Annex A – Protocol for the assessment of the public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU

A.1. Introduction

A.1.1.   Scope of this protocol

This document outlines the protocol for the scientific assessment of the public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU, which will be used as input for the scientific opinion of the EFSA Panel on Biological Hazards (BIOHAZ) on the public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU.

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). It describes how the mandate Terms of Reference (ToRs) were interpreted and translated into operational assessment questions (AQs) and sub-questions (SQs) and it illustrates the approach and methods planned for conducting the assessment.

The protocol was developed by the members of a working group (WG) and EFSA staff and was approved by the BIOHAZ Panel at their 162nd plenary meeting (7-8 June 2023).

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

Vibrio spp. are a group of common, Gram-negative, rod-shaped bacteria that are part of the natural microbiome of freshwater, estuarine and marine environments. A dozen of the over 160 recognised species[1] of *Vibrio* are potentially pathogenic to humans. *Vibrio cholerae* (O1 or O139 serogroups) is the aetiological agent of cholera while other pathogenic *Vibrio* species include *V. parahaemolyticus*, *V. vulnificus*, and non-O1, non-O139 *V. cholerae*. Vibrios grow in temperate and warm waters with moderate salinity (5−25 ppt). Under non-favourable conditions vibrios may enter the viable but non-culturable (VBNC) state, a state of reduced metabolic activity characterised by higher resistance to environmental stressors (Li et al., 2014).

Non-cholera *Vibrio* spp. cause vibriosis — infections normally acquired through exposure to sea water or consumption of raw/undercooked seafood. They may be occasionally detected also in other commodities, such as fermented foods, raw vegetables or ready-to-eat foods, but the factors influencing their transmission in the food chain is still a matter of discussion (Valero et al., 2021). The accumulation of vibrios in seafood, and in particular bivalves, followed by consumption of those products either raw or not fully cooked is an established route of human exposure. Other factors affecting exposure include storage and transportation at inappropriate temperatures, contamination by an infected food handler, or cross-contamination through contact with contaminated seafood or seawater (FAO and WHO, 2020, 2021). Further to this, *V. parahaemolyticus* displays the ability to form biofilms (Wang et al., 2022), underlying the need for cleaning and disinfection in seafood handling environments. Clinical manifestations are

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[1] https://lpsn.dsmz.de/search?word=vibrio

most commonly mild, self-limiting gastroenteritis, except for *V. vulnificus*, an opportunistic pathogen whose infection in individuals with predisposing health conditions (e.g. chronic liver diseases, hemochromatosis, immunocompromised) can rapidly lead to fatal septicaemia (Baker-Austin et al., 2018). Several other *Vibrio* species and some strains of *V. parahaemolyticus* and *V. vulnificus* can lead to vibriosis in aquatic animals and negatively impact animal production (de Souza Valente and Wan, 2021).

Current predictions show that the warming of marine waters as a result of global climatic change may have an impact on the transmission of these pathogens. In fact, it has been argued that this group of pathogens represents an important and tangible barometer of climate change in marine systems (Baker-Austin et al., 2016). Indeed, the association between the increase of sea surface temperatures (SST) in the Baltic area between 1982 and 2010 and the emergence of *Vibrio* infections clustered around the Baltic Sea area was observed (Baker-Austin et al., 2013) and was confirmed in correspondence with an extreme heat wave in northern Scandinavia during summer 2014 (Baker-Austin et al., 2016). As a consequence, the European Centre for Disease Prevention and Control (ECDC) started to monitor *Vibrio* growth in the Baltic Sea during summer through a near real-time model (i.e. the *Vibrio* suitability tool in a *Vibrio* map viewer[2]) that uses daily updated remote sensing data to examine environmental conditions such as SST and salinity. Alongside, climate change as a driver of emerging risk of *Vibrio*-infections through food has been raised by EFSA in the CLEFSA (CLimate change and Emerging risks for Food Safety) project. *Vibrio* spp., especially *V. parahaemolyticus* and *V. vulnificus*, were indicated among the 'biological hazards to human health' issues with a very high likelihood of emerging in Europe under a near-future climate scenario (EFSA, 2020).

Odeyemi (2016) reviewed the occurrence of *V. parahaemolyticus* in seafood based on studies reported in the 2003-2015 period; European studies reported prevalences from 7.8% for mussels (Normanno et al., 2006) to 77.8% for shrimps (Copin et al., 2012). Concentrations were also variable depending on studies and methods: in Italy, an average concentration of 77 CFU/g (range $3 - 1.8 \times 10^3$) was reported in bivalve shellfish (Suffredini et al., 2014) and 400 MPN/g (range $3 - 1.6 \times 10^5$) in crustacea (Caburlotto et al., 2016). Outbreaks of *V. parahaemolyticus* infections following consumption of contaminated raw or undercooked seafood have been reported around the world. In the EU, seven strong-evidence foodborne outbreaks caused by *V. parahaemolyticus* with 'Crustaceans, shellfish, molluscs and products thereof' as the suspected vehicle have been reported (2010-2020 period), causing 127 cases, of which 50 were hospitalised. Only a minority of *V. parahaemolyticus* environmental isolates are pathogenic to humans, pathogenicity being mainly associated with the production of haemolysins (thermostable direct haemolysin, TDH, or thermostable direct haemolysin-related haemolysin, TRH). Recent studies, however, demonstrated the absence of the genes codifying for TDH and TRH in approximately 10% of clinical isolates (Raghunath, 2015), highlighting the relevance of other possible virulence factors, such as type 3 and type 6 secretion systems, adhesion and iron uptake systems.

Vibrio vulnificus is the leading cause of seafood-related deaths in the United States and displays the highest fatality rate of any foodborne pathogen (Scallan et al., 2011); 51.6% of cases reported to the Food and Drug Administration (FDA) between 1992 and 2007 died (Jones and Oliver, 2009). *V. vulnificus* optimum salinity lays between 10 and 18 ppt; it is rarely isolated from waters with salinities >25 ppt, so occurrence and human infections are infrequent in such environments (e.g. the Mediterranean Sea). Although survival of *V. vulnificus* in cold water (<10°C) is achieved by entering the VBNC state, it is rarely isolated when water temperatures are lower than 13°C, so that most cases occur in the warmer summer months. Attempts to identify a single genetic marker for *V. vulnificus* virulence have failed. Clinical and environmental strains are often divided according to polymorphisms of *pilF* (pilus type IV), *vcg* gene, 16S RNA

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[2] https://geoportal.ecdc.europa.eu/vibriomapviewer/. The model used for the *Vibrio* viewer has been calibrated to the Baltic Region in Northern Europe and might not apply to other worldwide settings prior to validation.

gene, or capsular polysaccharide, however no single target has proved completely reliable (Roig et al., 2018).

Non O1-non O139 *V. cholerae* strains do not generally produce the cholera toxin typical of the O1/O139 serogroups but may be responsible for gastroenteritis cases varying from mild to serious in severity in association with the production of a heat-stable enterotoxin or the expression of other virulence factors (e.g. the El Tor haemolysin or the RtxA cytotoxin). Similar pathogenicity may be expressed by non-cholera-toxin-producing *V. cholerae* O1 and O139 strains. Occurrence of non-O1, non-O139 *V. cholerae* in seafood is well documented, with a prevalence of 5.6% in different products collected in Italy (Ottaviani et al., 2009), 11 to 16% in mussels from German production areas (Huehn et al., 2014), and with a sporadic detection in water from shellfish-growing areas in France (Cantet et al., 2013).

The issue of the public health risk associated with *Vibrio* spp. in seafood was considered, to different levels, in several documents. In 2001 the Scientific Committee on Veterinary Measures relating to Public Health published an opinion on *V. vulnificus* and *V. parahaemolyticus* in raw and undercooked seafood, concluding that the incidence of infections by these two species in Europe could not be quantified. Concern was raised, however, that the trends in international trade, consumption of raw seafood and number of susceptible persons may lead to an increase of infections (SCVM, 2001). Following this, risk assessments were developed by FAO/WHO on *V. vulnificus* in raw oysters (FAO and WHO, 2005a), *V. cholerae* O1 and O139 in warm water shrimp (FAO and WHO, 2005b), and *V. parahaemolyticus* in seafood (FAO and WHO, 2011). However, as modelling in these risk assessments was mainly based on data gathered in the United States, their application in areas where initial *Vibrio* concentrations, environmental conditions, and harvesting/post-harvesting practices may differ significantly, should be considered with caution. With regard to the EU area, in 2022, the German Federal Institute for Risk Assessment published a health risk assessment of the occurrence of *Vibrio* spp. in food, which remarked that food investigation should concentrate on *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*, and that the consumption of raw or insufficiently cooked food products is one of the most important factors for their transmission (BfR, 2022).

Risk assessments focused on the EU situation have not taken place since the previously mentioned opinion of the SCVM relating to Public Health published in 2001. This, together with the presence of pathogenic *Vibrio* spp. in European marine and freshwater and in seafood, the underreporting of human cases due to the lack of surveillance of non-cholerae *Vibrio* infections in the EU, the evidence of outbreaks of *Vibrio* infections following consumption of contaminated raw or undercooked seafood also in the EU, the absence of food safety criteria of *Vibrio* spp. in seafood, and the warming pattern of the SST in particular areas, underpin the need to propose a self-task mandate by the BIOHAZ panel on the public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU.

The BIOHAZ Panel is asked to issue a scientific opinion on the public health aspects of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1, non-O139 *Vibrio cholerae* (and other species whenever relevant) related to the consumption of seafood for the EU population. More specifically, EFSA is requested to address the following terms of reference (ToRs):

ToR1. To review, for the relevant *Vibrio* spp., the existing information on occurrence and concentration in seafood, available analytical methods, pathogenicity to humans and virulence factors, as well as antimicrobial resistance and persistence mechanisms in different environments

ToR2. To identify the factors in the aquatic environments and in seafood (including during production and processing) that influence occurrence and growth of the relevant *Vibrio* spp., and affect transmission of their virulence and resistance determinants

ToR3. To review the evidence on the impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in water environments and seafood

ToR4. To list and review prevention and control measures along the food chain for the relevant *Vibrio* spp.

ToR5. To review risk assessment modelling options for *Vibrio* spp. in seafood and to identify the knowledge gaps and data needed to perform a risk assessment on the public health impact of the relevant *Vibrio* spp. in seafood at the EU level

ToR6. To recommend areas for future research on *Vibrio* spp. in seafood and aquatic environments

A.1.3. Interpretation of the Terms of Reference of the mandate

The following has been clarified with the requestor:

- Seafood, for this assessment, is understood to be food of marine and animal origin. It encompasses a variety of food products including fish and fishery products, molluscs (bivalve molluscs, marine gastropods and cephalopods), crustaceans, echinoderms (e.g. sea urchins and holoturidae) and tunicates. Food products from inland aquaculture settings reproducing the marine environment will be included. Marine food of non-animal origin (e.g. seaweed) will not be considered in this risk assessment.
- The assessment will deal with *Vibrio* foodborne transmission through the consumption, by the EU population, of seafood as placed or intended to be placed on the EU market (thus including global trade). Hence, infections deriving from environmental, recreational and/or occupational exposure to vibrios will not be considered further in this risk assessment.
- The consumer's part in the risk assessment (e.g. storage, cross-contamination or (under)cooking during food preparation) will be addressed in ToR4 and 5.
- The assessment will cover *V. parahaemolyticus*, *V. vulnificus* and non-O1, non-O139 *V. cholerae*. Other species will be considered relevant if found in seafood placed or intended to be placed on the EU market and reliably identified as the cause of illness in humans in the EU through seafood consumption.

Clarifications for specific ToRs are listed below:

- For ToR1, it is understood that the occurrence and concentration of the relevant *Vibrio* spp. in seafood should cover both the detection/enumeration of the aforementioned species and, where achievable, the specific detection/enumeration of their pathogenic strains or of strains with characteristic features associated with pathogenicity or virulence.
- For ToR1, analytical methods will cover those used for the detection and enumeration of the different relevant *Vibrio* spp. including, where available, methods targeting pathogenic strains within a species. Methods addressing the identification and characterisation of virulence profiles and closely related strains of the different relevant *Vibrio* spp. will also be considered. Strengths and limitations of the described methods will be addressed.
- For ToR1, pathogenicity is defined as the ability of the relevant *Vibrio* spp. to cause disease in a human host and virulence as the degree to which a pathogen causes damage to the host. For the review of the pathogenicity and virulence factors of the relevant *Vibrio* spp. it is understood that features differentiating *Vibrio* strains with pandemic potential will be considered and addressed.

- For ToR1, antimicrobial resistance (AMR) is understood as the evidence of resistance to antimicrobial compounds in isolates of relevant *Vibrio* spp. taken from seafood or in clinical isolates associated with foodborne outbreaks or cases. Clinical isolates with no clear link with foodborne transmission will be excluded. Both phenotypic expression of resistance as well as genotypic information will be considered. Emerging AMRs that are regarded as a possible threat for the public will also be addressed.
- For ToR1, persistence is understood as the ability of a given organism to establish itself and remain within a given environment for a long term (long-term survival in the specific environment).
- For ToR2, factors are understood to refer to extrinsic and intrinsic factors that affect the presence or concentration (due to growth or inactivation), or that affect horizontal or vertical transmission of pathogenicity, virulence or resistance determinants in the relevant *Vibrio* spp. in aquatic environments and in food. Implicit factors affecting occurrence, growth and transmission of virulence/resistance determinants will also be addressed.
- For ToR2, the aquatic environments are considered in their whole and not limited to areas associated with seafood production (aquaculture or fishing). Furthermore, both seawaters and brackish waters will be considered. Seafood (see above) is covered in all its production stages, including the aquaculture process, harvesting/fishing, processing and transport up to retail level. Domestic handling and consumption practices are out of the scope of the ToR.
- For ToR3, the evidence on the impact of climate change is interpreted as the reporting of empirical observations or of modelling studies assessing the changes of the occurrence and/or concentration of the relevant *Vibrio* spp. (in water environments and/or in seafood) in correlation with one or more of the changes of the marine environment associated with global climate change (e.g. increase of SST associated with warming trends or extreme weather events).
- For ToR4, control measures for vibrios in seafood that are already in place (either locally or globally), as well as those experimentally tested for possible implementation will be included. The advantages and disadvantages of the prevention and control measures will be listed and, will include, when relevant and feasible, a qualitative evaluation of their efficacy (effect on prevalence/concentration of the relevant *Vibrio* spp. in seafood). Economic or environmental impacts or user acceptance will not be considered.
- For ToR5, the risk assessment modelling options for *Vibrio* spp. in seafood will be reviewed based on existing risk assessments, models and approaches and will cover identifying the data needed to perform a risk assessment on the public health impact of the relevant *Vibrio* spp. in seafood at the EU level. The characteristics of the available models will consider the methods, assumptions, data, model limitations, etc. Performing a risk assessment itself is not envisaged.

A.2. Problem formulation

A.2.1. Assessment question(s) and sub-questions and conceptual model

The ToRs of the mandate were translated into six AQs. The AQ1, AQ2, AQ3 and AQ5 were each further broken down into eight, two, three and three SQs, respectively. The AQs and SQs and 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 or SQs.
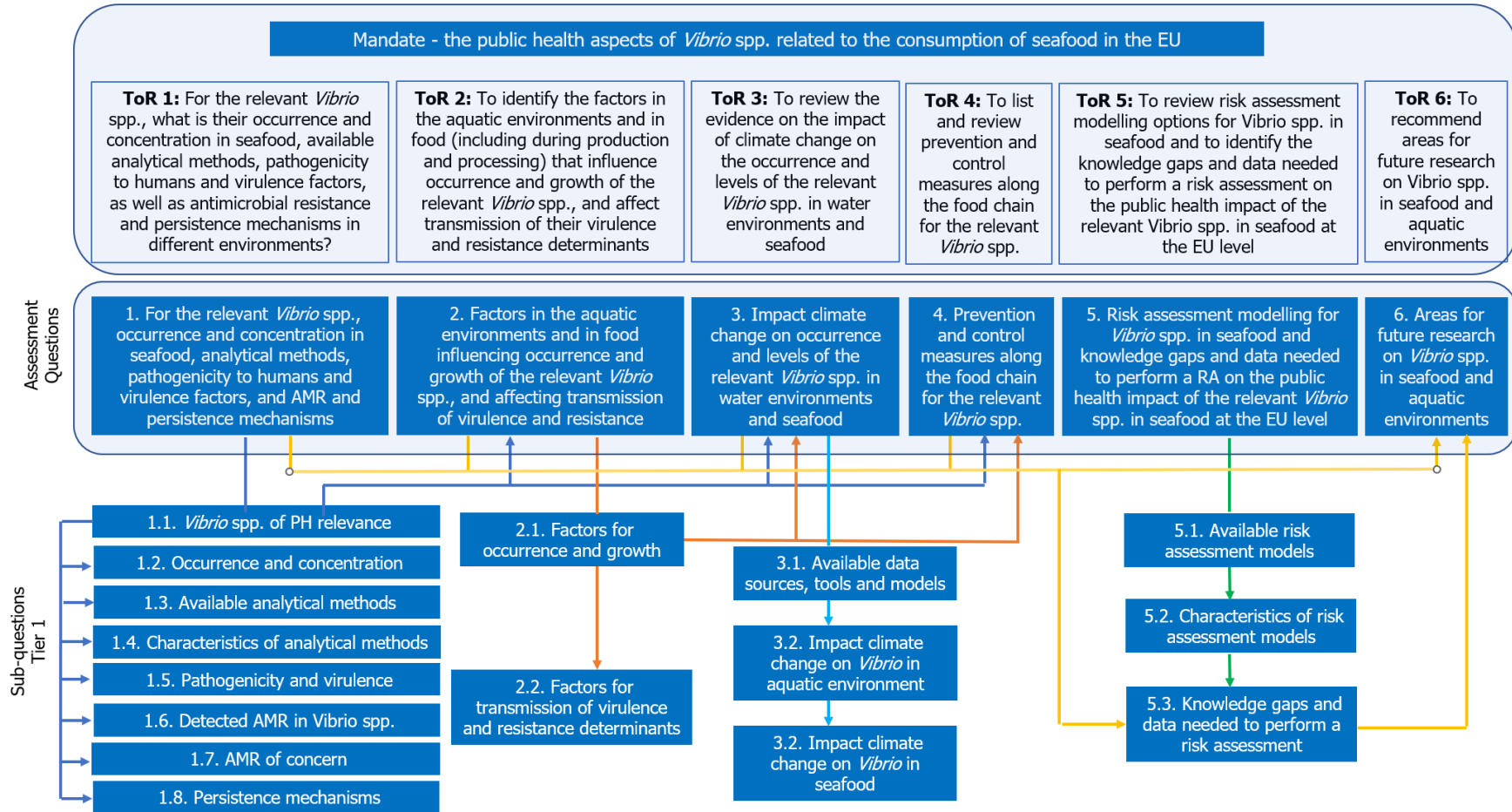
Figure A.1: The relationship between the assessment questions (AQs) and sub-questions (SQs).

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 and SQ 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 SQs and for accounting for the remaining uncertainty are provided in Table A.2 based on the conceptual model.

Table A.1: Assessment questions and sub-questions for the assessment of the public health aspects of *Vibrio* spp. related to the consumption of seafood in the EU.

Assessment question(s)	Sub-questions (SQ)	Overall approach	Method	Evidence needs and methods
AQ1/ For the relevant *Vibrio* spp., what is their occurrence and concentration in seafood, available analytical methods, pathogenicity to humans and virulence factors, as well as antimicrobial resistance and persistence mechanisms in different environments?	SQ1.1/ What are the *Vibrio* spp. of highest relevance for public health in the EU through consumption of seafood beyond *V. parahaemolyticus*, *V. vulnificus* and non-O1, non-O139 *V. cholerae*?	Qualitative	By using data extracted from databases other than literature and from literature	**a. Evidence needs/Eligibility criteria:** The aim is to retrieve information on the *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus* and non-O1, non-O139 *V. cholerae* and other) of highest relevance for public health in the EU through consumption of seafood. In the **general literature search to provide a cross-sectoral data source for the various AQ and SQ**, the eligibility criterion related to study characteristics is whether the record includes information on *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* non-O1, non-O139 or other relevant species) with regards to the topics covered under ToRs 1 to 5[3]). The eligibility criteria related to report characteristics are: • Language of the full text: English and Spanish • Time: 2010 onwards • Publication type: review or book (chapter) **b. Source of data/Search strategy:** <u>Source of data:</u> The data on foodborne outbreaks (FBO) in the EU will be extracted from the EFSA zoonoses database (both 'strong and weak evidence' FBO) without time restrictions and with *Vibrio* spp. as causative agent. Additionally, data will be extracted from the Rapid Alert System for Food and Feed (RASFF) database without time restrictions for the notification type 'food' and the hazard category 'pathogenic micro-organisms' (more specifically the hazard *Vibrio*); retrieved records will be screened for detected *Vibrio* spp. and notification type (alert being related to human cases/outbreaks). Further to this, data will be searched from the ProMED records (https://promedmail.org/) without time restriction for the posts reporting 'Vibrio' as keyword or within the post and will be screened for having occurred within the EU. Also, recent reports, articles and reviews will be consulted related to *Vibrio* FBOs/cases in the EU. In addition, the outcome of recent risk assessments undertaken by MSs and international bodies on the topic will be collected and screened for evidence of reported foodborne vibriosis. Furthermore, the results of the Food Standards Australia New Zealand (FSANZ) data request to EU MSs and international bodies, submitted in 2022, will be considered for further evidence[(a)].

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[3] Occurrence and concentration in seafood intended to be placed on the EU market, available analytical methods (detection, enumeration, identification, characterisation) in seafood, pathogenicity to humans and virulence factors (including *Vibrio* outbreaks for the three relevant species), AMR, persisting mechanisms in different environments, factors in the aquatic environments and in seafood that influence occurrence and growth of the relevant *Vibrio* spp., factors in the aquatic environments and in seafood that affect transmission of their virulence and resistance determinants, impact of climate change on the occurrence and levels of the relevant *Vibrio* spp. in water environments and seafood, prevention and control measures along the seafood chain for the relevant *Vibrio* spp., risk assessment modelling options for *Vibrio* spp. in seafood.

The information will be supplemented by other information based on the knowledge/expertise of the Working Group (WG) and Panel members.

Search strategy: The general (cross-sectoral) literature search to gather recent reviews will be carried out in the Web of Science™ Core Collection (SCI-Expanded, BKCI-S, ESCI, CCR-Expanded, IC) and will consider either in the title or topic: Vibrio or synonyms AND seafood or synonyms or in the title only Vibrio or synonyms (see Table A.4).

c. Data model to extract data from the selected databases/Study selection for inclusion/exclusion:

Data model: The extracted FBO datafile will be filtered considering time (from 2010 to 2021) and relevant food vehicles (i.e. the food(stuff) suspected of causing human cases) considered seafood or containing seafood. The available epidemiological evidence will then be summarised using number of outbreaks, number of cases, number of hospitalised cases and number of deaths categorised by *Vibrio* spp. Reporting per year and per country will be provided.

The extracted RASFF data will be filtered for time (from 2010 onwards) and the product categories bivalve molluscs and products thereof, cephalopods and products thereof, crustaceans and products thereof, fish and products thereof, gastropods. The information will be summarised using alert/notification per product category, *Vibrio* spp. and year. Information on detection of *Vibrio* spp. with specific virulence factors will be checked.

Study selection: The screening process will be undertaken by two reviewers in two steps: screening of (1) title/abstract (Ti/Ab) based on the review question to exclude irrelevant records, and then screening of (2) full-text documents based on the previously defined criteria related to report and study characteristics. It will be flagged during the screening on which topic(s) the review provides information.

d. Data check and validation (i.e. identification of the uncertainty in the evidence). The uncertainty will mainly be linked to the quality of data retrieved from the databases. A table will be elaborated including all the uncertainty sources.

e. Uncertainty sources and methods for prioritising them. Sources of uncertainty will be listed in a table without the need for prioritising them.

f. Evidence synthesis. The methods used for the synthesis will be qualitative and cover the various data sources. A list of *Vibrio* spp. that are found to be associated with seafood and with human disease in EU will be prepared, obtained from the sources of evidence, and the most relevant *Vibrio* species will be identified based on the available data and expert judgement .

g. Uncertainty analysis. The methods used for the uncertainty analysis will be qualitative.

SQ1.2/ Considering the relevant *Vibrio* spp. (from SQ1.1), what is their prevalence and concentration in seafood placed or intended to be placed on the EU market?	Qualitative or semi-quantitative	By using data extracted from databases other than literature and literature	**a. Evidence needs/Eligibility criteria.** The aim is to retrieve information on the occurrence (prevalence and concentration) of the relevant *Vibrio* spp. (from SQ1.1) in seafood placed or intended to be placed on the EU market.

b. Source of data/Search strategy: The data on the occurrence of *Vibrio* in food in the EU will be extracted from the EFSA zoonoses database without time restrictions.

Additionally, the recent reviews collected as described in SQ1.1 will be considered. Eligible records must include information on the occurrence of one of the relevant *Vibrio* spp. in seafood placed or intended to be placed on the EU market. Depending on the coverage of these reviews, e.g. the seafood and *Vibrio* species considered, as well as the time frame covered, an extensive literature search (ELS) will be conducted to retrieve observational studies. For experimental

studies, the eligibility criteria for the literature review related to study characteristics are:

(1) Occurrence data must originate from observational studies that could be cross-sectional or longitudinal where seafood samples have been sampled.

(2) Seafood, as finished product or during production/processing, must be sampled from farms, processing facilities, border control points, wholesale markets, retail establishments located in a European country. Seafood category/type and food chain stage where samples were taken must be clearly specified.

(3) Occurrence data must be given for one of the relevant *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus* and non-O1, non-O139 *V. cholerae* and other species if identified as relevant), thus with species identification (not only reporting *Vibrio* spp.).

(4) The study must present the results on prevalence and/or enumeration of the relevant *Vibrio* spp. in/on seafood. For prevalence, it should provide at least sample size and number of positive samples; and for enumeration, it should provide at least the sample size and a measure of concentration.

The eligibility criteria related to report characteristics are:

- Language of the full text: English only (or also including Spanish, French, Portuguese, Italian or German).
- Time: 2010 onwards.
- Publication type: primary research studies and reviews (to find additional primary research studies).

b. Search strategy. The search will be carried out in the Web of Science™ Core Collection (alternative: in the e-bibliographic databases PubMed, Web of Science (Core Collection, all databases), Scopus and SciELO) and will consider the general terms (sampling etc.) AND the hazard term (the relevant *Vibrio* species) AND the foodstuff terms (seafood and composite products) AND a country term (to restrict to Europe) (see Table A.4).

c. Study selection for inclusion/exclusion. The screening process will be undertaken in two steps: screening of (1) Ti/Ab based on the review question to exclude irrelevant records, and then screening of (2) full-text documents based on the previously defined criteria related to report and study characteristics. It will be flagged during the full-text screening when a study reports on antimicrobial resistance data.

d. Data extraction from included studies. Selected full-text documents will be screened by one reviewer to extract the relevant information, similarly to what has been described in https://zenodo.org/record/7504196#.Y-JzfnbMKUl. The data to be extracted are related to (i) general framing of the study, (ii) study characteristics relevant to explain heterogeneity in the outcomes; namely the nature of the seafood sampled and the method of detection/quantification, and (iii) outcome measures, detection and/or quantification. A data extraction model will be agreed beforehand.

e. Evidence appraisal. This will be done in a narrative way based on expertise of WG and BIOHAZ Panel members.

f. Uncertainty sources and methods for prioritising them. Sources of uncertainty will be listed in a Table without the need for prioritising them.

g. Evidence synthesis. The evidence retrieved will be synthesised by listing the data on the occurrence (prevalence and concentration) of the relevant *Vibrio* spp. in seafood on the EU market (from SQ1.1) according to seafood category and type (animal species). The synthesis will include country of the study, year(s) of sampling, description of the sampling point (stage of the food chain), sampling size and number of positive units, concentration measure (for quantitative analysis), the tested amount, a reference

to the method used. Where reported, data related to pathogenic strains (detection/quantitation) or to the identification of features associated with pathogenicity/virulence (e.g. serotype, sequence type, toxin genes, etc.) will be captured.

h. Uncertainty analysis. The methods used for the uncertainty analysis will be qualitative.

SQ1.3/ Considering the relevant *Vibrio* spp. (from SQ1.1), what are the available analytical methods for the detection, enumeration, and characterisation in seafood?	Qualitative	By using data extracted from literature	**a. Eligibility criteria.** The aim is to retrieve information on the analytical methods for the detection, quantification, and characterisation of the relevant *Vibrio* spp. (from SQ1.1) in seafood. Priority will be given to official control and standardized methods; analytical methods only experimentally tested will be briefly summarised.
SQ1.4/ Considering the available analytical methods (from SQ1.3), what are their characteristics and how do they contribute to risk assessment?	Qualitative	By using data extracted from literature	Firstly, ISO/CEN catalogues will be consulted. Other methods from national bodies (e.g. FDA BAM, Ministry for Primary Industries NZ, Health Products and Food Branch Canada, etc.) will also be included. Also, information shared by international bodies will be consulted. FAO/WHO reviews of methods will be considered in the discussion of the characteristics of the available methods.

Secondly, review articles, books, book chapter and reports will be considered as well as the results of the FSANZ data request to EU MSs and international bodies[a].

Finally, primary research studies will be considered using the data collected through the ELS as described in SQ1.2, in which the method of detection (and also quantification) will be extracted.

b. Search strategy. The reviews, books and book chapters would be collected from the search described in SQ1.1, while the primary research studies will be collected from the search described in SQ1.2.

c. Study selection for inclusion/exclusion. See in SQ1.1 and SQ1.2.

d. Data extraction from included studies. See in SQ1.1 and SQ1.2. The data to be extracted are related to general structure of the method, principle, scope, laboratory technique adopted, breadth of application, availability of performance characteristics (limit of detection-LOD, limit of quantification-LOQ, accuracy, etc) and framework of their collection (in-house, ILS).

e. Evidence appraisal. See SQ1.2

f. Uncertainty sources and methods for prioritising them. Sources of uncertainty will be listed in a Table without the need for prioritising them.

g. Evidence synthesis. For SQ1.3 the evidence retrieved will be synthesised by listing the methods used for the detection, enumeration, and characterisation of the relevant *Vibrio* species, and describing their use on different seafood types and/or in different contexts and summarising (when available) their performance characteristics. For SQ1.4 the characteristics of the analytical methods identified in SQ1.3, grouped per types, will be addressed, and summarised through a SWOT[4] analysis.

h. Uncertainty analysis. The methods used for the uncertainty analysis will be qualitative.

SQ1.5/ Considering the relevant *Vibrio* spp. (from SQ1.1), what is their pathogenicity to humans, and which are their virulence factors?	Qualitative	By using data extracted from literature	**a. Eligibility criteria.** The aim is to retrieve, for each of the relevant *Vibrio* spp. (from SQ1.1), information on: (1) the diseases induced in humans, indicating their features (e.g. infectious dose, incubation, length, symptoms and how often they are present, susceptibility or predisposing diseases, hospitalisation rate, remission, sequelae, fatality rate and cause of death); (2) the mechanisms through which the pathogenicity is exerted; (3) the virulence factors associated with each of the relevant *Vibrio* spp., their genetic determinants, and their occurrence in isolates from different sources.

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[4] Strengths, Weaknesses, Opportunities, and Threats.

For experimental studies, the inclusion criteria for the literature review related to study characteristics are:

1) Dealing with one of the relevant *Vibrio* spp. identified in SQ1.1;
2) Addressing the detection, identification or characterization of a pathogenicity factor or mechanism OR providing evidence – using in vivo or in vitro models – that the presence of the virulence factor is associated with pathogenic effects

The eligibility criteria related to report characteristics are:

- Language of the full text: English only;
- Time: 2010 onwards (as it is not useful to retrieve older methods);
- Publication type: original articles, research notes.

b. Search strategy. Depending on evidence requirements, experimental studies will be retrieved on pathogenicity and on virulence factors based on the knowledge of the WG members, through snowballing from the reviews/book chapters/reports retrieved in the cross-sectoral literature search, or through specific literature searches in the Web of Science™ Core Collection. For the latter, the search will consider in the title or topic: relevant *Vibrio* spp. (or synonyms) AND pathogenic* OR virulence OR toxin* OR (relevant synonyms) OR (list of factors identified by the WG members). Further to this, virulence genes of the relevant *Vibrio* spp. (SQ1.1) will be searched in VFDB (Virulence Factor Database, http://www.mgc.ac.cn/VFs/). The listed genes will be used to complete the previously defined searches.

c. Study selection for inclusion/exclusion. See in SQ1.1. Screening will be done at full-text level, while for the specific literature searches (if used), the screening process will be undertaken in two steps: screening of (1) Ti/Ab mainly based on inclusion criteria and (2) full-text documents to further identify records to be excluded based on criteria related to report characteristics (e.g. not in English).

d. Data extraction from included studies. Selected full-text documents (reviews or primary research studies) will be screened by one reviewer to extract the relevant information.

e. Evidence appraisal. This will be done in a narrative way based on expertise of WG and BIOHAZ Panel members.

f. Uncertainty sources and methods for prioritising them. Sources of uncertainty will be listed in a Table without the need for prioritising them.

g. Evidence synthesis. The evidence retrieved will be synthesized, for each of the relevant *Vibrio* spp., (1) by describing the human disease (or the different clinical manifestations) caused by the species (2) by listing the virulence factors, including information on their occurrence and distribution in clinical, environmental and food samples, when present, and on their genetic determinants and mechanisms. Virulence factors will be considered as such based on the concurrent evidence of detection/identification in one of the relevant *Vibrio* spp. and of their pathogenic effect (i.e. the presence of a gene alone is not sufficient to define a virulence factor).

h. Uncertainty analysis. The methods used for the uncertainty analysis will be qualitative.

SQ1.6/ Which are the most frequently detected antimicrobial resistances in the relevant *Vibrio* spp. (from SQ1.1) isolated	Qualitative	By using data extracted from literature and from	**a. Eligibility criteria**. The aim is to recognise the most frequently detected AMR of the relevant *Vibrio* spp. (from SQ1.1) in seafood (primary production or retail) and clinical isolates associated with FBOs or cases. Both phenotypic expression of resistances as well as genotypic information will be considered.

from seafood and from seafood-borne infections?		information request	Secondly, the genetic background of AMRs regarding their transmission/transfer to other bacteria will be identified. The focus will be on emerging resistances against antimicrobials of therapeutic relevance.
			b. Search strategy. The data will be mainly retrieved from review articles, books and book chapters as collected from the search strategy described in SQ1.1. Information from member states on AMR data of the relevant vibrios will be included, if available. For this, the EFSA AMR Network will be contacted asking if AMR data has been collected/reported for the three *Vibrio* spp. (specifically *V. parahaemolyticus*, *V. vulnificus* and non-O1, non-O139 *V. cholerae*), that stem from isolates from seafood (environmental samples, retail or production - e.g. primary production) and if so, to share available data, or the information on where to find them (e.g. national reports, public databases, outbreak investigations, etc.). The primary research studies will be collected through non-extensive searches and snowballing.
			c. Study selection for inclusion/exclusion. Same as in SQ1.5.
			d. Data extraction from included studies. Same as in SQ1.5.
			e. Evidence appraisal. Same as in SQ1.5.
			f. Uncertainty sources and methods for prioritising them. Same as in SQ1.5.
			g. Evidence synthesis. The evidence retrieved will be synthesised by listing antimicrobial resistance determinants of the relevant *Vibrio* spp. in a summarising table with main findings, including species, source of isolation, country and year of isolation, frequency of the AMR phenotype detected in the study, associated AMR determinants, type of reference (review/meta-analysis, primary research paper, report).
			h. Uncertainty analysis. The methods used for the uncertainty analysis will be qualitative.
SQ1.7/ Which of the detected antimicrobial resistances (SQ1.6) are of concern due to their possible horizontal transmission to other bacteria or for their emerging in the EU?	Qualitative	By using data extracted from literature	**a. Eligibility criteria**. The aim is to display the genetic background of AMRs, particularly in relation to the character - intrinsic or acquired through horizontal gene transfer – of the AMR genes. The genetic background of mobile elements will be shown regarding the transmission/transfer to other bacteria. Additionally, emerging resistance against antimicrobials of therapeutic relevance will be covered.
			b. Search strategy. At first, the data will be retrieved from review articles, books and book chapters as collected from the search strategy described in SQ1.1. Depending on evidence, primary research papers on transfer/mobility of genetic elements shall also be searched for using non-extensive searches and snowballing.
			c. Study selection for inclusion/exclusion. Same as in SQ1.5.
			d. Data extraction from included studies. Same as in SQ1.5.
			e. Evidence appraisal. Same as in SQ1.5.
			f. Uncertainty sources and methods for prioritising them. Same as in SQ1.5.
			g. Evidence synthesis. The evidence retrieved on genetic background will be summarised in a list, including the relevant AMR genes, the genetic element (e.g. plasmid, ICE, chromosomal), the occurrence in the relevant *Vibrio* spp. Discussion on genetic transfer will be included in the accompanying text. Information on emerging AMR resistances in the EU will be discussed in a narrative way.
			h. Uncertainty analysis. The methods used for the uncertainty analysis will be qualitative.
SQ1.8/ Considering the relevant *Vibrio* spp. (from SQ1.1), what are their persistence	Qualitative	By using data extracted	**a. Eligibility criteria.** The aim is to retrieve information on the different strategies that the relevant *Vibrio* spp. (from SQ1.1) use to persist in the environment, including the aquatic environment and

	mechanisms in different environments?		from literature	environments associated with food production/processing/transport. **b. Search strategy.** The data will be mainly retrieved from review articles, books and book chapters as collected from the search strategy described in SQ1.1, and from reports. The primary research studies will be collected through non-extensive searches and snowballing. **c. Study selection for inclusion/exclusion.** Same as in SQ1.5. **d. Data extraction from included studies.** Same as in SQ1.5. **e. Evidence appraisal.** Same as in SQ1.5. **f. Uncertainty sources and methods for prioritising them.** Same as in SQ1.5. **g. Evidence synthesis.** The evidence retrieved will be synthesised in a narrative way by describing all the bacterial mechanism that allow the bacteria to persist in the different environment. **h. Uncertainty analysis.** Same as in SQ1.5.
AQ2/ What are the factors in the aquatic environments and in seafood[5] that influence occurrence and growth of the relevant *Vibrio* spp., and affect transmission of their virulence and resistance determinants?	SQ2.1/ What are the factors in the aquatic environments and in food, including during production and processing that affect the presence or concentration of the relevant *Vibrio* spp. (from SQ1.1)?	Qualitative	By using data extracted from literature	**a. Eligibility criteria.** The aim is to retrieve information on the factors in the aquatic environments and in food, including during production and processing that influence presence or concentration (growth or inactivation) of the relevant *Vibrio* spp. (from SQ1.1). **b. Search strategy.** The data will be mainly retrieved from review articles, books and book chapters as collected from the search strategy described in SQ1.1, and from reports. The primary research studies will be collected through non-extensive searches and snowballing. **c. Study selection for inclusion/exclusion.** Same as in SQ1.5. **d. Data extraction from included studies.** Same as in SQ1.5. **e. Evidence appraisal.** Same as in SQ1.5. **f. Uncertainty sources and methods for prioritising them.** Same as in SQ1.5. **g. Evidence synthesis.** The evidence retrieved will be synthesised in a narrative way by listing the factors under the various categories (extrinsic, intrinsic, or implicit factors). **h. Uncertainty analysis.** Same as in SQ1.5.
	SQ2.2/ What factors in the aquatic environments and in food are considered to affect transmission of *Vibrio* virulence and resistance determinants?	Qualitative	By using data extracted from literature	**a. Eligibility criteria.** The aim is to retrieve information on the different factors that affect transmission of virulence factors and/or resistance determinants of the relevant *Vibrio* spp. (from SQ1.1) in the aquatic environment or in the food. **b. Search strategy.** Same as in SQ1.5. **c. Study selection for inclusion/exclusion.** Same as in SQ1.5. **d. Data extraction from included studies.** Same as in SQ1.5. **e. Evidence appraisal.** Same as in SQ1.5. **f. Uncertainty sources and methods for prioritising them.** Same as in SQ1.5. **g. Evidence synthesis.** The evidence retrieved will be synthesised in a narrative way by listing the factors that promote the transmission of virulence factors and resistance determinant in the different environments. A summary of their action mechanism will also be provided in the synthesis. **h. Uncertainty analysis.** Same as in SQ1.5.
AQ3/ What is the impact of climate change on the occurrence and	SQ3.1/ What data sources, tools and models are available or are under development to evaluate the impact	Qualitative	By using data extracted from databases	**a. Eligibility criteria.** The aim is to retrieve sources of information on (1) climate change, particularly in relation to the aquatic environments, (2) effect of climate change and associated events (oceanographic warming, salinity changes, extreme weather events,

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[5] including during production and processing.

levels of the relevant *Vibrio* spp. in water environments and seafood?	of climate change on the relevant *Vibrio* spp. in water environments and seafood and what are their limitations?		other than literature and literature	etc.) on changes in the presence and concentrations of the relevant *Vibrio* spp. (from SQ1.1) in the aquatic environment and in seafood.
	SQ3.2/ How is climate change affecting the occurrence and levels of the relevant *Vibrio* spp. in the aquatic environment at a global level and in Europe?	Qualitative	By using data extracted from databases other than literature and literature	**b. Source of data/Search strategy.** At first, general information on the effect of climate change on *Vibrio* spp. will be retrieved from review articles, books and book chapters as collected from the search strategy described in SQ1.1. Following, technical reports from international organization (e.g. European Environmental Agency, Intergovernmental Panel on Climate Change, etc.) and primary research papers on the topic will be collected based on the knowledge of the WG members, through snowballing from the reviews/book chapters/reports retrieved in the cross-sectoral literature search, or through specific literature searches in the Web of Science™ Core Collection or in NCBI.
	SQ3.3/ How is climate change affecting the occurrence and levels of the relevant *Vibrio* spp. in seafood at global level and in Europe?	Qualitative	By using data extracted from databases other than literature and literature	Primary research studies specifically addressing seafood-associated *Vibrio* outbreaks with a strong climatic component of the event will be collected through non-extensive searches and snowballing. Available data sources (oceanographic datasets and websites, e.g. Copernicus, NOAA, ECDC), and predictive models (mechanistic models, suitability models, etc.) will be compiled based on technical reports and on experts' knowledge. **c. Study selection for inclusion/exclusion.** Same as in SQ1.5. **d. Data extraction from included studies.** Same as in SQ1.5. **e. Evidence appraisal.** Same as in SQ1.5. **f. Uncertainty sources and methods for prioritising them.** Same as in SQ1.5. **g. Evidence synthesis.** The evidence retrieved will be synthesised visually, using *Vibrio* suitability projections for Europe (e.g. figures showing mapping of areas likely to sustain vibrios occurrence and growth in the future under different climate emission scenarios), and in a narrative way. In addition, specific case studies of past *Vibrio* outbreaks in Europe will be used to outline climatic/environmental conditions likely to drive risk. **h. Uncertainty analysis.** The methods used for the uncertainty analysis will be qualitative.
AQ4/ What are the prevention and control measures along the seafood chain for the relevant *Vibrio* spp.?	None	Qualitative	By using data extracted from literature	**a. Eligibility criteria.** The aim is to retrieve information on prevention and control measures along the seafood chain for the relevant *Vibrio* spp. Focus will be on those measures already in place while methods that have only been tested experimentally will only be summarised briefly. **b. Search strategy.** Reviews, books and book chapters will first be considered and will be collected from the search described in SQ1.1. More recent papers describing primary research studies will be collected, where needed, through non-extensive searches and snowballing. **c. Study selection for inclusion/exclusion.** Same as in SQ1.5. **d. Data extraction from included studies.** Same as in SQ1.5. **e. Evidence appraisal.** Same as in SQ1.5. **f. Uncertainty sources and methods for prioritising them.** Same as in SQ1.5. **g. Evidence synthesis.** The measures will be first listed based on the available information and a definition for each of the measures will be provided. The evidence retrieved will be synthesised in tables giving first an overview of the measures already in place and those measures that have only been tested experimentally. For those measures already in place, their advantages and disadvantages will be provided

AQ	SQ			
				and, if relevant and possible, a qualitative evaluation of their efficacy will be provided. **h. Uncertainty analysis.** Same as in SQ1.5.
AQ5/ What are the risk assessment modelling options for *Vibrio* spp. in seafood and which are the knowledge gaps and data needed to perform a risk assessment on the public health impact of the relevant *Vibrio* spp. in seafood at the EU level?	SQ5.1/ What are the available (semi-) quantitative risk assessments (QMRA) for the relevant *Vibrio* spp. (SQ1.1) in seafood or in specific seafood types/products?	Qualitative	By using data extracted from literature	The available QMRAs covering the relevant *Vibrio* spp. (SQ1.1) in seafood or in specific seafood types/products will be identified and listed based on non-extensive literature searches, snowballing, and based on the knowledge and expertise of the WG and Panel members.
	SQ5.2/ What are the characteristics of the available risk assessment models (listed in SQ5.1)?	Qualitative	The QMRAs identified in SQ5.1 will be characterised and summarised	The characteristics of each of the steps (hazard identification, exposure assessment, hazard characterisation and risk characterisation) in the identified QMRAs will be summarised and evaluated. The evaluation will address the objectives, the approach, methods used, assumptions, data, limitations, etc.
	SQ5.3/ Which are the knowledge gaps and data needed to perform assessments addressing the public health impact of relevant *Vibrio* spp. in different types of seafood at the EU level?	Qualitative	By using the outcome of SQ5.2 and ToR1 and 2	The outcome of SQ5.2 together with the outcome of ToR1 and ToR2 will be the basis for an analysis to identify the data and knowledge needed to perform assessments addressing the public health impact of relevant *Vibrio* spp. in relevant types of seafood at the EU level, taking different factors associated with risk into consideration (e.g. the number of foodborne cases, frequency of consumption, production volumes).
AQ6/ What are areas for future research on *Vibrio* spp. in seafood and aquatic environments?	None	Qualitative	Expert judgement	Based on the knowledge gaps identified when answering AQ5, research needs will be identified based on expert knowledge (WG and BIOHAZ Panel members) and prioritised based on expert group judgement.

Abbreviations: AQ, assessment question; SQ, sub-question.

[a]A questionnaire was circulated to EU MSs (EFSA MRA network) and international bodies by Food Standards Australia New Zealand (FSANZ) in 2022. Data providers granted access to use the available information also in this risk assessment. The questions were:

1) Is *Vibrio parahaemolyticus* and/or *V. vulnificus* **notifiable human diseases** in your country? Have you witnessed an increase in *Vibrio* species illness over the past 3 – 5 years? If so, do you consider that this is climate change related? Is there any substantive correlative evidence for this?
2) What *Vibrio*/**environmental monitoring** (e.g. sea water temperature, salinity etc.) in shellfish growing areas, if any, do you or your colleagues undertake? Has changes in domestic requirements in your country changed requirements for equivalence testing of imported seafood?
3) What **phenotypic methodologies (quantitative and presence/absence)** are currently being used for routine identification and testing of Vibrios in shellfish? Noting we've witnessed challenges with non-culturable *Vibrio* and issues with MPN methods.
4) Are environmental or human isolates **routinely sequenced**? Are there specific **genes** (other than *tdh* and *trh* for Vp) that are of interest from a risk perspective? Are sequences on a public or internal database and what metadata is uploaded associated with those sequences?

Table A.2: 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 1-AQ1	The various SQs are each addressing part of the AQ1. Combined they address the AQ1. The answer of SQ1.1 (*Vibrio* spp. of public health relevance) feeds into SQ1.2-1.8.	There is no need to plan beforehand.
ToR 2-AQ2	The SQs 2.1 and 2.2 are each addressing part of AQ2. Combined they address the AQ2.	There is no need to plan beforehand.
ToR 3-AQ3	Evidence integration across SQs is not needed as the SQs are organised in a logical sequence that requires the answer to the first SQ to feed into the next, until the assessment question is answered. In this case, the answer to SQ3.1 feeds into SQ3.2 and the answer to SQ3.2 feeds into SQ3.3.	There is no need to plan beforehand.
ToR 4-AQ4	Not applicable as there are no SQs	Not applicable
ToR 5-AQ5	Evidence integration across SQs is not needed as the SQs are organised in a logical sequence that requires the answer to the first SQ to feed into the next, until the assessment question is answered. In this case, the answer to SQ5.1 feeds into SQ5.2 and the answer to SQ5.2 feeds into SQ5.3.	The uncertainty will be determined to the degree that all relevant QMRAs are identified since this will be the basis for identifying knowledge and data gaps for future risk assessments.
ToR 6-AQ6	AQ6 considers the answers to AQs 1-5 to define the areas of future research	There is no need to plan beforehand.

Abbreviations: AQ, assessment question; SQ, sub-question; ToR, terms of reference.

Table A.3: Details of search strings for literature searches for the general cross-sectoral search on Vibrio in seafood using Web of Science™ Core Collection (SCI-Expanded, BKCI-S, ESCI, CCR-Expanded, IC) as of 23 March 2023.

Set number	Search query	No of records
#1	**TS**=(vibrio OR vibrios OR Vibrionaceae OR vibriosis OR parahaemolyticus OR vulnificus OR cholerae)	43,735
#2	**TS**=(seafood OR seafoods OR "sea food" OR "sea foods" OR crustacean* OR shellfish OR bivalve* OR mollusc* OR mollusk* OR fish* OR "fishery product*" OR "marine gasteropod*" OR cephalopod OR cephalopods OR crustacean OR crustaceans OR echinoderm OR echinoderms OR "sea urchin" OR "sea urchins" OR holoturid* OR tunicate OR tunicates OR urchin* OR crab* OR prawn* OR shrimp* OR lobster* OR "crayfish" OR crabfish OR crawfish OR langoustine OR scampi OR "clam" OR "clams" OR "carpet shell*" OR scallop* OR Pecten OR oyster* OR cockle OR cockles OR mussel OR mussels OR mytilus OR "Pen shell*" OR snail* OR abalone* OR Nassarius OR "whelk*" OR Bolinus OR "murex" OR ormer OR Haliotis OR "true limpet*" Patella OR Cellana OR Buccinum OR Concholepas OR conch* OR winkle* OR periwinkle* OR octopus OR squid* OR cuttlefish OR nautilus* OR Todarodes OR Loligo OR Sepia OR Paracentrotus OR Strongylocentrotus OR "Echinus esculentus" OR "sea cucumber*" OR "cukes" OR piure* OR pyura OR "sea violet*" OR "sea tulip*" OR "sea peach*" OR "sea pineapple*" OR "ice floe" OR "sea squirt*" OR gravad OR graved OR "gravad lax" OR gravlax OR sushi OR sashimi OR surimi OR ceviche OR caviar OR albacore OR amberjack OR anchovy OR anchovies OR angler OR argentine OR bacha OR barbel OR barracuda OR basa OR bass OR beluga OR bib OR bigeye OR blackfish OR bleak OR blenny OR bluefish OR "blue runner" OR "blue shark" OR bonito OR branzino OR bream OR brill OR burbot OR butterfish OR carp OR catfish OR catshark OR chub OR cod OR comber OR conger OR corb OR cutlassfish OR dab OR "danubian wels" OR dentex OR dogfish OR eel OR emperor OR flathead OR flounder OR "flying fish" OR forkbeard OR garfish OR garrick OR goby OR goldline OR grouper OR guitarfish OR gunard OR haddock OR hake OR halibut OR hammerhead OR herring OR hoki OR huss OR icefish OR "John dory" OR lamprey OR lanternfish OR leerfish OR ling OR "little tunny" OR lythe OR mackerel OR "mahi mahi" OR marlin OR megrim OR melva OR monkfish OR moonfish OR mullet OR needlefish OR oreo OR pacu OR pandoras OR panga OR pangasius OR parrotfish OR "parrot fish" OR perch OR picarel OR pike OR pilchard OR pilotfish OR "pilot fish" OR plaice OR pollan OR Pollack OR Pollock OR ponyfish OR porbeagle OR pout OR ray OR ribbonfish OR rigg OR rockfish OR rosefish OR sablefish OR sailfish OR salmon OR sandeel OR sardine OR sardinella OR scabbardfish OR scorpionfish OR "sea bass" OR seabream OR "sea bream" OR sheatfish OR "shi drum" OR sild OR sillago OR skipjack OR smelt OR smooth hound OR "smooth-hound" OR snapper OR snook OR sole OR sparling OR spearfish OR "St Peter's fish" OR stargazer OR stingray OR sturgeon OR "surgeon fish" OR swordfish OR tailor OR tench OR tilapia OR threadfin OR triggerfish OR trout OR tubefish OR tuna OR turbot OR tusk OR walleye OR weever OR whitebait OR whiting OR wrasse OR yellowtail)	3,400,724
#3	#1 AND #2	16,192
#4	#3 AND DocType=(BOOK OR REVIEW OR BOOK CHAPTER)	726
#5	#4 AND LA=English or Spanish	711
#6	#5 AND PY=2010-2023	536
#7	**TI**=(vibrio OR vibrios OR Vibrionaceae OR vibriosis OR parahaemolyticus OR vulnificus OR cholerae)	19,150
#8	#7 AND DocType=(BOOK OR REVIEW OR BOOK CHAPTER)	598
#9	#8 AND LA=English or Spanish	580
#10	#9 AND PY=2010-2023	394
#11	#10 NOT #6	235
#11	**#6 OR #11**	**771**

Table A.4: Details of search strings for literature searches for the search on the occurrence and concentration in seafood placed or intended to be placed on the EU market using Web of Science™ Core Collection (SCI-Expanded, BKCI-S, ESCI, CCR-Expanded, IC).

Set number	Search query	No of records
#1 (general)	**TS**=("microbial quality" OR "microbial safety" OR "microbiological quality" OR "microbiological safety" OR analyses OR analysis OR concentration OR contamination OR count* OR detection OR enumeration OR incidence OR investigation OR occurrence OR presence OR prevalence OR sampling OR survey*)	27,112,296
#2 (hazard)	**TS**=(vibrio parahaemolyticus OR vibrio vulnificus OR parahaemolyticus OR vulnificus OR ((vibrio OR cholerae) NEAR/3 (non-O1 OR non-O139 OR nonO1 OR nonO139)))	10,900
#3 (foodstuff)	**TS**=(seafood OR seafoods OR "sea food" OR "sea foods" OR crustacean* OR shellfish OR bivalve* OR mollusc* OR mollusk* OR fish* OR "fishery product*" OR "marine gasteropod*" OR cephalopod OR cephalopods OR crustacean OR crustaceans OR echinoderm OR echinoderms OR "sea urchin" OR "sea urchins" OR holoturid* OR tunicate OR tunicates OR urchin* OR crab* OR prawn* OR shrimp* OR lobster* OR "crayfish" OR crabfish OR crawfish OR langoustine OR scampi OR "clam" OR "clams" OR "carpet shell*" OR scallop* OR Pecten OR oyster* OR cockle OR cockles OR mussel OR mussels OR mytilus OR "Pen shell*" OR snail* OR abalone* OR Nassarius OR "whelk*" OR Bolinus OR "murex" OR ormer OR Haliotis OR "true limpet*" Patella OR Cellana OR Buccinum OR Concholepas OR conch* OR winkle* OR periwinkle* OR octopus OR squid* OR cuttlefish OR nautilus* OR Todarodes OR Loligo OR Sepia OR Paracentrotus OR Strongylocentrotus OR "Echinus esculentus" OR "sea cucumber*" OR "cukes" OR piure* OR pyura OR "sea violet*" OR "sea tulip*" OR "sea peach*" OR "sea pineapple*" OR "ice floe" OR "sea squirt*" OR gravad OR graved OR "gravad lax" OR gravlax OR sushi OR sashimi OR surimi OR ceviche OR caviar OR albacore OR amberjack OR anchovy OR anchovies OR angler OR argentine OR bacha OR barbel OR barracuda OR basa OR bass OR beluga OR bib OR bigeye OR blackfish OR bleak OR blenny OR bluefish OR "blue runner" OR "blue shark" OR bonito OR branzino OR bream OR brill OR burbot OR butterfish OR carp OR catfish OR catshark OR chub OR cod OR comber OR conger OR corb OR cutlassfish OR dab OR "danubian wels" OR dentex OR dogfish OR eel OR emperor OR flathead OR flounder OR "flying fish" OR forkbeard OR garfish OR garrick OR goby OR goldline OR grouper OR guitarfish OR gunard OR haddock OR hake OR halibut OR hammerhead OR herring OR hoki OR huss OR icefish OR "John	8,097,418

dory" OR lamprey OR lanternfish OR leerfish OR ling OR "little tunny" OR lythe OR mackerel OR "mahi mahi" OR marlin OR megrim OR melva OR monkfish OR moonfish OR mullet OR needlefish OR oreo OR pacu OR pandoras OR panga OR pangasius OR parrotfish OR "parrot fish" OR perch OR picarel OR pike OR pilchard OR pilotfish OR "pilot fish" OR plaice OR pollan OR Pollack OR Pollock OR ponyfish OR porbeagle OR pout OR ray OR ribbonfish OR rigg OR rockfish OR rosefish OR sablefish OR sailfish OR salmon OR sandeel OR sardine OR sardinella OR scabbardfish OR scorpionfish OR "sea bass" OR seabream OR "sea bream" OR sheatfish OR "shi drum" OR sild OR sillago OR skipjack OR smelt OR smooth hound OR "smooth-hound" OR snapper OR snook OR sole OR sparling OR spearfish OR "St Peter's fish" OR stargazer OR stingray OR sturgeon OR "surgeon fish" OR swordfish OR tailor OR tench OR tilapia OR threadfin OR triggerfish OR trout OR tubefish OR tuna OR turbot OR tusk OR walleye OR weever OR whitebait OR whiting OR wrasse OR yellowtail OR meal OR meals OR food OR foods OR "buffet meal*" OR "complex food" OR "frozen meal*" OR multiingredient OR "multi ingredient" OR "probiotic drink*" OR ready-to-eat OR RTE OR "ready meal" OR "ready prepared" OR "ready to eat" OR "sous vide" OR coleslaw OR composite* OR convenience OR cured OR dip OR dips OR dish OR dishes OR dressing* OR dumpling* OR fermented OR filling OR gravy OR hummus OR jam* OR kebab OR macerated OR marinad* OR marinate* OR mayonnaise OR pasta OR pizza OR pickled OR preserved OR pudding* OR puree* OR salsa OR salsas OR salted OR sandwich* OR sashimi OR ceviche OR sauce* OR smoked OR snack OR snacks OR soup OR soups OR stew* OR surimi OR sushi OR tofu* OR topping* OR vegan OR vegetarian OR chowder OR tahini)

#4 (countries)	**TS**=(Albania OR Andorra OR Armenia OR Austria OR Azerbaijan OR Belarus OR Belgium OR Bosnia and Herzegovina OR Bulgaria OR Croatia OR Cyprus OR Czech Republic OR Denmark OR England OR Estonia OR Finland OR France OR Georgia OR Germany OR Greece OR Hungary OR Iceland OR Ireland OR Italy OR Kazakhstan OR Kosovo OR Latvia OR Liechtenstein OR Lithuania OR Luxembourg OR Macedonia OR Malta OR Moldova OR Monaco OR Montenegro OR Netherlands OR North Macedonia OR Norway OR Poland OR Portugal OR Romania OR Russia OR San Marino OR Serbia OR Slovakia OR Slovenia OR Scotland OR Spain OR Sweden OR Switzerland OR Turkey OR Ukraine OR United Kingdom OR UK OR Vatican OR Wales OR Yugoslavia OR Europe* OR EU) OR **AD**=(Albania OR Andorra OR Armenia OR Austria OR Azerbaijan OR Belarus OR Belgium OR Bosnia and Herzegovina OR Bulgaria OR Croatia OR Cyprus OR Czech Republic OR Denmark OR England OR Estonia OR Finland OR France OR Georgia OR Germany OR Greece OR Hungary OR Iceland OR Ireland OR Italy OR Kazakhstan OR Kosovo OR Latvia OR Liechtenstein OR Lithuania OR Luxembourg OR Macedonia OR Malta OR Moldova OR Monaco OR Montenegro OR Netherlands OR North Macedonia OR Norway OR Poland OR Portugal OR Romania OR Russia OR San Marino OR Serbia OR Slovakia OR Slovenia OR Scotland OR Spain OR Sweden OR Switzerland OR Turkey OR Ukraine OR United Kingdom OR UK OR Vatican OR Wales OR Yugoslavia OR Europe* OR EU)	29,253,753
#5 (NOT term)	**TS**=("in vitro" OR "in-vitro" OR "challenge study" OR "essential oil*" OR antimicrobial* OR attribution OR biofilm* OR "plant extract" OR "extracts" OR feed OR livestock OR sanitiser OR sanitizer OR spiked OR "feed supplement*")	3,557,700
#6	#1 AND #2 AND #3	4,821
#7	#6 AND #4	1,009
#8	#7 AND LA=English OR Spanish OR French OR Portuguese OR Italian OR German	1,002
#9	#8 NOT #5	707
#10	#9 AND PY=2010-2023	442

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