DOMOIC ACID (DA) IN KING SCALLOPS (*Pecten Maximus*) PROCESSED IN SCOTLAND

INTRODUCTION

1. The Committee was asked to consider the available evidence to support shucking as a scientifically robust and effective method for managing the health risks associated with Amnesic Shellfish Poison (ASP) toxins in scallops at its March 2014 meeting (TOX/2014/10). At the time, it was noted that shucking (removal of the non-edible parts) carried out by appropriately trained individuals should eliminate a large proportion of domoic acid (DA) and epi-DA present in the scallop. Members however requested further analysis of the available data to explore the possibility of identifying an upper level for DA and epi-DA in whole scallops, at which consumption of a large portion of the shucked product would not result in intakes that exceeded the acute reference dose (ARfD) of 30 µg/kg bodyweight (bw) established by EFSA (2009). It was anticipated that such analysis might provide supportive evidence for assessing whether whole scallops (comprising both edible and inedible scallop tissues) with levels of ASP exceeding the maximum permitted limit (MPL) of 20 mg/kg DA and epi-DA could be sold to establishments capable of effective shucking.

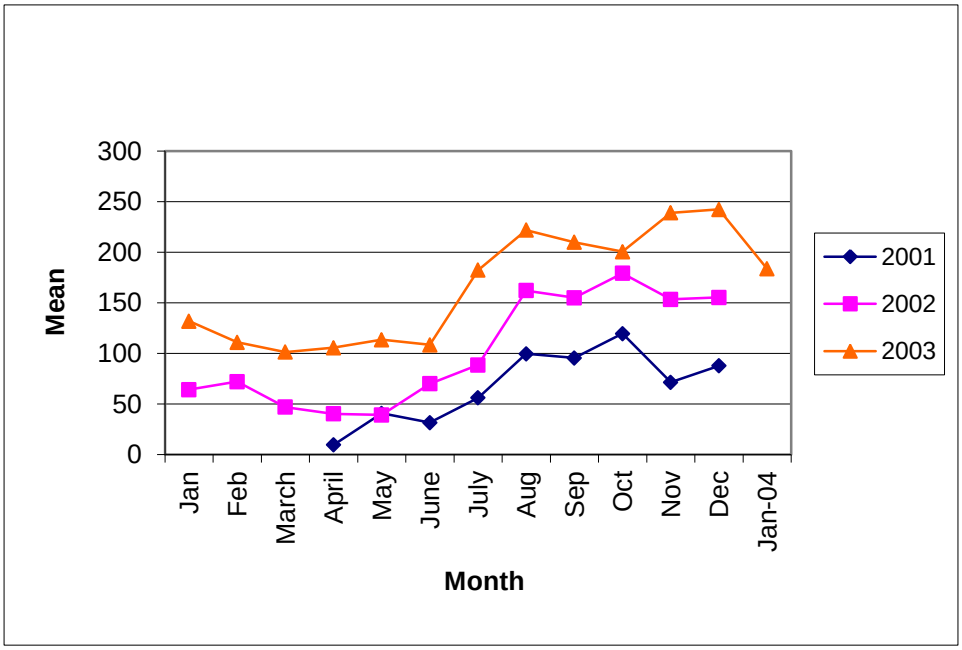
2. FSA Scotland provided the Secretariat with raw data from the 2001/02 study reported by McKenzie and Bavington (referred to as the 'Integrin study'; Annex C to TOX/2014/10), which provides DA levels in whole King Scallops and shucked component parts (adductor muscle, gonad and non-edible parts) in animals from the same batch (i.e. dredged from the same area on the same day). The Integrin study was based on King Scallops harvested from a single area of sea located near the island of Jura on the West Coast of Scotland. This area was chosen for the study because it was considered to be representative of the Scottish offshore King Scallop fishery, and furthermore some of the highest levels of DA in whole King Scallops had previously been recorded through official control monitoring in the area (see Annex C to TOX/2014/10). In addition to the data from the Integrin study, additional information on DA levels detected in scallops from the Scottish monitoring programme between 2001 and 2010 was provided. The DA levels reported in monitoring programs were predominantly for edible tissue in shucked animals and thus it was not possible to relate these to whole animal levels. Therefore only the data from the Integrin study were used in the current data analysis.

Variability in King Scallop DA levels

3. Marine biotoxins are known to show a non-homogeneous distribution in terms of time and geographical location (Ciminiello et al., 1999). The occurrence of DA and epi-DA is variable in time and some geographical areas are more affected than others by these toxins. Peak abundance of the

phytoplankton species responsible for producing DA is believed to occur between April to August (Scottish Executive, 2006). However, FSAS monitoring has detected the causative phytoplankton all year round, and in 2013 the largest bloom in Scottish waters occurred in October. A previous study (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) November to April. This study highlighted that although there was variation in the seasonality of DA uptake by King Scallops in different areas, that there was a general trend for King Scallops to show elevated levels of DA from May or June through to December. Figure 1 below, shows the mean levels of DA in whole King Scallops, identified in monitoring data relating to offshore areas across Scotland. This provides some evidence that elevated levels of DA are found towards the end of the year, when the Integrin study data analysed in this report was collected.

Figure 1: Mean level of DA in King Scallops sampled from different offshore areas in Scotland (McKenzie and McIntyre, 2004)



4. The size of the gonad contributes to significant variability in DA concentrations in King Scallops. The intestinal loop (which is the key source of DA) travels through the gonad, and can represent up to 6% of the gonadal volume (Campbell et al., 2001). The contents of this intestinal loop therefore have the potential to contribute to the toxin content of this organ. 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 gonad mass will vary depending on which stage an individual animal is at in its reproductive cycle (Ansell et al., 1991; UK National Reference Laboratory for Biotoxins, 2001). Spawning is associated with water temperature, and in Scottish waters is believed to occur most frequently during the Spring months (Marine

Scotland Science, personal communication). The gonad size is at its highest prior to spawning, and at this point relative DA concentrations will be lower due to the dilution effect of the increased gonad mass. After spawning, there is a significant reduction in gonad size, 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 also worth noting that it is much more difficult to free smaller gonads from the intestinal loop during the shucking process. This can further magnify the variation in DA levels, and highlights the importance of effective shucking practice in reducing toxin load.

Level that is unlikely to lead to exposures above the ARfD

5. Because DA has acute toxic effects, it is important to use a large portion size rather than long term average consumption in dietary exposure calculations. In the context of a series of evaluations of marine biotoxins in shellfish, EFSA selected a value of 400 g for the large portion size, initially based on data for shellfish consumption obtained from five European countries (France, Germany, Italy, The Netherlands and UK), for which the 95th percentile values ranged from 70 g to 465 g (EFSA, 2009). Analysis of additional data in 2010 led EFSA to confirm that the earlier estimate of a large portion size of 400 g shellfish meat was appropriate for protecting high level consumers against acute effects of marine biotoxins (EFSA, 2010).

6. EFSA noted that in order for a 60 kg adult to avoid exceeding the ARfD of 30 µg/kg bw, a 400 g portion of shellfish should not contain the sum of DA and epi-DA at more than 1.8 mg (corresponding to 4.5 mg/kg) shellfish meat (EFSA, 2009).

7. A study that was not available to EFSA investigated the weight of a scallop portion, in and out of home, in key European markets (Seafish, 2004). Sampling occurred in five countries of known high scallop consumption (UK, France, Italy, Spain and Belgium). Almost 1000 individual scallops were collected across the five countries for accurate laboratory determination of the weight of the scallops (both adductor muscle and gonad separately). These scallops were randomly selected in batches of ten from a mixture of retail outlets, with roughly equal numbers from each of the five countries. The UK data showed that restaurant portion weights were: mean = 147g; median = 133g and 99th percentile = 396g. Integrin noted that scallop portions comprise between 5 and 10 gonad combined with adductor muscles. The approximate average weight of edible tissue from the Integrin study (41 g see Table 1) from the serving size of 10 scallops, gives a large portion size of 410g which is consistent with the 99th percentile in the Seafish study and the value of 400 g used by EFSA for shellfish (EFSA, 2009 & 2010).

Table 1: Summary statistics on weights of scallops and its tissues; n=50

Tissue	Mean weight (g)	Median weight (g)	95th Percentile weight (g)
Adductor muscle	36.9	35.9	51.9
Gonad	4.2	3.9	7.6
'Rest-of-tissue'	33	32	47
Edible tissue (muscle & gonad)	41.1	38.4	57.4
Whole scallop	74	72	107

Data used for exposure modelling

8. The Integrin report noted that the adductor muscle should in principle contain no DA at all and that any DA present is a result of failure to remove all the gut tissue and/or because of contamination of the adductor muscle by fluids from the offal. As highlighted in para 5, elevated levels of DA can be found in the gonad due to its association with the intestinal loop.

9. Analysis of data in this paper focussed on the raw data obtained for two batches of scallops that were investigated in the Integrin study as follows:
(i) Trial one - a batch of King Scallops, sampled in November 2001, from a region which had been found to have scallop gonad DA levels above 20mg/kg . One sub-batch of 50 scallops was shucked to muscle, gonad, and rest-of-tissue. Each tissue from a scallop was given an identifier to allow summation of DA levels to provide a total DA level for the individual animal using the corresponding weight of the tissues and the whole animal.
(ii) Trial two - a batch of King Scallops, sampled in December 2001 from the same site as trial 1, which were randomly sorted into 6 bags of scallops. The sub-batches were then distributed to 5 processors (n=100 per plant) and to Integrin for shucking (50 scallops were supplied to Integrin for shucking but analytical data were only available for 32.
A batch is defined as all the scallops from a single harvesting area (monitored for biotoxins) landed to a single processor during one week. Thus a batch could comprise any number of scallops, depending on harvesting yields.

Trial 1 (batch sampled in November 2001)

10. Total DA levels in the 50 scallop samples from the sub-batch ranged from 210 to 459 mg/kg. The mean ratio of DA level in edible tissue (muscle and gonad) to the whole scallop was 0.25%; the median was 0.18% and the 95th percentile (P95) was 0.56%. Figures 2 to 4 compare the levels of DA in the edible tissues with the levels in the whole scallop, indicating a lack of correlation, with the possible exception of the gonads (R^2=6%[1], Figure 4). All

[1] **R^2** =the **coefficient of determination** which indicates how well data points fit a statistical model – sometimes simply a line or curve.

except one of the scallops had levels below 4.5 mg/kg for DA in the edible parts (adductor muscle and gonads combined).

11. Table 2 provides summary statistics on the concentrations of DA for 50 scallops (taken in November 2001 in the Integrin study). The mean gonad:adductor muscle ratio by weight is approximately 1:9 (Table 1). However according to Integrin, gonads can contribute up to 50 % of the edible meats. The ratio of mean, median and 95th percentile DA concentrations in adductor muscle relative to the corresponding level in whole animal is up to 0.4% (Table 2). Extending the latter comparison to total edible tissue results in a ratio of up to 0.76%. The ratios of mean (2.6%) and 95th percentile DA level (5.3%) in the gonad relative to DA level in the whole animal were an order of magnitude higher. The data confirm that the mean and 95th percentile DA level in adductor muscle and the total edible tissue (adductor muscle and gonads) derived from this analysis are below 4.5 mg/kg and hence unlikely to result in exceedance of the ARfD.

Table 2: Summary statistics on DA concentrations of scallops and its tissues; n=50

Tissue	Mean DA (mg/kg)	Median DA (mg/kg)	95th Percentile DA (mg/kg)
Adductor muscle	0.6	0.3	1.5
Gonad	8.5	6.8	22.4
'Rest-of-tissue'	731	728	951
Edible tissue (muscle & gonad)	1.4	1.1	3.2
Whole scallop	328	323	422

Trial 2 (batch sampled in December 2001)

12. The variation in DA levels following shucking by different processing plants and Integrin is shown in Figure 5 for adductor muscle and in Figure 6 for gonads.

Modelling of high-level exposure

13. Levels of DA identified in scallops from the Integrin study were subjected to statistical modelling for prediction of 95th percentile levels that may be encountered from a portion size range of between 8 and 12 scallops (edible parts only). The data used for the modelling were from samples taken in November 2001. The simulation intended to reflect high-end consumer exposure for a batch of 50 scallops with whole animal DA range of 210-459 mg/kg (corresponding to edible part mean concentration of 1.39 mg/kg) by taking account of scallop-scallop and inter-processor variability. The main elements of the model-based estimate were as follows:

- The weak positive correlation (R^2=6%) between gonad DA and whole-scallop DA (Figure 4) was used to predict mean gonad DA level for a whole animal DA level of 250 mg/kg.
- The uncorrected mean abductor muscle DA level was used, owing to the apparent lack of positive correlation between abductor muscle DA and whole-scallop DA (Figure 3).
- Scallop-to-scallop variability was estimated from the data, for both gonad and muscle portions.
- Inter-processor variability was estimated from the Trial 2 data.

The mean and variance components were combined to estimate the mean and standard deviation (SD) of DA concentration in a plate of scallops (post-shucking portion only). The estimated mean and SD were then combined to obtain an estimate of the 95th percentile concentration (Table 3) assuming that, at plate level (8-12 scallops), mean concentration follows an approximately normal distribution.

14. The calculations involve the following assumptions, which should be noted:

- The mean gonad:muscle weight ratio in the Trial 1 data is 1:9. This was assumed to be a constant ratio when forming the mean and SD of the composite edible portions. The data available do not enable us to gauge accurately what would happen for a batch with a larger gonad to muscle ratio.
- The scallops comprising the composite plates have been assumed to all be of the same weight: the coefficients of variation of gonad and muscle weights are 47% and 34% respectively.
- Since the Trial 1 data exhibit no apparent correlation between muscle DA and gonad DA, these have been assumed to be independent when forming the composite edible portions.

15. Table 3 demonstrates that the 95th percentile level estimated from different portions of scallops are below 4.5 mg/kg. Data show that the larger the portion, the lower the value of the high percentile, due to averaging of levels as a function of increasing number of individual scallops in a portion.

Table 3: DA Concentration estimates predicted for a range of scallop portions, based on data from Trial 1 (n=50) and gonad:muscle ratio by weight of 10:90

Number of scallops in portion	Mean DA concentration (mg/kg)	95th percentile DA concentration (mg/kg)
8	1.39	2.17
10	1.39	2.13
12	1.39	2.11

16. To address the fact that the gonad can sometimes contribute up to 50 % of the edible meats (though more usually 25 %); a scenario where the edible portion is split 50:50 between gonad and muscles was carried out for illustrative purposes. The calculations based on a mean DA concentration of 4.22 mg/kg for a plate of 8-12 scallops, resulted in 95th percentile DA

concentration range of 7.41 to 7.05 mg/kg. However, as noted in paragraph 4 gonad size and associated DA concentration could vary in relation to the spawning cycle.

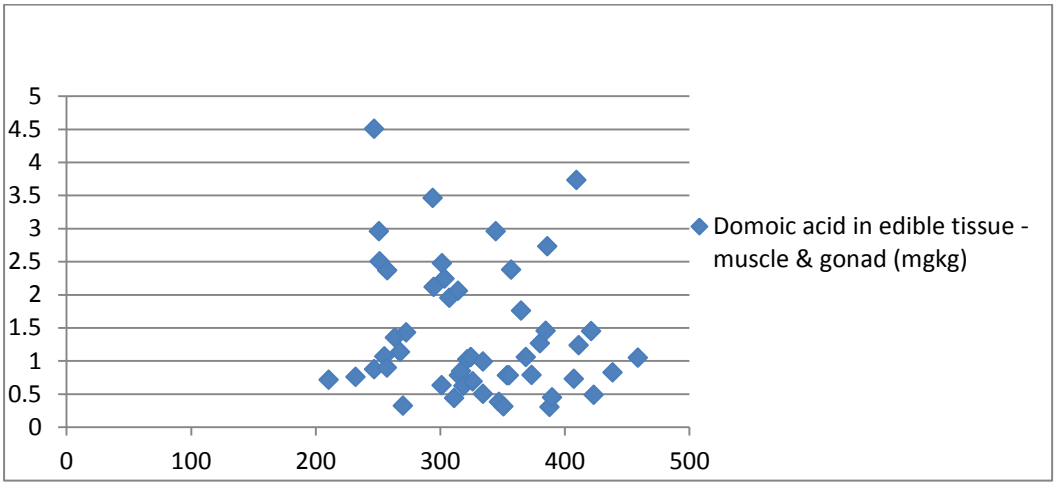
17. The data used in this modelling were for a batch of scallops that exhibited higher (210-459 mg/kg) whole animal DA levels. The Integrin study was conducted on scallops from a single area chosen because some of the highest levels of DA in whole King Scallops had previously been recorded through official control monitoring in the area. Therefore the data analysis carried out in this paper represents a period during which DA levels in King Scallops are anticipated are to be at the higher end .

Questions for the Committee

i. Based on the available data can the Committee identify a maximum level of DA in whole King Scallop, that would not lead to a risk to the consumer following effective shucking? In order to facilitate the development of potential risk management options, it would be useful if the Committee could provide values for products which are to be shucked to adductor only as well as adductor plus gonad.

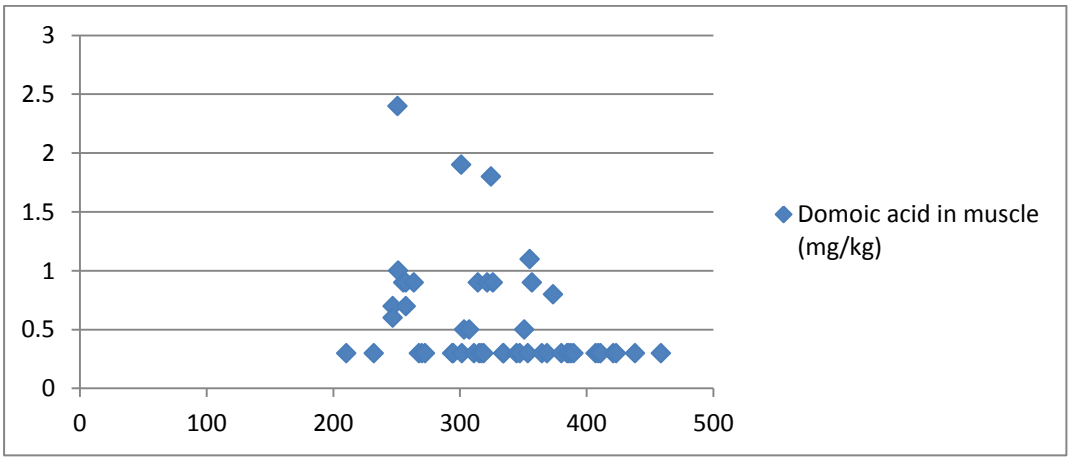
ii. If the available data are not adequate for this purpose, what additional studies would be required?

Figure 2: DA in edible tissue (muscle and gonad) vs DA in whole scallop (mg/kg); n=50



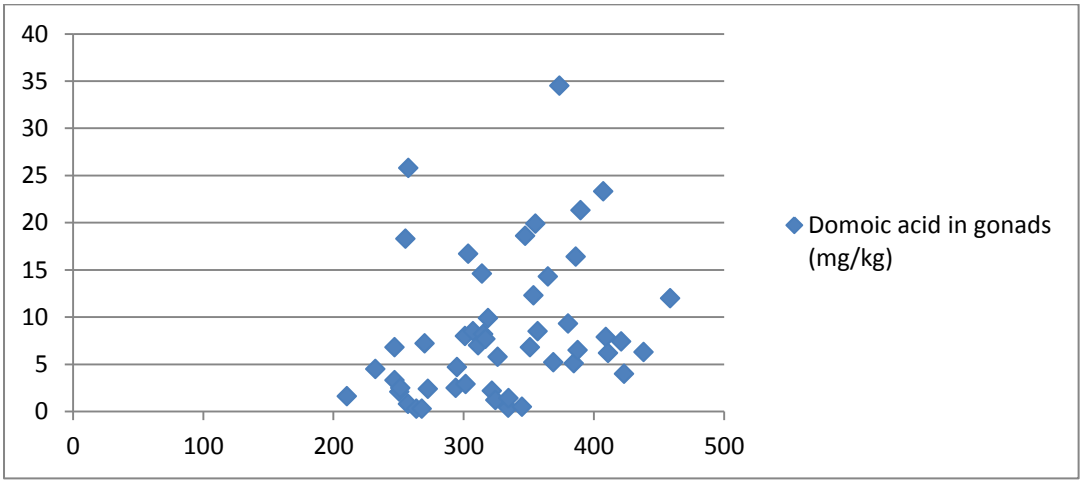
NB: Upper bound estimates (using an LOD of 0.3 mg/kg where the concentration of DA was zero)

Figure 3: DA in muscle vs DA in whole scallop (mg/kg); n=50



NB: Upper bound estimates (using an LOD of 0.3 mg/kg where the concentration of DA was zero)

Figure 4: DA in gonads vs DA in whole scallop (mg/kg); n=32



NB: Upper bound estimates (using an LOD of 0.3 mg/kg where the concentration of DA was zero)

Figure 5: Levels of DA (mg/kg) reported in adductor muscle samples taken from the same Batch shucked by 5 Processors (n=100) and Integrin (n=50)

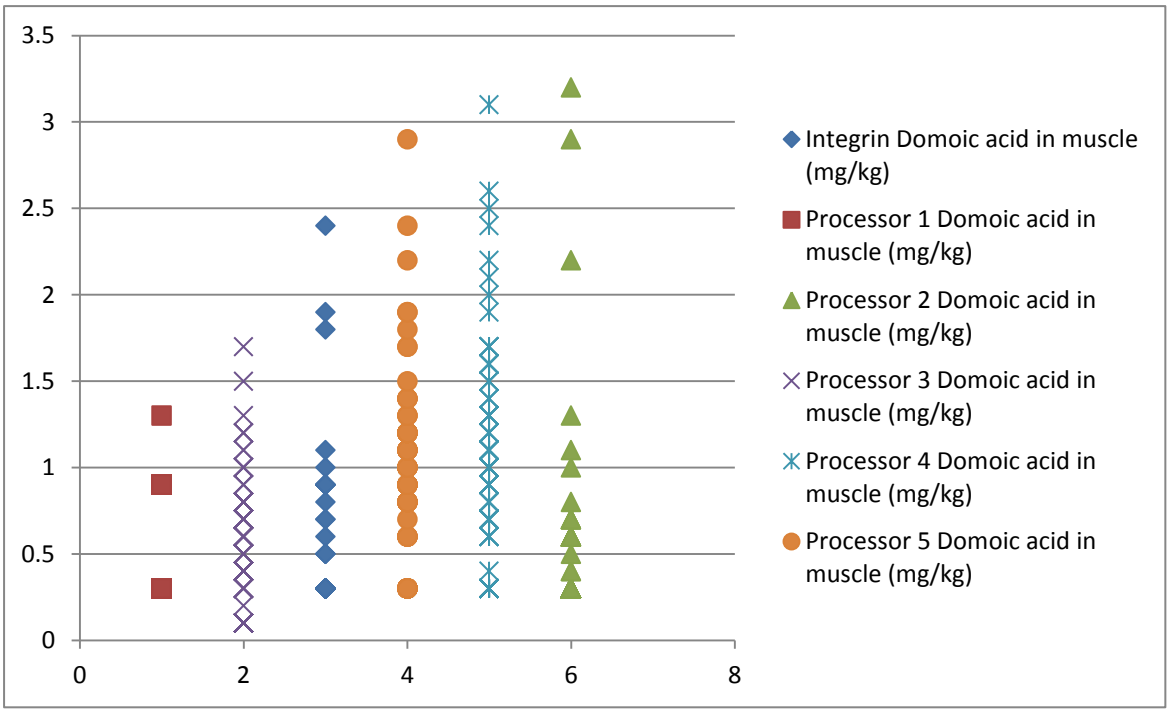
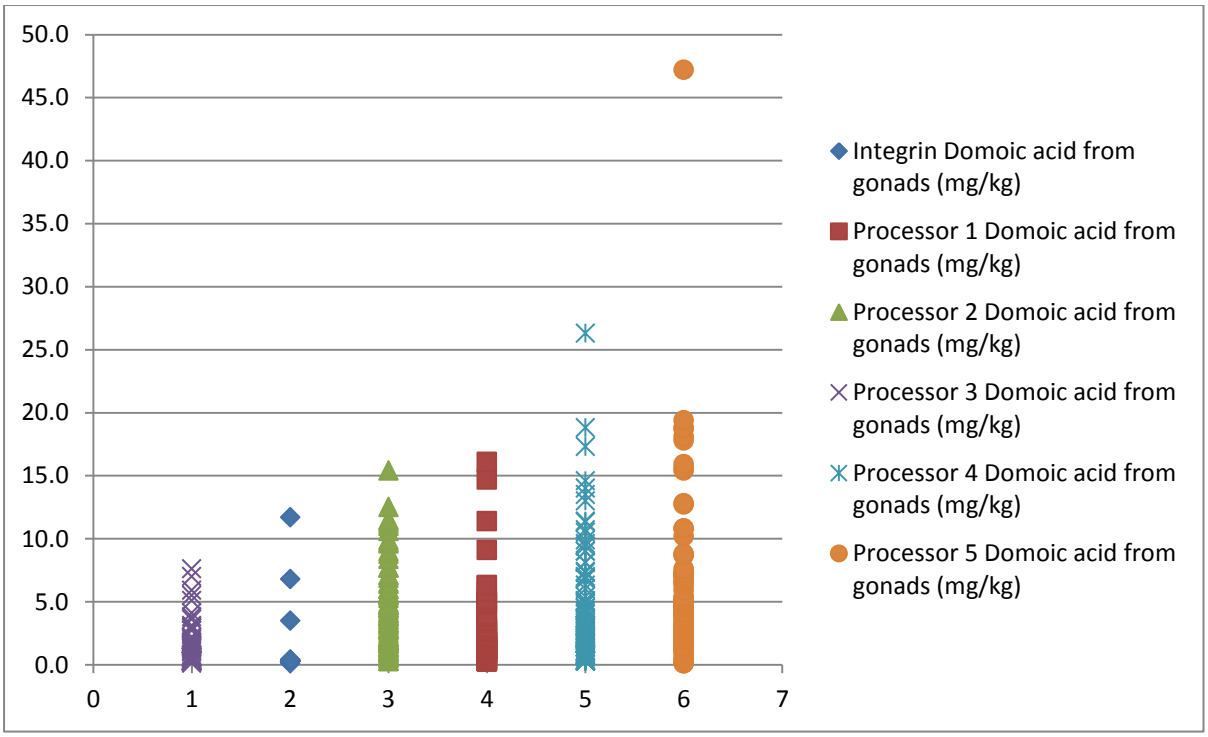


Figure 6 Levels of DA (mg/kg) reported in gonad samples taken from the same batch shucked by 5 Processors (n=100) and Integrin (n=32)



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