Annex to: Scientific opinion on the 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. doi:10.2903/j.efsa.2023.8093

Annex A – Protocol for the 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 (EFSA-Q-2022-00455)

A.1 Introduction

A.1.1 Scope of this protocol

This document outlines the protocol for the scientific assessment of 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. The protocol will be used as input for the scientific opinion of the EFSA Panel on Biological Hazards (BIOHAZ) on 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.

This protocol was developed with the aim of defining the methods for collecting data, appraising the relevant evidence, and analysing and integrating the evidence in light of the identified uncertainties. It was developed following the principles and process defined in a project that aimed to further improve EFSA's scientific assessment processes (EFSA, 2015) and based on the recommendations for protocol development described in the draft framework for protocol development for EFSA's scientific assessments (EFSA, 2020).

The protocol was drafted by the WG members and was approved by the BIOHAZ Panel at their 159th plenary meeting (25-26 January 2023).

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

Background: see scientific opinion.

ToR: The Commission requested EFSA to provide a scientific opinion concerning the efficacy of methods 2 to 5 and method 7 as set out in Regulation (EU) No 142/2011 to inactivate relevant pathogens when producing PAP 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.

A.1.3 Interpretation of the ToRs of the mandate

Initial clarification was requested to the EC on whether the Cat 3 material considered by the mandate should be only of the EU origin and not sourced from third countries, since the criteria to select relevant pathogens could differ. Following a posteriori decision by the WG on the criteria to select the relevant pathogens (see AQ1), which was explained to the EC, the requestor agreed fully with the criteria. No further clarification was requested on the source of the materials nor on the criteria for selection of relevant pathogens.

A.2 Problem formulation

The ToRs of the mandate were translated into five assessment question(s) (AQs). Their relationship is shown through the conceptual model shown in Figure A.1.

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

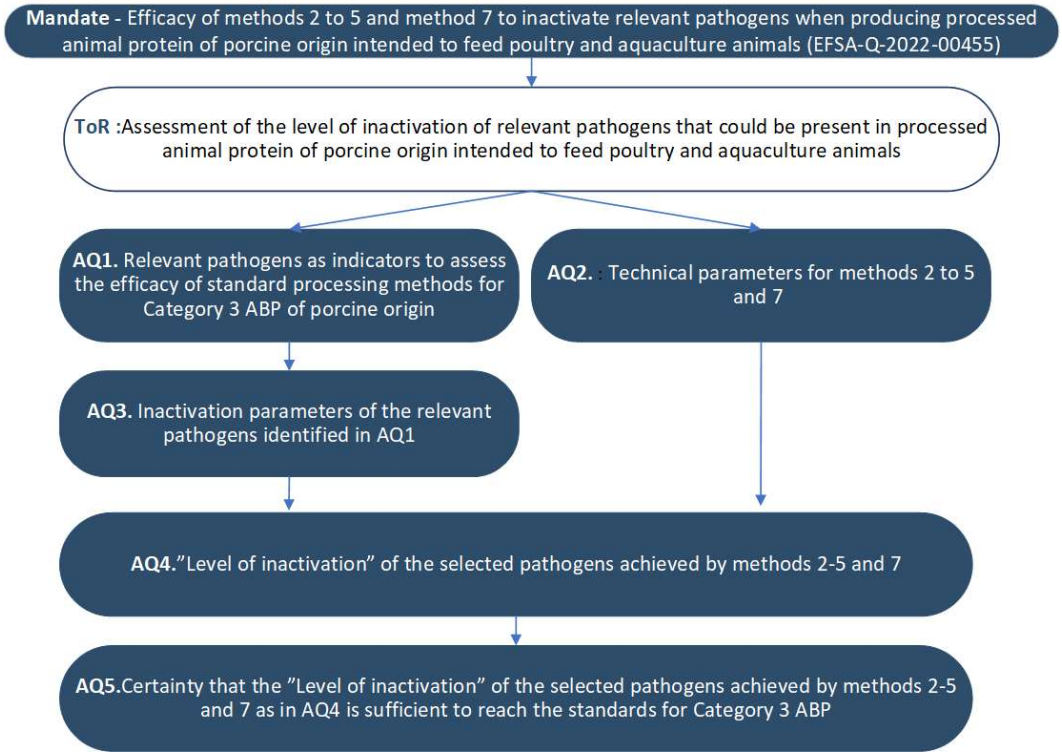

Figure A.1: The relationship between the assessment questions (AQs) for the assessment of the efficacy of methods 2 to 5 and method 7 to inactivate relevant pathogens when producing processed animal protein of porcine origin intended to feed poultry and aquaculture animals

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

The second step includes the overall approach, as well as the evidence needs and the methods, for answering each AQ including uncertainty analysis (i.e. the use of a literature review, data from databases, expert judgement or primary data collection). Table A.1 provides this information.

The methods that will be used for evidence integration across AQs and for accounting for the remaining uncertainty are provided in Table A2 based on the conceptual model.

Table A.1: Assessment questions and sub-questions 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

Step 1.1	**Step 1.2**	**Step 1.3**	**Step 2.1**	**Step 2.1**
Assessment questions (reflecting clarification of ToRs)	**Sub-assessment questions *(if needed)***	**Overall approach**	**Evidence needs**	**Description of method to be used**
AQ1: What relevant pathogens can be used as indicators to assess the efficacy of standard processing methods for Category 3 ABP of porcine origin?		Develop criteria for relevance Non-systematic biological hazard identification Extensive Literature search	Hazards listed in the EU legislation List of diseases in the WOAH terrestrial manual EFSA scientific opinions Extensive literature search	**A. Criteria for selection of BACTERIAL relevant pathogens**: -previous EFSA standards applied for Category 3 -legislation on alternative methods for composting and biogas -indicators for method 7 As a result, three indicators will be used: *Salmonella senftenberg, Enterococcus faecalis* and spores of *Clostridium perfringens* **B. Criteria for selection of VIRAL relevant pathogens:** An initial list of families of virus will be produced based on the criteria below and on the structure (non-enveloped) and the genomic structure (DNA). Among the ones included in the initial list, only the most thermo-resistant will be selected for AQ5, based on the outputs of AQ3. -To be included in the WOAH list of swine diseases and multiple species OR -To be included in the AHAW risk mitigation opinion OR -Identified in a literature search on virus presence in pig matrices AND ((To be present in the EU OR pose significant risk of introduction into the EU) AND are pathogens to humans or animals)) **Literature review for the presence of viral pathogens in porcine matrices** • Describe all eligibility criteria for study selection (i.e. the criteria related to study e.g. target population, intervention/exposure of interest, and the relevant outcomes and record characteristics e.g. time, language, publication type) The aim is to retrieve information on viral pathogens to "sus scrofa" species present/ detected/isolated in pigs from slaughterhouse onwards: pig carcases, porcine products, etc. (AQ1) Articles must report detection directly linked to hazards of interest: virus. Language of the full text: English only Time: 1990 onwards (and with no restrictions in case of limited data) Publication type: original article describing primary research studies, reviews articles or book (chapter) in case they provide sufficient evidence. Exclusion of conference abstracts • Provide the rationale for the choice of the eligibility criteria

				The screening process will be undertaken in two or three steps: screening of (1) Title and abstract to exclude irrelevant records. Questions to address during the screening Question 1: Does the paper report presence/detection/isolation in pigs, pig carcasses, porcine products, tissues, fluids, excreta, etc., of the "sus scrofa" species etc. from slaughterhouse onwards of a viral pathogen to pigs/poultry? Yes/No/Maybe (2) Full-text screening documents to further identify records to be excluded based on criteria related to report characteristics (e.g. not in English) and study characteristics considering whether the record contains information about the presence of viral pathogens in the matrices of interest (AQ1). Question 2: Is the full text available? Yes/No Question 3: Is the paper in English? Question 4: Does the paper report presence/detection/isolation in pigs, pig carcasses, porcine products, of the ""sus scrofa"" species etc. from slaughterhouse onwards of a viral pathogen to pigs/poultry? (3) data: extraction name of the pathogen (family and species) and matrix and prevalence where it was found Question 5: Please select the Family of the virus identified? Question 6: Please select the Genus of the virus identified? Question 7: Please select the Species of the virus identified? Question 8: Please select the matrix in which the virus has been identified? Question 9: Are there data on prevalence/load of the viruses in the reported matrices? Question 10: What is the prevalence/load of the viruses in the selected matrices? • Describe the final search strategy in the protocol, i.e. search string(s) including planned limitations See below Indicate the information sources (bibliographic databases and grey literature resources) that will be searched: Web of ScienceTM Core Collection (SCI-Expanded, BKCI-S, ESCI, CCR-Expanded, IC), and CAB Abstracts • Describe any other search approaches (e.g., citation indexes, handsearching): No other search approaches will be applied. Title and abstract for the screening • Indicate any software (e.g., for reference management) that will be use: DistillerSR for screening and Endnote for reference management and extraction of articles • Indicate the number of reviewers: 3 WG members and 3 EFSA staff • Describe the method for study selection e.g., in parallel or not. Parallel screening in batches, followed by full review of the shortlisted records • If applicable, describe how conflicts will be solved, if and what Artificial Intelligence techniques will be used • Ad hoc meetings will be held by the pair of reviewers to discuss and solve the discrepancies by reaching consensus. If needed, a third party (the chair of the working group) will decide the inclusion or not. • Indicate the software that will be used for screening paper DistillerSR • Describe the main characteristics of data model (i.e. what data will be extracted from the included studies)

Presence, detection or isolation of virus
Matrices where the virus was isolated/detected
If prevalence data are available, it will be also extracted

- Indicate how data will be extracted (e.g. by two independent reviewers in parallel or one reviewer extracting and one validating the process)
  Hits will be split into batches and distributed for pairs of reviewers. Double screening in parallel
- Indicate the software that will be used for data extraction
  Manually. Collated in tabular format

CAB Abstracts (Web of Science platform)

Set	Query	Concept
#8	**#6 AND PY= 1990-2023 and Journal Article or Book Chapters or Conference Proceedings or Bulletin or Correspondence or Journal Issue or Book or Miscellaneous or Thesis or Annual Report or Bulletin Article (Document Types)**	Exclusion of conference abstracts
#7	**#6 AND PY= 1990-2023**	Time limit applied
#6	#5 AND LA=English	Language applied
#5	#4 AND #3 AND #2 AND #1	Virus AND Detection AND Pigs AND Setting
#4	TS=((("processing" OR "production" OR "producing") NEAR/3 (chain* OR environment* OR facility* OR industr* OR line)) OR "processing plant*" OR "production plant*" OR "producing plant*" OR abattoir* OR slaughter*)	Setting
#3	TS=(pig OR pigs OR swine OR pork OR "porcine product" OR hog OR hogs OR "sus scrofa" OR "s scrofa" OR porcine OR offal)	Pigs
#2	TS=(detect* OR isolate* OR "isolation*" OR presence OR present OR persist* OR permanent OR occurrence OR recurrence OR recurrent OR colonise OR colonisation OR colonize OR colonization OR survival OR surviving OR survive* OR maintenance OR stability OR prevailed OR outbreak* OR prevalen* OR test*)	Detection
#1	TI=(virus* OR viral OR adenovir* OR PAdV OR "Swine Fever" OR "porcine reproductive and respiratory syndrome" OR PRRSV OR SIRSV OR PRRS OR PERS OR aujsezky OR "aujeszky's" OR pseudorabies OR "suid herpesvirus" OR SHV1 OR SuHV1 OR SHV-1 OR "SuHV 1" OR "japanese encephalit*" OR "porcine circovirus" OR PCV2 OR "PCV 2" OR "porcine rotavirus*" OR "porcine parvovirus*" OR PPV OR "vesicular exanthema" OR "porcine parainfluenza" OR "porcine respiratory coronavirus*" OR PRCV OR "hepatitis E" OR "Paslahepevirus balayani" OR "porcine teschovirus*" OR ((Teschen OR Talfan) NEAR/3 disease*) OR "Cholera hog" OR "hog	Virus

					cholera" OR "Transmissible gastroenteritis" OR TGEV OR rinderpest OR coxsackievirus* OR echovirus* OR enterovirus* OR "Foot and mouth disease*" OR "FMD virus*" OR (Neethling NEAR/3 (disease* OR infection*)) OR "Lumpy skin disease" OR "marek disease*" OR "mareks disease*" OR "marek's disease*" OR "Newcastle disease*" OR "Influenza A" OR "influenzavirus A" OR nipah OR "Porcine epidemic diarrhea" OR PED OR norovirus* OR (Norwalk NEAR/3 (disease* OR infection*)) OR pestivirus* OR reovir* OR rotavirus* OR "Swine Vesicular Disease*" OR "SVD virus*"OR "foot and mouth disease" OR "hoof and mouth disease" OR rinderpest OR morbillivirus* OR phlebovirus* OR CSF OR CSFV OR pestivirus* OR ASF OR asfivirus* OR "avian influenza" OR H5N1 OR H7N9 OR H5N8 OR H5N5 OR raby OR rabies OR "swine pox" OR swinepox OR suipoxvirus* OR "swine papillomavirus*" OR (getah NEAR/3 (disease* OR infection*)) OR GETV OR "Senecavirus A" OR SVA OR "Seneca valley fever" OR ("Torque Teno " NEAR/3 (infection* OR disease*)) OR TTV OR TTSuV OR "postweaning multisystemic wasting syndrome" OR "porcine encephalomyocardit*" OR "porcine cytomegalovirus*" OR "inclusion body rhinitis" OR "Lateral-shaking inducing neuro-degenerative agent" OR (Linda NEAR/3 (disease* OR infection*)) OR LindaV) OR AB=(virus* OR viral OR adenovir* OR PAdV OR "Swine Fever" OR "porcine reproductive and respiratory syndrome" OR PRRSV OR SIRSV OR PRRS OR PERS OR aujsezky OR "aujeszky's" OR pseudorabies OR "suid herpesvirus" OR SHV1 OR SuHV1 OR SHV-1 OR "SuHV 1" OR "japanese encephalit*" OR "porcine circovirus" OR PCV2 OR "PCV 2" OR "porcine rotavirus*" OR "porcine parvovirus*" OR PPV OR "vesicular exanthema" OR "porcine parainfluenza" OR "porcine respiratory coronavirus*" OR PRCV OR "hepatitis E" OR "Paslahepevirus balayani" OR "porcine teschovirus*" OR ((Teschen OR Talfan) NEAR/3 disease*) OR "Cholera hog" OR "hog cholera" OR "Transmissible gastroenteritis" OR TGEV OR rinderpest OR coxsackievirus* OR echovirus* OR enterovirus* OR "Foot and mouth disease*" OR "FMD virus*" OR (Neethling NEAR/3 (disease* OR infection*)) OR "Lumpy skin disease" OR "marek disease*" OR "mareks disease*" OR "marek's disease*" OR "Newcastle disease*" OR "Influenza A" OR "influenzavirus A" OR nipah OR "Porcine epidemic diarrhea" OR PED OR norovirus* OR (Norwalk NEAR/3 (disease* OR infection*)) OR pestivirus* OR reovir* OR rotavirus* OR "Swine Vesicular Disease*" OR "SVD virus*"OR "foot and mouth disease" OR "hoof and mouth disease" OR rinderpest OR morbillivirus* OR phlebovirus* OR CSF OR CSFV OR pestivirus* OR ASF OR asfivirus* OR "avian influenza" OR H5N1 OR H7N9 OR H5N8 OR H5N5 OR raby OR rabies OR "swine pox" OR swinepox OR suipoxvirus* OR "swine papillomavirus*" OR (getah NEAR/3 (disease* OR infection*)) OR GETV OR "Senecavirus A" OR SVA OR "Seneca valley fever" OR ("Torque Teno " NEAR/3 (infection* OR disease*)) OR TTV OR TTSuV OR "postweaning multisystemic wasting syndrome" OR "porcine encephalomyocardit*" OR	

					"porcine cytomegalovirus*" OR "inclusion body rhinitis" OR "Lateral-shaking inducing neuro-degenerative agent" OR (Linda NEAR/3 (disease* OR infection*)) OR LindaV) OR BD=(virus* OR viral OR adenovir* OR PAdV OR "Swine Fever" OR "porcine reproductive and respiratory syndrome" OR PRRSV OR SIRSV OR PRRS OR PERS OR aujsezky OR "aujeszky's" OR pseudorabies OR "suid herpesvirus" OR SHV1 OR SuHV1 OR SHV-1 OR "SuHV 1" OR "japanese encephalit*" OR "porcine circovirus" OR PCV2 OR "PCV 2" OR "porcine rotavirus*" OR "porcine parvovirus*" OR PPV OR "vesicular exanthema" OR "porcine parainfluenza" OR "porcine respiratory coronavirus*" OR PRCV OR "hepatitis E" OR "Paslahepevirus balayani" OR "porcine teschovirus*" OR ((Teschen OR Talfan) NEAR/3 disease*) OR "Cholera hog" OR "hog cholera" OR "Transmissible gastroenteritis" OR TGEV OR rinderpest OR coxsackievirus* OR echovirus* OR enterovirus* OR "Foot and mouth disease*" OR "FMD virus*" OR (Neethling NEAR/3 (disease* OR infection*)) OR "Lumpy skin disease" OR "marek disease*" OR "mareks disease*" OR "marek's disease*" OR "Newcastle disease*" OR "Influenza A" OR "influenzavirus A" OR nipah OR "Porcine epidemic diarrhea" OR PED OR norovirus* OR (Norwalk NEAR/3 (disease* OR infection*)) OR pestivirus* OR reovir* OR rotavirus* OR "Swine Vesicular Disease*" OR "SVD virus*"OR "foot and mouth disease" OR "hoof and mouth disease" OR rinderpest OR morbillivirus* OR phlebovirus* OR CSF OR CSFV OR pestivirus* OR ASF OR asfivirus* OR "avian influenza" OR H5N1 OR H7N9 OR H5N8 OR H5N5 OR raby OR rabies OR "swine pox" OR swinepox OR suipoxvirus* OR "swine papillomavirus*" OR (getah NEAR/3 (disease* OR infection*)) OR GETV OR "Senecavirus A" OR SVA OR "Seneca valley fever" OR ("Torque Teno " NEAR/3 (infection* OR disease*)) OR TTV OR TTSuV OR "postweaning multisystemic wasting syndrome" OR "porcine encephalomyocardit*" OR "porcine cytomegalovirus*" OR "inclusion body rhinitis" OR "Lateral-shaking inducing neuro-degenerative agent" OR (Linda NEAR/3 (disease* OR infection*)) OR LindaV)	

Web of Science Core Collection (Science Citation Index Expanded, Book Citation Index Expanded, Emerging Sources Citation Index, Current Chemical Reactions, Index Chemicus)

Set	Query	Concept
#7	**#6 AND PY=1990-2023**	Time limit applied
#6	#5 AND LA=English	Language applied
#5	#4 AND #3 AND #2 AND #1	Virus AND Detection AND Pigs AND Setting

				#4	TS=((("processing" OR "production" OR "producing") NEAR/3 (chain* OR environment* OR facility* OR industr* OR line)) OR "processing plant*" OR "production plant*" OR "producing plant*" OR abattoir* OR slaughter*)	
				#3	TS=(pig OR pigs OR swine OR pork OR "porcine product" OR hog OR hogs OR "sus scrofa" OR "s scrofa" OR porcine OR offal)	Pigs
				#2	TS=(detect* OR isolate* OR "isolation*" OR presence OR present OR persist* OR permanent OR occurrence OR recurrence OR recurrent OR colonise OR colonisation OR colonize OR colonization OR survival OR surviving OR survive* OR maintenance OR stability OR prevailed OR outbreak* OR prevalen* OR test*)	Detection
				#1	TS=(virus* OR viral OR adenovir* OR PAdV OR "Swine Fever" OR "porcine reproductive and respiratory syndrome" OR PRRSV OR SIRSV OR PRRS OR PERS OR aujsezky OR "aujeszky's" OR pseudorabies OR "suid herpesvirus" OR SHV1 OR SuHV1 OR SHV-1 OR "SuHV 1" OR "japanese encephalit*" OR "porcine circovirus" OR PCV2 OR "PCV 2" OR "porcine rotavirus*" OR "porcine parvovirus*" OR PPV OR "vesicular exanthema" OR "porcine parainfluenza" OR "porcine respiratory coronavirus*" OR PRCV OR "hepatitis E" OR "Paslahepevirus balayani" OR "porcine teschovirus*" OR ((Teschen OR Talfan) NEAR/3 disease*) OR "Cholera hog" OR "hog cholera" OR "Transmissible gastroenteritis" OR TGEV OR rinderpest OR coxsackievirus* OR echovirus* OR enterovirus* OR "Foot and mouth disease*" OR "FMD virus*" OR (Neethling NEAR/3 (disease* OR infection*)) OR "Lumpy skin disease" OR "marek disease*" OR "mareks disease*" OR "marek's disease*" OR "Newcastle disease*" OR "Influenza A" OR "influenzavirus A" OR nipah OR "Porcine epidemic diarrhea" OR PED OR norovirus* OR (Norwalk NEAR/3 (disease* OR infection*)) OR pestivirus* OR reovir* OR rotavirus* OR "Swine Vesicular Disease*" OR "SVD virus*"OR "foot and mouth disease" OR "hoof and mouth disease" OR rinderpest OR morbillivirus* OR phlebovirus* OR CSF OR CSFV OR pestivirus* OR ASF OR asfivirus* OR "avian influenza" OR H5N1 OR H7N9 OR H5N8 OR H5N5 OR raby OR rabies OR "swine pox" OR swinepox OR suipoxvirus* OR "swine papillomavirus*" OR (getah NEAR/3 (disease* OR infection*)) OR GETV OR "Senecavirus A" OR SVA OR "Seneca valley fever" OR ("Torque Teno " NEAR/3 (infection* OR disease*)) OR TTV OR TTSuV OR "postweaning multisystemic wasting syndrome" OR "porcine encephalomyocardit*" OR "porcine cytomegalovirus*" OR "inclusion body rhinitis" OR "Lateral-shaking inducing neuro-degenerative agent" OR (Linda NEAR/3 (disease* OR infection*)) OR LindaV)	Virus

				C. Criteria for selection of PARASTIC relevant pathogens: -(To be included in the WOAH list of swine diseases and multiple species OR -To be included in the AHAW risk mitigation opinion) Two parasites fulfil this criterial Taenia solium and Trichinella spp.
AQ2: What are the technical parameters (e.g., time, temperature, pressure, pH, particle size) for methods 2 to 5 and 7?	SAQ2.1: What are the technical parameters (e.g., time, temperature, pressure, pH, particle size) for methods 2,3,4 and 5?	Qualitative. Descriptive	Details of the technical parameters in terms of time, temperature, pressure, pH, particle size, of the considered methods	Review of Annexes III, IV and XIII of Commission Regulation (EU) 142/2011 for methods 2-5 for which the technical parameters are in the legislation. Description and presentation in tabular format of the technical parameters e.g., (e.g., time, temperature, pressure, pH, particle size) of methods 2 to 5. If technical parameters are explicitly defined in the legislation, they are the intended reference for the assessment and there is no uncertainty about them.
	SAQ2.2: What are the technical parameters for method 7 approved at national level in EU?	Qualitative. Descriptive	Details of the technical parameters of the mapped methods	Consultation with the association on the industrial standards applied for method 7 approved and currently implemented in the EU (where the legislation does not provide direct information on the technical parameters used). If they are not explicitly defined, there may be uncertainty about the selection of the parameters. This uncertainty will be described in the uncertainty analysis section. Only methods applied to porcine Category 3 material only or mixed with other material will be considered
AQ3: What are the inactivation parameters (temperature, time, pressure, pH) of the relevant pathogens identified in AQ1?		Qualitative. Descriptive	EFSA scientific opinions Extensive literature search	**A. Inactivation parameters of the selected BACTERIAL relevant pathogens**: *Salmonella senftenberg, Enterococcus faecalis:* source EFSA's OF/SI opinion (2021) Spores of *Clostridium perfringens*: extract data from scientific literature via a literature review on thermal inactivation in the form of time/temperature combinations. Analyse data using predictive models. **Literature review plan for inactivation parameters of spores of *Clostridium perfringens*:** • Research question: What are the thermal inactivation parameters for spores of Clostridium perfringens expressed in the form of D-values at certain temperatures, z-values, log reductions after certain temperature/time combinations? • Describe all eligibility criteria for study selection (i.e. the criteria related to study e.g. target population, intervention/exposure of interest, and the relevant outcomes and record characteristics e.g. time, language, publication type) The aim is to retrieve information on the thermal inactivation parameters for spores of Clostridium perfringens expressed in the form of D-values at certain temperatures, z-values, log reductions after

certain temperature/time combinations in multiple matrices (special emphasis in matrices of porcine origin and containing proteins)

- Language of the full text: English only
- Time: 1990 onwards (and with no restrictions in case of limited data)
- Publication type:
  Original article describing primary research studies, reviews articles or book (chapter) in case they provide sufficient evidence. Exclusion of conference Proceedings or Bulletin or Correspondence or Journal Issue or Book or Miscellaneous or Thesis or Annual Report or Bulletin Article (Document Types)
- Provide the rationale for the choice of the eligibility criteria
  The screening process will be undertaken in two or three steps: screening of
  (1) Title and abstract to exclude irrelevant records. Questions to address during the screening
  Question 1: Does the reference contain data on thermal inactivation parameters spores of Clostridium perfringens expressed in the form of D-values at certain temperatures, z-values, F-value, log reductions after certain temperature/time combinations or any other measurement? Answers: Yes/No/Maybe

  (2) Full-text screening documents to further identify records to be excluded based on criteria related to report characteristics
  Question 2: Is the full text available? Answers: Yes/No
  Question 3: Is the full paper in English? Answers: Yes/No
  Question 4: does the full paper contain data about thermal inactivation parameters of spores of Clostridium perfringens in the form of D-values at certain temperatures, z-values, F-value, log reductions after certain temperature/time combinations or any other measurement? Answers: Yes/No
  (3) Data extraction

Type of C. perfringens	Number of measurements of the parameters	D-value	Temperature for D-value	Z-value	F-value	Log10 reduction	Time/temperature for log10 reduction	Other
Spores or vegetative cell								

- Describe the final search strategy in the protocol, i.e., search string(s) including planned limitations
  See below
- Indicate the information sources (bibliographic databases and grey literature resources) that will be searched:
  Web of ScienceTM Core Collection (SCI-Expanded, BKCI-S, ESCI, CCR-Expanded, IC), and CAB Abstracts

- Describe any other search approaches (e.g., citation indexes, handsearching):
  No other search approaches will be applied.
- Indicate any software (e.g., for reference management) that will be use:
  DistillerSR for screening and Endnote for reference management and extraction of articles
- Indicate the number of reviewers
  3 WG members and 3 EFSA staff
- Describe the method for study selection e.g., in parallel or not.
- Parallel screening in batches, followed by full review of the shortlisted records in batches
- If applicable, describe how conflicts will be solved, if and what Artificial Intelligence techniques will be used
  Ad hoc meetings will be held by the pair of reviewers to discuss and solve the discrepancies by reaching consensus. If needed, a third party (the chair of the working group) will decide the inclusion or not.
- Indicate the software that will be used for screening paper
  DistillerSR
- Describe the main characteristics of data model (i.e., what data will be extracted from the included studies)
  D-values at z-values, F- values, log10 at T/T combinations
- Indicate how data will be extracted (e.g., by two independent reviewers in parallel or one reviewer extracting and one validating the process)
- Hits will be split into batches and distributed for pairs of reviewers. Double screening in parallel
- Indicate the software that will be used for data extraction
  Manually. Collated in tabular format

CAB Abstracts

Set	Query	Concept
#6	#5 AND PY= 1990-2023 and Journal Article or review papers or Book Chapters	Exclusion of conference abstracts, conference Proceedings or Bulletin or Correspondence or Journal Issue or Book or Miscellaneous or Thesis or Annual Report or Bulletin Article (Document Types)
#5	#4 and PY=1990-2023	Time

#4	#3 and LA=English	Language
#3	#1 AND #2	Hazard AND Inactivation
#2	TS=(Inactivat* OR reduction OR survival OR viability OR death* 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 resistan*" OR "heat resistan*" OR "thermal stress*" OR "heat stress*" OR "thermal performance*" OR "heat performance*" OR "temperature toleran*" OR "heat toleran*" OR "thermal toleran*" OR "time temperature" OR Lethality OR Bigelow OR "D value*" OR "z value*" OR "F value*" OR "Decimal reduction" OR Sterility OR Pasteuriz* OR Pasteuris* OR Steriliz* OR Sterilis*)	Inactivation
#1	TS=("Clostridium perfringens" OR "C. perfringens" OR "Cl. Perfringens" OR (perfringens AND spore*) OR "Clostridium welchi*" OR "Welchia perfringens" OR "C welchi*" OR "Cl. welchi*")	Hazard

Web of Science ((SCI-Expanded, BKCI-S, ESCI, CCR-Expanded, IC)

Set	Query	Concept
#6	#5 AND PY= 1990-2023 and Journal Article or Review Article or Book Chapters or Early Access	Exclusion of conference abstracts, conference Proceedings or Bulletin or Correspondence or Journal Issue or Miscellaneous or Thesis or Annual Report or Bulletin Article (Document Types)
#5	#4 AND PY=1990-2023	Time
#4	#3 AND LA=English	Language
#3	#1 AND #2	Hazard AND Inactivation
#2	TS=(Inactivat* OR reduction OR survival OR viability OR death* 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 resistan*" OR "heat resistan*" OR "thermal stress*" OR "heat stress*" OR "thermal performance*" OR "heat performance*" OR "temperature toleran*" OR "heat toleran*" OR "thermal toleran*" OR "time	Inactivation

				<table><tr><td></td><td>temperature" OR Lethality OR Bigelow OR "D value*" OR "z value*" OR "F value*" OR "Decimal reduction" OR Sterility OR Pasteuriz* OR Pasteuris* OR Steriliz* OR Sterilis*)</td><td></td></tr><tr><td>#1</td><td>TS=("Clostridium perfringens" OR "C. perfringens" OR "Cl. Perfringens" OR (perfringens AND spore*) OR "Clostridium welchi*" OR "Welchia perfringens" OR "C welchi*" OR "Cl. welchi*")</td><td>Hazard</td></tr></table> **B. Inactivation parameters of the selected VIRAL relevant pathogens:** From the listed viruses in AQ1, selection the most resistant one based on the structure (non-enveloped) and genomic characteristics (DNA). If inactivation parameters are not available in the EFSA's OF/SI opinion (2021), a literature review for the ascertainment of the inactivation parameters of the selected virus will be conducted. The literature, if needed, will be conducted using the same search strategy as the one for the inactivation parameters of spores of *Clostridium perfringens* (see above). **C. Inactivation parameters of the selected of PARASTIC relevant pathogens**: If any method 7 includes a chemical non-thermal treatment, then inactivation parameters will be considered. If all the treatments are thermal, considering the thermo-sensitivity of parasites, they will not be included in the answer to AQ3. Full inactivation will be assumed.
AQ4: What is the "level of inactivation" of the selected relevant pathogens achieved by methods 2-5 and 7?	SAQ4.1 What is the "level of inactivation" achieved by methods 2,3,4 and 5?	Quantitative	Details of the technical parameters in terms of time, temperature, pressure, PH, particle size, etc., of the considered methods	Since methods 2-5 provide holding times for fixed temperatures, estimates of the accumulated lethality (L) of the heat treatments for a given microorganism will be calculated, based on high thermal resistance (e.g. Clostridium perfringens spores). The lethality is a relative term that compares the microbial inactivation effect at a measured temperature profile to one minute at the reference temperature. Such calculations require the availability of D values and reference temperature (Tref), or the z value and Tref. Alternatively, the level of inactivation can be estimated using such heat resistant parameters and assuming a credible initial concentration of the relevant pathogens.
	SAQ4.2 What is the "level of inactivation" achieved for method 7?	Quantitative	Details of the technical parameters in terms of time, temperature, pressure, PH, particle size, etc., of the considered methods	- If robust technical parameters for the different applications of method 7 are available, the same approach as for the calculation of the F value for methods 2-5 will be applied. If there was variation in the implementation of method 7, the calculation of the inactivation would be done for a number of combinations of time/temperature. - If not, the sampling plans intended to ensure that certain level of safety has been attained by the given treatment will be used. It will be assumed that a sampling plan was designed in order to "approve" a "good lot of acceptable quality level AQL" with a confidence of 95%. In other words, lots of a quality level of at least AQL should be accepted 95% of the times they are monitored. In this way, the level of microbial inactivation attained by method 7 can be estimated by deriving AQL from a sampling plan and assuming a credible initial concentration of the pathogen in the batch. The difference between the two estimates will be the level of microbial inactivation.

			Microbiological criteria as in the legislation	The sampling plans are different for the three bacterial indicators according to Chapter III, Annex IV of Commission Regulation (EU) 142/2011 describes the standard processing methods of ABP. In method 7: "*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 25g: 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"*
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?		Search for evidence of inactivation of the biological hazards identified as the most thermal and pressure resistant in matrixes which might represent a good proxy for PAP	Standards applied by EFSA in Cat 3 ABP applications Alternative methods for biogas and composting (Com. Reg. (EU) 142/2011)	Expert judgment of the working group on the minimum level of inactivation required (standards to be applied): 5 $\log_{10}$ for *Salmonella Senftenberg*, 5 $\log_{10}$ for *Enterococcus faecalis* 5 $\log_{10}$ for spores of *Clostridium perfringens* 3 $\log_{10}$ for the selected thermoresistant virus Judgement by consensus using a facilitator EKE external to the working group

References

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