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## Assessment on the efficacy of methods 2 to 5 and method 7 set out in Commission Regulation (EU) No 142/2011 to inactivate relevant pathogens when producing processed animal protein of porcine origin intended to feed poultry and aquaculture animals

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### Abstract

An assessment was conducted on the level of inactivation of relevant pathogens that could be present in processed animal protein of porcine origin intended to feed poultry and aquaculture animals when methods 2 to 5 and method 7, as detailed in Regulation (EU) No 142/2011, are applied. Five approved scenarios were selected for method 7. *Salmonella* Senftenberg, *Enterococcus faecalis*, spores of *Clostridium perfringens* and parvoviruses were shortlisted as target indicators. Inactivation parameters for these indicators were extracted from extensive literature search and a recent EFSA scientific opinion. An adapted Bigelow model was fitted to retrieved data to estimate the probability that methods 2 to 5, in coincidental and consecutive modes, and the five scenarios of method 7 are able to achieve a 5 log<sub>10</sub> and a 3 log<sub>10</sub> reduction of bacterial indicators and parvoviruses, respectively. Spores of *C. perfringens* were the indicator with the lowest probability of achieving the target reduction by methods 2 to 5, in coincidental and consecutive mode, and by the five considered scenarios of method 7. An expert knowledge elicitation was conducted to estimate the certainty of achieving a 5 log<sub>10</sub> reduction of spores of *C. perfringens* considering the results of the model and additional evidence. A 5 log<sub>10</sub> reduction of *C. perfringens* spores was judged: 99–100% certain for methods 2 and 3 in coincidental mode; 98–100% certain for method 7 scenario 3; 80–99% certain for method 5 in coincidental mode; 66–100% certain for method 4 in coincidental mode and for method 7 scenarios 4 and 5; 25–75% certain for method 7 scenario 2; and 0–5% certain for method 7 scenario 1. Higher certainty is expected for methods 2 to 5 in consecutive mode compared to coincidental mode.

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**Amendment note:** On page 30 the text 'Freezing at  $-21^{\circ}\text{C}$  for 7 days... as amended' replaced the text 'The EU legislation... in pork'. Reference Lacour et al., 2013 has been added to the references list. This editorial correction does not materially affect the contents or outcome of this scientific output. To avoid confusion, the original version of the output has been removed from the EFSA Journal, but is available on request.

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## Summary

Following the partial revision of the feed ban introduced by Commission Regulation (EU) 2021/1372 the European animal by-product processing sector (EFPRA) asked the Commission to revise the standards for the production of processed animal protein (PAP) of porcine origin set out in Regulation (EU) No 142/2011. In accordance with specific requirements set out in this Regulation, to be placed on the market, PAP of mammalian origin intended to feed poultry and aquaculture animals must have been submitted to processing method 1 (pressure sterilisation) as set out in Chapter III of Annex IV to Regulation (EU) No 142/2011. However, the rendering industry produces PAP intended for pet food, fertilisers or fuel for combustion applying primarily methods 2 to 5 or method 7 as set out in the Regulation. Therefore, the Commission requested EFSA to provide a scientific opinion concerning the efficacy of methods 2 to 5 and method 7 to inactivate relevant pathogens when producing PAP of porcine origin intended to feed poultry and aquaculture animals. In particular, the assessment concerned the level of inactivation of relevant pathogens that could be present in PAP of porcine origin intended to feed poultry and aquaculture animals.

The Term of Reference was translated into five assessment questions (AQ). AQ1: what relevant pathogens can be used as indicators to assess the efficacy of standard processing methods for Category 3 ABP of porcine origin? AQ2: what are the technical parameters (e.g. time, temperature, pressure, pH, particle size) for methods 2 to 5 and 7? AQ3: what are the inactivation parameters (D values, z) of the relevant pathogens identified in AQ1? AQ4: what is the 'level of inactivation' of the selected relevant pathogens achieved by methods 2 to 5 and 7? AQ5: what is the certainty that the 'level of inactivation' achieved by methods 2 to 5 and 7, as in AQ4, is sufficient to reach the standards for Category 3 ABP of porcine origin? The approach to answer the ToR is described in the protocol (Annex A).

To identify what relevant pathogens (**AQ1**) (i.e. bacteria, parasites and viruses) can be used as indicators to assess the efficacy of the methods under assessment, a set of criteria were developed based on previous EFSA standards and scientific opinions, the EU legislation, the WOAHA list of swine diseases and an extensive literature search (for viruses only). As a result, *Salmonella* Senftenberg, *Enterococcus faecalis* and spores of *Clostridium perfringens* were selected as relevant bacterial pathogens in PAP of porcine origin. Relevant viral hazards in PAP of porcine origin identified were: porcine adenovirus (Adenoviridae), Torque Teno virus (Anelloviridae), porcine circovirus (Circoviridae), bocavirus and porcine parvovirus (Parvoviridae). *Taenia solium* and *Trichinella* spp were considered relevant parasitic pathogens in PAP of porcine origin.

The technical parameters of methods 2 to 5 (**AQ2**) are stated in Chapter III of Annex IV to Regulation (EU) No 142/2011: method 2, 100°C for at least 125 min, 110°C for at least 120 min and 120°C for at least 50 min; method 3, 100°C for at least 95 min, 110°C for at least 55 min and 120°C for at least 13 min; method 4, 100°C for at least 16 min, 110°C for at least 13 min, 120°C for at least 8 min and 130°C for at least 3 min; method 5, 80°C for at least 120 min and 100°C for at least 60 min. The Commission Regulation (EU) 142/2011 specifies that the core temperatures specified above may be achieved consecutively or through a coincidental combination of the time periods indicated. The coincidental mode was considered the worst-case scenario and selected for the assessment of the efficacy of methods 2 to 5 to inactivate relevant pathogens. For method 7, the legislation does not state time/temperature parameters. However, there are process parameters approved in different EU member states based on equipment used and treated raw materials. Based on the approved parameters provided by the industry, five scenarios of individual time/temperature profiles were selected for this assessment in order to apply for method 7 the same methodological approach than for methods 2 to 5: scenario 1, 80°C for at least 14 min; scenario 2, 95°C for at least 90 min; scenario 3, 115°C for at least 56 min; scenario 4, 125°C for at least 10 min; scenario 5, 133°C for at least 5 min.

The thermal inactivation parameters (**AQ3**) of spores of *C. perfringens* were retrieved through an extensive literature search (ELS), while those of *S. Senftenberg* and *E. faecalis* were collated in a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2021). The thermal inactivation parameters of the relevant viral families were retrieved through an ELS and from a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2021). Parvoviruses were selected as the most thermal resistant viral hazards for the assessment. For the parasites, the thermal resistance parameters were not retrieved because the thermal resistance of parasites is lower than that of the relevant bacterial and viral pathogens.

An adapted Bigelow model was fitted to the thermal inactivation parameters retrieved for the selected pathogens to estimate the probability that the time/temperature combinations of methods 2 to 5 as stated in the regulation, in both coincidental and consecutive mode, and of the five scenarios

of method 7, set for this assessment, are able to achieve a 5 log<sub>10</sub> reduction of bacterial indicators and a 3 log<sub>10</sub> reduction of parvoviruses (**AQ4**). *C. perfringens* was the indicator with the lowest probability of achieving the target 5 log<sub>10</sub> reduction by methods 2, 3, 4, 5 in coincidental mode and the five time/temperature combinations of method 7. The results of the model showed a probability of inactivation of at least 5 log<sub>10</sub> of spores of *C. perfringens* of over 0.99 for methods 2, 3 and 5, in both the coincidental and consecutive modes and of 0.92 for method 4 in consecutive mode. For method 4 in coincidental mode, the model estimated a probability of 0.066. For method 7, the model estimated a probability of 0.004, or below, of achieving the 5 log<sub>10</sub> reduction for scenarios 1, 4 and 5, and probabilities of 0.685 and 0.999 of achieving the same level of reduction for scenarios 2 and 3, respectively. For method 4, in coincidental mode, and scenarios 4 and 5 of method 7, the low probabilities are possibly associated with the fact that, when modelling, no extrapolation at temperatures other than those with experimental data available (105°C for spores of *C. perfringens*) was applied while the processing methods involved much higher temperatures (in the range 125–133°C). This would lead to an underestimation of the level of inactivation reached.

An expert knowledge elicitation (EKE) was conducted to elucidate what the probability is that a 5 log<sub>10</sub> reduction of *C. perfringens* spores is achieved, in more than 99% of cases, by application of the relevant processes (methods 2, 3, 4, 5 in coincidental mode and five t/T combinations of method 7) assuming that the processes are performed as prescribed and that the indicated process conditions are achieved (**AQ5**). Based on the EKE, the certainty of achieving a 5 log<sub>10</sub> reduction of *C. perfringens* spores (which also assure the target inactivation for the other relevant pathogens) was: 99–100% certain for methods 2 and 3 in coincidental mode; 98–100% certain for method 7 scenario 3; 80–99% certain for method 5 in coincidental mode; 66–100% certain for method 4 in coincidental mode and for method 7 scenarios 4 and 5; 25–75% certain for method 7 scenario 2; and 0–5% certain for method 7 scenario 1. Compared to the results of the EKE for methods 2–5 in coincidental mode, the same or higher certainty to achieve the 5 log<sub>10</sub> reduction of *C. perfringens* spores is expected when methods 2 to 5 are applied in consecutive mode.

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## 1. Introduction

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

Following the partial revision of the feed ban introduced by Commission Regulation (EU) 2021/1372<sup>1</sup> the European animal by-product processing sector (EFPPRA) asked the Commission to revise standards for the production of processed animal protein (PAP) of porcine origin set out in Point B (1) of Section 1 of Chapter II of Annex X to Regulation (EU) No 142/2011<sup>2</sup>.

PAP is defined in point 5 of Annex I of Regulation (EU) No 142/2011 as Category 3 materials referred to in Article 10 of Regulation (EC) No 1069/2009<sup>3</sup>. PAP must comply with general requirements for the processing and placing on the market set out in Chapter I Annex X of Regulation (EU) No 142/2011. In accordance with specific requirements for processing of PAP set out in Point B (1) of Chapter II of that Annex, PAP of mammalian origin must have been submitted to processing method 1 (pressure sterilisation) as set out in Chapter III of Annex IV to Regulation (EU) No 142/2011.

By way of derogation set out in Point B(1)(b) PAP of mammalian origin may have been submitted to any of the processing methods 1 to 5 or processing method 7, as set out in Chapter III of Annex IV to Regulation (EU) No 142/2011 when disposed of or used as a fuel for combustion or use in petfood.

In accordance with point 1(b) of Section 1 of Chapter II of Annex XI to Regulation (EU) No 142/2011, the above derogation is applicable also to PAP intended for the manufacturing of organic fertilizers and soil improvers (fertilizers).

Before publication of Commission Regulation (EU) 2021/1372, the use of PAP of mammalian origin was limited to the manufacturing of petfood, feed for aquaculture animals (since 2013), and fertilizers or as fuel for combustion, as described above.

After the partial revision of the feed ban, PAP of porcine origin may be also fed to poultry. However, following decades of a quasi-complete feed ban on protein of terrestrial animal's origin, rendering industry produces only PAP intended for petfood, fertilizers or fuel for combustion applying methods 2 to 5 or method 7. According to the information from EFPPRA, currently no EU operator applies method 1 for the processing of PAP of porcine origin.

The Commission would therefore like to explore the efficacy of methods 2 to 5 or method 7 to inactivate relevant pathogens when producing PAP of porcine origin intended to feed poultry and aquaculture animals.

#### Terms of Reference (ToR)

In the light of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002<sup>4</sup>, the Commission requests EFSA to provide a scientific opinion concerning the efficacy of methods 2 to 5 and method 7 to inactivate relevant pathogens when producing processed animal protein (PAP) of porcine origin intended to feed poultry and aquaculture animals.

In particular, the scientific opinion should comprise an assessment of the level of inactivation of relevant pathogens that could be present in processed animal protein of porcine origin intended to feed poultry and aquaculture animals.

### 1.2. Interpretation of the Terms of Reference

Initial clarification was requested to the European Commission on whether the Category 3 material considered by the mandate should be only of EU origin and not sourced from third countries, since the criteria to select relevant pathogens could differ. Subsequently, since the WG decided to work with indicators (see AQ1 of the protocol in Annex A) and the EC agreed with the approach (see

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

<sup>2</sup> 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.

<sup>3</sup> Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. OJ L 170, 25.6.2019, p. 1–114.

<sup>4</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1.

Section 3.2.1), the origin of the Category 3 material was not considered relevant. No further clarification was requested on the source of the materials nor on the criteria for selection of relevant pathogens.

The ToR has been translated into assessment questions (AQ) and sub-questions (SQ), as follows:

AQ1: What relevant pathogens can be used as indicators to assess the efficacy of standard processing methods as in Chapter III of Annex IV to Regulation (EU) No 142/2011, for Category 3 ABP of porcine origin?

AQ2: What are the technical parameters (e.g. time, temperature, pressure, pH, particle size) for methods 2 to 5 and 7 as in Chapter III of Annex IV to Regulation (EU) No 142/2011?

SQ2.1: What are the technical parameters (e.g. time, temperature, pressure, pH, particle size) for methods 2, 3, 4 and 5 as in Chapter III of Annex IV to Regulation (EU) No 142/2011?

SQ2.2: What are the technical parameters for methods 7 approved at national level in the EU?

AQ3: What are the inactivation parameters (D and z) of the relevant pathogens identified in AQ1?

AQ4: What is the 'level of inactivation' of the selected relevant pathogens achieved by methods 2 to 5 and 7?

SQ4.1 What is the 'level of inactivation' achieved by methods 2, 3, 4 and 5?

SQ4.2 What is the 'level of inactivation' achieved for method 7?

AQ5: What is the certainty that the 'level of inactivation' achieved by methods 2, 3, 4, 5 and 7, as in AQ4, is sufficient to reach the standards for Category 3 ABP of porcine origin?

Further processing of porcine PAP before they are included in feed for poultry and aquaculture may contribute to further reduction of relevant pathogens. These subsequent processing steps have not been considered in this assessment.

Any assessments conducted by the competent authorities to approve applications of method 7 have not been considered in this opinion, precluding any conclusion about the validity or appropriateness of such assessments.

### 1.3. Additional information (if appropriate)

#### 1.3.1. Technical parameters (time, temperature, pressure, pH, particle size) of methods 2 to 5 and 7 (AQ2)

Chapter III, Annex IV of Commission Regulation (EU) 142/2011 describes the standard processing methods of ABP, summarised in Figure 1, as follows (A. Processing method 1 (pressure sterilisation) and F. processing method 6 are not described):

#### **B. Processing method 2**

##### *Reduction*

- 1) *If the particle size of the animal by-products to be processed is more than 150 millimetres, the animal by-products must be reduced in size using appropriate equipment, set so that the particle size after reduction is no greater than 150 millimetres. The effectiveness of the equipment must be checked daily and its condition recorded. If checks disclose the existence of particles larger than 150 millimetres, the process must be stopped and repairs made before the process is resumed.*

##### *Time, temperature and pressure*

- 2) *After reduction the animal by-products must be heated in a manner, which ensures that a core temperature greater than 100°C is achieved for at least 125 min, a core temperature greater than 110°C is achieved for at least 120 min and a core temperature greater than 120°C is achieved for at least 50 min. The core temperatures may be achieved consecutively or through a coincidental combination of the time periods indicated.*
- 3) *The processing must be carried out in a batch system.*

### C. Processing method 3

#### Reduction

- 1) *If the particle size of the animal by-products to be processed is more than 30 millimetres, the animal by-products must be reduced in size using appropriate equipment, set so that the particle size after reduction is no greater than 30 millimetres. The effectiveness of the equipment must be checked daily and its condition recorded. If checks disclose the existence of particles larger than 30 millimetres, the process must be stopped and repairs made before the process is resumed.*

#### Time, temperature and pressure

- 2) *After reduction the animal by-products must be heated in a manner, which ensures that a core temperature greater than 100°C is achieved for at least 95 min, a core temperature greater than 110°C is achieved for at least 55 min and a core temperature greater than 120°C is achieved for at least 13 minutes. The core temperatures may be achieved consecutively or through a coincidental combination of the time periods indicated.*
- 3) *The processing may be carried out in batch or continuous systems.*

### D. Processing method 4

#### Reduction

- 1) *If the particle size of the animal by-products to be processed is more than 30 millimetres, the animal by-products must be reduced in size using appropriate equipment, set so that the particle size after reduction is no greater than 30 millimetres. The effectiveness of the equipment must be checked daily and its condition recorded. If checks disclose the existence of particles larger than 30 millimetres, the process must be stopped and repairs made before the process is resumed.*

#### Time, temperature and pressure

- 2) *After reduction the animal by-products must be placed in a vessel with added fat and heated in a manner which ensures that a core temperature greater than 100°C is achieved for at least 16 min, a core temperature greater than 110°C is achieved for at least 13 min, a core temperature greater than 120°C is achieved for at least 8 min and a core temperature greater than 130°C is achieved for at least 3 min. The core temperatures may be achieved consecutively or through a coincidental combination of the time periods indicated.*
- 3) *The processing may be carried out in batch or continuous systems.*

### E. Processing method 5

#### Reduction

- 1) *If the particle size of the animal by-products to be processed is more than 20 millimetres, the animal by-products must be reduced in size using appropriate equipment, set so that the particle size after reduction is no greater than 20 millimetres. The effectiveness of the equipment must be checked daily and its condition recorded. If checks disclose the existence of particles larger than 20 millimetres, the process must be stopped and repairs made before the process is resumed.*

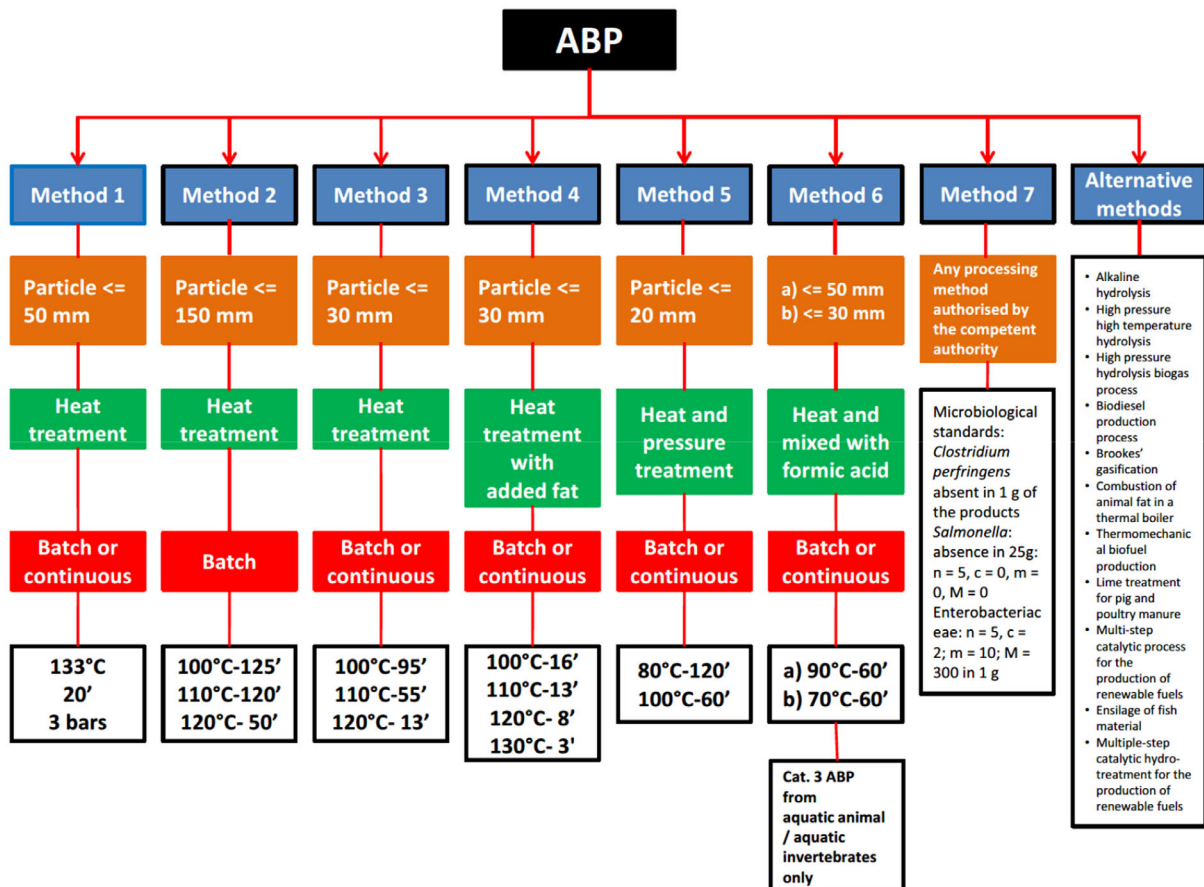
#### Time, temperature and pressure

- 2) *After reduction the animal by-products must be heated until they coagulate and then pressed so that fat and water are removed from the proteinaceous material. The proteinaceous material must then be heated in a manner which ensures that a core temperature greater than 80°C is achieved for at least 120 min and a core temperature greater than 100°C is achieved for at least 60 min.  
The core temperatures may be achieved consecutively or through a coincidental combination of the time periods indicated.*
- 3) *The processing may be carried out in batch or continuous systems.*



## G. Processing method 7

- 1) 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.
- 2) Details of the critical control points under which each processing plant satisfactorily complies with the microbiological standards must be recorded and maintained so that the operator and the competent authority can monitor the operation of the processing plant. The information to be recorded and monitored must include the particle size, and, as appropriate, the critical temperature, the absolute time, pressure profile, raw material feed rate and fat recycling rate.
- 3) By way of derogation from point 1, the competent authority may authorise the use of processing methods which have been approved prior to the date of entry into application of this Regulation, in accordance with Chapter III of Annex V to Regulation (EC) No 1774/2002.
- 4) The competent authority shall permanently or temporarily suspend the application of processing methods referred to in points 1 and 3, if it obtains evidence that any of the circumstances specified in point 1(a) or (b) have substantially changed.
- 5) The competent authority shall inform the competent authority of another Member State upon request about the information at its disposal under points 1 and 2 in relation to an authorised processing method.



**Figure 1:** Summary of the processing methods of ABP according to Commission Regulation (EU) No 142/2011. Source: adapted from the Spanish Renderers Association (ANAGRASA) website: <https://www.anagrasa.org/es/sector/preguntas-frecuentes/index.htm>

### 1.3.2. Authorised method 7 processes

The European Fat Processors and Renderers Association (EFPPA) shared with EFSA a total of 21 process parameters approved in the EU since 2004 for method 7 to support the assessment to answer the ToR of this mandate.

The provided parameters refer to both in-continuous and in-batch processes, with temperatures ranging between 85°C and 133°C and lasting between 2 and 255 min. The particle sizes of the processed materials range between < 20 and 50 mm and the maximum pressure applied is 3.5 bars. The raw materials for which method 7 has been approved include blood, hair and melted fat and originate from different animal species, including pigs. The process parameters were shared with EFSA confidentially and the WG selected five time/temperature scenarios out of them (see Section 2.3.1). The scenarios were selected to include the widest range of temperatures among those reported using starting material of porcine or mixed origin, in continuous and batch modes.

### 1.3.3. Processed animal proteins and feed

Processed Animal Protein (PAP) is defined in Annex I of Commission Regulation (EU) No 142/2011, 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 petfood, 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.*

According to Article 31 of Regulation (EC) No 1069/2009, animal by-products and derived products destined for feeding to farmed animals, excluding fur animals, may only be placed on the market provided:

- a) they are derived from Category 3 material other than:
  - 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 (Article 10 (n) Regulation (EC) No 1069/2009)
  - 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 (Article 10 (o) Regulation (EC) No 1069/2009)
  - catering waste (Article 10 (p) Regulation (EC) No 1069/2009)
- b) they have been collected or processed, as applicable, in accordance with the conditions for pressure sterilisation or other conditions to prevent risks arising to public and animal health in accordance with measures adopted pursuant to Article 15 of Regulation (EC) No 1069/2009 and any measures which have been laid down in accordance with paragraph 2 of Article 15; and
- c) they come from approved or registered establishments or plants, as applicable for the animal by-product or derived product concerned.

According to Section 1.A, Chapter II of Annex X of Commission Regulation (EU) No 142/2011 only animal by-products which are Category 3 material, or products which are derived from such animal by-products, other than the Category 3 materials referred to in Articles 10 n, o, p of Regulation (EC) No 1069/2009, may be used for the production of PAP.

In point 1.B,

- 1) PAP of mammalian origin must have been submitted to processing method 1 (pressure sterilisation) as set out in Chapter III of Annex IV. However,
  - a) porcine blood or fractions of porcine blood for the production of blood meal may have been submitted instead to any of the processing methods 1 to 5 or processing method 7 as set out in Chapter III of Annex IV, provided that in the case of processing method 7, a heat treatment throughout its substance at a temperature of 80°C has been applied;
  - b) processed animal protein of mammalian origin
    - i) may have been submitted to any of the processing methods 1 to 5 or processing method 7, as set out in Chapter III of Annex IV, provided that it is subsequently disposed of or used as a fuel for combustion;
    - ii) where it is exclusively destined for use in petfood, it may have been submitted to any of the processing methods 1 to 5 or processing method 7, as set out in Chapter III of Annex IV, provided that it is
      - transported in dedicated containers that are not used for the transport of animal by-products or feedingstuffs for farmed animals, and
      - consigned directly from a processing plant for Category 3 material to the petfood plant or to an approved storage plant, from where it is directly consigned to a petfood plant
- 2) Non-mammalian processed animal protein, with the exception of fishmeal, must have been submitted to any of processing methods 1 to 5 or processing method 7, as set out in Chapter III of Annex IV.
- 3) Fishmeal must have been submitted to: (a) any of the processing methods set out in Chapter III of Annex IV; or (b) another method which ensures that the product complies with the microbiological standards for derived products set in Chapter I of this Annex.

## 2. Data and methodologies

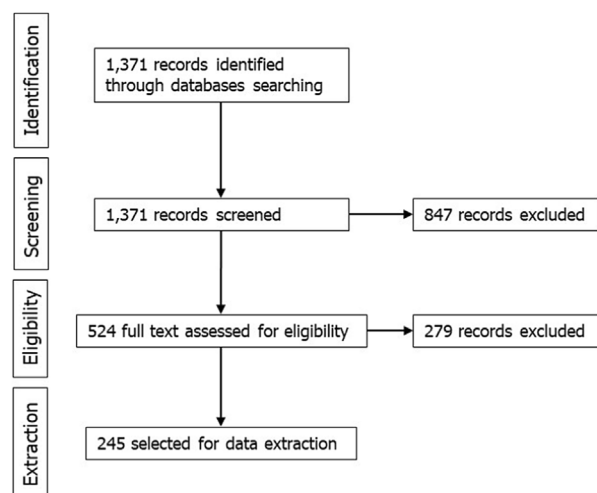
The approach to answer the ToR was defined in advance and is described in the protocol (Annex A). Protocol development followed the draft framework for protocol development for EFSA's scientific

assessments (EFSA, 2020). It covers both the problem formulation (i.e. what the assessment aims to address) and which methods will be used for addressing the problem. The problem formulation ('what') includes the clarification of the mandate (see further refined in Section 1.2) and consists of the steps (1) translation of the mandate into scientifically answerable AQ, (2) definition of the sub-questions (SQ) of each AQ, if needed, and their relationship (conceptual model) and (3) the selection of the approach for the assessment. The planning of the methods for conducting the assessment ('how') consists of (1) specifying the evidence needs and the methods for answering each AQ/SQ, including uncertainty analysis and (2) the methods for integrating evidence across AQ/SQ and addressing the remaining and overall uncertainty.

The methodologies applied for answering some AQ can be fully found in the protocol, while more details are provided below for methods used for other AQ.

## 2.1. Relevant pathogens that can be used as indicators to assess the efficacy of standard processing methods for Category 3 ABP of porcine origin (AQ1)

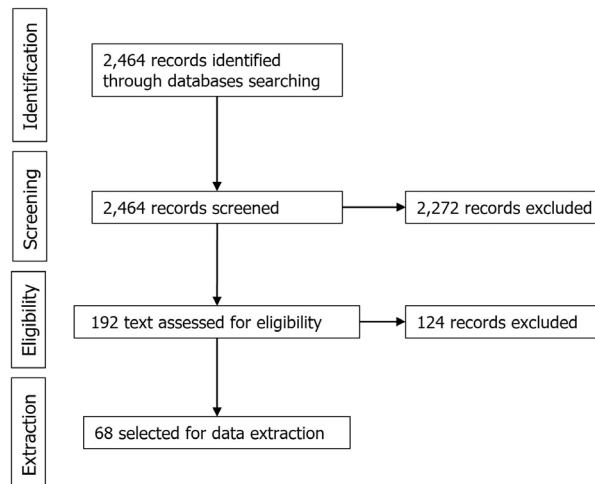
An extensive literature search (ELS) was carried out for the identification of viral hazards, which resulted in the extraction of 1,371 records after applying the search strategy described in the protocol (Figure 2). All of them were screened by title and abstract, with 524 being selected for full text screening, and the other 847 excluded because they did not meet the inclusion criteria. Out of the 524 selected for full text screening, and 245 were selected for data extraction. The other 279 were discarded because they were not in English, no full text was available, or they did not meet the eligibility criteria (see protocol in Annex A). Relevant data from the shortlisted 245 references were extracted in tabular format and are presented in Section 3.1.3.



**Figure 2:** Flow diagram of the literature review for viral hazard identification

## 2.2. Thermal inactivation parameters (D values) of the relevant biological hazards (AQ3)

An extensive literature search (ELS) was also performed for the identification of thermal inactivation parameters of spores of *Clostridium perfringens*, which resulted in the extraction of 2,464 records after applying the search strategy described in the protocol (Figure 3). All were screened by title and abstract, with 192 being selected for full text screening, and the other 2,272 excluded because they did not meet the inclusion criteria. Out of the 192 selected for full text screening, 68 were selected for data extraction. The other 124 were discarded because they were not in English, no full text was available, or they did not meet the eligibility criteria (see protocol in Annex A). Relevant data from the shortlisted 68 references were extracted. Of those, D-values from 18 references were selected for analyses and are described in tabular format in Section 3.2.1.



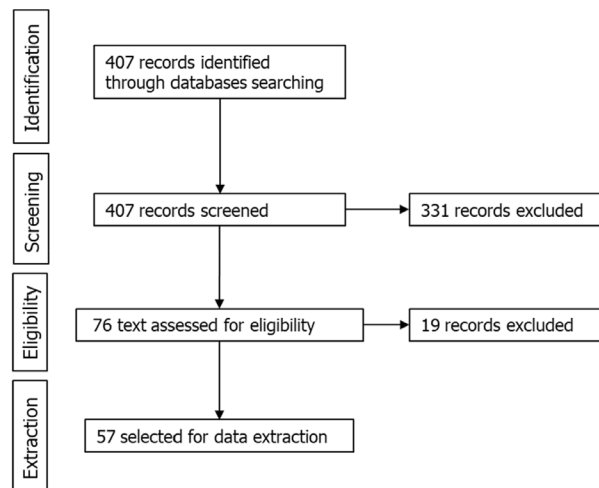
**Figure 3:** Flow diagram of the literature review for inactivation parameters of *Clostridium perfringens*

The search string used for the extensive literature search (ELS) for *C. perfringens* was revised to search for thermal inactivation parameters of the identified relevant viral pathogens (parvoviruses, circoviruses, anelloviruses and adenoviruses), as follows (not included in the protocol):

Title and abstract = [("thermal inactivat\*" OR "heat inactivat\*" OR "thermal reduction" OR "heat reduction" OR "thermal survival" OR "heat survival" OR "kill time" OR "thermal kinetic\*" OR "heat kinetic\*" OR "thermal destruction" OR "heat destruction" OR "thermal process\*" OR "thermal treatment\*" OR "heat treatment\*" OR "thermal resistanc\*" OR "heat resistanc\*" OR "thermal performance\*" OR "heat performance\*" OR "temperature toleranc\*" OR "heat toleranc\*" OR "thermal toleranc\*" OR "time temperature" OR "thermal lethality" OR "heat lethality" OR Bigelow OR "D value\*" OR "z value\*" OR "F value\*" OR "D-value\*" OR "z-value\*" OR "F-value\*" OR "Decimal reduction" OR Sterility OR Pasteuriz\* OR Pasteuris\* OR Steriliz\* OR Sterilis\*)] AND [(Parvovirus OR porcine parvovirus OR swine parvovirus OR bocavirus OR human bocavirus OR porcine bocavirus OR swine bocavirus OR parvoviridae OR parvovirinae OR Amdoparvovirus OR Artiparvovirus OR Aveparvovirus OR Bocaparvovirus OR Copiparvovirus OR Dependoparvovirus OR Erythroparvovirus OR Loriparvovirus OR Protoparvovirus OR Tetraparvovirus OR Circovirus OR porcine circovirus OR swine circovirus OR Cyclovirus OR circoviridae OR Torque teno sus virus OR Torque Teno virus OR torqueteno OR TTSuV OR Adenovirus OR porcine adenovirus OR swine adenovirus OR human adenovirus OR HAdV OR Adenoviridae OR Atadenovirus OR Aviadenovirus OR Ichtadenovirus OR Mastadenovirus OR Siadenovirus OR Testadenovirus)].

The search was conducted in Web of Science Core Collection (Science Citation Index Expanded, Book Citation Index Expanded, Emerging Sources Citation Index, Current Chemical Reactions, Index Chemicus) and CAB abstracts. The outputs were merged and duplicates removed. The search was restricted to journal articles, review papers or book chapters in English, for the period 1900–2023.

As a result, 407 references were screened for title and abstract (Figure 4), 76 being selected for full text screening, and the other 331 excluded because they did not meet the inclusion criteria. Out of the 76 selected for full text screening, 57 were selected for data extraction. The other 19 were discarded because they were not in English, no full text was available or they did not meet the eligibility criteria.



**Figure 4:** Flow diagram of the literature review for heat inactivation parameters of Parvoviridae, Circoviridae, Adenoviridae and Anelloviridae

For data extraction, the papers were screened for evidence of thermal inactivation (D-values, z-values,  $\log_{10}$  reductions or any other measurement) following heat treatment with defined temperature/time combinations. The information retrieved through literature search was complemented with thermal inactivation data available for the same viral families in previous EFSA Opinions (EFSA BIOHAZ Panel, 2021). The data from the different papers were used to extract D-values at different temperatures or, when this was not possible due to data limitations (e.g. less than three available measures), to estimate time to 1  $\log_{10}$  reduction based on the reduction achieved in a single point experiment. The following restrictions were applied to the data collected:

- considering the technical parameters of methods 2 to 5 and five scenarios of method 7 (see Section 1.3.1) and the differences in thermal inactivation of viruses under dry and moist heat conditions (Bräuninger et al., 2000; Sauerbrei and Wutzler, 2009), only studies dealing with moist heat inactivation were included;
- considering the technical parameters of methods 2 to 5 and five scenarios of method 7, only studies dealing with temperatures equal or above 50°C were considered;
- taking into account the evidence on higher thermal susceptibility of Parvovirus B19 compared to other animal parvoviruses (Blumel et al., 2002; Yunoki et al., 2003), data associated to this viral species were not included.

Papers that did not provide thermal inactivation as quantitative values (e.g. reporting of ‘total inactivation’) or in which heat treatment was combined with other inactivating treatments (e.g. chemical disinfection) were also excluded. Relevant data extracted from the shortlisted references are presented in Section 3.2.4.

## 2.3. ‘Level of inactivation’ achieved for methods 2–5 and 7 (AQ4)

### 2.3.1. Calculation of the level of inactivation ( $\log_{10}$ reduction)

Specific holding times at fixed temperatures are specified for methods 2 to 5 in Chapter III, Annex IV of Commission Regulation (EU) No 142/2011 are displayed in Table 1, and for the selected scenarios of method 7 in Table 2. Estimates of the accumulated lethality of the heat treatments were calculated for the target microorganisms: *C. perfringens* spores, *Salmonella* Senftenberg, *Enterococcus faecalis* and the most thermal-resistant virus family, Parvoviridae. The level of inactivation (L) ( $\log_{10}$  reduction) of the heat regimes was computed by Equation (1), derived from the Bigelow model (Bigelow, 1921)

$$L = \frac{\left( t_{\text{treat } 1} \times 10^{\frac{T_{\text{treat } 1} - T_{\text{ref}}}{z}} \right) + \left( t_{\text{treat } 2} \times 10^{\frac{T_{\text{treat } 2} - T_{\text{ref}}}{z}} \right) \dots + \left( t_{\text{treat } n} \times 10^{\frac{T_{\text{treat } n} - T_{\text{ref}}}{z}} \right)}{D_{\text{ref}}}, \quad (1)$$

where  $t_{treat\ 1}/t_{treat\ 2}/t_{treat\ n}$  and  $T_{treat\ 1}/T_{treat\ 2}/T_{treat\ n}$  are the corresponding holding times (min) and temperatures (°C) of the  $n$  heat treatments (see Tables 1 and 2);  $D_{ref}$  (min) is the reference  $D$  value (e.g. time to reach one  $\log_{10}$  reduction) at the reference temperature  $T_{ref}$  (°C); and  $z$  (°C) is the  $z$  value defined as the temperature increment needed for a 10-fold decrease in  $D$ .

Taking into consideration that the steps of the heat regimes can be consecutive or coincidental in the four methods 2 to 5 (Section 3.3.1),  $L$  was estimated in the two modes: using non-overlapping time–temperature profiles to represent the best-case scenarios and using overlapping time–temperature profiles that comply with the heat treatment requisites but would provide the lowest  $\log_{10}$  reduction (worst-case scenarios) (Table 1).

**Table 1:** Time–temperature profiles corresponding to methods 2–5 in consecutive and coincidental modes providing the best- and worst-case scenarios, respectively, in terms of microbial  $\log_{10}$  reduction

	Method 2	Method 3	Method 4	Method 5
<b>Consecutive (best-case scenario)</b>				
Step 1	100°C × 125 min	100°C × 95 min	100°C × 16 min	80°C × 120 min
Step 2	110°C × 120 min	110°C × 55 min	110°C × 13 min	100°C × 60 min
Step 3	120°C × 50 min	120°C × 13 min	120°C × 8 min	–
Step 4	–	–	130°C × 3 min	–
<b>Coincidental (worst-case scenario)</b>				
Step 1	100°C × 5 min	100°C × 40 min	100°C × 3 min	80°C × 60 min
Step 2	110°C × 70 min	110°C × 42 min	110°C × 5 min	100°C × 60 min
Step 3	120°C × 50 min	120°C × 13 min	120°C × 5 min	–
Step 4	–	–	130°C × 3 min	–

In addition, five scenarios of individual time/temperature profiles corresponding to applications of method 7 were assessed (Table 2). These five time–temperature scenarios were selected based on the process parameters approved in different MS, as provided by EFPR. As explained in the model assumptions, the effect of particle size was not taken into account in the models.

**Table 2:** Method 7 scenarios selected for the assessment

	Batch (B) or Continuous (C)	T (°C)	Time (min)
<b>Scenario 1</b>	C	80	14
<b>Scenario 2</b>	C	95	90
<b>Scenario 3</b>	B	115	56
<b>Scenario 4</b>	C	125	10
<b>Scenario 5</b>	B	133	5

The level of inactivation in terms of  $\log_{10}$  reductions attained was determined by performing simulations on Equation (1), using distributions for  $\log_{10}D_{ref}$  (Normal( $\mu_{\log_{10}D_{ref}}, \sigma_{\log_{10}D_{ref}}$ )) and  $z$  (Normal( $\mu_z, \sigma_z$ )), representing uncertainty about the heat resistance parameters of the target microorganism. The parameters  $\mu_{\log_{10}D_{ref}}$  and  $\sigma_{\log_{10}D_{ref}}$  are the mean and standard deviation of the logarithm base 10 of  $D_{ref}$ , respectively, whereas  $\mu_z$  and  $\sigma_z$  are the mean and standard deviation of the  $z$  value, respectively. Such parameters were estimated by fitting the Bigelow model (Bigelow, 1921) of the form shown in Equation (2),

$$\log_{10} D_{j(i)} = \log_{10} D_{ref\ j(i)} - \frac{(T - T_{ref})}{z} + \varepsilon_{j(i)}, \quad (2)$$

to literature data collected for each selected pathogen (see below the source of data and its selection). The subscripts  $j(i)$  denote the  $i$ th growth medium taken from the  $j$ th study; and  $\varepsilon_{j(i)}$  are the normally distributed residuals. Random effects with the growth medium as clustering variable were placed only in the intercept, therefore, affecting  $\log_{10}D_{ref}$  and not  $z$ . Since extrapolation out of the temperature range available in each data set was not applied, the maximum temperature of each data set (i.e. target microorganism × inactivation method) was established as the reference temperature  $T_{ref}$ . By

doing this, the level of inactivation was estimated without recourse to extrapolation (i.e. beyond the maximum temperature available). Since distributions about  $\log_{10}D_{ref}$  and  $z$  were built by extracting the fitted estimates and associated standard errors, these distributions represent the uncertainty about the true heat resistance parameters of the target microorganisms. Simulations were run considering the correlation between  $\log_{10} D_{ref}$  and  $z$ .

For each of the data sets, Bigelow models were fitted using the non-linear mixed effects as in the *nlme* library of the R software (R Core Team, 2021). Monte Carlo simulations with 500,000 iterations of Equation (1) were performed using the add-in tool for Microsoft Excel @RISK 7.6 (© 2018 Palisade Corporation).

### 2.3.2. Model assumptions

The model applied to assess the level of inactivation achieved by methods 2 to 5 and method 7 as stated in Regulation (EU) No 142/2011 did not take into consideration the differences among methods in terms of different maximum particle sizes, real temperatures profiles, differences in physicochemical properties of the substrates or impact of come-up times. It was assumed that the core temperatures of the profiles for each method (either coincidental or consecutive ones) were reached at the coldest point of the pig ABP material being processed. The impact of the come-up times (times of temperature increase or decrease before reaching the target temperature of each of the steps) on the level of inactivation was not considered, as it would mean an increased level of inactivation that cannot be quantified for each case.

It was also assumed that the survivor curves of the different reference microorganisms presented a linear shape. No shoulder or tailing effects were considered, as the  $D$  and  $z$  values were obtained from the original studies identified in the extensive literature search for each microorganism and in a previous scientific opinion (EFSA BIOHAZ Panel, 2021).

It was also assumed that the equipment used could reach the conditions specified in the legislation for all the methods. So, the evaluation performed is reliable as long as the distribution of the temperature in the equipment and the heat transfer/penetration of the matrix are taken into consideration and the  $T/t$  profile considered corresponds to the cold spot, to comply with the requirements of the Regulation (EU) No 142/2011 (i.e. minimum temperature in all material in the unit or in the reactor).

The models have been truncated so that no predictions could be obtained at temperatures higher than the maximum one for which experimental data are available. This led to an underestimation of the level of inactivation reached by some of the methods, and potentially to an underestimation of the certainty that the target  $\log_{10}$  reductions are achieved. Equally, previous assumptions led to the underestimation (not considering the come up) or both under/overestimation (non-linear behaviour) of the level of inactivation. All these assumptions were taken into account during the EKE.

### 2.3.3. Model data

#### **Salmonella Senftenberg**

Data on thermal inactivation of *S. Senftenberg* were obtained from the review made by Doyle and Mazzotta (2000), as in a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2021), including inactivation data for whole eggs, egg yolk, egg whites, raw milk, ground beef, nutrient broth and chocolate. The  $\log_{10}D_{ref}$  and  $z$  distributions to be used in the determination of the level of inactivation ( $L$ ) were obtained from this data set for methods 2, 3, 4 and scenarios of method 7. However, since the thermal treatment of method 5 is applied to defatted proteinaceous material, the  $\log_{10} D_{ref}$  and  $z$  distributions were in that case modelled using only the data for ground beef, egg whites, egg yolk, whole eggs and nutrient broth.

The parameters for  $\log_{10}D_{ref}$  and  $z$  for use in the simulations of level of inactivation by methods 2–4, 7 and method 5 were obtained by adjusting Equation (2) to each of the two data subsets. Level of inactivation distributions were then obtained as described above. Distributions of the level of inactivation were then obtained through simulation solving the Equation (1) for each of the methods (2–5) and the 5 scenarios of method 7 for the temperature profiles shown in Tables 1 and 2.



## ***Enterococcus faecalis***

Data on thermal inactivation of *E. faecalis* were obtained from Ugwuanyi et al. (1999) (digestion waste), Sörqvist (2003) (mixed liquid medium), Aguirre et al. (2009) (whole milk) and Saucier and Plamondon (2011) (ground beef), as in a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2021). For *E. faecalis*, all available thermal inactivation data were used to adjust Equation (2) in order to determine the means and standard errors to model the uncertainty distributions about  $\log_{10}D_{ref}$  and  $z$ . Distributions of the level of inactivation were then obtained as described above.

## **Spores of *Clostridium perfringens***

Data on  $D$  values of *C. perfringens* at different temperatures were obtained from Andersen et al. (2004) (Duncan and Strong medium), Brooks (2013) (fruit juice), Byrne et al. (2006) (pork luncheon roll), Craven (1990) (sodium phosphate buffer), Evelyn and Silva (2015) (beef slurry), Li et al. (2018) (laboratory medium), Ma et al. (2012) (DS medium), Orsburn et al. (2008) (DS medium), Paredes-Sabja et al. (2008) (DS medium), Raju and Sarker (2005) (DS medium), Sarker et al. (2000) (DS medium) and Soni et al. (2022) (beef gravy). Equation (2) was fitted to these data in order to determine the means and standard errors to model the uncertainty distributions about  $\log_{10}D_{ref}$  and  $z$ , to be used in the simulations for methods 2–4 and the 5 scenarios of method 7. For the thermal treatment of method 5, which is applied to defatted proteinaceous material, the  $\log_{10}D_{ref}$  and  $z$  distributions were modelled using the data from pork luncheon roll, beef slurry and beef gravy. Distributions of the level of inactivation were then obtained as described above.

## **Parvoviruses**

Data on thermal inactivation of parvoviruses were obtained from the outputs of the extensive literature search undertaken for the four families of virus selected by the hazard identification. The data from the different papers were used to extract  $D$ -values at different temperatures (see Table B.1 of Appendix B). Available thermal inactivation data were used to adjust Equation (2) in order to determine the means and standard errors to model the uncertainty distributions about  $\log_{10}D_{ref}$  and  $z$ . The same simulation procedure as described above was employed to obtain distributions of the level of inactivation for the heat treatment methods.

### **2.3.4. Use of inactivation parameters of spores of *Clostridium botulinum* and interpretation of extrapolation**

Considering that the model could underestimate for some methods the level of inactivation achieved for spores of *C. perfringens*, as no experimental data were retrieved for this pathogen at temperatures above 105°C, estimations were also made on the level of inactivation that the methods would produce for spores of *C. botulinum*, considering that spores of *C. botulinum* are more heat resistant than spores of *C. perfringens* or any other food-borne pathogenic spore-forming bacteria (Rosnes et al., 2012).

In previous EFSA ABP scientific opinions, it was agreed to accept only scientific evidence demonstrating a sufficient level of inactivation and to disregard results of extrapolation at higher temperatures than the experimental ones. This choice was made based on evidence indicating that extrapolation beyond the experimental range can lead to serious mistakes (Masana and Baranyi, 2000; Peleg, 2021). Furthermore, the risk of extrapolation beyond the experimental limits (EFSA BIOHAZ Panel, 2022) and the difficulties to develop accurate models when experimental bias takes place (Garre et al., 2023) have also been reported recently. As a result, extrapolation of thermal inactivation beyond the conditions (in our case, temperatures) of available experimental data was not considered. Therefore, for some of the methods under assessment, the reductions that can be reached at the actual treatment temperatures of the heat regime may be underestimated.

It is accepted though, that, if the process takes place at a higher temperature than the one published in the scientific literature, the reduction/inactivation achieved would be, at least equal to that demonstrated at the lower temperature.

In previous EFSA assessments of alternative ABP processing methods applied to Category 3 ABP, if the hazard identification considers spore-forming bacteria as relevant biological hazards, the required level of inactivation will be a 5  $\log_{10}$  reduction of spores from these bacteria, with the exception of spores of *C. botulinum* for which a 12  $\log_{10}$  reduction would be required to comply with a treatment

equivalent to 3 min at 121°C ( $F_0 = 3 \text{ min}$ )<sup>5</sup> (Section 3.1.1). At this time/temperature conditions a 5  $\log_{10}$  reduction of other less heat resistant spore forming bacteria, such as *C. perfringens*, would be also met.

There are heat inactivation data available for *C. botulinum* in a wide range of temperatures and substrates, including temperatures up to 140°C (Diao et al., 2014). Therefore, the accumulated lethality expressed in minutes at Tref 121.1°C (0.25 min) of can be estimated at a wider temperature range compared to that of *C. perfringens*, as the z value is considered to be 10°C (Lund and Peck, 2001).

*C. botulinum* could be then considered as a surrogate for the assessment of the level of inactivation achieved for other relevant spore-forming pathogens in Category 3 material, although it needs to be evaluated case by case.

## 2.4. AQ5: What is the certainty that the 'level of inactivation' achieved by methods 2, 3, 4, 5 and 7, as in AQ4, is sufficient to reach the standards for Category 3 ABP of porcine origin?

An expert knowledge elicitation (EKE) was performed to answer AQ5, based on the collected evidence and indicated uncertainties. The EKE question was specified as follows:

- What is the probability that a 5  $\log_{10}$  reduction of spores of *C. perfringens* is achieved, in more than 99% of cases, by application of each of the relevant processes (methods 2, 3, 4, 5 in coincidental mode and five t/T combinations of method 7 (see Table 3), assuming that the processes are performed as prescribed and that the indicated process conditions are achieved?

The EKE addressed the spores of *C. perfringens* only because it was shown it is the most thermal resistant among the four selected indicators (Section 3.3). The processes assessed for methods 2–5 were in coincidental mode, which represent the worst-case scenario. The processes assessed are displayed in Table 3.

**Table 3:** t/T combinations assessed in the EKE exercise

Process	t/T combinations
Method 2 – coincidental	100°C × 5' – 110°C × 70' – 120°C × 50'
Method 3 – coincidental	100°C × 40' – 110°C × 42' – 120°C × 13'
Method 4 – coincidental	100°C × 3' – 110°C × 5' – 120°C × 5' – 130°C × 3'
Method 5 – coincidental	80°C × 60' – 100°C × 60'
Method 7 – scenario 1	80°C × 14'
Method 7 – scenario 2	95°C × 90'
Method 7 – scenario 3	115°C × 56'
Method 7 – scenario 4	125°C × 10'
Method 7 – scenario 5	133°C × 5'

In the EKE question, the phrase 'in more than 99% of cases' refers to the potential variation in the performance of the relevant process/es. As the process/es is/are well defined, this variation was considered to be small. The 'probability' refers to the certainty that the  $\log_{10}$  reduction is achieved if this well-defined process is performed, and not to the certainty that the conditions of the methods are applied.

The EKE consisted of two steps: individual judgements and consensus judgements.

In Step 1, the experts provided individual judgements for each of the nine processes, taking into account the version of the draft opinion at the beginning of the process with the evidence on thermal inactivation of the *C. perfringens* spores (including the data and modelling results), the description of the processes, the integration of the evidence and the uncertainty table (Table 9), as well as the personal expertise and assessment of the uncertainties involved.

In Step 2, during an open session, the experts were asked to consider what a rational impartial observer (RIO) would judge, having considered the evidence, uncertainties, the individual judgements

<sup>5</sup> According to Commission Regulation (EU) 142/2011, Annex XIII, Chapter II, point 3 'canned petfood must be subjected to heat treatment to a minimum  $F_c$  value of 3'.

and having heard the discussion maintained by the experts. The objective was to reach consensus on the probability ranges that were considered to best represent the uncertainty on whether the 5 log<sub>10</sub> reduction of spores of *C. perfringens* is achieved, in more than 99% of cases, by application of each of the relevant processes. Detailed information on the EKE can be found in section 3.4 and Appendix D.

### 3. Assessment

#### 3.1. What relevant pathogens can be used as indicators to assess the efficacy of standard processing methods for Category 3 ABP of porcine origin? (AQ1)

To quantify the inactivation level of each relevant pathogen (bacteria, parasites and viruses) in raw materials submitted to methods 2 to 5 and 7 is very complex because the behaviour of microorganisms throughout processing or transformation methods is difficult to elucidate for every single species and/or strain. Moreover, pathogens are irregularly distributed in the raw materials and usually occur in low prevalence and concentration. Therefore, indicator microorganisms have been used. Indicator microorganisms typically represent the most resistant pathogens within specific categories. The effect of processing or transformation methods can therefore be assessed, as if these most resistant indicator microorganisms are inactivated, then more sensitive biological hazards can also be assumed to be inactivated.

##### 3.1.1. Criteria for selection of relevant bacterial pathogens

###### 3.1.1.1. Previous EFSA standards applied for Category 3 animal by-products

The EFSA scientific opinion on the evaluation of a multi-step catalytic co-processing hydro-treatment for the production of renewable fuels using Category 3 animal fat and used cooking oils (EFSA BIOHAZ Panel, 2022) defined the following standards to be applied for Category 3 material:

*In order to be considered at least equivalent to the processing methods approved in the legislation, the alternative methods for Category 3 ABP should be capable of reducing the concentration of the relevant pathogenic bacteria by at least 5 log<sub>10</sub> and the infectious titre of the relevant viruses by at least 3 log<sub>10</sub> (EFSA BIOHAZ Panel, 2005a). For chemical treatments, a reduction of viable stages of resistant parasites such as eggs of *Ascaris* sp. by at least 99.9% (3 log<sub>10</sub>) shall be required. The determination of the relevant pathogenic bacteria and viruses should be defined by the hazard identification, specific for the material to be treated. If the hazard identification considers spore-forming pathogenic bacteria to be relevant, the required level of inactivation will also be a 5 log<sub>10</sub> reduction of spores from these bacteria, with the exception of spores of *C. botulinum* for which a 12 log<sub>10</sub> reduction would be required, as for processing canned petfood. This is the expected reduction in *C. botulinum* spores after applying 121.1°C for 3 min, the minimum standard of a heat treatment for canned petfood. Given their well-described high level of resistance to thermal and chemical treatments, applicants may choose to directly use spores of pathogenic bacteria as primary indicators without carrying out a full hazard identification exercise.*

*If needed/appropriate, for both spore-forming and non-spore-forming bacteria and viruses, adequately justified alternative non-pathogenic indicator or surrogate organisms with at least the same level of resistance may be used, demonstrating an equivalent level of reduction in the substrate of interest.*

###### 3.1.1.2. Legislation on alternative methods for composting and biogas

Commission Regulation (EU) No 142/2011, in Section 2.1, Chapter III, Annex V describes the alternative transformation parameters for biogas and composting plants.

*The validation of the intended process referred to in point (c) must demonstrate that the process achieves the following overall risk reduction:*

- i) for thermal and chemical processes by:*
  - a reduction of 5 log<sub>10</sub> of *Enterococcus faecalis* or *Salmonella* Senftenberg (775 W, H<sub>2</sub>S negative),*
  - reduction of infectivity titre of thermoresistant viruses such as parvovirus by at least 3 log<sub>10</sub>, whenever they are identified as a relevant hazard; and*

ii) as regards chemical processes also by:

- a reduction of resistant parasites such as eggs of *Ascaris* sp. by at least 99.9% (3 log<sub>10</sub>) of viable stages.

### 3.1.1.3. Indicators for method 7

The three microorganisms for which microbiological standards are listed in legislation for processing method 7, as described in point G.1(i) of Chapter III, Annex IV of Commission Regulation (EU) 142/2011, are: *Salmonella*, *C. perfringens* and *Enterobacteriaceae*.

#### Conclusion

Upon consideration of the three listed criteria, three bacteria were selected to be used as indicators in this assessment: *S. Senftenberg*, *E. faecalis* and spores of *C. perfringens*.

### 3.1.2. Description of the selected relevant bacterial pathogens

#### *Salmonella Senftenberg*

*Salmonellae* are Gram-negative non-spore-forming motile rod bacteria. They are widespread in nature and found in food, soil, water, manure (Winfield and Groisman, 2003) and biological waste streams (Burtscher and Wuertz, 2003). The main reservoir of non-typhoidal *Salmonella* are animals, but they are well adapted to their surroundings and cycle between environmental matrices and living hosts. Certain serovars or strains of *Salmonella enterica* are noted for their high resistance to thermal treatments, relative to other *Salmonella* spp. or Gram-negative bacteria, the most prominent being *S. Senftenberg*, particularly the strain 775 W (Ng et al., 1969). In different model systems, this strain has shown D-values (times needed to reduce the bacterial population at a given temperature by 1 log<sub>10</sub> unit) around 10-fold to 20-fold higher than those of other serovars, such as *Salmonella* Typhimurium or *Salmonella* Enteritidis (Doyle and Mazzotta, 2000). *S. Senftenberg* is often used as an indicator organism to validate thermal treatments (Ng et al., 1969). The implication is that if a particular thermal process achieves a sufficient level of reduction for *S. Senftenberg* 775 W, it will also be effective against all *Salmonellae* and other Gram-negative non-spore-forming bacteria (Doyle and Mazzotta, 2000).

#### *Enterococcus faecalis*

*E. faecalis* is a member of the genus *Enterococcus* and is a Gram-positive non-spore-forming bacterium. It is described as an opportunistic pathogen, which particularly affects immunocompromised populations. *E. faecalis* is found in the gut of healthy humans but only reported in some warm-blooded animals, including dogs, and chickens (Pourcher et al., 1991; Wheeler et al., 2002). *E. faecalis* is identified as a heat-resistant microorganism, resulting in its successful application in process validation (Watcharasukarn et al., 2009). Indeed, *E. faecalis* often serves also as an indicator microorganism to characterise the performance of hygienisation processes (Sahlström, 2003). *E. faecalis* is the indicator organism that is mentioned in point 1 of Section 2 of Chapter III of Annex V of Commission Regulation (EU) No 142/2011. It serves as indicator microorganism for both Gram-positive and Gram-negative non-spore-forming bacteria, given the higher thermal tolerance that Gram-positive cocci generally show as compared with that of other non-spore-forming bacterial species.

#### Spores of *Clostridium perfringens*

*C. perfringens* is a Gram-positive square-ended anaerobic (microaerophilic) bacillus classified in Group III of the Family Bacillaceae (EFSA, 2005a,b). This non-motile member of the clostridia forms oval, central spores rarely seen in culture unless grown in specially formulated media, although the spores are produced readily in the intestine (EFSA, 2005a,b). *C. perfringens* is ubiquitous and widely distributed in soil, dust, vegetation and raw foods. It is part of the normal flora of the intestinal tract of humans and animals. *C. perfringens* was first recognised as being responsible for food poisoning in the 1940s and currently is a leading cause of food-borne illnesses. The symptoms consist of diarrhoea and abdominal cramps and appear 8–24 h following ingestion of large numbers of vegetative cells in temperature-abused protein-based foods. Cells sporulate in the small intestine, producing an enterotoxin. *C. perfringens* strains are classified into five toxicological types (A–E) based on the four major toxins produced (alpha (α), beta (β), epsilon (ε) and iota (ι)). Most of the strains produce α-toxin (lecithinase, phospholipase C). Only *C. perfringens* belonging to types A and C are able to cause human gastroenteritis. *C. perfringens* is one of the microbiological standards listed in the EU

Regulation 142/2011 for method 7 and should be absent in 1 g of product tested directly after the treatment.

### 3.1.3. Criteria for selection of relevant viral pathogens

Three criteria have been applied to select the relevant viral pathogens:

- 1) To be included in the WOAHP list of swine and multiple species diseases  
OR
- 2) to be included in the AHAW Scientific Opinion on the assessment of control measures of the Category A diseases of the Animal Health Law (AHL) (EFSA AHAW Panel, 2022)  
OR
- 3) to be identified in an ELS on virus presence in pigs AND (to be present in the EU OR pose significant risk of introduction into the EU AND being pathogens to humans or animals)

The following viral pathogens were included: foot and mouth disease virus, African swine fever virus, classical swine fever virus, because they are named in the AHL as category A listed diseases and considered in the Scientific Opinion on the assessment of control measures of the Category A diseases of the Animal Health Law (EFSA, 2022); porcine reproductive & respiratory syndrome virus, porcine epidemic diarrhoea virus, Aujeszky's disease virus, swine influenza virus, because they are named in the Annex II of Regulation (EU) 2016/429 or in the WOAHP list of swine diseases or diseases of multiple species (including swine). Senecavirus, as presenting a current threat to the EU due to the presence in the region.

For viral hazard identification, occurrence of the viral pathogens in pigs at slaughtering or in pig tissues or in pig products (i.e. considered fit for human consumption) was used as a proxy for their occurrence in Category 3 ABP. An ELS was conducted considering viruses occurring in pig husbandry in the EU or in third countries; viruses not detected in the EU and with a marginal likelihood of introduction in the EU through pig by-products (e.g. Ebola viruses) were not specifically considered in the search. Viruses were considered relevant if pathogenic to humans (e.g. through the introduction in the food chain) or to animals, including poultry, fish and pigs.

The result of the ELS and screening for the viral hazards is given in Table 4. Studies dealing with seroprevalence, experimental infections or with no detection of viral pathogens were excluded.

**Table 4:** Summary of the data extraction in the literature review for viral hazard identification

Family	Characteristics	Viral hazard	Matrix	References
Adenoviridae	Non-enveloped (linear) dsDNA	Porcine adenovirus (PAV)	Spleen	Kadoi et al. (1997)
			Swab slaughter line (bleeding)	Jones and Muehlhauser (2017a)
			Faeces, liver, muscle, Sausages	Berto et al. (2012)
			Faeces, liver, packaged meat, sausages	Di Bartolo et al. (2012)
			Nasal/faecal swabs pools	Hause et al. (2016a)
Anelloviridae	Non-enveloped (circular) ssDNA	Torque Teno virus (TTSuV)	Liver, serum, lung	de Arruda Leme et al. (2013)
			Liver, pork	Leblanc et al. (2014)
			Slaughterhouse collected fetuses	Martinez-Guino et al. (2010)
			Nasal/faecal swabs pools	Hause et al. (2016a)
		Torque Teno virus 1 (TTSuV1)	Bile, fresh pork liver sausages	Monini et al. (2016)
			Kidney	Ghosh et al. (2018)
			Liver	Da Silva et al. (2020)
			Blood	Luka et al. (2016)
			Lymph nodes	Huang et al. (2013)
Torque Teno virus 2 (TTSuV2)	Liver	Da Silva et al. (2020)		
Arteriviridae	Enveloped ssRNA+	Porcine reproductive and respiratory syndrome virus (PRRS)	Serum	Almeida et al. (2018)
			Meat sample from carcass, serum	Magar and Larochelle (2004)
			Tonsils	O'Sullivan et al. (2011)
			Lung	Hillen et al. (2014)
			Cranioventral lobe, dorsocaudal lobe, heart, kidney, liver, lymph nodes, small intestine, spleen, testis, tonsils	Ho et al. (1999)
			Nasal/faecal swabs pools	Hause et al. (2016a)
			Spray-dried porcine plasma	Blázquez et al. (2022)
Asfarviridae	Enveloped (linear) dsDNA	African Swine Fever (ASF)	Serum	Gallardo et al. (2011)
			Tissue, blood	Cho et al. (2022)
			Tissue	Abworo et al. (2017)
			Blood	Thomas et al. (2016)
			Tissue	Owolodun et al. (2010)
			Blood	Adedeji et al. (2022)

Family	Characteristics	Viral hazard	Matrix	References
			Liver, lymph nodes, spleen	Owolodun et al. (2007)
			Blood	Ebwanga et al. (2022)
			Blood	Luka et al. (2016)
			Not reported	Kong et al. (2021)
Astroviridae	Non-enveloped ssRNA+	Astrovirus (AstV)	Faeces	Machnowska et al. (2014)
		Porcine astrovirus	Faeces	Luo et al. (2011)
Caliciviridae	Non-enveloped ssRNA+	Porcine enteric calicivirus (PEC)	Faeces	Halaihel et al. (2010)
			Retail pork	Jones and Muehlhauser (2017b)
		Calicivirus	Nasal/faecal swabs pools	Hause et al. (2016a)
		European swine norovirus (NOV)	Faeces	Machnowska et al. (2014)
Circoviridae	Non-enveloped (circular) ssDNA	Porcine circovirus	Faeces	Laconi et al. (2020)
			Nasal/faecal swabs pools	Hause et al. (2016a)
		Porcine circovirus Type 1 (PCV1)	Serum	Quintana et al. (2001)
			Not reported	Csagola et al. (2008)
			Kidney, lung, lymph nodes, spleen	Hu et al. (2022)
			Liver	Da Silva et al. (2020)
		Porcine circovirus Type 2 (PCV2)	Tonsils	Saekhow et al. (2015)
			Not reported	Csagola et al. (2008)
			Lymph nodes	Ojok et al. (2013)
			Tissue	Jia et al. (2022)
			Lymph nodes	Laisse et al. (2018)
			Kidney, lung, lymph nodes, spleen	Hu et al. (2022)
			Kidney	Ghosh et al. (2018)
Faeces, kidney	Kleymann et al. (2020)			
Liver	Da Silva et al. (2020)			
Tonsils	O'Sullivan et al. (2011)			
Lung	Hillen et al. (2014)			
Lung	Yue et al. (2022)			
Plasma	Blazquez et al. (2019)			
Spray-dried porcine plasma	Blázquez et al. (2022)			

Family	Characteristics	Viral hazard	Matrix	References
		Porcine circovirus Type 2a (PCV2a)	Plasma	Shen et al. (2011)
		Porcine circovirus Type 2b (PCV2b)	Plasma	Shen et al. (2011)
		Porcine circovirus Type 3 (PCV3)	Tissue	Jia et al. (2022)
			Kidney, lymph nodes	Hu et al. (2022)
			Lung	Wen et al. (2018)
			Lymph nodes	Liu et al. (2019)
		Lung	Yue et al. (2022)	
Lung	Yue et al. (2022)			
Coronaviridae	Enveloped ssRNA+	Porcine epidemic diarrhoea virus (PEDV)	Swabs from lower floor of truck after animal unloading at slaughterhouse	Boniotti et al. (2018)
			Nasal/faecal swabs pools	Hause et al. (2016a)
			Spray-dried porcine plasma	Blázquez et al. (2022)
		Porcine hemagglutinating encephalomyelitis virus	Nasal/faecal swabs pools	Hause et al. (2016a)
Porcine deltacoronavirus (PDCoV)	Nasal/faecal swabs pools	Hause et al. (2016a)		
Flaviviridae	Enveloped ssRNA+	Classical swine fever (CSF)	Tissue	Sarma and Meshram (2008)
			Spleen	Rout and Saikumar (2016)
			Tissue	Rout et al. (2015)
			Tissue	Sarma et al. (2008)
			Intestine, kidney, lymph nodes, spleen, tonsils	Sarma et al. (2007)
		Atypical porcine pestivirus	Nasal/faecal swabs pools	Hause et al. (2016a)
Hepadnaviridae	Enveloped partially-dsDNA	Hepatitis B virus (HBV)	Bile, liver	Vieira et al. (2014)
Hepeviridae	Quasi-enveloped* (non-enveloped) ssRNA+	Hepatitis E virus (HEV)	Liver	Jori et al. (2016)
			Faeces	Jones and Johns (2012)
			Liver	Muller et al. (2017)
			Liver	Bouquet et al. (2011)
			Swab slaughter line	Lainšček et al. (2017)
			Faeces	Machnowska et al. (2014)
			Liver	Gutierrez-Vergara et al. (2015)
			Faeces	Lainšček et al. (2017)
			Blood, kidney, liver	Geng et al. (2019)
			Faeces	Di Martino et al. (2010)



Family	Characteristics	Viral hazard	Matrix	References
			Faeces	Cappai et al. (2018)
			Bile	Wang et al. (2015)
			Liver	Vonlanthen-Specker et al. (2021)
			Pork liver surface	Dzierzon et al. (2022)
			Liver	Traore et al. (2015)
			Liver	de Paula et al. (2013)
			Bile, liver	Gardinali et al. (2012)
			Blood	Sooryanarain et al. (2020)
			Bile, faeces, liver pork, meat	Intharasongkroh et al. (2017)
			Faeces liver sausages	Berto et al. (2012)
			Faeces, liver, packaged meat, sausages	Di Bartolo et al. (2012)
			Bile	dos Santos et al. (2011)
			Bile	Mughini-Gras et al. (2017)
			Faeces, liver	Chambaro et al. (2021)
			Bile	Amorim et al. (2018)
			Bile, bladder, faeces, liver, lymph nodes, plasma, tonsils	Leblanc et al. (2010)
			Diaphragm, faeces, liver	Chelli et al. (2021)
			Not reported, serum	de Souza et al. (2012)
			Blood, rectum swabs	Khounvisith et al. (2018)
			Liver	Feurer et al. (2018)
			Caecum content, liver, serum	Boxman et al. (2022)
			Liver	Temmam et al. (2013)
			Liver	Rose et al. (2011)
			Liver	Motoya et al. (2019)
			Bile, liver	Casas et al. (2011)
			Liver	Pellerin et al. (2022)
			Bile	Zhang et al. (2018)
			Faeces, liver	Forero et al. (2017)
			Blood, liver	Bigoraj et al. (2021)
			Blood	Boxman et al. (2017)
			Faeces, plasma	Leblanc et al. (2007)
			Liver	Wang et al. (2023)

Family	Characteristics	Viral hazard	Matrix	References
			Liver, swab slaughter line	Milojević et al. (2019)
			Pork liver surface	Li et al. (2011)
Herpesviridae	Enveloped (linear) dsDNA	Porcine cytomegalovirus (PCMV)	Lung	Tajima and Kawamura (1998)
Orthomyxoviridae	Enveloped segmented ssRNA-	Influenza A (H1N1, pandemic strain 2009)	Lung	Paladino et al. (2017)
			Nasal swab	Perera et al. (2013)
			Blood	Nokireki et al. (2013)
			Nasal swabs	Chen et al. (2021)
			Lung	Olaniyi et al. (2020)
			Nasal swabs	Rose et al. (2013)
			Nasal swabs	Takemae et al. (2017)
			Nasal swabs	Baudon et al. (2018)
			Serum/nasal swab	Baudon et al. (2015)
			Nasal swabs/tracheal swab	Cheung et al. (2022)
		H7N2 influenza virus	Lung	Kwon et al. (2011)
		H1N2 influenza virus	Nasal swabs	Qiao et al. (2014)
		Influenza virus	Nasal swabs	Osoro et al. (2019)
			Nasal/faecal swabs pools	Hause et al. (2016a)
			Lung	De Conti et al. (2021)
			Tracheal swab	Meseko et al. (2018)
			Tracheal swab	Amorim et al. (2013)
			Nasal swabs	Ducatez et al. (2015)
		Swine influenza virus (SIV)	Lung	Kwon et al. (2011)
			Lung	Florez et al. (2018)
Lung	Hillen et al. (2014)			
Nasal swabs	Papatsiros et al. (2015)			
Nasal swabs	Oladipo et al. (2013)			
Nasal swabs	Baudon et al. (2018)			
Spray-dried porcine plasma	Blázquez et al. (2022)			

Family	Characteristics	Viral hazard	Matrix	References
Paramyxoviridae	Enveloped ssRNA-	Porcine parainfluenza virus type 1 (PPIV-1)	Assorted tissue, bronchoalveolar lavage, lung, nasal swabs, nasal turbinate, oral fluid, respiratory swab	Park et al. (2019)
Parvoviridae	Non-enveloped (linear) ssDNA	Bocavirus	Faeces, lymph nodes, nasopharyngeal swab, serum	Lau et al. (2011)
			Inguinal lymph node, spleen, submandibular lymph node, tonsils	Liu et al. (2014)
			Liver	Da Silva et al. (2020)
			Nasal/faecal swabs pools	Hause et al. (2016a)
		Porcine boca-like virus (PBo-likeV)	Tonsils	Saekhow et al. (2015)
		Hokovirus	Nasal/faecal swabs pools	Hause et al. (2016a)
		Porcine parvovirus	Heart	Streck et al. (2013)
			Tonsils	Streck et al. (2013)
			Tonsils	Saekhow et al. (2015)
			Fetuses at abattoir	Mengeling et al. (1991)
			Follicular fluid	Pogranichniy et al. (2008)
			Kidney	Ghosh et al. (2018)
			Liver	Da Silva et al., 2020
			Nasal/faecal swabs pools	
		Spray-dried porcine plasma	Blázquez et al. (2022)	
		Porcine parvovirus 1 (PPV1)	Blood, lung	Thuy et al. (2021)
Porcine parvovirus 2 (PPV2)	Heart	Streck et al. (2013)		
	Tonsils	Streck et al. (2013)		
	Tonsils	Saekhow et al. (2015)		
	Blood	Thuy et al. (2021)		
	Lung	Thuy et al. (2021)		
Porcine parvovirus 3 (PPV3)	Tonsils	Streck et al. (2013)		
	Tonsils	Saekhow et al. (2015)		
	Blood	Thuy et al. (2021)		
	Lung	Thuy et al. (2021)		
Porcine parvovirus 4 (PPV4)	Tonsils	Streck et al. (2013)		
	Tonsils	Saekhow et al. (2015)		
	Lung	Thuy et al. (2021)		
Porcine parvovirus 7 (PPV7)	Lung	Chen et al. (2018)		

Family	Characteristics	Viral hazard	Matrix	References
Picobirnaviridae	Non-enveloped segmented dsRNA	Picobirnavirus	Nasal/faecal swabs pools	Hause et al. (2016a)
Picornaviridae	Non-enveloped ssRNA+	Encephalomyocarditis virus (EMCV)	Faeces	Machnowska et al. (2014)
		Enterovirus	Nasal/faecal swabs pools	Hause et al. (2016a)
		Foot-and-mouth-disease virus (FMDV)	Heart	Sharmila and Sherikar (2005)
		Kobuvirus	Nasal/faecal swabs pools	Hause et al. (2016a)
		Parecho-like virus	Nasal/faecal swabs pools	Hause et al. (2016a)
		Pasivirus	Nasal/faecal swabs pools	Hause et al. (2016a)
		Sapelovirus	Nasal/faecal swabs pools	Hause et al. (2016a)
			Intestinal content	Swati et al. (2020)
		Senecavirus A	Nasal/faecal swabs pools	Hause et al. (2016b)
			Nasal/faecal swabs pools	Hause et al. (2016b)
	Senecavirus A SVA-GD5-2018, SVA-GDSZ-2018	Lymph nodes	Jiang et al. (2021)	
unclassified Picornavirales		Posavirus	Nasal/faecal swabs pools	Hause et al. (2016a)
			Intestinal content	John et al. (2022)
			Swab slaughter line (bleeding)	Jones and Muehlhauser (2017a)
Reoviridae	Non-enveloped segmented dsRNA	Porcine rotavirus (RV)	Swab slaughter line (bleeding)	Jones and Muehlhauser (2017b)
		Rotavirus group A (GARV)	Faeces	Machnowska et al. (2014)
		Rotaviruses	Faeces	Halaihel et al. (2010)
Tobaniviridae	Enveloped ssRNA+	Torovirus	Nasal/faecal swabs pools	Hause et al. (2016a)

\*: 'Quasi-enveloped' viruses (Feng et al., 2014) are viruses historically considered as non-enveloped due to the features displayed at excretion and in the environment that, however, circulate in the bloodstream of an infected subject in a membrane-cloaked form. This attribute has been described, among others, in Hepatitis E virus (Takahashi et al., 2010).

Resistance to thermal and chemical processes is generally accentuated in non-enveloped viruses, rather than in enveloped ones (McDonnell, 2020). Therefore, the identification of relevant viral hazards focused on families displaying a viral and genome structure associated with higher thermal resistance (i.e. absence of envelope, DNA genome).

The following non-enveloped DNA viruses were therefore identified as relevant hazards:

- porcine adenovirus (Adenoviridae,
- Torque Teno virus (Anelloviridae,
- porcine circovirus (Circoviridae,
- bocavirus (Parvoviridae,
- porcine parvovirus (Parvoviridae.

### 3.1.4. Description of the pre-selected relevant viral pathogens

#### **Family Adenoviridae**

*Porcine adenovirus* (PAcV) is a species of the *Mastadenovirus* genus within the Adenoviridae family, with a double-stranded DNA genome of ~ 31–34 kbp. The virion displays an icosahedral symmetry with protruding fibres and a diameter of ~ 80 nm. Five serotypes divided in three species (*Porcine mastadenovirus A* with serotypes 1 to 3, *Porcine mastadenovirus B* with serotype 4 and *Porcine mastadenovirus C* with serotype 5) have been described to date (Benkő et al., 2022).

PAcV is considered a low-grade porcine pathogen, mostly associated with subclinical/mild and transitory infections and with limited impact on swine production. Clinical manifestations, where present, include gastrointestinal symptoms (diarrhoea, dehydration, etc.), particularly in association with PAcV-A or respiratory disease, encephalitis and reproductive disorders, including abortion in sows (Berto et al., 2012; Di Bartolo et al., 2012; Benfield and Hesse, 2019). While a possible role of PAcV in coinfections with other severe porcine pathogens has been hypothesized, some serotypes of PAcV have been significantly exploited for the development of recombinant vaccines towards economically relevant swine diseases (Tuboly and Nagy, 2001). The occurrence of the virus in pigs at the slaughtering time is variable, with a detection rate close to 100% in faeces and slaughtering environments and ranging from 1% to 40% in different pig tissues/organs (Berto et al., 2012; Di Bartolo et al., 2012).

#### **Family Anelloviridae**

The family Anelloviridae includes heterogeneous single-stranded circular DNA virus (2–3.9 kb), grouped in genera named following the letters of the Greek alphabet (*Alphatorquevirus*, *Betatorquevirus*, etc.). Anelloviruses were first described in 1997 (Nishizawa et al., 1997) and, based on genome structure and morphological similarity, were previously classified in the family Circoviridae. Two species of porcine anelloviruses have been reported, torque teno sus virus 1 (TTSuV1, with two genotypes) and torque teno sus virus 2 (TTSuV2, with three genotypes), which display low nucleotide identity (Cortey et al., 2011). TTSuV transmission is mainly faecal-oral, though vertical transmission has also been demonstrated. The infection by TTSuV is usually subclinical in swine and their pathogenic potential is still unclear, but a role in the development and the outcome of some diseases such as post-weaning multisystemic wasting syndrome has been hypothesized (Kekarainen and Segales, 2012). The detection rate of TTSuVs in swine populations is usually high, ranging from 17% in blood and lymph nodes to almost 100% in liver (Huang et al., 2013; de Arruda Leme, 2013; Leblanc et al., 2014).

#### **Family Circoviridae**

*Porcine circoviruses* (PCV) (genus *Circovirus*) are single-stranded circular DNA viruses with genomes of ~ 1.7–2.0 kb and a virion of 20–25 nm in diameter. Four circovirus genotypes have been detected in pigs: porcine circovirus 1 (PCV1), considered non-pathogenic; porcine circovirus 2 (PCV2, divided in four subtypes PCV2a, PCV2b, PCV2c, PCV2d), the predominant pathogen, responsible for the different syndromes collectively described as porcine circovirus-associated diseases (PCVADs); *porcine circovirus 3* (PCV3), described in 2015 and associated to porcine dermatitis and nephropathy syndrome (PDNS); and *porcine circovirus 4* (PCV4), discovered in 2019 and also associated to porcine dermatitis and nephropathy syndrome (PDNS). The PCVADs induced by PCV2 occur frequently in weaning piglets, leading to a progressive loss of weight and of body condition, with a pathogenicity mechanism not completely understood yet. The detection of PCV in pigs at slaughtering is commonly reported, with virus detection in plasma, lymph nodes, kidneys, spleen, lung and liver (Shen et al., 2011; Hu et al., 2022; Kleyman et al., 2020; Da Silva et al., 2020).

## Family Parvoviridae

*Porcine parvovirus* (genus *Protoparvovirus*) and bocavirus (genus *Bocaparvovirus*) are members of the family Parvoviridae, subfamily Parvovirinae, that includes the species infecting vertebrates. Parvoviridae are small (25–30 nm), resistant, non-enveloped viruses with linear, single-stranded DNA genomes of 4–6 kb. *P. parvovirus* (currently designated as ungulate protoparvovirus 1, PPV1) is the causative agent of the SMEDI syndrome (stillbirths, mummification, embryonic death and infertility), a highly impacting disease in pig husbandry. Infection of seronegative gilts or sows during pregnancy leads – depending on the stage of gestation – to the typical manifestations of the disease. In the last decade, several new porcine parvoviruses have been recognised (Cadar et al., 2013) and provisionally named *Porcine parvovirus 2 to 6* (PPV2, PPV3, etc.). Although the occurrence of the disease has significantly reduced due to vaccination, porcine parvoviruses are geographically ubiquitous, and they are frequently detected in slaughtered pigs (e.g. depending on the type of porcine parvovirus, 7–78% in tonsils, 55–60% in heart tissue, 8–68% in lungs, 34% in blood (Streck et al., 2013; Thuy et al., 2021)). *Porcine bocavirus* (PboV, reviewed in Aryal and Liu, 2021) was discovered in 2009 in Sweden in pigs suffering from post-weaning multisystemic wasting syndrome (PMWS) (Blomström et al., 2010). Six groups are described (PboV1 to PboV6V7V) based on VP1 sequence. Due to the detection of PboV in co-infection with other viruses, the pathogenesis of these viruses has not been determined yet, though its occurrence has been reported in several tissues, including lymph nodes, spleen, liver, as well as in faeces (Liu et al., 2014; Da Silva et al., 2020).

### 3.1.5. Criteria for selection of relevant parasitic pathogens

Two criteria have been applied to select the relevant parasitic pathogens:

To be included in the WOAHP list of swine and multiple species diseases OR to be included in the AHAW Scientific Opinion on the assessment of control measures of the Category A diseases of the Animal Health Law (EFSA, 2022).

Two parasites fulfil these criteria: *Taenia solium* and *Trichinella* spp.

### 3.1.6. Description of the selected relevant parasitic pathogens

#### ***Trichinella* spp.**

*Trichinella* constitutes a genus of worldwide distributed parasitic nematodes (roundworms). Most of the 13 genotypes described to date are zoonotic, with all of them being able to infect humans and domestic pigs (Cristóforo-Jorquera and Landaeta-Aqueveque, 2022). Other domestic and wild animals such as wild boars, horses, bears, rodents or foxes can also be reservoirs. Two life cycles have been described: the domestic cycle involves pigs and rodents as hosts, the sylvatic cycle includes wild mammals, birds and reptiles. Humans are infected by consumption of raw or undercooked contaminated meat, especially game meat and pork. In Europe, pigs, horses and wild boars are the main sources of infection. The transmission between non-human animals occurs by predation or carrion consumption. The infective larvae are located within the muscle cells and, after consumption, are liberated in the stomach to later become adult worms in the small intestine. Following mating, new larvae progenies move to the muscle. Depending on the number of infective larvae ingested, symptomatology ranges from mild (fatigue, weakness, fever, diarrhoea, muscle pains) to severe (heart or breathing problems and, rarely, death) (Franssen et al., 2021; Gabriel et al., 2023). Symptoms appear from 1 to 2 days after consumption until 2–8 weeks after treatment (European Centre for disease control (ECDC<sup>6</sup>)). While the incidence of trichinosis from pig consumption has decreased lately, that coming from game meat has simultaneously increased (Cristóforo-Jorquera and Landaeta-Aqueveque, 2022). Cooking, freezing and irradiation are the three admitted processing methods for *Trichinella* inactivation before human consumption (Franssen et al., 2021). Freezing at –21°C for 7 days is recommended by the International Commission on Trichinellosis (ICT) for complete inactivation of *T. spiralis* in pork (Franssen et al., 2019). The same combination of time and temperature combination achieves inactivation of muscle larvae of *T. spiralis* encapsulated in wild boar meat for 24 weeks (Lacour et al., 2013). However certain *Trichinella* species occurring in game and horses are resistant when freezing is carried out using the recommended temperature and time combinations, as

<sup>6</sup> ECDC, online. Trichinellosis. Available online: <https://www.ecdc.europa.eu/en/trichinellosis>

stated in Commission Regulation (EC) No 2075/2005, as amended.<sup>7</sup> A heat-inactivation model proposed cooking at 60°C for 10 min for *Trichinella* muscle larvae inactivation (Franssen et al., 2019, 2021). Prevention includes mandatory inspection of all slaughtered pigs and horses in the EU.

### **Taenia solium**

*T. solium* is a pork tapeworm found in pigs, as the intermediate host, and humans as the sole definitive host. Pigs are infected by ingesting tapeworm eggs from faeces, which subsequently develop in the muscles, eyes and the central nervous system. Human infection occurs by eating raw or undercooked infected pork containing viable tapeworm larvae (cysticerci). In its adult form *T. solium* causes taeniasis, whose symptomatology is usually absent or mild including abdominal pain, nausea, diarrhoea or constipation (WHO,<sup>8</sup> Gabriel et al., 2023). Additionally, ingestion of tapeworm eggs (via the faecal-oral route or contaminated food or water) may lead to infection with the larval parasite in the tissues (cysticercosis). Human cysticercosis affects muscles, skin, eyes and central nervous system. When cysts develop in the brain the disease is referred as to neurocysticercosis, able to cause seizures, convulsions, blindness and even death. *T. solium* is endemic in large parts of Asia, Latin America and sub-Saharan Africa (Jansen et al., 2021). Its control includes proper hygiene, preventive chemotherapy (Haby et al., 2020), treatment of pigs and meat inspection (Carabin and Traore, 2014). For elimination, *T. solium* cysticerci in pork are inactivated by freezing at –24 to –5°C for 1–4 days or cooking of pork meat at 60°C for a minimum period of time (Franssen et al., 2019).

The collection of the thermal resistance parameters for parasites was excluded 'a priori' since it is well known that the thermal resistance of parasites is lower than that of the bacterial and viral indicators selected.

## **3.2. What are the thermal inactivation parameters (D and z) of the biological hazards identified in AQ1? (AQ3)**

### **3.2.1. Spores of *Clostridium perfringens***

An extensive literature search (ELS) was performed to collect data on the thermal inactivation of spores of *C. perfringens* in different substrates. The search revealed a range of D and z parameters for this bacterium in different conditions. A total of 91 D-values were collected in the temperature range from 85 to 105°C. The information is summarised in Table A.1 of Appendix A.

### **3.2.2. *Salmonella* Senftenberg**

Data on thermal inactivation of *S. Senftenberg* were extracted from the review of studies on the thermal resistance of salmonellae by Doyle and Mazzotta (2000), collated in a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2021). The D values retrieved provide evidence that the physico-chemical characteristics of the ABP or the raw materials used in the thermal processes under assessment will impact on the levels of reduction achieved. Details of the data points used are displayed in table A2 of appendix A of the previous EFSA opinion (EFSA BIOHAZ Panel, 2021).

### **3.2.3. *Enterococcus faecalis***

Data on thermal inactivation (D-values) of *E. faecalis*, collated in a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2021), were used to estimate the times needed to inactivate 5 log<sub>10</sub> units as a function of the treatment temperature. Details of the data points used are displayed in table A1 of appendix A of the former EFSA opinion (EFSA BIOHAZ Panel, 2021).

### **3.2.4. Viruses**

A total of 11 D-values or point estimates of the time needed to achieve 1 log reduction were retrieved through literature search for Adenoviridae (Shirasaki et al., 2020; Peter and Kühnel, 2020; Maheswari et al., 2004; Tuladhar et al., 2012; Walker et al., 1982), 4 for Anelloviridae (Welch et al., 2006), 8 for Circoviridae (O'Dea et al., 2008; Welch et al., 2006) and 26 for Parvoviridae (Spillmann et al., 1987; Brauniger et al., 2000; Blumel et al., 2002; Paluszak et al., 2010; Elving et al., 2014; Huangfu et al., 2017;

<sup>7</sup> Commission Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat (Text with EEA relevance). OJ L 338, 22.12.2005, p. 60., as amended.

<sup>8</sup> <https://www.who.int/news-room/fact-sheets/detail/taeniasis-cysticercosis>

Gröner et al., 2018; Gemmell et al., 2021). Further 19 values (Lelie et al., 1987; Lund et al., 1996; Yunoki et al., 2003; Sahlström et al., 2008; Nims and Plavsic, 2013; Nims and Zhou, 2016) were retrieved for Parvoviridae from a previous EFSA Opinion (EFSA BIOHAZ Panel, 2021).

The comparison of data points obtained for the four viral families, showed a higher thermal susceptibility of Adenoviridae while, based on the few values available for Anelloviridae and Circoviridae, their heat resistance was comparable or slightly lower than that of Parvoviridae, confirming the high heat resistance of this latter viral family (Nims and Plavsic, 2013). Parvoviruses were therefore selected as the most thermal resistant viral hazards for the assessment.

The information related to the thermal resistance of members of the family Parvoviridae retrieved through the extensive literature search, including the matrices and the conditions applied in the different inactivation studies, is summarised in Table B.1 of Appendix B. Time required for 3 log<sub>10</sub> reduction of the four families of viruses at different temperatures are displayed in Figure B.1 of Appendix B.

### 3.3. What is the 'level of inactivation' achieved for methods 2–5 and 7? (AQ4)

#### 3.3.1. Methods 2–5 and 7

Table 5 compiles the heat resistance parameters (log<sub>10</sub>D<sub>ref</sub> and z) and their standard errors, which were obtained by fitting the Bigelow equation to the data retrieved for the target pathogens. According to these meta-analytical data, *C. perfringens* spores presented greater heterogeneity in the relationship between log<sub>10</sub>D<sub>ref</sub> and temperature (Figure 5) than *S. Senftenberg* (Figure 6), *E. faecalis* (Figure 7) and parvoviruses (Figure 8).

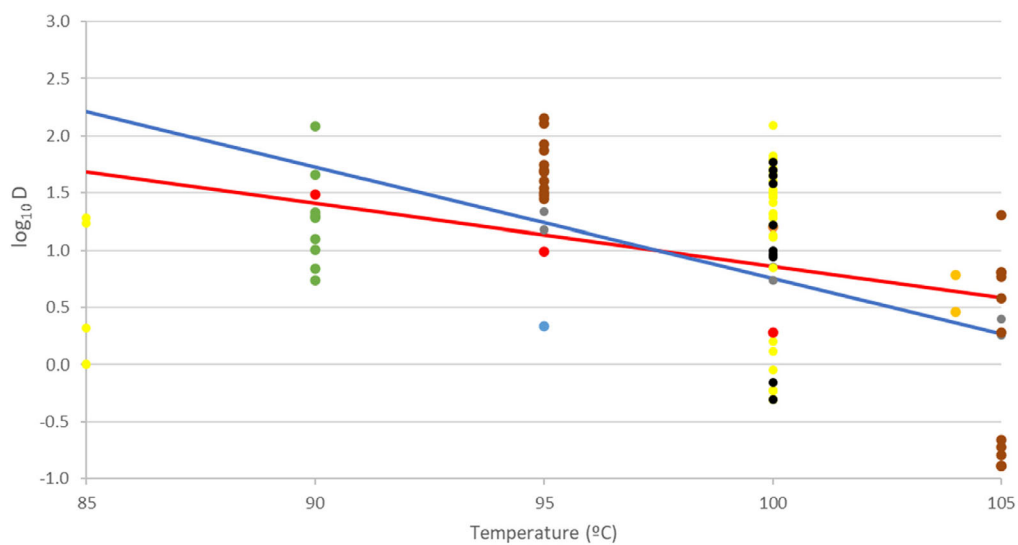
These graphs also show the fitted lines corresponding to the Bigelow model. In the cases of *C. perfringens* spores and *S. Senftenberg*, two model solutions were achieved and are presented in the graph: the red line for use in simulations pertaining to methods 2–4 and 7, and the blue line for the simulations pertaining to method 5. The red line represents a solution for data from studies reporting the highest thermal resistance in combination with matrices of a composition resembling that of processed animal protein. The blue line is a solution for matrices that are low in fat and high in protein. For both microorganisms, spores of *C. perfringens* and *S. Senftenberg*, the models combining matrices low in fat and high in protein provided lower log<sub>10</sub>D<sub>ref</sub> and z estimates. This means that in proteinaceous matrices, both microorganisms are less resistant to heat. For both *E. faecalis* and parvoviruses only one Bigelow model solution was attained since the low number and the nature of the matrices recovered did not make such an assessment possible. For easier comparison of the thermal resistance between the target pathogens, the mean predictions of the six Bigelow models fitted are shown in Figure 9. According to the log-linear inactivation data recovered, greater resistance in response to temperature increase is displayed by spores of *C. perfringens* followed by parvoviruses (lower slopes in Figure 9).

**Table 5:** Estimates and standard errors (SE) of the thermal inactivation parameters obtained from the data retrieved for spores of *Clostridium perfringens*, *Salmonella* Senftenberg, *Enterococcus faecalis* and parvoviruses used in the simulations. Correlation values between estimates and standard deviations (SD) of residuals are also shown

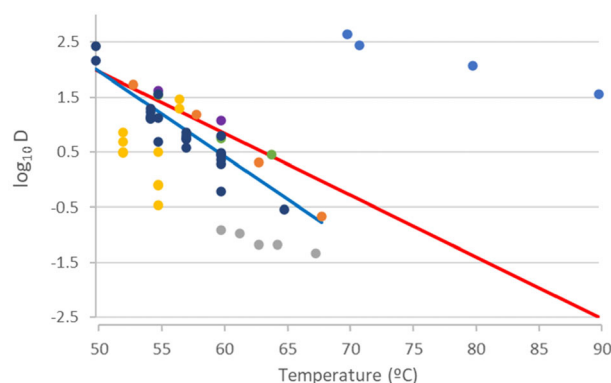
Microorganism	Log <sub>10</sub> D <sub>ref</sub>	SE (log <sub>10</sub> D <sub>ref</sub> )	z	SE (z)	Correlation	SD (residuals)
Spores of <i>Clostridium perfringens</i> (Methods 2–4, 7). Data: all matrices except water T <sub>ref</sub> = 105°C (N = 89)	0.647	0.128	19.19	5.549	0.798	0.729
<i>Clostridium perfringens</i> (Method 5) Subset: beef slurry, beef gravy, pork luncheon roll T <sub>ref</sub> = 105°C (N = 11)	0.268	0.129	10.28	1.041	0.404	0.125
<i>Salmonella</i> Senftenberg (Methods 2–4,7) Data: all matrices T <sub>ref</sub> = 90°C (N = 53)	-2.501	0.635	8.899	1.086	0.632	0.382



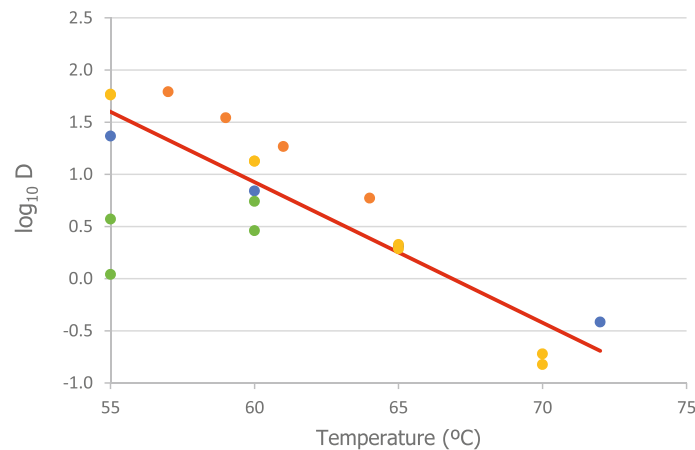
Microorganism	Log <sub>10</sub> D <sub>ref</sub>	SE (log <sub>10</sub> D <sub>ref</sub> )	z	SE (z)	Correlation	SD (residuals)
<i>Salmonella</i> Senftenberg (Method 5) Data: all matrices except chocolate and raw milk T <sub>ref</sub> = 68°C (N = 44)	-0.777	0.255	6.465	0.653	0.649	0.340
<i>Enterococcus faecalis</i> (All Methods) Data: all matrices T <sub>ref</sub> = 72°C (N = 20)	-0.692	0.217	7.421	0.667	0.622	0.263
Parvoviruses (All Methods) Data: all matrices T <sub>ref</sub> = 117°C (N = 42)	-1.522	0.342	15.00	1.462	0.915	0.534



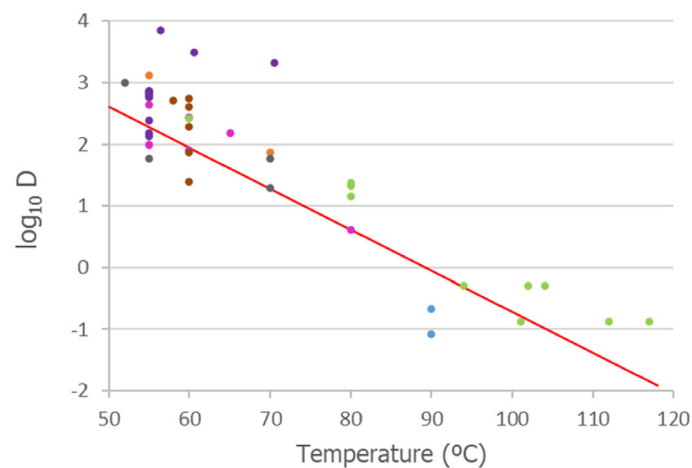
**Figure 5:** Scatter plot of the log<sub>10</sub> D [min] of *Clostridium perfringens* spores in different matrices versus temperature, showing Bigelow models for use in methods 2–4 and 7 (fitted to all data; red line) and method 5 (fitted to beef slurry, beef gravy and pork luncheon roll; blue line). Black: water or broth; orange: beef gravy; yellow: DS (Duncan and Strong medium); red: pork luncheon roll; grey: beef slurry; blue: fruit juice; brown: sodium phosphate buffer; dark orange: distilled water; green: not stated



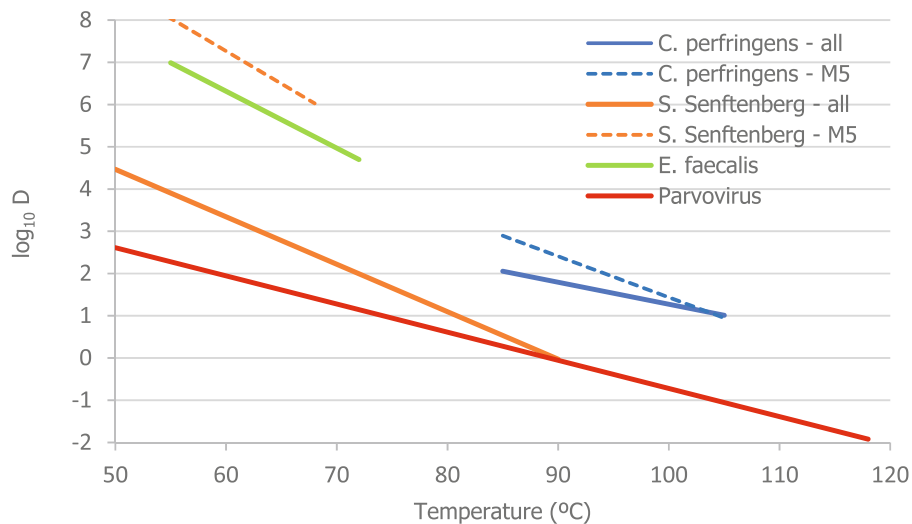
**Figure 6:** Scatter plot of the log<sub>10</sub> D [min] of *Salmonella* Senftenberg in different matrices versus temperature, showing Bigelow models for use in methods 2–4 and 7 (fitted to all data; red line) and method 5 (fitted to all data except chocolate and raw milk; blue line). Blue: chocolate; orange: egg whites; grey: raw milk; dark orange: egg whites; purple: egg yolks; light green: whole eggs; black: nutrient broth



**Figure 7:** Scatter plot of the  $\log_{10} D$  [min] of *Enterococcus faecalis* in different matrices versus temperature, showing the fitted Bigelow model for use in all methods. Blue: mixed liquid; orange: ground beef; dark orange: whole milk; green: digestion waste



**Figure 8:** Scatter plot of the  $\log_{10} D$  [min] of parvoviruses in different matrices versus temperature, showing the fitted Bigelow model for use in all methods. Pink: culture medium; blue: glucose concentrate; green: water; dark orange: saline solution; brown: plasma derivatives; purple: virus on filters; green: water; dark green: semi-solid medium



**Figure 9:** Predicted lines of the Bigelow models describing  $\log_{10} D$  [min] as a function of temperature for the four target pathogens. Thick lines denote models fitted to all available matrices, and dashed lines to proteinaceous matrices. The temperature range of the lines covers the range for which data were available, to prevent extrapolation

Table 6 summarises the probabilities of achieving the target reduction of selected bacterial and viral indicators when Methods 2 to 5, in both the coincidental and consecutive modes, and the five scenarios for Method 7, are applied.

**Table 6:** Probability to achieve the target inactivation standards for spores of *Clostridium perfringens*, *Salmonella* Senftenberg, *Enterococcus faecalis* and parvoviruses according to the results of the model

Method	Indicators	Target $\log_{10}$ reduction	Probability to achieve at least the target $\log_{10}$ reduction	Probability to achieve at least the target $\log_{10}$ reduction	Probability to achieve at least the target $\log_{10}$ reduction
			Mode: coincidental	Mode: consecutive	
<b>2</b>	Spores of <i>C. perfringens</i>	5	1.0000	1.0000	
	<i>S. Senftenberg</i>	5	1.0000	1.0000	
	<i>E. faecalis</i>	5	1.0000	1.0000	
	Parvoviruses	3	1.0000	1.0000	
<b>3</b>	Spores of <i>C. perfringens</i>	5	1.0000	1.0000	
	<i>S. Senftenberg</i>	5	1.0000	1.0000	
	<i>E. faecalis</i>	5	1.0000	1.0000	
	Parvoviruses	3	1.0000	1.0000	
<b>4</b>	Spores of <i>C. perfringens</i>	5	0.0660	0.9240	
	<i>S. Senftenberg</i>	5	1.0000	1.0000	
	<i>E. faecalis</i>	5	1.0000	1.0000	
	Parvoviruses	3	0.9963	0.9998	
<b>5</b>	Spores of <i>C. perfringens</i>	5	0.9970	0.9970	
	<i>S. Senftenberg</i>	5	1.0000	1.0000	
	<i>E. faecalis</i>	5	1.0000	1.0000	
	Parvoviruses	3	0.9984	0.9992	
<b>7 scenario 1</b>	Spores of <i>C. perfringens</i>	5			0.0000
	<i>S. Senftenberg</i>	5			0.9966
	<i>E. faecalis</i>	5			1.0000
	Parvoviruses	3			0.0735

Method	Indicators	Target log <sub>10</sub> reduction	Probability to achieve at least the target log <sub>10</sub> reduction	Probability to achieve at least the target log <sub>10</sub> reduction	Probability to achieve at least the target log <sub>10</sub> reduction
			Mode: coincidental	Mode: consecutive	
<b>7 scenario 2</b>	Spores of <i>C. perfringens</i>	5			0.6850
	<i>S. Senftenberg</i>	5			1.0000
	<i>E. faecalis</i>	5			1.0000
	Parvoviruses	3			0.9950
<b>7 scenario 3</b>	Spores of <i>C. perfringens</i>	5			0.9999
	<i>S. Senftenberg</i>	5			1.0000
	<i>E. faecalis</i>	5			1.0000
	Parvoviruses	3			1.0000
<b>7 scenario 4</b>	Spores of <i>C. perfringens</i>	5			0.0040
	<i>S. Senftenberg</i>	5			1.0000
	<i>E. faecalis</i>	5			1.0000
	Parvoviruses	3			0.9905
<b>7 scenario 5</b>	Spores of <i>C. perfringens</i>	5			0.0000
	<i>S. Senftenberg</i>	5			0.9999
	<i>E. faecalis</i>	5			0.9993
	Parvoviruses	3			0.9256

The results of the model show that for the four selected indicators, the lowest probability of achieving the target level of inactivation in all methods was estimated for the spores of *C. perfringens*. Thus, if the model predicts a high probability of 5 log<sub>10</sub> inactivation of *C. perfringens*, the probability will be even higher for the other indicators, becoming more likely to achieve the required standard of 5 log<sub>10</sub> or 3 log<sub>10</sub>.

The results of the model showed a probability of inactivation of at least 5 log<sub>10</sub> of spores of *C. perfringens* over 0.99 of the iterations, for methods 2, 3 and 5, in both the coincidental and consecutive modes and 0.92 for method 4 in consecutive mode. For method 4 in coincidental mode, the model estimated a probability of 0.066. For method 7, the model estimates a probability of 0.004 or below of achieving the 5 log<sub>10</sub> reduction for scenarios 1, 4 and 5, and probabilities of 0.685 and 0.999 for achieving the same level of reduction for scenarios 2 and 3, respectively.

Table 7 shows the outcomes of the simulations undertaken to determine the probability of achieving specific log<sub>10</sub> reductions against spores of *C. perfringens* considering different log reduction levels ranging between < 5 log<sub>10</sub> and > 12 log<sub>10</sub>.

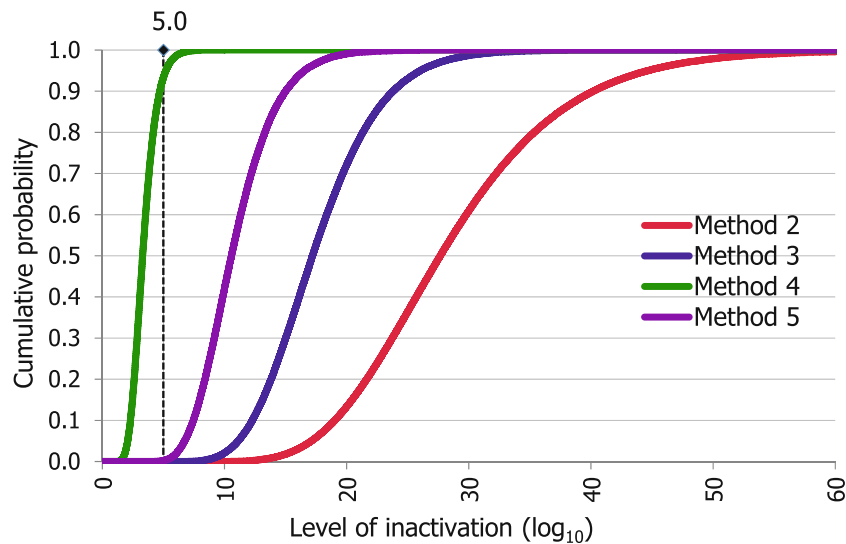
**Table 7:** Probability of achieving specific log<sub>10</sub> reductions in different ranges, and expected value of log<sub>10</sub> reduction (in brackets) of spores of *Clostridium perfringens* after application of coincidental and consecutive heat treatments

Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
<b>2</b>	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.002	0.000	
	L ≥ 12	0.998	1.000	
		(27.6)	(53.8)	
<b>3</b>	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.002	0.000	

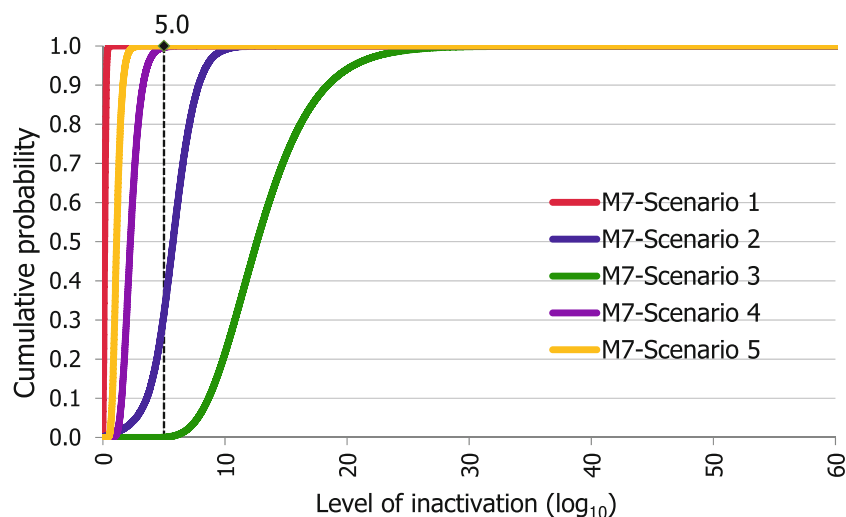
Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
	8 ≤ L < 10	0.019	0.000	
	10 ≤ L < 12	0.065	0.001	
	L ≥ 12	0.914	0.999	
		(17.3)	(27.1)	
<b>4</b>	L < 5	0.934	0.076	
	5 ≤ L < 6	0.051	0.147	
	6 ≤ L < 8	0.014	0.408	
	8 ≤ L < 10	0.001	0.257	
	10 ≤ L < 12	0.000	0.086	
	L ≥ 12	0.000	0.026	
		(3.30)	(7.39)	
<b>5</b>	L < 5	0.003	0.003	
	5 ≤ L < 6	0.016	0.015	
	6 ≤ L < 8	0.136	0.127	
	8 ≤ L < 10	0.265	0.256	
	10 ≤ L < 12	0.260	0.265	
	L ≥ 12	0.320	0.334	
		(10.7)	(10.8)	
<b>7, Scenario 1</b>	L < 5			1.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			0.000
				(0.157)
<b>7, Scenario 2</b>	L < 5			0.315
	5 ≤ L < 6			0.252
	6 ≤ L < 8			0.345
	8 ≤ L < 10			0.078
	10 ≤ L < 12			0.009
	L ≥ 12			0.001
				(6.1)
<b>7, Scenario 3</b>	L < 5			0.001
	5 ≤ L < 6			0.005
	6 ≤ L < 8			0.055
	8 ≤ L < 10			0.154
	10 ≤ L < 12			0.217
	L ≥ 12			0.568
				(12.6)
<b>7, Scenario 4</b>	L < 5			0.996
	5 ≤ L < 6			0.003
	6 ≤ L < 8			0.001
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			0.000
				(2.25)
<b>7, Scenario 5</b>	L < 5			1.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			0.000
				(1.12)

The  $\log_{10}$  reduction of *C. perfringens* spores achieved by the inactivation methods are also illustrated as cumulative distributions in Figures 10 and 11. The left skewness of these distributions (i.e. the further it lies to the right) is linked to a greater level of inactivation. The decreasing order of the estimated lethality is: method 2 > method 3 > method 5 > method 4. The probability of insufficient inactivation (lower than 5  $\log_{10}$ ) is associated with the left tail. In Figure 10, the vertical threshold of 5  $\log_{10}$  cycles was placed on the graph for comparison with the target reduction.

For method 7, the mildness of the heat regimes of scenarios 1 and 5 is evident in their respective lethality cumulative distributions in Figure 11. The latter shows that the lethality against *C. perfringens* spores is higher in scenario 3, followed by scenario 2, scenario 4, scenario 5 and scenario 1, being the last three scenarios on the left side of the threshold.



**Figure 10:** Cumulative probability of the lethality of the heat treatment methods 2, 3, 4 and 5 in coincidental mode against *Clostridium perfringens* spores



**Figure 11:** Cumulative probability of the lethality of the five scenarios of heat treatment method 7 against *Clostridium perfringens* spores

Tables C.1, C.2 and C.3 of Appendix C show the outcomes of the simulations undertaken to determine the probability of achieving specific  $\log_{10}$  reductions against *S. Senftenberg*, *E. faecalis* and parvoviruses considering different  $\log$  reduction levels, ranging between < 5  $\log_{10}$  (< 3  $\log_{10}$  for parvoviruses) to > 12  $\log_{10}$ . The  $\log_{10}$  reduction of parvoviruses achieved by the inactivation methods are also illustrated as cumulative distributions in Figures C.1 and C.2.

### 3.3.2. Comparative analysis with *Clostridium botulinum*

As explained in Section 2.3.4, in previous EFSA ABP scientific opinions, extrapolation at higher temperatures than the experimental ones was discouraged as an approach for estimating the lethality of alternative methods. Therefore, for some of the methods under assessment, the reductions that can be reached at the actual treatment temperatures of the heat regime may be underestimated.

In Section 3.1.1.1, criteria for the selection of relevant bacterial pathogens, it is highlighted that in previous EFSA assessments of alternative ABP processing methods applied to Category 3 ABP, if the hazard identification considers spore-forming bacteria as relevant biological hazards, a requirement of 12 log<sub>10</sub> reduction in *C. botulinum* spores, equivalent to a treatment at 121.1°C for 3 min, can be considered also effective to achieve a 5 log<sub>10</sub> reduction of other less heat resistant spore-forming bacteria, such as *C. perfringens*. The equivalent accumulated lethality expressed in minutes at Tref 121°C of methods 2 to 5 and the scenarios of method 7 considered in this opinion at temperatures other than the reference one for *C. botulinum* (121.1°C) was calculated considering a z-value of 10°C. The values reached would be:

- Method 2: 38.81 min
- Method 3: 10.09 min
- Method 4: 27.16 min
- Method 5: 0.46 min
- Method 7 scenario 1: 0.001 min
- Method 7 scenario 2: 0.22 min
- Method 7 scenario 3: 13.74 min
- Method 7 scenario 4: 24.54 min
- Method 7 scenario 5: 77.44 min

Therefore, all methods assessed, except method 5 and scenarios 1 and 2 of method 7, would exceed the requirement for *C. botulinum* (equivalent to > 3 min at 121°C in terms of lethality). Consequently, it would be expected that they will achieve at least a 5 log<sub>10</sub> reduction of other less heat resistant bacterial spores, such as those of *C. perfringens*. Considering the outputs of the model, method 4 coincidental and method 7 scenarios 4 and 5 would have a low probability of achieving the 5 log<sub>10</sub> reduction of *C. perfringens*, but they would achieve the requirements for *C. botulinum* (12 log reduction). This is due to the fact that extrapolation of the model at temperatures above 105°C for *C. perfringens* is not applied, while method 4 and scenarios 4 and 5 of method 7 involve treatments at much higher temperatures (in the range of 125–133°C). In these cases, the model is very likely underestimating the lethality achieved by the processing methods, and the fact that the methods are predicted to reach a sufficient level of reduction (> 12 log<sub>10</sub>) of spores of *C. botulinum* provides an indication that a sufficient level of reduction of the less heat resistant *C. perfringens* spores would be also achieved.

The opposite happens for method 5, for which the model predicts a probability of over 99% of achieving the 5 log<sub>10</sub> reduction of *C. perfringens*, whereas the requirement for *C. botulinum* is not met (equivalent to < 3 min at 121°C in terms of lethality). In this case, this indicates that the process is possibly not achieving a 12 log<sub>10</sub> reduction of *C. botulinum* spores, but it is producing a 5 log<sub>10</sub> reduction of spores of *C. perfringens*, identified as the relevant hazard to be considered in the assessment.

In scenarios 1 and 2 of method 7, the estimations show that the minimum level of inactivation required is possibly not achieved either for *C. perfringens* (5 log<sub>10</sub> reduction), or for *C. botulinum* (12 log<sub>10</sub> reduction). In these cases, the hygienic conditions and the nature of the material will ultimately determine the safety of the end product, as the effectiveness of those heat regimes is limited. It is important to highlight that the material treated by method 7 is subject to the safety/hygiene verification by the microbiological standards established in Chapter III, Annex IV of Commission Regulation (EU) 142/2011.

### 3.4. AQ5: What is the certainty that the 'level of inactivation' achieved by methods 2–3–4–5 and 7 as in AQ4 is sufficient to reach the standards for category 3 ABP?

The following EKE question was used to address AQ5: What is the probability that a 5 log<sub>10</sub> reduction of spores of *C. perfringens* is achieved, in more than 99% of the cases, by application of each of the relevant processes (methods 2, 3, 4, 5 in coincidental mode and five t/T combinations of

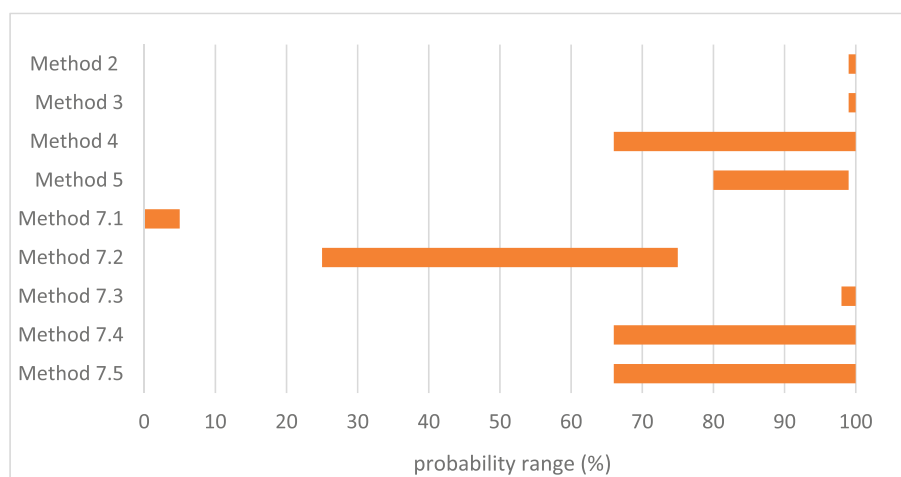
method 7), assuming that the processes are performed as prescribed and that the indicated process conditions are achieved?

The reason why only the spores *C. perfringens* are considered in the EKE question is because (according to the results of this assessment) they represent the most thermal resistant microorganism among the selected indicators. The coincidental mode of the methods 2 to 5 was assessed as it has been considered in this assessment as the worst-case scenario.

The results of the consensus judgements for each of the t/T combinations are given in Table 8 and Figure 12. Based on the results, a 5 log<sub>10</sub> reduction of *C. perfringens* spores was judged: 99–100% certain for methods 2 and 3 in coincidental mode; 98–100% certain for method 7 scenario 3; 80–99% certain for method 5 in coincidental mode; 66–100% certain for method 4 in coincidental mode and for method 7 scenarios 4 and 5; 25–75% certain for method 7 scenario 2; and 0–5% certain for method 7 scenario 1. The same or higher certainty to achieve the 5 log<sub>10</sub> reduction of *C. perfringens* spores is expected when methods 2 to 5 are applied in consecutive mode.

**Table 8:** Time/temperature (t/T) combinations for each of the processes (methods 2, 3, 4, 5 in coincidental mode and five t/T combinations of method 7) included in the EKE and results of the consensus judgement

Process	Combination t/T				Consensus judgement
<b>Method 2 – coincidental mode</b>	100°C × 5'	110°C × 70'	120°C × 50'	–	99–100%
<b>Method 3 – coincidental</b>	100°C × 40'	110°C × 42'	120°C × 13'	–	99–100%
<b>Method 4 – coincidental</b>	100°C × 3'	110°C × 5'	120°C × 5'	130°C × 3'	66–100%
<b>Method 5 – coincidental</b>	80°C × 60'	100°C × 60'	–	–	80–99%
<b>Method 7 – scenario 1</b>	80°C × 14'				0–5%
<b>Method 7 – scenario 2</b>	95°C × 90'				25–75%
<b>Method 7 – scenario 3</b>	115°C × 56'				98–100%
<b>Method 7 – scenario 4</b>	125°C × 10'				66–100%
<b>Method 7 – scenario 5</b>	133°C × 5'				66–100%



**Figure 12:** Probability ranges obtained in the EKE, indicating how certain the experts are that a 5 log<sub>10</sub> reduction of spores of *Clostridium perfringens* is achieved, in more than 99% of the cases, by application of each of the relevant processes (methods 2, 3, 4, 5 in coincidental mode and five t/T combinations of method 7), assuming that the processes are performed as prescribed and that the indicated process conditions are achieved



### 3.5. Uncertainty analysis

**Table 9:** Sources of uncertainty associated with the Aqs and their possible impact on the conclusions

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions (e.g. over/underestimation)
Identification of viral hazards	All viral hazards that may occur in the raw materials for production of pig PAP may not have been identified through the literature search. It is also possible that the occurrence of virus, in general, or certain families, in particular, have never been investigated in materials used for pig PAP production.	The lack of identification of a relevant virus is expected to impact the conclusions only if it would be a heat-resistant virus (e.g. non-enveloped DNA viruses). Parvoviruses are included in the assessment and are considered among the most heat-resistant viruses in nature, and it is unlikely that other more heat-resistant viruses, not considered in the opinion, could occur.
Technical parameters for methods 2 to 5	The Commission Regulation (EU) 142/2011 specifies that the core temperatures listed under each method must be achieved. Either in batch or continuous mode, the temperature in different sections of the equipment must be higher in order to ensure the minimum temperatures at the core.	As estimates were made considering core temperatures being achieved, the level of inactivation achieved by processing methods 2 to 5 may be in some cases underestimated outside the core.
Technical parameters for method 7	Method 7 requires compliance with microbiological criteria ensuring the non detection of <i>Salmonella</i> and <i>Clostridium perfringens</i> , and certain levels of <i>Enterobacteriaceae</i> , but processes approved in the EU since 2004 for method 7 have very different parameters in terms of temperature and time combinations.	Depending on the method applied, the inactivation of the indicator microorganisms in the raw material could be higher or lower than that calculated for the five scenarios included in the present opinion.
Data related. Identification of studies on the inactivation of the hazards	Relevant records for extraction of data on thermal inactivation of selected indicators were identified through literature searches. There is the possibility that some relevant studies were not identified or considered for data extraction.	Considering the randomness of the non-inclusion of potentially relevant studies, this source of uncertainty could lead to either an over or underestimation of the inactivation of the biological hazards.
Data related. Type of matrices used for the evaluation of the inactivation of the hazards	The data extracted on the thermal inactivation of hazards were sourced from experimental studies using different matrices, in most cases of a different nature to the materials used for pig PAP production. The different composition in terms of dry matter (total solid contents, $a_w$ ), fat content, etc. determines the capacity of hazards to survive under different conditions of time/temperature.	The ability of methods 2 to 5 and method 7 to achieve the targeted reductions in the materials used to produce pig PAP may be higher or lower than estimated. Most inactivation data used were derived from studies using liquid media or foods. As microbial inactivation by heat is lower in systems with lower $a_w$ , estimations from studies on liquid acidic media or on foods with high $a_w$ , this could result in an overestimation of the inactivation achieved by methods 2 to 5 and method 7.

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions (e.g. over/underestimation)
Data related. Strain of the hazard and enumeration method used to assess the level of inactivation of the hazards	The data extracted on the thermal inactivation of hazards were sourced from experimental studies using specific strains/isolates of the relevant hazards and different analytical methods, which, for viral hazards, are not standardised. It is uncertain whether those strains are representative of the behaviour of the whole species.	This source of uncertainty could lead to either over or underestimate the inactivation of the biological hazards.
Data related. Temperature measurement used for the inactivation of the hazards	In the studies, the temperature was measured in the substrate and resembled isothermal conditions. The accuracy of the temperature measurement may affect the thermal resistance estimation.	This source of uncertainty could lead to either an over or underestimation of the inactivation of the biological hazards.
Data related. Heating temperatures used for the inactivation of the hazards	The data retrieved on thermal inactivation of hazards contained information on certain heating temperatures, that in some cases were far from the temperatures under assessment. Extrapolations at temperatures above those for which experimental data was available were not performed.	No extrapolation at temperatures above those for which experimental data is available may lead to an underestimation of the level of inactivation achieved by the methods, especially for those methods that work at higher regime temperatures.
Model related. D-value estimation	Estimation of D values from primary research studies. D values were extracted, where available, from tables and text as in the published peer-reviewed literature without checking the primary data. For viruses, D values from data with low linearity and point estimates of time to 1 log <sub>10</sub> reduction at different temperatures were also included.	Underestimation or overestimation of D-values could lead to overestimation or underestimation of log <sub>10</sub> reductions. Uncertainty in D values introduces bidirectional uncertainty in the estimation of D <sub>ref</sub> and z.
Model assumptions	The model does not consider come up times. i.e. the additional inactivation due to heating and cooling times to the time/temperature combinations required for each process under assessment.	The model underestimates the level of inactivation achieved by each method, the underestimation being greater in the method/s with higher temperatures.
Use of <i>Clostridium botulinum</i> as surrogate for <i>Clostridium perfringens</i>	Use of thermal resistance data of <i>C. botulinum</i> spores, obtained in different substrates, conditions and methodologies, assuming that they will be, at least, equivalent to the thermal inactivation of <i>C. perfringens</i> spores in the conditions of the method evaluated.	Using D values at temperatures below those of some of the assessed methods (e.g. method 4 and method 7 scenarios 4 and 5) could lead to underestimation of the level of inactivation achieved.
Use of comparative analysis with <i>Clostridium botulinum</i> in the EKE	In the EKE both the model results and the results of the accumulated lethality in minutes at T <sub>ref</sub> 121°C of methods 2 to 5 and the scenarios of method 7, at temperature other than the reference one for <i>C. botulinum</i> (121.1°C), were used.	For some of the methods there was a discrepancy between the outputs of the model and of the EKE. The individual judgements may have over or underestimated the level of inactivation of the methods.

## 4. Conclusions

- Four relevant pathogens were selected as indicators to assess the efficacy of standard processing methods 2, 3, 4, 5 and 7 for Category 3 ABP of porcine origin: *S. Senftenberg*, *E. faecalis*, spores of *C. perfringens* and parvoviruses. It was agreed to assess whether the methods would reach a level of reduction of 5 log<sub>10</sub> for the bacterial indicators and 3 log<sub>10</sub> for the viral indicator. This approach has been considered a worst-case scenario, as Category 3 ABP are only sourced from animals slaughtered fit for human consumption or rejected but not showing signs of disease communicable to humans or animals.
- The time/temperature (t/T) parameters of processing methods 2, 3, 4 and 5 are stated in Chapter III, Annex IV of Commission Regulation (EU) 142/2011: method 2–100°C for at least 125 min, 110°C for at least 120 min and 120°C for at least 50 min; method 3–100°C for at least 95 min, 110°C for at least 55 min and 120°C for at least 13 min; method 4–100°C for at least 16 min, 110°C for at least 13 min, 120°C for at least 8 min and 130°C for at least 3 min; method 5–80°C for at least 120 min and 100°C for at least 60 min. The efficacy of these processing methods was assessed either when the core temperatures are achieved consecutively or through a coincidental combination of the time periods indicated. The latter has been considered as the worst-case scenario.
- For method 7, the legislation does not state time/temperature parameters. However, there are process parameters approved in different EU member states based on equipment used and treated raw materials. Based on the approved parameters provided by the industry, five scenarios of individual time/temperature profiles were selected for the scope of this assessment in order to apply for method 7 the same methodological approach followed for methods 2 to 5. The selected scenarios were: scenario 1–80°C for at least 14 min; scenario 2–95°C for at least 90 min; scenario 3–115°C for at least 56 min; scenario 4–125°C for at least 10 min; scenario 5–133°C for at least 5 min.
- The level of inactivation of the selected indicators was calculated using the parameters of the Bigelow model fitted to thermal inactivation parameters retrieved for the four indicators from a previous EFSA scientific opinion (EFSA BIOHAZ Panel, 2021 and/or from extensive literature searches. Matrices with different levels of fat and proteins were included in the data modelling.
- The model to estimate the level of inactivation of relevant pathogens was applied to limited experimental data and built up with certain assumptions that may result in an underestimation of the levels of reduction achieved, mostly for methods for which the temperature is above those for which thermal inactivation data are available.
- Considering the data retrieved from the literature and the model estimates for the four selected indicators, the lowest probability of achieving the target level of inactivation was estimated for the spores of *C. perfringens* for all methods.
- The results of the simulation showed a probability of inactivation of at least 5 log<sub>10</sub> of spores of *C. perfringens* of over 0.99 for methods 2, 3 and 5, in both coincidental and consecutive modes and 0.92 for method 4 in consecutive mode. For method 4 in coincidental mode, the model estimated a probability of 0.066. For method 7, the model estimated a probability of 0.004 or below of achieving the 5 log<sub>10</sub> reduction for scenarios 1, 4 and 5, and of 0.685 and 0.999 for achieving the same level of reduction for scenarios 2 and 3, respectively. The calculation of such probabilities was driven by the uncertainty about the thermal inactivation parameters of the target microorganisms, which could be attributed to the different strains assessed in the primary studies, the matrices and the experimental design.
- An expert knowledge elicitation (EKE) was conducted to elucidate what the probability is that a 5 log<sub>10</sub> reduction of *C. perfringens* spores is achieved, in more than 99% of cases, by application of the relevant processes (methods 2, 3, 4, 5 in coincidental mode and five t/T combinations of method 7) assuming that the processes are performed as prescribed and that the indicated process conditions are achieved. The information considered in the expert judgements was based on the information and data available in the draft opinion at the time of conducting the EKE, also considering evidence on inactivation of spores of *C. botulinum* as an additional surrogate. The latter is related to the underestimation of the model since the extrapolation at temperatures above 105°C for *C. perfringens* was not applied, while method 4 and scenarios 4 and 5 of method 7 involve treatments at much higher temperatures.

- Based on the EKE, the certainty of achieving a 5 log<sub>10</sub> reduction of *C. perfringens* spores (which also assure the target inactivation for the other relevant pathogens was:
  - 99–100% certain for methods 2 and 3 in coincidental mode; 98–100% certain for method 7 scenario 3;
  - 80–99% certain for method 5 in coincidental mode;
  - 66–100% certain for method 4 in coincidental mode and for method 7 scenarios 4 and 5;
  - 25–75% certain for method 7 scenario 2; and
  - 0–5% certain for method 7 scenario 1.
- Compared to the results of the EKE for methods 2–5 in coincidental mode, the same or higher certainty to achieve the 5 log<sub>10</sub> reduction of *C. perfringens* spores is expected when methods 2 to 5 are applied in consecutive mode.

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## Abbreviations

ABP	Animal by-products
AHAW	Animal Health and Animal Welfare
AHL	Animal Health Law
AQ	Assessment question
ASF	African Swine Fever
AstV	Astrovirus
BIOHAZ	biological hazards
BPV	bovine parvovirus
CPV	canine parvovirus
CSF	classical swine fever
DS	Duncan Strong
D value	The time (in minutes) of exposure at a given temperature that causes a one-log <sub>10</sub> or 90% reduction in the population of a specific microorganism
ECDC	European Centre for Disease Control
EFPPA	European Fat processors and Renderers Association
EKE	Expert Knowledge Elicitation
ELS	extensive literature search
EMCV	encephalomyocarditis virus
F	The time necessary to destroy a given number of microorganisms at a reference temperature, usually 121°C for spores or 60°C for vegetative cells
FMDV	Foot and Mouth disease virus
GARV	Rotavirus group A
HBV	Hepatitis B virus
HEV	Hepatitis E virus
L	lethality
MVM	Minute virus of mice
NOV	European swine Norovirus
PAP	Processed animal protein
PAdV	Porcine adenovirus
PBo-likeV	Porcine boca-like virus
PboV	Porcine bocavirus
PCAD	Porcine adenovirus
PCV	Porcine circovirus
PCMV	Porcine cytomegalovirus
PDCoV	Porcine deltacoronavirus
PDNS	Porcine dermatitis and nephropathy syndrome
PEC	Porcine enteric calicivirus
PMWS	Post-weaning multisystemic wasting syndrome
PPIV	Porcine parainfluenza virus
PPV	Porcine parvovirus
PRRS	Porcine respiratory and reproductive syndrome
SD	Standard deviation
SE	Standard error
SIV	Swine influenza virus
SQ	Sub-assessment question
t/T	Time/temperature
TTSuV	Torque Teno virus
WOAH	Organization of Animal Health and Welfare
z	The number of degrees the temperature has to be increased to achieve a tenfold (i.e. 1 log <sub>10</sub> ) reduction in the D-value

## Appendix A – Data extracted on thermal inactivation parameters of spores of *Clostridium perfringens*

**Table A.1:** Summary of the data extracted from the selected papers in the literature review for the identification of thermal inactivation parameters of spores of *Clostridium perfringens* expressed as D values

Temperature	D-value (minutes)	Matrix	Pre-treatment	Comment	Reference	
85	19	Duncan and Strong sporulation media	Spores produced at 37°C	Treatment in an immersed-coil heating apparatus to immediately heat at 85°C; strain with a chromosomal <i>cpe</i> gene	Andersen et al. (2004)	
	2.10					
	17		Spores produced at 42°C			
	1					
90	30.6	Pork luncheon roll		Treatment in a temperature-controlled water bath; Three-strain cocktail used	Byrne et al. (2006)	
95	9.7					
100	1.9					
95	35	Sodium phosphate buffer	Spores produced in in DS-MOPS pH 7.0	Recovery on BASE + L medium; treatment in glass capillary tubes	Craven (1990)	
105	1.9					
95	75		Spores produced in in DS-MOPS pH 7.5			
105	5.9					
95	48					
105	0.13		Spores produced in in DS-EPPS pH 7.5			
95	30					
105	0.19					
95	40					
105	3.8					
95	56					Spores produced in in DS-MOPS pH 8.0
105	0.13					
95	128		Spores produced in in DS-EPPS pH 8.0			
105	20					
95	84					
105	6.4					
95	50		Spores produced in in DS-EPPS pH 8.5			
95	144					
105	6.4					
95	50		Spores produced in in DS-EPPS pH 8.5			Recovery on BASE medium; treatment in glass capillary tubes
105	0.22					

Temperature	D-value (minutes)	Matrix	Pre-treatment	Comment	Reference
95	32		Spores produced in in DS-EPPS pH 8.0		
105	0.16				
95	28		Spores produced in in DS-MOPS pH 7.0		
105	0.13				
105	2.5	Beef slurry, 14% protein, 7% fat	Spores produced in modified Duncan-strong (DS) sporulation medium	Treatment in thin layer pouches (1-2 mm × 8 cm × 8 cm), submerged in oil bath. No info of actual temp in the centre is provided	Evelyn and Silva (2015)
100	7.1				
95	21.7				
105	1.8	Beef slurry	Spores produced in modified Duncan-strong (DS) sporulation medium	Treatment in thin layer pouches (1-2 mm × 8 cm × 8 cm), submerged in oil bath.	
100	5.5				
95	15				
100	0.5	Unclear, water or laboratory broth	In: Sarker MR, Shivers RP, Sparks SG, Juneja VK, McClane BA (2000) Comparative experiments to examine the effects of heating on vegetative cells and spores of <i>Clostridium perfringens</i> isolates carrying plasmid versus chromosomal enterotoxin genes. Appl Environ Microbiol 66: 3234–3240	Not indicated how it was done, values for several strains.	Li and McClane (2008)
	10				
	59.1				
	8.7				
	44.7				
	16.4				
	9.3				
	38				
	50				
	0.7				
100	13.5	MDS medium		Heat treatment very vague, immersion in boiling water of 10 mL tubes	
	16.6				
	40.3				
	20.8				
	20.5				
	33.4				
	7				
100	16	Distilled water	Spores produced in Duncan and Strong (DS) sporulation medium	Heat treatment in tubes, exposure not specified to 100°C, poor description of the methodology	Novak and Yuan (2003)

Temperature	D-value (minutes)	Matrix	Pre-treatment	Comment	Reference
90	120.6	Not specified, laboratory conditions	Spores produced in Duncan and Strong (DS) sporulation medium	Method not explained, in: Ando, Y., T. Tsuzuki, H. Sunagawa, and S. Oka. 1985. Heat resistance, spore germination, and enterotoxigenicity of <i>Clostridium perfringens</i> . Microbiol. Immunol. 29:317–326	Osburn et al. (2008)
	45.6				
	21.4				
	19.9				
	19				
	12.5				
	10.1				
	6.9				
5.5					
100	49.1	DS medium	Spores produced in Duncan and Strong (DS) sporulation medium	Heat treatment at 100°C, not clearly explained, submerged	Paredes-Sabja et al. (2008)
	19.2				
	45.2				
	28.8				
	52.2				
100	62	As Sarker et al. (2000)	Same as Sarker et al. (2000)	Heat treatment at 100°C, not clearly explained, submerged	Raju and Sarker (2005)
	61				
	0.5				
100	124	DS medium culture	Spores in Duncan and Strong sporulation	Flask technique or similar, temp fixed at 90 or 100°C, then spore suspension added, mixed and sampled at time intervals	Sarker et al. (2000)
	67				
	32				
	30				
	45				
	60				
	0.5				
	1.9				
	1.6				
	0.9				
	1.3				
	0.5				



Temperature	D-value (minutes)	Matrix	Pre-treatment	Comment	Reference
104	2.9	Commercial beef gravy	Not available	Data from Bradshaw JG, Peeler JT and Twedt RM, 1977. Thermal inactivation of ileal loop-reactive <i>Clostridium perfringens</i> type A strains in phosphate buffer and beef gravy. Applied Environmental Microbiology, 34, 280–284. Borosilicate glass tubes in oil bath	Soni et al. (2022)
	6.1				
100	50	DS medium culture	Spores produced in Duncan and Strong (DS) sporulation medium	Flask technique or similar, temp fixed at 100°C, then spore suspension added, mixed and sampled at time intervals	Raju and Sarker (2007)
	26				
	13				
95	2.16	Fruit juices	Not clarified, serious shortcomings, not specified that they use anaerobiosis for incubation	Kooiman tubes technique	Brooks (2013)

## Appendix B – Data extracted on thermal inactivation parameters of the family Parvoviridae

**Table B.1:** Summary of the data extracted from the relevant papers on thermal inactivation parameters of members of the family *Parvoviridae*

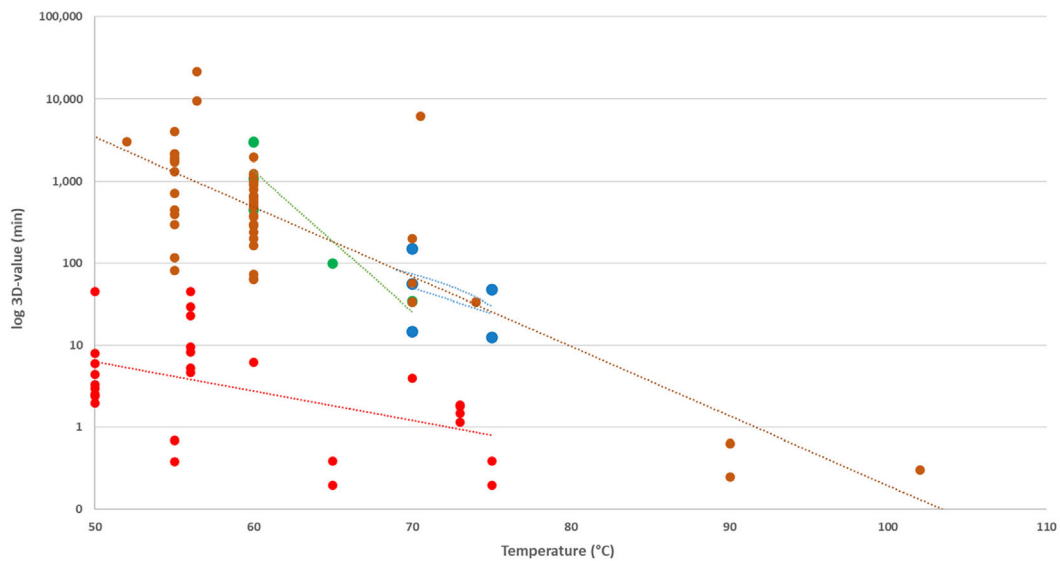
Virus	Temperature (°C)	D-value or point estimate of time to 1 log reduction (min)	Matrix	Experimental conditions	Reference
Bovine parvovirus (BPV)	60	433,0 <sup>(a)</sup>	Serum albumin	Suspension medium: plasma. Haden strain, titre: 10 <sup>8</sup> infectious units/mL. Albumin solution.	Brauniger et al. (2000)
	60	257,8 <sup>(a)</sup>	Distilled water	Suspension medium: AquaDest. Haden strain, titre: 10 <sup>8</sup> infectious units/mL. Albumin solution.	
	55	133,3; 576,0 <sup>(b)</sup>	Viruses on filters within solid carriers	Thermophilic fermentation. Virus (titre: 5.8 log TCID <sub>50</sub> /mL) adsorbed on filters placed within minced meat.	Paluszak et al. (2010)
	55	150,0; 640,0 <sup>(b)</sup>	Viruses on filters within solid carriers	Thermophilic fermentation. Virus (titre: 5.8 log TCID <sub>50</sub> /mL) adsorbed on filters placed within small meat carriers.	
	55	600,0; 720,0 <sup>(b)</sup>	Viruses on filters within solid carriers	Thermophilic fermentation. Virus (titre: 5.8 log TCID <sub>50</sub> /mL) adsorbed on filters placed within large meat carriers.	
	55	240,0; 720,0 <sup>(b)</sup>	Viruses on filters within solid carriers	Thermophilic fermentation. Virus (titre: 5.8 log TCID <sub>50</sub> /mL) adsorbed on filters placed within bone carriers.	
	55	100,0; 443,1 <sup>(b)</sup>	Culture medium	Thermophilic fermentation. Viral suspension (titre: 5.8 log TCID <sub>50</sub> /mL) in tubes.	
	56,4	7236,2 <sup>(a)</sup>	Viruses on filters	Anaerobic thermophilic digestion. Virus (Haden strain) adsorbed on filters (10 <sup>6</sup> PFU/filter).	
	60,6	3171,8 <sup>(a)</sup>	Viruses on filters	Aerobic thermophilic digestion. Pressure 15 kPa. Virus (Haden strain) adsorbed on filters (10 <sup>6</sup> PFU/filter). Inactivation rate constant: 0,454 ± 0,018/day	
	70,5	2087,0 <sup>(a)</sup>	Viruses on filters	Pasteurisation. Virus (Haden strain) adsorbed on filters (10 <sup>6</sup> PFU/filter). Inactivation rate constant: 0,69 ± 0,018/day	

Virus	Temperature (°C)	D-value or point estimate of time to 1 log reduction (min)	Matrix	Experimental conditions	Reference
Canine parvovirus (CPV)	60	193,5 <sup>(b)</sup>	Blood and plasma-derived products	Pasteurisation, albumin concentration 4%	Gröner et al. (2018)
	60	545,5 <sup>(b)</sup>	Blood and plasma-derived products	Pasteurisation. Stabilised intermediates in plasma manufacturing processes (standard conditions).	
	60	400,0 <sup>b</sup>	Blood and plasma-derived products	Pasteurisation. Stabilised intermediates in plasma manufacturing processes (stabiliser concentration increased to 110%).	
	58	517,2 <sup>(b)</sup>	Blood and plasma-derived products	Pasteurisation. Stabilised intermediates in plasma manufacturing processes (temperature reduced to 58°C).	
Minute virus of mice (MVM)	90	0,1; 0,2 <sup>(b)</sup>	Glucose concentrate solution	HTST pasteurisation in 50% glucose solution. Viral titre: 10 <sup>7</sup> TCID <sub>50</sub> /mL	Gemmell et al. (2021)
Porcine parvovirus (PPV)	60	73,7 <sup>(a)</sup>	Blood and plasma-derived products	Pasteurisation. NADL-2 strain, titre: 10 <sup>8</sup> TCID <sub>50</sub> /mL	Blumel et al. (2002)
	52	1006,2 <sup>(a)</sup>	Faecal suspension	Strain 893/76, titre: 10 <sup>7,2</sup> TCID <sub>50</sub> /g	Elving et al. (2014)
	55	1336,8 <sup>(a)</sup>	Saline solution	Strain 893/76, titre: 10 <sup>7,2</sup> TCID <sub>50</sub> /mL	
	70	74,2 <sup>(a)</sup>	Saline solution	Strain 893/76, titre: 10 <sup>7,2</sup> TCID <sub>50</sub> /mL	
	60	24,7 <sup>(a)</sup>	Blood and plasma-derived products	Pasteurisation. Viral titre: 4.88 log TCID <sub>50</sub> /mL	Huangfu et al. (2017)

(a): D value.

(b): Point estimates of time to 1 log reduction.

Figure B.1 Shows the time required for 3 log<sub>10</sub> reduction of the four families of viruses at different temperatures.



**Figure B.1:** Time in minutes to achieve 3 log<sub>10</sub> reduction of the pre-selected families of virus *parvoviruses* in different matrixes (mixed liquid, whole milk, ground beef, digestion waste) and temperatures obtained from the ELS (3-fold the estimated D value, assuming log-linear behaviour). Lines represent the fit of the Bigelow model to the corresponding data set. Blue: Circoviridae. Green: Anelloviridae. Red: Adenoviridae. Brown: Parvoviridae

## Appendix C – Results of the model for other indicators

The estimation of the level of inactivation and probabilities of inactivation by the heat treatments against *S. Senftenberg* as estimated by the model are displayed in Table C.1. Despite the most conservative approach applied by the model, the results showed a probability of inactivation of at least 5 log<sub>10</sub> of 100% for all methods and implementation modes.

**Table C.1:** Probability of achieving specific log<sub>10</sub> reductions in different ranges, and expected value of log<sub>10</sub> reduction (in brackets) of *Salmonella* Senftenberg after application of coincidental and consecutive heat treatments

Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
<b>2</b>	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
		(39,620)	(93,502)	
<b>3</b>	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
		(30,110)	(51,664)	
<b>4</b>	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.001	0.000	
	L ≥ 12	0.999	1.000	
		(5,071)	(12,678)	
<b>5</b>	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
		(718)	(1,077)	
<b>7, Scenario 1</b>	L < 5			0.000
	5 ≤ L < 6			0.001
	6 ≤ L < 8			0.001
	8 ≤ L < 10			0.002
	10 ≤ L < 12			0.002
	L ≥ 12			0.994
			(333)	
<b>7, Scenario 2</b>	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			1.000
			(28,526)	

Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
7, Scenario 3	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			1.000
				(17,750)
7, Scenario 4	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.001
	L ≥ 12			0.999
				(3,169)
7, Scenario 5	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.001
	L ≥ 12			0.999
				(1,585)

The estimation of the lethality and probabilities of inactivation by the heat treatments against *E. faecalis* as estimated by the model are displayed in Table C.2. Despite the most conservative approach applied by the model, the results showed a probability of 0.99 or above of an inactivation of *E. faecalis* equal or greater than 5 log<sub>10</sub>.

**Table C.2:** Probability of achieving specific log<sub>10</sub> reductions in different ranges, and expected value of log<sub>10</sub> reduction (in brackets) of *Enterococcus faecalis* after application of coincidental and consecutive heat treatments

Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
2	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
		(615)	(1,452)	
3	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
		(467)	(802)	
4	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.001	0.000	
	L ≥ 12	0.999	1.000	
		(78.8)	(197)	

Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
<b>5</b>	L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
			(590)	(886)
<b>7, Scenario 1</b>	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.001
	L ≥ 12			0.999
				(68.9)
<b>7, Scenario 2</b>	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			1.000
				(443)
<b>7, Scenario 3</b>	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			1.000
				(275)
<b>7, Scenario 4</b>	L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.001
	10 ≤ L < 12			0.002
	L ≥ 12			0.997
				(49.2)
<b>7, Scenario 5</b>	L < 5			0.001
	5 ≤ L < 6			0.002
	6 ≤ L < 8			0.010
	8 ≤ L < 10			0.024
	10 ≤ L < 12			0.040
	L ≥ 12			0.924
				(24.6)

The estimation of the lethality and probabilities of inactivation by the heat treatments against *Parvovirus* as estimated by the model are displayed in Table C.3. All combinations of methods and types of application have a probability of 0.99 or above to achieve an inactivation greater than 3 log<sub>10</sub> except for method 7 scenario 1 having a probability of 0.023 of achieving an inactivation greater than 3 log<sub>10</sub>.

**Table C.3:** Probability of achieving specific  $\log_{10}$  reductions in different ranges, and expected value of  $\log_{10}$  reduction (in brackets) of Parvovirus after application of coincidental and consecutive heat treatments

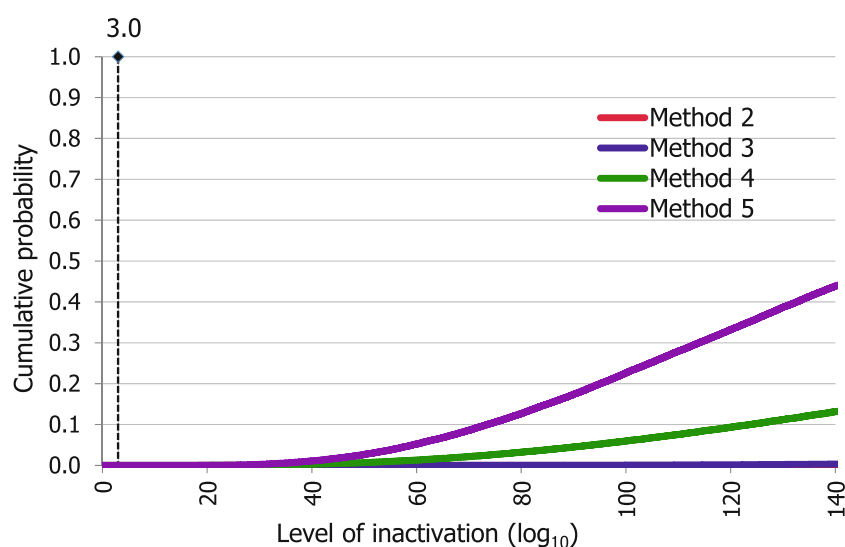
Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
<b>2</b>	L < 3	0.000	0.000	
	3 ≤ L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
			(2,470)	(3,332)
<b>3</b>	L < 3	0.000	0.000	
	3 ≤ L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
			(1,007)	(1,290)
<b>4</b>	L < 3	0.000	0.000	
	3 ≤ L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
			(330)	(553)
<b>5</b>	L < 3	0.000	0.000	
	3 ≤ L < 5	0.000	0.000	
	5 ≤ L < 6	0.000	0.000	
	6 ≤ L < 8	0.000	0.000	
	8 ≤ L < 10	0.000	0.000	
	10 ≤ L < 12	0.000	0.000	
	L ≥ 12	1.000	1.000	
			(154)	(160)
<b>7, Scenario 1</b>	L < 3			0.978
	3 ≤ L < 5			0.022
	5 ≤ L < 6			0.001
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			0.000
				(1,592)
<b>7, Scenario 2</b>	L < 3			0.000
	3 ≤ L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.001
	L ≥ 12			0.999
				(102)
<b>7, Scenario 3</b>	L < 3			0.000
	3 ≤ L < 5			0.000
	5 ≤ L < 6			0.000



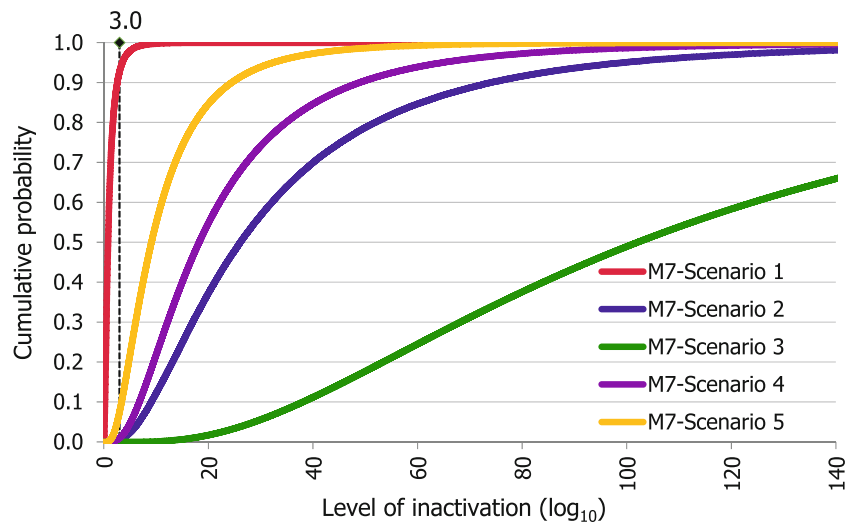
Method	Log <sub>10</sub> reduction	Probability of reduction in coincidental mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction in consecutive mode (Expected value of log <sub>10</sub> reduction)	Probability of reduction (Expected value of log <sub>10</sub> reduction)
7, Scenario 4	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.000
	L ≥ 12			1.000
				(1,370)
	L < 3			0.000
	3 ≤ L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
10 ≤ L < 12			0.000	
L ≥ 12			1.000	
			(332)	
7, Scenario 5	L < 3			0.000
	3 ≤ L < 5			0.000
	5 ≤ L < 6			0.000
	6 ≤ L < 8			0.000
	8 ≤ L < 10			0.000
	10 ≤ L < 12			0.001
	L ≥ 12			0.999
				(166)

The log<sub>10</sub> reduction of parvoviruses achieved by the inactivation methods are also illustrated as cumulative distributions in Figures C.1 and C.2. In this case, the lethality of the methods has the decreasing order method 2 > method 3 > method 4 > method 5. The probability of insufficient inactivation (lower than 3 log<sub>10</sub>) is associated with the left tail and when the expected value of the distribution is high (as is the case of methods 2, 3 and 4), such risks manifest themselves as rare events. In Figure C.1, the vertical threshold of 3 log<sub>10</sub> cycles was placed on the graph for comparison with the target reduction.

For method 7, the mildness of the heat regimes of scenario 1 is evident in the respective lethality cumulative distributions of Figure C.2. This graph shows that the lethality against parvoviruses is higher in scenario 3, followed by scenario 2, scenario 4 and scenario 5, with scenario 1 on the left side of the threshold.



**Figure C.1:** Cumulative probability of the lethality of the heat treatment methods 2, 3, 4 and 5 in coincidental mode against parvoviruses



**Figure C.2:** Cumulative probability of the lethality of the five scenarios of heat treatment method 7 (M7) against parvoviruses

## Appendix D – Report on expert knowledge elicitation

### Description and methodology

The EKE (expert knowledge elicitation) questions concerned one hazard (*C. perfringens* spores) and nine methods (processes), making a total of nine data points to be assessed (Table D.1).

The EKE question was specified as follows: 'What is the probability that a 5 log<sub>10</sub> reduction of spores of *Clostridium perfringens* is achieved, in more than 99% of cases, by application of each of the relevant processes (methods 2, 3, 4, 5 in coincidental mode and five t/T combinations of method 7), assuming that the processes are performed as prescribed and that the indicated process conditions are achieved?'

**Table D.1:** Processes assess in the EKE

Data point	Process	t/T combinations
1	Method 2 – coincidental	100°C × 5' – 110°C × 70' – 120°C × 50'
2	Method 3 – coincidental	100°C × 40' – 110°C × 42' – 120°C × 13'
3	Method 4 – coincidental	100°C × 3' – 110°C × 5' – 120°C × 5' – 130°C × 3'
4	Method 5 – coincidental	80°C × 60' – 100°C × 60'
5	Method 7A – scenario 1	80°C × 14'
6	Method 7B – scenario 2	95°C × 90'
7	Method 7C – scenario 3	115°C × 56'
8	Method 7D – scenario 4	125°C × 10'
9	Method 7E – scenario 5	133°C × 5'

It is assumed that the standard process is correctly performed, under the conditions indicated by the process parameters, and as described in the opinion. Variability in process performance is not to be considered in this assessment. However, even without any variation in process performance, the log<sub>10</sub> reduction achieved will vary to some extent from case to case. The question to answer is whether the target log<sub>10</sub> reduction will be achieved in more than 99% of cases, because 100% may be too unrealistic and would become dependent on 'exceptional cases'. Thus, the 'probability' in the question refers to uncertainty, not variability. Specifically, it expresses the degree of certainty that the target log<sub>10</sub> reduction will be achieved in more than 99% of the cases.

The EKE consisted of two steps:

- Step 1: individual judgements (4 April to 13 April 2023)
- Step 2: consensus judgement (19 April 2023)

The experts comprised six Working Group (WG) members developing the opinion, plus one EFSA scientist who was supporting the WG. The elicitation was facilitated by an elicitor (hearing expert). A member of the EFSA scientific staff was appointed as rapporteur.

The EKE section was recorded, only as a support to prepare the notes. This recording has been deleted to assure anonymity of the experts.

### Step 1: Individual judgements

Training was delivered to all participants on the general concept of probability, EFSA's approximate probability scale, uncertainty, variability and EKE.

During Step 1, the participants had 1 week to provide individual judgements for each of the nine processes, taking into account the version of the draft opinion at the beginning of the process (4 April 2023) with the evidence on thermal inactivation of the *C. perfringens* spores (including the data and modelling results), the description of the processes and the integration of the evidence and the uncertainty table, as well as the personal expertise and assessment of the uncertainties involved. To perform the individual judgements, the experts received by e-mail a spreadsheet with a template to provide their answers. They did not discuss their judgements with other experts at this stage.

The answer for each process was given as a probability range that reflects the expert's degree of certainty that the indicated log<sub>10</sub> reduction is achieved. These probability ranges could be one of those given in the approximate probability scale presented in EFSA's uncertainty guidance (Table D.2) or any

other. The participants were encouraged to give explanations of the reasons for each subjective probability range.

The options included in the template were:

**Table D.2:** Probability scores proposed to the experts

99–100% (almost certain)
95–99% (extremely likely)
90–95% (very likely)
66–90% (likely)
33–66% (about as likely as not)
10–33% (unlikely)
5–10% (very unlikely)
1–5% (extremely unlikely)
0–1% (almost impossible)
100% (certain)
50–100% (more likely than not)
0–50% (more unlikely than likely)
0–100% (inconclusive)
Other (to be defined by the participant)

## Step 2: Consensus judgement

The next step was to reach a consensus judgement for the datapoints of each of the nine processes during the open session in the WG meeting. It was explained that the consensus is not an average of the individual judgements, or a compromise where some experts defer to the judgement of other participants. The experts were asked to consider what a rational impartial observer (RIO) would judge, having considered the evidence, uncertainties, the individual judgements and having heard the discussion. Consensus can be any probability range, not necessarily one from the table with standard subjective probability ranges.

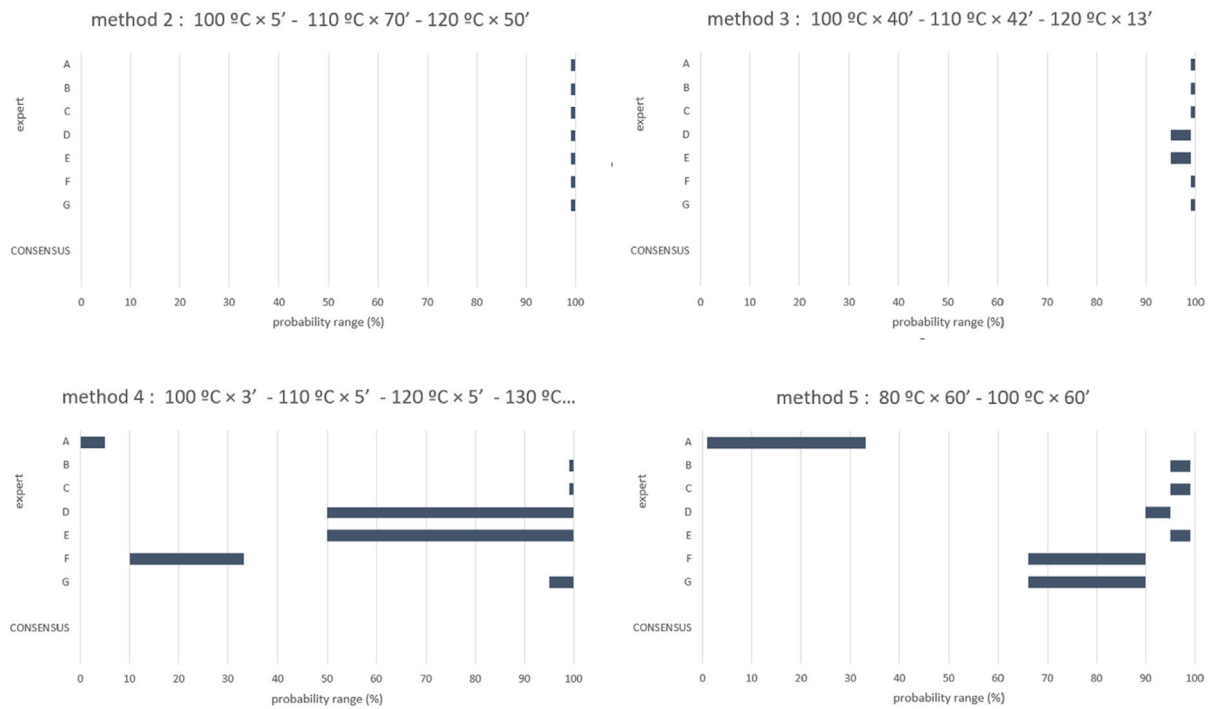
After getting an overview of the individual expert judgements obtained in step 1, at the beginning of the EKE session, the participants expressed the rationale behind their individual judgements, to clarify potential generic biases in their judgements.

The stepwise approach applied for each of the combinations consisted of the following actions:

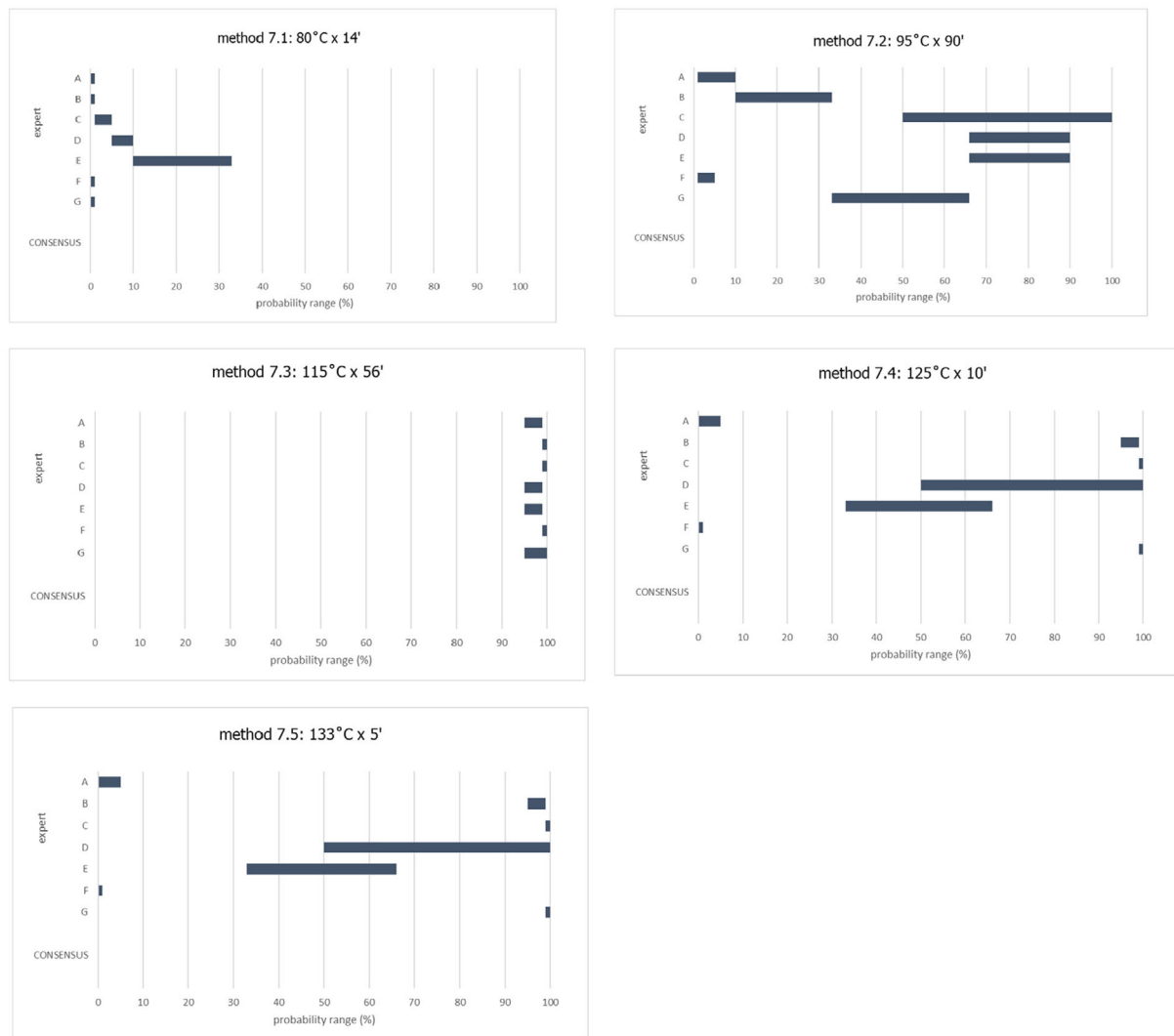
- Recall the processes and time/temperature combinations and target  $\log_{10}$  reduction (i.e.  $5 \log_{10}$ ).
- Display graphs showing expert ranges.
- Invite some experts (most deviating to explain the reasoning for their judgements).
- Invite experts to review/revise own judgements.
- Propose a consensus judgement and ask experts if this range reflects what a RIO would think. If needed, discuss and agree on the upper or lower range of the consensus judgement.
- Check that the notes taken have captured the key reasons for the consensus judgements. responses to the proposed consensus ranges for the indicators

## Results of Step 1: Individual judgements

Individual judgements were obtained from seven experts. The results are illustrated in Figures D.1 and D.2. Both figures show that there is large agreement between individual experts for some of the processes, and large disagreement for others. These results were discussed during Step 2 of the EKE.



**Figure D.1:** The individual elicited probability ranges by each of the seven experts for achieving a probability of  $5 \log_{10}$  reduction of spores of *Clostridium perfringens*, in more than 99% of cases, by application of methods 2, 3, 4, 5 in coincidental mode, assuming that the processes are performed as prescribed and that the indicated process conditions are achieved



**Figure D.2:** The individual elicited probability ranges by each of the seven experts for achieving a probability of 5  $\log_{10}$  reduction of spores of *Clostridium perfringens*, in more than 99% of cases, by application of the five t/T combinations of method 7, assuming that the processes are performed as prescribed and that the indicated process conditions are achieved

### Individual expert's rationales for the individual judgements, as discussed at the start of Step 2

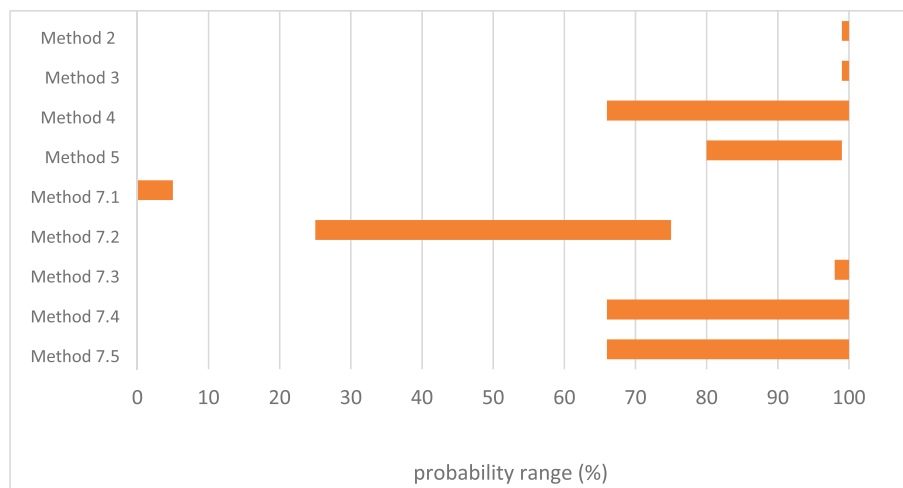
- Expert A based the judgements mainly on the output of the model with and without temperature extrapolation, was very strict, and considered the judgements made as too conservative. Inactivation of spores of *C. botulinum* was also considered.
- Expert B used the model outputs with and without extrapolation. This expert handled the data and has run the model with subsets of data and considers that the judgements therefore may be biased.
- Expert C based the judgement on the model output without extrapolation as primary source considering there could be underestimation. The expert also considered inactivation of spores of *C. botulinum* when the process used high temperatures and short times as in these cases the model output could be far from reality. The expert explained that spores of *C. botulinum* could be used as a surrogate for spores of *C. perfringens* as these are more heat resistant. The expert considered that a 12  $\log_{10}$  reduction of spores of *C. botulinum* is accepted by public health authorities (equivalent to 3 min at 121.1°C. If that is accomplished, the expert considered that at least a 5  $\log_{10}$  reduction of spores of *C. perfringens* is achieved.

- Expert D considered the output of the model in relation to the achievement of at least 5 log<sub>10</sub> and between 5 and 6 log<sub>10</sub> reduction of spores of *C. perfringens*.
- Expert E based the judgement on the output of the model without extrapolation and considered that the model underestimates the inactivation. The expert did not consider inactivation of spores of *C. botulinum*.
- Expert F based on judgement mainly on the output of the model.
- Expert G used the entire information from the scientific opinion, not only the output of the model with and without extrapolation, but also the distribution of the D-values at the relevant temperatures. The expert considered that the output of the model without extrapolation is underestimating the inactivation, depending on the method at hand (if the temperature is further from Dref, there is more underestimation).

## Results of the consensus for each combination

After discussion among the experts, consensus was achieved on the probability ranges that were considered to best represent the uncertainty on whether 5 log<sub>10</sub> reduction of spores of *C. perfringens* is achieved with each of the relevant processes. Consensus implied that the experts agreed that a RIO, considering the evidence and following the discussion, would conclude that the elicited probability range was appropriate.

Figure D.3 gives the outcome of the consensus reached for each process, while Table D.3 provides the main arguments for obtaining these ranges, for each of the nine processes.



**Figure D.3:** The consensus judgement for achieving a probability of 5 log<sub>10</sub> reduction of spores of *Clostridium perfringens*, in more than 99% of cases, by application of the t/T combinations of methods 2–5 and method 7, assuming that the processes are performed as prescribed and that the indicated process conditions are achieved

**Table D.3:** Summary of the rationale for the consensus of each process, as recorded by the rapporteur

Process	Summary of rationale as recorded by the rapporteur
<b>Method 2 – coincidental</b>  100°C × 5' – 110°C × 70' – 120°C × 50'	<p>There was a full agreement between individual experts for this process; all experts provided individual elicited probability ranges of 99–100%.</p> <p>Expert C explained that the model output, without extrapolation, predicts more than 10 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i>, based on the time/temperature profile of the treatment. A higher inactivation is expected as the actual process temperature would be higher.</p> <p>The elicitor proposed a <b>consensus judgement of 99–100%</b> and this was agreed.</p>
<b>Method 3 – coincidental</b>  100°C × 40' – 110°C × 42' – 120°C × 13'	<p>There was good agreement between individual experts for this process. Five experts provided individual elicited probability ranges of 99–100%, while two experts (expert D and E) provided ranges of 95–99%.</p> <p>Expert D gave a lower probability compared to method 2 considering that the processing time at 120°C is lower compared to the previous process.</p> <p>Expert C stated that the inactivation of spores of <i>C. perfringens</i> in method 3 would still be very high. The predicted inactivation, based on the model output, is beyond 6–8 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> and the actual processing temperature would be higher than that. Expert G confirmed that the probability was 100% for at least a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> and as the temperature is higher than the temperature used for Dref (i.e. 105°C), the inactivation is underestimated.</p> <p>Experts D and E, after hearing the arguments of the other experts, agreed to increase their probability range to 99–100%.</p> <p>The elicitor proposed a <b>consensus judgement of 99–100%</b> and this was agreed.</p>
<b>Method 4 – coincidental</b>  100°C × 3' – 110°C × 5' – 120°C × 5' – 130°C × 3'	<p>There was a large disagreement between individual experts for this process.</p> <p>Expert A (providing a probability range of 0–5%) considered the high probability (82.1%) in the model output that a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> would not be achieved. The expert considered extrapolation. The expert informed that the probability could be revised to values higher than 50% if also the inactivation of spores of <i>C. botulinum</i> would be considered.</p> <p>Expert G (providing a probability range of 95–100%), stated that the probability was about 18% for at least a 5 log<sub>10</sub> inactivation of <i>C. perfringens</i>, based on model predictions. There is a very high underestimation as high temperatures were used for short times. Even if <i>C. perfringens</i> is the focus, spores of <i>C. botulinum</i> are more resistant. Therefore, the expert is quite sure that if 5 log<sub>10</sub> inactivation of spores of <i>C. botulinum</i> would be met, it would also be the case for spores of <i>C. perfringens</i>. Therefore, this expert increased the probability range, but judged a slightly lower probability range compared to method 3 considering the time/temperature (t/T) combinations.</p> <p>Expert B (providing a probability range of 99–100%) considered the model output using extrapolation as high temperatures were used in this process and the model without extrapolation is 'truncated' at 105°C (Tref). As the model did not consider the temperature come-up time, the expert was certain that a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> is achieved.</p> <p>Expert C (providing a probability range of 99–100%) based the judgement on <i>C. botulinum</i>. The expert informed the group that the actual temperature is almost 30°C higher than Tref. An inactivation equivalent to more than 27 min at 121°C would be expected for spores of <i>C. botulinum</i>, sufficient to ensure 12 log<sub>10</sub> reductions of those spores (3 min is enough). Therefore, the expert considered that inactivation of spores of <i>C. perfringens</i> would be almost certain. However, the expert proposes to broaden the probability range to increase the uncertainty.</p> <p>Expert F (providing a probability range of 10–33%) only considered <i>C. perfringens</i> and found it unlikely that there would be a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i>, based on the model outputs.</p>



Process	Summary of rationale as recorded by the rapporteur
	<p>Expert A (providing a probability range of 0–5%) asked to agree on the extent to use the data on inactivation of spores of <i>C. botulinum</i> in the judgements. If so, it would become very likely to achieve a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i>.</p> <p>Expert B informed that the probability range could be lowered from 99–100% to 95–100%.</p> <p>Expert E stated that the reasoning to consider <i>C. botulinum</i> as a surrogate/indicator for <i>C. perfringens</i> needs to be well explained in the opinion. Also, the underestimation of the model should be better explained in the opinion. This was agreed by expert G.</p> <p>Expert D provided a large probability range (of 50–100%) as the probability of at least a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> is low (about 18%), but there is high probability to achieve between 5 and 6 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i>. The expert would stay on the right side of the probability scale but use a range wider than 95–100%.</p> <p>Expert B was asked to explain the model outputs. The expert recalled that the model was truncated at 105°C. When temperatures are above 105°C, there is more uncertainty and underestimation (but not clear to what extent).</p> <p>Expert E then proposed to have a large probability range (of 50–100%), which was agreed by expert F.</p> <p>The elicitor proposed 66% as lower limit of the probability range which was agreed. The upper limit for some experts was 100% and it was agreed to use this value as an upper limit considering also <i>C. botulinum</i> spores' inactivation.</p> <p>Thus, the agreed <b>consensus judgement was 66–100%</b>.</p>
<p><b>Method 5 – coincidental</b></p> <p>80°C × 60' – 100°C × 60'</p>	<p>There was again a large disagreement between individual experts for this process.</p> <p>Expert A (providing a probability range of 1–33%) based on discussions for other process, believes that spores of <i>C. botulinum</i> have low mortality here, so the expert is not positive to reach a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> in 99% of cases.</p> <p>Expert C (providing a probability range of 95–99%) explained that the predicted inactivation of the temperature profile is higher than 5 log<sub>10</sub> with a 99% probability. The actual temperature is within the limits where there are published data available, so the prediction is expected to be close to reality. The inactivation is more likely than not but would broaden the range provided.</p> <p>Expert B (providing a probability range of 95–99%) said that the models output can be trusted without extrapolation considering the temperatures used in this process. Considering additionally the come-up time, the provided range is believed still valid. This was agreed by expert E.</p> <p>Expert G (providing a probability range of 66–90%) believes the model overestimates the inactivation of spores of <i>C. botulinum</i> because the product would be quite dehydrated through the treatment.</p> <p>Expert F (providing a probability range of 66–90%) considered mainly the model outputs and expects explanations to be added in opinion.</p> <p>Expert A doubted about the upper range of 99% considering the model outputs. The elicitor explained that the model percentages are to be considered also uncertainties. The expert then agreed that 'more likely than not' should be the outcome. Adding uncertainty, the expert would agree with a probability range of 66–90%.</p> <p>The elicitor proposed 66% as lower limit of the probability range. According to expert B, it should be rather 90% as explained before, also considering dehydration of the product. Expert D agrees with 90% as lower limit considering the model output and the estimated inactivation of spores of <i>C. perfringens</i> between 5 and 6 log<sub>10</sub>.</p> <p>The agreed <b>consensus judgement was 80–99%</b>.</p>

Process	Summary of rationale as recorded by the rapporteur
<b>Method 7 – scenario 1</b> 80°C × 14'	<p>There was some level of agreement between individual expert judgements for this process. Most extreme were four experts providing individual elicited probability ranges of 0–1%, while one expert (expert E) provided a range of 10–33%.</p> <p>Expert E wanted to give a lower probability as well but then considered the underestimation of the model (through the come-up time). The expert would agree to lower the probability.</p> <p>Expert D explained that the probability was 100% for less than a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i>, and the probability is 0% when between 5 to 6 log<sub>10</sub>.</p> <p>Expert C considers that method is quite insufficient (based on the t/T profiles) to inactivate spores of <i>C. perfringens</i>, but it also needs to be considered that the data used in the model relies on highly resistant spores. Only if all the spore population would have low heat resistance, a 5 log<sub>10</sub> reduction could be accomplished.</p> <p>The agreed <b>consensus judgement was 0–5%</b>.</p>
<b>Method 7 – scenario 2</b> 95°C × 90'	<p>There was again a large disagreement between individual experts for this process, with experts A and F providing the lowest probability ranges.</p> <p>The model output yields a probability of 31.9% that a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> would not be achieved, based on the t/T profile of the method.</p> <p>Expert F would agree to increase the probability range. Expert A informed that there is no need to extrapolate, and <i>C. botulinum</i> inactivation is not to be used. The probability would be rather below 50%.</p> <p>Expert E (providing a probability range of 66–90%) would rather have a probability beyond 50% considering the under-estimation of the inactivation by the model.</p> <p>Expert C (providing a probability range of 50–100%) considers the probability more likely than not also considering there was no extrapolation needed. The expert would lower the upper limit to 90%.</p> <p>The elicitor questioned if the lower limit should be below or above 50%.</p> <p>According to expert B (providing a probability range of 10–33%), the range should be quite low (below 50%) as the mean inactivation of spores of <i>C. perfringens</i> is 7 log<sub>10</sub>.</p> <p>Expert G (providing a probability range of 33–66%), also considered that in most of the studies it needs more than 90' to achieve a 5D inactivation of spores of <i>C. perfringens</i>.</p> <p>The elicitor then asked about upper limit. Expert C agreed to lower it to 90%. Expert B proposes 66%.</p> <p>Expert F proposed to use 33–66% as probability range as this would reflect that there is a high uncertainty.</p> <p>The elicitor questioned whether the upper limit is acceptable considering the model output (68.1% that a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> would be achieved); hence the model must be overestimating.</p> <p>Expert E further questioned the divergence with method 5 where we were more certain. According to expert C the impact of a small change in temperature is significant.</p> <p>A higher value of the upper range (80%) was proposed to reflect uncertainty. Finally expert C proposed <b>25–75%, which was agreed as consensus judgement.</b></p>
<b>Method 7 – scenario 3</b> 115°C × 56'	<p>There was a high level of agreement between individual experts for this process; all experts provided high probability ranges.</p> <p>The model output yields a probability of 100% that a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> would be achieved, based on the t/T profile of the method.</p> <p>Expert C (providing a probability range of 99–100%) had a high level of confidence as the predicted inactivation based on the t/T profile is higher than 6–8 log<sub>10</sub> and considered that truncation was applicable as the actual temperature is higher. The method would reach 12 log<sub>10</sub> reduction of spores of <i>C. botulinum</i>, with an equivalent time at 121°C of 13.7 min.</p>

Process	Summary of rationale as recorded by the rapporteur
	<p>Expert F (providing a probability range of 99–100%) followed the same reasoning based on the t/T profile.</p> <p>Expert E (providing a probability range of 95–99%) considered that the t/T profile is a bit less stringent than method 2 and therefore gave somewhat lower values. The expert informed that the probability could be increased.</p> <p>Expert G (providing a probability range of 95–100%) said that 100% should be included in the range.</p> <p>Expert A (providing a probability range of 95–99%) had the same arguments.</p> <p>The <b>upper limit of 100% as consensus judgement</b> was agreed by all. There was no clear argument for underestimation of the model and <b>98% as lower limit</b> was agreed.</p>
<p><b>Method 7 – scenario 4</b></p> <p>125°C × 10'</p>	<p>There was a large disagreement between individual experts for this process, with experts A and F again providing the lowest probability ranges.</p> <p>The model output yields a probability of 98.5% that a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> would not be achieved, based on the t/T profile of the method. In this process higher temperatures are used, so there is an extrapolation effect.</p> <p>Expert G (providing a probability range of 99–100%), as in method 4, indicated that there is a huge underestimation in the model outputs as the process relies on high temperature/short time processing and considered inactivation of spores of <i>C. botulinum</i>. The expert would consider revising the probability range to the consensus reached for method 4.</p> <p>Expert C (providing a probability range of 99–100%) followed the same reasoning. When inactivation of spores of <i>C. botulinum</i> is achieved an inactivation equivalent to more than 24.7 min at 121°C would be expected, sufficient to ensure 12 log reductions of <i>C. botulinum</i>, the expert gave a high probability, similarly to method 4.</p> <p>Expert A (providing a probability range of 0–5%) agreed and would be fine to revise the range as agreed for method 4. Expert F (providing a probability range of 0–1%) agreed as well.</p> <p>Expert E (providing a probability range of 33–66%) is in middle of the probability range. The expert is uncertain, and the arguments are the same. The model predictions are high but there is a larger underestimation. The expert did not consider <i>C. botulinum</i>, but only the model output and underestimation.</p> <p>The elicitor asked if a comparable range to method 4, method 7 scenario 4 and method 7 scenario 5 would apply (66–100%) as the arguments are the same. Expert B (providing a probability range of 95–99%) would not include 100% as the highest temperature is different and there are more come-up times in method 4. Expert C believes that 100% is reasonable as these conditions would lead to a safe process (accepted as a safe for pet food in legislation).</p> <p>The elicitor proposed a <b>consensus judgement of 66–100%</b> and this was agreed</p>
<p><b>Method 7 – scenario 5</b></p> <p>133°C × 5'</p>	<p>The model output yields a probability of 100% that a 5 log<sub>10</sub> inactivation of spores of <i>C. perfringens</i> would not be achieved, based on the t/T profile of the method.</p> <p>The elicitor proposed for this process <b>the same consensus judgement as for method 7 scenario 5 (66–100%)</b> comparing the t/T profiles of both methods and the individual expert judgements. This was agreed.</p>

## **Annex A – Protocol for the assessment of the efficacy of methods 2 to 5 and method 7 to inactivate relevant pathogens when producing processed animal protein of porcine origin intended to feed poultry and aquaculture animals (EFSA-Q-2022-00455)**

Protocol for the assessment of the efficacy of methods 2 to 5 and method 7 to inactivate relevant pathogens when producing processed animal protein of porcine origin intended to feed poultry and aquaculture animals (EFSA-Q-2022-00455) is available under the Supporting Information section on the online version of the scientific output.