

SINGAPORE

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I. Introduction

The Agri-Food and Veterinary Authority (AVA) of Singapore is the agency responsible to ensure a resilient supply of safe food, safeguard the health of animals and plants; and facilitate agri-trade for Singapore. As Singapore is a small city state, much (about 90%) of its food is imported, including seafood / shellfish. A stringent monitoring programme is already in place for the screening of imported seafood/shellfish for biotoxins to ensure that they are safe for consumption.

On local production of food fish, Singapore has several coastal floating fish farms that supplies about 8 % of our food fish consumption. In recent years, these food fish farms have been affected by HAB occurrence, resulting in massive destruction of the food fish stock and large monetary losses to the livelihood of the local fish farmers.

The biotoxin monitoring programme that AVA implemented for the local fish farms is to provide early warnings to the local fish farmers and so that they can be prepared and implement measures to mitigate the effects of a HAB occurrence to a minimum. The AVA biotoxin monitoring programme has the following objectives:

- a) to monitor the imported seafood / shellfish for biotoxins,
- b) to monitor local farms and to ensure that contaminated products are not harvested and sold for human consumption.

All the biotoxins testing are carried out at the AVA's official food safety testing laboratory, the Veterinary Public Health Laboratory (VPHL).

II. Objectives and Goals

This ASEAN biotoxin monitoring project is to monitor products for the biotoxins Azaspiracids (AZA) 1,2 and 3, Amnesiac Shellfish Poisons (ASP) and Brevetoxins (PbTx) 2,3 and 6 in Green Mussels (*Perna viridis*). The results will provide useful database on the levels of these biotoxins in the seafood and for assessment if there is any food safety concern.

III. Survey Methodologies

a. Sampling Method, Sampling Site, Target Species (include scientific name), Number of Samples and Sampling Size

For sampling method, mussel samples were taken from various aquaculture farms near and around the coastal region of the Singapore waters. At least three samples were taken monthly under normal non-HAB alert situations. However, during any alert of HAB occurrence near the coastal food fish farms due to an elevated algae count, the sampling process will be increased. The target species used for this survey were Green Mussels (*Perna viridis*). Each time, the sampling size as per sample used was about 1.5 to 2kg of Green Mussels.

On average, 4 to 10 samples were tested for ASP, Brevetoxins and AZA each month according to AVA's surveillance program. The samples were taken randomly from local fish farms located off the shores of Singapore. No biotoxins (ASP, Brevetoxins and AZA) were detected in the Green Mussel (*Perna viridis*) samples surveyed during the period Jan 2015 – Dec 2017.

b. Method of Analysis (e.g. sample preparation method, analytical method used, quality control measures)

On arrival at the laboratory, the samples were de-shelled and the whole shellfish was homogenized for use in analysis. The extraction was carried out as in accordance to the laboratory's protocol for the various biotoxins – Azaspiracids (AZA) 1,2 and 3, Amnesiac Shellfish Poisons (ASP) and Brevetoxins (PbTx) 2,3 and 6 method. High Performance Liquid Chromatography coupled to a Triple Quadrupole Mass Spectrometer (LC-MS/MS). technique was used for the testing of the 3 groups of biotoxins. The methods used are adopted from the EC reference methods. All the methods are fully validated and accredited to ISO / IEC 17025.

c. Limit of Detection and Limit of Quantification

| Biotoxin | Limit of Detection (LOD) | Limit of Quantification (LOQ) |
|----------------|--------------------------|-------------------------------|
| AZA1 , 2 and 3 | 2ppb | 4ppb |
| ASP | 200ppb | 400ppb |
| Brevetoxins 2 | 160ppb | 200ppb |
| Brevetoxins 3 | 80ppb | 200ppb |
| Brevetoxins 6 | 160ppb | 200ppb |

d. National Regulatory Limits

The following limits were used:

| Marine Biotoxins | Guidelines applied |
|-----------------------|--------------------|
| AZA1 , 2 and 3 | 160µg/kg flesh |
| ASP | 20 mg/kg flesh |
| Brevetoxins 2,3 and 6 | 80µg/100g flesh |

IV. Results and Discussion

a. Participation in Inter-Laboratory Proficiency Testing and Results *(if any)*

The laboratory participated in 2 rounds of inter-laboratory proficiency testing (PT) programme organised by WEPAL, QUASIMEME for **ASP toxins** in May 2016 and Oct 2017 respectively. Reports of proficiency test results that were released on July 2016 and Feb 2017, showed that our testing results were within the z-score of 2 for the 2 PT rounds ie results were satisfactory

The laboratory also participated in 2 rounds of inter-laboratory proficiency testing programme for AZA1,2 and 3 toxins. organised by the same organiser in October 2016 and

Oct 2017 respectively . For the Oct 2016 round, results of 9 out of 10 analytes screened were within the z-score of 2. For the Oct 2017 round, results of 9 out of 11 analytes were within z-score of 2 i.e. satisfactory performance.

There are no PTs rounds currently for Brevetoxins 2,3,6.

Summary of Proficiency Testing (PT) for ASP and AZA

| PT Provider | Round | ASP | AZA1,2 and 3 toxins |
|---------------------|----------|------------------|---------------------|
| WEPAL, QUASIMEME | May 2016 | 3/3 satisfactory | - |
| | Oct 2017 | 3/3 satisfactory | - |
| WEPAL, QUASIMEME | Oct 2016 | | 9/10 satisfactory |
| | Oct 2017 | | 9/11 satisfactory |

b. Survey Results and Discussion

Table for Survey Results

| Sampling Location | Month & Year of Sampling (MM/YYYY) | Analyte Tested | No. of Samples Analysed | Min Concentration (ug/100g of meat) | Max Concentration (ug/100g of meat) | Average Concentration (ug/100g of meat) |
|---|------------------------------------|-------------------|-------------------------|-------------------------------------|-------------------------------------|---|
| Local farms off the shores of Singapore | Jan-Mar/2015 | ASP | 9 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 9 | | | |
| Local farms off the shores of Singapore | Apr-Jun/2015 | ASP | 22 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 22 | | | |
| | | AZA 1,2,3 | 22 | | | |

| | | | | | | |
|---|--------------|----------------------|----|--------------|--------------|--------------|
| Local farms off the shores of Singapore | Jul-Sep/2015 | ASP | 9 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 9 | | | |
| | | AZA 1,2,3 | 9 | | | |
| Local farms off the shores of Singapore | Oct-Dec/2015 | ASP | 13 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 13 | | | |
| | | AZA 1,2,3 | 13 | | | |
| Local farms off the shores of Singapore | Jan-Mar/2016 | ASP | 16 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 16 | | | |
| | | AZA 1,2,3 | 16 | | | |
| Local farms off the shores of Singapore | Apr-Jun/2016 | ASP | 12 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 8 | | | |
| | | AZA 1,2,3 | 12 | | | |
| Local farms off the shores of Singapore | Jan-Mar/2017 | ASP | 4 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 6 | | | |
| | | AZA 1,2,3 | 4 | | | |
| Local farms off the shores of Singapore | Apr-Jun/2017 | ASP | 33 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 30 | | | |
| | | AZA 1,2,3 | 33 | | | |

| | | | | | | |
|---|--------------|----------------------|-----|--------------|--------------|--------------|
| Local farms off the shores of Singapore | Jul-Sep/2017 | ASP | 38 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 38 | | | |
| | | AZA 1,2,3 | 38 | | | |
| Local farms off the shores of Singapore | Oct-Dec/2017 | ASP | 18 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 18 | | | |
| | | AZA 1,2,3 | 18 | | | |
| TOTAL | | ASP | 174 | Not Detected | Not Detected | Not Detected |
| | | Brevetoxins 2,3,6 | 169 | | | |
| | | AZA 1,2,3 | 165 | | | |

Discussion Results

A total of 174 shellfish samples were tested for ASP, 169 shellfish samples for Brevetoxins 2,3,6 and 165 shellfish samples for AZA1,2,3. ASP, Brevetoxins and AZA were not detected in all the samples tested.

d. Corrective Actions

Results of proficiency testing rounds have been good overall, with only 3 out of the 27 analytes screened outside of the satisfactory z-score of ± 2 . Corrective action have been taken and results of re-testing with newly purchased PT samples from the previous 2 rounds were within the acceptable z-score ie satisfactory performance.

V. Problems and Challenges Encountered

There is no PT provider providing PT rounds for Brevetoxins 2,3,6.

VI. Recommendations and Suggestions for Future Follow- Up Action(s)

1. To source for PT providers for Brevetoxins
2. To initiate a monitoring programme for Ciguatoxins in reef fish in order to assess if there is any food safety concern