Monitoring of Seafood for Marine Toxins

SINGAPORE

5-9 April 2018

WESTPAC training workshop on “Applying analytical method for detecting Ciguatoxins (CTXs) in fish”, 5-9 April 2018
Presentation Outline

- AVA’s Seafood control Strategies & Programmes
- Local Aquaculture
- Marine Toxins Monitoring Programme
- Capability for Ciguatoxin Detection
- Future plans
Agri-Food & Veterinary Authority (AVA)

- Ministry of National Development

Responsibilities

- Regulating import and export of all food
- Food factories and transportation of food up to just before retail
- Setting and enforcing food safety standards

“Safe food, health animals and plants for Singapore, trusted and respected globally”
AVA’s Seafood control Strategies & Programmes

Import
- Monitoring seafood imports
  - Monitors seafood import and conducts inspections and testing to prevent the marketing of unsafe seafood.
  - Products that do not meet these standards are refused entry.

Locally Farmed (Aquacultured)
- Monitoring of local shellfish harvesting areas
  - Provide early warning, close affected areas, etc.

Locally Processed
- Monitoring of processing facilities
  - Ensures that seafood processing facilities are built and operated according to standards and have in place effective quality assurance systems to protect consumers.
Main Fish Species Imported

Agri-Food & Veterinary Authority

- Sea Bream
- Turbot
- **Seabass**
- Pomfret
- Croaker fish
- Yellow tail
- Snakehead
- **Mackerel**
- **Snapper**
- Salmon

- Scad
- Catfish
- **Trevally**
- Tilapia
- Threadfin
- **Grouper**
- Goby
- Pompano
- Mullet
- Sweet fish

* Fish species that can cause Ciguatera fish poisoning

N.B. Based on 2017 import data.
Seafood - Import Requirements

- Licensing of Importer & permit requirement for each import
- Fish – low risk
- Molluscan shellfish & cooked shellfish – high risk
  - Shellfish sanitation programme
  - Health certificate required
Local Aquaculture Farms

Land-based (Freshwater) : 7

Coastal (Marine): 117

Fish, mussels, oysters, etc

About 6,000 tons per year
10% of fish supply
Fresh Water Food Fish Culture

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Fresh water pond

Locally farmed Freshwater Food Fish Species

- Snakehead
  *Channa micropeltes*
- Big Head Carp
  *Aristichthys nobilis*
- Marble Goby
  *Oxyeleotris marmoratus*
- Striped Catfish
  *Pangasius sutchi*
Foodfish species like groupers, seabass, snappers and milk fish, as well as green mussels.
AVA Food Testing Laboratory Facility

- AVA’s food safety testing facilities

Veterinary Public Health Laboratory (VPHC)

- Houses state-of-the-art facilities for a comprehensive range of microbiological and chemical analytical services
Test methodology
Agri-Food & Veterinary Authority

Current monitoring program:

Shellfish

- **PSP (Saxitoxin)**
  - ELISA as screening
  - Confirmation by LCMSMS

- **DSP (Okadaic acid)**
  - LCMSMS

- **ASP (Domoic acid)**
  - HPLC

- **PbTX (Brevetoxin)**
  - LCMSMS

Fish

- **TTX (Tetrodotoxin)**
  - LCMSMS
## Test methodology (2)

### Other Tests

<table>
<thead>
<tr>
<th>Toxins Type</th>
<th>Compound</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-toxins analysis of Brevetoxin-2, 3, 6/ Okadaic acid/ Domoic Acid</td>
<td>PbTX-2, 3, 6/ OA/ DA</td>
<td>LCMS/MS</td>
</tr>
<tr>
<td>Multi-toxins analysis of Brevetoxin-2, 3, 6/ Azaspiracid-1, 2, 3/ Domoic Acid</td>
<td>PbTX-2, 3, 6/ AZA-1, 2, 3/ ASP</td>
<td>LCMS/MS</td>
</tr>
<tr>
<td>Multi-toxins analysis of Azaspiracid-1, 2, 3/ Dinophysistoxin-1, 2/ Okadaic acid/ Pectenotoxin-2/ Yessotoxins/ Homo-Yessotoxins</td>
<td>AZA-1,2,3/ DTX-1,2/ OA/ PTX-2/ YTX/ homo-YTX</td>
<td>LCMS/MS</td>
</tr>
<tr>
<td>Microcystin-LR</td>
<td>MCLR</td>
<td>LCMS/MS</td>
</tr>
<tr>
<td>β-Methylamino-L-alanine (BMAA)</td>
<td>BMAA</td>
<td>LCMS/MS</td>
</tr>
</tbody>
</table>
Ciguatera Fish Poisoning Case

- 1 case of Ciguatera Fish Poisoning reported so far, in 2000 (could be because of under-reporting / not suspected clinically)
- Affected 2 women who had consumed fish in a restaurant
- Diagnosis is based on the typical clinical signs (gastrointestinal and neurological)
- Fish source traced back to Fiji (transhipment in AUS)
- Fish Species implicated: Kawakawa (*Deep Water Rock Cod*)
- Presence of CTX could not be confirmed by Mouse Bioassay and Cigua-Check
Ciguatera Fish Poisoning Case
Agri-Food & Veterinary Authority

This incident created quite a bit of publicity although it affects only 2 victims
Though reported cases of CFP is rare in Singapore, having reliable capability to detect CTX is important as:

a) Much of the Singapore’s food supply, including seafood, are imported;

b) Fish species imported and consumed in Singapore includes species which have been implicated in CFP (e.g rabbit fish, red snapper, grouper, etc)

c) AVA as the agency responsible for safe food supply, to be able to support any clinical diagnosis of ciguatera
Cigua-Check Test Kits and mouse bioassay
- not sensitive/specific

Chemical method using LCMS/MS

Challenge/limitation – lack of CTX congener standards
- CTX 1B (major congener) not available in the market
  CTX 3C is available from commercial company
Future Plans

- Collaboration with Partners / Research Institutes
- Participation in Inter-laboratory Proficiency Test Schemes
- Test Capabilities Development
  - Source for reference standards and materials to set up and validate method for detection of new emerging toxins
e.g. Spirolides (SPXs), gymnodimines (GYMs), pinnatoxins (PnTXs) and Pteriatoxins (PtTxs) in shellfish
Thank you

Http://www.ava.gov.sg
## Method Performance

### Test for

- **PSP**
  - **Mouse Bioassay**
    - LOD: 40 µg STX/100g tissue
    - LOQ: NA
    - Recovery (%): NA
    - Turn-around time: 1 day
  - **ELISA**
    - LOD: 4 ppb
    - LOQ: NA
    - Recovery (%): >85%
    - Turn-around time: ½ day
  - **LCMSMS (for confirmation)**
    - LOD: 25 ppb STX
    - LOQ: 50 ppb STX
    - Recovery (%): >60%
    - Turn-around time: 1 day
- **DSP**
  - **LCMSMS**
    - LOD: 5 ppb
    - LOQ: 10 ppb
    - Recovery (%): >70%
    - Turn-around time: 1 day
- **ASP**
  - **HPLC-DAD**
    - LOD: 0.25 ppm
    - LOQ: 0.5 ppm
    - Recovery (%): >75%
    - Turn-around time: 1 day
- **PbTX**
  - **LCMSMS**
    - LOD: 160 ppb (PbTX 2, PbTX 6)
      - 80 ppb (PbTX 3)
    - LOQ: 200 ppb
    - Recovery (%): >70%
    - Turn-around time: 1 day
- **TTX**
  - **LCMSMS**
    - LOD: 0.25 ppm
    - LOQ: 0.5 ppm
    - Recovery (%): >70%
    - Turn-around time: 1 day

* For Routine samples, based on 10 - 15 samples per test batch
<table>
<thead>
<tr>
<th>Test</th>
<th>Maximum Regulatory Limit (MRL)</th>
</tr>
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<tbody>
<tr>
<td>PSP</td>
<td>80 μg/100g</td>
</tr>
<tr>
<td>DSP</td>
<td>160 μg/kg</td>
</tr>
<tr>
<td>ASP</td>
<td>20 ppm</td>
</tr>
<tr>
<td>PbTX</td>
<td>80 μg/100g</td>
</tr>
<tr>
<td>TTX</td>
<td>Not Detected</td>
</tr>
<tr>
<td>AZA</td>
<td>160 μg/kg</td>
</tr>
<tr>
<td>PTX</td>
<td>160 μg/kg</td>
</tr>
<tr>
<td>YTX</td>
<td>3.75 mg/kg</td>
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