SCIENTIFIC OPINION

Evaluation of the shucking of certain species of scallops contaminated with domoic acid 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 the levels of domoic acid (DA) in whole scallops that would ensure that levels in edible parts are below the regulatory limit after shucking. This should include five species of scallops. In addition, EFSA was asked to recommend the number of scallops to be used in an analytical sample. To address these questions, EFSA received suitable data on DA for only one scallop species, *Pecten maximus*, i.e. data on pooled samples of edible and non-edible parts. A large part of the concentration levels was above the limit of quantification (LOQ) and only these data were used for the assessment. Shucking in most cases resulted in a strong decrease in the toxin levels. Statistical analysis of the data showed that levels in whole scallops should not exceed 24 mg DA/kg, 59 mg DA/kg and 127 mg DA/kg to ensure that levels in, respectively, gonads, muscle and muscle plus gonads are below the regulatory limit of 20 mg DA/kg with 99% certainty. Such an analysis was not possible for the other scallop species. In the absence of data from member states, published data of variations between scallops were used to calculate the sample size 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 DA levels in the area/lot were twofold below or above the regulatory limit for the highest reported coefficient of variance (CV) of 1.06. To predict with 95% certainty for levels between 15 and 27 mg DA/kg, a pooled sample of more than 30 scallops would have to be tested.

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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 domoic acid (DA) with a view to the production of edible parts meeting the safety requirements foreseen in the Union legislation. The request included 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 complying with the regulatory limit can still be placed on the market for two scallop species (*Pecten maximus*, *Pecten jacobaeus*) even if the whole animal exceeds the regulatory limit. In order to evaluate if a similar approach can be applied for other species, data on toxin levels in edible parts and whole scallops have been requested from member states. In addition, a sampling scheme ensuring reliable prediction on compliance/non-compliance of a lot or area with the established regulatory limit has been investigated, focusing on the number of scallops to be included in an analytical sample. Since no data on individual scallops were received, published information on variation in toxin levels between scallops was used.

Upon launching a call for data on concentrations of DA in whole animals and body parts of the species of concern, EFSA received data from several EU member states. However, only for one species, *Pecten maximus*, were the data suitable to answer the first question in the terms of reference from the European Commission. These data came from routine monitoring and were submitted by France and Ireland. Compared to the edible parts, which are the adductor muscle and the gonad, in most cases, the major part of the toxins was present in the non-edible parts of the scallops that are removed during shucking.

Of the data provided by Member States, 3% of the gonad samples measured exceeded the regulatory limit of 20 mg/kg, but none of the muscle samples, whereas for whole scallops 73% were not compliant.

Statistical analysis showed that levels in whole scallops should not exceed 24 mg DA/kg to ensure that levels in gonads are below the regulatory limits of 20 mg DA/kg with 99% certainty. For muscles only, the critical level in whole scallops would be 59 mg DA/kg. for muscle plus gonad combined, the critical concentration was calculated to be 127 mg DA/kg.

For an analysis of the sample sizes needed to conclude reliably on compliance/non-compliance of a lot/area, only published data on the variation between scallops were available, showing coefficients of variance (CVs) between 0.40 and 1.06. 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 DA in the area/lot were twofold below or above the regulatory limit ($<$ 10 or $>$ 37 mg/kg), based on the highest reported CV of 1.06. However, to predict with 95% certainty for levels between 15 mg DA/kg and 27 mg DA/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 samples. Also, the potential application of measurement uncertainty in relation to compliance should be considered. The CONTAM Panel noted that consumption of edible parts of scallops with a level at the regulatory limit (as few as four) may result in exceedance of the acute reference dose for DA. The major uncertainty in this assessment came from the fact that only a limited number of suitable data points were provided to answer the questions posed in the terms of reference and from only two member states. Furthermore, these data only covered one scallop species. It is unclear to what extent results can be extrapolated to other species. For *Pecten maximus*, 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 considered uncertainty were used based on the actual levels. Also, for determining the number of scallops that should be included in an analytical sample, the published data on variation between individual scallops were used.

The CONTAM Panel recommends updating the risk assessment on DA 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 domoic acid 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 that in any case 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 mollusc's 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) N° 853/2004, Annex III, Section VII, Chapter V, point 2[1] 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 kilogram".*

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 the risk assessment in 2009 demonstrated that, overall, it appears that all of the shellfish species considered can be contaminated with DA and epi-DA, but a high level of contamination is found only in scallops, with almost 60% of the samples above the EU regulatory limit.[2]

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

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[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. Domoic acid (https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2009.1181)

Member States, allows the adductor muscle and gonads of two species of scallops[3] 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. 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 favour extending 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.

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[3] *Pecten Maximus* and *Pecten Jacobaeus.*

1.1.3. Interpretation of the Terms of Reference

This Opinion focuses on DA. 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 limit was in principle the basis for the assessment and not the health-based guidance value (HBGV) previously derived in an EFSA Opinion (EFSA, 2009). However, the CONTAM Panel did evaluate the impact of consumption scenarios leading to exceedances of the regulatory limit on the risks for consumers by comparison with the Acute Reference Dose (ARfD). Data on the toxin levels in various parts of the scallops and on interindividual variations between levels within a batch[4] were used for the assessment. A brief summary on the adverse effects in humans and the HBGV 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, without any consideration of these different interpretations.

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.

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[4] A group of scallops or scallop parts produced and placed on the market as food by an approved establishment, which had been caught/harvested sufficiently close in time and space, to allow assumption of similar biotoxin risk.

Mantle

Gills

Adductor muscle

Gonad[1]

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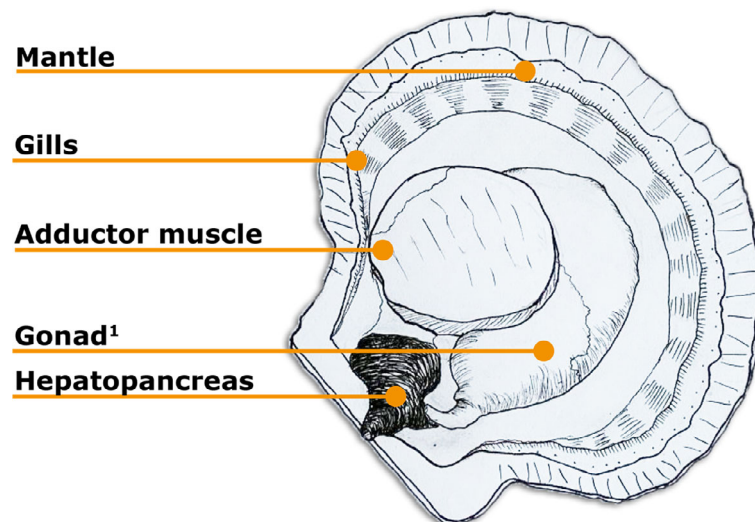
The term 'gonad' is used to refer to the anatomically distinct reproductive organ sometimes also referred to as 'roe'. Physiologically, this structure is involved in reproduction and also contains a loop of the intestine, which is significant in terms of toxin concentration for the structure. The term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

Figure 1: Anatomy of a scallop (simplified)

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'[5] in Regulation No 2073/2005[6]. 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 226/2002[7]). 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

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

[6] 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.

[7] 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 or 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 connotations, there may exist additional specific obligations for both FBOs and CAs designed to manage those specific risks (O'Mahony, 2018).

1.2.3.1. General food safety provisions

Regulation (EC) No 178/2002[8] on general principles and requirements of food law, and Regulation (EC) No 852/2004[9] 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.

1.2.3.2. 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: Summary of biotoxin health standards 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 equivalents 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 (14 July 2021), Pectenotoxins remain legally included in the DSP group; however, there exists a Member State agreement and an active Comitology process designed to result in legislative modification to remove them from the compounds to be analysed within regulatory risk management.

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

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.

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[8] 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.

[9] 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.

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.

1.2.3.3. 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 heath 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 molluscs[10] 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 heath standards and the operator obligations for handling such non-CPA *Pectinidae*.

1.2.3.4. 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, one particular legislative provision permits 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 a level below 250 mg/kg. In that case, levels in the edible parts should not exceed 20 mg/kg (Commission Decision 2002/226/EC). This should not be confused with the level of 4.6 mg/kg, that is used to reopen a closed fishing water (see Terms of Reference).

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[10] 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.

1.2.4. Mollusc sampling

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. However, for *Pecten maximus* and *Pecten jacobaeus* shucking is allowed if levels in whole scallop are above the regulatory limit but below 250 mg/kg in the whole scallop. In that case, where an area[11] was closed because levels in whole scallop exceeded the regulatory limit of 20 mg/kg, Commission Decision 2002/226/EC allows 'a restricted harvesting regime if two consecutive analyses of samples, taken between one and no more than seven days, show that the DA concentration in whole mollusc is lower than 250 mg/kg. In addition, the DA concentration in the parts intended for human consumption, which have to be analysed separately, must be 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'. As explained in the ToR, levels in edible parts put on the market should be below the regulatory limit of 20 mg/kg. The level of 4.6 mg/kg is for reopening the area and should ensure with high probability that levels in other portions from the same area are below the regulatory limit of 20 mg/kg.

FBOs are generally required to have their own checks where appropriate to verify adequate management of safety of their products. Operators operating in 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 DA (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

Domoic acid (DA) is produced mainly by marine red algae of the genus *Chondria* and diatoms of the genus *Pseudo-nitzschia* although other species are known to produce DA (FAO, 2004). DA is a cyclic amino acid with a proline ring, an imino group and three carboxylic acid groups (Quilliam, 2001) (Figure 2). The presence of the carboxylic acid groups is responsible for its water solubility and its relatively high polarity. Several isomers of DA (epi-domoic acid (epi-DA), (domoic acid C5′-diastereomer)) and isodomoic acids A, B, C, D, E, F, G and H (iso-DA A-H)) have been reported (Maeda et al., 1986; Wright et al., 1990a; Walter et al., 1994; Zaman et al., 1997; Holland et al., 2005). DA transforms into epi-DA through long-term storage (Figure 2) (Quilliam et al., 1989) and degrades and transforms to epi-DA and iso-DAs through exposure to ultra-violet light (Wright et al., 1990a,b; Wright and Quiliam, 1995; Djaoued et al., 2008). Epimerisation is also accelerated by heating (Quilliam, 2003a). In general, DA itself is stable and does not degrade at ambient temperatures. In shellfish, DA is heat stable and cooking does not destroy the toxin (McCarron et al., 2007). Depuration

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[11] In the context of scallops harvested outside of CPAs, refers to a geographical zone where the biotoxin risk of scallops harvested is regarded to be similar.

of DA in scallops depends on the species as well as on the environmental conditions and availability of nutrients. For example, in a study on *Argopecten purpuratus* depuration is fast as in less than 3 days 50% of DA is depurated (Álvarez et al., 2020). However, Blanco et al. (2002) described a slow depuration for *Pecten maximus* with a half-life of 150 days.

Domoic acid

Epi-domoic acid (Domoic acid C5′-diastereomer)
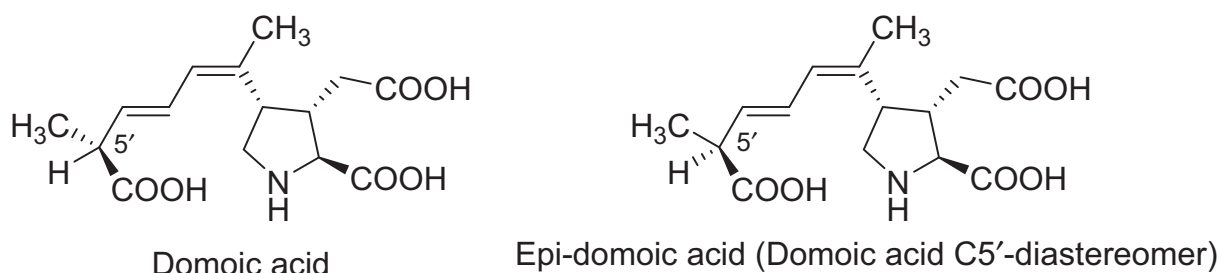
Figure 2: Structure of domoic acid and epi-domoic acid

1.2.6. Analytical methods

1.2.6.1. LC-UV EU Reference method

The EU reference method for the determination of DA is liquid chromatography (LC) coupled to ultra-violet detection (UV). DA can absorb UV light due to the presence of a conjugated double bond in the aliphatic side chain of the DA structure (see Figure 2). DA has a strong absorbance at 242 nm. This method went through several full collaborative trials and is therefore the reference method mentioned in EU legislation for the quantitative analysis of DA in shellfish (Regulation 853/2004/EC[1], and Regulation 2019/627[12]). This reference method has been inter-laboratory validated by the European Reference Laboratory for Marine Biotoxins (EURLMB). Briefly, it consists of the extraction with 50% aqueous methanol of a shellfish homogenate followed by the filtration of the extracts and injection into the LC-UV system. Additional clean-up steps before the analysis are not needed. However, a strong anion exchange solid phase extraction clean-up step can be used for removal of compounds that could interfere with the analysis. The LC-UV analysis is based on the separation of DA on a reverse phase LC column using either isocratic or gradient elution with an acidic mobile phase composition. The method is applicable for a wide variety of molluscan shellfish matrices, both fresh and cooked, such as mussels, clams, razor clams and cockles as well as finfish (i.e. anchovies). The LC-UV method (CEN 14176) is also described by the European Committee for Standardization (CEN, 2017).

1.2.6.2. Alternative methods

The EU legislation establishes that in addition to the EU reference method (LC-UV) mentioned above, other methods such as immunoassays can be used as alternatives or supplementary to the EU reference LC-UV 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. Such methods must be intra- and preferably inter-laboratory 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-UV reference method. The ASP-ELISA has been internationally validated by the International Association of Official Analytical Collaboration (AOAC International) becoming an official method of analysis (OMA) (AOAC, 2006). Therefore, the ASP-ELISA can be used as an alternative method for screening purposes.

Liquid chromatography coupled to tandem mass spectrometry using electrospray ionisation (LC–ESI–MS–MS) is also a valuable tool for rapid and sensitive determination of DA. Nevertheless, the LC-MS/MS methods have not been inter-laboratory validated and are currently not formally recognised as official alternative methods for the determination of DA. Furthermore, there are also other mass spectrometric methods as well as immunochemical methods (i.e. lateral flow devices) available, but also these methods are not formally recognised as an official alternative method.

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[12] Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls. OJ L 131, 17.5.2019, p. 51–100.

1.2.7. Hazard characterisation of domoic acid

The toxicity of DA is described below summarising a previous EFSA Opinion (EFSA, 2009). It is important to note that many other *in vivo* and *in vitro* toxicological studies on these compounds have been reported in the literature since the publication of this Opinion. However, these were not considered as a re-evaluation of the toxicity of DA 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 this previous Opinion.

DA provokes Amnesic Shellfish Poisoning (ASP) characterised by gastrointestinal symptoms (vomiting, diarrhoea or abdominal cramps) and/or neurological symptoms (confusion, loss of memory or other serious signs, such as seizures or coma) usually appearing in humans 24–48 h after consumption of contaminated bivalve molluscs. Neurotoxicity is the critical toxicological effect identified in experimental animals (rodents and non-human primates) as well as in humans. The toxic effects of DA are due to its high affinity binding and agonist activity on some types of glutamate receptors in the nervous system (non-N-methyl-D-aspartate-, alfa-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate- and kainate receptors) leading to cell death in certain regions of the brain (e.g. the hippocampus).

Genotoxicity data on DA were inconclusive. Since no data on the chronic effects of DA were available, a tolerable daily intake (TDI) could not be established. However, in view of the acute toxicity of DA, the CONTAM Panel decided to establish an acute reference dose (ARfD) based on human data on acute toxicity from an outbreak of DA poisoning in Canada in 1987, comprising of 107 cases. In the nine individuals with symptoms and where the DA doses could be estimated, six had mild symptoms such as vomiting, nausea, memory loss, dizziness and/or confusion (and had an exposure of about 0.9 mg/kg body weight (bw)). Three of the patients had severe symptoms and the two individuals with the highest exposure (around 4 mg/kg bw) had permanent neurological symptoms. The CONTAM Panel used the lowest observed adverse effect level (LOAEL) of 0.9 mg/kg bw applying an uncertainty factor of 30 (3 for using a LOAEL as reference point and 10 for consideration of interindividual variability) to derive an acute reference dose (ARfD) of 30 μg/kg bw. The CONTAM Panel noted that information on the relative potency of epi-DA *in vivo* was lacking but because the occurrence data did not make a distinction between the concentration of parent DA and epi-DA and results were reported as sum of DA and epi-DA (sum DA), the CONTAM Panel had to assume in its assessment that DA and epi-DA are equally toxic. Because DA can be converted to epi-DA during storage, the ARfD applies to the sum of DA and epi-DA.

1.2.8. Previous assessments by other risk assessment bodies

In 2001, the UK National Reference Laboratory (NRL) for Marine Biotoxins prepared a report on DA in *P. maximus* for the EU ASP working group (UKNRL MB, 2001). This report used research data from Spain, Ireland, Scotland and Northern Ireland, along with monitoring information from Scotland and Ireland. The analysis therefore considered a substantial database of DA concentrations, both individual animal and pooled[13] animal results which, having considered left-censorship, showed log-normal distribution. Detailed consideration was given to inter-animal variation both in whole-animal DA concentration and also for individual tissue compartments including adductor muscle and gonads. The variability of DA within whole scallops (range of median of the CV for different data sets, 18–68%) was less than the variability amongst gonad or adductor muscle, with variability in gonad and muscle reported as very similar (range of median of the CV for different data sets, 57–122% and 41–140%, respectively). Some data were also presented on spatial variability, based on repeated near-contemporaneous fishing hauls in the same area. In the available data sets, the majority of adductor muscles contained levels of DA which were either below detection limit or low (i.e. < 5 mg/kg), rarely exceeding 20 mg/kg. Adductor muscle contamination pathways were examined, with specific assessment of such contamination arising through either exposure to hepatopancreatic fluids during shucking or some non-muscle tissue such as kidney or anal tube remaining attached after shucking. The report considered a 10-animal pool to be a good representation of a typical portion/meal size, therefore reflective of consumer exposure. Detailed consideration was given to inter-pool variation arising from inter-animal variation. Using available data, notably inter-animal variability coefficients, statistical modelling considered various population median DA scenarios regarding likely distribution of sample DA results to calculate the likelihood of high DA, i.e. above 20 mg/kg, samples existing in

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[13] Combination of the units in a sample to create a single matrix for analysis with a single analytical result for that sample. A typical example is homogenate of all soft tissues of 10 individual scallops before analysis.

populations with those median levels. Conversely, for various probabilities that one such sample exceeding 20 mg/kg might exist in a population, the median DA level of the population was calculated. That assessment showed that for a 0.001 probability that a population might contain a single 10-animal pool with greater than 20 mg/kg DA, then the median 10-animal pool level in that population would be 4.6 mg/kg. This implies that populations with a median pool DA level below 4.6 mg/kg would have less than 1 in 1,000 probability of any individual pool exceeding the limit of 20 mg/kg. This assessment may have been a contributor to the provisions in the Commission Decision 226/2002, whereby a restricted harvesting regime may be deemed permissible based on several parameters including that the level in edible parts should be below 4.6 mg/kg.

In a study for the Food Standards Agency UK (FSA), McKenzie and Bavington (2002) examined the effect of commercial shucking in managing the risk of DA present in *P. maximus*. The overall conclusion was that shucking removes 99% of DA present in whole animals from the end-product. However, a significant variability in shucking efficacy was reported across different shucking establishments. Differences in the DA concentrations of the edible parts resulting from the commercial shucking of scallop from the same harvested batch in five different processors were described as highly significant ($p < 0.001$) albeit always less than 20 mg/kg for adductor muscle. Ultimate effectiveness in toxin removal was significantly influenced by final rinsing, particularly in poorly shucked scallop. The production of muscle-only edible part had more reliable physical removal of hepatopancreas than the production of muscle-plus-gonad (roe-on) edible part. Key aspects of good commercial shucking included discarding of broken scallop, and avoiding physical immersion of edible parts in scallop offal fluid during shucking. The authors posed concerns about the application of a lower limit of 5.6 mg/kg as an approach to managing risk of inter-animal variability, when they found that the most significant contributor to variability of DA levels in edible parts was shucking efficacy, as opposed to initial whole-animal concentration. The study recommends an end-product testing of the edible parts following shucking, either of muscle plus gonad together or of muscle and gonad analysed separately if marketed with potential to be consumed separately. In winter months, the increase in gonad size resulted in higher amounts of DA being accumulated in the gonads and in the adjacent digestive tract (which is part of the gonad preparation). This work also examined spatial and chronological inter-sample variability within an area, and found spatial variation related to harvest depth, with speculation on effect of water movement or scallop feeding. Authors offered the view that understanding such spatial variation and consistency could be used to better inform the definition of area with relatively consistent DA concentration, instead of basing such areas on the boundaries of traditional fishing grounds.

In 2014, the UK Food Standards Agency, Committee on Toxicity of chemicals in foods (COT, 2014) produced a statement on DA in king scallops (*P. maximus*). They noted the wide range of DA in whole scallops and the generally lower concentration in edible parts. COT expressed the view that the only way in which adductor muscles might contain any DA would be through poor shucking. They considered gonad mass variation, during animal reproductive cycle, with resultant effect on concentration and quantity of DA within gonad as analysed. With the potential for greater gonadal mass to dilute DA concentration, and lower gonadal mass to inflate concentration, the COT expressed concerns in interpreting changes in DA concentrations when analysing gonads alone. The COT noted UK scallop consumption data ranging from 15 to 120 g per consumer per day, and a further study indicating 396 g for the 99th percentile of UK consumption. They selected a value of a 400 g portion size for exposure assessment around an ARfD of 30 μg/kg bw of DA and epi-DA. Data on DA by geographical location and tissue type were considered, based on data from scallop areas as monitored in UK EFZ. Descriptive data showed highest results in whole animals where mean values across four areas were well in excess of 20 mg/kg, with lowest levels in muscle and somewhat higher levels in gonads. However, both of those edible parts remained well below 20 mg/kg despite high whole animal means. Statistical modelling was undertaken from available monitoring data to generate 100,000 notional 400 g portions of edible tissues (adductor muscle + gonad) and adductor muscle alone concluding that less than 1% of those modelled portions would result in exceedance of the ARfD. Sensitivity analysis, taking into account inter-processor shucking variability had the effect of increasing the DA concentrations in modelled portions. The model was also used to derive an upper level of DA in whole animals that would allow 400 g portions of adductor plus gonad or adductor alone not to result in exceedance of the ARfD. Variation across scallop areas was evident with the lowest acceptable whole animal value in the worst area shown at 162 mg/kg at 99th percentile and 129 mg/kg at 99.9th percentile for adductor muscle plus gonad, and generally higher for production of adductor muscle alone. Sensitivity analysis was undertaken, further modifying the model to include a processing factor

to account for operator shucking variability, and it showed the necessity for a fourfold reduction in acceptable whole-animal concentration to ensure similar protection against ARfD exceedance when producing muscle plus gonad with poorest shucking.

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 Science[14] (WoS) and Pubmed.[15] References were retrieved and managed by using Endnote.[16] 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 working group (WG) experts for their relevance regarding either the lipophilic marine biotoxins of concern (for the previous Opinion) and/or domoic acid (for the present 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',[17] including also government reports perhaps as part of national risk assessments, and considered for the assessment where relevant.

1.3.2. Occurrence data submitted to EFSA

1.3.2.1. Data collection and validation

At the time the mandate for the present Opinion from the European Commission was received, only few occurrence data on DA in scallops were available in the EFSA Chemical Occurrence Database. Therefore, additional occurrence data on DA (and OA-, AZA- and YTX group toxins reported in EFSA CONTAM Panel (2021) 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[18] in food and feed that opened on the 15 April 2020. 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 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.

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

[15] 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 from: https://pubmed.ncbi.nlm.nih.gov/

[16] Endnote X9, Thomson Reuters. Available from: https://endnote.com/

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

[18] https://www.efsa.europa.eu/en/consultations/call/call-continuous-collection-chemical-contaminants-occurrence-data

The data submission followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a,b) 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 16,369 analytical results were available on DA in the EFSA database. Data were provided by national competent authorities and designated laboratories from France, Germany, Denmark, 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 UK leaving the EU), no data from UK are available for the present assessment.

1.3.2.2. 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 (according to the terminology reported in Annex Table A1). Detailed information on data cleaning can be found in Annex Table A2. Briefly, 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. Moreover, 889 calculated results reported as 'Entire animal' for *Pecten maximus* were excluded due to the fact that levels were mathematically reconstructed rather than analysed. When the concentrations of the complementary body parts and their weights were available, these results were recalculated with formulas reported in Section 1.3.2.4. The presence of duplicates was excluded during data cleaning. When information on pooled samples was missing (e.g. the number of scallops), this information was sought from data providers and recoded accordingly. 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.

Results were then evaluated considering the analytical method applied for their generation. Only results produced with the official or equivalent methods (see Section 1.2.6.1) were considered; LOQ ranged from 0.2 to 1.9 mg/kg, no cut-off was applied based on method sensitivity. Recovery and information on results recovery correction was poorly reported. When missing, data on recovery were requested from the data providers and, when provided, added.

Sampling years ranged from 2002 to 2020 and no exclusion was made for sampling year.

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.

The left-censored data (analytical data reported below the limit of detection (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, 2010a,b) 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.

After data cleaning, a total of 15,459 analytical results on DA were present in the database.

1.3.2.3. 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 seven 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 Annex Table A1.

1.3.2.4. Methodology used for calculating toxin concentrations

When possible, the DA concentration in the entire animal was calculated by summing up the mass of DA of the different tissues divided by the total body weight according to the following equations:

$$\text{Entire animal concentration} = \frac{\sum_{i=n}^{m} \text{mass DA in tissue i}}{\text{Entire animal weight}}, \quad \text{(F1)}$$

where,

$$\text{Mass toxin in tissue i} = \text{tissue weight} \times \text{DA concentration in tissue}, \quad \text{(F2)}$$

$$\text{Entire animal weight} = \sum \text{tissue weight i}. \quad \text{(F3)}$$

Additionally, 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:

$$\text{'muscle plus gonad' concentaration} = \frac{\text{DA in gonad} + \text{mass DA in muscle}}{\text{gonad weight} + \text{muscle weight}}, \quad \text{(F4)}$$

where

$$\text{Mass DA in gonad} = \text{gonad weight} \times \text{DA concentration in gonad}, \quad \text{(F5)}$$

$$\text{Mass DA in muscle} = \text{muscle weight} \times \text{DA concentration in muscle}. \quad \text{(F6)}$$

1.3.2.5. Relation between concentrations in the edible parts and the entire animal

The CONTAM Panel also evaluated the data sets submitted applying a statistical approach based on the estimation of the distribution of the ratios between the levels encountered in the edible parts and the entire animal. This was only possible for *Pecten maximus* data from Ireland where DA levels were reported for the different edible parts and the rest of the animal as well as the weights of the parts, all based on pooled scallops, thus allowing a notional calculation of the concentration for the entire animal (See Section 1.3.2.4, equation F1). 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 fall. The analysis for each edible part separately considers only quantified data (left censored data were used for the final calculations). In the cases where the sum was used, data with DA quantified for at least one of the edible parts were included in the analysis. Subsequently, a distribution curve was derived for the whole set of samples, from which mean and median ratios were derived. These distribution curves 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. Calculations were performed for the ratio of the concentration in adductor muscle or gonads and the entire animal, as well as the ratio of the sum of adductor muscle plus gonads and the entire animal. Details are presented in Appendix A.1 (DA ratio between sum of edible parts and entire animal), A.2 (DA ratio between muscle and entire animal) and A.3 (DA ratio between gonad and entire animal).

Additional analyses were performed for the data submitted by France in which only concentrations in the combination of 'adductor muscles and gonads' and the entire animal could be quantified. Different subsamples of 10 scallops each coming from the same lot[19] were used to measure the respective concentrations in either whole scallops or edible parts coming from the same lot. This implies that concentrations measured in entire animal and the edible parts were coming from the same lot, but not from the same animals, establishing the ratio only at lot level. Considering that each subsample contains 10 scallops, it could be questioned if a subsample of 10 scallops provides a good

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[19] A group of scallops as primary products which had been caught/harvested sufficiently close in time and space to allow assumption of similar biotoxin risk.

representation of a lot. If both distributions, i.e. based on data from Ireland and France, are similar, this would indicate that ratios across different countries provide similar results, and also that 10 scallops provide a reasonably good representation of the lot. In addition, the two data sets were used to estimate the robust relations between concentrations reported in the muscle plus gonads and the entire scallops. A plot overlaying the scatter from both data sets and their respective relationship is shown to illustrate their similarities (see Figure 3 in Section 2.1.4.1).

To account for the uncertainty in the estimation of the quantiles coming from the estimated distribution, the LB of a 95th confidence interval was estimated for the 99th percentile of the fitted distribution and used as the maximum toxin level that would be allowed in the entire animal still ensuring that the edible parts have toxin levels below 20 mg DA/kg. Considering that the estimated quantile has been obtained using maximum likelihood methods, it can be assumed that a normal distribution applies and with this a normal approximation can be used to obtain the confidence interval for the 99th percentile $(e^{\hat{\sigma}^2} - 1) \cdot e^{2 \cdot \hat{\mu} + \hat{\mu}^2}$ 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.

1.3.2.6. Sample size estimation

The approach proposed by Matsushima et al.(2018) was followed and 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. Assuming a log-normal distribution ensures that such low values can still be observed. 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/lot as compliant or not. The approach also considers the variation reported in several published studies with coefficients of variation (CVs) ranging from 0.4 up to 1.06 (Bogan et al., 2007a,b,c). The method uses the confidence bounds (LB and UB of the 95% confidence interval) to identify the number of scallops needed to result in a 95% confidence depending on the mean value observed and the variations used to classify an area/batch as compliant or non-compliant. The approach proposed by El-Shaarawi and Lin (2007) was used to estimate the confidence interval for a Log-Normal random variable. Details are presented in Appendix B.

2. Assessment

2.1. Occurrence data submitted to EFSA

After data validation and cleaning (as detailed in Section 1.3.2.2), 15,459 results on DA in scallops were present in the EFSA database.[20] Sampling years spanned from 2002 to 2020 (Table 2), with the majority of results provided by Ireland followed by France.

Table 2: Number of analytical results (after data cleaning) on domoic acid in scallops, reported to EFSA in relation to sampling year and data providers[a]

| Sampling year | Reporting Country | | | | |
	France	Germany	Denmark	Ireland	Total
2002	–	–	–	65	65
2003	–	–	–	646	646
2004	109	–	–	629	738
2005	47	–	–	468	515
2006	.	–	–	541	541
2007	27	–	–	485	512
2008	40	–	–	494	534
2009	9	–	–	567	576
2010	10	–	–	735	745
2011	15	–	–	1,035	1,050

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[20] Occurrence data on scallops are available on the EFSA Knowledge Junction community on Zenodo (https://doi.org/10.5281/zenodo.5102830).

Sampling year	Reporting Country				
	France	Germany	Denmark	Ireland	Total
2012	284	–	–	1,086	1,370
2013	264	–	–	1,288	1,552
2014	15	–	–	1,214	1,229
2015	30	–	–	913	943
2016	34	–	30	1,110	1,174
2017	46	–	–	1,018	1,064
2018	40	1	–	950	991
2019	6	3	–	861	870
2020	–	–	–	344	344
Total	**976**	**4**	**30**	**14,449**	**15,459**

(a): See Annex Table A2 and Section 1.3.2.2 for details on data cleaning.

(b): −: no data.

2.1.1. Distribution of analytical results across different food types

An overview of available data reported for different species is presented in Table 3. The majority of data were on *Pecten maximus* (n = 15,021), followed by *Aequipecten opercularis* (n = 406). A minimal number of data were submitted on *Chlamys islandica* (n = 30) and on *Pecten jacobaeus* (n = 2). No data were available on *Mimachlamys varia*. Several data providers analysed different body parts of scallops as part of monitoring campaigns. Sometimes animals were dissected into specific and complementary body parts belonging to the same animal, the same pool or to the same lot. A detailed description is reported in Section 2.1.4.

Table 3: Number of analytical results on domoic acid according to species and body parts as present in the EFSA database

Species	Matrix	France	Germany	Denmark	Ireland
Aequipecten opercularis	Adductor muscle	–	–	–	311
	Adductor muscle and Gonad	–	–	–	14
	Entire animal	–	–	–	60
	Gonad	–	–	–	21
Chlamys islandica	Adductor muscle	–	–	30	–
Pecten jacobaeus	Adductor muscle	–	2	–	–
Pecten maximus	Adductor muscle	41	1	–	6,560
	Adductor muscle and gonad	428	1	–	–
	Entire animal	437	–	–	36
	Entire animal excluding hepatopancreas	14	–	–	–
	Entire animal excluding gonad and adductor muscle	–	–	–	910
	Gonad	40	–	–	6,537
	Hepatopancreas	16	–	–	–

−: no data.

2.1.2. Analytical methods and limits of quantification

Only results obtained with the EU reference method for DA or equivalent methods (LC-MS/MS) were considered. The majority of results were produced with HPLC-UV and only a few with LC-MS/MS which has a slightly higher sensitivity (for LOQs, see Table 4 below). The LODs can be found in Annex Table A3.

For the majority of the data (14,449 results reported by Ireland), a recovery of 109% was reported and results were already corrected for that. For ca. 1,000 results submitted by France information on recovery was not available and concentration values were thus not corrected for recovery. This is a source of uncertainty.

Table 4: Overview of number of samples (N), left-censored data and reported LOQs for domoic acid in scallops (mg/kg)

Method	N	Left-Censored	LOQ						
			Min	P25	Mean	Median	P75	P95	Max
HPLC-UV	15,425	59%	0.5	0.8	0.81	0.8	0.8	1	1.9
LC-MS/MS	34	97%	0.2	0.3	0.36	0.3	0.3	–	1.34

HPLC-UV: high-performance liquid chromatography with UV detector; LC-MS/MS: liquid chromatography with mass spectrometry; LOQ: limit of quantification, Max: maximum; Min: minimum; N: number of samples; P25: 25th percentile; P75: 75th percentile P95: 95th percentile.

2.1.3. Occurrence data on domoic acid in scallops

Descriptive statistics for DA in scallops at the lower and upper bounds are presented in 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 analysis, descriptive statistics were weighted for sample number.

Table 5 reports some descriptive statistics for different body parts in several species, along with the percentage of samples with levels above 20 mg DA/kg. While 73% of the reported results exceeded 20 mg DA/kg in 'entire body' in *Pecten maximus* (with a mean content of 60 mg/kg), only 2.8% of the 'gonad' samples (with a mean content of 4.24–4.47 LB-UB mg DA/kg) exceeded this limit. None of the 'remaining edible parts' samples exceeded the limit.

Data not sufficient to reconstruct the concentration in the 'entire animal' were not used for the assessment as these were not suitable to answer the Terms of Reference.

Only part (n = 2,265) of the data reported by Ireland, belonging to 755 batches, allowed the mathematical reconstruction of the content of DA in the entire animal based on the formula detailed in Section 1.3.2. Out of the totality of French data, 778 samples pertaining to 389 lots could be matched and used. These results are further described in Section 2.1.4. The other results could not be used for the present assessment and are no more described in this document.

Table 5: Number of samples, fraction of left-censored data, percentage of samples above the limit of 20 mg/kg (where applicable) and mean concentration of DA (mg/kg) (weighted for number of pooled samples)

Species	Matrix	n	LC %	Samples above 20 mg/kg (%)	Mean LB	Mean UB	P95 LB	P95 UB
Aequipecten opercularis	Adductor muscle	311	99	0	0.0	0.3	0.0	0.3
	Gonad	21	52	0	1.1	1.2	–	–
	Adductor muscle and gonad	14	100	0	0.0	0.3	–	–
	Entire animal	60	78	0	0.6	0.9	3.1	3.1
Chlamys islandica	Adductor muscle	30	100	0	0.0	0.3	–	–
Pecten jacobaeus	Adductor muscle	2	50	0	0.1	0.2	–	–
Pecten maximus	Adductor muscle	6,602	89	0	0.3	0.7	2.5	2.5
	Gonad	6,577	39	3	4.2	4.5	15.3	15.3
	Hepatopancreas	16	0	–	644	644	–	–
	Adductor muscle and gonad	429	45	0	1.4	1.8	4.5	4.5
	Entire animal	473	3	73	60	60	208	208
	Entire animal excluding gonad and adductor muscle	910	13	–	166	166	542	542
	Entire animal excluding hepatopancreas	14	43	–	2.7	3.0	–	–

LC: left censored; n: number of samples; P95: 95th percentile; LB: lower bound; UB: upper bound; –: not applicable.

2.1.4. Data on concentrations used for the assessment

2.1.4.1. Description of the data submitted by Ireland

Ireland reported detailed information on complementary body parts of *Pecten maximus*. Animals were dissected in 'adductor muscle', 'gonad' and 'entire animal excluding gonad and adductor' for a total of 2,265 samples belonging to 755 batches; for all of them, tissues of 12 animals of the same batch were pooled before further sample preparation and analysis of DA concentrations. Results of each matrix were reported separately. Only results of samples for which all complementary body parts were reported were considered and no imputation was performed for missing results. Moreover, sample weights were reported, and this allowed the reconstruction of the content of DA in the entire animal and in the edible parts, using the equations reported in Section 1.3.2.4. Most of these samples originated from Official Control Biotoxin Monitoring of CPAs (n = 734) and some from non-CPAs (n = 21) in the North Atlantic.

Figure 3 presents scatter plots of DA concentrations in gonads (Figure 3a) and adductor muscles (Figure 3b) vs. 'entire body' at the LB. The concentration of DA in this set of *Pecten maximus* batches ranged from 0 up to 406 mg/kg in the 'entire animal', from 0 to 56 mg/kg in 'gonad' and from 0 to 16 mg/kg in the 'adductor muscle'.

Based on the levels in gonad and adductor muscle, levels of DA in the combined edible parts were calculated, using the tissues weights (Figure 3c). The highest concentration in the 'adductor muscle plus gonad' was 21 mg DA/kg.
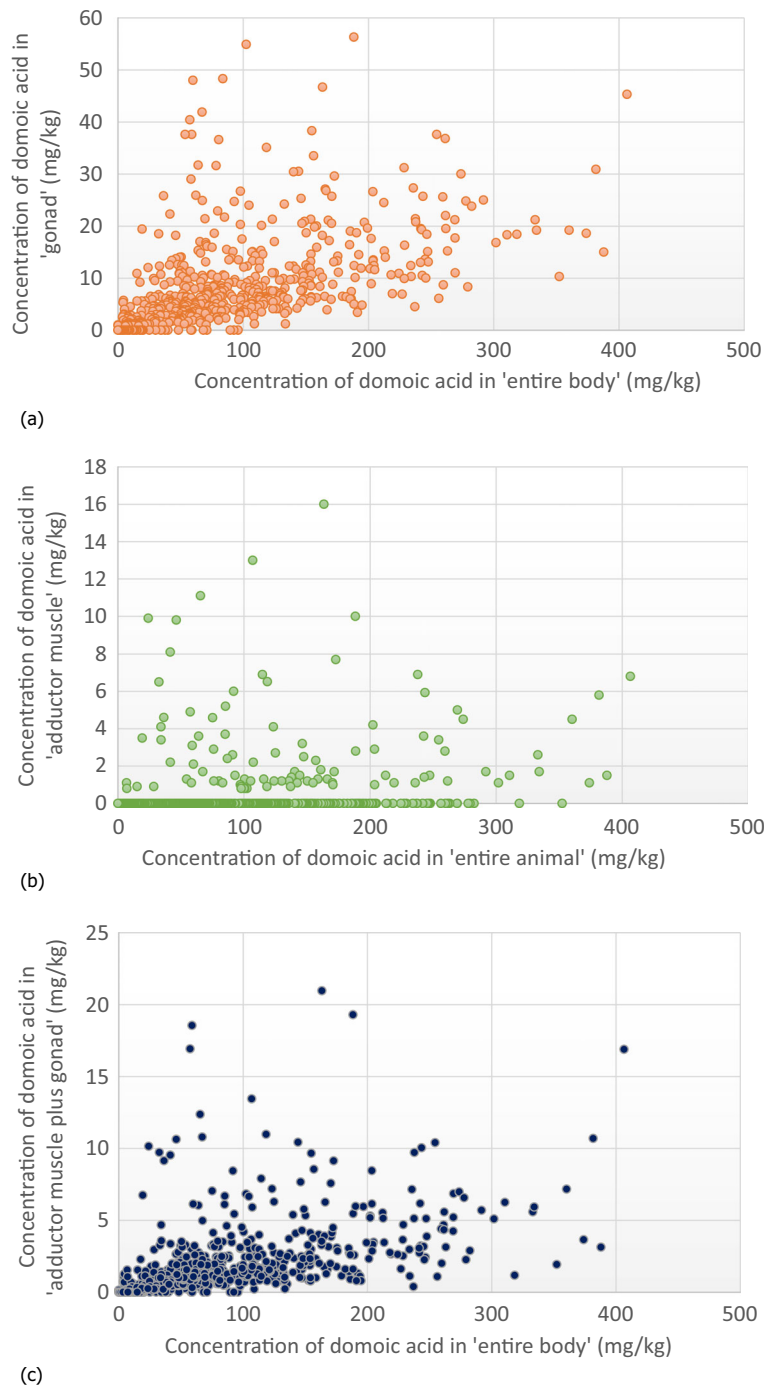
Figure 3: Scatter plot of concentration of domoic acid at the lower bound in (a) 'gonad' and (b) 'adductor muscle' and (c) 'muscle plus gonad' vs. concentration in entire body as reported by Ireland for *Pecten maximus* (pooled)

2.1.4.2. Description of the data submitted by France

France also reported data on *Pecten maximus* only. In this case, data on body parts did not come from the same pool of animals, but from subsamples belonging to the same lot. Once in the laboratory, the lot was divided into several subsamples, each of the latter comprising at least 10 individuals. Subsequently, a pools of 10 scallops was analysed to estimate domoic acid content in the 'entire animal', while other subsamples of the same lot were used to prepare analytical samples of the edible parts ('adductor muscle and gonad' and/or 'adductor muscle' and/or 'gonads') to determine the domoic acid content of these specified edible parts.

From the totality of the data submitted by France (n = 976), concentrations in 'entire animal' and 'adductor muscle and gonad' could be matched for 389 lots that were therefore selected for the current risk assessment (see Figure 4).

For this set of data, the DA concentration in the 'entire animal' ranged at the LB from 0 up to 304 mg/kg, while in 'adductor muscle and gonad' it ranged from 0 up to 13 mg/kg.
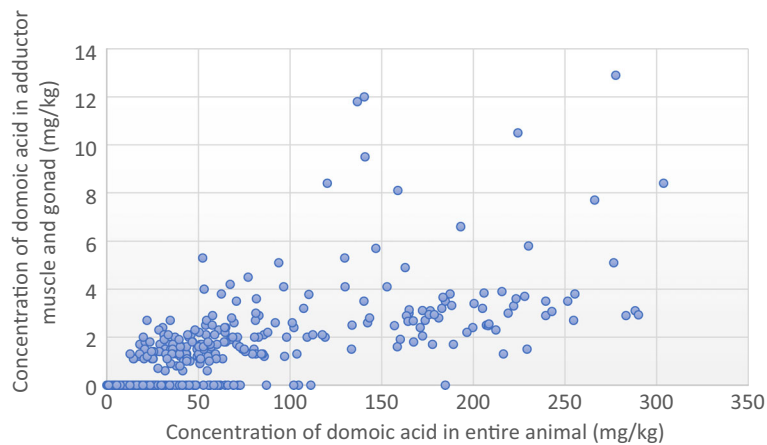
Figure 4: Scatter plot of concentrations of domoic acid in 'adductor muscle and gonad' versus entire animal, in animals belonging to the same lot, as submitted by France

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

The CONTAM Panel calculated the ratio of the toxin 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.

2.1.5.1. Ratios based on toxin levels

For data submitted by Ireland, the calculated UB levels in the 755 samples of entire animal ranged between 0.3 and 407 mg DA/kg. The toxin concentrations in the 97 muscle samples where DA could be quantified varied between 0.8 and 16 mg DA/kg. The range for the ratios was between 0.003 and 0.41. For the 547 quantified samples of gonads, the concentrations varied between 0.8 and 56.3 mg DA/kg. The range for the ratio varied between 0.009 and 1.29. Two samples were excluded because levels in muscle and the remaining part of the scallop were below the LOQ and in the gonad just above. Three other samples with levels in gonads of 5.3, 5.7 and 19.4 mg/kg showed ratios above 1. Based on quantified levels in one or both edible parts, the levels ranged between 0.1 and 21 mg/kg (n = 547). The ratios between the concentration of DA in edible parts (muscle plus gonad) and whole scallop varied between 0.002 and 0.422.

For the data submitted by France, estimated UB levels in whole scallops ranged between 0.2 and 304 mg DA/kg, overlapping with the ranges observed in the data submitted by Ireland. For quantified levels in edible parts (gonad plus muscle), it ranged between 0.6 and 12.9 mg/kg. The ratio between the quantified concentrations of DA in the edible parts (muscle and gonad) and the whole scallop was 0.01 and 0.12.

2.1.5.2. Ratios based on distributions

Several distributions were fitted to the data submitted by France and Ireland accounting for their censoring nature (i.e. levels of one or more edible parts < LOQ in all samples). The analysis was performed for adductor muscle, for gonads and for gonads plus adductor muscle. Since there were enough quantified data and to reduce the uncertainty, samples with non-quantified levels or, in the case of the combined edible parts from Ireland, for both tissues (muscles and gonad) were excluded.

The resulting distributions allowed estimation of the 99th percentile for the ratios and with this also the expected concentration in whole scallops that would still lead to compliant levels for adductor

muscle plus gonad, for adductor muscle or for gonad. The respective analyses are presented in Appendices A.1–A.3.

For answering the first question in the ToR, only samples for which levels in muscle, gonads and other parts were reported could be included in the statistical analysis. Especially for the data submitted by Ireland this meant that only a small part of the data could be used. Considering the relatively small size of the data sets submitted by Ireland and France, the 95th confidence interval was obtained for the 99th percentile 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 6 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 6: Summary of the findings to estimate concentration thresholds in the whole scallop for the most conservative result − estimated ratios based on the data sets[a] submitted by France and Ireland

Toxin	Parts	Data	Ratios		Critical concentrations in the whole scallop (mg/kg)	
			P99	**UB P99**	**at P99**	**at UB P99**
DA	Adductor muscle and gonads	FRIRL	0.110.14	0.120.16	179143	163127
	Adductor muscle	IRL	0.26	0.34	76	59
	Gonads	IRL	0.56	0.83	36	24

FR: France; IRL: Ireland; DA: domoic acid; P99: 99th percentile from the distribution of the ratios and upper bound P99 referring to the upper bound of the 95% confidence interval for the P99 for the ratio; UB: upper bound.

(a): Subset of data used for assessment is described in Sections 2.1.4.1 and 2.1.4.2.

For gonads and adductor muscle combined, it follows that a concentration of 127 mg DA/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 20 mg DA/kg.

For adductor muscle only, a concentration of 59 mg DA/kg in the entire animal should not be exceeded to ensure that the concentration in muscle does not exceed the regulatory limit of 20 mg DA/kg.

For gonads only, a concentration of 24 mg DA/kg in the entire animal should not be exceeded to ensure that the concentration in gonad does not exceed the regulatory limit of 20 mg DA/kg.

The analysis carried out to compare the relationship between concentration levels in gonads plus adductor muscle and those in the entire animal, based on data submitted by Ireland with those submitted by France shows that when robust regression is used, the estimated relationship is very similar with the data from the two different countries (see Figure 5 below).
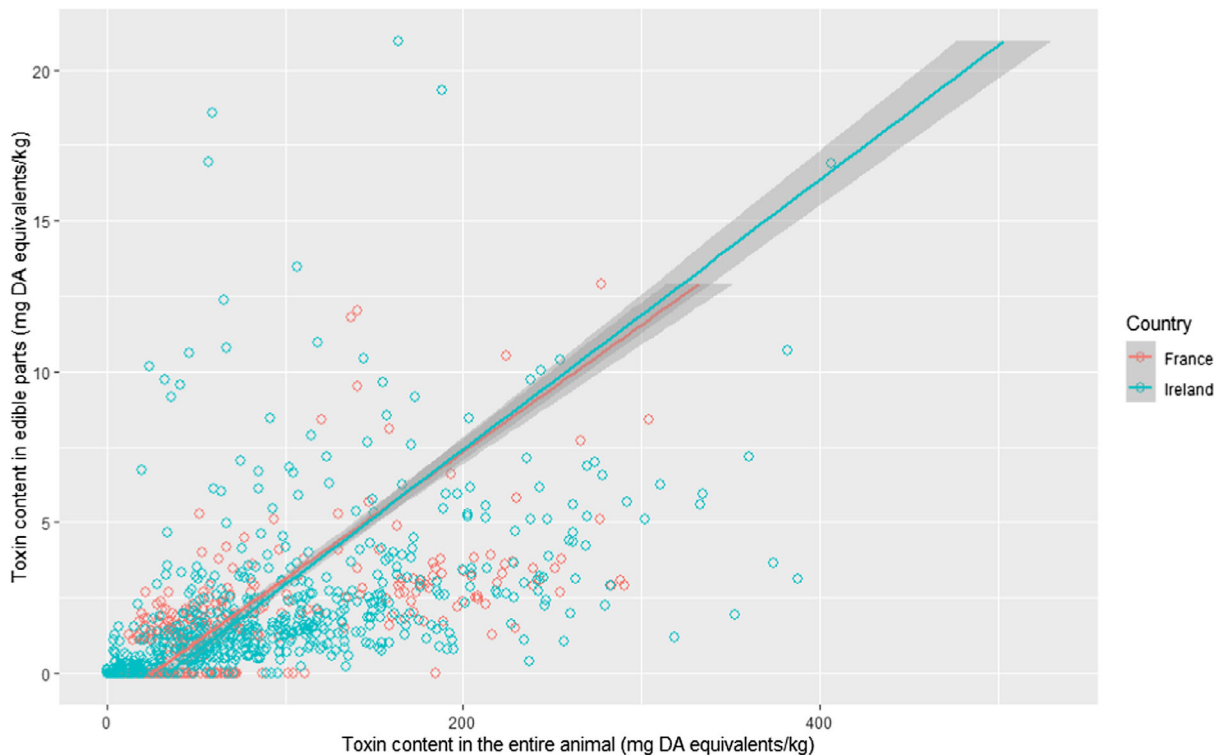
Figure 5: Scatter plot for the lower bound data submitted by France and Ireland together with their corresponding robust regression fits and confidence interval

For all other scallop species than *Pecten maximus*, the data submitted were not sufficient to carry out such statistical analysis.

2.1.6. Public literature 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, dealing with farmed animals with levels likely to be 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.

Campbell et al. (2001) investigated the distribution of DA in tissues from in total 170 scallops (*P. maximus*) from three different regions in Northern Scotland using LC-MS/MS. Mean $\pm$ SE concentrations in muscle, gonad and remaining parts of animals were 0.6 ± 0.114, 9.58 ± 0.722 and 669 ± 45.7 mg DA/kg wet weight, respectively. Toxin concentrations in the latter compartment accounted for 99% of the total DA burden.

Blanco et al. (2002) investigated the depuration of DA in naturally contaminated *P. maximus* in four body parts. The scallops were harvested from the Ría de Pontevedra, Spain and placed in a tank during the depuration study. The authors studied the digestive glands, gonad plus kidney, gills plus mantle plus foot and the adductor muscle. Samples of six individuals were taken at the four different time points studied for the depuration rates. The concentration of DA was determined with HPLC-UV. Of the studied body parts, DA was present for more than 94% in the hepatopancreas at all time points studied. The authors concluded that in their model, there was no evidence for transfer from the most toxic fraction, the digestive gland, to the other fractions during depuration.

Blanco et al. (2006) studied the depuration in *P. maximus* from rafts and natural beds in the Ría de Arousa, Spain. In total, 372 scallops were investigated from three sampling locations at five sampling dates. The scallops were dissected in three groups: the digestive gland, gonad plus adductor muscle plus kidney plus foot and gills plus mantle. The depuration was accelerated with the scallops placed on the rafts. From all the studied samples, the digestive gland counted for 95% of the toxin burden.

Bogan et al. (2006) studied DA concentrations in *P. maximus* from seabed and longline cultures in the Clew Bay at the west coast of Ireland. During a 1-year period at 10 time points, 12 scallop samples were collected and analysed with LC-UV. For each scallop, the hepatopancreas was analysed individually as well as composite samples of gonad and adductor muscle for each sample group and each date. The DA concentration (mean ± SD) in the hepatopancreas was 326.4 ± 169.9 mg/kg for seabed scallops and 313.9 ± 214.6 mg/kg for longline scallops. While the DA concentration in the composite gonad samples was 5.0 ± 2.1 mg/kg for seabed and 3.8 ± 1.9 mg/kg for longline scallops. For all composite samples of adductor muscle, the toxin levels were below the LOD of 0.1 mg/kg. The CV of DA concentration in hepatopancreas of longline scallops ranged from 11.7% to 55.5% whereas that of seabed scallops ranged from 17.8% to 49.5%. The mean CV of DA concentration in hepatopancreas of longline was 42.3% and seabed scallops 31.0%.

Bogan et al. (2007a) dissected from 69 sampling sites within an area located at the southeast coast of Ireland, 12 scallops (*Pecten maximus*) from each sampling station (total n = 782). For each type of dissected tissue, composite samples were prepared for analysis. From the pooled samples of each of the 69 stations the concentrations (mean ± SD) were 40.6 ± 30.8 mg/kg (whole tissue), 345.7 ± 299.6 mg/kg (hepatopancreas), 15.1 ± 10.8 mg/kg (gonad), 5.4 ± 5.7 mg/kg (adductor muscle) and 15.7 ± 14.1 mg/kg (remainder). Corresponding CVs were for whole scallop 75.9%, hepatopancreas 86.7%, gonad 71.3%, adductor muscle 106.2% and remainder 89.7%. This represents the variation between the pooled samples from the 69 locations and not the individual scallops.

In another study, Bogan et al. (2007b) reported DA levels in *Pecten maximus* scallops in each of the size groups with lengths small (70–85 mm), medium (85–100 mm), large (100–115 mm) and very large (> 115 mm) harvested at Clew Bay at the west coast of Ireland. The DA concentration was determined in the hepatopancreas of 12 individual scallops and in composite samples of gonad and adductor muscle, in triplicate for each size group. The hepatopancreas accounted for more than 95% of the toxin burden followed by the gonad with 1–2% and only trace levels in the adductor muscle. The highest DA concentration recorded in the hepatopancreas of an individual scallop was 1348.1 mg/kg while the highest concentration in gonad composite sample was 9.59 mg/kg and that in the adductor muscle composite sample did not exceed the LOQ of 1 mg/kg. The CV of the DA concentration in the hepatopancreas in the sample batches ranged from 12.5% to 40.3% for very large scallops and from 22.7% to 82.5% for small scallops.

Bogan et al. (2007c) studied eight scallop fishing locations near the Isle of Man. For each location at three occasions, 12 individual scallops (*Pecten maximus*) were collected. For each scallop the total tissue weight was recorded and after dissection the weight of the hepatopancreas, gonad and adductor muscle. The DA concentration was determined in the individual hepatopancreas samples and in the composite gonad and adductor muscle sample. The highest mean DA concentration in hepatopancreas was 296.3 mg/kg, in gonad 27.8 mg/kg and in adductor muscle 7.3 mg/kg. The CV of the DA concentrations in the hepatopancreas in the sample batches from the different fishing locations ranged from 16.1% to 70.0%.

Amzil et al. (2009) reported first detection of DA in *Pecten maximus* harvested from non-CPAs in Bay de Seine, Normandy. A DA concentration exceeding the limit of 20 mg/kg in whole animals was detected at the beginning of the monitoring and intended scallop fishing season and continued, for 10 months. Causative *Pseudo-nitzchia* phytoplankton was not detected through that time, with authors speculating that an algal bloom had occurred during the closed season before monitoring began, and describing the prolonged presence in scallops due to slow depuration. Additional DA contamination of scallops was detected further West (Brittany) and then further Southwest (Loire-Atlantique) along the French coast following this event. The distribution of DA in different scallop organs was reported for 3 sample series, with no toxin detected in muscle in any of the three. In two of those, DA concentrations in whole animals were less than the regulatory limit at 15.2 and 4.3 mg/kg, respectively, and 100% of the DA was reported to be within the digestive gland. In the other sample series with a whole animal level of 28.3 mg/kg, 96.6% of DA was reported in the digestive gland with the remaining 3.4% in gonad and nothing exceeding the detection limit in muscle. This work also graphically reports inter-individual animal variability in 5 sample series of 10 scallops with a notable finding of greater inter-individual variability for higher contamination levels. A homogenate of 10 animals harvested from the same area at the same time was reported to yield a mean DA result close to the mean of each of the 10 individual animal concentrations.

Mauriz and Blanco (2010) undertook an *in vitro* investigation of the molecular basis for prolonged depuration of DA from the enteric tract of *Pecten maximus* earlier described by Blanco et al. (2006). The 2010 study concluded that DA was retained in the cytosol of enteric cells not bound to any

substance or receptor. This conclusion differed from the cell receptor binding with which prolonged DA depuration had been associated in pacific razor clams (*Siliqua patula*), and curiously correlated with unbound cytosolic DA in mussels which do not show prolonged DA depuration. The authors of the 2010 paper therefore hypothesised reduced expression or functionality of cell membrane transporter excretion mechanisms involved in the excretion of DA from scallop enteric cells.

Reboreda et al. (2010) applied various post-harvest interventions and examined their ability to decrease the content of biotoxins in bivalve molluscs containing concentrations exceeding the regulatory limit on whole-animal basis. In the case of DA in the scallop species *Pecten jacobaeus* with an initial whole-animal mean concentration of 112.5 mg/kg, three interventions were studied. Those were examined both when applied as individual processes, and when combined sequentially. The processes comprised one that the authors refer to as evisceration or hepatopancreas ablation, which included removal of the digestive system and therefore 'shucking' as defined in this Opinion presumably to produce the muscle plus gonad edible part. Other processes included a specific thermal processing regimen shown to have PSP-detoxification utility, and freezing to −20°C and then thawing. Considered individually, all three processes produced significant ($p < 0.05$) reduction in DA content with evisceration being the only process which alone produced a reduction below the legal limit to reach a mean of 1.98 mg/kg. Thermal processing alone, and freezing alone reached 35.33 and 50.00 mg/kg, respectively, thereby not enough to achieve compliance. DA reduction from freezing/ thawing was more profound when assessed on scallop-flesh alone, with less apparent reduction when post-thaw intervalvular fluid was included in the analysis. Combining these detoxification processes produced additive reductions with evisceration becoming even more effective when combined with thermal processing to bring DA from 112.5 mg/kg below the LOD (1.6 mg/kg). Freezing/thawing and then thermal processing also reduced the concentration below the legal limit to a mean of 7.25 mg/kg. The authors concluded that evisceration is the best option to detoxify DA from scallops, but should be combined with some other process releasing water (and thereby anything in solution such as DA) from the tissue to give adequate public health assurance when the initial DA concentration is very high.

Smith et al. (2006) examined different storage conditions during transport or storage of scallops (*P. maximus*) and particularly gonads, and the apparent effect on DA concentration as analysed on wet-weight basis. Despite significant variation amongst the DA concentrations of subsamples subjected to particular storage treatment, the study shows the toxin concentration to be higher when sample transport before analysis is longer and warmer. Samples stored for 2–3 days at 12°C had a significantly higher concentration than samples stored at 4°C and analysed within 48 h. The authors consider natural post-harvest dehydration of the tissues to create an apparent increase in concentration as the major contributor to this effect, and also comment on ongoing production of toxin by plankton present in the gonadal intestinal loop. The study also examined typical extraction reagents and found storage in aqueous methanol to be associated with a significantly lower concentration of DA than shown when extracts were stored in refrigerated citrate buffer.

The dynamics of accumulation and excretion of DA were studied *in vitro* by Wohlgeschaffen et al. (1992) in one mussel species (*Mytilus edulis*) and one scallop species (*Placopecten magellanicus*). DA accumulation in mussels was shown to be relatively inefficient with much of the ingested toxin lost to solution during mollusc feeding. The scallops showed slower accumulation than mussels with dramatic inter-animal variation in accumulation efficiency. Scallops however retained the DA dramatically longer than mussels following removal of the toxin source, with consequent implications for whole-animal marketing.

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 domoic acid. They were obtained from pooled samples used for routine monitoring. The data offered the opportunity to study the distribution among the tissues but not between individual scallops. However, data on variation in the levels between scallops have been published. Suitable data for answering the questions from the EC were obtained for only one scallop species, *Pecten maximus* 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 domoic acid 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 toxins are mainly present and which generally leads to the removal of most of the DA. In the case of *Pecten maximus*, both the adductor muscle and the gonads, or their combination, are considered as the edible parts. The data sets obtained from France and Ireland allowed to some extent addressing the question on the distribution of the toxin 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 *Pecten maximus*. Since there was a sufficient number of quantified levels, only these were used for the statistical analysis in order to reduce the uncertainty. For the sum of muscle and gonads, this applied to samples where at least one of the tissues showed a level of DA above the LOQ. For each sample, the ratios between edible parts (muscle, gonad or the sum of these) and entire animals were calculated, for levels in muscle or gonad provided by Ireland or those on edible parts provided by France, resulting in single data points. For the calculated levels in edible parts, based on data on individual tissues from Ireland, an interval was obtained for parts of the samples. 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 6 (Section 2.1.5).

In the data set on 755 samples of *Pecten maximus* obtained from Ireland, toxin concentrations of 'adductor muscle', 'gonads' and the combined 'hepatopancreas and mantle' were determined. Based on the weights provided for muscle, gonads and the other tissues (statistics reported in Annex Table A5), it was possible to calculate the toxin level in the entire animal (as reported in Section 1.3.2.4 on methodology used for the assessment). The quantified levels in muscle varied between 0.8 and 16 mg/kg, those in gonads between 0.8 and 56.3 mg/kg. Based on these levels, the ratio between muscle and whole scallop varied between 0.003 and 0.41 and for gonads between 0.009 and 1.29. For muscle, this resulted in an LB level for the P99 in whole scallops of 59 mg/kg and for gonads of 24 mg/kg.

For muscle plus gonads, the data submitted by Ireland showed levels between 0.1 and 21 with ratios between 0.002 and 0.42. This resulted in an LB level for the P99 in whole scallops of 127 mg/kg. The quantified levels submitted by France on combined muscle and gonads ranged between 0.6 and 12.9 mg/kg, the ratios between 0.01 and 0.12. Based on the distribution of the ratios, an LB level for the P99 in whole scallops of 163 mg/kg was calculated. It should be noted that even when sold together, muscle and gonads may be consumed in separate portions.

Overall, this implies that the lowest calculated level is the obtained for data on gonads, being 24 mg/kg, which is the level in whole scallops that would ensure that levels in gonads and thus also other parts remain below the regulatory limit of 20 mg/kg.

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 domoic acid 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.

No data on individual scallops were provided by Member States. Therefore, this information was obtained from the literature (Bogan et al., 2007a,b,c), in order to answer this second question. However, in most cases this applied only for levels in the hepatopancreas and not in whole scallops or edible parts. For hepatopancreas CVs of 0.40, 0.70 and 0.83 were selected, for adductor muscle a CV of 1.06, the highest CV reported. It was noted that the latter CV was based on the variation between levels in pooled samples collected from 69 different sites within a certain area. Assuming that these CVs also apply to whole scallops, it was estimated for a number of true levels around the limit of 20 mg DA/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 6 (and Appendix B), in the range of 18–22 (CV of 0.40), or 15–27 (CV of 1.06) mg/kg, this would require the pooling of more than 30 scallops in an analytical sample. This is 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. Based on the highest CV, sample sizes of 10 or less would give a 95% correct classification at actual levels of ≤ 10 or ≥ 37 mg/kg (i.e. 50% or 185% of the regulatory limit, respectively). In these calculations, the potential application of a measurement uncertainty before deciding on non-compliance was not taken into account. Assuming a measurement uncertainty of 30%, a value based on an interlaboratory validation of the HPLC/UV method in which six matrices have been included and in which an interlaboratory validation range of 14–28% was observed (EURLMB, 2021), only test results higher than 29 mg DA/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.
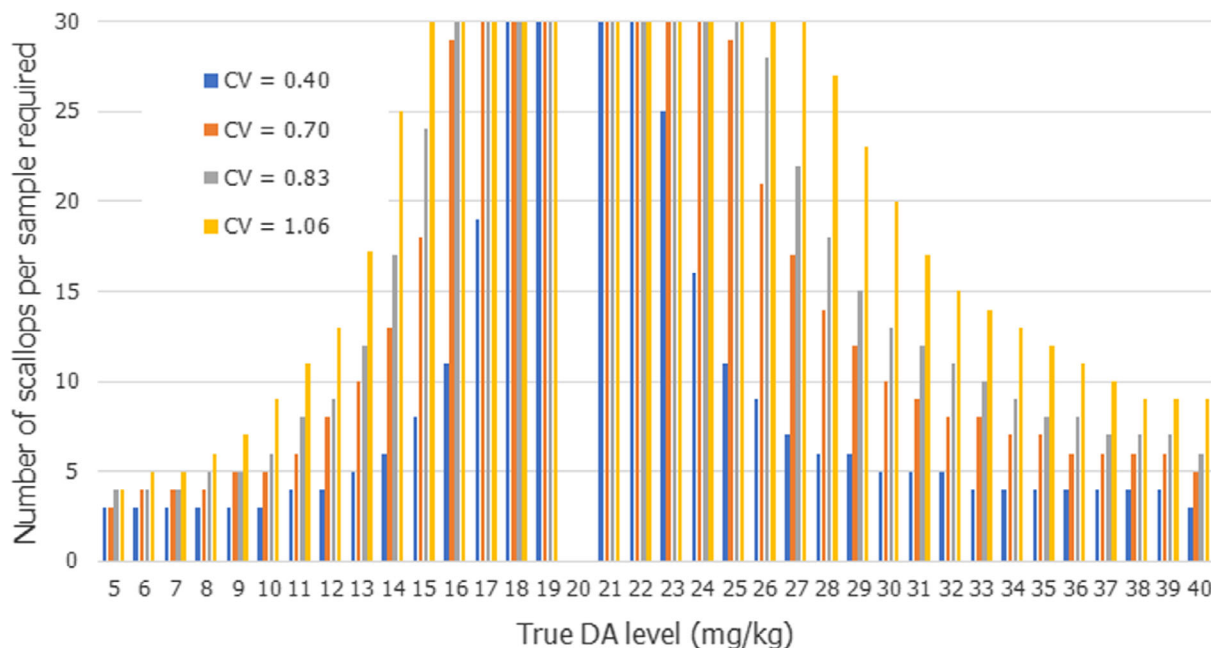
Numbers above 30 were not estimated and are presented as 30. Coefficients of variance (CVs) are based on data reported in the literature for domoic acid levels in hepatopancreas (0.40, 0.70 and 0.83) and adductor muscle (1.06) of *Pecten maximus*.

Figure 6: 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 noncompliance (if level above the regulatory limit), at a given concentration in the area or lot

As already mentioned, data on whole scallops, adductor muscle or gonads, or adductor muscle plus gonads were not available. It is evident that the major part of the toxin is in the hepatopancreas but whether this implies that CVs for whole scallops and edible parts are similar, is unclear.

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 Ireland (see Annex, Table A5) was 28 g. Based on the available data, an average weight of edible parts per scallop of 28 g was assumed. As mentioned in Section 1.2.7, the ARfD for DA of 30 µg/kg bw is based on an intake of 900 µg/kg bw causing mild effects in humans and a UF of 30. This ARfD is equivalent to an absolute amount of 1,800 µg DA for a person of 60 kg bw. This implies that consumption of edible parts of four scallops with a level equal to the regulatory limit of 20 mg/kg, would result in an intake of 2,240 ($28 \times 4 \times 20$) µg DA, clearly exceeding the ARfD. Gonads show much lower weights and a much larger number with a level equal to the regulatory limit is required to exceed the ARfD.

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); however, it was focused on the quantification of the uncertainties related to the sampling strategy.

2.3.1.   Quality of the obtained data

For answering the questions in the ToR, EFSA took into account mostly results obtained with the recommended HPLC-UV method.

For the majority of the data (2,265 results reported by Ireland), a recovery of 109% was reported and results were already corrected for that. For 976 results submitted by France, information on recovery was not available and concentration values were thus not corrected for recovery. This is a source of uncertainty.

It is expected that this would affect the DA levels in the non-edible parts more than those in muscle and gonads, since recoveries in the non-edible parts are in practice lower *due to matrix effe*cts. This implies that the observed ratios of levels in edible parts and whole scallops might likely be over or underestimated when results are not corrected for recovery.

The recovery may also affect the reported 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 is unlikely to affect 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.

Based on the recovery reported by Ireland, it is noted that not correcting for recovery could result in overestimation of the levels and thus in false non-compliant results. However, it is unclear if recoveries could also be lower than 100% and as such result 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 *Pecten maximus*, i.e. data on levels in whole scallops or different parts of the scallops. It remains unclear if the results could be extrapolated to other species.

2.3.3.   Ratios between levels in edible parts and whole scallops

Data obtained on DA 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 (89%) and, to a lesser extent, gonads (39%) were below the LOQ. As a result, ratios on measured levels were only available for part of the samples but overall, the numbers were high enough to use only data on measured levels. Only for combining the data on muscle and gonads, LC-UV data were used in case the other tissue contained a quantified level. In general, the obtained ranges were rather small. On the other hand, for the data from France, levels in the edible parts were measured in a different subsample as those in whole scallops. However, it was noted that the data from Ireland on muscle and gonads measured separately showed a similar distribution as those from France where levels in the combined muscle and gonads were measured.

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 variation is determined by the variability observed in the reported data.

Considering *Pecten maximus*, levels in gonads and muscles combined, the calculated P99s were 179 and 143 mg/kg for the data submitted by France and Ireland, respectively. 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 0.12 and 0.16 for the data from France and Ireland, respectively. This highest ratio of 0.16 corresponds to a critical concentration in whole scallops of 127 mg DA/kg. Of the samples with detectable levels in edible parts, 14 pooled samples showed a ratio higher than the 0.16, with ratios up to 0.42.

For DA in adductor muscle from *Pecten maximus* (pooled samples), a critical concentration of 76 mg/kg was calculated (P99). The upper bound of the 95% confidence interval for the ratio was 0.34, resulting in a critical level of 59 mg/kg in the entire animal. Due to the smaller number of samples with quantified levels as compared to gonads, this level is lower than that for the sum of muscle and gonads, despite the lower levels in adductor muscle. Only 1 pooled sample with detectable levels in muscle exceeded this ratio of 0.34, being 0.41.

For DA in gonads from *Pecten maximus* (pooled samples), a critical concentration of 36 mg/kg in was calculated (P99). The upper bound of the 95% confidence interval for the ratio was 0.83, resulting in a critical level of 24 mg/kg in the entire animal. Three pooled samples with detectable levels exceeded this ratio of 0.83 with ratios up to 1.3.

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

In the absence of data on individual scallops provided by the member States, it was decided to use data reported in the literature. Most of these describe the variation in the hepatopancreas, showing the highest levels within the scallop. One paper (Bogan et al., 2007a) showed CVs for different tissues, with the highest CV for the adductor muscle. As a worst case this CV of 106% was used in the calculations of the number of samples required in an analytical sample. It was noted that this CV actually applied to the variation between 69 different samples of 12 scallops each within a certain area, rather than individual scallops. It most likely results in an underestimation of the CV because of the pooling of samples. The other assumption was that the variation in the hepatopancreas is representative for that in whole scallops which adds to the uncertainty.

3. Conclusions

Scallops can be contaminated with shellfish toxins, such as domoic acid. Shucking will remove the most contaminated parts (hepatopancreas) and thus most of the toxins. For DA in *Pecten maximus* and *Pecten jacobaeus* this practice is allowed if levels in edible tissues are below the regulatory limit.

- EFSA received data from several member states, but only for one species, *Pecten maximus*, were the data suitable to answer one of the two questions in the terms of reference from the European Commission. These were provided by only two member states, France and Ireland and came from routine monitoring of scallops. In some cases, the edible parts were only analysed if levels in whole scallops (France) exceeded the regulatory limit of 20 mg DA/kg.
- Compared to the edible parts, which are the adductor muscle and gonad, the major part of the DA was present in the non-edible parts of the scallops that are removed during shucking. Of the data provided by Member States, 3% of the gonads measured exceeded the regulatory limit but none of the muscle samples, whereas for whole scallops 73% were not compliant.
- In the case of gonads, levels and the percentage of samples containing detectable levels (mean UB 4.5 mg/kg, 39% left censored) was higher than for adductor muscle (mean UB 0.7 mg/kg, 89% left censored). As a result, ratios between levels in gonads and whole scallops were higher than those for muscle or the two tissues combined.
- In order to ensure that the level in gonads plus muscle does not exceed the regulatory limit of 20 mg/kg, statistical analysis showed that the level in whole scallops of *Pecten maximus* should not exceed 127 mg DA/kg.
- In order to ensure that the level in the adductor muscle does not exceed the regulatory limit of 20 mg/kg, statistical analysis showed that the level in whole scallops of *Pecten maximus* should not exceed 59 mg DA/kg.
- In order to ensure that the level in the gonads does not exceed the regulatory limit of 20 mg/kg, statistical analysis showed that the level in whole scallops of *Pecten maximus* should not exceed 24 mg DA/kg.
- In the absence of data on levels in individual scallops, the variance reported in literature was used to calculate the number of scallops required in a sample to ensure with 95% confidence on whether the level in scallops in an area or lot is correctly predicted to be compliant/non-compliant. This showed that for the highest reported coefficient of variance (CV), 10 scallops per sample would be sufficient if levels the area/lot were two-fold below or above the regulatory limit (< 10 mg/kg or > 37 mg/kg). However, to predict with a 95% certainty for levels between 15 and 27 mg/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 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 edible parts of four scallops with a level at the regulatory limit of 20 mg/kg may result in exceedance of the acute reference dose for DA of 30 μg/kg bw.

4.   Recommendations

- The CONTAM Panel recommends an update of the full risk assessment (hazard and exposure) on domoic acid.
- For evaluation of ratios of toxins in different body parts of scallops, more data are needed on the levels of domoic acid in edible parts vs. whole scallops of various species.

Documentation provided to EFSA

Data on occurrence of domoic acid in scallops were kindly provided by: Dave Clarke of the Shellfish Safety Department of the Marine Institute (Ireland) on 29 May 2020; Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer) on 29 May 2020; 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

AM	adductor muscle
ANSES	Agency for Food, Environmental and Occupational Health and Safety
ARfD	Acute Reference Dose
ASP	Amnesic shellfish poison
AZA	azaspiracid
bw	body weight
CA	competent authority
CONTAM	EFSA Panel on Contaminants in the Food Chain
COT	Committee on Toxicity
CPA	classified production area
CV	coefficient of variance
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 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
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
NOEL	no-observed-effect level
OA	okadaic acid
PP	protein phosphatase
PSP	paralytic shellfish poisoning
SD	standard deviation
SOP	standard operation procedure
TDI	tolerable daily intake
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 edible parts and entire animal

A.1. Ratio between edible part (adductor muscle plus gonads) and entire animal

A.1.1. Introduction

The data submitted by Ireland and France for DA 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 will remain under the legislative limit of 20 mg DA/kg.

A.1.2. Ratio between edible part and entire animal for domoic acid

The concentrations reported by Ireland for adductor muscle (AM) and gonads (G) were combined 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:

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

where Conc refers to concentration and Weight to the weight of the animal part. For the data submitted by France, the concentration of the edible parts and entire animal were reported for subsamples of each lot (more information provided below). The resulting concentrations of the edible part of the animal and the entire animal were censored if one of the parts were < LOQ. The concentration of DA in the edible part was divided by the concentration in the entire animal.

The ratio is then defined as:

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

The lower bound for the numerator (concentration of edible parts) and denominator (concentration of the entire animal) is defined the lower bound of the ratio $\left(\text{Ratio [DA] LB} = \frac{\text{Conc [EP] LB}}{\text{Conc [EA] LB}} \right)$, while the upper bound levels for the numerator and the denominator were used to define the ratio's upper bound $\left(\text{Ratio [DA] UB} = \frac{\text{Conc [EP] UB}}{\text{Conc [EA] UB}} \right)$. In the case that the denominator is zero, then a value smaller than the minimum concentration value for the lower bound different from zero is used in order to avoid an infinite lower bound ratio. The final data used are a censored interval defined by a lower and upper bound. It is clear that the resulting sum would generate censored observations, unless for the specific edible parts of the scallop were quantifiable. For Ireland, the samples used were observations for pooled scallops, while for France, the samples in edible and entire animals came from different subsamples from the same lot.

A.1.2.1. Data submitted by France

The data on 389 pooled *Pecten maximus* scallops submitted by France were analysed. It is important to highlight that different subsamples of pooled scallops were used to measure the concentration in edible parts and the entire animal but that the subsamples were coming from the same lot and each pool consisted of 10 scallops.

The data plotted before were now used to fit models that account for censoring considering different distributions for the ratio and the best fitted distribution and the characteristics on the curve fit are presented below.

[1] "The AIC value for the log-normal fit is: −992.6"
[1] "The AIC value for the exponential fit is: −931.8"

```
## [1] "The AIC value for the gamma fit is: −985.3"
## [1] "The AIC value for the weibull fit is: −975.6"
## [1] "The AIC value for the normal fit is: −837.1"
## [1] "The AIC value for the logistic fit is: −873"
## Fitting of the distribution 'lnorm 'By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## meanlog −3.8087655 0.04070507
## sdlog 0.6663829 0.03132593
## Loglikelihood: 498.3238 AIC: −992.6476 BIC: −984.7204
## Correlation matrix:
## meanlog sdlog
## meanlog 1.0000000 −0.2374139
## sdlog −0.2374139 1.0000000
```

Figure A.1 shows the distribution of the ratios between the domoic acid levels in edible parts and entire animals

Empirical and theoretical CDFs

CDF: cumulative distribution function.
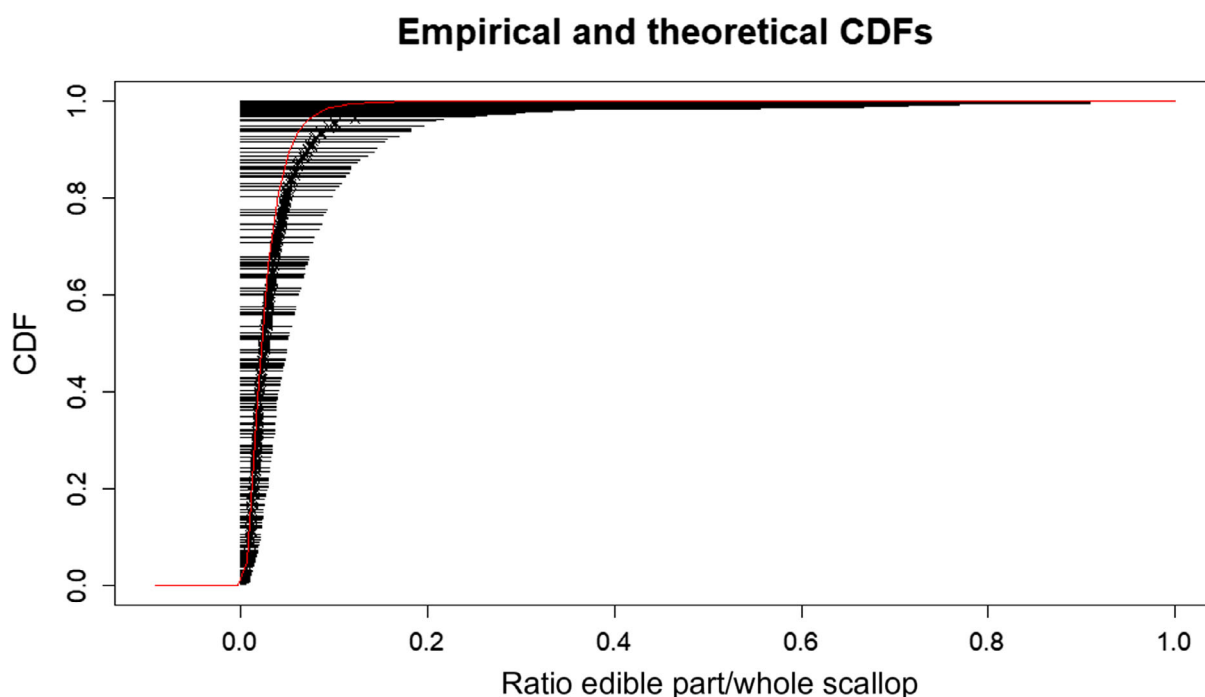
Figure A.1: Distribution of the ratios between the levels in edible parts (muscle plus gonad) and whole scallop for pooled scallops from the same lot submitted by France, for each sample ranging between the ratio based on lower bound and those based on upper bound levels

Considering that a large number of reported results for at least one of the edible parts was quantifiable, it was decided to focus only on those observations for which the lower bound of the edible part was above zero, consequently reducing the uncertainty in the estimates. The analysis was performed using only observations for which the lower bound of the ratio was larger than zero, leaving 221 out of 389 observations to estimate the best distribution fitting the data. The resulting distribution is shown in Figure A.2.

```
## [1] "The AIC value for the log-normal fit is: −1,177.7"
## [1] "The AIC value for the exponential fit is: −1,056.9"
## [1] "The AIC value for the gamma fit is: −1,165.5"
## [1] "The AIC value for the weibull fit is: −1,147.1"
## [1] "The AIC value for the normal fit is: −1,078.3"
```

[1] "The AIC value for the logistic fit is: −1,095.3"
Fitting of the distribution 'lnorm 'By maximum likelihood on censored data
Parameters
estimate Std. Error
meanlog −3.5717696 0.03996614
sdlog 0.5941393 0.02825997
Loglikelihood: 590.8374 AIC: -1177.675 BIC: −1170.879
Correlation matrix:
meanlog sdlog
meanlog 1 0
sdlog 0 1

Empirical and theoretical CDFs
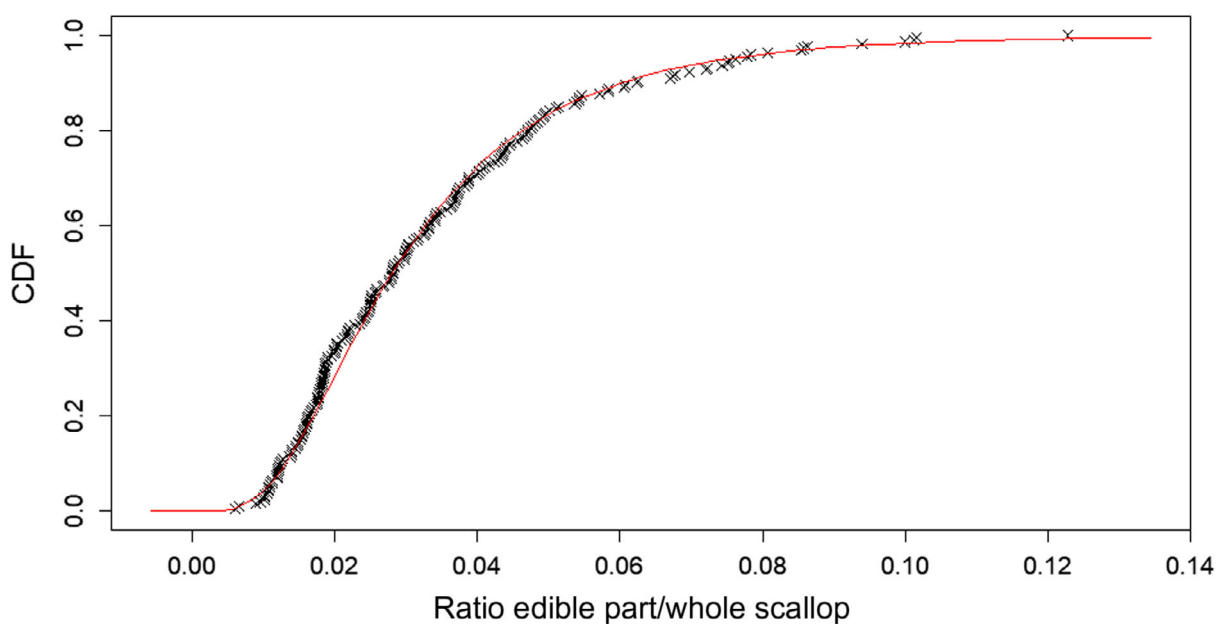
CDF: cumulative distribution function.

Figure A.2: Distribution of the ratios between the levels in edible parts (muscle plus gonad) and whole scallop for pooled scallops from the same lot submitted by France, for each sample ranging between the ratio based on LB levels and those based on UB levels

Samples with detected levels for domoic acid in the edible parts show ratios with lower bounds larger than zero. The ratios ranged between 0.01 and 0.12. The mean ratio for these data is 0.034 and the median 0.028.

A.1.2.2.   Data submitted by Ireland

The data submitted by Ireland on *Pecten maximus* were analysed in order to have enough information to estimate the parameters and to select the best fitting distribution to the censored data.

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 following the observed data.

[1] "The AIC value for the log-normal fit is: 2,103.1"
[1] "The AIC value for the exponential fit is: 2,312.7"
[1] "The AIC value for the gamma fit is: 2,289.6"
[1] "The AIC value for the weibull fit is: 2,313.6"
[1] "The AIC value for the normal fit is: 3,279.3"
[1] "The AIC value for the logistic fit is: 2,775.1"
Fitting of the distribution 'lnorm 'By maximum likelihood on censored data

```
## Parameters
## estimate Std. Error
## meanlog −3.8471933 0.03365856
## sdlog 0.8038461 0.02438048
## Loglikelihood: −1,049.547 AIC: 2103.093 BIC: 2,112.341
## Correlation matrix:
## meanlog sdlog
## meanlog 1.00000000 −0.06680809
## sdlog −0.06680809 1.00000000
```

Figure A.3 shows the distribution of the ratios between the levels in edible parts and entire animals.

Empirical and theoretical CDFs
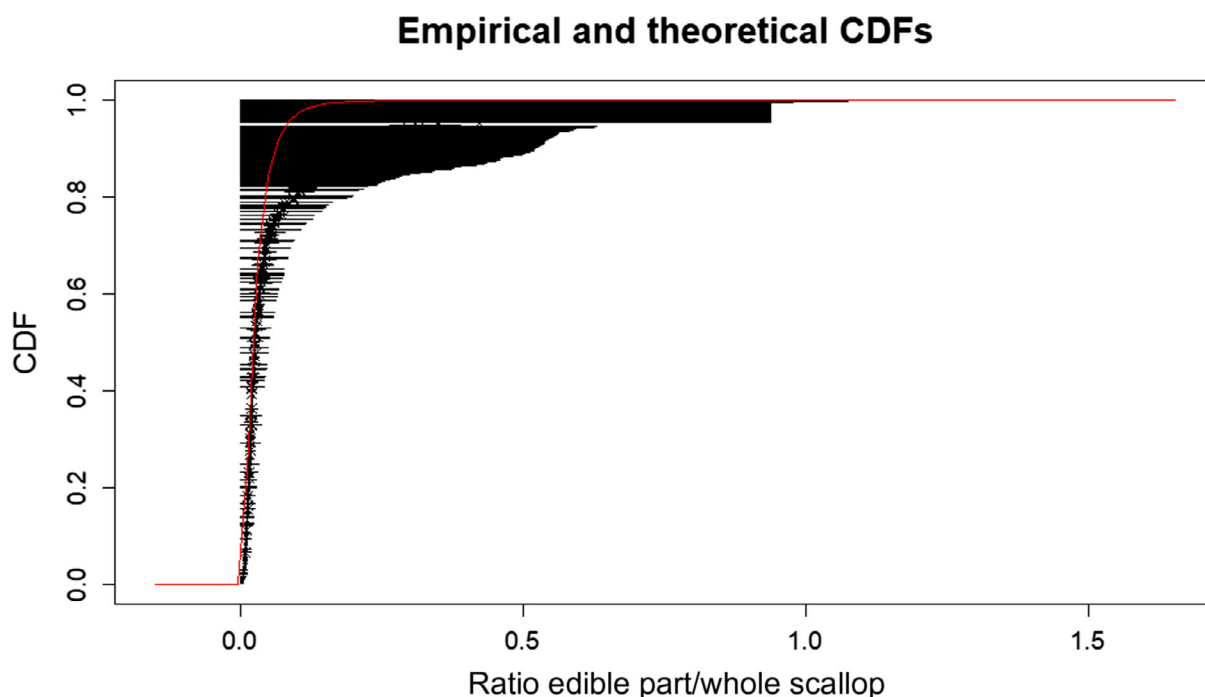
CDF: cumulative distribution function.

Figure A.3: Distribution of the ratios between the levels in edible parts (muscle plus gonad) and whole scallop for pooled scallops from Ireland, for each sample ranging between the ratio based on lower bound levels and those based on upper bound levels

Similarly, as for the data provided by France a large number of observations reported quantified concentrations for one or both of the parts (adductor muscle and/or gonads). Thus, the analysis was performed using only observations for which the lower bound of the ratio was larger than zero. In addition, two samples were removed with levels in gonads just above the LOQ but in other parts below the LOQ, thus resulting in high ratios. The analysis was performed considering 547 out of 755 observations to estimate the best distribution fitting the data. The resulting distribution is shown in Figure A.4.

```
## [1] "The AIC value for the log-normal fit is: 1,994.7"
## [1] "The AIC value for the exponential fit is: 2,228.8"
## [1] "The AIC value for the gamma fit is: 2,192.3"
## [1] "The AIC value for the weibull fit is: 2,226.5"
## [1] "The AIC value for the normal fit is: 3,017.4"
## [1] "The AIC value for the logistic fit is: 2,597.2"
## Fitting of the distribution 'lnorm' By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## meanlog −3.7769906 0.03360594
## sdlog 0.7793144 0.02391619
```
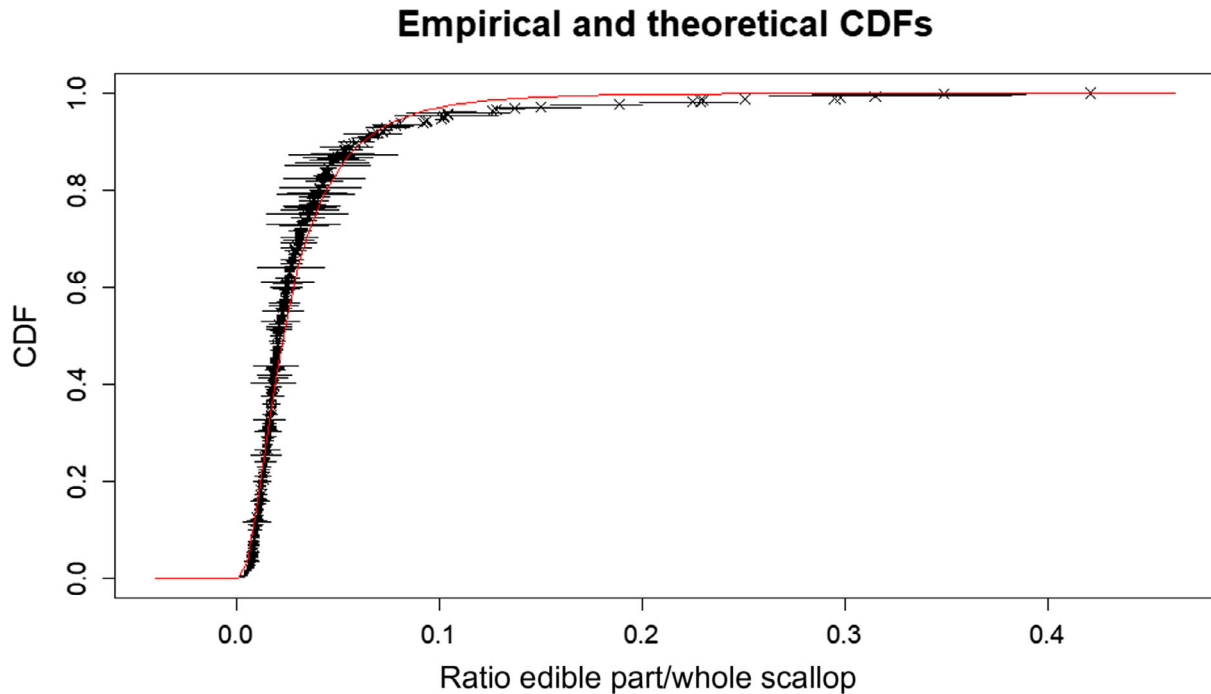
```
## Loglikelihood: −995.3506 AIC: 1994.701 BIC: 2003.31
## Correlation matrix:
## meanlog sdlog
## meanlog 1.000000000 −0.008216381
## sdlog −0.008216381 1.000000000
```

Empirical and theoretical CDFs

CDF: cumulative distribution function.

Figure A.4: Distribution of the ratios between the levels in edible parts (muscle plus gonad) and whole scallop for pooled scallops from Ireland, for each sample ranging between the ratio based on lower bound and those based on upper bound levels

The samples with detected levels for both parts or those for which the lower bound is larger than zero (i.e. the concentration of DA was at least for one part quantifiable) were used to estimate the distribution of the ratios, considering that there is enough information about the distribution of ratios for the edible part. The ratio for the data from Ireland varied between 0.002 and 0.42, with a mean of 0.031 and a median of 0.023.

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 in Figure A.5.
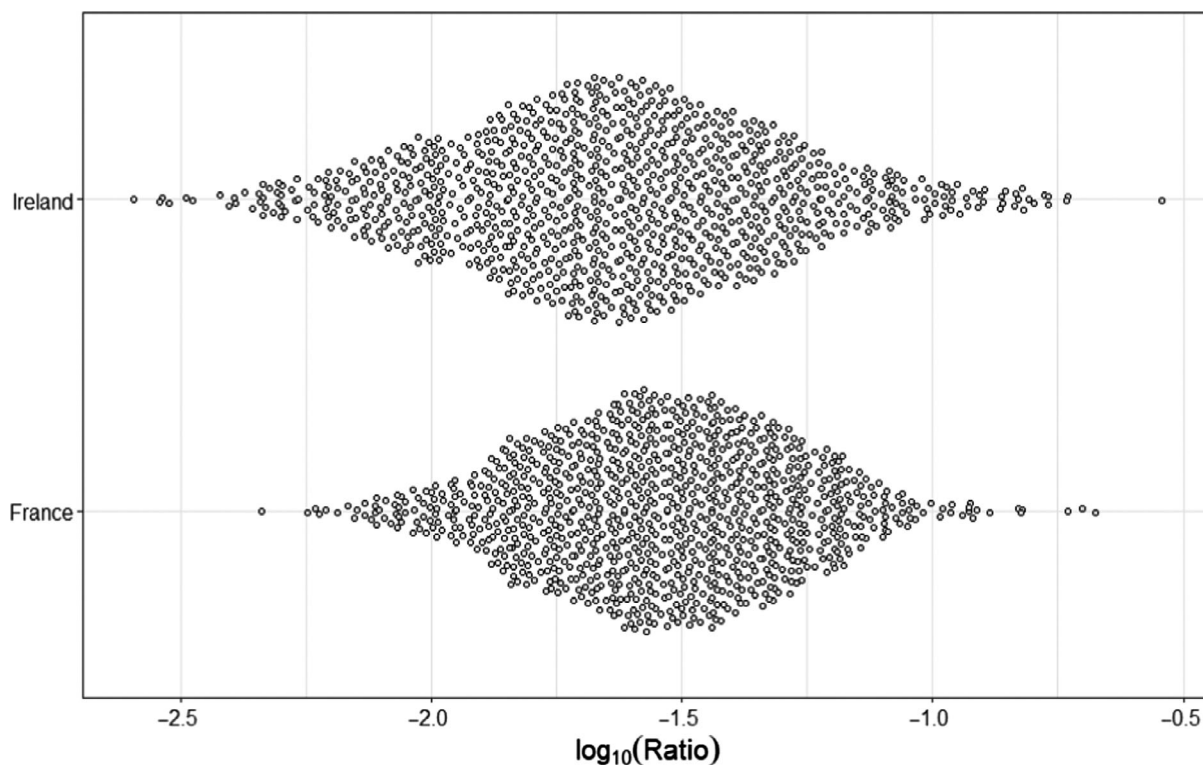
Figure A.5: Distribution of the ratios for data from France and Ireland

The resulting fitted distributions are compared in Figure 6, showing that the data submitted by France have similar mean and median ratios of concentrations than those obtained from the data submitted by Ireland. The data reported by Ireland show larger upper bound values for the censored intervals than those for the data reported by France, but the difference is negligible. It should be highlighted how close the estimated central tendencies of the distributions are. The ratio of the medians for the data from Ireland compared to France is 0.814, which is clearly indicating how close the central tendencies of both distributions are.

Another way to demonstrate that both data sets show a similar pattern is to overlay the data for both countries in a scatter plot together with the robust regression fitted models in order to downward the effect of potential outliers and with this explore if the relationship shown by both data sets are similar. It is shown in Figure A.6 that the overall trends down weighting effect of potential outliers are similar for both data sets with overlaying confidence bands along the entire range of concentrations.
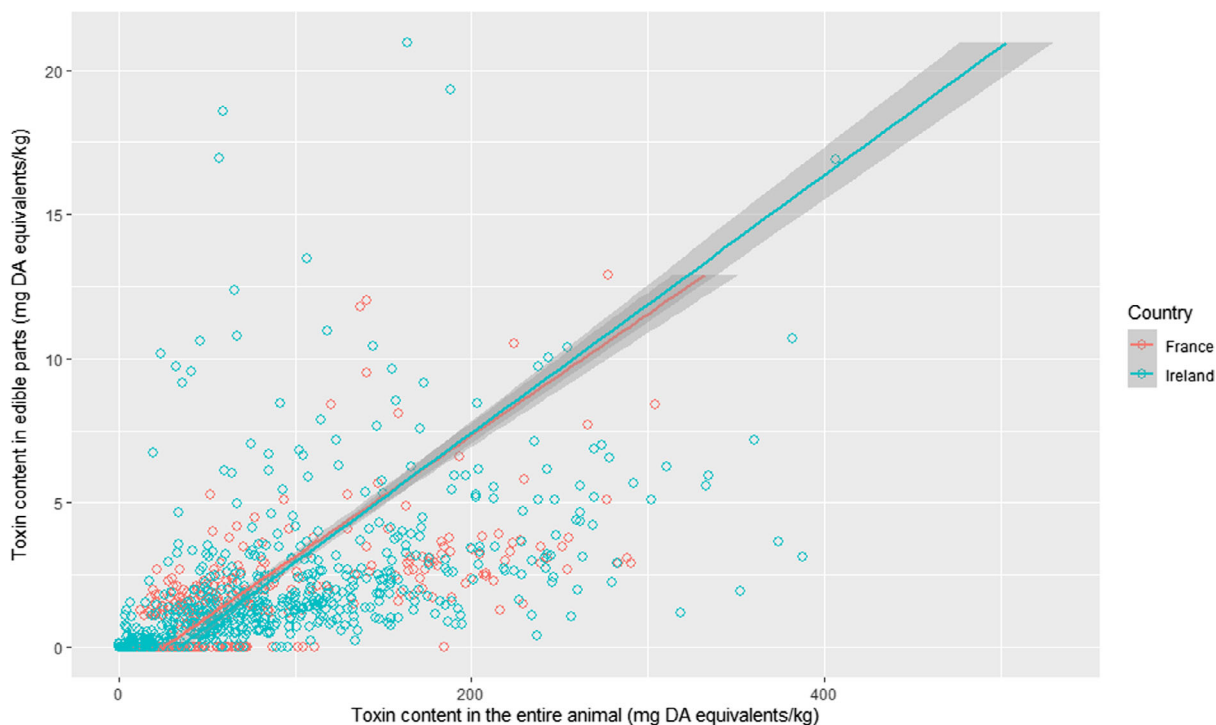
Figure A6: Scatter plot for the lower bound data submitted by France and Ireland together with their corresponding robust regression fits and confidence interval

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

The ratio was used to estimate what the concentration in the whole scallop would be assuming the limit of 20 mg DA/kg in the edible part.

The calculations were done as follows:

$$\text{Ratio (Percentile)} = \frac{\text{Edible part concentration}}{\text{Whole scallop concentration}},$$

$$\text{Maximum whole scallop concentration} = \frac{\text{Regulatory limit edible part}}{\text{Ratio (Percentile)}} = \frac{20 \text{ mg DA/kg}}{\text{Ratio (Percentile)}}.$$

Taking the two data sets and the 99th, 95th or 50th percentiles of the ratios (fail-safe hypothesis), the maximum concentration in the whole scallop is as described in Table A.1.

Table A.1: Highest concentration in whole scallops that ensures that the level in edible parts is below the regulatory limit of 20 mg/kg based on the P99, P95 and median ratios

Data set	Median	P95	P99
France	712	268	179
Ireland	874	242	143

P95: 95th percentile; P99: 99th percentile.
Note: Values calculated based on quantified levels only.

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 information from the two data sets (submitted by France and Ireland). Thus, the uncertainties around the 99th quantile values for the entire animal of 179 and 143 mg DA/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 163 mg DA/kg. The expected LB of toxin concentration considering the data reported by Ireland for the whole scallop would be 127 mg DA/kg.

It follows that the maximum concentration in the entire animal should not exceed 127 mg DA/kg in order reach a 99% certainty that the concentration of the edible parts will not exceed the regulatory limit of 20 mg DA/kg.

A.2. Ratio between adductor muscle and entire animal

A.2.1. Introduction

The data from Ireland on DA were analysed to study the ratio of the concentration in adductor muscle and the entire animal. The distribution of the ratios was estimated considering the censored observations and this will be used to estimate the threshold for the entire animal that should ensure that the concentration in the adductor muscle part remains under the regulatory limit of 20 mg DA/kg.

A.2.2. Ratio between adductor muscle and entire animal for domoic acid

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

$$\text{Ratio [DA]} = \frac{\text{Conc [AM]}}{\text{Conc [EA]}}.$$

The lower bound for the numerator (adductor muscle) and denominator (concentration of the entire animal) in the ratio defined the lower bound of the ratio $\left(\text{Ratio [DA] LB} = \frac{\text{Conc [AM] LB}}{\text{Conc [EA] LB}}\right)$, while the upper bound levels for the numerator and the denominator were used to define the ratio's upper bound $\left(\text{Ratio[DA] UB} = \frac{\text{Conc [AM] UB}}{\text{Conc [EA] UB}}\right)$.

In the case that the level in the whole scallop (denominator) was zero, then a value smaller than the minimum concentration value for the lower bound different from zero was used in order to avoid an infinite ratio. The final data used is a censored interval defined by a lower and upper bound if the numerator or the denominator are not quantified, in case that both were quantified then lower and upper bound ratios were equal. For Ireland, the data used were observations for pooled samples of scallops.

A.2.2.1. Data submitted by Ireland

The data submitted by Ireland on *Pecten maximus* were analysed in order to have enough information to estimate the parameters and to select the best fitting distribution to the censored data.

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 following the observed data.

```
## [1] "The AIC value for the log-normal fit is: 16.6"
## [1] "The AIC value for the exponential fit is: 728.2"
## [1] "The AIC value for the gamma fit is: 12.5"
## [1] "The AIC value for the normal fit is: 2,593.4"
## [1] "The AIC value for the logistic fit is: 1,808.7"
## Fitting of the distribution 'gamma' By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## shape 0.07952205 0.01014496
## rate 11.44054869 2.09469965
## Loglikelihood: −4.250703 AIC: 12.50141 BIC: 21.75484
## Correlation matrix:
## shape rate
## shape 1.0000000 0.6313495
## rate 0.6313495 1.0000000
```

Figure A.7 shows the distribution ratios between the domoic acid levels in adductor muscle and entire animal.

Empirical and theoretical CDFs
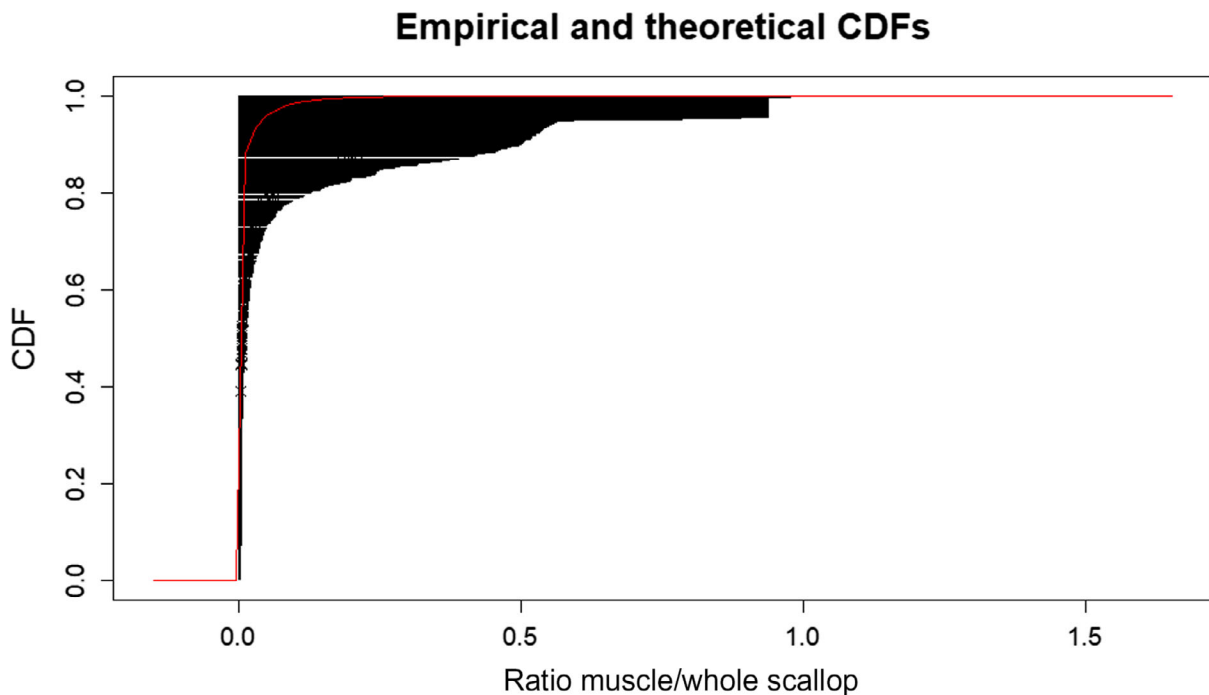
CDF: cumulative distribution function.

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

Considering that a large number of reported results for adductor muscle was quantifiable, it was decided to focus only on those observations for which the lower bound of the adductor muscle part was above zero, and consequently reducing the uncertainty in the estimates. The analysis was thus performed using only observations for which the lower bound of the ratio was larger than zero, leaving 97 out of 755 observations to estimate the best distribution fitting the data. The resulting distribution is shown in Figure A.8.

```
## [1] "The AIC value for the log-normal fit is: −460.7"
## [1] "The AIC value for the exponential fit is: −427.4"
## [1] "The AIC value for the gamma fit is: −427.6"
## [1] "The AIC value for the weibull fit is: −432.4"
## [1] "The AIC value for the normal fit is: −263.4"
## [1] "The AIC value for the logistic fit is: −305.6"
## Fitting of the distribution 'lnorm' By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## meanlog −3.920604 0.11289905
## sdlog 1.111927 0.07983139
## Loglikelihood: 232.3704 AIC: −460.7408 BIC: −455.5913
## Correlation matrix:
## meanlog sdlog
## meanlog 1.000000e+00 −3.202021e-11
## sdlog −3.202021e-11 1.000000e + 00
```
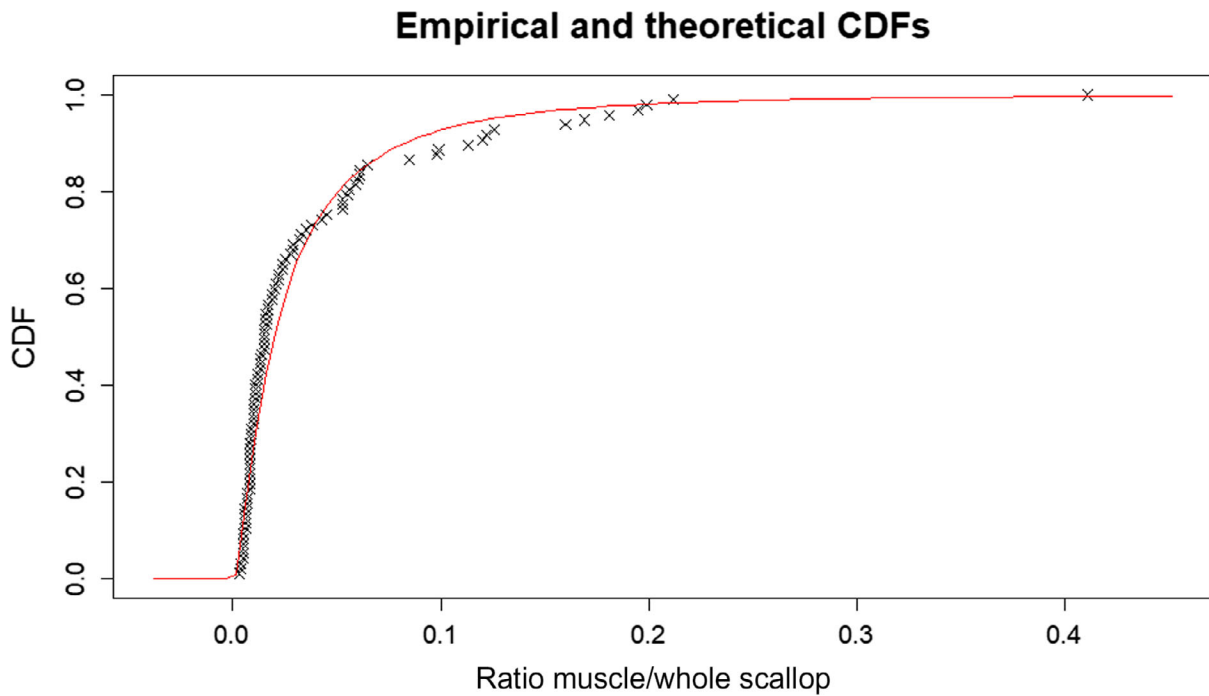
Empirical and theoretical CDFs

CDF: cumulative distribution function.

Figure A.8: Distribution of the ratios between the levels in muscle and whole scallop for pooled scallops from Ireland, for those samples in which the LB levels are equal to the UB levels

The samples with detected levels in adductor muscle show ratios with lower bounds larger than zero meaning that DA was found and quantified in that part. The range of ratios varied between 0.003 and 0.41. The mean ratio for the data from Ireland is 0.037 and the median is 0.02.

A.2.3.   Distribution of ratios based on the data from Ireland

The resulting estimated parameters were used to generate artificial data in order to visualise the ratios from Ireland when using censored or quantified concentrations. The expected scatter considering the estimated distributions is shown below in Figure A.9 below.
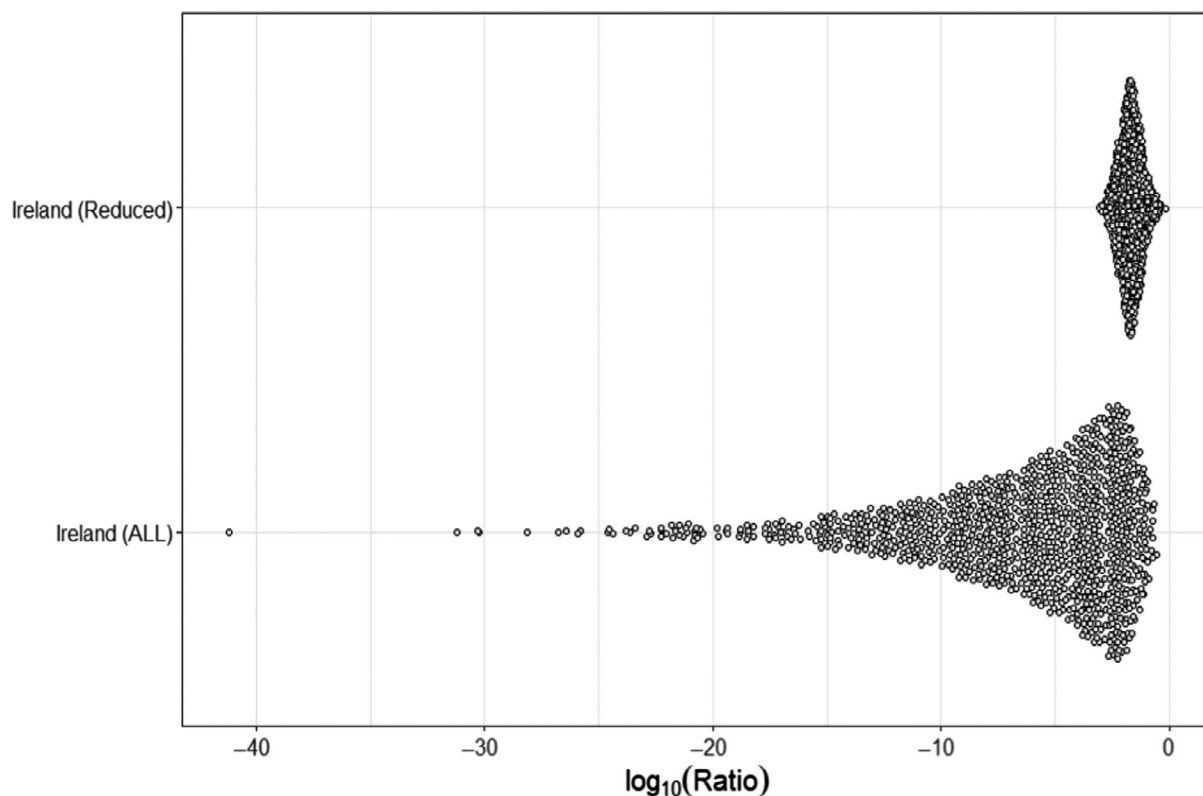
Figure A.9: Distribution of the ratios for data from Ireland considering all observations (ALL) and only those where the lower bound is larger than zero (Reduced)

The resulting fitted distribution shows the generated data for both best fitting models, clearly showing that the reduced data leads to higher ratios but with much smaller precision, as the interest is in the higher end of the tail (namely the 99th quantile), the distribution for the reduced data will be used in the subsequent analysis.

A.2.4. Estimation of the limit concentration for the whole scallop

The ratio was used to estimate what would be the concentration in the whole scallop that would correspond to the limit of 20 mg DA/kg in adductor muscle.

The calculations are done as follows:

$$\text{Ratio (Percentile)} = \frac{\text{Edible part concentration}}{\text{Whole scallop concentration}},$$

$$\text{Maximum whole scallop concentration} = \frac{\text{Regulatory limit edible part}}{\text{Ratio (Percentile)}} = \frac{20 \text{ mg DA/kg}}{\text{Ratio (Percentile)}}.$$

Taking the data set from Ireland (levels in adductor muscle larger than zero) and the 99th, 95th or 50th percentiles of the ratios (fail-safe hypothesis), the maximum concentration in the whole scallop is as shown in Table A.2.

Table A2: Highest concentration in whole scallops that ensures that the level in muscle is below the regulatory limit of 20 mg/kg based on the P99, P95 and median ratios

Dataset	Median	P95	P99
Ireland	1,009	162	76

P95: 95th percentile; P99: 99th percentile.
Note: Values calculated based on quantified levels only.

A.2.5. Determination of threshold to be used in the sampling plan

The estimation of the maximum concentration in the whole scallop is based on information from the data set (submitted by Ireland). Thus, the uncertainties around the 99th quantile values for the entire animal of 76 mg DA/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 on the adductor muscle part.

The expected LB toxin concentration considering the data reported by Ireland for the whole scallop would be 59 mg DA/kg.

It follows that the maximum concentration in the entire animal should not exceed 59 mg DA/kg in order to reach a 99% certainty that the concentration of the adductor muscle part will not exceed the regulatory limit of 20 mg DA/kg.

A.3. Ratio between gonad and entire animal

A.3.1. Introduction

The data from Ireland on DA 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 that should ensure that the concentration in the gonads part remains under the regulatory limit of 20 mg DA/kg.

A.3.2. Ratio between gonads and entire animal for domoic acid

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

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

The lower bound for the numerator (gonads) and denominator (concentration of the entire animal) in the ratio defined as the lower bound of the ratio $\left(\text{Ratio [DA] LB} = \frac{\text{Conc [EP] LB}}{\text{Conc [EA] LB}}\right)$, while the upper bound levels for the numerator and the denominator were used to define the ratio's upper bound $\left(\text{Ratio [DA] UB} = \frac{\text{Conc [G] UB}}{\text{Conc [EA] UB}}\right).$

In the case that the denominator was zero, a value smaller than the minimum concentration value for the lower bound different from zero was used in order to avoid an infinite ratio. The final data used are a censored interval defined by a lower and upper bound if the numerator or the denominator is not quantified, in case that both are quantified then the lower and upper bound will be equal. For Ireland, the samples used were observations for pooled samples of scallops.

A.3.2.1. Data submitted by Ireland

The data submitted by Ireland on *Pecten maximus* were analysed to estimate the parameters and to select the best fitting distribution to the censored data.

The range of ratios (LB to UB) were determined for each sample and subsequently several distributions were fitted to the data in order to select the best fitting distribution following the observed data.

```
## [1] "The AIC value for the log-normal fit is: −933.3"
## [1] "The AIC value for the exponential fit is: −860"
## [1] "The AIC value for the gamma fit is: −880.8"
## [1] "The AIC value for the weibull fit is: −863.8"
## [1] "The AIC value for the normal fit is: −251.6"
## [1] "The AIC value for the logistic fit is: −585"
## Fitting of the distribution 'lnorm 'By maximum likelihood on censored data
```
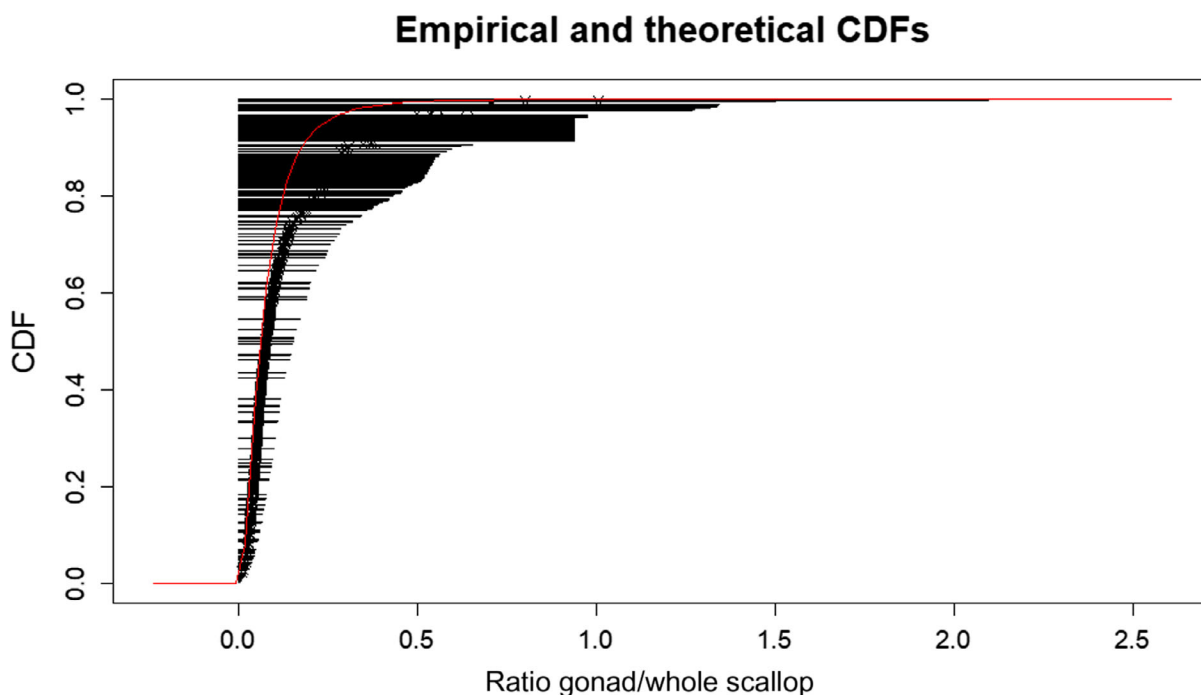
```
## Parameters
## estimate Std. Error
## meanlog −2.8061160 0.0394556
## sdlog 0.8281474 0.0295056
## Loglikelihood: 468.6706 AIC: −933.3412 BIC: −924.5641
## Correlation matrix:
## meanlog sdlog
## meanlog 1.000000 −0.125583
## sdlog −0.125583 1.000000
```

Figure A.10 describes the distribution of the ratios between the levels in gonad and whole scallop.

Empirical and theoretical CDFs

CDF: cumulative distribution function.

Figure A.10: Distribution of the ratios between the levels in gonad and whole scallop for pooled scallops obtained from Ireland, for each sample ranging between the ratio based on lower bound and those based on upper bound levels

Considering that there is enough information to build the probability distribution for the ratio, it was decided to focus only on those observations for which the lower bound of the adductor muscle part was above zero, thus reducing the uncertainty in the estimates. Therefore, the analysis was performed using only observations for which the lower bound of the ratio was larger than zero, leaving 547 out of 755 observations to estimate the best distribution fitting the data. In addition, two samples were removed with levels in gonads just above the LOQ but in other parts below the LOQ, thus resulting in high ratios. The resulting distribution is shown in Figure A.11.

```
## [1] "The AIC value for the log-normal fit is: −1,340.4"
## [1] "The AIC value for the exponential fit is: −1,146.5"
## [1] "The AIC value for the gamma fit is: −1,199.9"
## [1] "The AIC value for the weibull fit is: −1,161.8"
## [1] "The AIC value for the normal fit is: −539.2"
## [1] "The AIC value for the logistic fit is: −828.7"
## Fitting of the distribution 'lnorm 'By maximum likelihood on censored data
## Parameters
## estimate Std. Error
## meanlog −2.4071056 0.03360976
```

sdlog 0.7860661 0.02376552
Loglikelihood: 672.1982 AIC: −1,340.396 BIC: −1,331.787
Correlation matrix:
meanlog sdlog
meanlog 1 0
sdlog 0 1

Empirical and theoretical CDFs
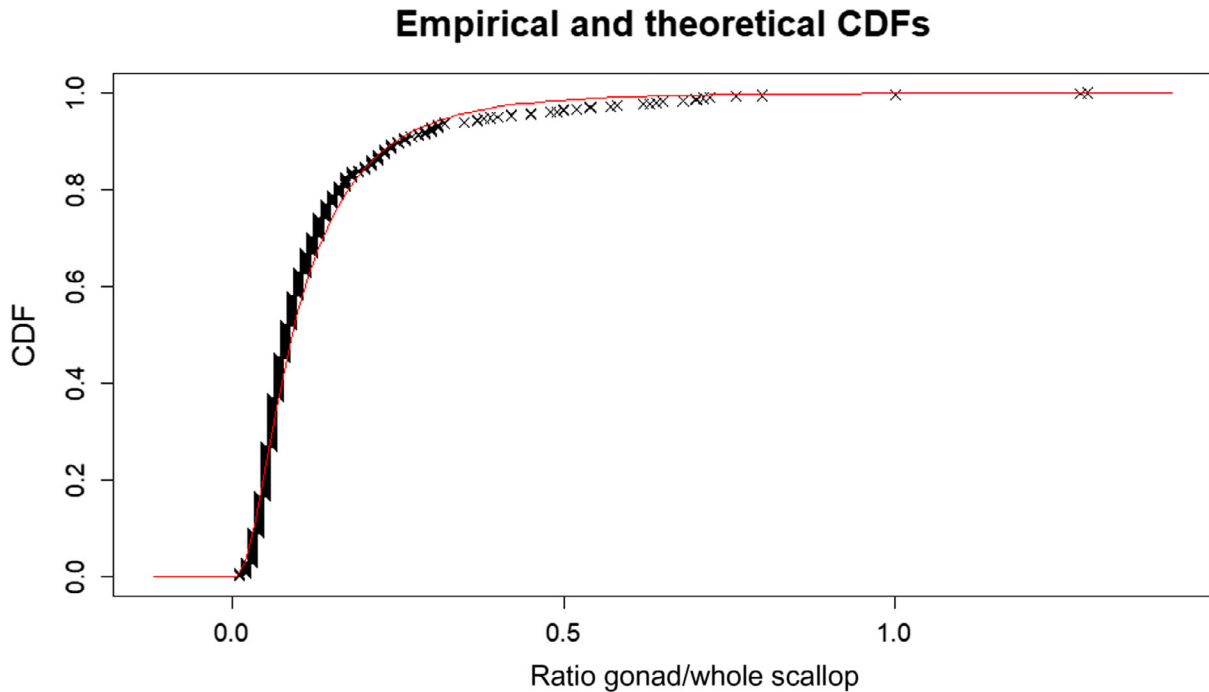
CDF: cumulative distribution function.

Figure A.11: Distribution of the ratios between the levels in gonad and whole scallop for pooled scallops from Ireland, for samples where the LB levels are equal to the UB levels

For the samples with detected levels in gonads the range of ratios varied between 0.01 and 1.29. The mean ratio for the Irish data is 0.123 and the median is 0.09.

A.3.2. Distribution of ratios based on the data from Ireland

The resulting estimated parameters were used to generate artificial data in order to visualise the ratios from Ireland when using all or only the quantified concentrations. The expected scatter considering the estimated distributions is shown below in Figure A.12.
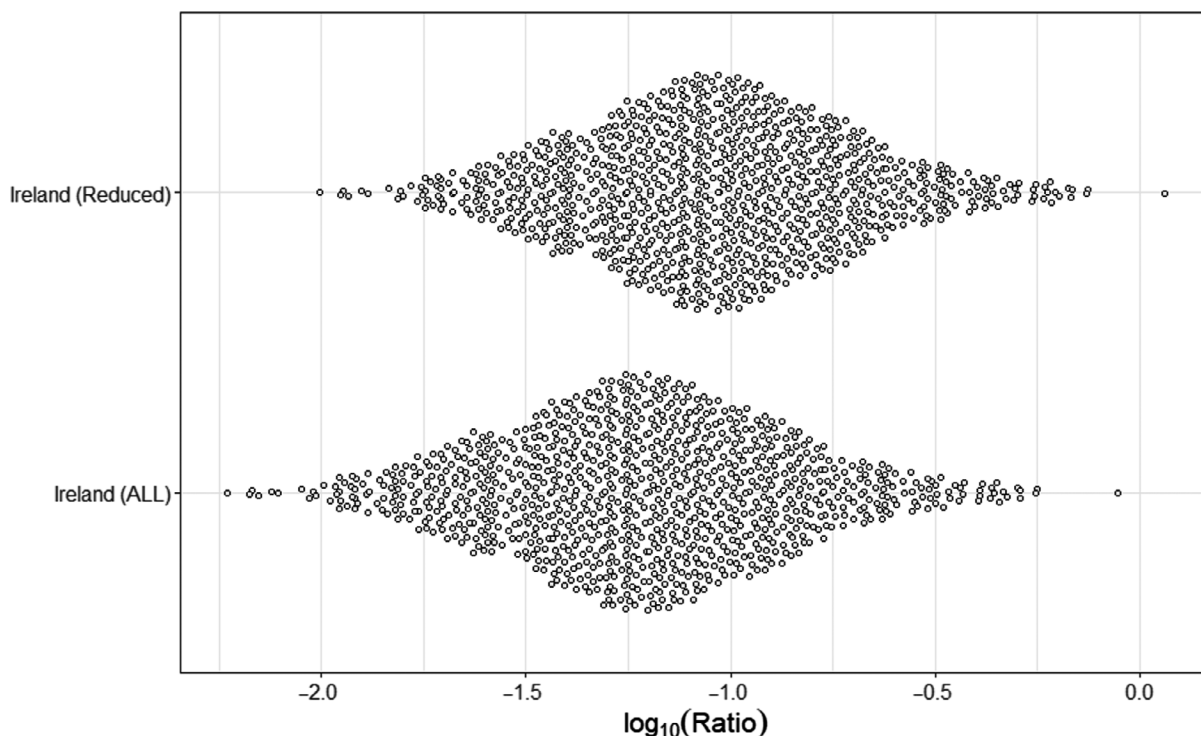
Figure A.12: Distribution of the ratios for the data from Ireland considering all observations (ALL) and only those where the lower bound is larger than zero (Reduced)

The resulting fitted distribution shows the generated data for both best fitting models, clearly showing that the reduced data leads to slightly higher ratios and also with slightly smaller precision. As the interest is in the higher end of the tail (namely the 99th quantile), the distribution for the reduced data will be used in the subsequent analysis.

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

The ratio was used to estimate what would be the concentration in the whole scallop that would correspond to the limit of 20 mg DA/kg in gonads.

The calculations were carried out as follows:

$$\text{Ratio (Percentile)} = \frac{\text{Edible part concentration}}{\text{Whole scallop concentration}},$$

$$\text{Maximum whole scallop concentration} = \frac{\text{Regulatory limit edible part}}{\text{Ratio (Percentile)}} = \frac{20 \text{ mg DA/kg}}{\text{Ratio (Percentile)}}.$$

Taking the data set from Ireland and the 99th, 95th or 50th percentiles (median) of the ratios (fail-safe hypothesis), the resulting critical concentrations in the whole scallop are shown in Table A.3. Based on only quantified levels, the level in whole scallops based on the P99 ratio should be lower than 54 mg/kg.

Table A.3: Highest concentration in whole scallops that ensures that the level in gonads is below the regulatory limit of 20 mg/kg based on the P99, P95 and median ratios. Values calculated based on quantified levels only

Data set	Median	P95	P99
Ireland	222	61	36

P95: 95th percentile; P99: 99th percentile

A.3.5. Determination of threshold to be used in the sampling plan

The estimation of the maximum concentration in the whole scallop is based on information from the data set (only data submitted by Ireland). Thus, the uncertainties around the 99th quantile values for the entire animal of 36 mg DA/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 gonads.

The expected LB toxin concentration considering the data reported by Ireland for the whole scallop would be 24 mg DA/kg for the data considering only quantified levels.

It follows that the maximum concentration in the entire animal should not exceed 24 mg DA/kg in order to reach a 99% certainty that the concentration of the gonads will not exceed the regulatory limit of 20 mg DA/kg.

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

B.1. Methodology

The aim of this appendix is to present the required sample size of an analytical sample to predict with a certain precision whether a lot exceeds the regulatory limit. In the absence of data provided by Member States, the CONTAM Panel decided to use published data, considering the data to be log-normally distributed using the confidence interval calculation for the mean.

B.1.1. Log-Normal distribution

The approach proposed by Matsushima et al. (2018) was extended considering that the concentration of toxins follows a 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. The approach also considers the variation reported in the literature. The method uses the confidence bounds (lower and upper bounds 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 percent confidence depending on the mean value observed and the variations shown in the literature.

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. It is assumed 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 $\hat{M} = E(y) = e^{\bar{x} + \frac{s^2}{2}}$, 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:

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

the estimated precision of the log of the mean would be depending on:

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

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

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

This means 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 being:

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

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

B.1.2. Results

B.1.2.1. Log-normal distribution

B.2.2.1.1. Scenarios: number of samples

In this case, different scenarios of expected means are considered (ranging from 5 to 35 mg/kg), which are used to calculate the number of samples required in order to be 95% confident that the true level in the area under investigation is compliant or not, meaning that the mean is expected to be below of above the prespecified threshold of 20 mg/kg. It is also possible for a specific scenario to consider the highest coefficient of variation observed to visualise the confidence intervals for different numbers of scallops to estimate the concentration of the toxins. The number of samples needed for each coefficient of variation reported in the literature (Bogan et al., 2007a,b,c) and used are presented in Table B.1 below.

Table B.1: Number of scallops per sample required for four reported coefficients of variance in order to predict with 95% certainty that a sample is compliant (if true level below 20 mg/kg) or not compliant (if true level above 20 mg/kg)

True level (mg/kg)	CV 0.40	CV 0.70	CV 0.83	CV 1.06
5	3	3	4	4
6	3	4	4	5
7	3	4	4	5
8	3	4	5	6
9	3	5	5	7
10	3	5	6	9
11	4	6	8	11
12	4	8	9	13
13	5	10	12	18
14	6	13	17	25
15	8	18	24	> 30
16	11	29	> 30	> 30
17	19	> 30	> 30	> 30
18	> 30	> 30	> 30	> 30
19	> 30	> 30	> 30	> 30
21	> 30	> 30	> 30	> 30
22	> 30	> 30	> 30	> 30
23	25	> 30	> 30	> 30
24	16	> 30	> 30	> 30
25	11	29	> 30	> 30
26	9	21	28	> 30
27	7	17	22	> 30
28	6	14	18	27
29	6	12	15	23
30	5	10	13	20
31	5	9	12	17
32	5	8	11	15
33	4	8	10	14
34	4	7	9	13

True level (mg/kg)	CV 0.40	CV 0.70	CV 0.83	CV 1.06
35	4	7	8	12
36	4	6	8	11
37	4	6	7	10
38	4	6	7	9
39	4	6	7	9
40	3	5	6	9

CV: coefficient of variance.

Another way of deciding the number of scallops needed to be sampled would be to consider a specific number of scallops in a sample and estimate the probability that the mean would exceed the threshold of 20 mg/kg for different reported CVs. Table B.2 shows the probability of a false compliant (true level below 20 mg/kg) or false non-compliant (true level above 20 mg/kg) result for 10 scallops per sample depending on the CV. This shows that with a CV of 0.40 the chance of a false compliant result of less than 5% is obtained for levels of 26 mg/kg or higher. With the highest reported CV of 1.06, this applies to levels almost twofold the regulatory limit of 20 mg/kg. There is also a considerable chance of false non-compliant results when the true level approaches the regulatory limit.

Table B.2: Probability of a false compliant or false non-compliant result for 10 scallops per sample

True level (mg/kg)	CV 0.40	CV 0.70	CV 0.83	CV 1.06
5	0.00012	0.00012	0.00024	0.00108
6	0.00012	0.00017	0.00058	0.00255
7	0.00012	0.00049	0.00142	0.00531
8	0.00012	0.00125	0.00315	0.01030
9	0.00012	0.00279	0.00650	0.01859
10	0.00018	0.00595	0.01257	0.03148
11	0.00061	0.01202	0.02303	0.05031
12	0.00154	0.02292	0.03984	0.07625
13	0.00411	0.04136	0.06510	0.11005
14	0.01047	0.07027	0.10059	0.15175
15	0.02536	0.11239	0.14741	0.20082
16	0.05724	0.16921	0.20533	0.25598
17	0.11752	0.24013	0.27268	0.31545
18	0.21533	0.32224	0.34662	0.37730
19	0.34865	0.41071	0.42358	0.43940
21	0.35564	0.41497	0.42730	0.44231
22	0.23728	0.33801	0.36050	0.38861
23	0.15109	0.27120	0.30102	0.33948
24	0.09357	0.21506	0.24935	0.29526
25	0.05726	0.16920	0.20533	0.25598
26	0.03505	0.13245	0.16842	0.22140
27	0.02165	0.10351	0.13788	0.19123
28	0.01357	0.08086	0.11280	0.16503
29	0.00868	0.06328	0.09233	0.14248
30	0.00567	0.04971	0.07569	0.12306
31	0.00376	0.03917	0.06221	0.10641
32	0.00256	0.03104	0.05129	0.09212
33	0.00175	0.02474	0.04241	0.07992
34	0.00124	0.01981	0.03520	0.06946
35	0.00085	0.01597	0.02937	0.06051
36	0.00062	0.01296	0.02459	0.05283

True level (mg/kg)	CV 0.40	CV 0.70	CV 0.83	CV 1.06
37	0.00050	0.01057	0.02066	0.04622
38	0.00031	0.00870	0.01747	0.04057
39	0.00030	0.00718	0.01480	0.03569
40	0.00018	0.00597	0.01261	0.03148

CV: Coefficient of variance.

Table B.3 shows the probability of a false compliant or false non-compliant result for 20 scallops in a sample depending on the CV. This shows that at the lowest CV of 0.40, the level with a less than 5% chance on a false compliant result is reduced from 26 to 24 mg/kg. For the highest CV this is reduced to 30 mg/kg, i.e. 1.5-fold the regulatory limit.

Table B3: Probability of a false compliant or false non-compliant result for 20 scallops per sample

True level (mg/kg)	CV 0.40	CV 0.70	CV 0.82	CV 1.06
5	0.00000	0.00010	0.00011	0.00012
6	0.00007	0.00011	0.00011	0.00012
7	0.00007	0.00011	0.00011	0.00012
8	0.00007	0.00011	0.00011	0.00039
9	0.00008	0.00011	0.00012	0.00126
10	0.00008	0.00011	0.00056	0.00351
11	0.00009	0.00057	0.00196	0.00868
12	0.00009	0.00192	0.00562	0.01909
13	0.00009	0.00606	0.01429	0.03786
14	0.00045	0.01658	0.03232	0.06817
15	0.00241	0.03980	0.06502	0.11243
16	0.01145	0.08353	0.11735	0.17136
17	0.04360	0.15426	0.19127	0.24358
18	0.12840	0.25286	0.28452	0.32556
19	0.28795	0.37242	0.39046	0.41271
21	0.29726	0.37842	0.39565	0.41691
22	0.15174	0.27334	0.30308	0.34116
23	0.06871	0.18975	0.22574	0.27474
24	0.02865	0.12748	0.16426	0.21819
25	0.01140	0.08353	0.11731	0.17133
26	0.00449	0.05377	0.08263	0.13335
27	0.00176	0.03416	0.05760	0.10312
28	0.00072	0.02160	0.03991	0.07932
29	0.00031	0.01361	0.02755	0.06082
30	0.00018	0.00861	0.01899	0.04652
31	0.00006	0.00542	0.01311	0.03558
32	0.00006	0.00348	0.00910	0.02723
33	0.00006	0.00224	0.00631	0.02086
34	0.00006	0.00142	0.00441	0.01601
35	0.00006	0.00098	0.00308	0.01233
36	0.00006	0.00061	0.00219	0.00948
37	0.00006	0.00043	0.00157	0.00737
38	0.00006	0.00031	0.00112	0.00574
39	0.00006	0.00018	0.00081	0.00448
40	0.00006	0.00018	0.00057	0.00351

CV: Coefficient of variance.

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

The Annex is available as Excel files on the EFSA Knowledge Junction community on Zenodo, at: https://doi.org/10.5281/zenodo.5102830