Annex to:

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Annex A – Protocol for the assessment of the efficacy and safety of high pressure processing of food

A.1. Introduction

A.1.1. Introduction and scope of this protocol

This document outlines the protocol for the scientific assessment of the efficacy and safety of high pressure processing (HPP) of food, which will be used as input for the scientific opinion of the EFSA Panel on Biological Hazards (BIOHAZ) on the efficacy and safety of HPP of food

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 144th plenary meeting (10-11 March 2021).

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

EFSA is asked to deliver a scientific opinion on the efficacy (reduction of the levels of food-borne pathogens) and safety of HPP of food. Quality issues and organoleptic properties are not part of this mandate.

More specific, EFSA is asked:

ToR1. To assess the efficacy and microbiological and chemical safety of the use of HPP when applied to relevant foodstuffs, and in particular:

a. To provide an overview of the foods to which HPP is or could be applied along with the processing conditions (e.g. pressure, time, temperature).

b. To list the intrinsic and extrinsic factors that may influence the efficacy of HPP.

c. To evaluate the potential chemical and microbiological food safety risks in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods with the purpose to increase microbiological food safety, if any (e.g. pasteurisation of juices).

ToR2. To assess the efficacy of HPP when applied to raw milk and raw colostrum from ruminants, and in particular:

a. To recommend minimum requirements as regards time and pressure of the HPP, and other factors if relevant, for the control of *Mycobacterium*, *Brucella*, *Listeria monocytogenes*, *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC), to achieve an equivalent efficacy to that of pasteurisation;

b. To propose appropriate indicators to verify the efficacy of HPP, either as part of the validation and verification in the HPP facility and/or in the end-product on the market;

c. if data allow, to provide a comparative assessment of the risk to human health that could derive from the consumption of HPP-treated vs. raw vs. pasteurised vs. UHT-treated milk or colostrum.

ToR3. To assess the efficacy of HPP when applied to foods known to cause human listeriosis (e.g. RTE smoked or gravid fish, soft and semi-soft cheese and cooked meat products and (blanched) frozen vegetables such as peas or corn that are consumed without prior cooking) and in particular:

a. To recommend minimum requirements as regards time and pressure of the HPP, and other factors if relevant, to reduce significantly *L. monocytogenes* levels (e.g. by a certain log reduction or reduction of the probability of illness per serving), and assuming that the parameters influencing the growth of *L. monocytogenes* remain unchanged (e.g. shelf-life and storage conditions);

b. To assess the efficacy on other relevant pathogens when applying the minimum requirements identified in a.

A.2. Problem formulation

A.2.1. Clarification of the ToRs

The following has been clarified with the requestor:

- High-pressure homogenisation (HPH; also called dynamic high-pressure homogenisation, dynamic-HPH) is out of scope.
- Two or more subsequent cycles of pressure treatment are out of scope.
- This SO will consider HPP as a non-thermal treatment and will not consider treatments causing an increase in product temperature above 45°C.
- HPP can be used for a variety of purposes. The main reason to apply HPP to a food matrix is the non-thermal inactivation of pathogenic and spoilage vegetative microorganisms[1] in order to increase microbiological safety and shelf-life of the processed food with, in general, minimal impact on thermally sensitive attributes (e.g. nutrients and vitamins). This scientific opinion will focus on the use of HPP for microbial inactivation, particularly of pathogenic vegetative bacteria, with the aim of improving

[1] The inactivation of bacterial spores it is only achieved when high pressure is combined with a thermal treatment, the so called pressure-assisted thermal processing (PATP) or high pressure-assisted thermal sterilization (HPTS), a technology/equipment which is not industrially implemented at large-scale (Moller, B; Dönitz, E; Jung-Erceg, P; Matser, A; and Vollebregt, M (2018) Roadmap High Pressure Thermal Sterilisation (HPTS). I3 Food project report. https://www.isi.fraunhofer.de/content/dam/isi/dokumente/ccv/2018/Roadmap-High-Pressure-Thermal-Sterilisation.pdf)

food safety. Quality issues including sensory properties, as well as nutritional aspects are out of scope.

- HPP can be applied at different points in the food processing and preservation chain. It can be applied to intact or minimally processed raw materials or fresh products (e.g. milk, fruit juices, smoothies, dips, sauces, etc.) and after the food is exposed to re-contamination after a lethal treatment (e.g. heat pasteurisation, cooking, etc.) due to post-process handling, e.g. slicing of cooked meat products, preparation of ready-to-eat meals. This is usually referred as a post-lethality treatment.
- For most of the conventional HPP applications, foods are packaged before the treatment (in-pack HPP), which is a discontinuous batch-based process. For liquid food, semi-continuous HPP (also known as in-bulk HPP) is also possible followed by ultra clean/aseptic packaging.

Clarifications for specific ToRs are listed below:

- Specifically, for **ToR1a**, the food categories/foods that are treated with HPP worldwide with the purpose to increase microbiological food safety will be considered, placing the focus on those foods that are being commercially processed by HPP in the EU.
- For **ToR1c**, the microbiological food safety risks are not those for which the efficacy (pathogen reduction) is being evaluated. Instead it is referring to a physiological/biochemical/genetic effect on a pathogen that could result in an increased risk (e.g. potential activation of spores or prions). The potential concerns will be contextualized as they might be common to many other treatments, not specific for HPP. The whole duration of the shelf-life of the foods is to be considered in the assessment of the potential chemical and microbiological food safety risks.
- For **ToR2**, other relevant pathogens may be added, e.g. *Campylobacter*. The end point in the assessment is the raw milk or colostrum for direct consumption. Its further use in other dairy products (e.g. for cheese or yoghurt production) is out of scope. The efficacy of the processing conditions is of relevance (not the post-processing contamination) considering the minimum t/T requirements for pasteurisation and UHT treatment of milk from legislation (as reference condition).
- For **ToR3**, the focus is on those foods known to cause human listeriosis in the EU, not on foods that could potentially cause listeriosis (based on the risk factors associated with the processing conditions, exposure to contamination, growth supporting characteristics, etc.) but without recorded cases/outbreaks in EU.

A.2.2. Assessment questions based on the interpretation of the mandate

Step 1 consists of the translation of the mandate into assessment question(s) (AQs) (step 1.1) and the definition of the sub-questions (SQs) (step 1.2) of each assessment question and their relationship (conceptual model).

Tables A1-A3 provide, for each of the ToR, the translation of the mandate into AQs as included in the second column (step 1.1), while the SQs are included in the third column (step 1.2). Their relationship is shown in Figure A1.

The approach for each SQ, i.e. whether to apply a quantitative, qualitative or semi-quantitative approach, has been specified in the fourth column (step 1.3). There was no need to prioritise SQs over others.

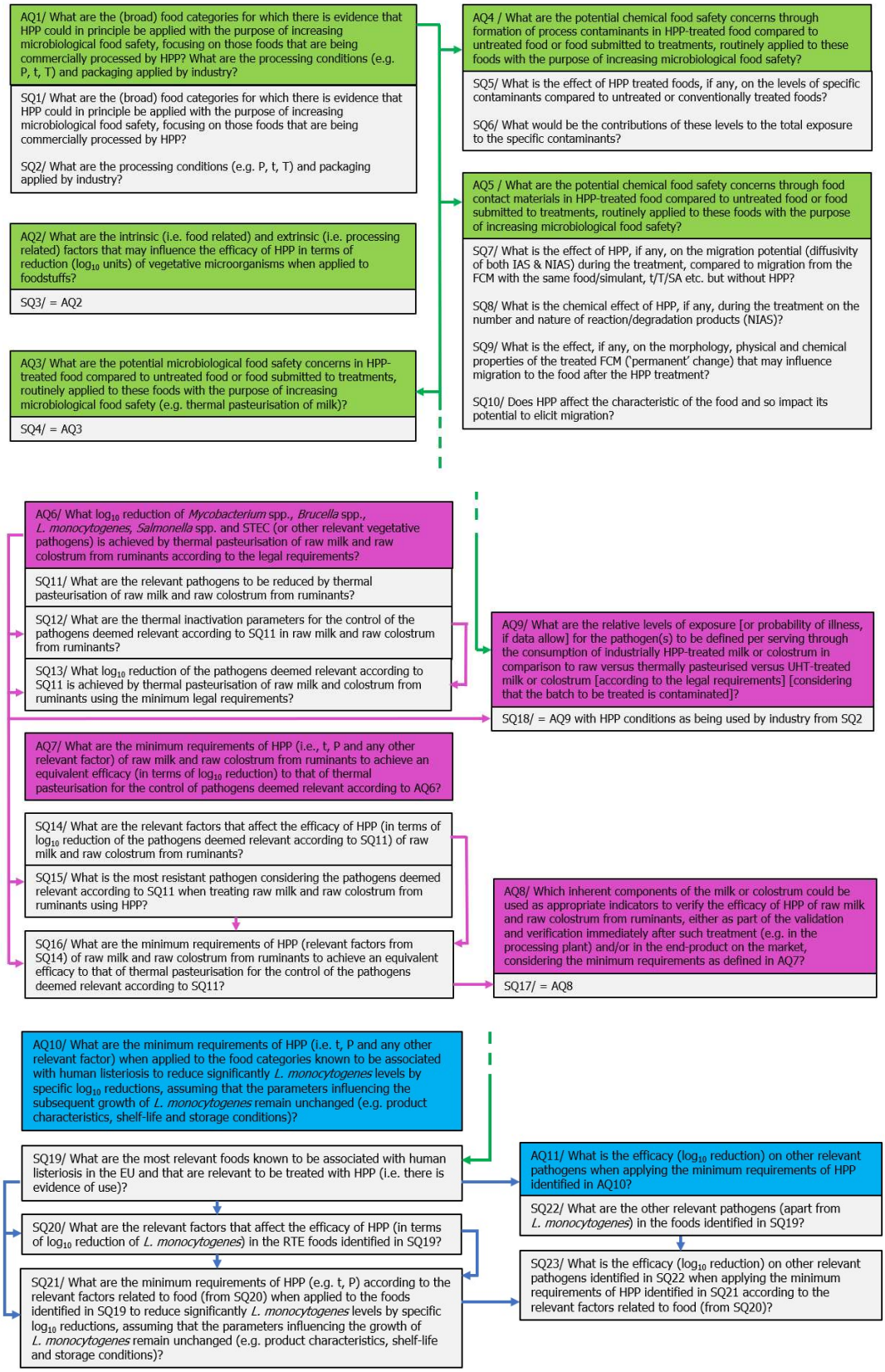

Figure A1: The relationship between the sub-questions (SQs) for each assessment question (AQ)

A.3. Methods for conducting the assessment

The second step includes the overall approach (step 2.1) as well as the evidence needs and the methods (step 2.2) for answering each SQ including uncertainty analysis (i.e. the use of a literature review, data from databases, expert knowledge or primary data collection). Tables A1-A3 provide this information in the fifth (step 2.1) and sixth (step 2.2) columns.

The methods that will be used for evidence integration across SQs and for accounting for the remaining uncertainty is provided in Table A4 based on the conceptual model.

Table A1: Assessment questions and sub-questions for ToR1 to assess the efficacy and microbiological and chemical safety of the use of HPP when applied to relevant foodstuffs

ToR	Step 1.1. AQ	Step 1.2. SQ	Step 1.3. Approach	Step 2.1. Overview method	Step 2.1. Evidence needs and methods
ToR 1a/ To provide an overview of the foods to which HPP is or could be applied along with the processing conditions (e.g. P, t, T)	AQ1/ What are the (broad) food categories for which there is evidence that HPP could in principle be applied with the purpose of increasing microbiological food safety, focusing on those foods that are being commercially processed by HPP? What are the processing conditions (e.g. P, t, T) and packaging applied by the industry?	SQ1/ What are the (broad) food categories for which there is evidence that HPP could in principle be applied with the purpose of increasing microbiological food safety, focusing on those foods that are being commercially processed by HPP?	Qualitative	Literature review Primary data collection (questionnaire*)	**a. Eligibility criteria:** The aim is to retrieve information on the food categories/foods that are treated with HPP worldwide with the purpose to increase microbiological food safety. The focus would be on those foods that are being commercially processed by HPP. This will be derived through a literature review and a questionnaire that will be sent to CA, establishment and equipment providers. The eligibility criteria for the literature review related to study characteristics are: – Population: any food that is being treated by HPP with the aim to increase microbiological food safety – Intervention: treatment with HPP – Outcome: none specific as there is no need to capture the pathogen reduction – Setting: industrial (for questionnaire), pilot (experiments using industrial equipment in non-industrial settings) and laboratory (for literature review) Those related to report characteristics are: – Language of the full text: all languages – Time: 2010 onwards – Publication type: review or book (chapter) **b. Definition of the search strategy**: The search would consider in the title or topic: HPP or synonyms AND food (or various types). **c. Methods for selecting studies for inclusion/exclusion**: The screening process will be undertaken in two or three steps: screening of (1) Ti, (2) Ab and (3) full-text 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 info about the use of HPP only of food products to improve food safety. All data gathered through the questionnaire will be included. **d. Methods for extracting data from included studies**. Selected full-text documents will be screened by one reviewer to extract the relevant information needed to answer SQ1. Data obtained in the questionnaires will be included. **e. Methods for appraising evidence**. There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative/narrative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ2/ What are the processing	Qualitative	Literature review	**a. Eligibility criteria:** The aim is to retrieve information on the window of HPP processing conditions used to treat food categories/foods worldwide with the purpose to

		conditions (e.g. P, t, T) and packaging applied by industry?		Primary data collection (questionnaire*)	increase microbiological food safety. This will be captured from the literature search described in SQ1. It will also be informed through a questionnaire (see SQ1). **b. Definition of the search strategy**: Selected full-text documents for SQ1 will be screened to retrieve relevant information regarding the processing conditions (e.g. P, t, T) (literature review). Additionally, a questionnaire would be used to gather information about the type of foods industrially processed with HPP along with their processing conditions (primary data collection). **c. Methods for selecting studies for inclusion/exclusion**: See SQ1. All data gathered through the questionnaire will be included as well as relevant data from research papers. **d. Methods for extracting data from included studies**. Data will be extracted using pre-defined tables. **e. Methods for appraising evidence**. There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
ToR 1b/ To list the intrinsic and extrinsic factors that may influence the efficacy of HPP	AQ2/ What are the intrinsic (i.e. food related) and extrinsic (i.e. processing related) factors that may influence the efficacy of HPP in terms of reduction (log_{10} units) of vegetative microorganisms when applied to foodstuffs?	SQ3/ = AQ2	May range from quantitative to qualitative depending on the factor	Literature review	**a. Eligibility criteria:** Eligible studies should fulfil the following criteria: (i) report inactivation of vegetative microorganisms by HPP at mild Ts (< 45°C), (ii) deal with bacterial pathogens or non-pathogens, since their inactivation is influenced by the same factors, (iii) be conducted in food or in laboratory media. The study design must include at least two or more levels of an intrinsic or extrinsic factor as well as a control (untreated product), to allow evaluation of the effect of that factor on inactivation. The major factors will be listed in a quantitative way and the additional ones in a qualitative way. **b. Definition of the search strategy**: The starting point will be the general search for review papers from SQ1 on HPP inactivation of vegetative bacteria. **c. Methods for selecting studies for inclusion/exclusion**: The records (reviews) from the Ti/Ab screening in SQ1 will be screened first at Ti/Ab level and then full text level for studies on HPP inactivation of vegetative bacteria. The reference list of these review papers will be screened for additional relevant information based on the eligibility criteria. If needed, an additional search may be done, depending on the completeness of the available evidence. **d. Methods for extracting data from included studies**. Data will be extracted using predefined tables reporting: (a) intrinsic factors: pH (+ food type or acid used), a_w (+

					food type or solute used), (b) extrinsic factors: P, T, t, (c) bacteria used, (d) information on reduction (D_P-value or $\log_{10}$ reduction), (e) any other relevant information **e. Methods for appraising evidence**. There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **Protocol deviation** According to the established protocol (point a), for a study to be included, the study design had to include at least two or more levels of an intrinsic or extrinsic factor as well as a control (untreated product). The major factors would be listed in a quantitative way and the additional ones in a qualitative way. According to point (d), data should have been extracted using predefined tables. However, the approach used was narrative and mainly based on the general search for review papers from SQ1, including the review of existing meta-analysis. The rationale is that this descriptive approach was considered sufficient for the risk manager considering also that a more detailed description of the impact of factors will be provided for the specific food commodities in ToR2 and 3. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
ToR 1c/ To evaluate the potential chemical and microbiological food safety risks in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods with the purpose to increase microbiological	AQ3/ What are the potential microbiological food safety concerns in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods, with the purpose of increasing microbiological food safety (e.g. thermal pasteurisation of milk)?	SQ4/ = AQ3	Qualitative	Literature review Primary data collection (questionnaire*)	**a. Eligibility criteria:** Eligible studies should report on microbiological risks generated or increased specifically by HPP treatment. They should fulfil the following criteria: (i) deal with the effect of HPP on foodborne pathogens including vegetative and spore-forming bacteria, viruses, mycotoxin-producing moulds, protozoa and prions, (ii) report an increased or new risk, or a physiological/biochemical/genetic effect on a pathogen that could result in an increased risk, compared to the food that was not HPP treated, as opposed to the risk reduction that is normally the goal of HPP treatment. This will be informed through a literature search and a questionnaire (see SQ1). **b. Definition of the search strategy**: A two-step strategy will be followed: (i) the records (reviews) from the Ti/Ab screening in SQ1 will be screened first at Ti/Ab level and then full text level to identify specific HPP associated concerns (as defined above) and (ii) subsequently a targeted search will be conducted with specific search strings for each specific concern identified (e.g. related to prions) or snowballing would be used for other concerns (e.g. spore activation). **c. Methods for selecting studies for inclusion/exclusion**: The results of the general search will be narrowed down in several steps: (i) select review papers meeting

food safety, if any (e.g. thermal pasteurisation of juices)					(or possibly meeting) eligibility criteria (formulated under a) based on Ti/Ab, (ii) collect and scan selected review papers (full text) for desired information, (iii) identify specific research papers with the desired information from the literature list of the review papers and (iv) collect selected research papers and confirm whether they contain the desired information that meets the eligibility criteria. The results of the targeted literature study will be screened to confirm whether they contain the desired information that meets the eligibility criteria. **d. Methods for extracting data from included studies**. Data will be extracted using a predefined table reporting: (i) nature of the risk (e.g. induction of virulence genes, spore activation…), (ii) type of evidence (methods used…), (iii) matrix: food type, laboratory medium, (iv) qualitative, semi-quantitative, quantitative. **e. Methods for appraising evidence.** There is no need to plan beforehand. **Protocol deviation** According to the established protocol (point d), data should have been extracted using predefined tables. However, the approach used was narrative as there were few eligible studies. **f. Sources of uncertainty and definition of the methods for prioritising them**. No need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
	AQ4/ What are the potential chemical food safety concerns through formation of process contaminants in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods with the purpose of increasing microbiological food safety?	SQ5/ What is the effect of HPP treated foods, if any, on the levels of specific contaminants compared to untreated or conventionally treated foods?	Qualitative	Literature review Primary data collection (questionnaire*)	**a. Eligibility criteria:** The aim is to retrieve information reporting or reviewing on chemical changes during the HPP compared to non-treated or conventional processes and that are related to contaminants. This will be informed through a literature search and a questionnaire (see SQ1). **b. Definition of the search strategy**: Keywords:("polychlorinated naphthalene" OR PCN OR heterocyclic amines OR polycyclic amines OR acrylamide OR mcpd OR monochloropropanodiol OR chloropropanodiol OR monochloropropanediol OR chloropropanediol OR mineral oil OR aflatoxin* OR glycoalkaloid* OR HMF OR grayanotoxin* OR perfluoroalkyl substance* OR PFAs OR Ochratoxin A OR OTA OR chlorinated paraffin* OR quinolizidine alkaloid* OR cyanogenic glycoside* OR "perfluorooctane sulfonic acid" OR "perfluorooctanoic acid" OR dioxin* OR "dioxin-like PCB" OR diacetoxyscirpenol OR fumonisin OR "opium alkaloid" OR moniliformin OR "monochloropropane diol" OR furan OR methylfuran OR "hydrocyanic acid" OR deoxynivalenol OR zearalenone OR tetrodotoxin OR TTX OR TTX analogue OR "T2 toxin" OR "HT2 toxin" OR Erucic acid OR Malachite green OR monochloropropanediol OR MCPD
		SQ6/ What would be the contributions of these levels to the total exposure to the specific contaminants?	Qualitative		

					OR phorbol ester OR tetrahydrocannabinol OR THC OR nitrofuran OR chlorate OR acrylamide OR nickel OR mycotoxin OR Chloramphenicol OR perchlorate OR beauvericin OR enniatin OR methylmercury OR chromium OR "Tropane alkaloid" OR sterigmatocystin OR endocrine disruptor OR mercury OR PCDD/F OR DL-PCB OR "Brominated Flame Retardant" OR BFR OR Ergot alkaloid OR "Mineral Oil Hydrocarbon" OR "Brominated Phenol" OR citrinin OR phomopsin OR Tetrabromobisphenol A OR TBBPA OR "Pyrrolizidine alkaloid" OR "Alternaria toxin" OR Hexabromocyclododecane OR HBCDD OR "Polybrominated Diphenyl Ether" OR PBDE OR glycerine OR PBB OR marine biotoxin OR Brevetoxin OR mercury OR PCDD/F OR DL-PCB OR Ciguatoxin OR Cyclic imine OR spirolide OR gymnodimine OR pinnatoxin OR pteriatoxin OR Lead OR Melamine OR Palytoxin OR "high viscosity white mineral oil" OR Arsenic OR "Domoic acid" OR Pectenotoxin OR Uranium OR Saxitoxin OR Nitrite OR Cadmium OR Yessotoxin OR Gossypol OR Azaspiracid OR "Polycyclic Aromatic Hydrocarbon" OR Perfluorooctane sulfonate OR PFOS OR perfluorooctanoic acid OR PFOA OR Nitrate OR diclazuril OR nicarbazin OR robenidine OR decoquinate OR halofuginone hydrobromide OR okadaic acid OR "Ethyl carbamate" OR hydrocyanic acid OR hormone residue or OR nitrosamines OR "reaction product" OR "potential reaction product" OR metabolite* OR "metabolite* of contaminant*" OR contaminant* OR "chemical food safety risk*" OR impurit* OR food safety OR risk assessment OR risk OR toxic compound OR toxic* OR adverse OR undesirable OR harm* OR safe OR safety OR hazard OR chemical) AND ("High pressure process*" OR "high hydrostatic pressure process*" OR "ultra-high-pressure process" OR "ultra high pressure process" or "high hydrostatic pressure process*" or "hydrostatic pressure process") **c. Methods for selecting studies for inclusion/exclusion**: A two-step screening is followed: (i) the retrieved articles will be screened first at Ti/Ab level for information on chemical substances that are modified or produced due to HPP and (ii) subsequently the selected records will be screened at the full text level for information that is related to contaminants. **d. Methods for extracting data from included studies**. No need to plan beforehand. **e. Methods for appraising evidence.** There is no need to plan beforehand. The evidence from the selected records related to contaminants will be synthesised in a narrative way. **f. Sources of uncertainty and definition of the methods for prioritising them**. Uncertainty will be noted, with a quantitative analysis when possible/necessary. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. No need to plan beforehand.
	AQ5/ What are the potential chemical food safety concerns through food contact	SQ7/ What is the effect of HPP, if any, on the migration potential (including	Semi-quantitative	Literature review	**a. Eligibility criteria:** The aim is to retrieve information on the effect of HPP on the packaging and ultimately on the migration potential to food or food simulants.

	materials (FCM) in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods with the purpose of increasing microbiological food safety?	diffusivity and partitioning effects) of FCM substances during the treatment, compared to migration from the FCM with the same food/simulant, t/T/SA etc. but without HPP?		Expert knowledge from WG and US-FDA Primary data collection (questionnaire*) Collection of information from the EFSA-MS FCM network	Eligibility criteria related to study characteristics are: HPP treatments of food contact materials (with or without packaged food (simulants)); migration; FCM physicochemical characteristics; reaction products; food and packaging. Databases: Web of science Core Collection and Scopus. Cut-off date: fundamental studies on migration behavior (from 1990 to October 2020). English, primary articles, review, books, proceedings. Review papers will be examined but no conclusions will be drawn from them without recourse to an evaluation of the original research papers (without the 1990 cut-off date). Evaluate what is found and cited, then go further back if needed. **b. Definition of the search strategy**: The experts will agree on a conceptual framework of the factors that determine the identity and quantity (concentration) of chemicals migrating from FCM. This conceptual framework will be used to help identify in a reproducible way, those key features in the references (Ti/Ab) that would call for their inclusion/exclusion on the basis of relevance to the ToR. Additionally, the questionnaire* will be used to gather information about the type of foods industrially processed with HPP along with their processing conditions (primary data collection). The EFSA-MS FCM Network will be consulted on the same type of information as in the questionnaire. Finally, expert knowledge from the US-FDA will be considered through a technical hearing with them. **c. Methods for selecting studies for inclusion/exclusion**: The screening process will be undertaken in two/three steps: (1) screening of Ti and/or (2) Ab and (3) evaluation of full-text documents to further identify records to be included/excluded based on criteria related to report characteristics and study characteristics. The Ti/Ab (1 & 2) will be screened blind to eliminate the possibility of bias, i.e. without knowledge of the identity of the authors, the country in which the study was conducted, nor the journal and date in which the findings were published. The papers will be allocated in a random fashion to the reviewers and a format for the reporting of the screening will be agreed, with a section available for short notes on the justification of inclusion/exclusion at this screening stage. After the screening process (1 & 2) to identify the relevant papers, the aim is to then evaluate the published information (3) and address the main question: "What is the effect of HPP treatment on the food packaging and ultimately on chemical migration to food" addressing the four SQs. Papers will be rated for the relevance (high, medium, low) of the investigation aims and the materials and methods used and rated also for the reliability (high, medium, low) of the main reported findings. Each paper will be summarised by the evaluator in a short narrative text with a description/tabulation of the main qualitative/quantitative findings. **d. Methods for extracting data from included studies**.
		SQ8/ What is the chemical effect of HPP, if any, during the treatment on the number and nature of reaction/degradation products (non-intentionally added substances, NIAS) in/from the FCM?	Qualitative		
		SQ9/ What is the effect, if any, on the morphology, physical and chemical properties of the treated FCM ('permanent' change) that may influence migration to the food after the HPP treatment?	Semi-quantitative		
		SQ10/ Does HPP affect the characteristics of the food and so impact its potential to elicit migration?	Qualitative		

					The individual summaries from step 3 will be discussed on the information available for the different end-points identified in sub-questions 1-4. The goal of the discussions will be to have an agreed synthesis of all of the information and provide answers to the SQs and the main FCM migration question. Areas of uncertainty will be noted, with quantitative analyses when possible/necessary. If needed, cited papers will be added to step 3. Information/data from the studies selected after discussion will be synthesised in a table reporting data on FCM type, HPP conditions (P, T, t), migration conditions (migrant, T, t, SA, simulant), results, relevance and reliability). **e. Methods for appraising evidence.** There is no need to appraise the studies if a review paper or book chapter because no conclusions were drawn from them, without recourse to an evaluation of the original papers. **f. Sources of uncertainty and definition of the methods for prioritising them**. No need to plan beforehand **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative **h. Methods for analysing uncertainties**. No need to plan beforehand.

Ab = abstracts; AQ = assessment question; a_w = water activity; CA = competent authority; FCM = food contact material; HPP = high pressure processing; NIAS = non-intentionally added substances; P = pressure; SA = surface area; SQ = sub-question; T = temperature, t =time; Ti= titles; ToR = term of reference; US-FDA = US Food and Drug Administration.

*Also, for ToR1, a questionnaire will be used to gather information about the type of foods industrially processed with HPP along with their processing conditions (~ primary data collection). This questionnaire would contain the food category, food subtype, whether the HPP treatment performed by the FBO or by an "external service provider" (TOLL), the primary and secondary reason for using the HPP technology for the commodities listed (e.g. increase product safety (i.e. inactivate pathogenic microorganisms) and how the desired effect is reached, extend product shelf-life (i.e. inactivating spoilage bacteria), the various processing conditions used: (such as the target pressure (in MPa), treatment time (once target pressure reached) (in minutes), initial water temperature (water temperature before HPP treatment) (in °C), rate of pressurisation/depressurisation) and the packaging material used.

Table A2: Assessment questions and sub-questions for TOR2 to assess the efficacy of HPP when applied to raw milk and raw colostrum from ruminants

ToR	**Step 1.1. AQ**	**Step 1.1. SQ**	**Step 1.3. Approach**	**Step 2.1. Overview method**	**Step 2.1. Evidence needs and methods**
ToR 2a/ To recommend minimum requirements as regards time and pressure of the HPP, and other factors if relevant,	AQ6/ What $\log_{10}$ reduction of *Mycobacterium* spp., *Brucella* spp., *L. monocytogenes*, *Salmonella* spp. and STEC (or other relevant vegetative	SQ11/ What are the relevant pathogens to be reduced by thermal pasteurisation of raw milk and raw colostrum from ruminants?	Qualitative	Literature review Data from databases	**a. Eligibility criteria/evidence needs:** Epidemiological link, or occurrence in raw milk, requiring treatment for elimination or reduction at acceptable levels. **b. Definition of the search strategy/database**: Review of the previous BIOHAZ panel opinion on the public health risks related to the consumption of raw drinking milk (EFSA BIOHAZ Panel, 2015). Data on 'strong evidence' FBO from 2008 to 2015 will be extracted from the EFSA FBO database. **c. Methods for selecting studies for inclusion/exclusion/data model**: Relevant information will be obtained from the previous EFSA opinion (EFSA BIOHAZ Panel,

for the control of *Mycobacterium* spp., *Brucella* spp., *L. monocytogenes*, *Salmonella* spp. and STEC, to achieve an equivalent efficacy to that of pasteurisation	pathogens) is achieved by thermal pasteurisation of raw milk and raw colostrum from ruminants according to the legal requirements?				2015). In the case of data from FBO databases, data on FBO with milk as vehicle will be extracted. **d. Methods for extracting data from included studies**. Tables summarising available epidemiological evidence, including hazard name, number of outbreaks, cases and hospitalized cases. Further information in other fields (such as vehicle information and FBO-related factors) will be consulted. **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ12/ What are the thermal inactivation parameters for the control of the pathogens deemed relevant according to SQ11 in raw milk and raw colostrum from ruminants?	Semi-quantitative	Literature review	**a. Eligibility criteria:** The aim is to retrieve information from experimental studies reporting pathogen-specific thermal inactivation parameters (D_T and z_T-values) or log_{10}-reductions in raw milk/colostrum. The eligibility criteria related to study characteristics are listed below considering that the record needs to contain info about thermal treatment of milk or colostrum (any species) to evaluate the impact of T on the pathogens deemed relevant according to SQ11. – Population: Priority will be given to cow milk but milk from other animal species will be considered and discussed should the evidence for some of the pathogen be scarce. – Intervention: heat treatment – Outcome: thermal inactivation parameters and/or log_{10} reductions of the pathogens deemed relevant according to SQ11 – Setting: industrial, pilot and laboratory **b. Definition of the search strategy**: Data will be gathered by means of literature review and data from databases (i.e. "Lemgo D- and z-value Database for Food": https://www.th-owl.de/fb4/ldzbase/index.pl?link=New%20Search&Script=1), in case this gives complementary information. The search would consider in the title or topic: (milk or colostrum) AND (the relevant pathogens) AND (thermal inactivation parameters such as D-value OR z-value OR F-value OR "decimal reduction time" OR "decimal reduction dose" OR "thermal death time") **d. Methods for extracting data from included studies**. Pre-defined table reporting: pathogen, strain, D_T-value, time to x-log_{10} reduction, T (of D_T-value), z_T-value, log_{10} reduction, media, reference, notes (anything relevant). **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand.

					g. Methods for synthesising evidence. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ13/ What log_{10} reduction of the pathogens deemed relevant according to SQ11 is achieved by thermal pasteurisation of raw milk and raw colostrum from ruminants using the minimum legal requirements?	Quantitative	Calculation	Pathogen-specific parameters obtained from SQ12 will be used to estimate the log_{10} reduction of the pathogens deemed relevant according to SQ11 when raw milk and colostrum are pasteurised according to the legal requirements of 72°C for 15 s and 60°C for 30 min. **Protocol deviation** The estimation of the log_{10} reduction of the relevant pathogens was also used to evaluate, for each relevant hazard, whether the specific log_{10} reductions recommended by international agencies (i.e. 5, 6, 7 and 8 log_{10} reductions) are achieved using the minimal legal requirements (T/t combinations) for thermal pasteurisation of milk. The rationale is that according to the D_T and Z_T values available in the literature for the thermal inactivation of the relevant hazards, the expected reductions caused by regulated pasteurisation treatments for most of the pathogens assessed (except for *Brucella* and MAP) cause extremely high log_{10} reductions, e.g. up to 20 log_{10} units. As such, to enable comparison of reductions caused by thermal pasteurisation among all relevant pathogens (including those showing very high and other showing limited reductions) and also between thermal pasteurisation and HPP, it was deemed appropriate to also consider lower magnitude of reductions. The selection of such lower magnitude was based on the recommended performance criteria (PC) by different international agencies in publicly available documents.
	AQ7/ What are the minimum requirements of HPP (i.e. t , P and any other relevant factor) of raw milk and raw colostrum from ruminants to achieve an equivalent efficacy (in terms of log_{10} reduction) to that of thermal pasteurisation for the	SQ14/ What are the relevant factors that affect the efficacy of HPP (in terms of log_{10} reduction of the pathogens deemed relevant according to SQ11) of raw milk and raw colostrum from ruminants?	Qualitative	Literature review	**a. Eligibility criteria:** Similar to SQ3=AQ2 but focus will be narrowed down to relevant milk/colostrum-specific factors **b. Definition of the search strategy**: Narrative review of relevant review papers, book chapters. **c. Methods for selecting studies for inclusion/exclusion**: Literature review using the same string as for ToR 2/b Relevant reference (e.g. book chapters) from WG members knowledge. **d. Methods for extracting data from included studies**. n.a. **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand.

	control of the pathogens deemed relevant according to AQ6?				**g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ15/ What is the most resistant pathogen considering the pathogens deemed relevant according to SQ11 when treating raw milk and raw colostrum from ruminants using HPP?	Semi-quantitative	Literature review	**a. Eligibility criteria:** Ideally, the most resistant pathogens will be identified by means of a literature review aimed at capturing the pathogen-specific log_{10} reductions in comparable conditions (i.e. t/P, media). **b. Definition of the search strategy**: The search would consider in the title or topic: (HPP or synonyms) AND (milk or colostrum) **c. Methods for selecting studies for inclusion/exclusion**: After preliminary screening of Ti/Ab, studies are retained if the pathogen-specific parameters are reported and were obtained from HPP treatment of liquid milk/colostrum. **d. Methods for extracting data from included studies**. Pre-defined table will be used reporting pathogen, strain, D_P-value, P (of D_P-value), z_P-value, log_{10} reduction, media, reference, notes (anything relevant) **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be primarily quantitative **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ16/ What are the minimum requirements of HPP (relevant factors from SQ14) of raw milk and raw colostrum from ruminants to achieve an equivalent efficacy to that of thermal pasteurisation for the control of the pathogens deemed relevant according to SQ11?	Quantitative	Calculation	To answer this SQ the evidence needed are the thermal (SQ13) and HPP (SQ14) inactivation parameters, for the most resistant pathogen (per SQ15). With these data it is possible to estimate what HPP conditions would lead to log_{10} reduction that is comparable to that of the heat treatment. Sources of uncertainty and definition of the methods for prioritising them. There is no need to plan beforehand. **Protocol deviation** If the target log_{10} reductions were achieved (or exceeded), HPP equivalent conditions were derived for all relevant pathogens (and thus not only for the most resistant pathogen) for each PC and further identified in relation to the highest PC achieved by thermal. The rationale is that this would allow for the risk manager to have more flexibility as the outcome would allow to consider the minimum requirements for each relevant pathogen.

ToR 2b/ To propose appropriate indicators to verify the efficacy of HPP, either as part of the validation and verification in the HPP facility and/or in the end-product on the market	AQ8/ Which inherent components of the milk or colostrum could be used as appropriate indicators to verify the efficacy of HPP of raw milk and raw colostrum from ruminants, either as part of the validation and verification immediately after such treatment (e.g. in the processing plant) and/or in the end-product on the market, considering the minimum requirements as defined in AQ7?	SQ17/ = AQ8	May range from quantitative to qualitative depending on the type of indicator	Literature review	**a. Eligibility criteria:** The aim is to retrieve information about appropriate indicators (that need to be inherent components of the milk or colostrum from ruminants) that could be used to verify the efficacy of HPP of raw milk/raw colostrum from ruminants using the HPP conditions that will be an outcome of SQ14. The eligibility criteria related to study characteristics are listed below considering that the record needs to contain info about the use of HPP on milk or colostrum (any species) OR the use of HPP on a milk component that could serve as indicator for pasteurisation. - Treatment of milk or colostrum (with focus on bovines, but also other species) with HPP - Any setting would be eligible industrial, pilot and laboratory - Any indicator that is an inherent component of milk or colostrum from ruminants will be eligible Those related to report characteristics are: - Language of the full text: no restriction - Time: no restriction **b. Definition of the search strategy**: The search will consider in the title or topic: (HPP or synonyms) AND (milk or colostrum) (same search as for SQ13) **c. Methods for selecting studies for inclusion/exclusion**: The screening process will be undertaken in two steps: (1) screening of Ti and/or (2) Ab and (3) screening of full-text documents to further identify records to be excluded based on criteria related to report characteristics (e.g. some specific languages) and study characteristics: - The records that refer to the effects of HPP in milk or colostrum components will be selected: enzymes, proteins, fat, etc. - The records made with milk of any animal species and with any fat content will be included in the selection. - Records in which the effect studied on the component is evaluated on other dairy products other than milk (e.g. cheese or yoghurt) will be excluded from the selection. **d. Methods for extracting data from included studies**. Data will be extracted using pre-defined tables reporting Compound / type of milk (species, fat content) / HPP treatment parameters: P, T, t / Type of effect (e.g. inactivation, changes in structure, etc.) and stability over time / Detection method / Kinetic parameters: D_P, z_P or similar / Any other relevant information. **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.

ToR 2c/ if data allow, to provide a comparative assessment of the risk to human health that could derive from the consumption of HPP-treated vs. raw vs. pasteurised vs. UHT-treated milk or colostrum	AQ9/ What are the relative levels of exposure [or probability of illness, if data allow] for the pathogen(s) to be defined per serving through the consumption of industrially HPP-treated milk or colostrum in comparison to raw versus thermally pasteurised versus UHT-treated milk or colostrum [according to the legal requirements] [considering that the batch to be treated is contaminated]?	SQ18/ = AQ9 with HPP conditions as being used by industry from SQ2	Quantitative	Calculation	Comparisons of the probability of exposure to P from consumption of raw milk, pasteurised milk, UHT milk and industrially HPP treated milk will be done by means of quantitative probabilistic modelling. The QMRA model will be informed by: - Initial fixed level of contamination - Kinetic inactivation parameters collected as part of SQ13 and SQ15 - Selected scenarios of t/T conditions of storage from literature review - Pathogen-specific growth models with relevant parameters (e.g. specific growth rate, duplication rate etc…) gathered from literature or databases for predictive microbiology (i.e. ComBase) with priority to experiments carried out using the appropriate media for the scope of the QMRA (i.e. raw milk, pasteurised milk, UHT milk). Should this not be available, feasibility of using data from broth media or equivalent will be assessed on a case-by-case basis - Serving size (literature or EFSA Food Consumption database) Cumulative distributions will be used for the probability of exposure to P from consumption of raw milk, pasteurised milk, UHT milk and HPP treated milk. Scenario analysis and/or sensitivity analysis and/or second order plots will be carried out to separate sources of uncertainty and variability. **Protocol deviation** The model used for the comparative exposure assessment considered the probability of contaminated servings immediately after HPP treatment and not at the moment of consumption (as data were lacking to assess the impact of storage on the population of bacteria ingested by the consumer). As for SQ13, the comparison with thermal pasteurisation was also done here considering the PC recommended by international agencies. Scenario analysis was used to cover the variability in levels of initial contamination of the pathogens and the number of surviving bacteria after treatment.

Ab = abstracts; AQ = assessment question; FBO = food-borne outbreak; HPP = high pressure processing; n.a. = not applicable; P = pressure; QMRA = quantitative microbial risk assessment; SQ = sub-question; STEC = Shiga toxin-producing *E. coli*; T = temperature, t = time; Ti = titles; ToR = term of reference; UHT = ultra-high temperature.

Table A3: Assessment questions and sub-questions for TOR3 to assess the efficacy of HPP when applied to foods known to cause human listeriosis (e.g. RTE smoked or gravid fish, soft and semi-soft cheese and cooked meat products and (blanched) frozen vegetables such as peas or corn that are consumed without prior cooking)

ToR	**Step 1.1. AQ**	**Step 1.1. SQ**	**Step 1.3. Approach**	**Step 2.1. Overview method**	**Step 2.1. Evidence needs and methods**

ToR 3a/ To recommend minimum requirements as regards time and pressure of the HPP, and other factors if relevant, to reduce significantly *L. monocytogenes* levels (e.g. by a certain log reduction or reduction of the probability of illness per serving), and assuming that the parameters influencing the growth of *L. monocytogenes* remain unchanged (e.g. shelf-life and storage conditions)	AQ10/ What are the minimum requirements of HPP (i.e. t, P and any other relevant factor) when applied to the food categories known to be associated with human listeriosis to reduce significantly *L. monocytogenes* levels by specific log_{10} reductions, assuming that the parameters influencing the subsequent growth of *L. monocytogenes* remain unchanged (e.g. product characteristics, shelf-life and storage conditions)?	SQ19/ What are the most relevant foods known to be associated with human listeriosis in the EU and that are relevant to be treated with HPP (i.e. there is evidence of use)?	Qualitative	Literature review Data from databases	**a. Eligibility criteria/evidence needs:** The aim is to retrieve information on the RTE foods known to cause human listeriosis in the EU and that are relevant to HPP either because they are already commercialized, or literature provides some evidence that they can be used. **b. Definition of the search strategy/database**: Review of the previous BIOHAZ opinions on the *L. monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU (EFSA BIOHAZ Panel, 2018) and on the public health risk posed by *L. monocytogenes* in frozen fruit and vegetables including herbs, blanched during processing (EFSA BIOHAZ Panel, 2020). Data on 'strong evidence' FBO from 2008 to 2019 will be extracted from the EFSA FBO database. **c. Methods for selecting studies for inclusion/exclusion/data model**: Relevant information will be obtained from the previously mentioned BIOHAZ opinions (EFSA BIOHAZ Panel, 2018, 2020). In the case of FBO data, only data on FBO caused by *Listeria* will be filtered and relevant information will be extracted on *L. monocytogenes* in relevant food categories. **d. Methods for extracting data from included studies/data check and validation**. Tables summarizing available epidemiological evidence, including food (sub)category, number of outbreaks, cases, hospitalized cases, deaths. **e. Methods for appraising evidence.** There is no need to plan beforehand. **e. Sources of uncertainty and definition of the methods for prioritising them**. No need to plan beforehand. **f. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **g. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ20/ What are the relevant factors that affect the efficacy of HPP (in terms of log_{10} reduction of *L. monocytogenes*) in the RTE foods identified in SQ19?	Quantitative to qualitative	Literature review	**a. Eligibility criteria:** The aim is, based on the info from SQ3, to identify the factors relevant for the selected most relevant foods known to cause human listeriosis in the EU and are relevant to be treated with HPP (from SQ19). The study characteristics: - Challenge test (the pathogen needs to be inoculated at known level and controlled conditions) - Done in real food matrix, for which factors need to be described (preferably from quantitative perspective): food type and related factors (pH, a_w, fat, preservatives) and HHP technological conditions - Providing potential inactivation (log_{10} reduction, kinetic parameter and/or mathematical model) Report characteristics are: - Language of the full text: no restriction - Time: no restriction - Geographic: EU/Worldwide

					b. Definition of the search strategy: The search would consider in the title or topic: HPP AND (selected most relevant foods causing listeriosis including RTE cooked meat products, gravid fish and soft and semi-soft cheese) AND *L. monocytogenes* AND inactivation (and synonyms). **c. Methods for selecting studies for inclusion/exclusion**: The screening process will be undertaken in two steps: screening of (1) Ti/Ab and (2) full-text documents to further identify records to be excluded based on criteria related to report characteristics (e.g. not in English) and study characteristics. Foreseen exclusion: papers dealing with laboratory media experiments, unless they can help in identifying (qualitatively) a relevant factor. **d. Methods for extracting data from included studies**. Data will be extracted using pre-defined tables. Besides food related and HPP technological conditions, other relevant issues to be collected: microbial strain (and conditions for preparing the inoculation culture) and analysis (immediately after HPP, after some time (sublethal damage overestimating the efficacy). **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative (potentially with some quantitative reference to the effect of a given factor if the literature provides enough information). **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ21/ What are the minimum requirements of HPP (e.g. t, P) according to the relevant factors related to food (from SQ20) when applied to the foods identified in SQ19 to reduce significantly *L. monocytogenes* levels by specific log_{10} reductions, assuming that the parameters influencing the subsequent growth of *L. monocytogenes* remain unchanged (e.g. product characteristics, shelf-life and storage conditions)?	Quantitative Semi-quantitative	Literature review	**a. Eligibility criteria:** The aim is to identify combination of HPP processing parameters and food (food characteristics) associated with a given log_{10} reduction. Data gathered from the studies retrieved from SQ20 search will be used. **b. Definition of the search strategy**: Literature string as in SQ20 **c. Methods for selecting studies for inclusion/exclusion**: After preliminary screening of Ti/Ab, studies are retained based on the HPP conditions needed to reduce (and keep low) relevant pathogens in the selected RTE foods during the whole shelf-life. **d. Methods for extracting data from included studies**. Data will be extracted using pre-defined tables. **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.

ToR 3b/ To assess the efficacy on other relevant pathogens when applying the minimum requirements identified in a.	AQ11/ What is the efficacy (log_{10} reduction) on other relevant pathogens when applying the minimum requirements of HPP identified in AQ10?	SQ22/ What are the other relevant pathogens (apart from *L. monocytogenes*) in the foods identified in SQ19?	Qualitative	Literature review Data from databases	**a. Eligibility criteria/evidence needs:** The aim is to retrieve information on the RTE foods identified in SQ19 and to determine if there are other relevant pathogens incriminated in foodborne outbreaks in the EU. **b. Definition of the search strategy/database**: Data on 'strong evidence' FBO from 2008 to 2019 will be extracted from the EFSA FBO database. **c. Methods for selecting studies for inclusion/exclusion/data model**: Only food/food categories identified in SQ19 will be included in the search. In the case of data from FBO databases, only data on FBO with food/food categories identified in SQ19 will be filtered **d. Methods for extracting data from included studies/data check and validation**. Tables summarizing available epidemiological evidence, including food (sub)category, FBO agent, number of outbreaks, cases and hospitalized cases. **e. Methods for appraising evidence.** Three is no need to plan beforehand **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.
		SQ23/ What is the efficacy (log_{10} reduction) on other relevant pathogens identified in SQ22 when applying the minimum requirements of HPP identified in SQ21 according to the relevant factors related to food (from SQ20)?	Quantitative and semi-quantitative	Literature review	**a. Eligibility criteria:** The aim is to gather data on the quantitative effect on microbial reduction for the other relevant pathogens identified in SQ20. Data gathered from the studies retrieved from SQ19 search will be used to determine which is the microbial reduction. Study characteristics: – Challenge test (the pathogen needs to be inoculated at known level and controlled conditions) – Done in real food matrix, for which factors need to be described (preferably from quantitative perspective): food type and related factors (pH, a_w, fat, preservatives) and HHP technological conditions – Providing potential inactivation (log_{10} reduction and/or mathematical model) Foreseen exclusion: Records dealing with laboratory media experiments Report characteristics are: – Language of the full text: no restriction – Time: no restriction – Geographic: EU **b. Definition of the search strategy**: The search would consider in the title or topic: HPP AND RTE food AND Name of 'other pathogens' (and synonyms) **c. Methods for selecting studies for inclusion/exclusion**: Screening for relevance (Ti/Ab) and screening for eligibility (full text).

					d. Methods for extracting data from included studies. Data will be extracted using pre-defined tables. **e. Methods for appraising evidence.** There is no need to plan beforehand. **f. Sources of uncertainty and definition of the methods for prioritising them**. There is no need to plan beforehand. **g. Methods for synthesising evidence**. The methods used for the synthesis will be qualitative. **h. Methods for analysing uncertainties**. There is no need to plan beforehand.

Ab = abstracts; AQ = assessment question; a_w = water activity; FBO = food-borne outbreak; HPP = high pressure processing; P = pressure; RTE = ready-to-eat; SQ = sub-question; t =time; Ti = titles; ToR = term of reference.

Table A4: Integration of evidence across sub-questions and remaining overall uncertainty

ToR/AQ	Step 2.2. Integration of evidence between sub-questions	Step 2.2. Addressing overall uncertainty
ToR 1a-AQ1/ What are the (broad) food categories for which there is evidence that HPP could in principle be applied with the purpose of increasing microbiological food safety, focusing on those foods that are being commercially processed by HPP? What are the processing conditions (e.g. P, t, T) and packaging applied by industry?	SQ1, SQ2: The evidence from SQ1 and SQ2 will be combined in a narrative, qualitative way.	There is no need to plan beforehand.
ToR 1b-AQ2/ What are the intrinsic (i.e. food related) and extrinsic (i.e. processing related) factors that may influence the efficacy of HPP in terms of reduction (log_{10} units) of vegetative microorganisms when applied to foodstuffs?	SQ3: There is only one SQ so there is no need for evidence integration across SQs.	There is no need to plan beforehand.
ToR 1c-AQ3/ What are the potential microbiological food safety concerns in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods with the purpose of increasing microbiological food safety (e.g. thermal pasteurisation of milk)?	SQ4: There is only one SQ so there is no need for evidence integration across SQs.	There is no need to plan beforehand.
ToR 1c-AQ4/ What are the potential chemical food safety concerns through formation of process contaminants in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods with the purpose of increasing microbiological food safety?	SQ5, SQ6: The evidence from SQ5 and SQ6 will be combined in a narrative, qualitative way.	There is no need to plan beforehand.
ToR 1c-AQ5/ What are the potential chemical food safety concerns through food contact materials in HPP-treated food compared to untreated food or food submitted to treatments, routinely applied to these foods with the purpose of increasing microbiological food safety?	SQ7, SQ8, SQ9, SQ10: The evidence from SQ7, 8, 9 and 10 will be reported in a narrative, qualitative or semi-quantitative way when possible, either separately (e.g. for SQ8 and 10) or combined.	There is no need to plan beforehand.
ToR 2a-AQ6/ What log_{10} reduction of *Mycobacterium* spp., *Brucella* spp., *L. monocytogenes*, *Salmonella* spp. and STEC (or other relevant vegetative pathogens) is achieved by thermal pasteurisation of raw milk and raw colostrum from ruminants according to the legal requirements?	SQ11, SQ12, SQ13: The outcome of SQ11 (relevant pathogens) directly feeds into SQ12 in which the thermal inactivation parameters of the relevant pathogens will be derived. Then, the thermal inactivation parameters calculated in SQ12 will be used to calculate the log_{10} reduction of those pathogens using thermal pasteurisation (SQ13 answering AQ6).	There is no need to plan beforehand.
ToR2a-AQ7/ What are the minimum requirements of HPP (i.e. t and P and any other relevant factor) of raw milk and raw colostrum from ruminants to achieve an equivalent efficacy (in terms of log_{10} reduction) to that of thermal pasteurisation for the control of the pathogens deemed relevant according to AQ6?	SQ14, SQ15, SQ16: The relevant factors (outcome of SQ14) and most resistant pathogens (SQ15) considering those derived in SQ11 will be used to calculate the minimum requirements of HPP (SQ16 answering AQ7) that are equivalent to those of thermal pasteurisation (AQ6).	There is no need to plan beforehand.
ToR 2b-AQ8/ Which inherent components of the milk or colostrum could be used as appropriate indicators to verify the efficacy of HPP of raw milk and raw colostrum from ruminants, either as part of the validation and verification immediately after such treatment (e.g. in the processing plant) and/or in the end-product on the market, considering the minimum requirements as defined in AQ7?	SQ17: There is only one SQ so there is no need for evidence integration across SQs.	There is no need to plan beforehand.
ToR 2c-AQ9/ What are the relative levels of exposure [or probability of illness, if data allow] for the pathogen(s) to be defined per serving through the consumption of industrially HPP-treated milk or colostrum in comparison to raw versus thermally pasteurised versus UHT-treated milk or colostrum [according to the legal requirements] [considering that the batch to be treated is contaminated]?	SQ18: There is only one SQ so there is no need for evidence integration across SQs.	There is no need to plan beforehand.

ToR 3a-AQ10/ What are the minimum requirements of HPP (i.e. t and P and any other relevant factor) when applied to the food categories known to be associated with human listeriosis to reduce significantly *L. monocytogenes* levels by certain log_{10} reductions, assuming that the parameters influencing the subsequent growth of *L. monocytogenes* remain unchanged (e.g. product characteristics, shelf-life and storage conditions)?	SQ19, SQ20, SQ21: The minimum requirements of HPP (SQ21) according to the relevant factors related to food (from SQ20) when applied to the most relevant foods known to be associated with human listeriosis in the EU and that are relevant to be treated with HPP (from SQ19) will be calculated in SQ21 with the aim to reduce significantly *L. monocytogenes* levels by certain log_{10} reductions.	There is no need to plan beforehand.
ToR 3b-AQ11/ What is the efficacy (log_{10} reduction) on other relevant pathogens when applying the minimum requirements of HPP identified in AQ10?	SQ22, SQ23: After defining in SQ22 the other relevant pathogens (apart from *L. monocytogenes*) in the foods known to be associated with human listeriosis in the EU and that are relevant to be treated with HPP (from SQ19), in SQ23 the efficacy on these other pathogens when applying the minimum requirements of HPP identified in SQ21 will be estimated.	There is no need to plan beforehand.

AQ = assessment question; HPP = high pressure processing; P = pressure; SQ = sub-question; STEC = Shiga toxin-producing *E. coli*; ToR = term of reference; t = time.

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