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

First draft statement on domoic acid in King Scallops (pecten maximus)

Introduction

1. The Committee on Toxicity (COT) was asked to consider the evidence that was available to support shucking (removal of the non-edible parts) as a scientifically robust and effective method for managing the health risks associated with Amnesic Shellfish Poison (ASP) toxins in King Scallops which is caused by the water-soluble cyclic amino acid domoic acid (DA). This required analysis of data on the distribution of DA in different tissues of whole King Scallops for identification of an upper level for DA and epi-DA in whole scallops, at which consumption of a large portion of the shucked product (i.e. the edible parts) would not result in intakes that exceeded the acute reference dose (ARfD) of 30 µg/kg bodyweight (bw) established by EFSA in 2009. It was anticipated that such a level would provide the basis for future risk management decisions. Paper TOX/2014/10, and a subsequent paper (TOX/2014/18) which provided further analysis of the available data, were discussed by the COT in March and May 2014 respectively. The COT proposed that the analyses on all available data should be incorporated into a COT statement.

2. Annex A contains a first draft statement, taking into account the previous COT discussions.

Questions

3. Members are invited to comment on the structure and content of the first draft statement.

Secretariat
August 2014

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

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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 effective removal of these tissues by a process known as shucking can significantly reduce the levels that remain in the edible parts (adductor muscle and gonad). For scallops, the legislation requires that shucking must be carried out at an establishment which is approved to carry out the process. Live bivalve molluscs, including scallops, that are placed on the market, 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 (9 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

[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 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 was where some of the highest levels of DA that had previously been measured in official monitoring of whole King Scallops had been found. Similarly, Campbell et al. (2001) reported data

on DA tissue levels 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). Mean DA tissue levels were also calculated for the combination of all three sites studied by Campbell et al. (2001), and using all data from both studies. The observed differences in DA tissue levels between the studies are likely to reflect biological variation, but could also be affected by differences in the efficiency of shucking and measurement error.

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)
[+]Combined data for three sites from Campbell et al. (2001), n=170; range of DA levels in whole scallops: 1-1568 mg/kg	294 (250-354)	9.6 (5.1-13.6)	0.6 (0.2-0.9)	1.9 (1.0-2.5)
[++]McKenzie and Bavington (2002) and Campbell et al (2001) combined, n=220; range of DA levels in whole scallops: 1-1568 mg/kg	302 (250-354)	9.3 (5.1-13.6)	0.5 (0.2-0.9)	1.8 (1.0-2.5)

[+] Mean DA was derived by applying weights according to a ratio of 40:30:100. [++]Mean DA was derived by applying weights according to a ratio of 40:30:100:50

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

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

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 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
Combined data from 3 sites in Campbell et al. (2001), n=170	3.2	3.5	3.9	4.2	4.9
Area J5 McKenzie and Bavington (2002) , n=50	1.8	1.9	2.1	2.2	2.4
Combined data from McKenzie and Bavington (2002) plus Campbell et al (2001) studies n=220,	2.9	3.2	3.6	3.9	4.5

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 99.5th centile. However, at the highest centile considered in the analysis (99.9th), 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. The mean DA concentrations for simulated portions of edible tissue that were derived using the combination of all data, were at or below 4.5 mg/kg at every centile considered.

However, simulations involving the combination of DA data for edible tissue from the three sites within the Campbell et al (2001) study resulted in levels exceeding 4.5 mg/kg at the 99.9th centile.

18. Scallops from the Campbell et al. (2001) trial were obtained from different sites and sub-sites from the West Coast of Scotland. Thus, by combining data from these locations, it would not be possible to attribute modelled DA tissue levels to a specific batch of scallops. For the same reason, combining data from the studies by McKenzie and Bavington (2002) and Campbell et al (2001) would lead to uncertainty about the relevance of overall DA levels to a specific location, or a time period. Scallops from each study may have been subjected to different shucking standards which may further complicate interpretation of DA tissue levels obtained by modelling combined data.

19. 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 99.5th centile and above), none of the data sets (assessed separately or in combination) 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, number of samples taken and whole scallop mean (and range) of DA level mg/kg	**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
Combined data from 3 sites in Campbell et al. (2001), n=170	1.8	2.0	2.3	2.6	3.4
Area J5 McKenzie and Bavington (2002) , n=50	0.7	0.8	0.9	0.9	1.0
Combined data from McKenzie and Bavington (2002) plus Campbell et al (2001) studies n=220	1.5	1.9	2.1	2.3	3.1

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

20. McKenzie and Bavington (2002) also investigated the influence of shucking by different processors 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).

21. The sensitivity analysis also explored how the proportion of gonadal tissue could influence modelled DA concentrations in 400g portions of edible tissue. 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.

22. 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 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

23. 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.

24. Table 4 summarises the findings according to the batch of scallops from which measurements came. The dataset from Campbell et al (2001) for Loch

Slogachan gave the lowest average DA levels in whole animals which would be needed to ensure that the ARfD was unlikely to be exceeded.

Table 4. 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	712	464	431	396	375
Loch Sligachan site in Campbell et al. (2001), n=30	403	203	182	162	147
Area J5* in Campbell et al. (2001), n=100	755	484	442	400	375
Combined data from 3 sites in Campbell et al. (2001), n=170	690	416	378	337	314
Area J5 McKenzie and Bavington (2002) , n=50	1205	821	769	716	684
Combined data from McKenzie and Bavington (2002) plus Campbell et al (2001) study, n=220	770	463	419	375	346

25. 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 much higher DA levels might be acceptable in whole animals if only adductor muscle were consumed. In that case, the lowest DA levels in whole animals to ensure that the ARfD was not exceeded were again derived from data for the Loch Sligachan site (Table 5).

26. 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.

Table 5. 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,	6461	3421	2980	2601	2429
Loch Sligachan site in Campbell et al. (2001),n=30,	573	220	199	173	151
Area J5 in Campbell et al. (2001), n=100	2807	1521	1357	1190	1103
Combined data from 3 sites in Campbell et al. (2001), n=170,	2205	746	673	579	515
Area J5 McKenzie and Bavington (2002) , n=50	3934	2109	1917	1736	1622
Combined data from McKenzie and Bavington (2002) plus Campbell et al (2001) study, n=220	2487	914	723	642	581

Conclusions

27. DA levels in whole scallops are variable, with higher concentrations in the gonad than in adductor muscle

28. 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.

29. With efficient shucking, the maximum concentration of DA in whole scallops that would not lead to exceedance of the ARfD when consuming a 400g portion of adductor muscle plus gonad was estimated to be 182 mg/kg at the 99th centile and 147 mg/kg at the 99.9th centile.

30. With efficient shucking, the maximum concentration of DA in whole scallops that would not lead to exceedance of the ARfD when consuming a 400g portion of adductor muscle alone was estimated to be 199 mg/kg at the 99th centile and 151 mg/kg at the 99.9th centile.

31. A sensitivity analysis indicated that poor shucking has the potential to increase the DA concentration by approximately 4-fold in edible tissue comprising adductor muscle plus gonad, and 2.6-fold in adductor muscle only.

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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 proportion, p, of the total edible content of each scallop.

2. A simulated 400g portion was generated by randomly selecting n muscle DA concentrations (m_i) and n gonad DA concentrations (g_i) from a specified dataset. 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:

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

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

$$\text{Batch DA} \times 4.5 / DA_k$$

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. Equation 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. Thus, the concentration of DA in the simulated portion was then modelled as:

$$\Sigma [g_i \times f_g \times p + m_i \times f_m \times (1-p)]/n$$

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

2. 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) – see Table 1. 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 2 below.

Table 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 O% 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 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 safe level 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	1	12	0.7	0.7	0.8	0.9	1.0
0%	1	1	10	0.7	0.8	0.9	0.9	1.1
0%	1	1	8	0.7	0.8	0.9	1.0	1.2
0%	2.57	1	12	1.7	1.9	2.1	2.2	2.5
0%	2.57	1	10	1.8	2.0	2.2	2.4	2.7
0%	2.57	1	8	1.9	2.1	2.4	2.5	2.9
0%	1	4.94	12	0.7	0.7	0.8	0.9	1.0
0%	1	4.94	10	0.7	0.8	0.9	0.9	1.0
0%	1	4.94	8	0.7	0.8	0.9	1.0	1.1
0%	2.57	4.94	12	1.7	1.9	2.1	2.2	2.5
0%	2.57	4.94	10	1.8	2.0	2.2	2.4	2.7
0%	2.57	4.94	8	1.9	2.1	2.3	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.