

Annex to: EFSA's BIOHAZ Panel Scientific opinion "Re-evaluation of certain aspects of the EFSA Scientific Opinion of April 2010 on risk assessment of parasites in fishery products, based on new scientific data. Part 1: ToRs1-3". doi:10.2903/j.efsa.2024.8719

© European Food Safety Authority, 2024

## **Annex A – Protocol for the re-evaluation of certain aspects of the EFSA Scientific Opinion of April 2010 on risk assessment of parasites in fishery products, based on new scientific data.**

### **A.1 Introduction**

#### **A.1.1. Scope of this protocol**

This document outlines the protocol for the scientific assessment to be performed for the scientific opinions of the EFSA Panel on Biological Hazards (BIOHAZ) on the "re-evaluation of certain aspects of the EFSA Scientific Opinion of April 2010 on risk assessment of parasites in fishery products, based on new scientific data". These opinions will be referred as "Parasites in fishery products" scientific opinions, Part 1 (addressing ToR1-ToR3, see A.1.2.) and Part 2 (addressing ToR4, see A.1.2.).

This protocol was developed with the aim of defining the methods for data collection, appraising the relevant evidence, and analysing and integrating the evidence considering the identified uncertainties. In doing so the principles and process defined in a 'Principles and process for dealing with data and evidence in scientific assessment' (EFSA, 2015), and the recommendations for protocol development described in the draft framework for protocol development for EFSA's scientific assessments (EFSA, 2020) were followed.

The protocol was drafted by the WG members and was approved by the BIOHAZ Panel at their 163th plenary meeting (13/07/ 2023).

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

In 2010 EFSA published a scientific opinion on risk assessment of parasites in fishery products<sup>1</sup>. EFSA was requested in particular to analyse three aspects:

1. Assessment of food safety concerns due to possible allergic reactions from parasites in fishery products;
2. Alternative treatments for killing viable parasites and comparison with freezing method;
3. Criteria for when fishing grounds (wild-farmed) fishery products do not present a health hazard (Atlantic salmon in particular). The EFSA conclusions were taken into account when modifying part D of Annex III, Section VIII, Chapter III to Regulation (EC) No 853/2004 (Commission Regulation (EU) N°1276/2011).

<sup>1</sup> [https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2010.1543#:~:text=EFSA%20concluded%20that%20human%20fishery,hypersensitivity\)%20reaction%20against%20parasite%20antigens](https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2010.1543#:~:text=EFSA%20concluded%20that%20human%20fishery,hypersensitivity)%20reaction%20against%20parasite%20antigens)

The current part D of Annex III, Section VIII, Chapter III to Regulation (EC) No 853/2004 mandates the following:

1) Food business operators placing on the market the following fishery products derived from finfish or cephalopod molluscs:

(a) fishery products intended to be consumed raw; or

(b) marinated, salted and any other treated fishery products, if the treatment is insufficient to kill the viable parasite;

must ensure that the raw material or finished product undergo a freezing treatment in order to kill viable parasites that may be a risk to the health of the consumer.

The freezing treatment is not carried out for fishery products:

(a) that have undergone or are intended to undergo before consumption a heat treatment that kills the viable parasites. In the case of parasites other than trematodes the product is heated to a core temperature of 60 °C or more for at least one minute;

(b) that have been preserved as frozen fishery products for a sufficiently long period to kill the viable parasites;

(c) from wild catches, provided that: (i) there are epidemiological data available indicating that the fishing grounds of origin do not present a health hazard with regard to the presence of parasites; and (ii) the competent authority so authorises;

(d) derived from fish farming, cultured from embryos and have been fed exclusively on a diet that cannot contain viable parasites that present a health hazard, and one of the following requirements is complied with:

(i) have been exclusively reared in an environment that is free from viable parasites; or

(ii) the food business operator verifies through procedures, approved by the competent authority, that the fishery products do not represent a health hazard with regard to the presence of viable parasites.

Before placing on the market fishery products which have not undergone the freezing treatment or which are not intended to undergo before consumption a treatment that kills viable parasites that present a health hazard, a food business operator must ensure that the fishery products originate from a fishing ground or fish farming which complies with the specific conditions referred to in one of those points. This provision may be met by information in the commercial document or by any other information accompanying the fishery products.

ParaFishControl was an EU H2020-funded project that aimed to increase the sustainability and competitiveness of the European aquaculture industry by improving our understanding of fish-parasite interactions and by developing innovative solutions and tools for the prevention and mitigation of the most harmful parasitic species affecting the main European farmed fish species. The project started in 2015 and finished in 2020 and was undertaken by a consortium of 29 partners (public and private) from 13 countries. The project team reported that there were no zoonotic parasites in farmed seabass, farmed seabream, turbot and sea caged rainbow trout. They concluded that the risk related to zoonotic Anisakidae was negligible. These findings provide the basis for amending the current legislation.

Other studies demonstrated that farmed fish were found to be less infected in comparison with wild fish (2%) but not *Anisakis* free. Farmed fish is in general reported to be considerably less infected and therefore a very limited food safety risk, but guaranteeing nematode free fish is not currently possible.

In addition, the EFSA 2010 Opinion concluded that “no sea fishing grounds can be considered free of *A. simplex* larvae”, and that “all wild caught seawater and freshwater fish must be considered at risk of containing viable parasites of human health hazard if these products are to be eaten raw or almost raw”. Furthermore, the BIOHAZ Panel recommended the collection of systematic data on the complete life cycle, geographical and seasonal distribution, prevalence, intensity, and anatomical location of parasites of public health importance in wild caught fishery products.

The European Union One Health 2020 Zoonoses report elaborated by EFSA and ECDC reported that in 2020, *Anisakis* caused two outbreaks, both reported by Spain, involving six individuals. No outbreaks were reported in 2019. The causative agent was not identified at the species level.

#### TERMS OF REFERENCE:

EFSA is asked to update certain aspects of its Scientific Opinion of April 2010 on risk assessment of parasites in fishery products based on **any new scientific evidence that may have become available since then**. In particular, EFSA is requested to review and assess:

1. The occurrence of parasites of public health importance in fishery products derived from the most relevant farmed fish species in the EU (in particular, but not limited to, Atlantic salmon, seabass, farmed seabream and turbot).
2. Diagnostic methods for the detection of parasites of public health importance in fishery products from such farmed fish species.
3. Technical developments and new scientific data available in relation to killing viable parasites of public health importance in fishery products, in particular treatments other than freezing.
4. Whether any particular species of wild caught fish originating from specific fishing grounds could be regarded as not representing a health hazard with regards to the presence of parasites of public health importance.

The request implies to provide one scientific opinion addressing terms of reference 1-3 of this request by 31 March 2024 and a second scientific opinion addressing term of reference 4 by 31 December 2024.

#### **A.1.3. Interpretation of the ToRs of the mandate**

The following has been clarified with the requestor:

- Whereas the Legal definition according to (EC) No 853/2004 for "Fishery products" means all seawater or freshwater animals (except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles and frogs) whether wild or farmed and including all edible forms, parts and products of such animals, for the current opinion, only finfish species will be covered.
- ToR 1 covers aquaculture within the European Union/European Free Trade Association

(EU/EFTA), the Faroe Islands and United Kingdom (UK)<sup>2</sup>. Fish farmed outside these European areas/countries are excluded.

- ToR2 and ToR3 cover detection and inactivation methods, respectively, used for finfish (both wild caught and farmed fish). ToR4 covers all fishing grounds for caught fish that could be sold in the EU/EFTA market.
- The previous opinion will be the basis for the assessment (to be summarized in the new opinions). The WG will focus on new information and data generated since then. Accordingly, the information/literature/data to be revised will cover the period from 2010 to date.
- Allergies will not be covered by the current assessment.

Additional clarification done by the working group members:

- In the context of this opinion, the parasites considered of public health importance will be those parasites that are known to be zoonotic or are potentially zoonotic (updating those considered for EFSA, 2010).
- Only parasites that infect fish are considered. Parasites that do not infect fish but are found in polluted waters (e.g., *Cryptosporidium*, *Giardia*, *Toxoplasma*) and may be present in their gastrointestinal tract and subsequently cross-contaminate fish during processing are excluded.
- Relevant fish species will be defined considering data from the EU/EFTA aquaculture production and EU/EFTA consumption, and thus their economic importance.
- With regards to the fishery products, only the edible parts of the finfish will be considered.
- In ToR1, for the **occurrence of parasites in finfish**, epidemiological data on prevalence (= number of infected fish / number of analysed fish \*100, in %), mean abundance (= total number of parasites (of a particular species or group) recorded in a set of analysed fishes/ number of analysed fish) and mean intensity (= total number of parasites (of a particular species or group) recorded in a set of analysed fishes / number of infected fishes) (according to Bush and al., 1997) as well as data on presence/absence (in some studies this may be all that is reported) will be considered.
- In ToR2, both the visual-based detection methods and molecular-based detection/identification methods will be considered.
- In ToR3, treatments for the inactivation and/or removal of viable public health important parasites in fishery products will be considered. Treatments may be of a chemical or physical nature as well as combination treatments.
- In ToR4, fishing grounds described by FAO<sup>3</sup> from marine, brackish and freshwater will be considered.

<sup>2</sup> EFTA: includes Iceland, Liechtenstein, Norway and Switzerland. Faroe Islands (Denmark Kingdom) do not belong to the EU. Formal relations between the EU and the Faroe Islands are currently based on three separate bilateral agreements dealing with fisheries, trade in goods and scientific and technological cooperation. The United Kingdom (UK) left the European Union on 31 January 2020. These last two areas will be included based on their relevance in aquaculture production and imports into the EU.

<sup>3</sup> [https://fish-commercial-names.ec.europa.eu/fish-names/fishing-areas\\_en](https://fish-commercial-names.ec.europa.eu/fish-names/fishing-areas_en)

## **A.2. Problem formulation**

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

The ToRs of the mandate were translated into 4 assessment question(s) (AQs). AQ1 was further broken down into associated 3 sub-questions (SQs), as shown in Table A1, where the approach (quantitative, semi-quantitative or qualitative) is also provided. There was no need to prioritise AQs or SQs.

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

The second step includes the overall approach (step 2.1), as well as the evidence needs and the methods (step 2.1) for answering each AQ and SQ including uncertainty analysis (i.e., the use of a literature review, data from databases, expert judgement and/or primary data collection). Table A.1 provides this information in the fifth (step 2.1a) and sixth (step 2.1b) columns.

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

**Table A.1.** Assessment questions and sub-questions for the assessment of Parasites in fishery products

ToR	Step 1.1. AQ	Step 1.2. SQ	Step 1.3. Approach	Step 2.1(a) Overview method	Step 2.1(b) Evidence needs and methods
<p>ToR1 The <b>occurrence of parasites of public health importance</b> in fishery products derived from the <b>most relevant farmed fish species in the EU</b> (in particular, but not limited to, Atlantic salmon, seabass, farmed seabream and turbot).</p>	<p>AQ1: What is the occurrence of parasites of public health importance in fishery products derived from the most relevant farmed finfish species in EU/EFTA?</p>	<p>SQ1.1: Which are the most relevant farmed fish species produced in the EU/EFTA in addition to Atlantic salmon, seabass, farmed seabream and turbot that may be infected with parasites of public health importance?</p> <p>SQ1.2: Which are the parasites of public health importance that could infect the most relevant farmed finfish species in the EU/EFTA (from SQ1.1)?</p> <p>SQ1.3: Considering SQ1.1 and SQ1.2, what is the occurrence of parasites of public health importance in fishery products derived from the most relevant farmed finfish species in the EU/EFTA (from SQ1.1)?</p>	<p>Qualitative No sub-question is prioritized over any other</p>	<p>Database review (SQ1.1) Literature review (SQ1.2., 1.3) Expert judgement</p>	<p><b>a. Evidence needs/Eligibility criteria:</b> The aim is to retrieve information on the occurrence/ epidemiology of the fish parasites of public health importance in the farmed fish species of major importance with regards to their production/consumption in the EU/EFTA. For this, information on the farmed fish species that may be infected with zoonotic or potential zoonotic parasites (SQ1.1) and information on these parasites (SQ2) including the fish species they may be associated with (e.g., occurrence (presence/absence), and epidemiological data if available, SQ3) will be collected. The scientific opinion 2010 will be summarized to describe the state of the art as starting point for this opinion. The information will be updated, through a literature review and/or review of available databases.</p> <p>To address <b>SQ1</b>, public available data on fish consumption and production (e.g., reports from FAO and/or EU statistics reports) will be reviewed.</p> <p>For <b>SQ2 and SQ3</b>, literature reviews will be done as indicated below and in b).</p> <p>The eligibility criteria for the literature review related to the study characteristics for SQ2 and SQ3 are:</p> <ul style="list-style-type: none"> <li>- <b>Population:</b> publications in the peer reviewed or grey literature that describes farmed fish and associated zoonotic or potential zoonotic parasites in the EU/EFTA.</li> <li>- <b>Outcome:</b> epidemiology of the parasites associated with the fish species considered as relevant (as defined for SQ1.1. and SQ1.2.</li> </ul>

				<p>Eligibility criteria related to report characteristics, which are applicable to all AQs are:</p> <ul style="list-style-type: none"> <li>- <u>Language of the full text</u>: English</li> <li>- <u>Time</u>: 2010 onwards</li> <li>- <u>Publication type</u>: peer reviewed paper, industry report, review or book (chapter), project deliverables, etc.</li> </ul> <p><b>b. Search strategy:</b></p> <p>A literature search will be carried out in, relevant databases such as the Web of Science™ Core Collection Pubmed, Scopus and/or Google Scholar to retrieve information. The search strategy to be used is shown in Appendix A1.</p> <p>A general search (e.g., fish parasites and food, food safety) <b>valid for all AQs</b>, as well as more specific and targeted searches combining terms related to specific parasites and associated fish species with farmed/aquaculture (AQ1) will be conducted.</p> <p>Footnote chasing in which selected references will be screened for cited literature and for literature citing the reference will be also performed to supplement the list of references derived from the primary search.</p> <p>Apart from the literature search, relevant documents including published reported from national and international agencies and project reports (e.g., ParaFishControl), will also be identified and reviewed. The information will be supplemented by other information based on the knowledge/expertise of the Working Group and Panel members.</p> <p><b>c. Study selection for inclusion/exclusion.</b></p> <p>The screening process will be undertaken in two steps: screening of (1) titles, abstracts and keywords and then (2) full-text documents to further identify records to be included/excluded</p>
--	--	--	--	---

Parasites in fishery products, Part 1

					<p>based on the relevance of the information and data provided.</p> <p><b>d. Data extraction from included studies.</b> Selected full-text documents will be screened with one reviewer (member of the WG) extracting the information required to answer the SQs.</p> <p><b>e. Evidence appraisal.</b> The information gathered will be initially appraised by the WG members extracting the information/data and later appraised in a narrative way based on the expertise of the WG and Biohazard Panel members.</p> <p><b>f. Uncertainty sources and methods for prioritising them.</b> There are multiple sources of uncertainty and these will be captured in the uncertainty table including 'impact of the uncertainties on the conclusions'. These will not be prioritised.</p> <p><b>g. Evidence synthesis.</b> The methods used for synthesizing evidence will be qualitative.</p> <p><b>h. Uncertainty analysis.</b> The methods used for uncertainty analysis will be qualitative.</p>
<p>ToR2: Diagnostic methods for the detection of parasites of public health importance in fishery products from such farmed fish species.</p>	<p>AQ2: What testing methods are currently available and may be available in the near future to test the fish species for the parasites identified in the answer to ToR1?</p>		<p>Qualitative</p>	<p>Literature review</p>	<p><b>a.Evidence needs/Eligibility criteria:</b> The aim is to retrieve information on the detection methods currently used to test specific fish species for zoonotic or potential zoonotic parasites (as identified in the response to ToR1) with particular emphasis on methods developed since the 2010 Opinion. This will be undertaken by literature review. The eligibility criteria related to study characteristics are:</p> <ul style="list-style-type: none"> <li>- <b>Population:</b> publications in the peer reviewed or grey literature that describes the methods currently used and those at the 'close to market' development phase, to test specific fish species for zoonotic or potential zoonotic parasites.</li> <li>- <b>Outcome:</b> The outcome of this review will be information on the methods currently used</li> </ul>



Parasites in fishery products, Part 1

					<p>to test fish for zoonotic or potential zoonotic parasites, developments in detection technologies since 2010 and new 'near to market' innovations.</p> <p>The eligibility criteria related to the report characteristics are the same explained above for AQ1.</p> <p><b>b. Search strategy:</b> The general search strategy described above for AQ1, including the use of general keywords related to detection methods, will be followed. More information is shown in Appendix A1.</p> <p><b>c. to h:</b> the same methodology will be used as for AQ1 described above.</p>
<p>TOR3. Technical developments and new scientific data available in relation to killing viable parasites of public health importance in fishery products, in particular treatments other than freezing.</p>	<p><b>AQ3.</b> What technical developments and new scientific data for inactivation and/or removal of viable parasites (identified in the answer to ToR1) in fishery products, in particular treatments other than freezing, have been described since the EFSA BIOHAZ 2010 scientific opinion?</p>		Qualitative	Literature review	<p><b>a. Evidence needs/Eligibility criteria.</b> The aim is to retrieve information on the treatments, other than freezing, that are available to inactivate, reduce infectivity or remove zoonotic or potential zoonotic parasites in fish currently (as identified in the response to ToR1). This will be undertaken using a literature review.</p> <p>The eligibility criteria for the literature review related to study characteristics are:</p> <ul style="list-style-type: none"> <li>- <b>Population:</b> any publication in the peer reviewed or grey literature that describes the methods, other than freezing, currently available to inactivate, reduce infectivity or remove zoonotic or potential zoonotic parasites in fish</li> <li>- <b>Outcome:</b> The outcome of this review will be information on the methods available to inactivate, reduce infectivity or remove parasites of public health importance in fish.</li> </ul> <p>The eligibility criteria related to the report characteristics are the same explained above for AQ1.</p> <p><b>b. Search strategy:</b></p>

Parasites in fishery products, Part 1

					<p>The general search strategy described above for AQ1, including the use of general keywords related to inactivation and removal of fish parasites, will be followed. More information is shown in Appendix A1.</p> <p><b>c to h:</b> the same methodology will be used as for AQ1 described above.</p>
<p>TOR4. Whether any particular species of wild caught fish originating from specific fishing grounds could be regarded as not representing a health hazard with regards to the presence of parasites of public health importance.</p>	<p>AQ4: Are there any particular species of wild caught fish originating from specific fishing grounds, where fish consumed in the EU/EFTA are caught, that are free of parasites of public health importance?</p>				<p><b>a. Evidence needs/Eligibility criteria:</b> The aim is to retrieve information on whether there are any particular finfish species originating from any specific fishing grounds that are free from zoonotic or potential zoonotic parasites and fish from which can be regarded as not representing a fish borne parasitic health hazard for humans. This will be undertaken using a review of the scientific and grey literature.</p> <p>The eligibility criteria for the literature review related to study characteristics are:</p> <ul style="list-style-type: none"> <li>- <u>Population</u>: publications in the peer reviewed or grey literature that provides information on zoonotic parasites in specific wild caught fish species from specific fishing grounds</li> <li>- <u>Outcome</u>: The outcome of this review will be information on zoonotic parasites in specific fish species from specific fishing grounds since 2010.</li> </ul> <p>The eligibility criteria related to the report characteristics are the same explained above for AQ1.</p> <p><b>b. Search strategy:</b></p> <p>The general search strategy described above for AQ1, including the use of general keywords related wild fish caught and in specific fishing grounds, will be followed. More information is shown in Appendix A1.</p> <p><b>c to h:</b> the same methodology will be used as for ToR1 described above.</p>

AQ = assessment question; SQ = sub-question.

**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	Evidence integration across SQs is not needed as the SQs are organized 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 SQ1.1 feeds into SQ1.2 and the answer to SQ1.2 feeds into SQ1.3.	There is no need to plan beforehand.
ToR 2-AQ2	Not applicable as there are no SQs	Not applicable
ToR 3-AQ3	Not applicable as there are no SQs	Not applicable
ToR 4-AQ4	Not applicable as there are no SQs	Not applicable

AQ = assessment question; SQ = sub-question.

## References

- EFSA (European Food Safety Authority), 2015. Principles and process for dealing with data and evidence in scientific assessments. *EFSA Journal* 2015;13(5):4121, 35 pp. doi:10.2903/j.efsa.2015.4121.
- EFSA (European Food Safety Authority), 2020. Draft framework for protocol development for EFSA's scientific assessments EFSA supporting publication 2020:EN-1843. 46 pp. doi:10.2903/sp.efsa.2020.EN-1843.
- EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2008. Scientific Opinion of the Panel on Biological Hazards on a request from the Health and Consumer Protection, Directorate General, European Commission on Microbiological Risk Assessment in feedingstuffs for foodproducing animals. *The EFSA Journal* (2008) 720, 1-84.

## Appendix A:

### Search strings:

**General searches using broad range keywords and their combinations (eg. PubMed, Web of Science)** will be done. Eg.:

(fish parasites) AND

- keywords related to food (eg. food, muscle, fillets, seafood).
- keywords related to mitigation options (mitigation, viability, infectivity, inactivation, killing, larvicidal, anthelmintic, pathogenic potential, pathogenicity, virulence, food treatment).
- keywords related to diagnostic methods (diagnostic methods/tests, identification methods, molecular detection tools, test, detection, qPCR, PCR, sequencing, nuclear target sequences, mitochondrial DNA)
- keywords related to wild caught (wild caught, wild catch, fishery grounds)

Additionally, **specific targeted searches** will be done combining keywords related to the fish species and parasites identified for SQ1.1 and SQ1.2.