

ADOPTED: 27 January 2021

doi: 10.2903/j.efsa.2021.6422

Evaluation of the shucking of certain species of scallops contaminated with lipophilic toxins with a view to the production of edible parts meeting the safety requirements foreseen in the Union legislation

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Abstract

EFSA was asked by the European Commission to provide information on levels of lipophilic shellfish toxins in whole scallops that would ensure levels in edible parts below the regulatory limits after shucking, i.e. removal of non-edible parts. This should include the okadaic acid (OA), the azaspiracid (AZA) and the yessotoxin (YTX) groups, and five species of scallops. In addition, EFSA was asked to recommend the number of scallops in an analytical sample. To address these questions, EFSA received suitable data on the three toxin groups in two scallop species, *Aequipecten opercularis* and *Pecten maximus*, i.e. data on individual and pooled samples of edible and non-edible parts from contamination incidents. The majority of the concentration levels were below limit of quantification (LOQ)/limit of detection (LOD), especially in adductor muscle but also in gonads. Shucking in most cases resulted in a strong decrease in the toxin levels. For *Pecten maximus*, statistical analysis showed that levels in whole scallops should not exceed 256 µg OA eq/kg or 217 µg AZA1 eq/kg to ensure that levels in gonads are below the regulatory limits of 160 µg OA or AZA1 eq/kg with 99% certainty. Such an analysis was not possible for yessotoxins or any toxin in *Aequipecten opercularis* and an assessment could only be based on upper bound levels. To ensure a 95% correct prediction on whether the level in scallops in an area or lot is correctly predicted to be compliant/non-compliant, it was shown that 10 scallops per sample would be sufficient to predict with 95% certainty if levels of OA-group toxins in the area/lot were 25% below or above the regulatory limit. However, to predict with a 95% certainty for levels between 140 and 180 µg OA eq/kg, a pooled sample of more than 30 scallops would have to be tested.

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Keywords: lipophilic marine biotoxins, scallops, shucking, okadaic acid, azaspiracids, yessotoxins

Requestor: European Commission

Question number: EFSA-Q-2020-00245

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at <https://ess.efsa.europa.eu/doi/doiweb/doisearch>.

Acknowledgements: The Panel wishes to thank the following hearing experts for the support provided to this scientific output: Laurent Guillier, Marina Nicolas and Micheal O'Mahony. The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk D, Bignami M, Bodin L, del Mazo J, Grasl-Kraupp B, Hogstrand C, Chipman KJ, Leblanc J-C, Nebbia CS, Nielsen E, Ntzani E, Petersen A, Sand S, Schwerdtle T, Vleminckx C, Wallace H, Martinez AG, Gerssen A, Tubaro A, Cascio C, Abrahantes JC, Steinkellner H and Hoogenboom LR, 2021. Scientific Opinion on the evaluation of the shucking of certain species of scallops contaminated with lipophilic toxins with a view to the production of edible parts meeting the safety requirements foreseen in the Union legislation. *EFSA Journal* 2021;19(3):6422, 66 pp. <https://doi.org/10.2903/j.efsa.2021.6422>

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) evaluated the impact of the shucking of certain species of scallops contaminated with lipophilic toxins with a view to the production of edible parts meeting the safety requirements foreseen in the Union legislation. The request included three groups of marine biotoxins namely the okadaic acid (OA), the azaspiracid (AZA) and the yessotoxin (YTX) groups in five species of scallops, namely *Pecten maximus*, *Pecten jacobaeus*, *Chlamys islandica*, *Aequipecten opercularis* and *Mimachlamys varia*.

In scallop production, shucking means next to removal of the shell, also the removal of some of the soft tissue leaving only the edible parts, i.e. adductor muscle and gonad for consumption. Data show that contamination with domoic acid (a hydrophilic toxin) occurs mainly in the hepatopancreas and soft tissue and thus gonad and adductor muscle can still be placed on the market for certain scallop species even if the whole animal exceeds the regulatory limit but only if they comply with a prescribed limit for these parts. In order to evaluate if a similar approach can be applied for lipophilic toxins and for other species, ratios for toxin levels in edible parts and whole scallops have been investigated. In addition, a sampling scheme ensuring compliance of a batch or area with established regulatory levels has been investigated, focusing on the number of scallops to be included in an analytical sample.

Upon launching a call for data on concentrations of the toxins of concern in whole animals and body parts of the species of concern, EFSA received data from several EU member states. However, only for two species, *Aequipecten opercularis* and *Pecten maximus*, the data were suitable to answer the questions in the terms of reference from the European Commission. These data came from investigations following up contamination incidents (France) or elevated levels in non-edible parts (Ireland) and are not coming from a random sampling surveillance scheme and thus are not representative for the average levels of toxins found in scallops. Compared to the edible parts, which are the adductor muscle and for *Pecten maximus* also the gonad, the major part of the toxins was present in the non-edible parts of the scallops that are removed during shucking.

Based on data from both individual scallops submitted by France and pooled scallops submitted by Ireland, it could be concluded that the toxin levels in adductor muscles from *Aequipecten opercularis* were all below the detection limits and that in most cases (96–100%), this also applied to samples of adductor muscles of *Pecten maximus*. In the case of gonad samples from *Pecten maximus*, a higher fraction contained detectable levels of toxins.

For gonads of *Pecten maximus*, statistical analysis showed that levels in whole scallops should not exceed 256 µg OA eq/kg or 217 µg AZA1 eq/kg to ensure that levels in gonads are below the regulatory limits of 160 µg OA or AZA1 eq/kg with 99% certainty. For muscle plus gonad, the critical concentration would be higher, due to the lower and often non-detectable (97% left censored) levels in adductor muscle (342 µg OA eq/kg based on data from Ireland). For muscles only, data were not suitable for such an assessment and this could only be based on the ratios for the UB levels in muscle and whole scallop (range 0.01–0.23 for data from France and 0.1–1.0 for data from Ireland).

Also for yessotoxins or any toxin in *Aequipecten opercularis*, such an analysis was not possible and an assessment could only be based on upper bound levels. For OA group toxins, this showed an average ratio of muscle to whole animal of 0.017 (ranging from 0.008 to 0.037), and for YTX group toxins of 0.37 (ranging from 0.21 to 0.56). In the case of YTX, the levels in whole scallops were far below the regulatory limit of 3.75 mg/kg.

The data show that for both OA group and AZA group toxins, there is in general a strong reduction in toxin levels after shucking, but that occasionally levels in gonads are rather similar to those in the whole scallop. For YTX-group toxins, the levels in whole scallops were too low to allow such a conclusion.

For an analysis on the sample sizes needed to conclude on compliance/non-compliance of a lot/area, only the data on individual scallops obtained from France related to contamination incidents in four areas could be used. The analysis showed that to ensure a 95% correct prediction on whether the level in scallops in an area or lot is correctly predicted to be compliant/non-compliant, 10 scallops per sample would be sufficient if levels of OA-group toxins in the area/lot were 27% below or above the regulatory limit (< 110 µg OA eq/kg or > 210 µg OA eq/kg). However, to predict with a 95% certainty for levels between 140 and 180 µg OA eq/kg, a pooled sample of more than 30 scallops would have to be tested.

The Panel noted that there is a relatively high probability of either over- or underestimating the true level in the batch (lot/area) when too few scallops are included in the pooled sample and also

considering the potential application of a measurement uncertainty incurred with the analytical measurements. The CONTAM Panel noted that consumption of scallops with a level at the regulatory limit (as few as three or four, depending on species) may result in exceedance of the acute reference dose for okadaic acid and azaspiracid group toxins.

The major uncertainty in this assessment came from the fact that only a limited number of suitable data points to answer the questions posed in the terms of reference were provided and from only two member states. Furthermore, these data only covered part of the toxin groups and only part of the scallop species. It is unclear to what extent results can be extrapolated to other toxin groups and species. For those scallop/toxin combinations, where data were available and levels in the edible parts were not all below detection limits, a statistical approach was applied to determine the critical level in whole scallops and to account for uncertainties in the data. For data sets with detected levels in the whole scallop, the range of ratios and especially the highest ratio based on upper bound levels was estimated and methods to consider ~~ed~~ uncertainty were used based on the actual levels. Also for determining the number of scallops that should be included in an analytical sample, the uncertainties in the data sets were taken into account but the numbers clearly depend on the actual variation in the levels in an area/lot.

The CONTAM Panel recommends updating the risk assessments on OA, YTX and AZA group toxins including a re-evaluation of the relative potencies of their different analogues. For evaluation of ratios of toxins in different body parts of scallops, more data collected from contamination incidents are needed on the levels of lipophilic shellfish toxins in edible parts vs. whole scallops of various types.

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1. Introduction

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

1.1.1. Background

Scallops are bivalve molluscs which are found in all of the world's oceans. Many species of scallops are highly prized as a food source, and some are farmed as aquaculture. Scallops are commercialised in different forms: entire, only the adductor muscle, the muscle with the gonad or only the gonad. They can be commercialised with or without shells.

According to the EU legislation, live bivalve molluscs can be harvested only from classified production areas. However, in the case of scallops, they can also be harvested outside classified production areas due to their motility. The current rules require in any case that when data from official monitoring programmes enable competent authorities to classify fishing grounds, the requirements for classified production areas apply also to scallops.

Classified production areas are production zones whose location and boundaries are fixed by the competent authorities, normally close to shore. They are classified according to their microbiological quality, not linked to biotoxins. The EU legislation requires competent authorities to monitor the classified areas for plankton in the water and biotoxin concentrations in molluscs flesh. This monitoring allows the authorities to close production areas and prohibit the harvest of bivalve molluscs from them when regulated biotoxin limits are exceeded.

For scallops harvested outside classified production areas, which are normally offshore and therefore not regularly monitored, EU legislation requires official controls to take place on land, in fish auctions, dispatch centres and processing establishments before they are placed on the market for human consumption.

Scallops filter water to get their nutrients and thus may accumulate marine biotoxins from micro-organisms in concentrations that may create a risk for consumers. For scallops, the main hazard is Amnesic Shellfish Poisoning (ASP), caused by the consumption of shellfish contaminated with the marine neurotoxin domoic acid; however, they can also accumulate other toxins such as lipophilic toxins or Paralytic Shellfish Poisoning (PSP) toxins.

For the human health risks from scallops related to marine biotoxins, the EU legislation contains food safety obligations on both Food Business Operators and Competent Authorities.

The EU legislation sets regulatory limits for marine biotoxins. Regulation (EC) No 853/2004, Annex III, Section VII, Chapter V, point 2¹ states that live bivalve molluscs '*must not contain marine biotoxins in total quantities (measured in the whole body or any part edible separately) that exceed the following limits:*

- a) for paralytic shellfish poison (PSP), 800 micrograms of saxitoxin-2HCl equivalents per kilogram;
- b) for amnesic shellfish poison (ASP), 20 milligrams of domoic acid per kilogram;
- c) for okadaic acid, dinophysistoxins and pectenotoxins together, 160 micrograms of okadaic acid equivalents per kilogram;
- d) for yessotoxins, 3,75 milligrams of yessotoxin equivalent per kilogram; and
- e) for azaspiracids, 160 micrograms of azaspiracid equivalents per kilogram².

As marine biotoxins concentrate differently in different parts of the scallop, the level of contamination can be determined by either testing the whole body of the animal or testing its different parts separately and subsequently aggregating the results to calculate the total amount of biotoxins present in the entire animal.

With regard to domoic acid (DA), data provided to EFSA for its risk assessment in 2009 demonstrated that, overall, it appears that all of the shellfish species considered can be contaminated with DA and epi-DA, a high level of contamination is found only in scallops, with almost 60% of the samples above the EU regulatory limit² Domoic acid (<https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2009.1181>).

Taking into account that the data show that DA contamination in scallops occurs mainly in hepatopancreas and soft tissue, Commission Decision 226/2002/EC, which is still applied by some

¹ Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

² See Table 4 of EFSA opinion on Marine biotoxins in shellfish.

Member States, allows the adductor muscle and gonads of two species of scallops³ The muscle and gonad placed on the market must comply with the prescribed limit (20 mg/kg). To commercialise these scallops, the Decision imposes strict control conditions related to harvesting, transport and documentation and establishes strict handling conditions in on-land establishments specially authorised to remove the hepatopancreas. A restricted harvesting regime of molluscs with a domoic acid concentration in the whole body higher than 20 mg/kg can be initiated if two consecutive analyses of samples, taken between one and no more than seven days, show that the domoic acid concentration in whole mollusc is lower than 250 mg/kg and that the domoic acid concentration in the parts intended for human consumption, which have to be analysed separately, is lower than 4.6 mg/kg. The analyses of the entire body will be performed on a homogenate of 10 molluscs. The analysis on the edible parts will be performed on a homogenate of 10 individual parts.

Some Member States would favour extending this approach, under different control conditions, to other scallop species. Recently, some Member States asked the Commission to evaluate the possibility of applying shucking not only to *Pecten maximus* and *Pecten jacobaeus* but also to *Chlamys islandica*, *Aequipecten opercularis* and *Mimachlamys varia* contaminated with DA exceeding in the whole body the maximum limits foreseen in the Union legislation. This would imply that these species of scallops could be harvested and their edible parts (adductor muscle and gonads) placed on the market if in compliance with the established limits in Regulation (EC) No 853/2004.

Moreover, some Member States would be in favour to extend the possibility of shucking, under different conditions, to other scallop species and to other marine biotoxins. Recently some Member States asked the Commission to evaluate the possibility to apply shucking to *Pecten maximus*, *Pecten jacobaeus*, *Chlamys islandica*, *Aequipecten opercularis* and *Mimachlamys varia*, with lipophilic toxins in the whole body exceeding the maximum limits foreseen by the EU legislation. This would imply that such species of scallops could be harvested and their edible parts (adductor muscle and gonads) placed on the market for human consumption if in compliance with the established limits.

The French National Reference Laboratory for Marine Biotoxins (belonging to the French Agency for Food, Environmental and Occupational Health and Safety – ANSES) carried out a study on the efficiency of shucking of scallops (*Pecten maximus* and *Aequipecten opercularis*) contaminated by lipophilic toxins (okadaic acid and DTX2).

1.1.2. Terms of reference

EFSA is asked to provide a scientific opinion on the practice of shucking in certain species of scallops (*Pecten maximus*, *Pecten jacobaeus*, *Chlamys islandica*, *Aequipecten opercularis* and *Mimachlamys varia*) containing domoic acid and the lipophilic toxins covered by the Union legislation in the whole body, or in any part edible separately, exceeding the level authorised under the current EU rules and its possible public health impact.

EFSA is requested to:

- 1) propose what would be the upper limit concentration of lipophilic toxins in whole body scallops (belonging to the listed species) that would still result in compliant edible parts (adductor muscle and gonads separately and combined) after shucking;
- 2) given different possible scenarios of levels of lipophilic toxin and occurrence of contamination in individual animals, EFSA is asked to provide recommendations on sampling schemes (batch based for either whole body or shucked scallop) that would ensure that all subsets of pooled samples will be compliant if a sample is analysed by a laboratory and found compliant;
- 3) propose what would be the upper limit concentration of DA in whole body scallops (belonging to the listed species) that would still result in compliant edible parts (adductor muscle and gonads separately and combined) after shucking;
- 4) given different possible scenarios of levels of DA and occurrence of contamination in individual animals, EFSA is asked to provide recommendations on sampling schemes (batch based for either whole body or shucked scallop) that would ensure that all subsets of pooled samples will be compliant if a sample is analysed by a laboratory and found compliant;

³ *Pecten Maximus* and *Pecten Jacobaeus* with an ASP toxin content in their whole body of up to 250 mg/kg to be placed on the market after removal of hepatopancreas and soft tissues.

1.1.3. Interpretation of the Terms of Reference

This opinion focuses on the lipophilic shellfish toxins, and only on those included in the current EU legislation, except for pectenotoxins (PTX), which will be deregulated. This means that the opinion focuses on okadaic acid (OA), dinophysistoxins 1, 2 and 3 (DTX1, DTX2, DTX3), the yessotoxins YTX, homoYTX, 45-hydroxy YTX and 45-hydroxy homoYTX and the azaspiracids AZA1, AZA2 and AZA3. It should be noted that DTX3 covers a group of acylated forms of OA, DTX1 and DTX2, which are hydrolysed to these toxins prior to analysis. Following the terms of reference (ToR) from the European Commission (EC), the opinion will focus on the scallops *Pecten maximus* (Great scallop, King scallop, St. James' shell or escallop), *Pecten jacobaeus* (Mediterranean scallop), *Chlamys islandica* (Iceland scallop), *Aequipecten opercularis* (Queen scallop) and *Mimachlamys varia* (Variegated scallop). To deal with the request of the EC, the legal limits were in principle the basis for the assessment and not the health-based guidance values (HBGV)s previously derived in EFSA opinions (EFSA, 2008a,b, 2009). However, the CONTAM Panel did evaluate the impact of exceedances of the regulatory limit on the risks for consumers by comparison with the acute reference doses (ARfDs) for the different toxins. Data on the toxin levels in various parts of the scallops and on interindividual variations between levels within a batch were used for the assessment. A brief summary on the adverse effects in humans and HBGVs is part of the opinion. However, following the Terms of Reference provided by the EC, neither an exposure assessment nor a risk characterisation were performed.

The CONTAM Panel has a defined mandate, with specific terms of reference to answer, not including legal interpretations. Although the different interpretations were discussed it was agreed that it is to answer the questions posed in the Terms of Reference.

1.2. Additional information

1.2.1. Terminology

A 'Scallop' comprises both an exoskeleton and internal soft tissue. However, the term 'scallop' in food production and food labelling, is used to refer to food products containing just certain scallop parts or subsets of the whole animal.

Similarly, within a scientific, legislative and food production context, the phrase 'entire animal' is frequently used to refer to all soft tissues, even though this is not the entire animal with the exoskeleton. This convention is adapted in this opinion.

The term 'gonad', colloquially 'roe', refers to the anatomically distinguishable reproductive organ. However, the body part which appears macroscopically as a relatively discrete tissue type with well-defined boundaries, actually includes both the reproductive organ and an internal loop of intestine, therefore not just gonad tissue. This is a relevant consideration when assessing biotoxin concentrations since toxins may actually be present in this part of the intestine rather than the gonad. This opinion adapts the convention of using the term 'gonad' to refer to this entire structure.

The phrase 'edible parts' generally refers to both the adductor muscle, with adduct being an anatomical term referring to movement of a body part towards the central axis of the body or in the case of a scallop to close the scallop. Gonads may also be consumed and are frequently included within the concept of edible parts or shucked scallop meat, particularly for larger scallops such as *Pecten maximus*. However, for smaller species such as *Aequipecten opercularis*, the concept of edible parts, more commonly comprises adductor muscle only, without gonad. The components which are deemed edible parts are not explicitly regulated and ultimately matter for operators, subject to their legal obligations, so scenarios may arise, e.g. commercial market preference or biotoxin contamination in gonad, where edible parts include less or more than these industry norms.

A further consideration of this terminology is that of 'parts edible separately'. Amongst the data provided and in the scientific literature, there exist approaches of describing biotoxin levels in either a homogenate of muscle and gonad, or all edible parts analysed separately.

The detailed anatomy of scallops is seldom realised at a commercial level. Whilst there may be orders of magnitude of difference between the bioaccumulation of biotoxin in organs such as hepatopancreas and stomach, these tissues are generally amalgamated into a generic category of 'remaining flesh', which reflects all of the material removed from the animal and resultant food product, and disposed of as waste animal by-product, in the process of shucking. In larger species such as *Pecten maximus*, this is all body parts other than the muscle and the gonad. Figure 1 shows a simplified diagram of a scallop.

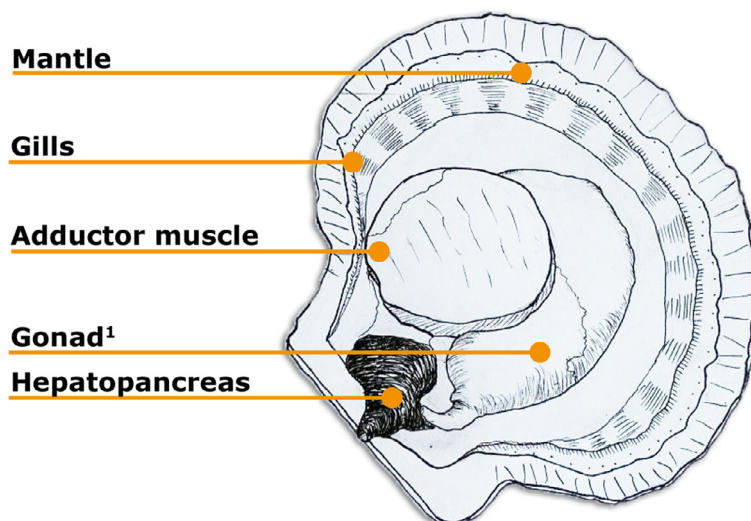


Figure 1: Anatomy of a scallop (simplified)

¹The term 'gonad' is used to refer to the anatomically distinct reproductive organ sometimes also referred to as 'roe'. Physiologically, this structure is involved in reproduction and also contains a loop of the intestine, which is significant in terms of toxin concentration for the structure. The term 'gonad' is used in this opinion to refer to the structure as recognised macroscopically and commercially harvested, therefore also including some of the intestine.

1.2.2. Definition of shucking

Whilst scallops may be placed on the market as live fresh entire animals, a significant aspect of scallop marketing and processing is the preparation method referred to as 'shucking', whereby only selected tissues of the scallop are offered as food.

Linguistically, the word 'shuck' has a meaning tending towards the removal of the outer hard shell with retention of inner soft parts. However, in scallop production shucking generally means removal of some soft tissues in addition to removing the shell. Therefore, as an interpretation of the terms of reference, the present Opinion regards the concept of shucking to mean the following as regards scallops:

- Opening of outer shell, and detaching soft tissues from both shells
- Evisceration to remove and discard hepatopancreas, intestines and mantle
- Leaving adductor mussel and gonad, or just adductor muscle alone
 - These parts are actively rinsed with water
 - These parts may be replaced in the half-shell for commercial presentation

EU food safety legislation does not define shucking. Shucking is, however, mentioned along with shelling and cooking, as a combined descriptor group of products subject to a 'microbiological criterion'⁴ in Regulation No 2073/2005⁵. There is no mention of shucking in the biotoxin-specific legislation, but there is mention of parts edible separately (Regulation No 853/2004), and also mention of removal of hepatopancreas, soft tissues and other contaminated parts (Decision No 226/2002⁶). The process of shucking kills the animal if it had been alive, thereby moving the product from the legal category of 'live bivalve mollusc' to the category of 'fishery product'. Furthermore, EU food legislation appears to accept the labelling of scallop adductor muscle alone or adductor muscle with gonad, within

⁴ 'Microbiological criterion' defines the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of microorganisms and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area or batch.

⁵ Commission Regulation (EC) No 2073 of the European Commission of 15 November 2005 on microbiological criteria of foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

⁶ Commission Decision 2002/226/EC of 15 March 2002 establishing special health checks for the harvesting and processing of certain bivalve molluscs with a level of amnesic shellfish poison (ASP) exceeding the limit laid down by Council Directive 91/492/EEC. OJ L 75, 16.3.2002, p. 65–66.

or without a half-shell i.e. shucked scallops, quite simply as 'scallop' even though much of the animal is not present.

1.2.3. EU legislation on biotoxin controls in bivalve molluscs

EU Legislation sets out food safety obligations in terms of what is required of food business operators (FBO), and what is required of Competent Authorities (CA). In both instances, these requirements are set out in broad general terms and in the case of foods and production systems with specific hazard considerations there may exist additional specific obligations for both FBOs and CAs designed to manage those specific risks (O'Mahony, 2018).

General food safety provisions

Regulation (EC) No 178/2002⁷ on general principles and requirements of food law, and Regulation (EC) No 852/2004⁸ on the hygiene of foodstuffs, create general obligations for all operators including primary producers, producing or placing food on the market in Europe. In the context of scallop production, primary producers are the wild-catching fishermen or occasionally fish farmers, and this primary phase of the food chain is subject to general hygiene requirements through structures and systems.

Specific biotoxin regulatory risk management provisions

EU legislation recognises the risk of biotoxins in live bivalve molluscs (LBMs) and establishes specific regulatory risk management framework placing specific obligations on both FBOs and CAs. A central concept is that of harvesting bivalve molluscs, whether farmed or wild, from a Classified Production Area (CPA), which is a geographically defined area of seawater assessed initially and monitored on ongoing by the CA to assess safety of molluscs harvested from the area. Some of the classification concepts such as Class A, B or C pertain to anthropogenic inputs and microbial food safety hazards, but such CPAs are also the unit of control for biotoxin purposes. CAs are obliged to establish monitoring programmes looking at parameters including toxin-producing plankton in the water, and biotoxin concentration in the molluscs. CAs are obliged to make decisions based on ongoing monitoring, and in the case of biotoxins, close production areas to prohibit harvesting when concentrations exceed regulatory limits. Regulation (EC) No 853/2004 obliges FBOs to ensure that live bivalve molluscs do not exceed specified biotoxins limits. Table 1 provides a summary of regulatory limits of biotoxins as laid down in Regulation (EC) No. 853/2004.

Table 1: Summary of biotoxin regulatory limits prescribed in Regulation (EC) No 853/2004 for live bivalve molluscs, measured in the entire body or any part edible separately

Biotoxin group	Abbreviation	Regulatory limits
Paralytic Shellfish Poison	PSP	800 micrograms of saxitoxin 2HCl equivalent per kilogram
Amnesic Shellfish Poison	ASP	20 milligrams of domoic acid per kilogram
Okadaic acid, Dinophysistoxins and (Pectenotoxins)^(a) together	DSP	160 micrograms of okadaic acid equivalents per kilogram
Yessotoxin	YTX	3.75 milligrams of yessotoxin equivalent per kilogram
Azaspiracid	AZA	160 micrograms of azaspiracid equivalents per kilogram ^(b)

(a): At the time of adoption of this Opinion, Pectenotoxins remain legally included in the DSP group, however, a draft Commission Delegated Regulation proposing their removal from chapter V of Section VII of Annex III to Regulation (EC) No 853/2004 has been discussed and agreed with member states and will enter into force mid-2021.

(b): Must be read as azaspiracid-1 (AZA1) equivalents per kilogram.

⁷ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

⁸ Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.

Primary-producing FBOs are obliged to only harvest within CPAs, which are not closed for health reasons including biotoxin risks. Following harvest, LBM primary products must be accompanied until taken-in by a specific registration document including biotoxin-open status.

FBOs handling or processing LBMs later in the food chain require specific approval for such activities, based on structural hygiene requirements, adequacy of systems to identify and manage risks in foods produced for such activities. Activities such as purification of LBMs or dispatching of LBMs on to the market as live animals for direct human consumption, require operator systems to ensure compliance with LBM biotoxin limits. Processing activities such as cooking, shucking or freezing of molluscs in order to place molluscan fishery products on the market, require operator systems to ensure these fishery products are derived from LBMs that meet the biotoxin limits. CAs are also explicitly required to have control systems including laboratory analysis, at all stages of production and processing, to verify levels of marine biotoxins do not exceed safety limits.

Scallop-specific biotoxin regulatory provisions

Scallops are bivalve molluscs, thereby subject to all of the preceding general and specific requirements for FBOs and CAs around biotoxins. In the case of scallops, within the specific obligations there are two additional considerations:

- Catching outside of classified production areas, e.g. in offshore wild fishing grounds
- Shucking to remove biotoxin-accumulating body parts from food placed on the market

The CPA concept has a clear resonance for static farmed inshore molluscs. Scallops may be wild caught in areas which are classified e.g. for other molluscan species, or specifically for scallops, and this production is therefore subject to the CPA official motoring regimen. However, the CPA concept is more difficult to apply to the scenario for catching wild motile animals in offshore fishing grounds. Such fisheries are also conducted across the jurisdictions of CAs, with vessels of one EU MS (the 'flag state') catching scallops in the exclusive fishery zone (EFZ) of another MS (the 'coastal state') and perhaps landing to a further MS (the 'port state'), and possible placing on the market in yet another MS. Accordingly, Regulation (EC) No 853/2004 allows operators to harvest certain LBMs, notably including *Pectinidae*, from outside CPAs. Such LBMs should meet all of the requirements mentioned for CPA molluscs, including biotoxin health standards and registration documents indicating harvest area. However, there is no obligation for the CA to define or monitor the sea area from which scallop harvest has occurred. Regulation (EC) No 853/2004 requires operators of approved dispatch centres or processing establishments, through which such LBMs must pass, to have a system of own checks to verify compliance with the necessary health standards including biotoxin limits. Commission Delegated Regulation (EU) 2019/624 on specific rules for the performance of official controls on the production of meat and for production and relaying areas of live bivalve molluscs⁹ derogates from CA responsibility to classify production areas from which these LBMs are harvested. However, the derogation requires that official controls are carried out at the auction halls dispatch centres or processing establishments, verifying compliance with health standards and the operator obligations for handling such non-CPA *Pectinidae*.

Legislation on shucking to remove biotoxin-accumulating parts

Scallops have a physical size and anatomy which facilitates the identification of body parts, and removal of some of those parts, as a viable commercial process. Knowledge of differential accumulation of biotoxins in organs and tissues creates potential for a regimen where biotoxin-safe parts are preferentially marketed for human consumption, with biotoxin-unsafe parts removed and disposed. EU legislation acknowledges the potential for an edible parts-only regimen where safe compliant food may be extracted from animals which had exceeded a biotoxin limit when assessed as an entire animal.

There exist different interpretations of the extent of the legislative allowance for shucking as an approach to managing biotoxin risk to produce biotoxin compliant edible parts from entire scallops exceeding a biotoxin limit.

In the case of DA, it is permitted under strict conditions to shuck *Pecten maximus* and *Pecten jacobaeus* scallops with levels in the whole scallop exceeding the regulatory limit of 20 mg/kg but with

⁹ Commission Delegated Regulation (EU) 2019/624 of 8 February 2019 concerning specific rules for the performance of official controls on the production of meat and for production and relaying areas of live bivalve molluscs in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council. OJ L 131, 17.5.2019, p. 1–17.

a level below 250 mg/kg. In that case, levels in the edible parts should not exceed 4.6 mg/kg (Commission Decision 2002/226/EC). This does not apply to other scallop species or toxins.

1.2.4. Biotoxin sampling and laboratory analysis

The legislation places an explicit onus on CAs to establish sampling plans for the purposes of monitoring CPAs. Such sampling should take place at regular intervals with geographical distribution of sampling points and sampling frequency to ensure representativeness of the CPA. Sampling plans to check for the presence of toxin-producing plankton, should take into account variations in the presence of such plankton, and be responsive to suggestions of toxin accumulation through more intensive sampling. Sampling plans monitoring toxins in LBMs should generally be at weekly frequency during harvesting periods, unless lower frequency is indicated by risk assessment.

The reopening of CPAs that have been closed due to plankton or toxins, requires at least two consecutive analytical results separated by at least 48 h being below the regulatory limits.

FBOs are generally required to have their own checks where appropriate to verify adequate management of safety of their products. Operators operating approved establishments accepting scallops harvested outside classified production areas are specifically obliged to have a system of own checks to monitor compliance with biotoxin levels. Operators shucking scallops harvested under a restricted harvest (Commission Decision 2002/226/EC) regimen are obliged to test each batch of end-product edible parts.

Regulation (EU) No 2019/627 obliges CAs to have control systems including laboratory analysis to verify compliance with biotoxin limits for LBMs through the entire food chain. Similarly, CAs are obliged to establish monitoring arrangements to control compliance of fishery products, and controls of fishery products should take place to ensure that fishery products are not containing toxin levels dangerous to human health. The Regulation also obliges CAs to use stipulated recognised laboratory methods for detection of marine biotoxins for each of the regulated biotoxin groups. FBOs are required to use these methods where appropriate.

The only specified sample size for biotoxin analysis is set out when an MS chooses to apply the optional derogation for domoic acid (Decision (EC) No 226/2002/EC) for a restricted harvest shucked parts marketing regimen. That requires a homogenate of 10 entire animals to assess whole animal status and a homogenate of the edible parts of 10 animals to assess edible parts status, including FBO checks of end-product batches.

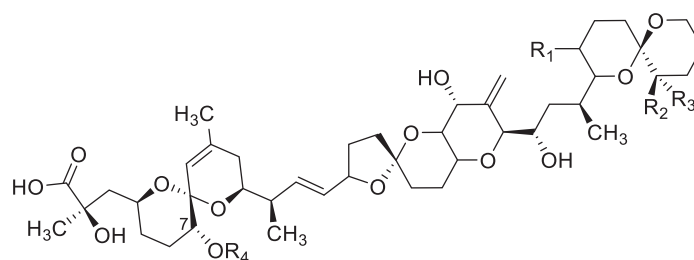
1.2.5. Chemistry

Okadaic acid and its derivatives

Both okadaic acid (OA) and its derivatives dinophysistoxin-1 (DTX1) and -2 (DTX2) are polyether compounds (see Figure 2). These toxins have lipophilic properties and accumulate in the hepatopancreas of shellfish. The toxins are temperature stable and when frozen (-20 to -80°C) they are stable for several months. At temperatures above 100°C , they start to degrade significantly (FAO, 2004; Yasumoto and Murata, 1990; McCarron et al., 2007). Any of these toxins can be esterified at the 7-hydroxy position with an acyl chain length of C_{14} to C_{22} , containing between 0 and 6 unsaturated carbon-carbon bonds. These acylated forms are known collectively as DTX3 (cited in Hallegraef et al., 1995; Wright, 1995; EU/SANCO, 2001). It is important to recognise that a significant portion of the toxin content is present as the DTX3 form. The most predominantly described acyl moiety for DTX3 detected is the hexadecanoic (palmitic, $\text{C}_{16:0}$) acid. The lipophilicity of DTX3 is increased as compared to the unacylated toxins (OA, DTX1 and DTX2).

In general, OA, DTX1 and DTX2 are products of toxin-producing microalgae (Wright, 1995) while DTX3 is suggested to be a metabolic product of OA, DTX1 and DTX2 produced within the shellfish itself. Suzuki et al. (1999) demonstrated the transformation of DTX1 to 7-O-acyl-DTX1 in the scallop *Patinopecten yessoensis*.

The stability of the DTX3 is comparable to OA, DTX1 and DTX2. However, under alkaline conditions, at elevated temperatures or in the presence of enzymes such as lipase and cholesterol esterase, DTX3 is deacylated to the parent toxin, i.e. OA, DTX1 or DTX2 (cited in EU/SANCO, 2001). The half-life for OA in mussels was estimated to be 7–10 days. However, and as expected, the depuration rates will depend on the oceanic characteristics and depuration rates in other regions will be influenced by the local dynamics and can be up to one month (Blanco et al., 2019; Marcaillou-Le Baut et al., 1993). Figure 2 shows the chemical structures of OA and DTX1, 2, 3.



Toxin	R ₁	R ₂	R ₃	R ₄
OA	CH ₃	H	H	H
DTX1	CH ₃	CH ₃	H	H
DTX2	H	H	CH ₃	H
DTX3 (acylated forms of OA, DTX1 and DTX2)	H/CH ₃	H/CH ₃	H/CH ₃	fatty acid

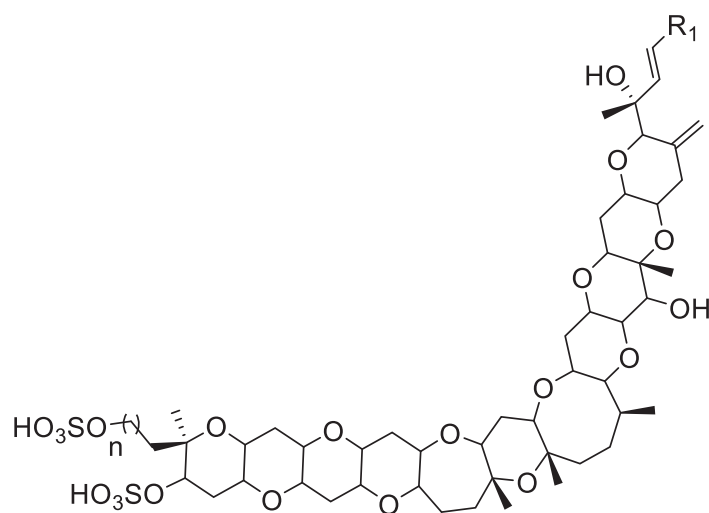
DTX: dinophysistoxin; OA: okadaic acid; R: rest.

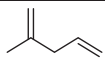
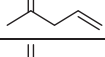
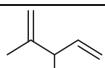
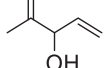
Figure 2: Chemical structures of OA and DTX1, 2, 3 (Larsen et al., 2007)

Yessotoxins

Like OA and its analogues, yessotoxins (YTXs) are polyether compounds (see Figure 3). However, YTXs consist of 11 contiguously transfused ether rings, an unsaturated side chain and two sulfonic acid groups (Samdal, 2005).

Miles et al. (2005) revealed the presence of an array of more than 90 YTX analogues in one algae isolate of *Protoceratium reticulatum*. Only a few dozens of YTXs have been fully identified. The structures of the YTXs that are mentioned in EU legislation are shown in Figure 3. Although there are many different YTX analogues, they seem not to be affected by heat, since clean-up procedures involving heating do not result in a decrease in the toxin amount (Alfonso et al., 2007). Furthermore, under both acidic and mild alkaline conditions, YTX is stable (Gerssen et al., 2009). Most of the YTXs found in microalgae are also found in shellfish and are initially concentrated in the hepatopancreas. There is evidence that YTXs are metabolised in shellfish based on the observation that 45-hydroxy YTX and carboxy YTX occur in much higher proportions in shellfish as compared to microalgae, and the proportions vary between various shellfish species (FAO/IOC/WHO, 2004b). The half-life for YTXs was estimated to be 20–24 days in Blue mussels (Aasen et al., 2005) and 49 days in Greenshell™ mussels (Mackenzie et al., 2002). Figure 3 shows the structures of EU-regulated yessotoxins.



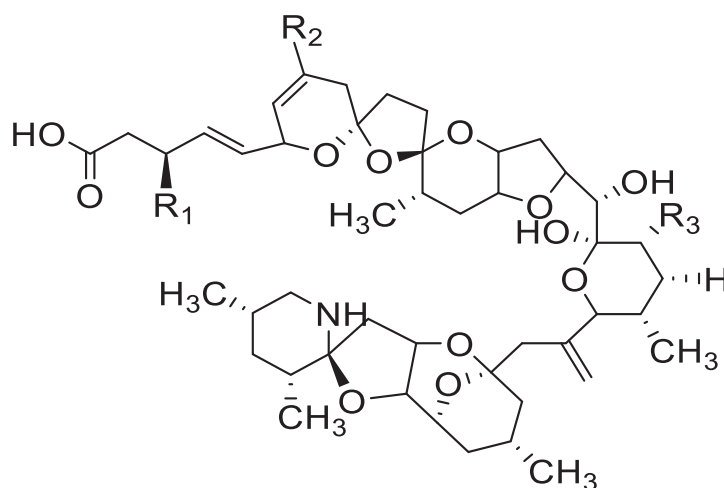
Toxin	Number of methyl groups	R ₁
YTX	1	
homoYTX	2	
45-hydroxy YTX	1	
45-hydroxy homoYTX	2	

R: rest; YTX: yessotoxin.

Figure 3: EU regulated yessotoxins

Azaspiracids

Azaspiracids (AZAs) are nitrogen containing polyether toxins comprising a unique spiral ring assembly containing a heterocyclic amine (piperidine) and an aliphatic carboxylic acid moiety (FAO, 2005). Over 50 AZAs have been described in the literature (Rehmann et al., 2008; Kilcoyne et al., 2014, 2015a,b; Krock et al., 2015) of which some are found only in microalgae. Of all AZAs described in literature, AZA1 and AZA2 are the most widely found in shellfish, but beside these two also AZA3 is regulated (See Figure 4). AZAs decompose or rearrange above a temperature of 100°C. Under acidic or alkaline conditions, AZAs are degraded rapidly. Among all analogues, AZA3 appears to be the most easily degraded analogue. This might explain why it occurs at lower concentrations than AZA1 and AZA2 in shellfish. Other non-regulated AZAs that might be present in shellfish can convert to the regulated AZA1, 2 and 3 after heating of shellfish, e.g. the conversion of AZA17 towards AZA3 (McCarron et al., 2009; Kilcoyne et al., 2015a,b). The same metabolites are also formed within shellfish without heating, however, at a slower conversion rate (Jauffrais et al., 2012). The half-life of azaspiracids in mussels was found to be comparable with OA and DTXs, approximately 10 days (Jauffrais et al., 2012). Figure 4 shows the structures of EU-regulated azaspiracids.



Toxin	R ₁	R ₂	R ₃
AZA1	H	H	CH ₃
AZA2	H	CH ₃	CH ₃
AZA3	H	H	H

AZA1: azaspiracid 1; AZA2: 8-methylazaspiracid; AZA3: 22-desmethyl-azaspiracid.

Figure 4: EU regulated azaspiracids

1.2.6. Analytical methods

LC-MS/MS EU reference method

Liquid chromatography (LC) coupled to mass spectrometry (MS) and in particular its coupling with tandem mass spectrometry (LC-MS/MS) has been the chemical approach selected as the most adequate tool for the determination of lipophilic toxins in shellfish. The LC-MS/MS method is therefore the reference method mentioned in EU legislation for the quantitative analysis of lipophilic toxins in shellfish (Regulation (EC) No 853/2004 and Regulation (EU) 2019/627). This reference method has been interlaboratory validated by the European Reference Laboratory for Marine Biotoxins (EURLMB). Briefly, it consists of the extraction with methanol of a shellfish homogenate followed by the filtration of the extracts and further injection into the LC-MS/MS system. Additional clean-up steps before the LC-MS/MS analysis are not needed. Only for the determination of the esterified forms of OA, DTX1 and DTX2 (namely DTX3), an alkaline hydrolysis step is incorporated. The LC-MS/MS analysis is based on the separation of the toxins on a reverse phase LC column using gradient elution with either acidic or alkaline mobile phase conditions. The method is applicable for a wide variety of molluscan shellfish matrices, both fresh and cooked, such as mussels, clams, razor clams and cockles and can be applied for the following toxins: OA, DTX1, DTX2, DTX3; PTX1 and PTX2; YTX, 45-OHYTX, homoYTX and 45-OH homoYTX and AZA1, AZA2 and AZA3. In order to test for compliance with the established EU regulatory limits, toxicity equivalency factors (TEFs), as recommended by EFSA (EFSA, 2008a,b, 2009), must be applied. Table 2 shows the EFSA TEFs for lipophilic toxins.

Table 2: EFSA toxicity equivalency factors (TEFs) for lipophilic toxins

Toxin group	Analogue	TEF
OA group	OA	1
	DTX1	1
	DTX2	0.6
PTX group	PTX1	1
	PTX2	1

Toxin group	Analogue	TEF
AZA group	AZA1	1
	AZA2	1.8
	AZA3	1.4
YTX group	YTX	1
	HomoYTX	1
	45-OH YTX	1
	45-OH homoYTX	0.5

AZA: azaspiracid, DTX: dinophysistoxin OA: okadaic acid; TEF: toxicity equivalency factor; PTX: pectenotoxin; YTX: yessotoxin.

Alternative methods

The EU Legislation establishes that in addition to the EU reference method (LC-MS/MS) mentioned above, other methods such as immunoassays and functional assays (e.g. the PP2A protein phosphatase inhibition assay) can be used as alternatives or supplementary to the EU reference LC-MS/MS method, following specific requirements specified in the EU legislation. The alternative methods must detect at least the analogues indicated in the EU Legislation and must fulfil performance criteria stipulated by the EURL LC-MS/MS method. Such methods must be intra- and preferably interlaboratory validated, and successfully tested under a recognised proficiency test scheme. The alternative method should provide equivalent level of public health protection compared to the EU LC-MS/MS reference method. The protein phosphatase inhibition assay (PP2A) is the only alternative method currently recognised, but it can only be used for the detection of the OA group toxins (OA and DTXs).

1.2.7. Hazard characterisation of lipophilic marine biotoxins

The toxicity of the lipophilic toxins of concern in this Opinion is described below summarising previous EFSA opinions on OA, YTX and AZA (EFSA, 2008a,b, 2009). It is important to note here that many other *in vivo* and *in vitro* toxicological studies on these compounds were reported in the literature after the publication of these opinions. However, these were not considered as a re-evaluation of the toxicity of these compounds was outside of the remit for the present Opinion and thus this section is limited to presenting an overview of the data as reported in these previous opinions.

Okadaic acid and its derivatives

Okadaic acid (OA) and its derivatives (DTX1, DTX2 and DTX3) provoke Diarrhoeic Shellfish Poisoning (DSP) characterised by diarrhoea, nausea, vomiting and abdominal pain, usually appearing in humans shortly after consumption of contaminated bivalve molluscs.

Inhibition of serine/threonine phosphoprotein phosphatases (PPs) is assumed to be the mode of action of OA group toxins. DTX3 cannot directly bind to PPs and a hydrolysis step is necessary to transform DTX3 into its corresponding precursors (OA, DTX1 or DTX2).

No chronic toxicity/carcinogenicity studies were reported for OA and its analogues. However, OA is considered to be a tumour promoter in rodents. The *in vivo* relevance of the *in vitro* genotoxicity found with OA is unclear. No genotoxicity data were available for DTX1, DTX2 and DTX3. The data on the chronic effects of OA were insufficient to establish a tolerable daily intake (TDI). However, an acute reference dose (ARfD) of 0.3 µg OA eq/kg body weight (bw) was established based on a lowest observed adverse effect level (LOAEL) for adverse effects in humans of 0.8 µg OA eq/kg body weight (bw) for adults and by applying an uncertainty factor (UF) of 3 (EFSA, 2008a). TEFs for OA and its derivatives were established using results from intraperitoneal (*i.p.*) experiments in mice. As shown also in Table 2, these are: OA = 1, DTX1 = 1, DTX2 = 0.6, while for DTX3, the TEF values were equal to those of the corresponding unesterified toxins (OA, DTX1 and DTX2).

Yessotoxins

Since YTX was initially reported together with OA, it was included within the DSP toxins. Subsequent studies revealed that YTX does not share the same mechanism of action of OA and its analogues on the serine/threonine PPs and is not diarrhoeic. For this reason, it was no longer classified with OA as a DSP toxin, but still included in the group of lipophilic biotoxins. No human poisoning cases were reported in the EFSA opinion of 2008 (EFSA, 2009). The CONTAM Panel identified four

major molecular processes of relevance for YTX toxicity: i) the modulation of calcium movements among different cellular compartments, ii) the modulation of cellular adenosine 3',5'-cyclic monophosphate (cAMP) levels, iii) the alteration of protein disposal by preventing endocytosis and degradation of the protein fragment produced after the proteolytic attack and iv) apoptosis. No data on the chronic toxicity, carcinogenicity and genotoxicity of YTXs were available. The establishment of a TDI was not possible, due to the lack of long-term *in vivo* toxicity studies. In animal experiments, the heart was a major target of YTX toxicity, and an ARfD of 25 µg/kg bw was derived based on a no observed adverse effect level (NOAEL) for acute cardiotoxicity of 5 mg/kg in mice and applying a UF of 200. TEFs were based on the same potency after *i.p.* exposure for YTX, 1-homoYTX, 45-hydroxy YTX = 1, while that for 45-hydroxy-1a homoYTX was 0.5 (see Table 2).

Azaspiracids

AZAs cause nausea, vomiting, diarrhoea and stomach cramps in humans. The gastro-intestinal effects might be a consequence of alteration of cytoskeletal and focal adherens structures and the E-cadherin system, with disruption of cell–cell and cell–matrix interactions and a perturbation of the intestinal barrier function. No genotoxicity data have been reported for AZAs. A TDI was not established because of the limited and inadequate data on the chronic effects in animals or humans. An ARfD of 0.2 µg AZA1 eq/kg bw was established based on an LOAEL in humans of 113 µg AZA1 eq per person (1.9 µg AZA1 eq/kg bw for a 60 kg adult) and applying and UF of 9. Based on lethality after *i.p.* administration in a limited number of mice, *interim* TEFs of 1.0, 1.8 and 1.4 for AZA1, AZA2 and AZA3, respectively, were established (EFSA, 2008b).

1.2.8. Previous assessments on shucking of scallops by other risk assessment bodies

In 2008, AFFSA published an assessment of the impact of shucking on lipophilic toxins (AFFSA Department of Risk Evaluation, 2008). AFFSA expressed concern about potentially high toxin levels in edible tissues of shucked animals and concluded that for evaluation of the adequacy of shucking and subsequent sampling, additional information on levels and distribution in a contaminated production area, quantitative distribution in animal tissues and quantitative effectiveness of shucking would be needed and that there were insufficient data to assess sanitary shucking for species outside of those mentioned in Decision (EC) 2002/226.

The ANSES NRLMB has published results of the effectiveness of sanitary shucking in scallops sampled from wild fishing grounds during naturally occurring DSP contamination events. Parts of these data were provided to EFSA and used in the current assessment.

In 2015, they reported a study on the efficacy of shucking of *Pecten maximus* (ANSES NRLMB, 2015). In 2017, they published a similar analysis on *Aequipecten opercularis* (ANSES NRLMB, 2017). In these studies, scallops were shucked to create two matrices, and the lipophilic toxin concentration for each matrix was determined. Using the mass of each matrix, concentrations in entire animals were mathematically reconstructed. In addition, statistical modelling was applied to assess the likelihood of that the concentrations in muscle exceed the LOQ or even the regulatory limit of 160 µg/kg.

In the *Pecten maximus* study (ANSES NRLMB, 2015), scallops from three different fishing areas, over two different time periods were analysed, as part of a natural contamination event in 2014. Following shucking, muscle and gonad, and the remaining flesh were analysed. Mean DSP levels in batches of whole scallops from areas 1, 2 and 3 were 86, 97 and 180 µg OA eq/kg, respectively. Corresponding mean concentrations in the remaining flesh (i.e. the parts removed by shucking) were 170, 202 and 372 µg OA eq/kg, respectively. Of 27 scallops where whole animal concentrations exceeded 160 µg/kg, only 5 had quantifiable DSP concentrations in muscle and gonads. Applying mathematical models to calculate the distribution of DSP toxins in the muscle and gonad, showed that there was no statistical difference between the DSP concentrations in that matrix regardless whether the whole animal was above or below the regulatory limit of 160 µg/kg. Data modelled from the two sample batches which had detectable toxin levels in muscle and gonad showed that there was an infinitesimal probability (2.1×10^{-6}) of exceeding the regulatory limit in one batch and zero probability in the other.

In the *Aequipecten opercularis* study (ANSES NRLMB, 2017), two sample batches of scallops comprising 79 and 43 animals, respectively, were obtained 8 days apart, from one area with a DSP contamination event in 2016. Concentrations in adductor muscle and remaining tissues were analysed. Whole animal concentrations exceeded 160 µg OA eq/kg in all animals analysed. Concentrations of

almost 500 and 1,000 µg OA eq/kg were found in the removed parts of sample batches 1 and 2, respectively, with whole-animal concentrations over 300 and nearly 600 µg OA eq/kg. Adductor muscle concentrations were all below the LOQ with only 1.3% of sample batch 2 above the LOD. Statistical modelling showed an extremely low probability (5×10^{-13}) of exceeding a concentration of 10 µg OA eq/kg in muscle of animals with that distribution in whole animals, and a probability of exceeding the limit of 160 µg OA eq/kg being too low to estimate.

1.3. Data and methodologies

1.3.1. Collection and appraisal of data collected from public literature

On 28 May 2020, a literature search has been carried out for studies available in the public domain and potentially relevant for the present assessment. The databases used were Web of Science¹⁰ and Pubmed¹¹ References were retrieved and managed by using Endnote.¹² The following search terms and Boolean operators were applied for the searches in both databases (no time limits were applied):

TOPIC: *Chlamys islandica* OR Iceland scallop OR *Aequipecten opercularis* OR Queen scallop OR *Mimachlamys varia* OR Variegated scallop OR *Pecten jacobaeus* OR Mediterranean scallop OR Pilgrim's scallop OR *Pecten maximus* OR Great scallop OR king scallop OR St. James shell OR great Atlantic scallop OR scallop*

AND

TOPIC: Okadaic acid OR dinophysistoxin* OR DTX OR yessotoxin OR YTX OR AZA OR azaspiracid* OR domoic acid OR amnesic shellfish poison OR ASP OR DA

After removal of duplicates, a total of 271 publications resulted from both databases. These publications were then evaluated by the WG experts for their relevance regarding either the lipophilic marine biotoxins of concern (for the present opinion) and/or domoic acid (for the second opinion) and/or for their relevance with regard to interspecies and interindividual differences (both whole animals and animal parts) in toxin content. A total of 82 publications were considered as potentially relevant, applying one or more of the above criteria and thus further considered for the two opinions.

During the development of the opinion, additional publications were collected by applying a 'snowballing approach',¹³ including also government reports and considered for the assessment where relevant.

1.3.2. Occurrence data submitted to EFSA

Data collection and validation

At the time the mandate for the present Opinion from the EC was received, only few occurrence data on lipophilic toxins in scallops were available in the EFSA Chemical Occurrence Database. Therefore, additional occurrence data on DA- and OA-, AZA- and YTX group toxins (detailed in Table 3) in scallops were sought by the EFSA Evidence Management Unit (DATA) and collected within the framework of a call for continuous collection of chemical contaminant occurrence data.¹⁴ EU national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders were invited to submit data on scallops by 25 May 2020. Data received after that date were not included in the data set used for evaluation for this Opinion.

Data providers were not asked to report whether the data came from wild-caught or farmed scallops. However, the vast bulk (99.97%) of the 2017 EU Scallop production has been identified as wild-caught (EUMOFA, 2020). Considering the absence of the UK data in the present Opinion which accounted for 92% of the small amount of EU-28 scallop aquaculture in 2017, the data considered in the present Opinion are very likely to be almost exclusively from wild scallops.

¹⁰ Web of Science (WoS), formally ISI Web of Knowledge, Thomson Reuters. [http://thomsonreuters.com/thomson-reuters-web-of-science/\(WoS\)](http://thomsonreuters.com/thomson-reuters-web-of-science/(WoS))

¹¹ A free resource supporting the search and retrieval of biomedical and life sciences literature with the aim of improving health—both globally and personally. Available online: <https://pubmed.ncbi.nlm.nih.gov/>

¹² Endnote X9, Thomson Reuters. Available from: <https://endnote.com/>

¹³ Identifying articles that have been cited in articles found in a search (see Jalali and Wohlin, 2012).

¹⁴ <https://www.efsa.europa.eu/en/consultations/call/call-continuous-collection-chemical-contaminants-occurrence-data> in food and feed that opened on 15 April 2020.

The data submission followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a, 2013) and the occurrence data were managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and on 'Data analysis of food consumption and occurrence data'. The data collection was in alignment with the latest chemical monitoring guidance (EFSA, 2020) with modifications related to sampling procedures in shellfish, including instructions to achieve a harmonised use of FoodEx2 terms to better detail scallop species and anatomic parts (as reported in Annex Table A1).

By the time of the data extraction (9 June 2020), a total of 16,845 analytical results were available on lipophilic toxins in the EFSA database as reported in Table 3. Data were provided by national competent authorities and designated laboratories from Spain, France, Germany, Denmark, Belgium, Ireland and The Netherlands. The vast majority of the data were submitted by Ireland and France. As the data call was launched in 2020 (after the UK leaving the EU), no data from the UK are available for the present assessment.

Table 3: Number of analytical results present on lipophilic toxins in scallops in the EFSA database at the moment of extraction (before data cleaning)

Toxin	Abbreviation	n
Okadaic acid	OA	1,969
Dinophysistoxin 1	DTX1	1,934
Dinophysistoxin 2	DTX2	1,934
Azaspiracid 1	AZA1	1,518
8-methylazaspiracid	AZA2	1,398
22-desmethyl-azaspiracid	AZA3	1,398
Yessotoxin	YTX	1,734
Homo yessotoxin	HomoYTX	1,674
45-hydroxy yessotoxin	45-OH YTX	1,643
45-hydroxy homo yessotoxin	45-OH homoYTX	1,643

n: number of analytical results.

Data analysis and cleaning

Data were thoroughly evaluated, followed by cleaning and validation steps. As a first step, accuracy of classification of scallop species and anatomical parts according to the FoodEx2 terms was checked (see Annex A, Table A1). For data collected before 2020, when food reclassification was not possible based on reported information, specific clarification requests were issued to data providers and samples re-coded accordingly (resulting in the reclassification of 139 scallop samples). Moreover, four samples (associated with 14 analytical results) were excluded because either no information of body part or species could be retrieved, or samples did not contain only scallops.

Results were then evaluated considering the analytical method applied for their generation. Where needed, data providers were asked to provide details on methods used and upon clarification analytical methods were reclassified or better classified as official protocols based on the EU reference method for lipophilic toxins (EURLMB SOP¹⁵) or equivalent methods.

When information on recovery was missing, this was requested from the data providers and values were added when provided. Data sets produced with methods with poor recovery (e.g. as low as 53% for OA) or quality criteria not according to the EURLMB SOP were excluded. Only results produced with the EU reference method for lipophilic toxins or equivalent methods based on LC-MS were kept in the database. Data referred to in this opinion as OA, DTX1 and DTX2 were confirmed to be on totals, indented as the 'sum of the free and the acyl-derivatives' according to the EU reference method for lipophilic toxins.

Ten results associated with a high LOQ of 500 µg/kg for the YTX group (method based on LC-HR-MS) were excluded. For 20 not left-censored analytical results, the LOQ was missing and was added based on data provided by the respective laboratories.

Duplicates were excluded during data cleaning. Sampling years ranged from 2013 to 2020.

¹⁵ Available online: https://www.aesan.gob.es/en/CRLMB/web/public_documents/seccion/crlmb_standard_operating_procedures.htm

Official control biotoxin monitoring of CPAs is done on a risk basis, generally at a weekly frequency. Official control biotoxin monitoring of scallops harvested outside of CPAs should also be carried out on a risk-based frequency. In some risk management strategies for scallops from outside CPAs, official monitoring is reactive to the areas fished and subsequent landings by fishermen, while in others, there is proactive sampling of particular fishing grounds. CAs are also obliged to have risk-based controls of biotoxin compliance throughout the molluscan fishery product food chain after harvest. Much of the data were derived from such risk-based official control monitoring. However, the samples reported by France for both *Pecten maximus* and *Aequipecten opercularis* were collected after unusual episodes of contamination of scallops by lipophilic toxins that occurred in the summers of 2014 and 2016 (ANSES NRLMB, 2017) as detailed in Section 2.1.4.

When information on pooled samples was missing (e.g. the number of scallops), this information was sought from data providers and the information was recoded accordingly. For one data set, the data providers declared that the number of animals per sample was 10–15 units. In this case samples were assigned a number of 12.5 units. When information on the number of units used for one pooled sample could not be retrieved, an imputation to 10 units per pooled sample was performed (this was the case for 310 results).

The left-censored data (analytical data reported below the LOD/LOQ) were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009). This method is also indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option for the treatment of left-censored data. According to this guidance, the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). At the LB, results below the LOQ or LOD were replaced by zero, at the UB the results below the LOD were replaced by the LOD and those below the LOQ were replaced by the value reported as LOQ.

Detailed information on data cleaning can be found in the Annex, Table A2. After data cleaning, a total of 16,134 analytical results on 1,879 samples were present in the database.

Food classification

Occurrence data were classified according to the 'Exposure hierarchy' of the FoodEx2 classification system (EFSA, 2015). This is based on a food list of 2,673 entries, referred as 'basic FoodEx2 code', aggregated into food groups and broader food categories in a hierarchical parent-child relationship (up to 7 levels). In addition, a catalogue of 28 'facets' is available in order to describe further characteristics of the foods, such as physical state (e.g. powder, liquid) or processing technology (e.g. grinding, milling, crushing). For the purpose of this opinion, ad hoc facets were used to classify scallop species and their body parts as reported in the Annex, Table A1.

Methodology used for calculating toxin equivalents and toxin concentrations

The results for the occurrence of single analytes reported for lipophilic toxins in scallops and parts of scallops were converted to total concentrations for each toxin group, using the corresponding toxicity equivalency factors (TEFs) for OA, AZA and YTX groups (see also Table 2) according to the following formulas:

$$\text{OA group concentration } [\mu\text{g OA equivalents/kg}] = \text{OA concentration } [\mu\text{g OA/kg}] \cdot 1 + \text{DTX1 concentration } [\mu\text{g DTX1/kg}] \cdot 1 + \text{DTX2 concentration } [\mu\text{g DTX2/kg}] \cdot 0.6 \quad (\text{F1})$$

$$\text{AZA group concentration } [\mu\text{g AZA equivalents/kg}] = \text{AZA1 concentration } [\mu\text{g AZA1/kg}] \cdot 1 + \text{AZA2 concentration } [\mu\text{g AZA2/kg}] \cdot 1.8 + \text{AZA3 concentration } [\mu\text{g AZA3/kg}] \cdot 1.4 \quad (\text{F2})$$

$$\text{YTX group concentration } [\text{mg YTX equivalents/kg}] = \text{YTX concentration } [\text{mg YTX/kg}] \cdot 1 + \text{homoYTX concentration } [\text{mg homoYTX/kg}] \cdot 1 + 45\text{OH YTX concentration } [\text{mg 45OH YTX/kg}] \cdot 1 + 45\text{OH homoYTX concentration } [\text{mg 45OH homoYTX/kg}] \cdot 0.5 \quad (\text{F3})$$

The toxin concentration for the entire animal was calculated by summing up the mass of toxin of the different tissues divided by the total body weight according to the following equations:

$$\text{Entire animal concentration} = \frac{\sum_{i=n}^m \text{mass toxin in tissue } i}{\text{Entire animal weight}} \quad (\text{F4})$$

where

$$\text{Mass toxin in tissue } i = \text{tissue weight} \times \text{toxin group concentration in tissue} \quad (\text{F5})$$

$$\text{Entire animal weight} = \sum \text{tissue weight } i \quad (\text{F6})$$

The toxin concentration for the 'muscle plus gonad' was calculated by summing up the mass of toxin of the different edible tissues divided by their weight according to the following equations:

$$\text{'muscle plus gonad' concentration} = \frac{\text{Mass toxin in gonad} + \text{mass toxin in muscle}}{\text{gonad weight} + \text{muscle weight}} \quad (\text{F7})$$

where

$$\text{Mass toxin in gonad} = \text{gonad weight} \times \text{toxin group concentration in gonad} \quad (\text{F8})$$

$$\text{Mass toxin in muscle} = \text{muscle weight} \times \text{toxin group concentration in muscle} \quad (\text{F9})$$

Relation between concentrations in the edible part and the entire animal

The CONTAM Panel also evaluated the data sets with a statistical approach. This was only possible for *Pecten maximus* where in some cases toxins were detected in edible parts. For each sample, the ratios between concentrations found in edible parts (muscle, gonad or the sum of muscle and gonad) were calculated both for LB and UB levels, thus giving for each sample an interval in which the true ratio could be established. Subsequently, a distribution curve was derived for the whole set of samples, from which mean and median ratios were derived (see Appendices A.1, A.2 and A.3). In addition, this allowed an estimation of the levels in the whole scallop that would guarantee that the level in the edible parts would be below the regulatory limit with a certain confidence.

The data submitted by Ireland and France for OA were analysed to study the ratio of the concentration in gonads and the entire animal, as well as the ratio of the sum of adductor muscle plus gonads and the entire animal. For adductor muscle only, too few samples contained detected levels to obtain reliable estimates of the expected distribution. Considering that the LB and UB were the same for all censored observations, this did not allow an estimation of the parameters of the potentially underlying distribution generating the observed data. The distribution of the ratios was estimated considering censoring and the estimated distribution was then used to estimate the threshold for the entire animal ensuring that the concentration in each of the edible parts, as well as the sum, remain under the regulatory limit of 160 µg/kg. Details are presented in Appendix A.1 (OA ratio between edible parts and entire animal and A2 (OA ratio between muscle and gonad combined and entire animal).

In order to account for the uncertainty in the estimation of the quantiles coming from the estimated distribution, the LB of a 95th confidence interval would be estimated for the 99th quantile of the fitted distribution and used as the maximum toxin level that would be allowed in the entire animal and still ensure that the part/parts analysed, have toxin levels below 160 µg OA eq/kg. Considering that the estimated quantile has been obtained using maximum likelihood methods, it can be assumed that it follows a normal distribution and with this a normal approximation can be used to obtain the 99th quantile confidence interval. The standard deviation to construct the confidence interval was estimated from the parameters from the log-normal distribution as follows: $(e^{\hat{\sigma}^2} - 1) \cdot e^{2\hat{\mu} + \hat{\sigma}^2}$, where $\hat{\mu}$ and $\hat{\sigma}^2$ are the estimated parameters of the best fitting log-normal distribution. The UB value of the ratio was then used to estimate the critical level in whole scallops that would ensure that levels in edible parts were below the regulatory limit.

It was not possible to estimate such ratios for YTX because of a lack of information. For both edible parts (muscle, gonad) for YTX, the calculated ratio to entire animal was between zero and one for all observations. For AZA for adductor muscle, all observations were censored starting all from zero, and therefore, reliable estimations for that body part were not possible. A ratio between AZA concentrations in gonad and entire animal could be established (for details, see Appendix A.3) but only results submitted by Ireland could be used for these estimations as France did not provide data on AZA.

Sample size estimation

The approach proposed by Matsushima et al. (2018a,b) was followed but adapted considering that the concentration of toxins in scallops follows a log-normal distribution instead of a normal distribution as assumed by the authors. A log-normal distribution was assumed because concentration values are only positive and in general their distribution is skewed, and lower concentrations are more likely to be observed than very high ones. The method considers the precision of the estimation of the mean concentration as the key element to define the number of scallops needed to classify an area as compliant or not. The approach also considers the variation observed in the four French sampling areas.¹⁶ The method uses the confidence bounds (LB and UB of the 95% confidence interval) to identify the number of scallops needed as they depend on the number of scallops, the expected mean and the variation expected to conclude with 95 per cent confidence depending on the mean value observed and the variations shown in the four areas used to classify the area/batch as compliant or non-compliant. The approach proposed by El-Shaarawi and Lin (2007) is used to estimate the confidence interval for a log-normal random variable. Details on the approach and results are presented in Appendix B.

2. Assessment

2.1. Occurrence data submitted to EFSA

After data validation and cleaning (as detailed in Sections 2.2.1 and 2.2.2), a total of 16,134 analytical results on 1,879 samples were present in the database for lipophilic toxins in scallops shown in Table 4¹⁷ Sampling years spanned from 2013 to 2020 (see Table 5 below), with the majority of results provided by Ireland followed by France.

Table 4: Number of analytical results (after data cleaning) on lipophilic toxins in scallops reported to EFSA^(a)

Toxin	Reporting country			
	France N	Germany N	Denmark N	Ireland N
OA ^(b)	549	3	30	1,296
DTX1 ^(b)	549	3	30	1,296
DTX2 ^(b)	549	3	30	1,296
AZA1	13	3	30	1,294
AZA2	13	3	30	1,294
AZA3	13	3	30	1,294
YTX	549	3	30	1,038
homoYTX	549	3	30	1,038
45-OH YTX	549	3	30	1,038
45-OH homoYTX	549	3	30	1,038

AZA: azaspiracid, DTX: dinophysistoxin; N: number of samples, OA: okadaic acid; YTX: yessotoxin.

(a): See Annex, Table A2 and Section 2.2.2 for details on data cleaning.

(b): Data in this opinion on OA, DTX1 and DTX2 were confirmed to be on totals, intended as the sum of the free and the acyl-derivatives according to the EU reference method for lipophilic toxins (EURLMB SOP).

Table 5: Number of analytical results (after data cleaning) on lipophilic toxins in scallops, reported to EFSA in relation to sampling year

Reporting country	2013	2014	2015	2016	2017	2018	2019	2020	Total
France	0	2,044	0	1,708	60	70	0	0	3,882
Germany	0	0	0	0	0	10	20	0	30
Denmark	0	0	0	300	0	0	0	0	300

¹⁶ Note that these sampling areas are not corresponding to CPAs.

¹⁷ Occurrence data on scallops are available on the EFSA Knowledge Junction community on Zenodo at: <https://doi.org/10.5281/zenodo.4472866>

Reporting country	2013	2014	2015	2016	2017	2018	2019	2020	Total
Ireland	888	1,958	1,396	1,549	2,381	1,486	1,496	768	11,922
Total	888	4,002	1,396	3,557	2,441	1,566	1,516	768	16,134

2.1.1. Distribution of analytical results across different food types

An overview of available data reported for different species is presented in Table 6. The majority of data were on *Pecten maximus* (n = 13,806), followed by *Aequipecten opercularis* (n = 1,888). A minimal number of data were submitted on *Chlamys islandica* (n = 300) and *Pecten jacobaeus* (n = 10). No data were available on *Mimachlamys varia*. Several data providers analysed different body parts of scallops, sometimes as part of monitoring campaigns (mainly in the case of adductor muscle and entire animal) or because of tailored studies undertaken on animals fished after contamination events. In the latter case, animals were dissected in specific and complementary body parts (e.g. single individuals of *Aequipecten opercularis*) were dissected in 'Hepatopancreas and mantle and Gonad' and 'Adductor muscle' in the data set submitted by France and analysed separately. A further description of the data is found in Section 2.1.4.

Table 6: Number of analytical results according to species, body parts and toxins as present in the EFSA database

Species	Matrix	OA group			AZA group			YTX group			
		OA	DTX1	DTX2	AZA1	AZA2	AZA3	YTX	homoYTX	45-OH YTX	45-OH homoYTX
<i>Aequipecten opercularis</i>	Adductor muscle	132	132	132	10	10	10	132	132	132	132
	Adductor muscle and Gonad	5	5	5	5	5	5	5	5	5	5
	Entire animal	1	1	1	1	1	1	1	1	1	1
	Gonad	2	2	2	2	2	2	2	2	2	2
	Hepatopancreas and Mantle and Gonad	122	122	122	NR	NR	NR	122	122	122	122
<i>Chlamys islandica</i>	Adductor muscle	30	30	30	30	30	30	30	30	30	30
<i>Pecten jacobaeus</i>	Adductor muscle	1	1	1	1	1	1	1	1	1	1
<i>Pecten maximus</i>	Adductor muscle	115	115	115	104	104	104	73	73	73	73
	Adductor muscle and Gonad	127	127	127	1	1	1	127	127	127	127
	Entire animal	32	32	32	32	32	32	31	31	31	31
	Entire animal excluding gonad and adductor muscle	634	634	634	632	632	632	482	482	482	482
	Gonad	518	518	518	509	509	509	455	455	455	455
	Hepatopancreas	10	10	10	NR	NR	NR	10	10	10	10
	Hepatopancreas and Mantle	126	126	126	NR	NR	NR	126	126	126	126
	Mantle	10	10	10	NR	NR	NR	10	10	10	10
<i>Pecten spp.</i>	Entire animal	13	13	13	13	13	13	13	13	13	13

AZA: azaspiracid, DTX: dinophysistoxin; OA: okadaic acid; NR: not reported; YTX: yessotoxin.

2.1.2. Analytical methods and limits of quantification

Only results obtained with the EU reference method for lipophilic toxins or equivalent methods were considered. All the reported results were obtained with LC-MS/MS-based methods. An overview of reported LOQs is shown in Table 7, while LODs can be found in the Annex, Table A3. For DTX1, AZA3, homoYTX, 45-OH YTX and 45-OH homoYTX, results were 100% left-censored.

Table 7: Overview of the percentage of left-censored data, number of samples (N) and descriptive statistics for LOQs reported for to lipophilic toxins in scallops ($\mu\text{g}/\text{kg}$)

Toxin	Left-censored	LOQs							
		N	Min	Q1	Mean	Median	Q3	P95	Max
OA	67%	1,878	2.7	10	13	15	15	15	75
DTX1	100%	1,878	4.8	10	13	15	15	15	73
DTX2	83%	1,878	2.5	10	13	15	15	15	81
AZA1	74%	1,340	2	5	6	5	5	5	30
AZA2	81%	1,340	1.9	5	6	5	5	5	30
AZA3	100%	1,340	2	5	6	5	5	5	30
YTX	92%	1,620	3.3	5	131	200	200	200	200
homoYTX	100%	1,620	3.3	5	131	200	200	200	200
45-OH YTX	100%	1,620	3.3	5	131	200	200	200	200
45-OH homoYTX	100%	1,620	3.3	5	131	200	200	200	200

AZA: azaspiracid, DTX: dinophysistoxin, LOQ: limit of quantification, Max: maximum; Min: minimum; N: number of samples; OA: okadaic acid; Q1: 25th percentile; Q3: 75th percentile P95: 95th percentile; YTX: yessotoxin.

Some data sets were corrected by recovery, while others were not. The reported recovery ranged between 72% (reported for homoYTX and 45-OH homoYTX in adductor muscle) and 109% (e.g. for DTX2 in adductor muscle).

2.1.3. Occurrence data on lipophilic toxins in scallops

Descriptive statistics for lipophilic toxins in scallops at the lower and upper bounds are presented in the Annex, Table A4. Since, depending on the species, body parts and data providers, a different number of animals were pooled into a homogenised sample prior to sample analysis, descriptive statistics were weighed for sample number. Only data reported by France pertained to single animals. Descriptive statistics (weighted by number of units per sample) for lipophilic toxins in scallops expressed by toxin group (formulas reported in Section 1.3.2) as μg toxin equivalents/kg are reported in the Annex, Table A5.

In cases where the data received were not sufficient to reconstruct the concentration in the entire animal, these were not suitable to answer the terms of reference and were therefore not used for the assessment (10,030 out of a total of 16,134 data points). From the 10,030 data points provided by Ireland, 8,989 were on gonads and 'entire animal excluding gonad and adductor muscle'. Thus, the level in the whole scallop could not be calculated, because for these samples neither the levels of toxins nor the weights of the adductor muscle were provided. Other data points excluded were based on levels only in whole scallops ($n = 456$) or muscle only ($n = 310$).

2.1.4. Data on concentrations used for the assessment

Only for a subset of samples, data were supplied for all of the body parts along with sufficient information on the relative contribution of these parts, i.e. the weights of the different parts. In those cases, it was possible to reconstruct the concentration in the entire animal. Such data were only submitted by France and Ireland. The formulas applied for the mathematical reconstruction of concentration levels in the entire animal are reported in Section 1.3.2. This was possible for a subset of 6,104 analytical results that were selected for the present assessment and are further described in the following sections. The analytical data submitted by France pertained to individual animals while those by Ireland were based on pooled samples. Individual and pooled samples are representing different sources of variation and could therefore only be considered separately in the assessment.

Description of the data submitted by France

France reported data on individuals of *Aequipecten opercularis* and *Pecten maximus*. Following episodes of contamination of *Pecten maximus* and *Aequipecten opercularis* by okadaic acid lipophilic toxins (autumn 2014 in Eastern Cotentin, Normandy; and summer 2016 in North West Cotentin, Normandy), the FR NRLMB was commissioned by the General Directorate of Food of the Ministry of Agriculture, Agri-Food and Forestry to carry out a study on the distribution of these toxins in the shellfish.

In total 136 individuals of *Pecten maximus*, sampled in three contaminated areas of the Baie-de-Seine in November 2014, and 122 individuals of *Aequipecten opercularis*, from two different lots sampled in August 2016, were analysed for regulated lipophilic toxins. Figure 5 shows a map with the different sampling areas.

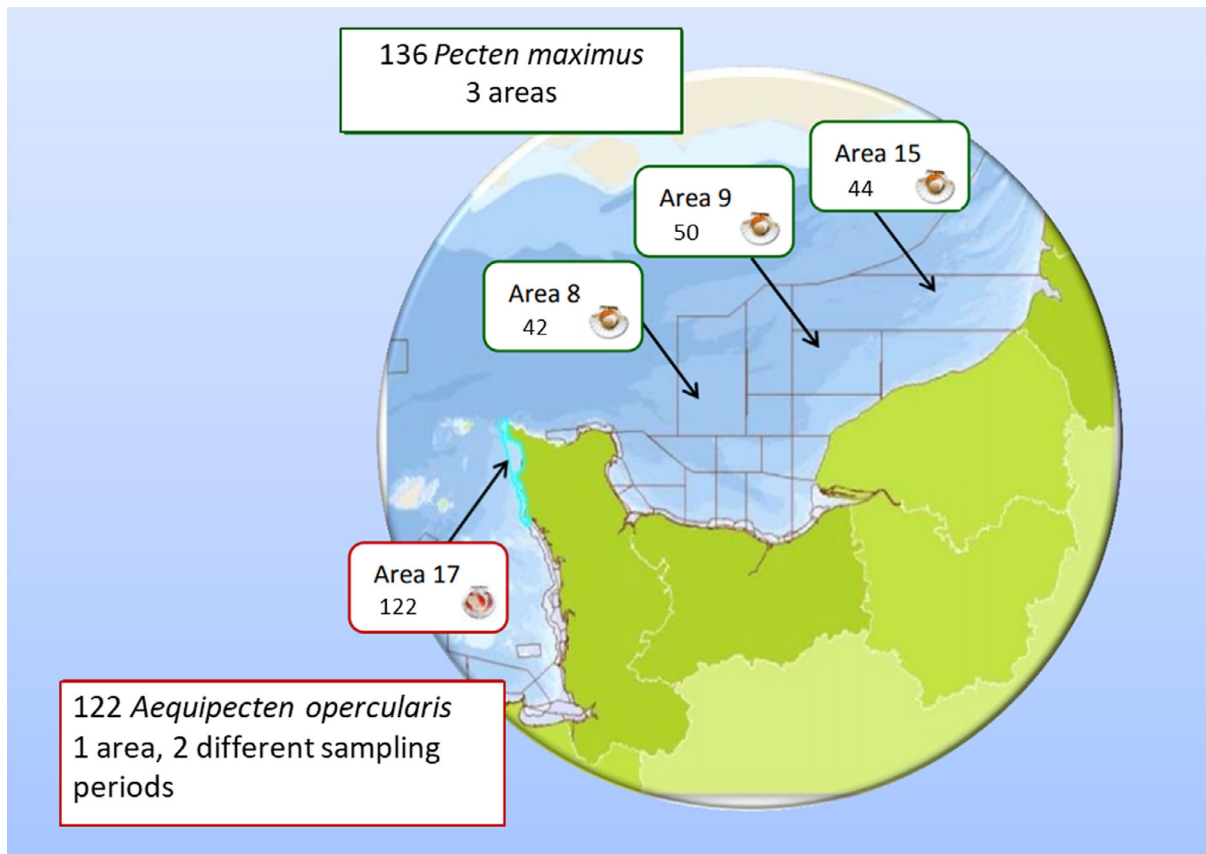


Figure 5: Map of the sampling areas for the data received from France (Adapted from Nicolas et al., 2017)

Analyses involved two different parts of each individual, namely 'roe-on' (adductor muscle and gonad in the case of *Pecten maximus*)/'roe-off' (adductor muscle only in the case of *Aequipecten opercularis*) and 'rest of the animal'. For 10 scallops of *Pecten maximus* also the muscle and gonads, as well as the remaining parts were dissected and analysed separately. Individual animals were dissected into complementary body parts as detailed in Table 8 and the concentration of lipophilic toxins (OA and YTX group) was analysed and reported separately for each matrix. Furthermore, the weights of body parts of each individual scallop were submitted to EFSA and this allowed calculating back the mass of toxins in each body part and a mathematical reconstruction of the concentration that had been present in the entire animals' soft tissue. Summary statistics of weights of different body parts are reported in the Annex, Table A6.

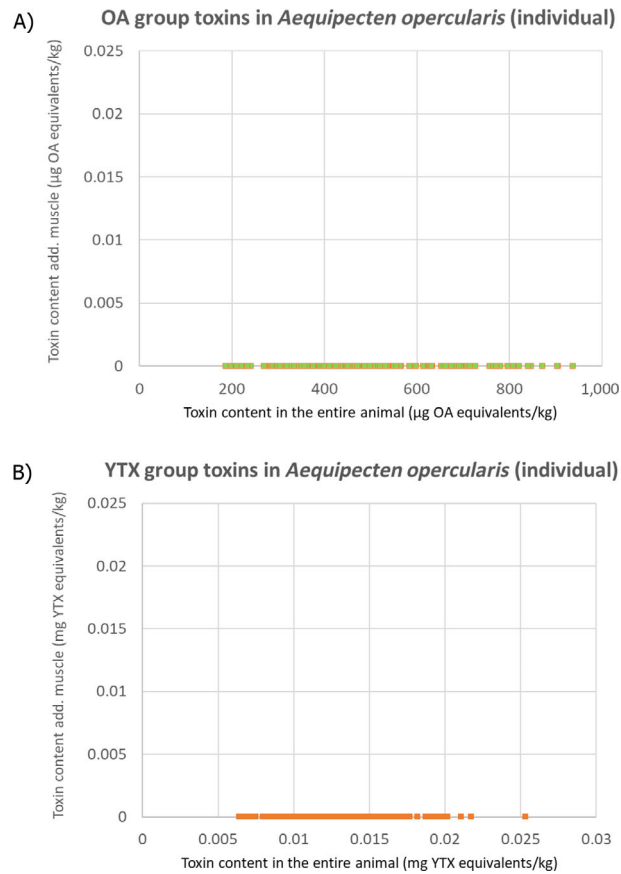
Table 8: Overview of the number of results from individual samples as reported by France

Species	Body part	AZA	OA	YTX
		N	N	N
<i>Aequipecten opercularis</i>	Adductor muscle	NR	122	122
	Hepatopancreas and mantle and gonad	NR	122	122
<i>Pecten maximus</i>	Adductor muscle	NR	10	10
	Adductor muscle and gonad	NR	126	126
	Gonad	NR	10	10
	Hepatopancreas	NR	10	10
	Hepatopancreas and mantle	NR	126	126
	Mantle	NR	10	10

AZA: azaspiracid, N: number; NR = not reported; OA: okadaic acid; YTX: yessotoxin.

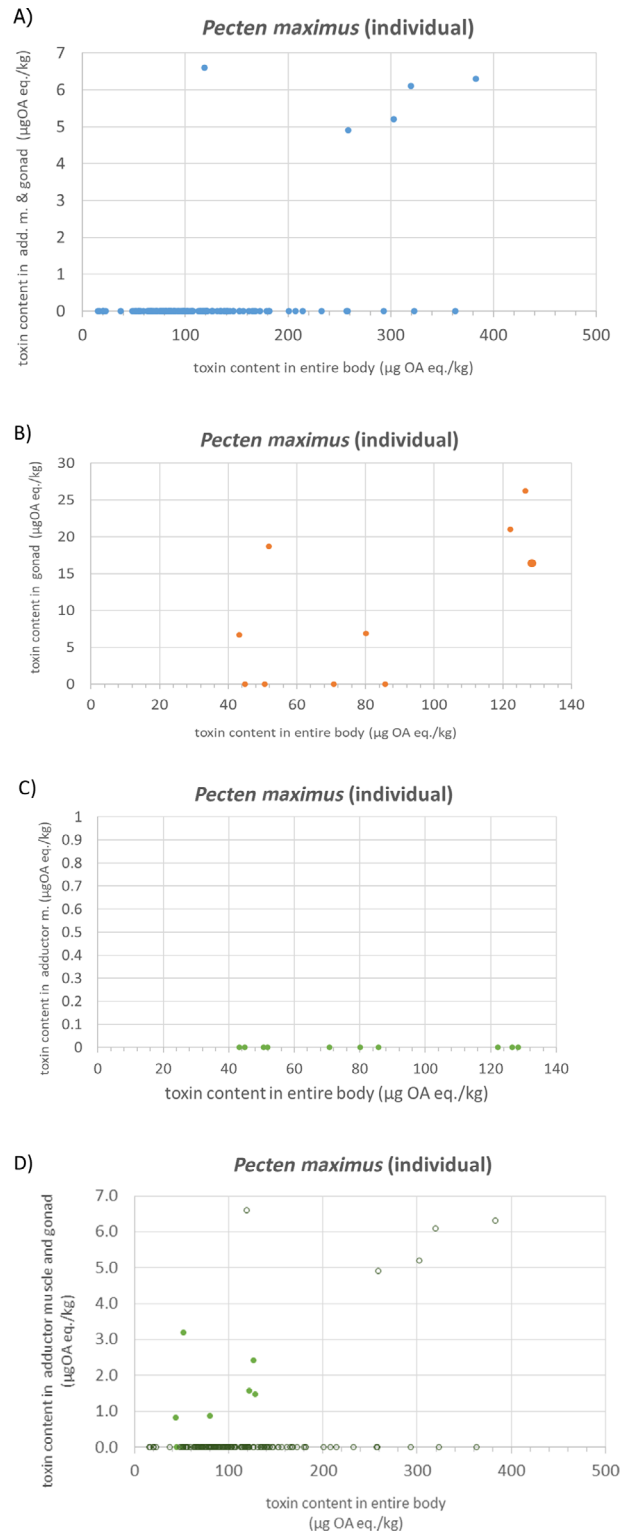
For this data set, the content of toxins in *Aequipecten opercularis* (n = 122) in the entire animal ranged from 187 to 938 µg OA eq/kg for the OA group and from 0.006 to 0.025 mg YTX eq/kg for the YTX group at the LB. Irrespective of the content of lipophilic toxins in the entire body, adductor muscles were always left-censored both for the OA- and the YTX group (see Figure 6).

For *Pecten maximus* in this data set, the content of lipophilic toxins (n = 136) in the entire animal ranged from 15 to 383 µg OA eq/kg for the OA group (at LB). All samples were left-censored for the YTX group. Out of 126 samples, only five had measurable levels in 'Adductor muscle and gonad' for OA group toxins (see Figure 7A), up to 6.6 µg OA eq/kg at the LB. For the 10 animals for which 'gonad' and 'adductor muscle' were analysed separately, the content of OA group toxins in the entire body ranged from 45 to 130 µg OA eq/kg for the LB. The 'gonad' samples showed a content up to 26.2 µg OA eq/kg for a sample with 128 µg OA eq/kg in the entire body (Figure 7B), while the levels in 'adductor muscle' were always left-censored (Figure 7C). Based on the weights, the levels in adductor muscle and gonads were also calculated for these 10 scallops and added to the other 126 results (see Figure 7D).



LB: lower bound; OA: okadaic acid; YTX: yessotoxin.

Figure 6: Toxin content (at the LB) in adductor muscle of *Aequipecten opercularis* (Y-axis) vs content in the entire body (X-axis) as reported by France in 122 individual scallops for the OA group (A) and the YTX group (B)



(A) in 'adductor muscle' and 'gonad' (Y-axis) vs content in the entire body (X-axis) in 126 individuals, or (B) in 'gonad' vs. 'entire body' and (C) 'adductor muscle' vs 'entire body' in 10 individuals. (D) shows the calculated values for the muscle plus gonad as filled green points in addition to those already shown in (A).

Figure 7: Okadaic acid group toxin content (at the lower bound) in single individuals of *Pecten maximus* reported by France in different body compartments (see above)

Description of the data submitted by Ireland

Ireland reported data on 93 batches of pooled *Pecten maximus* parts originating from Official Control Biotoxin Monitoring of North Atlantic CPAs. Information on the origin of the data submitted by Ireland has been provided but is not described here as considered not relevant for the assessment. Animals were dissected in 'adductor muscle', 'gonad' and 'entire animal excluding gonad and adductor'. Tissues of 12 animals of the same batch were pooled and analysis of concentrations of the OA-, AZA- and YTX group were carried out as described in Table 9. Results of each matrix were reported separately. Only results for samples for which all complementary body parts were analysed were considered and no imputation was done for missing results.

Table 9: Overview of the numbers of results from pooled samples as reported by Ireland

Species	Body parts	AZA	OA	YTX
		N	N	N
<i>Pecten maximus</i>	Adductor muscle	91	93	58
	Gonad	91	93	58
	Entire animal excluding gonad and adductor muscle	91	93	58

AZA: azaspiracid, N: number of samples; OA: okadaic acid; YTX: yessotoxin.

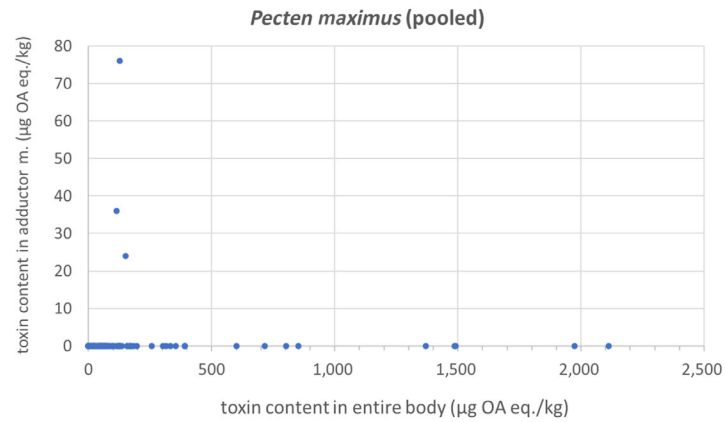
As for the database submitted by France, the sample weights were acquired (statistics reported in Annex A6) and it was possible to calculate the concentration for the entire animal, using equations F1, F2 and F3 (as described in Section 1.3.2) with the only difference that in this case the concentrations in the entire body and in all other tissues related to a group of 12 pooled animals instead of one individual animal.

At the LB, the content of toxins in the entire animal ranged from 0 to 2,117 μg OA eq/kg for the OA group, from 0 to 139 AZA1 eq/kg for the AZA group while 100% of the results were left-censored for the YTX group.

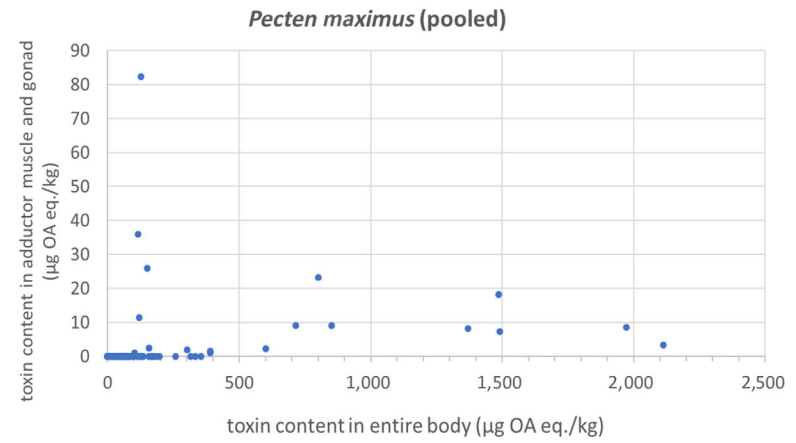
The majority of adductor muscle samples (97%) was left-censored for the OA group except for three samples with a content of 76, 36 and 24 μg OA eq/kg (Figure 8A). For the AZA group, irrespective of the content of lipophilic toxins in the entire body, levels in adductor muscles were always left-censored (see Figure 8D).

For gonad 18 samples with detectable levels were reported for the OA group, being up to 224 μg OA eq/kg, and 19 samples for the AZA group with up to 58 μg AZA1 eq/kg (Figures 8B and E).

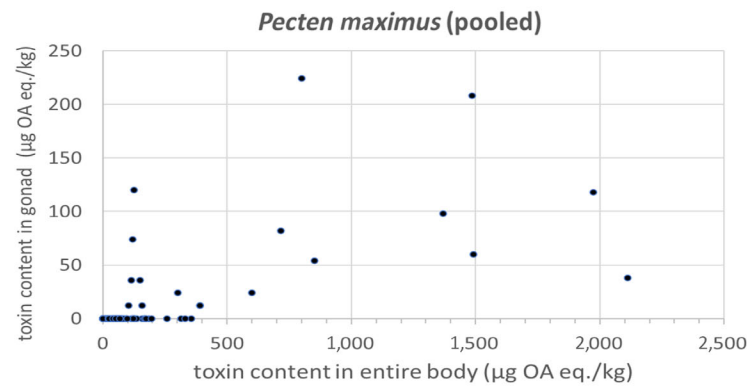
Based on the levels in gonad and muscle, also the levels of OA- and AZA group toxins in the combined edible parts was calculated, using the different weights of the tissues (Figures 8C and F).



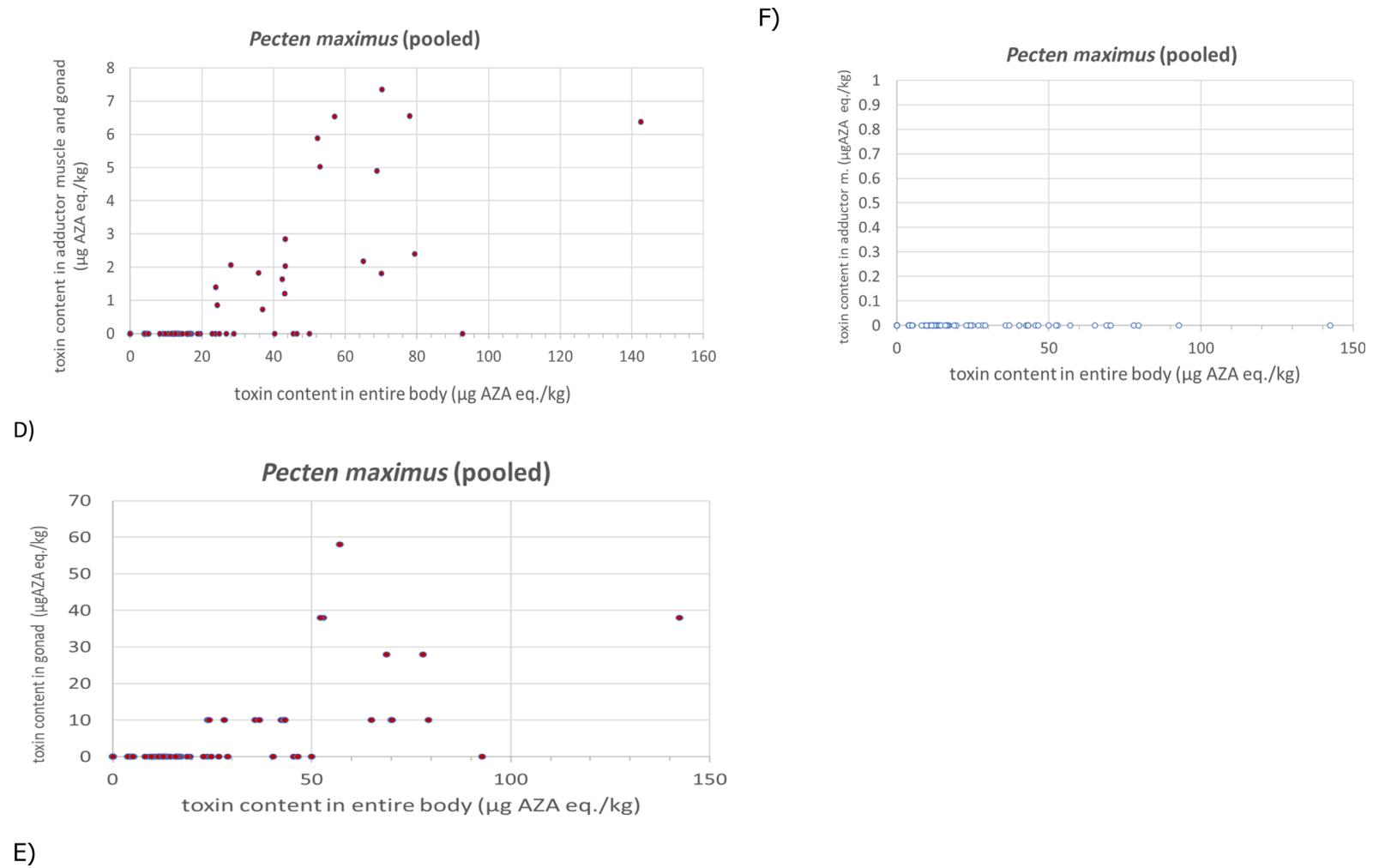
A)



C)



B)



(A–C): OA group toxin content at the LB (Y-axis) in adductor muscle (A), and gonad (B) and gonad plus adductor muscle (C) vs content in the entire body (X-axis) in pooled samples of *Pecten maximus* reported by Ireland. Each data point represents a pooled sample of 12 units). **(D–F): AZA** group toxin content at the lower bound (Y-axis) in adductor muscle (D), and gonad (E) and gonad plus adductor muscle vs content in the entire body (X-axis) in pooled samples of *Pecten maximus* reported by Ireland (each data point represents a pooled sample of 12 units)

Figure 8: Okadaic acid group toxin content and Azaspiracid group toxin content reported by Ireland in samples of *Pecten maximus*

2.1.5. Estimation of toxin concentration ratios between edible parts and whole scallop

The CONTAM Panel calculated the ratio of the toxin group concentration in the edible parts (muscle, gonad or the sum of muscle and gonad) and the concentration calculated for the entire body. In order to calculate these ratios, two approaches were followed: the first based on the reported data and the second based on the distributions obtained from the reported data. Further details can be found in Section 1.3.2.

Ratios based on toxin levels

In *Aequipecten opercularis*, toxins were only detected in the remaining tissue ('hepatopancreas, mantle and gonad'), meaning that all data on 'adductor muscle' were left-censored. For OA group toxins, the estimated toxin equivalents based on LOQ/LOD in muscle varied between 7.1 and 11.7 µg OA eq/kg. The estimated UB levels in the entire animal ranged between 191 and 942 µg OA eq/kg, i.e. were all above the regulatory limit of 160 µg OA eq/kg. Based on the UB levels, the ratio between the concentration of OA group toxins in muscle and whole scallop varied between 0.01 and 0.04.

YTX concentrations in the entire animal at the LB ranged between 0.006 and 0.025 mg YTX eq/kg, i.e. were far below the regulatory limit of 3.75 mg YTX eq/kg. However, based on the estimated UB levels in toxin equivalents using the LOQ/LOD of the applied method, the ratio between the concentration in muscle and whole scallop ranged from 0.2 to 0.6. These high ratios are most likely very conservative, due to the low toxin levels in the scallops, which were far below the regulatory limits and close to the LOQ/LOD of the analytical method used.

For the data submitted by France on 126 individual *Pecten maximus* scallops, estimated UB levels in whole scallops ranged between 18 and 385 µg OA eq/kg for the OA group toxins whereas in 'gonad and adductor muscle', only five samples showed a detectable level. Those five samples showed ratios for the LB concentrations between 'adductor muscle plus gonad' and whole scallop ranging from 0.016 to 0.056. In the case of the 10 samples in which the tissues were further separated, no toxins from the OA group were detected in the muscle (< 4 µg OA eq/kg), but six gonad samples contained LB levels between 7 and 26 µg OA eq/kg (UB 4–29 µg/kg; n = 10), as compared to LB levels in the entire scallop of 43–128 µg OA eq/kg (UB 47–132 OA µg eq/kg). The ratio between the LB concentration of OA in gonad and the whole scallop was 0.36 or lower. The fact that only gonad was contaminated may be explained by the fact that the gonad contains a part of the intestine, which cannot be removed during shucking (see Section 1.2.2).

For the OA group toxins in pooled *Pecten maximus* scallops, data submitted by Ireland, the estimated LB levels in whole scallops ranged from 0 to 2,112 µg OA eq/kg, whereas in adductor muscle only, 3 out of 93 samples showed quantifiable levels. For these three samples, with LB levels of 24, 36 and 76 µg OA eq/kg (all below the limit of 160 µg OA eq/kg), respectively, the ratios between LB concentrations in muscle and whole scallop were 0.16, 0.31 and 0.60, respectively. For gonads, 18 out of 93 samples showed a detectable level, ranging between 12 and 224 µg OA eq/kg at the LB. For these samples, ratios between LB concentrations in gonad and whole scallop ranged between 0.018 and 0.95. When combining gonads and muscle, 18 samples showed a detectable level ranging between 0.9 and 82 µg OA eq/kg. For the whole data set, LB levels ranged between 0 and 82 µg OA eq/kg. The ratio for samples with detectable levels ranged from 0.002 to 0.65 (based on LB levels).

For the AZA group in these pooled samples submitted by Ireland, the estimated LB levels in the entire scallops varied between 0 and 142 µg AZA1 eq/kg. Levels in adductor muscles were all below detection limits and the UB level was 8 µg AZA1 eq/kg in all these samples. In gonads, the levels in 19 out of 91 samples were above the LOQ and ranged between 10 and 58 µg AZA1 eq/kg at the LB. This resulted in ratios between LB concentrations in gonad and whole scallop ranging between 0.13 and 1.

For YTX in these pooled samples reported by Ireland, all levels were below the LODs/LOQs (see Section 2.1.4), resulting in a mean UB level of 0.175 mg YTX eq/kg in whole scallops but also adductor muscle and gonad. Consequently, these data could not be used for the assessment.

Ratios based on distributions

Several distributions were fitted to the data submitted by France and Ireland accounting for their censoring nature (levels of one or more toxins in the group < LOQ in all samples). The analysis was performed for gonads plus adductor muscle, as well as for gonads only. The resulting distributions allowed estimation of the 99th quantiles for the ratios and with this also the expected concentration in

whole scallops that would still lead to compliant levels for gonads or gonad plus adductor muscle. The respective analyses are presented in Appendices A.1–A.3.

Considering that the estimation is based on a limited data set collected by Ireland and France, the 95th confidence interval was obtained for the 99th quantile in each of the analyses presented in Appendices A.1–A.3. The UB value of the confidence interval for the P99 ratio was then used to calculate the critical level in whole scallops that would ensure that the level in the edible part is below the regulatory limit. Table 10 shows the calculated UB for the P99 ratios and critical levels in whole scallops for those tissues where the data sets allowed such a calculation.

Table 10: Summary of the findings to estimate concentration thresholds in the whole scallop for the most conservative result – estimated ratios based on the data sets submitted by France and Ireland

Toxin	Parts	Data	Ratios		Critical concentrations in the whole scallop ($\mu\text{g}/\text{kg}$)	
			P99	UB P99	At P99	At UB P99
OA	Adductor muscle and gonads	FR	0.05	NC	3,258	NC
		IRL	0.34	0.47	471	342
	Gonads	FR	0.60	0.97	268	165
		IRL	0.44	0.63	362	256
AZA	Gonads	IRL	0.51	0.74	312	217

AZA: azaspiracids; FR: France; IRL: Ireland; OA: okadaic acid; P99: 99th percentile from the distribution of the ratios and UB P99: refers to the upper bound of the 95% confidence interval for the P99 for the ratio.

For gonads and adductor muscle combined, it follows that a concentration of 342 μg OA eq/kg in the entire animal should not be exceeded to ensure that the concentration in these edible parts does not exceed the regulatory limit of 160 OA eq/kg.

For gonads only, a concentration of 165 μg OA eq/kg in the entire animal should not be exceeded to ensure that the concentration in gonad does not exceed the regulatory limit of 160 OA eq/kg.

For the AZA group toxins, only the data on gonads allowed this calculation, based on the data in pooled samples submitted by Ireland. Based on this, it follows that a concentration of 217 μg AZA1 eq/kg in the entire animal should not be exceeded to ensure that the concentration in the gonads would not exceed the regulatory limit of 160 AZA 1 eq/kg.

For other toxin/tissue combinations such a calculation based on distribution was not possible, since all data levels in edible parts were below the LOQ. In such cases, the ratios of the UB levels could be considered as the starting point. For OA group toxins in muscle of *Aequipecten opercularis*, these ratios varied between 0.01 and 0.04. For YTX group toxins in this species this ratio was between 0.21 and 0.56. Especially for the YTX toxins, the levels in whole scallop were far below the regulatory limit and close to the LOQ of the method. As a result, the estimated ratios based on UB levels are very conservative.

This conservatism is also observed for adductor muscle of *Pecten maximus*. For OA levels, the ratios based on UB levels ranged from 0.03 to 0.09 and 0.01–1.0 for the data submitted by France and Ireland, respectively. For AZA, this was 0.06–1.0 based on data submitted by Ireland. For YTX such calculations were not possible since no toxins were detected in any of the tissues.

2.1.6. Public literature potentially relevant for the present opinion

As described in Section 1.3.1, a systematic literature search and evaluation was carried out in order to include information from peer-reviewed studies in the present assessment. Only a few studies dealt with the impact of shucking on toxin levels. Although the data from these studies could not be used for the present assessment (e.g. not dealing with the species of concern, not dealing with farmed animals likely less varying or not providing information about toxin concentrations of whole animal vs. body parts), overall, these corroborate the findings in the present assessment and are therefore briefly summarised below.

Furey et al. (2003) investigated AZA contents in *Pecten maximus* in Irish waters. 75% of the samples were positive for AZA ($> 10 \mu\text{g}/\text{kg}$) and 50% exceeded the EU regulatory limit of 160 μg AZA1 eq/kg. Contamination of the muscle and gonad was consistently low ($< 30 \mu\text{g}/\text{kg}$) while AZA was found mainly in the digestive gland (concentrations not provided by the authors).

Madigan et al. (2006) studied DSP concentrations in *Pecten fumatus* in Australian waters. Toxin concentrations in the entire scallops were 23, 510, 18, < LOD and 410 µg/kg for PTX-2, PTX-2 seco acid, OA, OA released by hydrolysis and total DSTs, respectively. The corresponding figures for the adductor muscle + gonad were < LOD, 37, 14, < LOD and 14, respectively. These low values confirmed previous studies showing that the viscera contain the highest amounts of DSTs.

López-Rivera et al. (2010) investigated AZA concentrations in scallops (*Argopecten purpuratus*) in Chilean waters. While AZA1 and AZA2 were detected in the hepatopancreas, AZAs were not detected in remaining tissue of scallops.

Matsushima et al. (2018a,b) investigated the distribution of OA, DTX1 and DTX3 amongst tissues and individual animals of farmed Japanese scallops (*Patinopecten yessoensis*). Of the total DTX1 detected in seven different trials, 97.1–98.6% was found in tissues reported as hepatopancreas (Matsushima et al., 2018b) or digestive gland (Matsushima et al., 2018a). The corresponding figures were 0.5–1.5% in the gonad, 0.0–1.0% in the mantle and 0.0–1.2% in the gills while DTX1 was not detected in the muscle. That tissue distribution was in line with earlier studies showing both PTX and DST content being much higher in digestive gland of *Pecten yessoensis* than in other tissues (Matsushima et al., 2015). DTX1 concentrations in the hepatopancreas/digestive gland of 30 individual scallops were measured in 4 trials and showed a normal distribution of the concentrations in this tissue. In order to assess how many individual samples might be necessary to accurately reflect DTX1 content in a population, statistical resampling with and without bootstrap replacement was carried out. That analysis showed that a sample comprising of eight scallops would have a mean falling within ± 20% of the 30-animal population mean with a probability of 98%. The number of samples increased to 11 scallops when bootstrap replacement was applied.

2.2. Responses from the CONTAM Panel to the questions in the Terms of Reference

The data provided by EU Member States confirm that scallops can be contaminated with lipophilic shellfish toxins. They were obtained from routine monitoring but in particular from follow-up actions on contaminated populations. This offered the opportunity to study the distribution among the tissues and between individual scallops. Suitable data were obtained for only two scallop species, *Pecten maximus* and *Aequipecten opercularis* and only from two Member States, France and Ireland.

2.2.1. Response to question 1 of the Terms of Reference

Question 1: EFSA is requested to propose what would be the upper limit concentration of lipophilic toxins in whole body scallops (entire animal) that would still result in compliant edible parts (adductor muscle and gonads separately and combined) after shucking.

Shucking refers to the removal of certain parts of the scallops in which the lipophilic toxins are mainly present and which generally leads to removal of most, if not all, lipophilic toxins. In the case of *Pecten maximus*, both the adductor muscle and the gonads, or their combination, are considered as the edible parts. In the case of the much smaller *Aequipecten opercularis*, only the adductor muscle is consumed, and the gonad is removed with the other parts. The data sets obtained from France and Ireland allowed to some extent addressing the question on the distribution of the toxins between edible parts and the whole scallop. A statistical approach was applied to estimate the highest level in the scallop that would ensure that the level in the edible parts would be below the regulatory limit for a specific toxin group. Since exceedance of the limit should be prevented, the P99 was selected as a conservative approach. This approach was only possible for some toxins in *Pecten maximus* where in some cases toxins were detected in edible parts. For each sample, the ratios between edible parts (muscle, gonad or the sum of these) and entire animals were calculated both for LB and UB levels, thus giving for each sample an interval in which the true ratio could be. Subsequently, a distribution function was estimated for the whole set of samples, from which mean and median ratios were derived (see Section 2.1.5 and Appendices A.1–A.3). The resulting levels in the whole scallop that would guarantee that the level in the edible parts would be below the regulatory limit with a certain confidence, are shown in Table 10 (Section 2.1.5).

In the data set on 122 individual *Aequipecten opercularis* scallops obtained from France, toxin concentrations of 'adductor muscle' and the combined 'hepatopancreas, mantle and gonad' were determined. Based on the weights provided for muscle and the other tissues, it was possible to calculate the toxin level in the entire animal (as reported in Section 1.3.2 on methodology used for the

assessment). In this data set, OA- and YTX group toxins were analysed, but not the AZA group toxins. Since toxins were only detected in the remaining tissue (or in 'hepatopancreas, mantle and gonad') and not in 'adductor muscle', no statistical analysis of the data was possible. The estimated toxin equivalents in muscle based on LOQ/LOD varied between 7.1 and 11.7 μg OA eq/kg. For OA, the estimated UB levels in the entire animal ranged between 191 and 942 μg OA eq/kg, i.e. were all above the limit of 160 μg OA eq/kg. Based on the UB levels, the ratio between muscle and whole scallop varied between 0.01 and 0.0437 for the OA group. YTX concentrations in the muscle were all below the LOQ, those in entire animal at the LB ranged between 0.006 and 0.025 mg YTX eq/kg, i.e. far below the limit of 3.75 mg YTX eq/kg. This data set could not be used for a statistical approach. Based on the estimated UB levels in toxin equivalents, using the LOQ/LOD of the applied method, the ratio between the concentration in muscle and whole scallop ranged from 0.2 to 0.6 for the YTX group. These ratios are relatively high, most likely due to the low levels in the scallops, being close to the LOQ/LOD of the method.

For *Pecten maximus*, several data sets were obtained. France provided data on individual scallops for which either 'adductor muscle and gonad', as well as the 'hepatopancreas and mantle' ($n = 126$) were analysed or, for a small number of scallops, the separated 'adductor muscle', 'gonad' and 'hepatopancreas and mantle' ($n = 10$). Again, only data on OA and YTX group toxins were provided. The data set submitted by Ireland comprised of pooled samples (12 scallops per sample) with analytical results on AZA ($n = 91$), OA ($n = 93$) and YTX ($n = 58$). In these samples reported by Ireland, toxin levels in 'adductor muscle' and 'gonads' and 'remainder tissue' were analysed separately.

For OA group toxins in muscle + gonads or gonads only, the distribution of the ratios could be evaluated and was used to determine the level in whole scallops that should ensure that levels in edible parts are below the regulatory limit. For muscle + gonads, the data submitted by Ireland showed higher ratios and this resulted in an LB level for the P99 of 342 μg OA eq/kg. For gonads only, the lowest value was determined from the data set submitted by France, being 165 μg OA eq/kg, but this was based on only 10 samples. For the data set submitted by Ireland, the corresponding value was 256 μg OA eq/kg.

For AZA group toxins, a similar approach could be applied but only on the gonads. This resulted in an LB level for the P99 of 217 μg AZA1 eq/kg.

Data on YTX were all left-censored and could therefore not be used to answer the questions in the Terms of Reference.

Overall, the data show that shucking results in non-detectable levels in practically all cases in muscles and in most cases also in the gonads. However, in some cases, toxins are detected in edible parts and the ratios between the toxin levels in whole scallop and the muscle or gonad can be rather small. There are uncertainties regarding the relation between the levels in whole scallop and 'edible parts'. In this context, it needs to be pointed out that the toxins assessed all cause acute effects and that exceedance of the regulatory limits may result in adverse effects in the consumer (see Section 2.2.3).

2.2.2. Response to question 2 of the Terms of Reference

Question 2: EFSA is requested to give different possible scenarios of levels of lipophilic toxin and occurrence of contamination in individual animals, EFSA is asked to provide recommendations on sampling schemes (batch based for either whole body or shucked scallop) that would ensure that all subsets of pooled samples will be compliant if a sample is analysed by a laboratory and found compliant.

The data set submitted by France on individual scallops allowed answering this second question, because it provided information on the variation of the levels in a contaminated area. However, this applies only for the estimated levels in the total scallops and not for muscle and gonads because the number of scallops with detectable levels in these tissues was too small. In addition, it was only possible to answer the second question for OA but not for YTX and AZA because data on the latter toxins in individual scallops were too limited.

For the OA group, the actual data were derived from four different areas and time periods where a contamination episode allowed a more detailed study on individual scallops. For each area, the coefficient of variation (CV) was calculated, being between 39 and 50% for *Pecten maximus* and 37% for *Aequipecten opercularis*. Using these CVs, it was estimated for a number of hypothetical mean levels around the limit of 160 μg OA eq/kg how many individual scallops should be included in the sample to ensure with a 95% certainty that the sample tested is compliant (level < limit) or

non-compliant (level > limit). As shown in Figure 9, in the range of 140–190 OA eq/kg, this would require the pooling of more than 30 scallops in an analytical sample. This was deemed unfeasible for practical reasons and implies that if the actual levels are in this range, there is a considerable probability of a false compliant or false non-compliant test result. For levels < 130 and > 190 OA eq/kg, respectively, 11–18, and 15–25 animals per sample would be sufficient. Sample sizes of < 10 would give a 95% correct classification at actual levels of ≤ 120 or ≥ 210 μg OA eq/kg. In these calculations, the potential application of a measurement uncertainty before deciding on non-compliance was not taken into account. With e.g. a measurement uncertainty of 30%, only test results higher than 230 μg OA eq/kg would be considered as non-compliant. However, in practice there is no obligation to apply measurement uncertainty in the official control of marine biotoxins in shellfish.

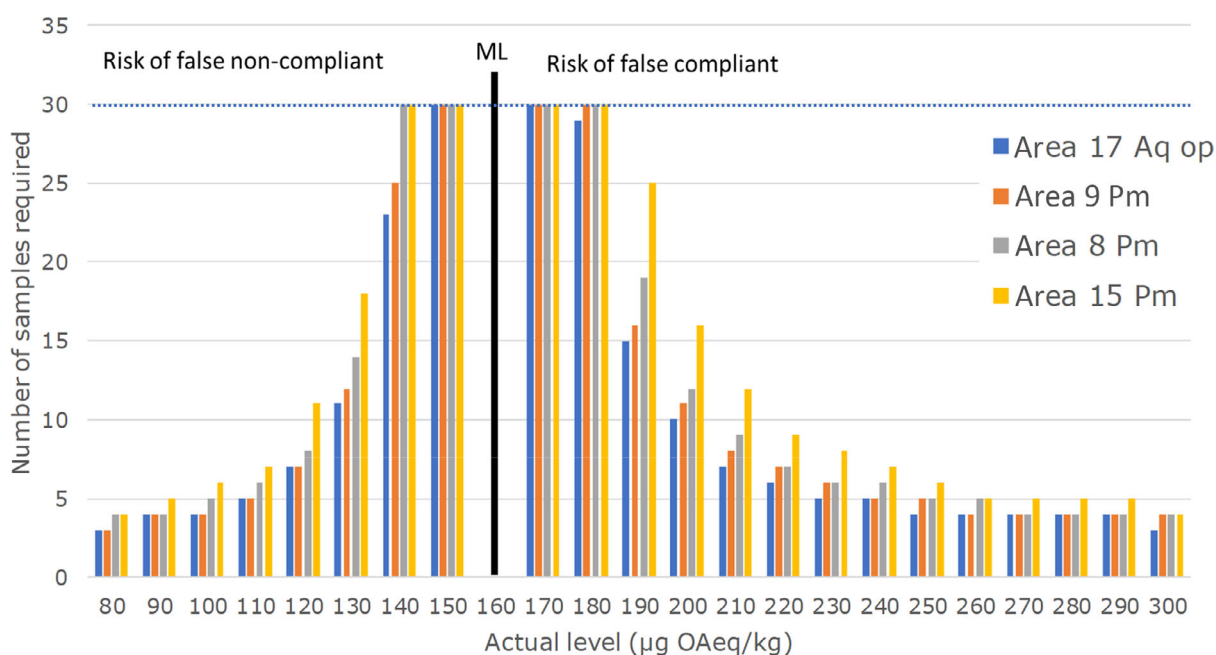


Figure 9: Number of samples required for a pooled analytical sample to ensure with 95% certainty a correct conclusion on either compliance (if level below the regulatory limit) or non-compliance (if level above the regulatory limit), at a given concentration in the area or lot. Numbers above 30 were not estimated and are presented as 30. For *Aequipecten opercularis* (Aq op), one area was sampled, for *Pecten maximus* (Pm) three.

As already mentioned, available data on adductor muscle or gonads, or adductor muscle plus gonads were not considered suitable for similar calculations for these edible parts. In addition, the observed ratio between edible tissues and whole scallops was too variable to assume similar CVs for these tissues.

2.2.3. Risks associated with scallop consumption

The fact that around the regulatory limit there is a considerable chance of false compliant results raises the question if this could imply a risk for the consumer. In the case of *Pecten maximus*, the weight of the adductor muscle plus gonads, as derived from the data submitted by France and Ireland (see Annex, Table A6) was 31 and 35 g, respectively.

Based on the available data, an average weight of edible parts per scallop of 33 g was assumed. As mentioned in Section 1.2.7, the ARfD for OA group toxins is 0.3 μg OA eq/kg bw, based on an intake of 50 μg causing effects in humans and an UF of 3. This ARfD is equivalent to an absolute amount of 18 μg OA equivalents for a person of 60 kg bw. This implies that consumption of about three scallops with a level equal to the limit of 160 μg eq/kg, would result in an intake close to the ARfD ($3 \times 33 \times 0.16 = 16$ μg).

For AZA group toxins, the ARfD is 0.2 μg AZA1 eq/kg bw, based on acute effects observed in humans at 113 μg AZA1 equivalents per person and using a UF of 9 (see Section 1.2.7). This ARfD is equivalent to an absolute amount of 12 μg AZA1 equivalents for a person of 60 kg. This implies that

consumption of two scallops with a level equal to the limit of 160 AZA1 $\mu\text{g eq/kg}$, would result in an intake close to the ARfD ($2 \times 33.5 \times 0.16 = 11 \mu\text{g}$).

For the YTX group, the ARfD is 25 $\mu\text{g YTX eq/kg bw}$, based on effects in mice with an NOAEL of 5 mg/kg bw and a UF of 200 (see Section 1.2.7). This ARfD is equivalent to an absolute amount of 1.5 mg for a person of 60 kg. This implies that consumption of 12 scallops with a level equal to the limit of 3.75 mg eq/kg, would result in exceedance of the ARfD ($11 \times 33 \times 0.00375 = 1.5 \text{ mg}$).

These calculations show that especially for toxins of the OA- and AZA group, consumption of only a few scallops exceeding the regulatory limit may result in intakes above the ARfD, thus potentially resulting in acute effects in the consumer. As shown above, uncertainties in the sampling and the measurement, but also the application of measurement uncertainty in the decision on compliance would result in levels that are considerably higher than the regulatory limit and thus an intoxication of consumers.

2.3. Uncertainty analysis

The evaluation of the inherent uncertainties in the present assessment was performed following the principles laid down in the guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018), taking into account the specific nature of this Opinion.

2.3.1. Quality of the obtained data

EFSA took into account only results obtained with the recommended LC/MS reference method or similar methods. Part of those data, however, were not corrected for the recovery. It is expected that this would affect the levels in the non-edible parts more than those in muscle and gonads, since recoveries in the edible parts are in practice lower due to matrix effects. This implies that the observed ratios of levels in edible parts and whole scallops might likely be overestimated when results are not corrected for recovery.

The recovery may also affect the variation (CV) in the levels in individual scallops, used for estimating the number of scallops to be included in a test sample. However, without labelled standards, it is not possible to determine the recovery for individual samples and levels could only be corrected by applying the same recovery for all samples in an analytical series. The latter would result in higher levels, but it is not expected a significant effect on the % CV. Overall, the question is if and how in a larger data set, the % CV would be affected by not applying correction for recovery.

It is noted that not correcting for recovery could result in underestimation of the levels and thus in false compliant results.

2.3.2. Extrapolation to other scallops

EFSA was asked to look at five different scallop species but obtained only suitable data for *Aequipecten opercularis* and *Pecten maximus*, i.e. data on either different parts of the scallops and/or on individual scallops. It remains unclear if the results could be extrapolated to other species, although a Japanese study on another scallop species showed similar results.

2.3.3. Ratios between levels in edible parts and whole scallops

Data obtained on OA group toxins were used to estimate the ratio between edible parts (muscle and/or gonads) and the whole scallops. In most cases, the levels in the muscle and, to a lesser extent, gonads were below the LOQ, and in all of these cases only some of the toxins in the group, levels were above the LOQ. This implies that even for samples with detected levels, LB levels are possibly underestimated. Ratios on measured levels were only available for a limited number of samples and based on only some of the toxins in the group. To overcome this, the LOQs were taken into account, resulting for each sample in a range that covered the lowest (often 0) and highest possible ratio. In general, these ranges were broader for scallops with low levels in the whole scallop, being close to the LOQs. These ratios were modelled and the curve was used to determine a P99 value for the ratio and the level in whole scallops that should ensure that the level in edible products will not exceed the regulatory limit. As a last step in this approach also the uncertainty in the variabilities observed for the data sets was taken into account. This procedure therefore includes the uncertainties of the data to ensure that if other data were collected for this purpose the estimated quantile would likely vary and its variations is determined by the variability observed in the reported data. A potential uncertainty is

the fact that the fitted curve in most cases appeared to underestimate the ratios for the few samples with detected levels for one or more of the toxins within the group.

For *Aequipecten opercularis*, only the muscle is consumed and levels of OA group toxins were all below the LOQ. These could not be used for calculating a ratio, except that it must be lower than 0.037 based on the LOQs, i.e. UB levels in muscle and whole scallop (range 0.008–0.037).

Considering *Pecten maximus*, for OA, levels in gonads and muscles combined, the calculated P99s were 3,258 and 471 µg OA eq/kg, respectively, for the data submitted by France and Ireland. The highest upper bound of the 95% confidence interval for the P99 ratio was then evaluated to account for uncertainties in the data set, being a ratio of 0.47. This corresponds to a critical concentration in whole scallops of 342 µg OA eq/kg. Of the samples with detectable levels in edible parts, one pooled sample showed a ratio of 0.65, being higher than the 0.47.

For OA group toxins in gonads from *Pecten maximus* (pooled samples), a critical concentration of 256 µg OA eq/kg was calculated 95% confidence interval was 256 µg OA eq/kg, corresponding to a ratio of 0.625. Only one pooled sample with detectable levels exceeded this ratio, being 0.95. For the data set submitted by France, gonads of only 10 individual scallops were analysed, resulting in a critical concentration of 268 µg OA eq/kg (ratio of 0.6), considering the limited number of observations available to estimate the distribution, the 95% confidence interval upper bound ratio was not used.

Data on AZA group toxins in *Pecten maximus* were obtained for pooled samples. Levels in muscle were all below the LOQ and could not be modelled. For gonads, a critical concentration of 217 µg AZA1 eq/kg was calculated, based on an upper bound value for the P99 ratio implying a ratio of 0.74. Three samples showed ratios similar or higher than this 0.74, i.e. 0.72, 0.73 and 1.0.

YTX group toxins were detected in individual scallops but not in the muscles. Levels in *Pecten maximus* were far below the regulatory limits, probably because the incidents were actually due to OA group toxins. In fact, levels were close to the LOQs and as a result the ratios based on UB levels were close to 1.

The approach takes into account also the samples with non-detected levels, resulting in P99 values for the ratios and corresponding levels in whole scallops that should ensure that levels in edible parts are below the regulatory limit. Especially for OA group toxins in muscle plus gonad, a comparison between the data submitted by France and Ireland could be made, showing quite a large difference in the calculated critical concentrations, 3,258 vs 471 µg OA eq/kg. For other tissues and toxins, such comparisons were difficult or impossible. It was also noted that in some cases, the ratios obtained for the few detectable levels exceeded those based on the calculated P99 values.

The question remains if these observations can be extrapolated to other contamination cases, other toxins and other scallops.

2.3.4. Variation among individual scallops

EFSA obtained results on OA group toxins in 122 individual *Aequipecten opercularis* scallops from one contaminated area in one specific year. In addition, data on 136 individual *Pecten maximus* scallops from three contaminated areas in one year were obtained. These scallops were sampled during contamination incidents with OA group toxins. In all these scallops also YTX group toxins were detected in the non-edible parts, but levels were considered too low to provide reliable data on the variation between individual scallops around the limit.

The variation in the levels of OA group toxins in individual whole scallops from these four areas was rather similar, being 37, 39, 42 and 50%, the first one being for *Aequipecten opercularis*, the latter three for *Pecten maximus*. However, it is unclear if during routine sampling from a contaminated area scallop samples could be mixed with those from a non-contaminated area, which could potentially result in a much larger variation in the levels found. In addition, the data are not sufficient to conclude whether the variation may be different at other locations and contamination incidents with other types of algae. These uncertainties affect in particular the question on the number of scallops to be included in an analytical sample.

3. Conclusions

Scallops can be contaminated with lipophilic shellfish toxins, such as toxins belonging to the okadaic acid, azaspiracid and yessotoxin groups.

- EFSA received data from several member states, but only for two species, *Aequipecten opercularis* and *Pecten maximus*, were the data suitable to answer the questions in the terms

of reference from the European Commission. These data came from investigations following contamination incidents or increased levels in non-edible parts and are not representative for the average levels of toxins found in scallops. They were provided by only two member states, France and Ireland.

- Compared to the edible parts, which are the adductor muscle and for *Pecten maximus* also the gonad, the major part of the toxins was present in the non-edible parts of the scallops that are removed during shucking.
- Based on data from both individual scallops (submitted by France) and pooled scallops (submitted by Ireland)
 - Toxin levels (OA- and YTX-group toxins, no data on AZA-group toxins) in adductor muscles from *Aequipecten opercularis* were all below the detection limits.
 - In most cases (97% for OA-group toxins, 100% for AZA- and YTX-group toxins), this also applied to adductor muscles of *Pecten maximus*.
- In the case of gonads from *Pecten maximus*, a higher fraction contained detectable levels of toxins (23% and 19% for OA- and AZA-group toxins, respectively; 0% for YTX but levels in whole scallops far below regulatory limit).
- In order to ensure that the level in edible parts does not exceed the regulatory limit, a potential cut-off for whole scallops for *Aequipecten opercularis* can only be based on LOQs of the method, i.e. using UB levels. For OA group toxins, this showed an average ratio of muscle to whole animal of 0.017 (ranging from 0.008 to 0.037), and for YTX group toxins of 0.37 (ranging from 0.21 to 0.56). In the case of YTX, the levels in whole scallops were far below the regulatory limit.
- For OA group toxins in muscle plus gonad, and gonad of *Pecten maximus*, the data allowed a statistical approach and the calculation of a level in whole scallops that should ensure with 99% certainty that levels in edible parts are below the regulatory limit. Based on these calculations, the CONTAM Panel selected the following values for whole scallops:
 - 342 µg OA eq/kg to ensure compliant levels in adductor muscle plus gonad
 - 256 µg OA eq/kg to ensure compliant levels in gonads
- For AZA group toxins, only a value based on levels in gonads could be calculated, being for whole scallops:
 - 217 µg AZA1 eq/kg to ensure compliant levels in gonads.
- The data show that for both OA- and AZA-group toxins, there is, in general, a strong reduction in toxin levels after shucking, but that occasionally levels in gonads are rather similar to those in the whole scallop.
- Only the data on individual scallops obtained from France on contamination incidents in four areas could be used to estimate the number of scallops needed to be analysed to conclude on compliance/non-compliance of a lot/area.
- The analysis showed that to ensure with 95% confidence on whether the level in scallops in an area or lot is correctly predicted to be compliant/non-compliant, 10 scallops per sample would be sufficient if levels of OA-group toxins in the area/lot were 27% below or above the regulatory limit (< 110 µg OA eq/kg or > 210 µg OA eq/kg). However, to predict with a 95% certainty for levels between 140 and 180 µg OA eq/kg, a pooled sample of more than 30 scallops would have to be tested.
- There is a relatively high probability of either over- or underestimating the true level in the batch (lot/area) when too few scallops are included in the pooled sample. Potential error in the estimate is aggravated by application of the measurement uncertainty that results in a cut-off higher than the limit (up to 30%).
- The CONTAM Panel noted that consumption of as few as three or four scallops (depending on species) with a level at the regulatory limit may result in exceedance of the acute reference dose for OA and AZA group toxins.

4. Recommendations

- The CONTAM Panel recommends updating the risk assessments on OA, YTX and AZA group toxins including a re-evaluation of the relative potencies of their different analogues.

- For evaluation of ratios of toxins in different body parts of scallops, more data collected from contamination incidents are needed on the levels of lipophilic shellfish toxins in edible parts vs. whole scallops of various types.

Documentation provided to EFSA

Data on occurrence of lipophilic toxins in scallops were kindly provided by: Dave Clarke of the Shellfish Safety Department of the Marine Institute (Ireland) on 29 May 2020; Marina Nicolas of the National Reference Laboratory for Marine Biotoxins (France) on 29 May 2020; the Food Safety Authority of Ireland (FSAI) (Ireland) on 19 December 2012, 6 November 2013, 10 January 2017, 10 October 2018; Federal Agency for the Safety of the Food Chain (FASFC) (Belgium) on 21 September 2018, 26 November 2019, 4 June 2020; The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on 21 September 2018, 27 November 2019; the National Institute for Public Health and the Environment (RIVM) (the Netherlands) on 29 May 2020; Danish Technical University (DTU) (Denmark) on 28 May 2020; Federal Office of Consumer Protection and Food Safety (BVL) (Germany) on 3 June 2020.

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Abbreviations

AFFSA	French food safety agency
AIC	Akaike information criterion
AM	adductor muscle
ANSES	Agency for Food, Environmental and Occupational Health and Safety
ARfD	Acute Reference Dose Acute Reference Dose
ASP	Amnesic shellfish poison
AZA	azaspiracid
bw	body weight
CA	competent authority
cAMP	cellular adenosine 3',5'-cyclic monophosphate
CONTAM	EFSA Panel on Contaminants in the Food Chain
CDF	Cumulative distribution function
COT	Toxicity of the Food Chain
CPA	classified production area
CV	coefficient of variation
DA	domoic acid
DSP	diarrhoeic shellfish poison
DSTs	Diarrhetic Shellfish Toxins
DTX	dinophysistoxin
EA	entire animal
EFZ	exclusive fishery zone
EP	edible parts
EUMOFA	European Market Observatory for fisheries and aquaculture

EURLMB	European Reference Laboratory for Marine Biotoxins
FAO	Food and Agriculture Organization of the United Nations
FBO	food business operator
FSA	UK Food Standards Agency
G	gonads
HBGV	health-based guidance values
HR-MS	High resolution mass spectra
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
LB	Lower bound
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
LOQ	Limit of quantification
LC-MS/MS	liquid chromatography coupled with tandem mass spectroscopy
LBM	live bivalve molluscs
MS	Member State
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRLMB	The French National Reference Laboratory for Marine Biotoxins
OA	okadaic acid
PP	protein phosphatase
PSP	paralytic shellfish poisoning
PTX	pectenotoxin
SD	Standard deviation
SOP	Standard operation procedure
TDI	tolerable daily intake
TEF	toxicity equivalence factor
ToR	Terms of Reference
UB	Upper bound
UK	United Kingdom
WHO	World Health Organization
WG	working group
WoS	web of science
YTX	yessotoxin

Appendix A – Ratios between body parts and entire animal

A.1. Okadaic acid toxin in scallops – ratio between edible part and entire animal

A.1.1. Introduction

The data submitted by Ireland and France for okadaic acid group toxins (OA) were analysed to study the ratio of the concentration in adductor muscle and gonads (edible parts) and the entire animal. The distribution of the ratios was estimated considering the censored observations and this was used to estimate the threshold for the entire animal in order to ensure that the concentration in the edible part would remain under the legislative limit of 160 µg OA eq/kg.

A.1.2. Ratio between edible and entire animal for okadaic acid

The concentrations reported for adductor muscle (AM) and gonads (G) were summed in order to obtain the concentration in the edible part of the scallop to be used in the numerator of the ratio. The weights of the edible parts were used to obtain the concentration, in the same way as it was used to reconstruct the entire animal concentration (Conc[EA]). The concentration of the edible part Conc[EP] is reconstructed as follows:

$$\text{Conc[EP]} = \text{Conc[AM]} \times \text{Weight[AM]} + \text{Conc[G]} \times \text{Weight[G]}$$

where Conc refers to concentration and Weight to the weight of the animal part divided by the weight of the edible parts of the animal (muscle plus gonad). The resulting concentrations of the edible part of the animal and the entire animal were in all cases censored for one or more derivatives (< LOD, or between LOD and LOQ). The concentration of OA toxin in the edible part was divided by the concentration in the entire animal. The ratio is then defined as:

$$\text{Ratio[OA]} = \frac{\text{Conc[EP]}}{\text{Conc[EA]}}$$

The LB for the numerator (concentration of edible parts) and denominator (concentration of the entire animal) in the ratio defined the LB of the ratio $\left(\text{Ratio[OA]}_{\text{LB}} = \frac{\text{Conc[EP]}_{\text{LB}}}{\text{Conc[EA]}_{\text{LB}}}\right)$, while the UB levels for the numerator and the denominator were used to define the ratio's UB $\left(\text{Ratio[OA]}_{\text{UB}} = \frac{\text{Conc[EP]}_{\text{UB}}}{\text{Conc[EA]}_{\text{UB}}}\right)$. In the case that the denominator is zero, then a value smaller than the minimum concentration value for the LB different from zero is used in order to avoid an infinite UB ratio. The final data used is a censored interval defined by a lower and upper bound, given that the OA group concentration is a composite measurement of okadaic acid and its derivatives. It is clear that the resulting sum would in general generate censored observations, unless for the specific scallop part measurements for all derivatives were quantifiable. For Ireland, the samples used were observations for pooled scallops.

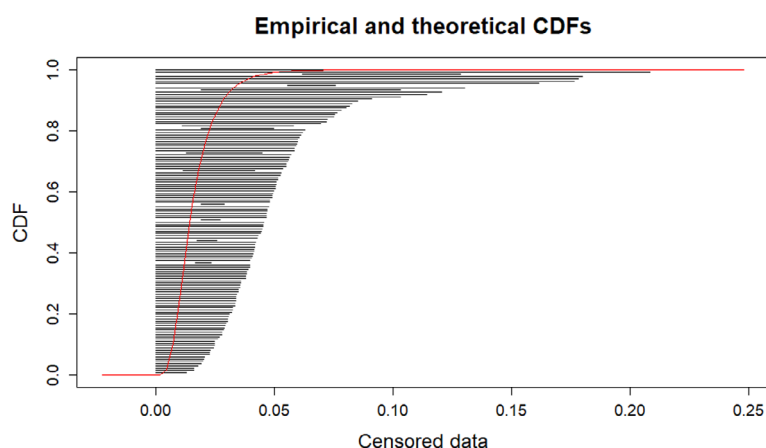
A.1.2.1. Data set submitted by France

The data on 136 individual *Pecten maximus* scallops submitted by France for one sampling year were analysed considering that it provided a good representation in terms of variation. Previous analysis showed that the median and mean ratios are similar to the ones in *Aequipecten opercularis* scallops collected in 2016. Considering that only non-quantifiable results were reported for that year, the data reported for the first sampling year were used in the analysis. Figure A1 shows the range of ratios for each sample. The data were subsequently fitted using several distributions and the best fitting distribution based on the Akaike information criterion (AIC) (the smaller the better the fit) was selected and the characteristics on the curve fit are presented below. Figure A.1 shows the distribution of the ratios between the levels in muscle plus gonad and whole scallop for individual scallops from France, for each sample ranging between the ratio based on LB levels and those based on UB levels.

```

## [1] "The AIC value for the log-normal fit is: 62.8"
## [1] "The AIC value for the exponential fit is: 65.6"
## [1] "The AIC value for the gamma fit is: 65.8"
## [1] "The AIC value for the normal fit is: 65.8"
## Fitting of the distribution 'lnorm' By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## meanlog -4.255585 0.162867
## sdlog 0.533900 0.105462
## Loglikelihood: -29.40758 AIC: 62.81515 BIC: 68.64046
## Correlation matrix:
## meanlog sdlog
## meanlog 1.0000000 -0.7769354
## sdlog -0.7769354 1.0000000

```



CDF: cumulative distribution function.

Figure A.1: Distribution of the ratios between the levels in muscle plus gonad and whole scallop for individual scallops from France, for each sample ranging between the ratio based on LB levels and those based on UB levels

Samples with detected levels for at least one toxin in the edible parts show ratios with LBs larger than zero and in general a small interval. The ratios ranged between 0 and 0.23. The mean ratio for the data submitted by France on *Pecten maximus* is 0.0164 and the median is 0.0142.

A.1.2.2. Data set submitted by Ireland

The data submitted by Ireland on *Pecten maximus* for the years 2013, 2014, 2015, 2016 and 2018 were analysed as a whole in order to have enough information to estimate the parameters and to select the best fitting distribution to the censored data. Figure 7C in Section 2.1.4 of the main text of the opinion shows the LBs for the calculated concentrations of edible parts and the entire animal.

The range of ratios (LB to UB) was determined for each sample and subsequently several distributions were fitted to the data in order to select the best fitting distribution (smallest AIC) following the observed data. Figure A.2 shows the distribution of the ratios between the levels in muscle plus gonad and whole scallop for pooled scallops submitted by Ireland, for each sample ranging between the ratio based on LB levels and those based on UB levels.

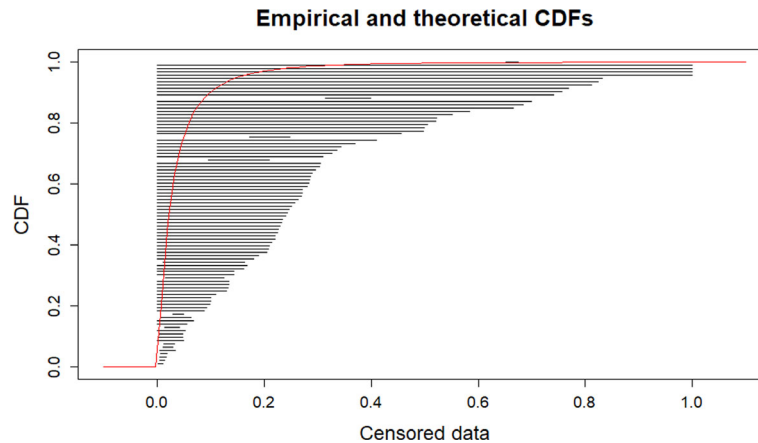
```

## [1] "The AIC value for the log-normal fit is: 76.4"
## [1] "The AIC value for the exponential fit is: 91.1"
## [1] "The AIC value for the gamma fit is: 88.1"
## [1] "The AIC value for the weibull fit is: 84.1"
## [1] "The AIC value for the normal fit is: 188.4"
## [1] "The AIC value for the logistic fit is: 156.1"
## Fitting of the distribution 'lnorm' By maximum likelihood on censored data
## Parameters

```



```
## estimate Std. Error
## meanlog -3.841822 0.2793856
## sdlog 1.187572 0.1700162
## Loglikelihood: -36.19097 AIC: 76.38195 BIC: 81.44714
## Correlation matrix:
## meanlog sdlog
## meanlog 1.0000000 -0.5150377
## sdlog -0.5150377 1.0000000
```



CDF: cumulative distribution function.

Figure A.2: Distribution of the ratios between the levels in muscle plus gonad and whole scallop for pooled scallops submitted by Ireland, for each sample ranging between the ratio based on LB levels and those based on UB levels

The 18 samples with detected levels for at least one toxin in one of the edible parts show ratios with LBs larger than zero and in general a smaller interval. The range of ratios varied between 0 and 0.8. The mean ratio for the data submitted by Ireland is 0.0434 and the median is 0.0215.

A.1.3. Distribution of ratios based on the data submitted by France and Ireland

In order to compare the estimated distributions for both data sets, the resulting estimated parameters are used to generate artificial data in order to visualise the difference between both countries. The expected scatter considering the estimated distributions is shown below (Figure A.3).

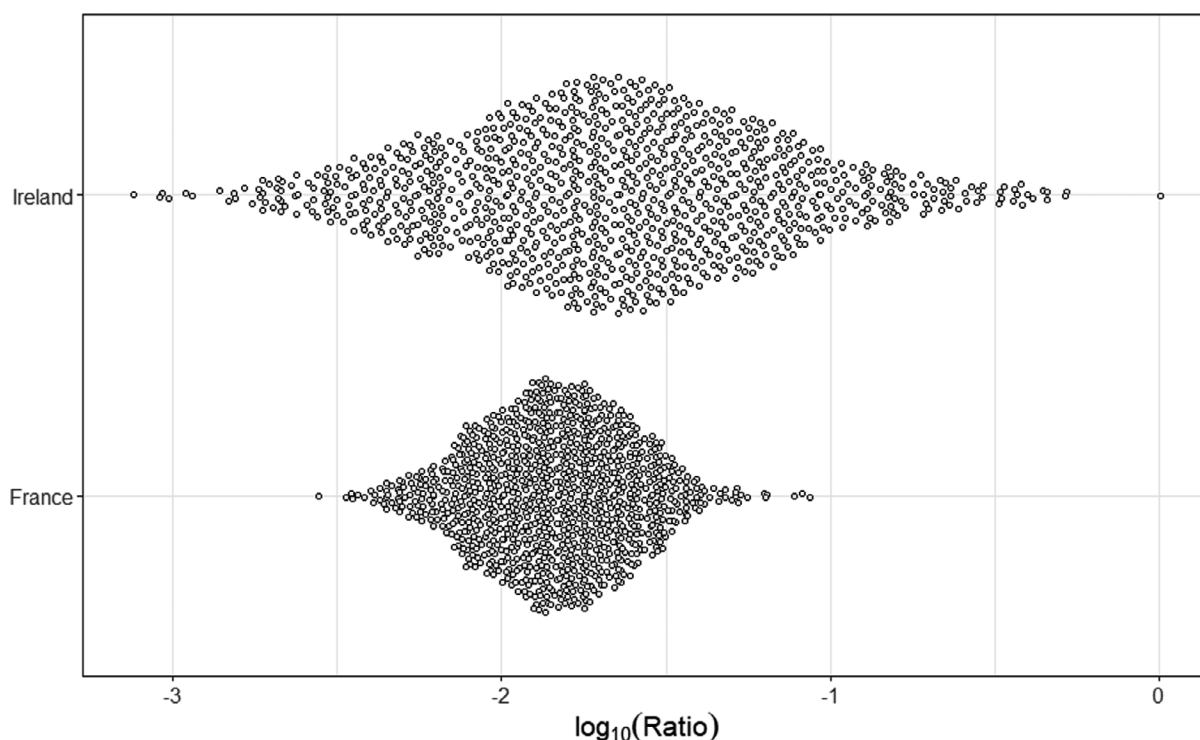


Figure A.3: Distribution of the ratios for the data submitted by France and Ireland

The resulting fitted distributions are compared in Figure A.3, showing that the 2014 French samples have smaller mean and median ratios of concentrations than those obtained from the data submitted by Ireland in the period between 2013 and 2018. The data reported by Ireland show larger UB values for the censored intervals than those for the data reported by France, but the best-fitting distribution shows the impact of not having accurately measured ratios to drive the estimation process. Considering that all ratios for both data sets are to some extent censored, the results should be interpreted with caution. It should be highlighted how close the estimated central tendencies of the distributions are, while the precision difference is clearly evidenced in this plot (the scatter of points represent the variation estimated from the data, which is clearly showing more variation in the data submitted by Ireland than the one observed in the data submitted by France). The ratio of the medians for the data submitted by Ireland compared to those by France is 1.5, which is clearly indicating how close the central tendencies of both distributions are.

A.1.4. Estimation of the limit concentration for the whole scallop

The estimated distribution of the ratio was then used to estimate what would be the concentration in the whole scallop that would correspond to the limit of 160 μg OA eq/kg in the edible part. Taking the data submitted by Ireland and the 99th, 95th or 50th percentiles of the ratios (fail-safe hypothesis) and fixing the concentration in the edible parts to be equal to the limit (160 OA eq/kg), the maximum concentration in the whole scallop would be:

Data set	Median	P95	P99
France	11,280	4,687	3,258
Ireland	7,458	1,057	471

A.1.5. Determination of threshold to be used in the sampling plan

The estimation of the maximum concentration in the whole scallop is based on limited information from the two data sets (submitted by France and Ireland). Thus, the uncertainties around the 99th quantile values for the entire animal of 3,258/471 μg OA eq/kg need to be considered. This ensures that if a different data set would be used, the resulting fitted distribution and the corresponding 99th

quantile value would also differ. Therefore, the variability of the estimated distribution was used to construct the 95% confidence interval for the 99th quantile and the LB was used as a precautionary measure to ensure compliance of the edible parts.

The expected LB of toxin concentration considering the data reported by France for the whole scallop would be 3,134 µg OA eq/kg. The expected LB of toxin concentration considering the data reported by Ireland for the whole scallop would be 342 µg OA eq/kg.

It follows that the maximum concentration in the entire animal should not exceed 342 µg OA eq/kg in order to reach a 99% certainty that the concentration of the edible parts will not exceed the regulatory limit of 160 µg OA eq/kg.

A.2. Okadaic acid toxin in scallops – ratio between gonads and entire animal

A.2.1. Introduction

The data submitted by Ireland and France for okadaic acid group toxins (OA) were analysed to study the ratio of the concentration in gonads and the entire animal. The distribution of the ratio was estimated considering the censored observations and this was used to estimate the threshold for the entire animal in order to ensure that the concentration in the edible part would remain under the legislative limit of 160 µg OA eq/kg.

A.2.2. Ratio between gonads and entire animal for okadaic acid

The concentrations reported for gonads (G) was used in the numerator of the ratio and they were divided by the concentration in the entire animal. The ratio is then defined as:

$$\text{Ratio[OA]} = \frac{\text{Conc[G]}}{\text{Conc[EA]}}$$

The LB for the numerator (concentration of G) and denominator (concentration of the entire animal) in the ratio defining the LB of the ratio, while the UBs for the numerator and the denominator were used to define the ratio's UB. In case that the denominator was zero, a value smaller than the minimum concentration value for the LB different from zero was used in order to avoid having an infinitely small UB.

For Ireland, the samples used are pooled observations of scallops. The final data used were interval censored defined by a lower and upper bound, given that the OA group concentration is a composite measurement of OA group toxins which are in general not all detected. It is clear that the resulting sum would in general generate censored observations, unless for the specific scallop part all measurements were quantifiable.

A.2.2.1. Data set submitted by France

The 10 data points for gonads submitted by France on *Pecten maximus* are only from the sampling year 2014 and were used to estimate the distribution of the ratios. Subsequently the data were fitted using several distributions, where the exponential distribution produced the best fit (smallest AIC) and it is presented in Figure A.4.

```
## [1] "The AIC value for the log-normal fit is: 52.4"
## [1] "The AIC value for the exponential fit is: 49.2"
## [1] "The AIC value for the gamma fit is: 51.1"
## [1] "The AIC value for the weibull fit is: 51"
## [1] "The AIC value for the normal fit is: 55.2"
## [1] "The AIC value for the logistic fit is: 55.1"
## Fitting of the distribution 'exp' By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## rate 7.70827 2.450979
## Loglikelihood: -23.58782 AIC: 49.17563 BIC: 49.47822
```

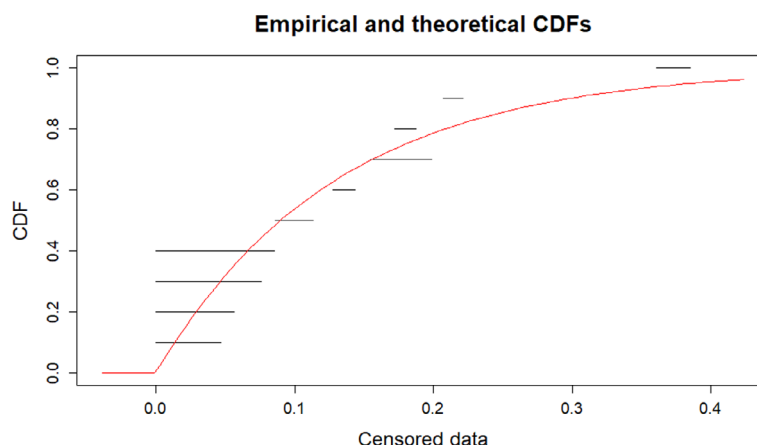


Figure A.4: Distribution of the ratios between the LB and UB based levels in gonads and whole scallop for 10 individual scallops from France. CDF: cumulative distribution function

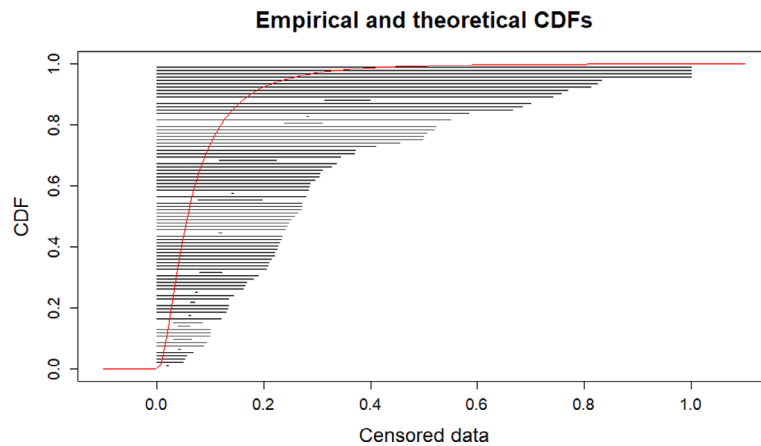
The ratios ranged between 0 and 0.38. The mean ratio for the data submitted by France is 0.13 and the median 0.09. It is clear that although the information on gonads is limited, the estimation for the ratio considering this specific edible part can be better estimated due to the fact that although information is censored for all observations, the data clearly indicate a pattern. The resulting distribution should be interpreted with caution, considering that only 10 observations were used.

A.2.2.2. Data set submitted by Ireland

The data on *Pecten maximus* submitted by Ireland for the years 2013–2016 and 2018 were analysed as a whole in order to have enough information to estimate the parameters and to select the best fitting distribution to the censored data. Figure 7B in Section 2.1.4 of the main text of the opinion shows the LBs for the concentrations of gonads and the entire animal.

The range of ratios (LB to UB) was determined for each sample and subsequently several distributions were fitted, resulting the log-normal distribution the best fitting distribution (see Figure A.5).

```
## [1] "The AIC value for the log-normal fit is: 136.1"
## [1] "The AIC value for the exponential fit is: 143.4"
## [1] "The AIC value for the gamma fit is: 144.7"
## [1] "The AIC value for the weibull fit is: 143"
## [1] "The AIC value for the normal fit is: 239"
## [1] "The AIC value for the logistic fit is: 192.7"
## Fitting of the distribution 'lnorm' By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## meanlog -2.8711304 0.1962781
## sdlog 0.8838297 0.1327842
## Loglikelihood: -66.02636 AIC: 136.0527 BIC: 141.0963
## Correlation matrix:
## meanlog sdlog
## meanlog 1.0000000 -0.5997536
## sdlog -0.5997536 1.0000000
```



CDF: cumulative distribution function.

Figure A.5: Distribution of the ratios between the levels in gonads and whole scallop for pooled scallops submitted by Ireland

The intervals in Figure A.5 show the LB and UB ratios for each sample, being in general much smaller for gonad samples where one or more toxins were detected. The range of ratios was between 0 and 1. The mean ratio for the data submitted by Ireland is 0.0837 and the median is 0.0566.

A.2.3. Distribution of ratios for data submitted by France and Ireland

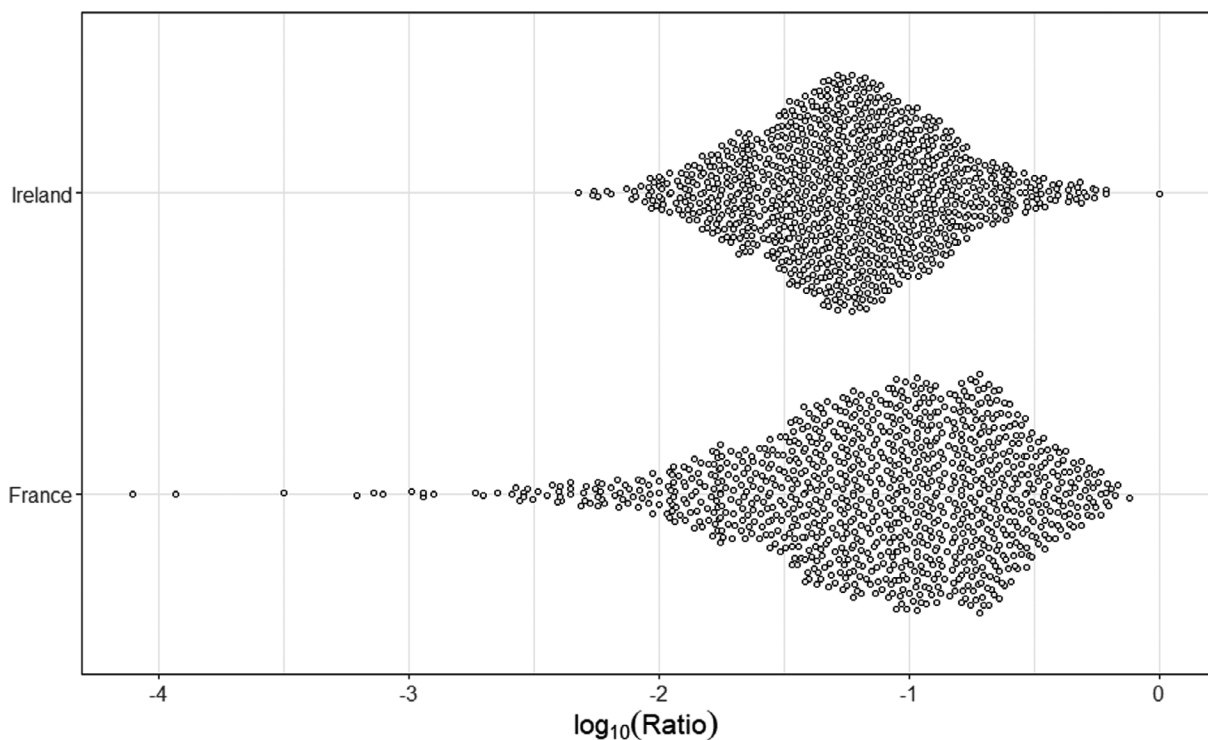


Figure A.6: Distribution of the ratios for the data from France and Ireland

The resulting fitted distributions are compared in Figure A.6, showing that the French samples have larger mean and median ratios for the concentrations than those obtained from the data submitted by Ireland. The data reported by Ireland show larger UB values for the censored intervals than those observed for the data reported by France, but the best fitting distribution shows the impact of not having accurately measured ratios and the limited amount of data to drive the estimation process. Considering that all ratios for both data sets are censored, the results should be interpreted with

caution. The ratio of the medians of the data submitted by France as compared to those from Ireland is 1.6, which is clearly indicating the differences between the central tendencies of both distributions.

A.2.4. Estimation of the limit concentration in whole scallops

The estimated distribution of the ratio was then used to estimate what should be the maximum concentration in the whole scallop that would ensure compliance with the limit of 160 µg/kg in the edible part. Taking the data set submitted by France and Ireland and the 99th, 95th or 50th percentiles of the ratios (fail-safe hypothesis) and fixing the concentration in the edible parts to be equal to the limit (160), the maximum concentration in the whole scallop would be:

Data set	Median	P95	P99
France	1,779	412	268
Ireland	2,825	660	362

A.2.5. Determination of threshold to be used in the monitoring of whole scallops

The estimation of the maximum concentration in the whole scallop is based on limited information from the two data sets (submitted by France and Ireland). Thus, the uncertainties around the 99th quantile values for the entire animal of 268/362 µg OA eq/kg need to be considered. This ensures that if a different data set would be used the resulting fitted distribution and the corresponding 99th quantile value would also differ. Therefore, the variability of the estimated distribution was used to construct the 95% confidence interval for the 99th quantile and the LB was then used as a precautionary measure to ensure compliance of the edible parts.

The expected LB of toxin concentration considering the data reported by France for the whole scallop would be 165.4 µg OA eq/kg. The expected LB of toxin concentration considering the data reported by Ireland for the whole scallop would be 256 µg OA eq/kg.

It follows that the maximum concentration in the entire animal should not exceed 165 µg OA eq/kg in order to reach a 99% certainty that the concentration of the edible parts is not exceeding the threshold of 160 µg/kg.

A.3. Azaspiracid toxin in scallops – ratio between gonad and entire animal

A.3.1. Introduction

The data submitted by Ireland and France for azaspiracid group toxins (AZA) were analysed to study the ratio of the concentration in gonads and the entire animal. The distribution of the ratios was estimated considering the censored observations and this was used to estimate the threshold for the entire animal in order to ensure that the concentration in the gonad, as one of the edible part, remains under the regulatory limit of 160 µg AZA1 eq/kg.

A.3.2. Ratio between gonad and entire animal for AZA

The concentrations reported for gonads (G) was used in the numerator of the ratio and they were divided by the concentration calculated for the entire animal. The ratio is then defined as:

$$\text{Ratio}[AZ] = \frac{\text{Conc}[G]}{\text{Conc}[EA]}$$

The LB for the numerator (concentration of G) and denominator (concentration of the entire animal) defined the LB of the ratio, while the UB for the numerator and the denominator was used to define the ratio's UB. In the case that the denominator was zero, a value smaller than the minimum concentration for the LB different from zero was used in order to avoid having an infinitely small UB ratio.

For Ireland, the data were obtained for pooled samples of scallops (n = 12). The final data used were interval censored defined by a lower and upper bound, given that the AZA group concentration is

a composite measurement of AZA1 and its derivatives. The resulting sum would in general generate censored observations, unless for the specific scallop parts all measurements on individual toxins were quantifiable.

A.3.2.1. Data set submitted by France

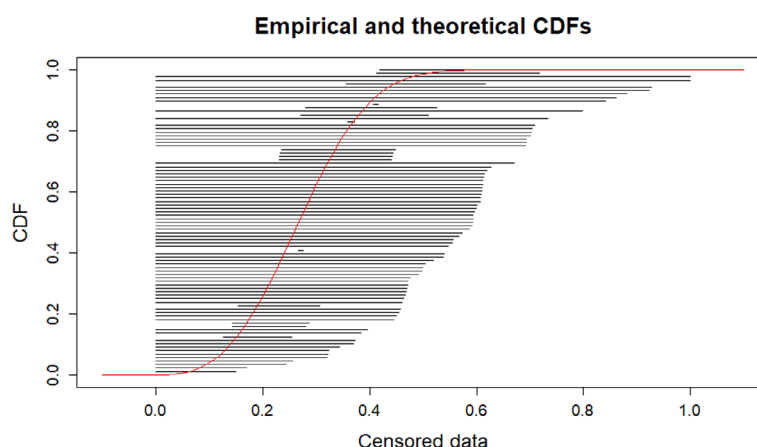
No data on AZA were submitted by France.

A.3.2.2. Data set submitted by Ireland

The data on *Pecten maximus* submitted by Ireland for the years 2013–2016 and 2018 were analysed as a whole in order to have enough information to estimate the parameters and to select the best fitting distribution to the censored data. Figure 7E in Section 2.1.4 of the main text of the opinion shows the LBs for the measured and calculated concentrations, respectively, of gonads and the entire animal.

The range of ratios (LB to UB) were determined for each sample (Figure A.7) and subsequently several distributions were fitted where the Weibull distribution resulted to be the best fitting distribution (smallest AIC).

```
## [1] "The AIC value for the log-normal fit is: 72.4"
## [1] "The AIC value for the exponential fit is: 88.3"
## [1] "The AIC value for the gamma fit is: 71.1"
## [1] "The AIC value for the weibull fit is: 69.8"
## [1] "The AIC value for the normal fit is: 70.6"
## [1] "The AIC value for the logistic fit is: 72.2"
## Fitting of the distribution 'weibull' By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## shape 2.9003931 0.59978084
## scale 0.3027778 0.02402845
## Loglikelihood: -32.91325 AIC: 69.82651 BIC: 74.78118
## Correlation matrix:
## shape scale
## shape 1.0000000 0.5688935
## scale 0.5688935 1.0000000
```



CDF: cumulative distribution function.

Figure A.7: Distribution of the ratios between the levels in gonad and whole scallop for pooled scallops submitted by Ireland, for each sample ranging between the ratio based on LB levels and those based on UB levels

Samples with detected levels for at least one toxin in the gonads show ratios with LBs larger than zero and in general a small interval. The range of ratios varied between 0 and 1.0. The mean ratio for the data submitted by Ireland is 0.27 and the median is 0.27.

A.3.3. Distribution of estimated ratios for the data submitted by Ireland

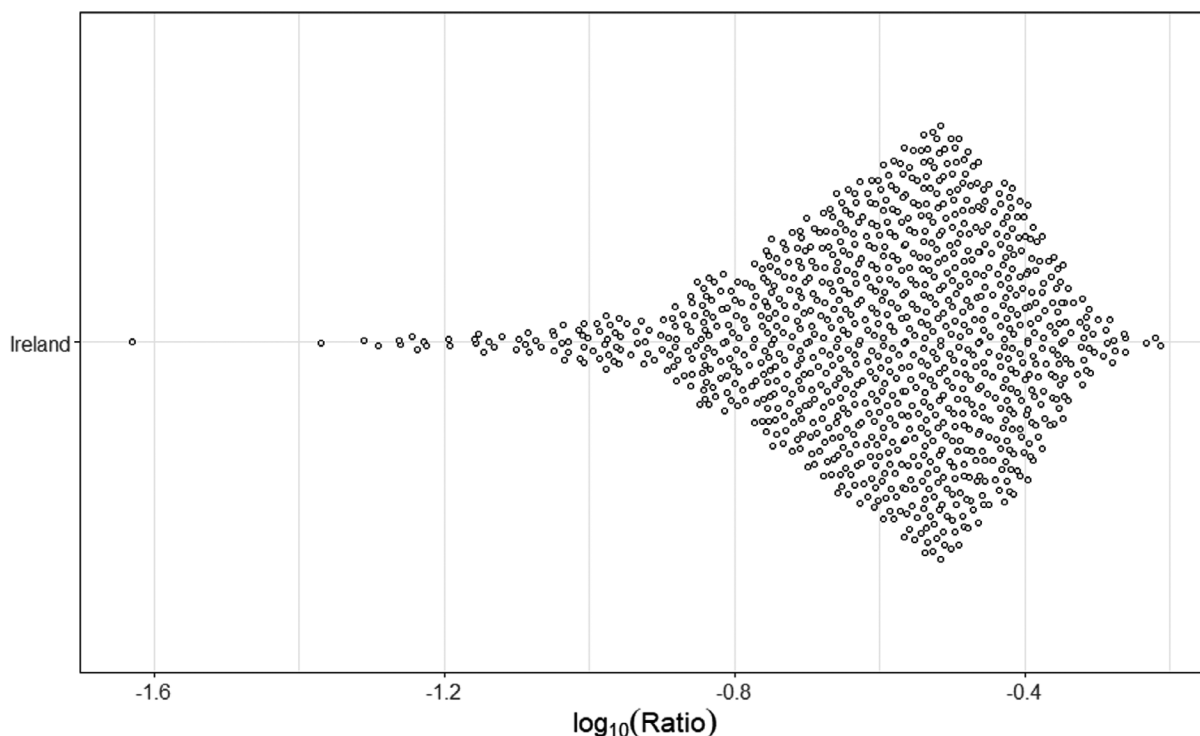


Figure A.8: Distribution of the ratios for levels in gonads and whole scallop based on the data submitted by Ireland

A.3.4. Estimation of the limit concentration for the whole scallop

The estimated distribution of the ratio was then used to estimate what should be the maximum concentration in the whole scallop that would correspond to a level equal to the limit of 160 μg AZA1 eq/kg in the gonads. Taking the data set submitted by Ireland and the 99th, 95th or 50th percentiles of the ratios (fail-safe hypothesis) and fixing the concentration in the edible parts to be equal to the limit (160 AZA1 eq/kg), the maximum concentration to respect in the whole scallop should be:

Data set	Median	P95	P99
Ireland	599.6	362	312.1

A.3.5. Determination of threshold to be used in the monitoring of whole scallops

The estimation of the maximum concentration in the whole scallop is based on limited information from the data set submitted by Ireland. Thus, uncertainties around the 99th quantile values for the entire animal of 312.1 μg AZA1 eq/kg need to be considered. This ensures that if a different data set would be used the resulting fitted distribution and the corresponding 99th quantile value would also differ. Therefore, the variability of the estimated Weibull distribution is used to construct the 95% confidence interval for the 99th quantile and the LB is then used as a precautionary measure to ensure compliance in the edible parts.

The expected LB of toxin concentration considering the data reported by Ireland for the whole scallop would be 217 μg AZA1 eq/kg.

We can conclude that the maximum concentration in the entire animal should not exceed 217 μg AZA1 eq/kg in order to reach a 99% certainty that the concentration of the edible parts is not exceeding the regulatory limit of 160 μg AZA1 eq/kg.

Appendix B – Sample size needed for the estimation of mean toxin concentration in scallops: Statistical considerations

B.1. Data exploration

In order to establish the precision that could be achieved for several sample sizes (number of scallops per analytical sample, varying from 3 to 20), the standard deviations for the OA group for the individual samples submitted by France for the four different areas were estimated (Table B.1). In areas 8, 9 and 15, this related to *Pecten maximus*, in area 17 to *Aequipecten opercularis*. In these samples, the levels in the whole scallop were calculated from the weights and levels in the analysed parts, as described in the main text.

Table B1: Number of animals and concentrations of toxins in French sampling areas

AREA	n	meanTTMB	sdTTMB	maxTTMB
Area 8	42	82.6	35.0	166.8
Area 9	50	97.2	37.5	202.3
Area 15	44	167.6	83.7	384.0
Area 17	122	498.6	183.1	939.9

sd: standard deviation; max: maximum; TTMB: total tissue concentration at middle bound.

There are clear differences between the areas in terms of mean and variation, but the differences between the three ways to deal with left censored data can be considered negligible, in the rest of the report, the approach that uses the mean imputation for left censored observations will be considered.

B.1.1. Methodology

The aim of this document is to present the sample size needed to achieve certain precision considering the data to be normally distributed or log-normally distributed, using the confidence interval calculation for the mean considering both assumptions.

B.2.2. Normal distribution

In this section, the approach proposed by Matsushima et al. (2018a,b) to estimate the expected relative deviations from the sample mean is presented. Assuming that y is the concentration of a toxin in scallops and that it is normally distributed with parameters μ and σ^2 representing the mean and variance. Thus,

$$y \sim \text{Normal}(\mu, \sigma^2).$$

Then, the $1 - \alpha$ confidence interval for the mean of y denoted as \bar{y} would be:

$$\bar{y} - Z_{1-\frac{\alpha}{2}} \times \sqrt{\text{VAR}(\bar{y})} \leq \mu \leq \bar{y} + Z_{1-\frac{\alpha}{2}} \times \sqrt{\text{VAR}(\bar{y})}.$$

Which can be written in function of σ as follow:

$$\bar{y} - Z_{1-\frac{\alpha}{2}} \times \sqrt{\frac{\sigma^2}{n}} \leq \mu \leq \bar{y} + Z_{1-\frac{\alpha}{2}} \times \sqrt{\frac{\sigma^2}{n}}.$$

Considering that σ is unknown, the $Z_{1-\frac{\alpha}{2}}$ can be replaced by $t_{1-\frac{\alpha}{2}}(v)$, representing the quantile for the student distribution with v being the degrees of freedom ($n - 1$) and σ by its estimate (s) as follows:

$$\bar{y} - t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{n}} \leq \mu \leq \bar{y} + t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{n}}$$

The estimated precision of the mean would be depending on:

$$t_{1-\frac{\alpha}{2}}(v) \times \frac{s}{\sqrt{n}}$$

Meaning that for a given sample size, the precision is defined by the estimated standard deviation, the number of samples used and the value of the quantile of the student distribution. In order to determine the expected percentage relative deviations from the sample mean, the following equation could be used:

$$\frac{100 \times t_{1-\frac{\alpha}{2}}(v) \times \frac{s}{\sqrt{n}}}{\bar{y}}$$

B.2.3. Log-normal distribution

The approach proposed by Matsushima et al. (2018a,b) is used considering that the concentration of toxins follows log-normal distribution instead of the distribution assumed by the authors (normal). The method considers the precision of the estimation of the mean concentration as the key element to define the number of scallops needed to classify an area as compliant or not compliant. The approach also considers the variation observed in the four French sampling areas. The method uses the confidence bounds (lower and upper bounds of the 95th confidence interval) to identify the number of scallops needed to conclude with 95% confidence that the area/batch is compliant or not, depending on the mean value observed and the variations shown in the four areas.

In this section, the approach proposed by El-Shaarawi and Lin (2007) will be presented and used to estimate the confidence interval for a log-normal random variable. Assuming that y is the concentration of a toxin in scallops and that it is log-normally distributed with parameters μ and σ^2 representing the mean and variance. Thus,

$$y \sim \log - \text{Normal}(\mu, \sigma^2).$$

The mean of y is $M = E(y) = e^{\mu + \frac{\sigma^2}{2}}$ and an estimate would be $\hat{M} = e^{\bar{x} + \frac{s^2}{2}}$, where \bar{x} and s are the sample mean and variance of the log transformed data.

Then, the $1 - \alpha$ confidence interval for the log of the mean of $\log(\hat{M})$ would be:

$$\bar{x} + \frac{s^2}{2} - t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{2} + \frac{s^4}{2(n-1)}} \leq \log(M) \leq \bar{x} + \frac{s^2}{2} + t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{2} + \frac{s^4}{2(n-1)}}$$

The estimation precision of the log of the mean would be depending on:

$$t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{2} + \frac{s^4}{2(n-1)}}$$

In order to obtain a confidence interval for the mean instead of the log of the mean, then a back transformation needs to be applied meaning that the expression would be:

$$\hat{M} \times e^{-t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{2} + \frac{s^4}{2(n-1)}}} \leq M \leq \hat{M} \times e^{t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{2} + \frac{s^4}{2(n-1)}}}$$

Meaning that for a given sample size the precision is defined by the estimated standard deviation, the number of samples used and the value of the quantile of the student distribution. It could also be interpreted as the factor inflating the estimated mean, indicating the percentage that could go below and above the estimated mean, in this specific situation, it would be:

$$LB = 100 \times (1 - e^{-t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{2} + \frac{s^4}{2(n-1)}}}),$$

$$UB = 100 \times (e^{t_{1-\frac{\alpha}{2}}(v) \times \sqrt{\frac{s^2}{2} + \frac{s^4}{2(n-1)}}} - 1).$$

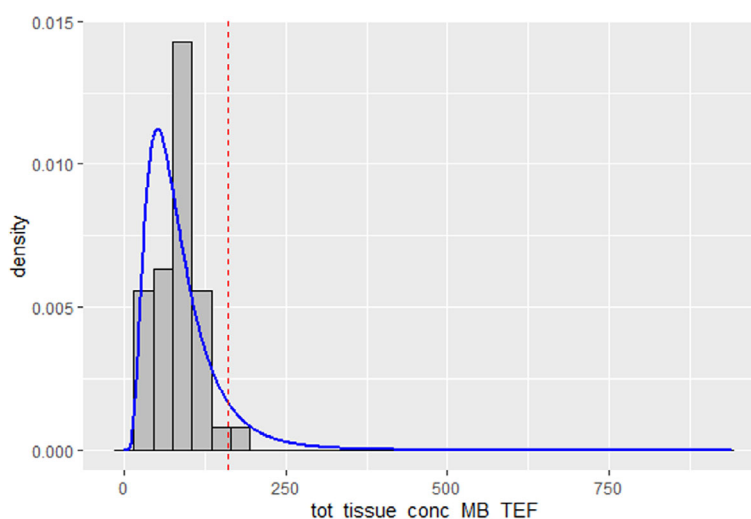
B.2.4. Results

B.2.4.1. Log-normal distribution

In order to estimate the percentage relative deviation for the log-normal case, the data for the four French sampling areas were used to estimate the log-normal distribution parameters and the estimated parameters were used to derive the relative deviations. The results for each area are presented in the subsequent sections.

B.2.4.1.1. Area 8: Log-normal

The estimated log mean when fitting the log-normal distribution to the observed data is 4.282 and the estimated log standard deviations is 0.581.



MB: middle bound; TEF: toxicity equivalence factor.

Figure B.1: Log-normal distribution of the MB levels in whole scallops in area 8

Table B.2: The precision achieved for Area 8

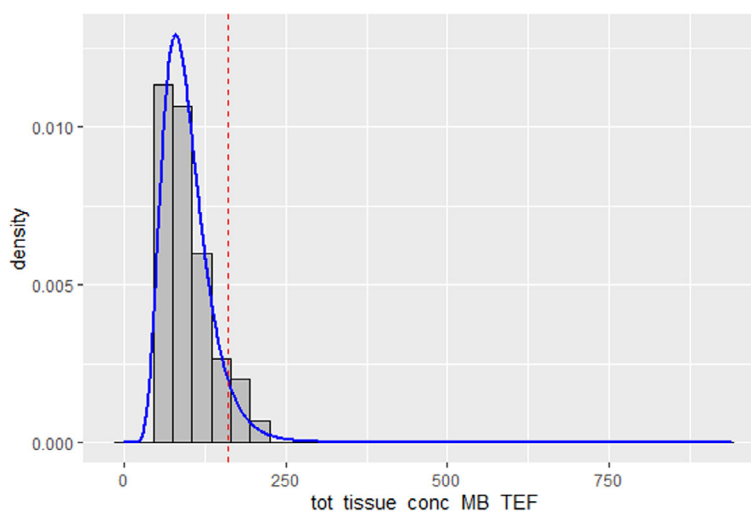
Sample size	t(0.05)	Deflation lower	Inflation upper	Mean	Mean lower bound	Mean upper bound
3	4.3	80.1	402.5	85.69	17.05	430.6
4	3.18	64.03	178	85.69	30.82	238.2
5	2.78	54.76	121.1	85.69	38.76	189.4
6	2.57	48.73	95.05	85.69	43.93	167.2
7	2.45	44.42	79.93	85.69	47.62	154.2
8	2.36	41.15	69.91	85.69	50.43	145.6
9	2.31	38.54	62.71	85.69	52.67	139.4
10	2.26	36.4	57.24	85.69	54.5	134.7
11	2.23	34.6	52.91	85.69	56.04	131
12	2.2	33.06	49.39	85.69	57.36	128
13	2.18	31.72	46.46	85.69	58.51	125.5
14	2.16	30.54	43.96	85.69	59.52	123.4

Sample size	t(0.05)	Deflation lower	Inflation upper	Mean	Mean lower bound	Mean upper bound
15	2.14	29.49	41.82	85.69	60.42	121.5
16	2.13	28.54	39.94	85.69	61.23	119.9
17	2.12	27.69	38.29	85.69	61.97	118.5
18	2.11	26.91	36.81	85.69	62.63	117.2
19	2.1	26.19	35.49	85.69	63.25	116.1
20	2.09	25.54	34.29	85.69	63.81	115.1
21	2.09	24.93	33.21	85.69	64.33	114.2
22	2.08	24.36	32.21	85.69	64.81	113.3
23	2.07	23.84	31.3	85.69	65.27	112.5
24	2.07	23.35	30.46	85.69	65.69	111.8
25	2.06	22.88	29.67	85.69	66.08	111.1
26	2.06	22.45	28.95	85.69	66.45	110.5
27	2.06	22.04	28.27	85.69	66.81	109.9
28	2.05	21.65	27.64	85.69	67.14	109.4
29	2.05	21.29	27.04	85.69	67.45	108.9
30	2.05	20.94	26.48	85.69	67.75	108.4

t: Student distribution.

B.2.4.1.2. Area 9: Log-normal

The estimated log mean when fitting the log-normal distribution to the observed data is 4.509 and the estimated log standard deviations is 0.362.



MB: middle bound; TEF: toxicity equivalence factor.

Figure B.2: Log-normal distribution of the MB levels in whole scallops in area 9

Table B.3: The precision achieved for Area 9

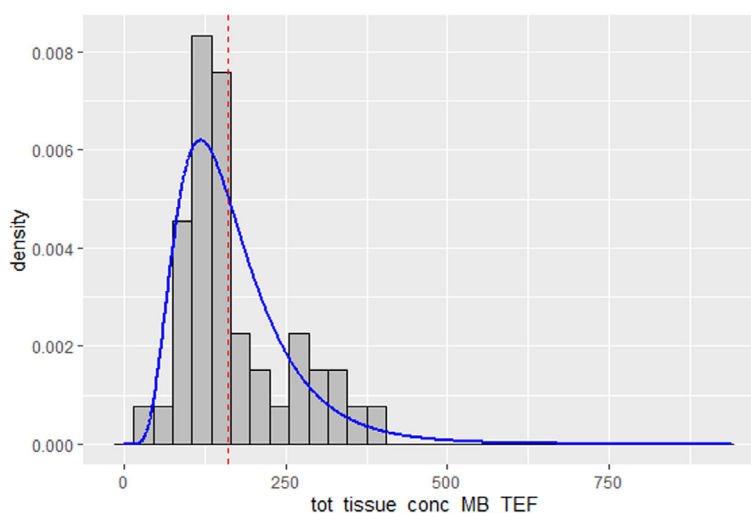
Sample size	t(0.05)	Deflation lower	Inflation upper	Mean	Mean lower bound	Mean upper bound
3	4.3	61.07	156.9	97.04	37.77	249.3
4	3.18	45.19	82.45	97.04	53.18	177.1
5	2.78	37.38	59.69	97.04	60.77	155
6	2.57	32.63	48.44	97.04	65.37	144
7	2.45	29.37	41.59	97.04	68.54	137.4

Sample size	t(0.05)	Deflation lower	Inflation upper	Mean	Mean lower bound	Mean upper bound
8	2.36	26.96	36.9	97.04	70.88	132.8
9	2.31	25.07	33.46	97.04	72.71	129.5
10	2.26	23.55	30.8	97.04	74.19	126.9
11	2.23	22.28	28.67	97.04	75.42	124.9
12	2.2	21.21	26.91	97.04	76.46	123.2
13	2.18	20.28	25.44	97.04	77.36	121.7
14	2.16	19.47	24.17	97.04	78.15	120.5
15	2.14	18.75	23.07	97.04	78.84	119.4
16	2.13	18.11	22.11	97.04	79.47	118.5
17	2.12	17.53	21.25	97.04	80.03	117.7
18	2.11	17	20.49	97.04	80.54	116.9
19	2.1	16.52	19.79	97.04	81	116.2
20	2.09	16.08	19.17	97.04	81.43	115.6
21	2.09	15.68	18.59	97.04	81.82	115.1
22	2.08	15.3	18.07	97.04	82.19	114.6
23	2.07	14.96	17.58	97.04	82.53	114.1
24	2.07	14.63	17.14	97.04	82.84	113.7
25	2.06	14.33	16.72	97.04	83.14	113.3
26	2.06	14.04	16.33	97.04	83.41	112.9
27	2.06	13.77	15.97	97.04	83.67	112.5
28	2.05	13.52	15.63	97.04	83.92	112.2
29	2.05	13.28	15.31	97.04	84.15	111.9
30	2.05	13.05	15.01	97.04	84.37	111.6

t: Student distribution.

B.2.4.1.3. Area 15: Log-normal

The estimated log mean when fitting the log-normal distribution to the observed data is 5.006 and the estimated log standard deviations is 0.485.



MB: middle bound; TEF: toxicity equivalence factor.

Figure B.3: Log-normal distribution of the MB levels in whole scallops in area 15

Table B.4: The precision achieved for Area 15

Sample size	t(0.05)	Deflation lower	Inflation upper	Mean	Mean lower bound	Mean upper bound
3	4.3	72.91	269.1	167.9	45.48	619.6
4	3.18	56.37	129.2	167.9	73.24	384.8
5	2.78	47.51	90.51	167.9	88.12	319.8
6	2.57	41.92	72.18	167.9	97.5	289
7	2.45	38	61.29	167.9	104.1	270.8
8	2.36	35.05	53.97	167.9	109	258.5
9	2.31	32.73	48.65	167.9	112.9	249.6
10	2.26	30.84	44.58	167.9	116.1	242.7
11	2.23	29.25	41.35	167.9	118.8	237.3
12	2.2	27.9	38.7	167.9	121	232.8
13	2.18	26.73	36.48	167.9	123	229.1
14	2.16	25.7	34.59	167.9	124.7	225.9
15	2.14	24.79	32.95	167.9	126.3	223.2
16	2.13	23.97	31.52	167.9	127.6	220.8
17	2.12	23.23	30.25	167.9	128.9	218.7
18	2.11	22.55	29.12	167.9	130	216.8
19	2.1	21.94	28.11	167.9	131	215.1
20	2.09	21.37	27.19	167.9	132	213.5
21	2.09	20.85	26.35	167.9	132.9	212.1
22	2.08	20.37	25.58	167.9	133.7	210.8
23	2.07	19.92	24.87	167.9	134.4	209.6
24	2.07	19.5	24.22	167.9	135.1	208.5
25	2.06	19.1	23.61	167.9	135.8	207.5
26	2.06	18.73	23.05	167.9	136.4	206.6
27	2.06	18.38	22.52	167.9	137	205.7
28	2.05	18.05	22.03	167.9	137.6	204.8
29	2.05	17.74	21.56	167.9	138.1	204.1
30	2.05	17.44	21.13	167.9	138.6	203.3

t: Student distribution.

B.2.4.1.4. Area 17: Log-normal

The estimated log mean when fitting the log-normal distribution to the observed data is 6.142 and the estimated log standard deviations is 0.382.

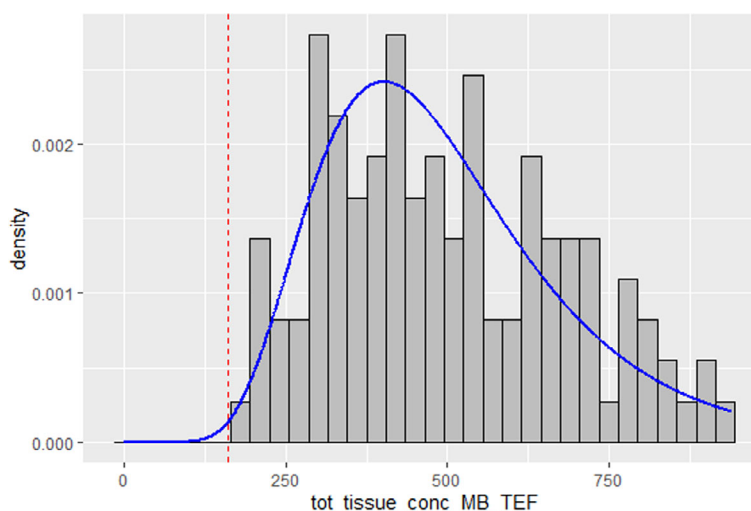


Figure B.4: Log-normal distribution of the MB levels in whole scallops in area 17

Table B.5: The precision achieved for Area 17

Sample size	t (0.05)	Deflation lower	Inflation upper	Mean	Mean lower bound	Mean upper bound
3	4.3	63.24	172	500	183.8	1360
4	3.18	47.14	89.16	500	264.3	945.9
5	2.78	39.11	64.22	500	304.5	821.2
6	2.57	34.2	51.97	500	329	759.9
7	2.45	30.82	44.55	500	345.9	722.8
8	2.36	28.31	39.48	500	358.5	697.5
9	2.31	26.35	35.77	500	368.3	678.9
10	2.26	24.76	32.9	500	376.2	664.6
11	2.23	23.43	30.61	500	382.9	653.1
12	2.2	22.31	28.72	500	388.5	643.6
13	2.18	21.34	27.13	500	393.3	635.7
14	2.16	20.49	25.77	500	397.6	628.9
15	2.14	19.74	24.6	500	401.3	623
16	2.13	19.07	23.56	500	404.7	617.9
17	2.12	18.46	22.64	500	407.7	613.3
18	2.11	17.91	21.82	500	410.5	609.2
19	2.1	17.41	21.08	500	413	605.5
20	2.09	16.95	20.41	500	415.3	602.1
21	2.09	16.52	19.8	500	417.4	599
22	2.08	16.13	19.23	500	419.4	596.2
23	2.07	15.77	18.72	500	421.2	593.6
24	2.07	15.42	18.24	500	422.9	591.2
25	2.06	15.1	17.79	500	424.5	589
26	2.06	14.8	17.38	500	426	586.9
27	2.06	14.52	16.99	500	427.4	585
28	2.05	14.26	16.63	500	428.8	583.2
29	2.05	14	16.28	500	430	581.5
30	2.05	13.76	15.96	500	431.2	579.9

t: Student distribution.

B.2.3. Scenarios: number of scallops per sample

In this case, different scenarios considering several mean levels in a or batch or area (80–300 μg OA eq/kg) are used to calculate the number of scallops needed in an analytical sample in order to be 95% confident that the area/batch under investigation is compliant or not, meaning that the detected level is expected to be below of above the regulatory limit of 160 μg OA eq/kg. Table 6 shows the outcome of these calculations. It is shown that if the numbers are far above or far below the limit of 160 μg OA eq/kg only few scallops per sample are required but if the true level is between 130 and 200, more than 10 scallops per sample are required and in the range 140–180 even more than 30 scallops. These calculations do not take into account the measurement uncertainty (generally around 30%) applied by the laboratory to decide with 95% certainty that a sample is non-compliant.

Table B.6: Number of scallops to include in an analytical sample to predict with 95% confidence that the area/batch is compliant (true level below the limit) or non-compliant (true level above the limit)

True level ($\mu\text{g}/\text{kg}$)	CV0.37	CV0.39	CV0.42	CV0.5
80	3	3	4	4
90	4	4	4	5
100	4	4	5	6
110	5	5	6	7
120	7	7	8	11
130	11	12	14	18
140	23	25	30	> 30
150	> 30	> 30	> 30	> 30
170	> 30	> 30	> 30	> 30
180	29	> 30	> 30	> 30
190	15	16	19	25
200	10	11	12	16
210	7	8	9	12
220	6	7	7	9
230	5	6	6	8
240	5	5	6	7
250	4	5	5	6
260	4	4	5	5
270	4	4	4	5
280	4	4	4	5
290	4	4	4	5
300	3	4	4	4

CV: coefficient of variation.

Note: The calculations are based on the CVs observed for data on four areas provided by France.

It is also possible for a specific scenario to visualise the confidence intervals for different number of scallops to estimate the concentration of the toxins, considering the highest coefficient of variation observed. The number of samples needed for each coefficient of variation observed in the French areas is used and presented in the tables below.

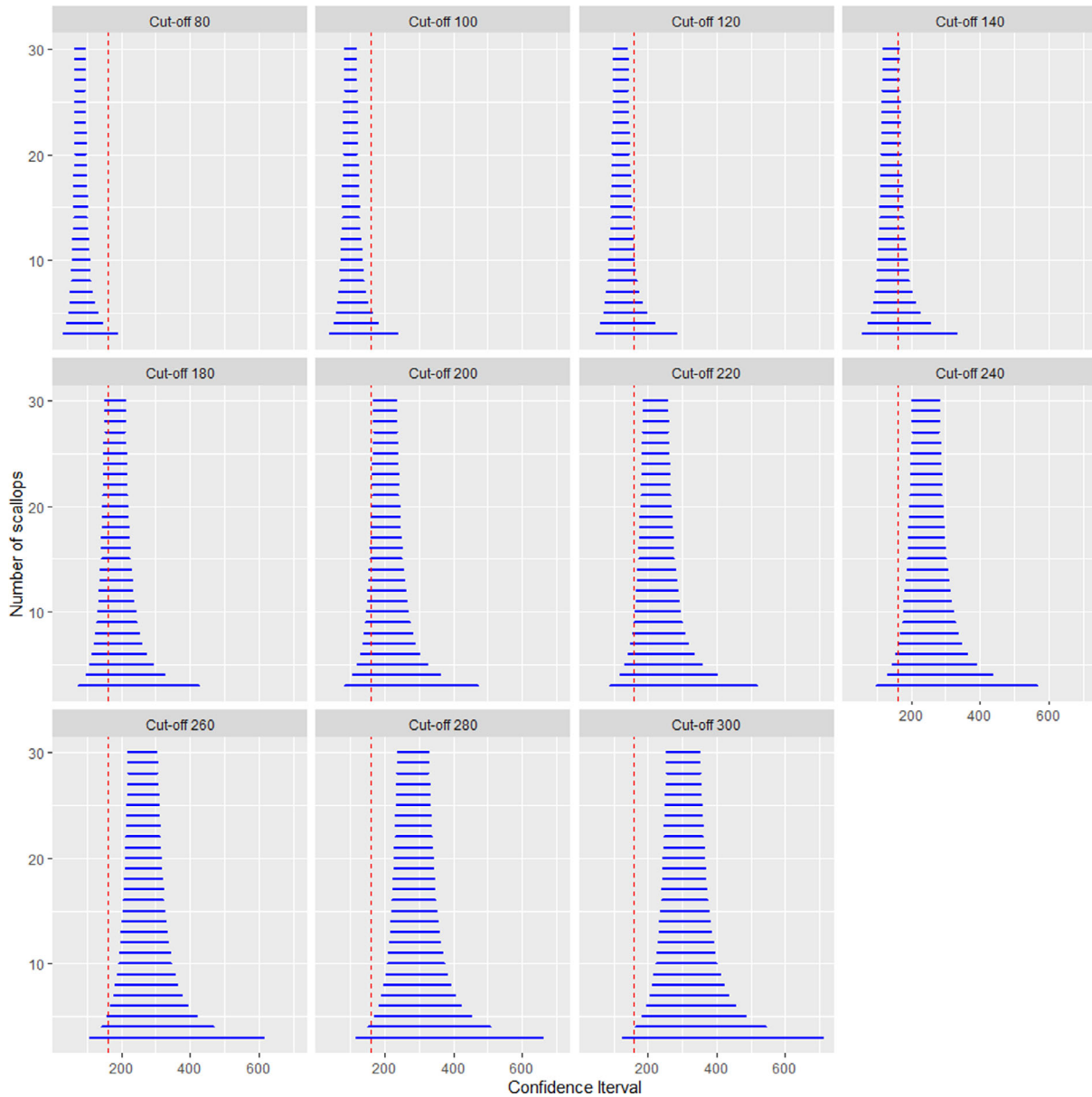


Figure B.5: Range of the actual levels in an analytical sample for varying numbers of scallops per sample at different true levels ('cut-off' in this graph) in a batch/area (80–300 μg OA eq/kg), based on a coefficient of variation of 0.5

The previous plot shows the confidence interval for different numbers of scallops to be analysed considering different mean concentration and a coefficient of variation of 0.5, being the largest observed in the French areas. The vertical dashed line represents the limit of 160 μg OA eq/kg. This can be used to derive the number of scallops needed in a sample to conclude with 95% confidence that the area is compliant or not.

B.2.4. Probability of being above or below the regulatory threshold when 10 scallops are analysed in a sample

Another way of evaluating the number of scallops included in a sample would be to consider 10 scallops per sample and estimate the probability that the level in the analytical sample would exceed the threshold of 160 μg OA eq/kg, below the resulting probabilities of being above or below the threshold depending on the mean level in the batch/area. This shows that in the range 130–200, there is more than 5% chance that the level in the test sample results in a wrong conclusion on compliance/non-compliance (disregarding measurement uncertainty).

Table B.7: Probability of a false non-compliant (true level below the limit) or false compliant (true level above the limit) result for 10 scallops per sample, given CVs based on the four areas sampled by France

Mean concentration ($\mu\text{g}/\text{kg}$)	CV0.37	CV0.39	CV0.42	CV0.5
80	0.00015	0.00016	0.00027	0.00087
90	0.00043	0.00056	0.00102	0.00274
100	0.00146	0.00197	0.00337	0.00782
110	0.00527	0.00676	0.01052	0.02098
120	0.01772	0.02155	0.03038	0.05118
130	0.05401	0.06182	0.07817	0.11089
140	0.14037	0.15153	0.17299	0.21023
150	0.29633	0.30516	0.32111	0.34640
170	0.30747	0.31592	0.33115	0.35521
180	0.16893	0.18024	0.20160	0.23777
190	0.08691	0.09669	0.11629	0.15277
200	0.04369	0.05065	0.06546	0.09597
210	0.02212	0.02660	0.03674	0.05987
220	0.01147	0.01428	0.02089	0.03747
230	0.00618	0.00786	0.01216	0.02370
240	0.00346	0.00450	0.00725	0.01526
250	0.00198	0.00268	0.00446	0.01000
260	0.00121	0.00164	0.00282	0.00666
270	0.00076	0.00100	0.00178	0.00457
280	0.00050	0.00064	0.00122	0.00314
290	0.00033	0.00043	0.00084	0.00225
300	0.00024	0.00032	0.00056	0.00163

CV: coefficient of variation.

B.2.5. Probability of being above or below the regulatory threshold when 20

Another way of evaluating the number of scallops included in a sample would be to consider 20 scallops per sample and estimate the probability that the level in the analytical sample would exceed the threshold of 160 μg OA eq/kg, below the resulting probabilities of being above or below the threshold depending on the mean level in the batch/area. This shows that in the range 140–180 μg OA eq/kg, there is more than 5% chance that the level in the test sample results in a wrong conclusion on compliance/non-compliance (disregarding measurement uncertainty).

Table B.8: Probability of a false non-compliant (true level below the limit) or false compliant (true level above the limit) result for 20 scallops per sample, given CVs based on the four areas sampled by France

Mean concentration ($\mu\text{g}/\text{kg}$)	CV0.37	CV0.39	CV0.42	CV0.5
80	0.00010	0.00011	0.00007	0.00007
90	0.00007	0.00008	0.00006	0.00008
100	0.00009	0.00007	0.00009	0.00025
110	0.00009	0.00019	0.00044	0.00163
120	0.00120	0.00175	0.00337	0.00919
130	0.01025	0.01324	0.02050	0.03911
140	0.06026	0.06910	0.08741	0.12296
150	0.22085	0.23180	0.25198	0.28480
170	0.23472	0.24540	0.26495	0.29652

Mean concentration ($\mu\text{g}/\text{kg}$)	CV0.37	CV0.39	CV0.42	CV0.5
180	0.08391	0.09408	0.11444	0.15208
190	0.02506	0.03049	0.04275	0.06992
200	0.00687	0.00908	0.01471	0.02996
210	0.00181	0.00265	0.00492	0.01238
220	0.00054	0.00078	0.00165	0.00503
230	0.00012	0.00026	0.00054	0.00209
240	0.00006	0.00006	0.00018	0.00085
250	0.00006	0.00006	0.00006	0.00036
260	0.00006	0.00006	0.00006	0.00018
270	0.00006	0.00006	0.00006	0.00006
280	0.00006	0.00006	0.00006	0.00006
290	0.00006	0.00006	0.00006	0.00006
300	0.00006	0.00006	0.00006	0.00006

CV: coefficient of variation.

Annex A – Summary statistics on occurrence and consumption data and exposure assessment results

The Annex is available as Excel file on the EFSA Knowledge Junction community on Zenodo, at: <https://doi.org/10.5281/zenodo.4472866>