamples is ground in preparation for analysis. A further subsample of 10 g will undergo sedimentation and 3 g will finally be used for the LM analysis. A very small final amount is used for LM.

For the qPCR another aliquot of 10 g is taken, from which two samples of 100 mg are used for the DNA extraction.

In order to obtain the separation of more dense parts, (minerals and therefore bone and fish scale fragments), starting from a portion of 10 g, extraction with tetrachloroethylene (specific gravity 1.62) is carried out. The portion for extraction is 3 g in the case of fishmeal or other pure animal material, mineral ingredients or premixes which generate more than 10% sediment. A conical glass separation funnel with a capacity of 250 mL, or a conical bottomed settling beaker, should be used.

Following extraction with tetrachloroethylene the sample will divide into sediment and flotate. Microscopic slides should be prepared from the sediment and, depending on the operator's choice, from either the flotate or the raw material. If sieving has been used during the sample preparation, the two resulting fractions (the fine and the coarse one) should be prepared. Test portions of fractions spread on slides must be representative of the whole fraction. Microscopic slides should be mounted with an adequate mounting medium in accordance with the SOP established by the EURL-AP and published on its website (Frick et al., 2013).

Sediment: If more than 5% of the sediment consists of particles > 0.5 mm, sieve at 0.25 mm and examine the two resulting fractions.

Flotate (or raw material portion of at least 5 g of the 50 g ground subsample): if more than 5% of the flotate or of the raw material consists of particles > 0.5 mm, sieve at 0.25 mm and examine the two resulting fractions.

Slide preparation

The minimum numbers of slides to be observed at each step of the observation protocol shall be strictly respected unless the entire fraction material does not permit to reach the stipulated slide number. No more than six slides per determination shall be observed.

First step: Prepare three slides from sediment and one slide from flotate or raw material. If more than five particles of animal origin of the same nature (i.e. fish or terrestrial) are detected, the sample is declared positive.

Second step: If no particles, or up to five particles of animal origin are detected, one more slide from the sediment has to be examined. If more than five particles of animal origin of the same nature (i.e. fish or terrestrial) are detected, the sample is declared positive.

Third step: If no particles or up to five particles of animal origin are detected so far, one more slide from flotate or raw material has to be examined. If more than five particles of animal origin of the same nature (i.e. fish or terrestrial) are detected, the sample is declared positive.

If no particles of animal origin are detected the sample is declared negative.

If between one and five particles of animal origin are detected a second determination has to be performed from a new 50 g subsample. A maximum of three determinations can be carried out each time, starting from a new 50 g subsample.

Number of determinations and expression of results

When reporting the results, the laboratory has to report on which type of material the analysis has been carried out (sediment, flotate or raw material) and how many determinations have been undertaken.

A result is considered positive only if more than five particles are counted (this is a kind of a 'rough LOD'). The problem is that the method cannot distinguish muscle cells of different species.

If following a first determination, no animal particle is detected, the result of the analysis is considered as negative.

If following a first determination, the total number of animal particles of a given nature (i.e. terrestrial animal or fish) detected ranges from 1 to 5, a second determination has to be performed

from a new 50 g subsample. If, following this second determination, the number of animal particles of this given nature detected ranges from 0 to 5, the result of the analysis should report that this low level presence, being below the limit of detection of the microscopic method, means that a risk of false positive result cannot be excluded. Then, a third determination shall be carried out from a new 50 g sub-sample. Nevertheless, if following the first and the second determination, the sum of the particles of a given nature detected over the two determinations is higher than 15, no additional determination is necessary and the result of the analysis shall be directly as positive.

If, following the third determination, the sum of the animal particles of a given nature detected over the three determinations is higher than 15, the result of the analysis is considered as positive.

If, following the third determination, the sum of the animal particles of a given nature detected over the three determinations is lower than 15, the result of the analysis the result of the analysis shall be reported as non-conclusive, as this low level presence, being below the limit of detection of the microscopic method, means that a risk of false positive result cannot be excluded.

If following a first determination more than five animal particles of a given nature (i.e. terrestrial animal or fish) are detected, the result of the analysis is considered as positive.

qPCR-based method

The procedure of analysis by qPCR involves the following steps of analysis:

A DNA extraction step

The DNA extraction step provides DNA for subsequent qPCR-based detection methods. The method has to yield DNA of sufficient quality and quantity from a feed sample as the matrix of analysis. A specific EURL-AP SOP for DNA extraction is available and accessible from the EURL-AP webpage (Benedetto et al., 2014). The extraction consists of a lysis step that brings the DNA into solution. The liberated DNA is recovered using silica-coated magnetic beads. This allows the purification of DNA from several other compounds such as proteins, glycosidic compounds or carbohydrates.

The SOP is based on two representative test portions per sample of 100 mg (100–105 mg) yielding two independent DNA extracts which can be used for qPCR analysis, and controls to be included are also described. The SOP includes two procedures: a semi-automated one and a manual one. Due to the fact that the qPCR results can be dependent on the DNA extraction method used, it is mandatory that DNA extracts used with the EURL-AP validated qPCR methods are produced according to this SOP. The method was used in the implementation study organised by the EURL-AP to assess its implementation by the NRL network (Fumière et al., 2012).

A DNA amplification step

This step consists of the specific amplification and detection of DNA from five ruminant species. This step starts from the DNA extracts that were obtained from two independent test portions taken from the sample and the protocol is described on the EURL-AP SOP 'Detection of ruminant DNA in feed using qPCRs' as mentioned above. For the detection of ruminant DNA, an extremely abundant nuclear DNA target of 85/86 base pairs is amplified using two specific primers. qPCR products are measured during each cycle by means of an oligonucleotide probe labelled with two fluorescent dyes: FAM as a reporter dye at its 5′ end and TAMRA as a quencher dye at its 3′ end. For the determination of the threshold, careful analysis of the signals is required, and as detailed on the SOP, the threshold should be set in the exponential increase phase and at a level higher than any fork effect. The measured fluorescence signal passes the threshold value after a certain number of cycles. This threshold cycle is called the 'Ct' value or Cq.

This Cq value is compared to a predetermined cut-off figure to establish if the PCR result is positive or negative. The determination of such cut-offs is as well detailed in the SOP, and an Excel file to support this is available on the EURL-AP website. In the SOP, the qPCR platform is considered as the combination of a thermocycler and the reagents used to perform a qPCR. The platform is machine-specific and cut-off values established for one machine cannot be transferred to another machine without re-determining the cut-off value.

As mentioned in the Commission Regulation (EU) No 51/2013 as well as in the SOP '*Detection of ruminant DNA in feed using real-time P*CR', it is crucial that the master mix used is fit-for-purpose and does not contain bovine serum albumin (BSA) or other reagents liable to lead to false results due to presence of animal DNA. An alternative master mix may be used if comparable performance has been demonstrated by a pretest. A dossier describing the experiments done to prove the equivalence of the alternative master mix must be communicated for approval to the EURL-AP via the NRL. The list of

approved master mixes on the EURL-AP website (eurl.craw.eu) will be updated accordingly. So far the EURL-AP recommends the use of one of the following mastermixes: 'Universal mastermix' reference number DMML-D2-D600 (Diagenode, Liège, Belgium) and 'qPCR MasterMix' reference number RT-QP2X-03 (Eurogentec, Seraing, Belgium). Using other mastermixes might lead to both false positive and false negative results if such mastermix has not been tested and proven equivalent to the recommended ones.

Interpretation of results

The interpretation of results involves:

1) The analysis of the shape of the amplification curve, which should show a typical increase of fluorescence.
2) The analysis of the controls that should also give the expected results as detailed on the SOP. Such controls include positive and negative controls for DNA extraction, to check for DNA extraction efficiency and absence of contamination respectively, and positive and negative controls of amplification to check for qPCR efficiency and the absence of contamination during qPCR respectively. If one of the controls does not meet the expected criteria, the qPCR run should be repeated and if the problem remains the extraction and/or qPCR step (depending of which control has failed) should be repeated with new reagents. If the results from the controls are as expected, and if a qPCR result from a sample is positive at least for one of the dilutions, the test portion will be considered as positive.
3) With the purpose of proving that negative results are due to the absence of target DNA and not to the inhibition of the qPCR reaction, an inhibition control is also used. In such case, a known amount of target not exceeding 100 copies per well shall be analysed in two different conditions: without any DNA source other than the added target, and in presence of the DNA extract that is analysed for inhibition. Both should give positive results to dismiss the possibility of inhibitors being present in the test portion being evaluated.

In order to determine the cut-off point, a calibration curve of the qPCR is built up using the European reference material consisting of certified amounts of plasmids (reference material ERM-AD482 ruminant pDNA calibrant - JRC-IRMM, 2015) bearing the target on which the qPCR is focused (the set consists of three vials with different plasmid concentrations). The reference material contains positive material ranging from 40 to 680 ruminant DNA copies. The standard curve is only designed for samples with a low level content of ruminant DNA. Using the reference material, the Ct value is calculated. If the number of cycles that produces a positive signal is lower than the Ct value set as the cut-off, the sample is declared positive. The calculation of the cut-off requires the production of results from four separate runs with 12 qPCR replicates, using three different plasmid calibrants from the reference material (In total 48 qPCR replicates of each calibrant). According to the instructions, the calculation must result in a cut-off between 9 and 11 ruminant DNA copies.

The validation of a diagnostic method does not require the determination of an exact limit of detection (LOD) but this performance parameter can be set as being below or equal to a certain value (CCMAS, 2010). It has been accepted that the benchmark established in the Commission Directive 2003/126/EC on the Sensitivity of microscopy that, dependent on the nature of the material of animal origin, can detect levels of contamination lower than 0.1% of mass fraction, is also achieved by the ruminant qPCR. However, the application of the qPCR method to samples with different matrices and with different level of processing does not allow a direct correlation to be established between the results from the qPCR method (expressed in the number of DNA copy numbers) and the level of contamination based on percentage of mass fraction (w/w).

Appendix E – Questionnaire on the controls of the feed ban

The Commission would like to request EFSA to update the 2011 "Scientific Opinion on the revision of the QRA of BSE risk posed by processed animal proteins (PAP)" (See: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.1947/epdf) based on more recent data to be used for the assumptions.

We therefore kindly ask you to complete the included table to the extent possible. We are very much aware that it might not be possible to submit all information in detail.

We will of course also collect information submitted through RASFF.

Please reply at the latest by 15 September 2017 to kris.de-smet@ec.europa.eu

Reply submitted by (country): . . . . . .

1. Controls for ruminant PAP carried out in non-ruminant PAP in 2015

1.1 Number of tests carried out on the presence of ruminant PAP: . . .**..**

1.2 Number of samples in which ruminant PAP was detected: . . .**.**

1.3 If any information is available on the source of the ruminant PAP detected, please provide:

. . . . . .

3.4 If any information is available on the amount (e.g. traces, x%, < x%) of the ruminant PAP detected, please provide:

. . . . . .

2. Controls of for ruminant PAP carried out in non-ruminant PAP in 2016

2.1 Number of tests carried out on the presence of ruminant PAP: . . .**..**

2.2 Number of samples in which ruminant PAP was detected: . . .**.**

2.3 If any information is available on the source of the ruminant PAP detected, please provide:

. . . . . .

If any information is available on the amount (e.g. traces, x%, < x%) of the ruminant PAP detected, please provide:

. . . . . .

3. Controls for ruminant PAP of feed intended for aquaculture in 2016

3.1 Number of tests carried out on the presence of ruminant PAP: . . .**..**

3.2 Number of samples in which ruminant PAP was detected: . . .**.**

3.3 If any information is available on the source of the ruminant PAP detected, please provide:

. . . . . .

3.4 If any information is available on the amount (e.g. traces, x%, < x%) of the ruminant PAP detected, please provide:

. . . . . .

4. Controls for ruminant PAP of feed intended for aquaculture in 2016

4.1 Number of tests carried out on the presence of ruminant PAP: . . .**..**

4.2 Number of samples in which ruminant PAP was detected: . . .**.**

4.3 If any information is available on the source of the ruminant PAP detected, please provide:

. . . . . .

4.4 If any information is available on the amount (e.g. traces, x%, < x%) of the ruminant PAP detected, please provide:

. . . . . .

5. Controls for non-authorised PAP in feed, other than feed intended for aquaculture, in 2015

5.1 Number of tests carried out on the presence of non-authorised PAP: . . .**..**

5.2 Number of samples in which non-authorised PAP was detected: . . .**.**

5.3 Number of samples in which the species of origin of the detected non-authorised PAP was (sum of 3 bullets should equal 5.2):

- Ruminants or multispecies including ruminants: . . . . . .
- Exclusively non-ruminants: . . . . .**,** if available specify source:. . . . . .
- Not found/not investigated: . . . . . .

5.4 If any information is available on the amount (e.g. traces, x%, < x%) of the ruminant PAP detected, please provide:

. . . . . .

6. CONTROLS FOR NON-AUTHORISED PAP IN FEED, OTHER THAN FEED INTENDED FOR AQUACULTURE, IN 2016

6.1 Number of tests carried out on the presence of non-authorised PAP: . . .**..**

6.2 Number of samples in which non-authorised PAP was detected: . . .**.**

6.3 Number of samples in which the species of origin of the detected non-authorised PAP was (sum of 3 bullets should equal 6.2):

- Ruminants or multispecies including ruminants: . . . . . .
- Exclusively non-ruminants: . . . . ., if available specify source:. . . . . **..**
- Not found/not investigated: . . . . . .

6.4 If any information is available on the amount (e.g. traces, x%, < x%) of the ruminant PAP detected, please provide:

. . . . . .

If you have any other information (including scientific publications) that might relevant for the revision of the QRA, please add.

Appendix F – Results of the questionnaire survey on feed ban official controls in the EU

Table F.1: Summary of answers received to a questionnaire on feed ban official controls in the EU Member States in 2015 and 2016

			EU[(a)]
Controls for ruminant PAP in non-ruminant PAP	**2015**	Number of tests	1,971
		Number of positives	28
	2016	Number of tests	1,839
		Number of positives	13
Controls for ruminant PAP in feed for aquaculture animals	**2015**	Number of tests	654
		Number of positives	40
	2016	Number of tests	634
		Number of positives	17
Controls for non-authorised PAP in feed other than aquafeed	**2015**	Number of tests	25,261
		Number of positives	24
	2016	Number of tests	22,898
		Number of positives	12

(a): Data from Malta have not been received.

Table F.2: Number of samples collected by 14 MS in 2015 and 2016 for the monitoring of the feed ban (1)

			BE	BG	CZ	DK	DE	EE	IE	EL	ES	FR	HR	IT	CY	LV
Controls for rum PAP in non-rum PAP	**2015**	**No of tests**	22	239	4	5	37	2	0	28	106	46	0	1	1	15
		No of positives	0	11	0	0	10	0	0	0	2	2	0	0	0	0
	2016	**No of tests**	42	179	8	8	39	2	0	43	119	45	0	16	1	18
		No of positives	0	1	0	0	10	0	0	0	0	0	0	0	0	0
Controls for rum PAP in feed for aquaculture animals	**2015**	**No of tests**	41	6	0	8	19	1	N.A.	17	28	140	0	255	22	15
		No of positives	1	0	0	0	0	0	N.A	3	0	2	0	29	3	1
	2016	**No of tests**	39	1	0	18	29	0	N.A.	19	8	138	0	269	18	12
		No of positives	1	1	0	0	1	0	N.A.	2	0	4	0	7	0	0
Controls for non-authorised PAP in feed other than aquafeed	**2015**	**No of tests**	966	222	217	309	2,646	87	1,209	166	1,155	653	164	1,873	351	96
		No of positives	2	0	1	0	2	0	0	1	2	1	0	1	0	0
	2016	**No of tests**	802	214	149	313	2,663	89	1,222	136	1,055	757	119	1,973	346	94
		No of positives	1	0	0	0	1	0	0	3	1	1	0	0	0	0

BE: Belgium. BG: Bulgaria. CZ: the Czech Republic DK: Denmark. DE: Germany. EE: Estonia. IE: Ireland. EL: Greece. ES: Spain. FR: France. HR: Croatia. IT: Italy. CY: Cyprus. LV: Latvia.

Table F.3: Number of samples collected by 14 MS and totals in 2015 and 2016 for the monitoring of the feed ban (2)

			LT	LU	HU	MT	NL	AT	PL	PT	RO	SI	SK	FI	SE	UK	EU28
Controls for rum PAP in non-rum PAP	**2015**	**No of tests**	11	0	9		0	611	0	7	663	2	21	94	0	47	1,971
		No of positives	0	0	0		0	1	0	0	0	2	0	0	0	0	28
	2016	**No of tests**	9	0	7		0	515	0	3	676	1	23	48	0	37	1,839
		No of positives	1	0	0		0	0	0	0	0	1	0	0	0	0	13
Controls for rum PAP in feed for aquaculture animals	**2015**	**No of tests**	3	0	28		0	5	23	19	0	2	7	12	1	2	654
		No of positives	0	0	0		0	0	0	0		0	0	1	0	0	40
	2016	**No of tests**	2	0	13		0	2	43	5	1	5	7	3	0	2	634
		No of positives	0	0	0		0	0	0	0	0	1	0	0	0	0	17
Controls for non-authorised PAP in feed other than aquafeed	**2015**	**No of tests**	83	42	592		985	606	2,950	976	1,416	81	261	291	118	6,746	25,261
		No of positives	3	0	0		3	2	4	0	0	0	0	0	0	2	24
	2016	**No of tests**	90	44	542		976	513	1,839	532	1,556	83	217	227	91	6,256	22,898
		No of positives	0	0	0		2	0	2	0	0	0	0	0	0	1	12

LT: Lithuania. LU: Luxembourg. HU: Hungary. MT: Malta. NL: The Netherlands. AT: Austria. PL: Poland. PT: Portugal. RO: Romania. SI: Slovenia. SK: Slovakia. FI: Finland. SE: Sweden. UK: the United Kingdom.

Appendix G – Summary of the RASFF notifications of EU Member States in 2015 and 2016

Table G.1: Summary of RASFF notifications of EU Member States in 2015 and 2016 as a result of feed ban official controls
Search criteria | **Notified from** 1/1/2015 | **Notified till** 31/12/2016 | **Category** TSEs

Product category	Date	Reference	Notification	Notification type	Notified by basis	Countries concerned	Subject	Action taken	Risk decision
Compound feeds	19/1/2015	2015.0059	Information for follow-up	Official control on the market	Italy	Bosnia and Herzegovina (D), Greece (D), Italy (WO)	Presence of ruminant DNA in complete feed for trout from Italy	Withdrawal from the market	Not serious
Compound feeds	26/1/2015	2015.0079	Information for follow-up	Official control on the market	Bulgaria	Bulgaria (D), Germany (0), Netherlands	Presence of ruminant DNA in feed for fish from Germany, via the Netherlands	Seizure	Not serious
Compound feeds	10/3/2015	2015.0287	Information for follow-up	Official control on the market	Latvia	Commission Services, Estonia (D), Latvia (D), Poland (0)	Presence of ruminant DNA in complete feed for fish from Poland	Withdrawal from the market	Not serious
Compound feeds	24/3/2015	2015.0360	Information for follow-up	Official control on the market	Finland	Estonia (D), Finland (D), Italy (0), Latvia (D)	Presence of ruminant DNA in fishmeal from Italy	Informing recipients	Not serious
Compound feeds	24/412015	2015.0518	Information for follow-up	Official control on the market	Cyprus	Cyprus (D), Germany (0), Italy (0), Spain (0)	Presence of ruminant DNA in fish feed manufactured in Italy, with raw material from Germany and Spain		Not serious
Compound feeds	9/6/2015	2015.0720	Information for follow-up	Official control on the market	Italy	Bulgaria (D), Italy (D/O), Portugal (D/O), Spain (D/0)	Presence of ruminant DNA in complete feed for trout from Italy, with raw material from Spain and Portugal	No action taken	Not serious
Compound feeds	26/6/2015	2015.0832	Information for follow-up	Official control on the market	Italy	Albania (D), Bulgaria (D), Germany (0), Italy (0), Spain (0)	Presence of ruminant DNA in complete feed for trout from Italy, with raw material from Germany and Spain		Not serious
Compound feeds	9/7/2015	2015.0880	Information for follow-up	Official control on the market	Italy	Italy (D), Spain (0)	Presence of ruminant DNA in complete feed for aquaculture from Spain	Seizure	Not serious

Product category	Date	Reference	Notification	Notification type	Notified by basis	Countries concerned	Subject	Action taken	Risk decision
Compound feeds	15/7/2015	2015.0920	Information for follow-up	Official control on the market	Cyprus	Commission Services, Cyprus (D), Germany, Italy (0), Portugal (0), Spain (0)	Presence of ruminant DNA in complete feed for fish manufactured in Italy, with raw material from Spain and Portugal, via Germany	Official detention	Not serious
Compound feeds	21/7/2015	2015.0949	Information for follow-up	Official control on the market	Italy	Bulgaria, Commission Services, Croatia (D). Germany (0), Greece (D), Italy (0), Portugal (0). Slovenia (D), Spain (0)	Presence of ruminant DNA in complete feed for trout manufactured in Italy, with raw material from Germany, Italy, Spain and Portugal	Withdrawal from the market	Not serious
Compound Feeds	3/8/2015	2015.1006	Information for follow-up the market	Official control on	Belgium	Belgium (D), Commission Services. Germany (0), Netherlands	Presence of ruminant DNA in fish feed from Germany		Not serious
Compound feeds	10/8/2015	2015.1032	Information for attention	Official control on the market	Italy	Germany (0), Italy (D/O), Pakistan, Portugal (0), Spain (0)	Presence of ruminant DNA in complete feed for trouts from Italy, with raw material from Germany, Spain and Portugal	Withdrawal from recipient (s)	Not serious
Compound feeds	7/9/2015	2015.1146	Information for follow-up	Official control on the market	Cyprus	Cyprus (D), Italy (D/0), Spain (D)	Presence of ruminant DNA in feed for seabream/dorada from Italy, via Spain	Official detention	Not serious
Compound feeds	11/9/2015	2015.1158	Information for follow-up	Official control on the market	Italy	Commission Services, Italy (D), Spain (0)	Presence of ruminant DNA in fish feed for trout from Spain	Detained by operator	Not serious
Compound feeds	12/11/2015	2015.1427	Information for attention	Official control on the market	Spain	Portugal (D), Spain (0)	Presence of ruminant DNA in fish feed from Spain		Not serious

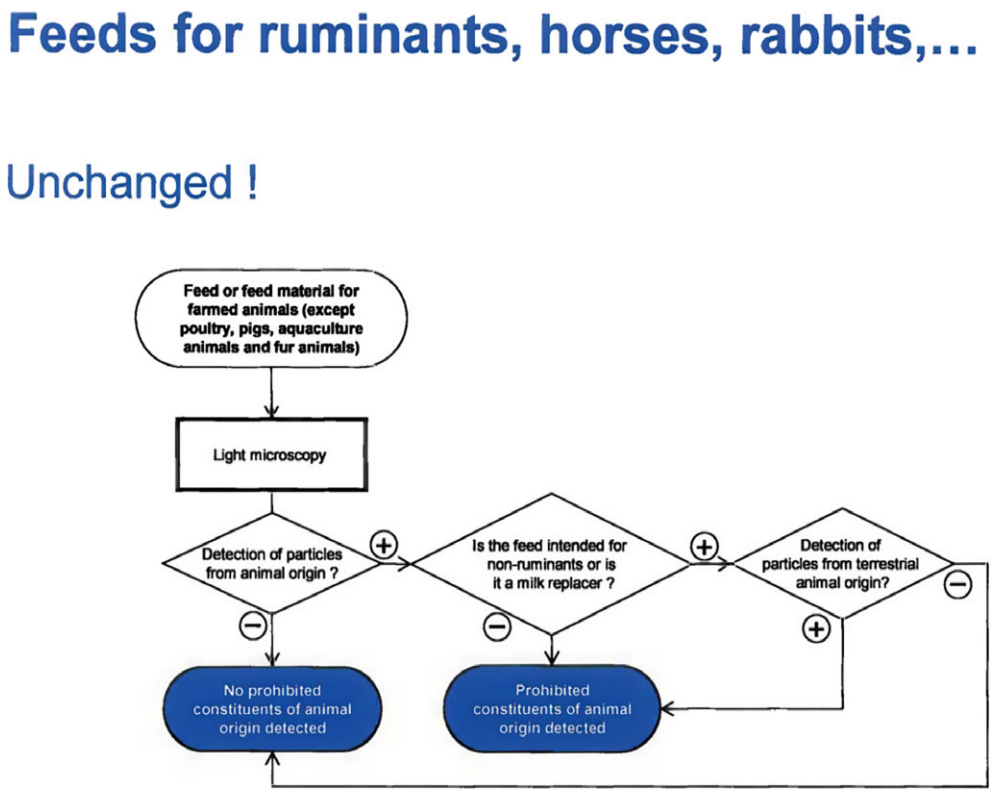
Product category	Date	Reference	Notification	Notification type	Notified by basis	Countries concerned	Subject	Action taken	Risk decision
Compound feeds	1/6/2016	2016.0709	Information for follow-up	Official control on the market	Italy	Czech Republic, Germany (D/O), Hungary (0), Italy (D), Poland (D)	Presence of ruminant DNA in complete feeds for fish from Germany, manufactured in Hungary	Withdrawal from the market	Not serious
Compound feeds	30/9/2016	2016.1346	Information for follow-up	Official control on the market	Greece	Germany (0), Greece (D), Italy (D)	Presence of ruminant DNA in fish feed from Germany	No stock left	Not serious
Compound feeds	21/11/2016	2016.1597	Information for follow-up	Official control on the market	Slovenia	Austria (D), Croatia (D), Czech Republic (D), Denmark (0), Germany (D), Poland (D), Romania (D), Slovakia (D), Slovenia (D)	Presence of ruminant DNA in fish feed from Denmark	Withdrawal from recipient (s)	Not serious
Feed materials	18/3/2015	2015.0327	Information for follow-up	Official control on the market	Italy	Armenia, Greece, Italy (0), Slovenia (D)	Presence of ruminant DNA in feed for trouts from Italy	Withdrawal from the market	Not serious
Feed materials	19/3/2015	2015.0330	Information for follow-up	Official control on the market	Italy	Armenia (D), Greece (D), Italy (D/O)	Presence of ruminant DNA in complete feed for trout from Italy	Withdrawal from the market	Not serious
Feed materials	7/5/2015	2015.0559	Information for follow-up	Official control on the market	Spain	Bulgaria, Romania (D), Spain (0)	Presence of ruminant DNA in processed animal protein from Spain		Not serious
Feed materials	5/6/2015	2015.0702	Information for follow-up	Official control on the market	Italy	Italy (D), Spain (0)	Presence of ruminant DNA in processed animal protein from Spain		Not serious
Feed materials	28/7/2015	2015.0975	Information for follow-up	Official control on the market	France	France (D), Spain (0)	Presence of ruminant DNA in fishmeal from Spain	No action taken	Not serious
Feed materials	16/10/2015	2015.1299	Information for follow-up	Official control on the market	France	France (D), Spain (0)	Presence of ruminant DNA in fishmeal from Spain		Not serious

Product category	Date	Reference	Notification	Notification type	Notified by basis	Countries concerned	Subject	Action taken	Risk decision
Feed materials	31/5/2016	2016.0701	Information for follow-up	Official control on the market	Belgium	Belgium (D), France (O)	Presence of ruminant dna in fish feed from France	No action taken	Not serious
Feed materials	17/6/2016	2016.0786	Information for follow-up	Official control on the market	Belgium	Belgium (O), Germany, Thailand (D), Vietnam (D)	Presence of ruminant dna in processed animal protein cat. 3 from Belgium		Not serious
Feed materials	27/6/2016	2016.0835	Information for follow-up	Official control on the market	Italy	Germany (O), Italy (D)	Presence of ruminant dna in complete feeds for aquaculture from Germany	Detained by operator	Not serious
Feed materials	3/8/2016	2016.1043	Information for follow-up	Official control on the market	Italy	China (D), Denmark (O), Finland (D), France (D), Italy (D), Sweden (D), Switzerland (D)	Presence of ruminant dna in feed materials from Denmark		Not serious
Feed materials	29/9/2016	2016.1342	Information for follow-up	Official control on the market	Belgium	Belgium (O), Germany, Thailand (D)	Presence of ruminant dna in fish processed animal protein from Belgium	Informing authorities	Not serious

O: origin; D: destination.

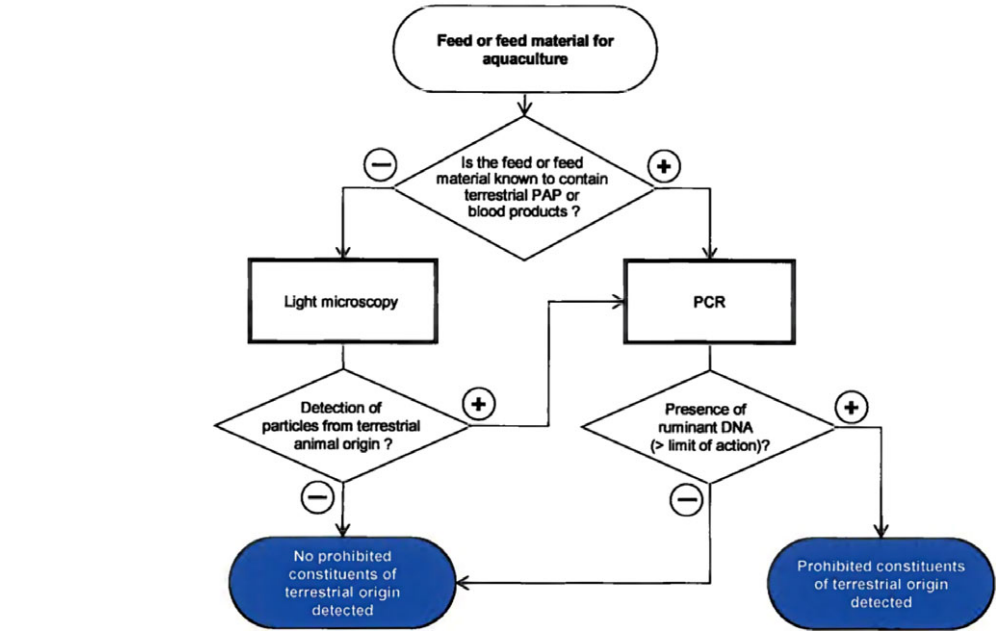
Appendix H – EURL-AP diagnostic protocols for detection of constituents of animals origin in feed

Diagrams of the 'possible combinations of methods for the disclosure of prohibited processed animal proteins in feed and feed ingredients', as included in Annex I of the letter of clarification to ToR1 and ToR3 of the mandate, produced by the EURL-AP.



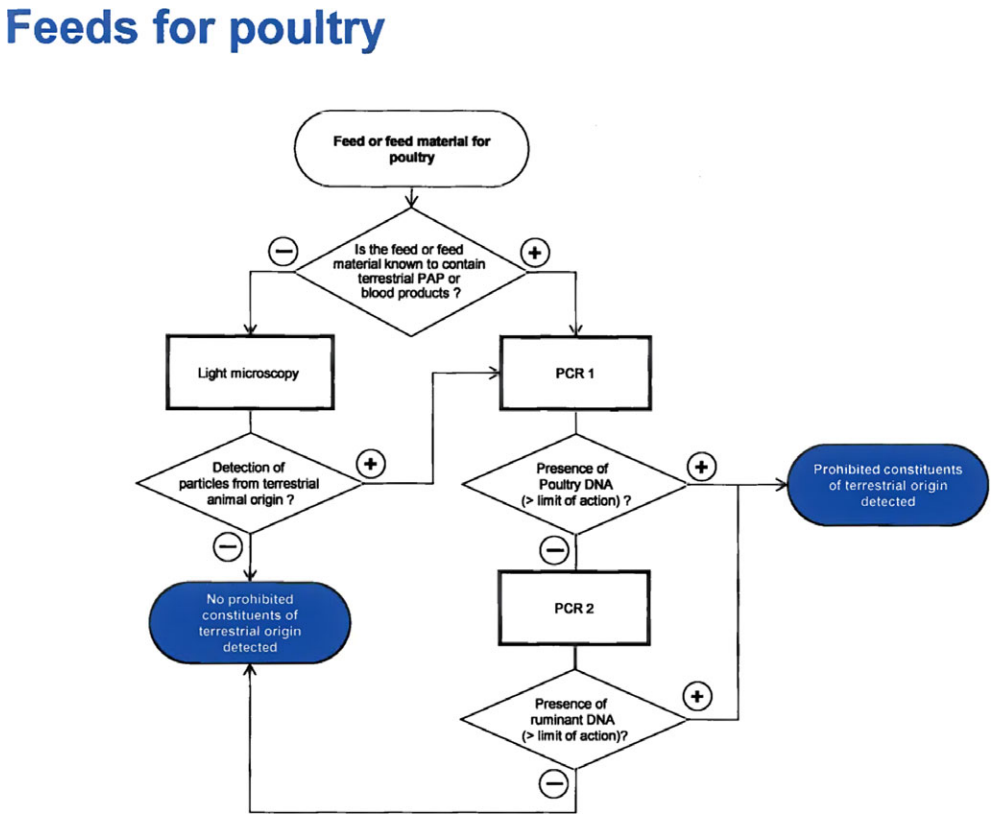
Note: +: yes; −: no.

Figure H.1: EURL-AP diagnostic protocol for detection of constituents of animals origin in feed for ruminants, horses, rabbits, . . .



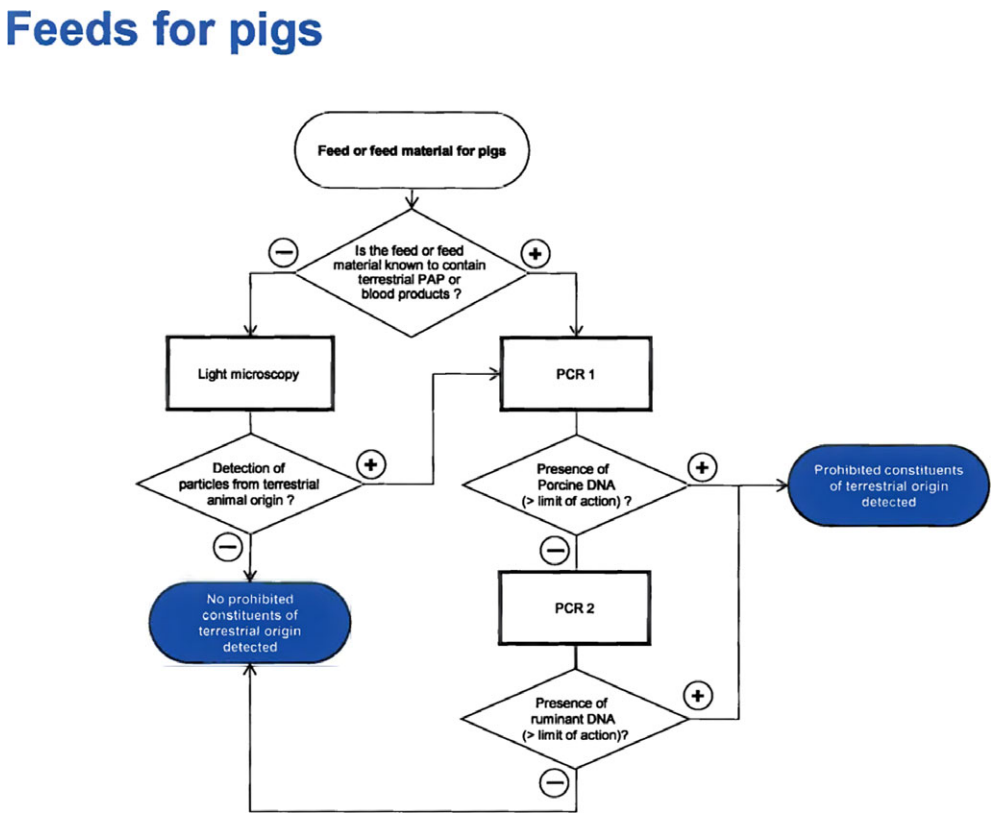
Note: +: yes; −: no.

Figure H.2: EURL-AP diagnostic protocol for detection of constituents of animals origin in feed for aquaculture



Note: +: yes; −: no.

Figure H.3: EURL-AP diagnostic protocol for detection of constituents of animals origin in feed for poultry



Note: +: yes; −: no.

Figure H.4: EURL-AP diagnostic protocol for detection of constituents of animals origin in feed for pigs