COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Statement on domoic acid in King Scallops (*Pecten Maximus*).

Introduction

1.   The COT was asked to consider the evidence that was available to support shucking (removal of inedible parts) as a scientifically robust and effective method of managing the health risks associated with Amnesic Shellfish Poison (ASP) toxins in scallops. The main toxin of concern is the water-soluble cyclic amino acid, domoic acid (DA). The Committee was asked whether, from data on the distribution of DA in different tissues from King Scallops, it was possible to identify a level of the compound in batches of whole animals that would pose minimal risk to consumers following shucking by trained workers.

Regulatory Context

2.   Controls on the sale of scallops in the UK are mandated by EU law (Regulation (EC) No 854/2004, which lays down specific rules for the organisation of official controls on products of animal origin intended for human consumption). The legislation sets legal limits for marine biotoxins. DA in scallops is present mainly in tissues that are not usually consumed (hepatopancreas, mantle and gills), and which are removed by a process known as shucking. The extent of residual contamination in the parts that remain depends on the efficiency of shucking. For scallops, the legislation requires that shucking must be performed at an establishment which is approved to carry out the process. Furthermore, live bivalve molluscs that are placed on the market, including scallops, must not contain total concentrations of DA (measured in the whole animal or any edible part separately) that exceed 20 mg per kg shellfish flesh. This legal requirement means that establishments which are approved to process and dispatch scallops are not permitted to place whole scallops on the market which exceed the legal limit of 20 mg/kg, regardless of whether they are intended to be shucked further (e.g. in a catering establishment).

3.   Routine shucking (by approved processors) has been consistently applied to the vast majority of whole scallops landed in the UK, irrespective of toxin levels at source. Due to the higher concentrations of DA in the tissues that are usually removed by shucking, official control verification frequently detects whole scallops that exceed the legal limit. Therefore the enforcement regime for biotoxins in live bivalve molluscs has restricted the market for scallops which are supplied whole (live, in-shell).

ASP Toxins

4.   A number of DA isomers have been reported, including epi-DA, domoic acid C5'-diastereoisomers and eight isodomoic acids, but DA is the predominant compound found in UK shellfish. DA is produced by species of *Pseudonitzschia* phytoplankton and can be accumulated by filter feeding shellfish. Clinical symptoms, which tend to occur 24-48 hours after ingestion of contaminated shellfish, can be both gastrointestinal (vomiting, diarrhoea or abdominal cramps) and neurological (confusion, memory loss, brain damage and even seizure or coma).

5.   DA is neurotoxic in experimental animals, including rodents and non-human primates, and in humans. The toxic effects of DA are mediated through its high affinity binding and agonist action on some types of glutamate receptors, which can affect neuronal function and lead to cell death in certain regions of the brain (e.g. the hippocampus) (EFSA, 2009).

6.   No cases of ASP poisoning in the UK have been reported in the scientific literature. However, since there is no formal reporting system, this cannot be viewed as conclusive evidence that such poisoning has not occurred. EFSA noted that the few data on exposures to DA associated with toxicity in humans (nine individuals) indicated that severe and irreversible effects occurred at doses in the region of 4 mg/kg b.w. (EFSA 2009). The lowest observed adverse effect level (LOAEL) for mild symptoms and signs was 0.9 mg/kg b.w. EFSA established an acute reference dose (ARfD)[1] on the basis of this LOAEL, applying an uncertainty factor of 3 for extrapolation from a LOAEL to a NOAEL, and a factor of 10 for human variability and also because the investigation of affected individuals had not used sensitive methods for detection of neurotoxic effects. This resulted in an ARfD of 30 µg DA/kg b.w. Because DA can be converted to epi-DA during storage, the ARfD applies to the sum of DA and epi-DA. It was noted that individuals with impaired renal function

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[1] An ARfD is an estimate of the amount of a substance that can be ingested in a period of 24 h or less without appreciable health risks to the consumer, on the basis of all known facts at the time of the evaluation.

are more susceptible to DA and therefore the ARfD might not be sufficiently protective for such people (EFSA, 2009). The COT agreed that the EFSA ARfD should be applied in this assessment.

Variability in King Scallop DA levels

7.   ASP toxins are not evenly distributed throughout the tissues of the scallop. DA concentrates in the digestive tissues (including the hepatopancreas), with much lower concentrations in the adductor muscle and gonad. The digestive (and other) tissues of scallops are removed by shucking before consumption, leaving only the 'scallop meat' comprising adductor muscle (white meat) and gonad (orange meat, also known as the "coral", which sometimes is also discarded). The adductor muscle should in principle contain no DA, and any DA present results from failure to remove all of the gut tissue and/or contamination of the adductor muscle by fluids from the offal (McKenzie and Bavington 2002).

8.   The occurrence of DA and epi-DA in scallops varies in time, and some geographical areas are more affected than others by these toxins (Ciminiello et al., 1999). Monitoring by the Food Standards Agency (FSA) in Scotland has detected the causative phytoplankton all year round, and in 2013 the largest bloom in Scottish waters occurred in October. A study by McKenzie and McIntyre (2004) indicated that in Scottish waters affected by blooms of DA-producing phytoplankton, King Scallops generally show a pattern of approximately six months of DA uptake (May to October) followed by approximately six months of DA release (November to April). Although there was variation in the seasonality of DA uptake by King Scallops in different areas, there was a general tendency for King Scallops to show elevated levels of DA from May or June through to December.

9.   The size of the gonad contributes significantly to the variability of DA concentrations in King Scallops. The intestinal loop (which is the key source of DA) passes through the gonad, and can constitute up to 6% of the gonadal volume (Campbell et al., 2001). This intestinal loop thus can contribute to the toxin content of the gonad. The gonad is a complex and dynamic organ, subject to a rapid change in size during its spawning cycle, and the contribution of the intestinal loop to gonadal mass varies depending on the stage which an animal has reached in its reproductive cycle (Ansell et al., 1991; Gallacher et al., 2001). Spawning is associated with water temperature, and in Scottish waters is believed to occur most frequently during the spring (Marine Scotland Science, personal communication). The size of the gonad is greatest just before spawning, and at this point relative DA concentrations will be lower due to the dilution effect of the greater gonadal mass. After spawning, there is

a significant reduction in the size of the gonad, leading to an overall increase in DA concentration. This variability makes it very difficult to interpret changes in DA levels by measuring concentrations in the gonad alone. The concentrations measured in whole animals are much less sensitive to this seasonal variation, as the proportional difference in mass due to spawning is much lower in the whole animal. It is more difficult to free smaller gonads from the intestinal loop during the shucking process, and this can increase variation in the DA levels in shucked scallops.

Consumption of scallops

10.   Because DA has acute toxic effects, it is important to base dietary exposure calculations on a large portion size rather than what would be considered high long-term average consumption. Between the years 2008 and 2011, the UK National Diet and Nutrition Survey (NDNS) included only six adults who reported consumption of scallops **(**Bates et al., 2012). The daily quantity of scallops eaten by the six NDNS participants over the period of the survey diary ranged from 15 to 120 g.

11.   In the context of a series of evaluations of marine biotoxins in shellfish, EFSA selected a value of 400 g for a large portion size, based on data for shellfish consumption obtained from five European countries including the UK, for which the 95th percentile values ranged from 70 g to 465 g (EFSA, 2009 and 2010). A study not available to EFSA investigated the weight of a scallop portion, in and out of the home, in key European markets including the UK (Seafish, 2004). The 99th percentile weight of a scallop portion for the UK was estimated at 396 g which is consistent with the figure of 400 g used by EFSA as a large portion for shellfish meat. Therefore 400 g is also taken to represent a large portion size in this statement.

12.   EFSA noted that for a 60 kg adult to avoid exceeding the ARfD of 30 µg/kg b.w., a 400 g portion of shellfish should not contain a combined total of DA and epi-DA of more than 1.8 mg. This corresponds to a concentration of 4.5 mg/kg edible shellfish meat (EFSA, 2009).

DA levels in scallop tissues

13.   McKenzie and Bavington (2002) reported data on concentrations of DA (excluding epi-DA and other isomers) in different tissues from 50 King Scallops sampled during 2001/2. The King Scallops were harvested from a single area of sea (referred to as the J5 area) located near the island of Jura on the West Coast of Scotland, which was considered to be representative of the Scottish offshore King Scallop fishery, and which was where some of the highest levels of DA had been found in official monitoring of whole King Scallops. Similarly, Campbell et al. (2001)

reported data on tissue levels of DA (excluding epi-DA and other isomers) in 170 King Scallops from three harvest sites in Scotland that had been closed due to high DA concentrations in gonadal tissue, following sampling in December 1999. The harvest sites were Tobermory Bay (10 samples from each of four sub-sites up to 1200 metres apart), Loch Sligachan (10 scallops from each of three sub-sites, up to 300 metres apart) and area J5 (20 scallops from each of five sub-sites, up to 12000 metres apart). Differences between sub-sites in the DA levels in gonad, adductor muscle and all other tissue were reported (Campbell et al., 2001).

14.   Table 1 summarises the mean DA tissue levels that were reported by Campbell et al (2001) for different geographical locations, and those from the study by McKenzie and Bavington (2002). DA levels in whole King Scallops at the three sites studied by Campbell et al. (2001) were more variable than in the McKenzie and Bavington (2002) study.

15.   These data confirm the lower levels of DA in adductor muscle than gonad. King Scallops from area J5 in the Campbell et al. (2001) study demonstrated the highest DA concentrations in whole animals, and similar whole animal DA levels were reported by McKenzie and Bavington (2002) for this area. King Scallops from area J5 reported by Campbell et al. (2001) also had the highest mean DA concentrations in gonad and total edible tissue (i.e. adductor muscle plus gonad).

Table 1. DA concentrations by geographical location and tissue

Source of data, number of samples taken and range of DA concentrations in whole King Scallops	Mean DA concentration mg/kg (95% confidence interval)			
	Whole scallops	Gonad	Adductor muscle	Gonad + adductor muscle
Tobermory Bay site in Campbell et al. (2001), n=40; range of DA levels in whole scallops: 18-1237 mg/kg	224 (103-346)	8.2 (3.7-12.7)	0.2 (0.1-0.2)	1.4 (0.5-2.4)
Loch Sligachan site in Campbell et al. (2001), n=30; range of DA levels in whole scallops: 26-401 mg/kg	160 (77-243)	6.8 (0.4-13.2)	1.3 (0.4-2.9)	1.8 (0.5-3.1)
Area J5 in Campbell et al. (2001) , n=100; range of DA levels in whole scallops: 1-1568 mg/kg	363 (279-447)	11 (5.7-16.2)	0.6 (0.3-0.8)	2.2 (1.0-3.3)
Area J5 McKenzie and Bavington (2002) study, n=50; range of DA levels in whole scallops: 210-459 mg/kg	328 (311-345)	8.5 (6.3-10.6)	0.4 (0.2-0.5)	1.4 (0.9-1.5)

Derivation of edible tissue DA levels for a 400g portion by statistical modelling

16.   The data on levels of DA in King Scallops from McKenzie and Bavington (2002) and Campbell et al. (2001) were analysed further to determine DA levels in edible tissue (adductor muscle and gonad) that might make up a 400g portion. Statistical modelling involved the generation of 100,000 simulated edible portions of 10 scallops by random sampling with replacement[2] from the datasets of DA measurements for individual animals (Annex 1 provides further details). Mean concentrations of DA were then calculated for each of these simulated portions. Table 2 shows high centiles of the distribution of these mean concentrations across the 100,000 simulated portions, according to the dataset(s) from which the simulated samples were derived. This modelling allowed the variability in DA levels associated with adductor muscle and gonadal tissue to be taken into account when estimating the concentrations that might occur in a 400g edible portion. The tissue DA levels presented in Table 2 can be compared to the reference level of 4.5 mg/kg, which is

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[2] Random sampling with replacement implies that each individual data point in the simulated sample is selected at random from the total dataset of DA measurements. Thus, by chance, the same data point might occur more than once in a given simulated sample.

the highest concentration that can be present in a large portion of edible tissue before the EFSA ARfD is exceeded.

Table 2. Modelled DA concentrations (mg/kg) in 400g portions of edible tissue (adductor muscle and gonad) of King Scallops at various centiles according to source of data.

Source of data, and number of samples in dataset	DA concentration (mg/kg) in a 400g portion of edible tissue (adductor muscle and gonad)				
	95th centile	97.5th centile	99th centile	99.5th centile	99.9th centile
Tobermory Bay site in Campbell et al. (2001), n=40	2.2	2.3	2.5	2.7	3.0
Loch Sligachan site in Campbell et al. (2001), n=30	3.5	3.9	4.4	4.9	5.6
Area J5 in Campbell et al. (2001), n=100	3.4	3.7	4.1	4.4	4.9
Area J5 McKenzie and Bavington (2002), n=50	1.8	1.9	2.1	2.2	2.4

17.   None of the mean DA levels in simulated portions exceeded 4.5 mg/kg at or below the 99th centile, regardless of the dataset on which they were based. DA data from Loch Sligachan (whole animal DA range of 26-401 mg/kg) gave mean DA levels in simulated portions that slightly exceeded 4.5 mg/kg at the 99.5[th] centile. However, at the highest centile considered in the analysis (99.9[th]), only the simulated portions based on DA levels in edible tissue from Tobermory Bay site and from McKenzie and Bavington (2002) were below 4.5 mg/kg.

18.   Simulated tissue DA levels were also obtained for 400g portions of adductor muscle only (Table 3). With the exception of estimates based on data for Loch Slingachan (at 99.5[th] centile and above), none of the data sets resulted in DA levels exceeding 4.5mg/kg at any of the centiles considered.

Table 3. Modelled DA concentration (mg/kg) in a 400g portion of adductor muscle from King Scallops at various high centiles

Source of data, and number of samples in dataset	DA concentration (mg/kg) in a 400g portion of adductor muscle				
	95th centile	97.5th centile	99th centile	99.5th centile	99.9th centile
Tobermory Bay site in Campbell et al. (2001), n=40	0.3	0.3	0.4	0.4	0.5
Loch Sligachan site in Campbell et al. (2001), n=30	3.3	3.6	4.2	4.8	5.6
Area J5[*] in Campbell et al. (2001), n=100	1.1	1.2	1.4	1.5	1.7
Area J5 McKenzie and Bavington (2002), n=50	0.7	0.8	0.9	0.9	1.0

Influence of shucking and the proportion of gonad in edible tissue on modelled DA levels in edible tissues of King Scallops

19.   McKenzie and Bavington (2002) also investigated the influence of shucking on DA levels in edible tissues, comparing the results from different processors with those obtained by best practice. Data from that investigation were used in a sensitivity analysis conducted for this statement (Annex 2). The sensitivity analysis also explored how the proportion of gonadal tissue could influence modelled DA concentrations in 400g portions of edible tissue.

20.   The data presented in Annex 2 show that for each centile, scenarios characterised by poor shucking, and those which assumed higher proportions of gonad in edible tissue, led to higher modelled DA levels in 400g portions of edible tissue.

21.   Centiles of the distribution of DA levels in modelled portions of scallop meat (either adductor muscle only or in combination with gonad) may also be influenced by the number of scallops that make up the edible portion. Data showing the influence of this parameter, in isolation or in combination with other parameters (gonad content and processing by shucking), were also investigated in the sensitivity analysis (Annex 2). Varying the number of scallops (from 8 to 12) in a modelled 400g portion had less impact than the other parameters on the modelled concentration of DA at a given centile.

Derivation of an upper level of DA in whole scallops that would make exceedance of the EFSA ARfD unlikely

22.   To explore upper levels for DA in batches of whole scallops that would give reasonable reassurance against toxicity from consumption of a large edible portion, the COT estimated the average DA concentrations in whole animals that would lead to exceedance of the EFSA ARfD in only a small fraction of 400g portions of edible tissues (Annex 1). These concentrations were derived by multiplying the mean DA concentrations in the batches of whole scallops for which measurements of DA concentrations were available (Table 1) by 4.5 mg/kg (the concentration in a 400g portion corresponding to the ARfD), and dividing by various high percentiles of the distribution of DA concentrations in 400g edible portions simulated using data from the same batch.

23.   Table 4 summarises the findings according to the batch of scallops from which measurements came. The dataset from Campbell et al (2001) for Loch Sligachan gave the lowest upper limit for average DA levels in whole animals which would be needed to ensure that the ARfD was unlikely to be exceeded.

Table 4. Highest average concentrations of DA in whole scallops that would not lead to exceedance of the EFSA ARfD in specified proportions of simulated 400g portions of adductor muscle and gonad.

Source of data and number of samples taken	Concentration of DA (mg/kg) in whole scallops at which specified percentiles of the distribution of DA concentrations in 400g edible portions will not exceed the ARfD:				
	95%	97.5%	99%	99.5%	99.9%
Tobermory Bay site in Campbell et al. (2001), n=40	465	431	397	375	334
Loch Sligachan site in Campbell et al. (2001), n=30	203	183	162	147	129
Area J5[*] in Campbell et al. (2001), n=100	483	442	399	375	332
Area J5 McKenzie and Bavington (2002), n=50	822	770	717	684	622

24.   The analysis in Table 4 was repeated for derivation of a similar level of DA in whole scallops if the 400g portions comprised only adductor muscle – i.e. reflecting a situation in which gonadal tissue was removed before consumption (Table 5). Comparison of Tables 4 and 5 indicates that higher DA levels might be acceptable in whole animals if only adductor muscle were consumed. In that case, the lowest limit

for average DA levels in whole animals to ensure that the ARfD was not exceeded was again derived from data for the Loch Sligachan site (Table 5).

Table 5. Highest average concentrations of DA in whole scallops that would not lead to exceedance of the EFSA ARfD in specified proportions of simulated 400g portions of adductor muscle.

Source of data and number of samples taken	Concentration of DA (mg/kg) in whole scallops at which specified percentiles of the distribution of DA concentrations in 400g edible portions will not exceed the ARfD				
	95%	97.5%	99%	99.5%	99.9%
Tobermory Bay site in Campbell et al. (2001), n=40,	3427	2985	2606	2434	2059
Loch Sligachan site in Campbell et al. (2001), n=30,	221	199	173	151	129
Area J5 in Campbell et al. (2001), n=100	1521	1357	1190	1103	938
Area J5 McKenzie and Bavington (2002), n=50	2110	1918	1738	1623	1420

25.   As discussed previously, the sensitivity analysis that applied data from McKenzie and Bavington (2002) (Annex 2) showed the potential impact of certain parameters (e.g. efficiency of shucking and proportion of gonad) on DA tissue levels in edible portions. These factors would also need to be taken into account in determining a mean concentration of DA in whole animals that would give adequate reassurance that the ARfD was unlikely to be exceeded in a large edible portion. Where shucking was poor, the whole animal DA levels in Tables 4 and 5 would need to be reduced by a factor of approximately 4 for the adductor muscle plus gonad, or approximately 2.6 for adductor muscle only, in order to achieve the same levels of protection against exceedance of the ARfD.

Major sources of uncertainty

26.   Scallops from the Campbell et al. (2001) trial and McKenzie and Bavington (2002) study were obtained from the West Coast of Scotland. The differences between the two studies in tissue levels of DA are likely to reflect biological variation (including differences between the sites and sub-sites from which the scallops were sampled), measurement error, and perhaps also differences in the efficiency of shucking. Furthermore, scallops from both studies were harvested during winter months, while DA levels in scallops are subject to seasonal variation and the extent of algal bloom in the area. Thus, the measured concentrations may not be representative of those in other locations and at other times of year. However, this

should not have seriously compromised the accuracy of estimated upper limits for average DA concentrations in whole animals that would protect against exceedance of the ARfD by consumption of a large edible portion of scallops, since those limits depended only on ratios of concentrations and not their absolute values.

27.   Both the Campbell et al. (2001) trial and McKenzie and Bavington (2002) study measured only DA, and not epi-DA or other minor isomers. However, DA is the predominant isomer and the relationship between average concentration in a batch and centiles of concentrations in large portions should be similar for DA as for the combination of DA and epi-DA.

28.   Experimental data from five processors demonstrated that variation in the efficiency of shucking could have important impacts on tissue levels of DA in scallops (McKenzie and Bavington 2002). However, the performance of the processors studied may not have been representative of all trained workers. Thus, in extreme situations, the impact of poor shucking might be greater than that observed.

29.   Also important is the statistical uncertainty that arises because the numbers of scallops that were measured in studies by Campbell et al. (2001) and McKenzie and Bavington (2002) were fairly small, and as illustrated by the histograms in Annex 3, the distributions of DA concentrations in gonad and adductor muscle were skewed. In particular, outlying high concentrations of DA occurred both in adductor muscle (area J5 and the Loch Sligachan site in the study by Campbell et al (2001)) and in gonad (Loch Sligachan site, Campbell et al 2001). This could lead to imprecision particularly when estimating high centiles of DA concentrations in large portions of edible tissue.

30.   Some reassurance is provided by the broad similarity of estimated upper limits for average DA concentrations derived independently from samples of scallops collected at four different geographical locations (Table 4). The values estimated to protect at the 99.9[th] centile ranged from 147 to 684 mg/kg.

31.   To explore further the uncertainties relating to outlying high DA values, the simulations used to derive Tables 4 and 5 were repeated with a) exclusion and b) duplication of the scallop with the highest concentrations of DA in each of the four geographical locations. The results of this sensitivity analysis are summarised in Annex 4. The effect of duplicating the scallop with the highest concentration of DA in the sampling bases was relatively small (a reduction of <20% in the upper limit to prevent exceedance of the ARfD at the 99.9[th] centile).

Conclusions

32.   A wide range of DA levels is found in whole scallops. The concentration of DA in edible parts (gonad and adductor muscle) is lower than in the whole animal.

33.   Simulation of large edible portions using data from batches of King Scallops that had been harvested at various locations in Scotland indicated that fewer than 1% of such portions would contain DA concentrations sufficient to cause exceedance of the ARfD.

34.   With efficient shucking, the maximum average concentration of DA in whole scallops that would protect against exceedance of the ARfD when consuming a 400g portion of adductor muscle plus gonad was estimated to be 162 mg/kg at the 99th centile and 129 mg/kg at the 99.9^{th} centile.

35.   With efficient shucking, the maximum average concentration of DA in whole scallops that would prevent exceedance of the ARfD when consuming a 400g portion of adductor muscle alone was estimated to be 173 mg/kg at the 99th centile and 129 mg/kg at the 99.9^{th} centile.

36.   These estimates are subject to some uncertainty, principally because of the limited number of data points used in their estimation and their skewed distribution.

37.   Analysis indicates that inefficient shucking, even by approved processors, may lead to concentrations of DA in edible tissue (comprising adductor muscle plus gonad) that are at least 4-fold higher than can be achieved by best practice. This figure was derived from comparisons of only five processors, and thus is also subject to some uncertainty.

38.   The simulations that are presented in this statement should assist risk managers in deciding whether they can select an upper limit for average DA in batches of scallops that would ensure adequate protection against ASP toxins when shucking is carried out by trained processors.

COT Statement 2014/05
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Annex 1

Derivation of DA tissue levels in a 400g portion of scallops by statistical modelling

1.   The expected distribution of DA concentrations in 400g portions of edible scallop was derived by random simulation. It was assumed that a 400g portion of edible scallop was derived from n scallops, each contributing 0.4/n kg of the total, and that the gonad made up a varying proportion, p_i, of the total edible content of each scallop.

2.   A simulated 400g portion was generated by randomly selecting n scallops from a specified dataset, the concentrations of DA in the ith scallop being m_i in muscle and g_i in the gonad. This selection was carried out with replacement such that an individual data point might in some cases contribute more than once to the same simulated portion. The concentration of DA in the simulated portion was then modelled as:

$$\Sigma \ [g_i \times p_i + m_i \times (1 - p_i)]/n \quad (1)$$

where p_i = $\dfrac{\text{weight of gonad in scallop i}}{\text{weight of muscle plus gonad in scallop i}}$

3.   100,000 400g portions of edible scallop were simulated to characterise the overall distribution of DA concentrations across simulated portions, and the 95th, 97.5^{th}, 99th, 99.5^{th} and 99.9^{th} this distribution were derived.

Identification of whole animal DA level at which up to 95%, 97.5%, 99%, 99.5% and 99.9% of 400g edible portions would not exceed the EFSA ARfD

4.   It was assumed that a given centile of the modelled distribution of DA concentrations in 400g edible portions was proportional to the mean concentration of DA in whole animals in the batch of scallops which provided the data on muscle and gonad concentrations that were used to generate the simulated portions.

5.   The aim was that at a given centile (k), the DA concentrations in a 400g portion should be ≤ 4.5 mg/Kg (established as a level that would not result in exceedance of the EFSA ARfD). Thus, the highest average whole animal DA level that would ensure no exceedance at the kth centile was calculated as:

Batch DA x 4.5 /DA_k

where Batch DA was the mean whole animal concentration of DA in the batch of scallops from which measurements were used to derive simulated 400g edible portions, and DA_k was the kth centile of the distribution of DA concentrations in simulated portions.

Annex 2

Sensitivity analysis showing the impact of gonad percentage, shucking and the number of scallops in a 400g portion of scallops on modelled DA tissue concentrations

1.   Formula 1 (from Annex 1), representing the model for deriving DA concentration in a simulated edible portion, was modified to include a processing factor for gonad (f_g) and another for adductor muscle (f_m), so that the effectiveness of shucking on tissue DA levels in a portion could be investigated. In addition, the influence of gonad content on the DA concentration of edible portions was investigated by varying p (the proportion of gonad in an edible portion). Thus, the concentration of DA in the simulated portion was then modelled as:

$$\Sigma\ [g_i\ x\ f_g\ x\ p + m_i\ x\ f_m\ x\ (1\text{-}p)]/n$$

The values assigned to f_g , f_m and p for this sensitivity analysis are set out in Table A2.1 below.

2.   DA levels in a modelled edible portion of scallops (either adductor muscle only, or adductor muscle in combination with gonad) might also be influenced by the number of scallops that made up a 400g portion. The effect of assuming either 8, 10 or 12 scallops in a 400g portion of adductor muscle plus gonad (or adductor muscle only) on tissue DA levels in simulated edible portions was investigated both in isolation, and in combination with changes in other parameters (gonad content and processing by shucking). The results of this sensitivity analysis are provided in Table A2.2 below.

Table A2.1. The levels used in the sensitivity analysis for each model parameter

Parameter in model	The levels considered
Average proportion of edible scallop by weight that is gonad	Variation in this parameter is seen between scallops. • 0% (Data shown for 0% gonad as a ratio of total edible portion is calculated on the basis of DA levels that are associated with muscle tissue only i.e. reflecting a situation when gonad is removed by shucking) • 10% • 25% • 50%
Processing factor for gonad	The parameter is based on empirical data from comparisons of shucking by best practice with the performance of five processors (McKenzie and Bavington 2002). Thus, using a gonad processing factor based on a ratio of worst case DA levels to those from best practice allows adjustment in the modelling to take into account the impact of variability in processing on DA levels: • A multiplier of 1 (equivalent to best practice, and implying no processing effect) • A multiplier of 4.97 (to represent worst case practice – a value established from data in McKenzie and Bavington (2002)
Processing factor for adductor muscle	Just as the DA levels in gonads varied between processors, so did the concentrations in adductor muscles (McKenzie and Bavington 2002). The approach taken for gonad was repeated for adductor muscle and the following multipliers applied: • A multiplier of 1 (equivalent to best practice, and implying no processing effect) • A multiplier of 2.57 (to represent worst case practice – a value established from data in McKenzie and Bavington (2002)
Number of scallops	The portion size in the model was fixed at 400g of edible scallop tissue. However, as the weight of edible tissue varies between scallops, so will the number of scallops needed to make up an 400g portion. The numbers of scallops considered in the modelling were as follows: • 8 x 50g • 10 x 40g • 12 x 33.3g

Table A2.2: Sensitivity analysis showing the impact of gonad content, processing and the number of scallops in a 400g portion on DA concentration in simulated 400g portions of edible tissue. Levels that exceed the EFSA ARfD of 4.5 mg/kg are shaded for ease of interpretation

Gonad as % of edible tissue by weight*	Muscle processing factor	Gonad processing factor	Number of scallops in 400g portion	DA concentration (mg/kg) in a 400g portion of edible tissue				
				95th centile	97.5th centile	99th centile	99.5th centile	99.9th centile
0%	1		12	0.7	0.7	0.8	0.9	1.0
0%	1		10	0.7	0.8	0.9	0.9	1.1
0%	1		8	0.7	0.8	0.9	1.0	1.2
0%	2.57		12	1.7	1.9	2.1	2.2	2.5
0%	2.57		10	1.8	2.0	2.2	2.4	2.7
0%	2.57		8	1.9	2.1	2.4	2.5	2.9
10%	1	1	12	1.6	1.7	1.8	1.9	2.1
10%	1	1	10	1.7	1.8	1.9	2.0	2.2
10%	1	1	8	1.7	1.9	2.0	2.1	2.3
10%	2.57	1	12	2.5	2.6	2.8	3.0	3.2
10%	2.57	1	10	2.6	2.7	3.0	3.1	3.4
10%	2.57	1	8	2.7	2.9	3.1	3.3	3.7
10%	1	4.94	12	**6.4**	**6.8**	**7.3**	**7.6**	**8.3**
10%	1	4.94	10	**6.6**	**7.0**	**7.6**	**8.0**	**8.7**
10%	1	4.94	8	**6.8**	**7.3**	**8.0**	**8.4**	**9.3**
10%	2.57	4.94	12	**7.0**	**7.4**	**8.0**	**8.3**	**9.0**
10%	2.57	4.94	10	**7.2**	**7.7**	**8.3**	**8.7**	**9.4**
10%	2.57	4.94	8	**7.5**	**8.0**	**8.6**	**9.1**	**10.0**
25%	1	1	12	3.4	3.6	3.8	4.0	4.4
25%	1	1	10	3.5	3.7	4.0	4.2	**4.6**

| 25%	1	1	8	3.6	3.9	4.2	4.4	**4.8**
25%	2.57	1	12	3.9	4.2	4.4	**4.6**	**5.0**
25%	2.57	1	10	4.0	4.3	**4.6**	**4.8**	**5.3**
25%	2.57	1	8	4.2	4.5	**4.8**	**5.1**	**5.6**
25%	1	4.94	12	**15.4**	**16.4**	**17.6**	**18.4**	**20.1**
25%	1	4.94	10	**15.9**	**17.0**	**18.4**	**19.3**	**21.4**
25%	1	4.94	8	**16.4**	**17.8**	**19.3**	**20.4**	**22.7**
25%	2.57	4.94	12	**15.9**	**16.9**	**18.1**	**18.9**	**20.8**
25%	2.57	4.94	10	**16.3**	**17.5**	**18.8**	**19.8**	**21.9**
25%	2.57	4.94	8	**17.0**	**18.3**	**19.8**	**20.9**	**23.0**
50%	1	1	12	**6.3**	**6.7**	**7.2**	**7.5**	**8.3**
50%	1	1	10	**6.5**	**7.0**	**7.5**	**7.9**	**8.7**
50%	1	1	8	**6.7**	**7.3**	**7.9**	**8.4**	**9.2**
50%	2.57	1	12	**6.6**	**7.0**	**7.5**	**7.9**	**8.7**
50%	2.57	1	10	**6.8**	**7.3**	**7.8**	**8.2**	**9.1**
50%	2.57	1	8	**7.1**	**7.6**	**8.3**	**8.7**	**9.6**
50%	1	4.94	12	**30.4**	**32.4**	**34.8**	**36.5**	**40.0**
50%	1	4.94	10	**31.3**	**33.6**	**36.2**	**38.0**	**42.1**
50%	1	4.94	8	**32.5**	**35.1**	**38.1**	**40.3**	**44.9**
50%	2.57	4.94	12	**30.7**	**32.7**	**35.1**	**36.8**	**40.3**
50%	2.57	4.94	10	**31.6**	**33.9**	**36.6**	**38.5**	**42.7**
50%	2.57	4.94	8	**32.9**	**35.3**	**38.4**	**40.3**	**45.2**

*0% reflects a scenario in which gonadal tissue is removed. In this circumstance the gonad processing factor is irrelevant.

Annex 3

Histograms for individual DA concentration (mg/kg) in gonad tissue by site
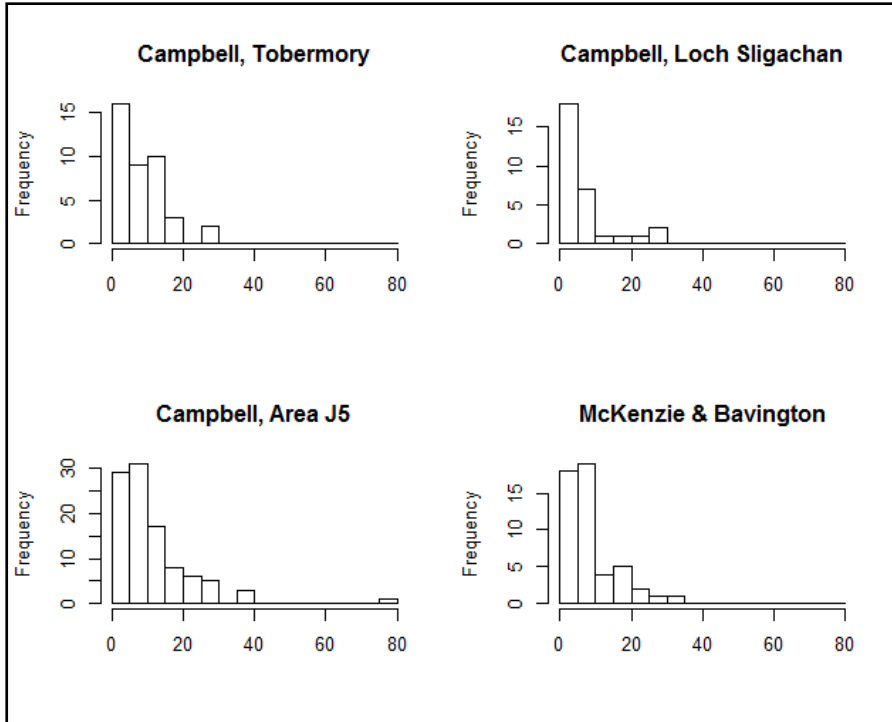
Histograms for individual DA concentration (mg/kg) in adductor tissue by site
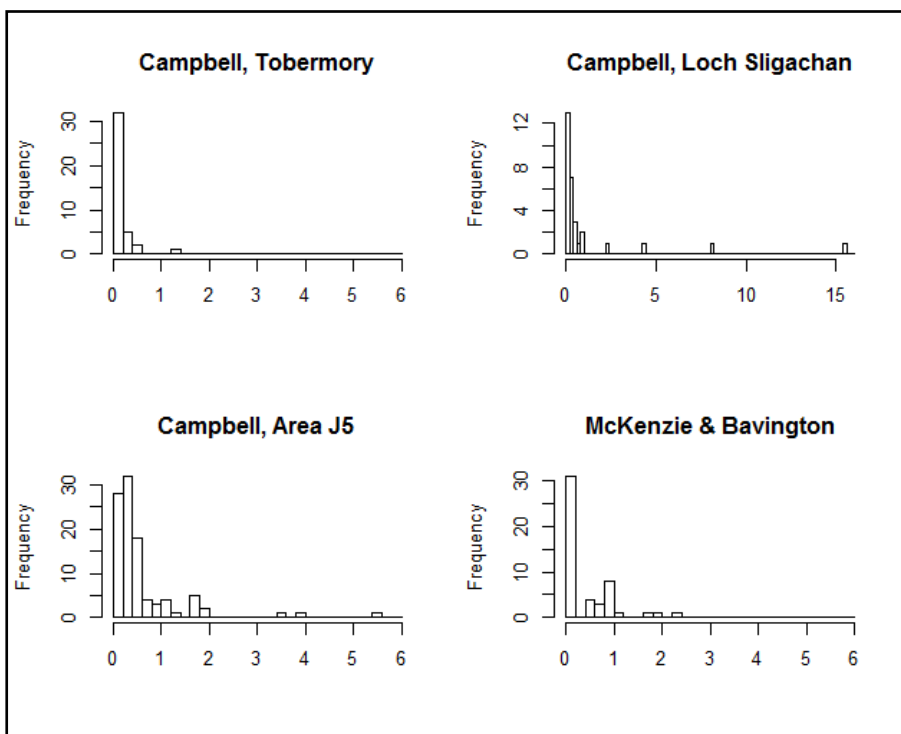
Annex 4

Table A4.1. Highest average concentrations of DA in whole scallops that would prevent exceedance of the EFSA ARfD in specified proportions of simulated 400g portions of <u>adductor muscle and gonad</u>: Effect of excluding or duplicating the highest concentration of DA in the sampling base used for simulation.

Source of data	Average concentration of DA (mg/kg) in whole scallops at which specified percentiles of the distribution of DA concentrations in 400g edible portions will not exceed the ARfD														
	95%			97.5%			99%			99.5%			99.9%		
	All data	Excluding the max DA level	Duplicating the max DA level	All data	Excluding the max level	Duplicating the max DA level	All data	Excluding the max DA level	Duplicating the max level	All data	Excluding the max DA level	Duplicating the max DA level	All data	Excluding the max level	Duplicating the max DA level
Tobermory Bay site in Campbell et al. (2001)	465	540	417	431	504	385	397	467	354	375	446	336	334	408	301
Loch Sligachan site in Campbell et al. (2001)	203	316	170	183	289	148	162	262	134	147	242	125	129	212	107
Area J5 in Campbell et al. (2001)	483	539	444	442	499	404	399	458	365	375	432	343	332	386	303
Area J5 McKenzie and Bavington (2002)	822	889	770	770	837	721	717	782	668	684	749	638	622	686	578

Table A4.2. Highest average concentrations of DA in scallops that would prevent exceedance of the EFSA ARfD in specified proportions of simulated 400g portions of <u>adductor muscle</u>: Effect of excluding or duplicating the outlying highest concentration of DA in the sampling base used for simulation.

Source of data	Concentration of DA (mg/kg) in whole scallops at which specified percentiles of the distribution of DA concentrations in 400g edible portions will not exceed the ARfD														
	95%			97.5%			99%			99.5%			99.9%		
	All data	Excluding the max DA level	Duplicating the max DA level	All data	Excluding the max DA level	Duplicating the max DA level	All data	Excluding the max DA level	Duplicating the max DA level	All data	Excluding the max DA level	Duplicating the max DA level	All data	Excluding the max DA level	Duplicating the max DA level
Tobermory Bay site in Campbell et al. (2001)	3427	5285	2686	2985	4903	2453	2606	4508	2132	2434	4273	1953	2059	3859	1693
Loch Sligachan site in Campbell et al. (2001)	221	391	180	199	356	154	173	307	140	151	272	129	129	231	109
Area J5 in Campbell et al. (2001)	1521	1782	1364	1357	1612	1203	1190	1445	1065	1103	1342	983	938	1153	841
Area J5 McKenzie and Bavington (2002) study	2110	2432	1910	1918	2214	1730	1738	2005	1548	1623	1878	1456	1420	1667	1279