Marine Biotoxins and Food Safety

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OUTLINE

- Marine Biotoxins: Introduction
- Transition from animal tests to chemistry
- Current situation on the control of marine biotoxins in the EU
- Challenges and future perspectives
- Emerging toxins in the EU
EURL FOR MARINE BIOTOXINS

EUROPEAN REFERENCE appointed by EU Commision (DGSANTE)

SPANISH REFERENCE appointed by Spanish Competent Authority (AECOSAN)

Vigo
Harmful algal blooms and marine biotoxins

Natural contamination: Proliferation of toxic phytoplankton

BIOLOGICAL RISK
Phytoplankton control
Shellfish Toxins

Saxitoxin

Domoic Acid

Pectenotoxin

Okadaic Acid

Azaspiracid

Yessotoxin

MW (H-form) [M-H] - [M-2H]

1142.5  1141.5  570.3
1158.5  1157.5  578.3
1102.5  1101.5  550.3
1156.5  1155.5  577.3
1172.5  1171.5  585.3
1062.5  1061.5  530.5
1174.5  1173.5  586.3
1188.5  1187.5  593.5
1050.5  1049.5  524.3
Marine toxins/Seafood Safety/ Human Health

- Phytoplankton & Bacteria
  - Birds
  - Finfish
  - Marine Mammals
  - Human
  - Bivalves
  - Crustaceans
Difficulties in implementing alternative non-animal methods:

1. Long time success of animal methods.

2. Ease of use of animals - no need for highly trained staff.

3. Chemical/ instrumental methods can be expensive especially to set up.
The Mouse Bioassay

The long-standing reference method for shellfish toxins

Mouse Bioassay for PSP Toxins

- An AOAC method that has been used over 50 years for protection against PSP
- Intraperitoneal (IP) injection of 0.1M HCl extract of shellfish tissues into 20 g mouse
- Measurement: time to death

Disadvantages

- Poor detection limit
- Poor precision (± 20-30%)
- Poor accuracy at low levels
- No information on toxin(s) present
- Subject to false positives
- Animal rights concerns (banned in some countries)

Mouse Bioassay for DSP Toxins

- The mouse bioassay for DSP has never been very successful.
- Several protocols exist and none of these have ever been validated. As new lipophilic toxins appeared, attempts were made to extend the method with more modifications.

MBA for DSP suffers from:

- serious false positive and false negative problems.
- poor sensitivity
- poor correlation with human oral toxicity
ALTERNATIVE METHODS

Based on mechanism of action
PHYSICO-CHEMICAL METHODS
LIQUID CHROMATOGRAPHY/DETECTION MODES

LC/UV

LC/FLD

LC-HRMS

LC-MS/MS

IDENTIFICATION/QUANTITATION/CONFIRMATION
Current Situation in the EU Legislation

HPLC /FLD
Official method for PSP

HPLC /UV
Ref. method for ASP

LC-MS/MS
Reference method for LPTs (Dec. 2014)

EU Ref. Method SOON!!
The transition from MBA to Chemistry
Most recent changes

Absence of CODEX Criteria (Type IV)

CODEX criteria Type III (Reference)
Analytical challenges of the transition

Method Validation

- Precision
- Accuracy
- Limit of Detection
- Limit of Quantitation
- Specificity
- Linearity
- Range
- Robustness

Harmonised Criteria

Harmonised accreditations

From LC-MS/MS to HRMS
Measurement of the Uncertainty of the analytical result

Recent developments in measurement uncertainty

EURLMB Working group harmonization of the measurement of the uncertainty among EU-NRLs
### EU Legislation

<table>
<thead>
<tr>
<th>EU Legislation</th>
<th>Marine Biotoxin</th>
<th>Regulatory Limit</th>
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</thead>
<tbody>
<tr>
<td>REGULATION (EC) No 853/2004</td>
<td>Okadaic Acid, dinophysistoxins and pectenotoxins together</td>
<td>160 micrograms of okadaic acid equivalents per kilogram</td>
</tr>
<tr>
<td></td>
<td>Azaspiracids</td>
<td>160 micrograms of azaspiracid equivalents per kilogram</td>
</tr>
<tr>
<td>REGULATION (EU) No 786/2013</td>
<td>Yessotoxins</td>
<td>3,75 milligram of yessotoxin equivalent per kilogram</td>
</tr>
</tbody>
</table>

### Toxicological Issues

- **OA equivalents??**
- **MBA inheritance**
- **TEF**
  - 
  - Toxicity Equivalent Factor
  - IP/ORAL
- **Are the TEFs adequate?**

### How to express results?

- **Are the TEFs adequate?**
Future perspectives
Harmonization of phytoplancton control
Future Perspectives
Detecting new or unknown toxins

Reg (EU) 15/2011. After the period established in point B(1) of this Chapter (31 December 2014), the mouse bioassay shall be used only during the periodic monitoring of production areas and relaying areas for detecting new or unknown marine toxins on the basis of the national control programmes elaborated by the Member States.
EMERGING TOXINS IN THE EU

Climatic changes

Fisheries activity

International trade

Oversea Traffic increase

Emerging toxins

Scientific Opinion on marine biotoxins in shellfish – Emerging toxins: Ciguatoxin group (July 2010)
Identification and confirmation of Ciguatoxins in the EU

Ciguatoxin

Seriola

Cell Assay

LC-MS/MS and HRMS

- Development of standards and reference materials
- Development of sample pretreatment protocols
- Development and optimization of screening and confirmation methods
Citotoxicity assay/ LC-MS/MS

Canary Islands

MeOH FRACTION

STANDARD C-CTX1

SCREENING

CONFIRMATION
SUMMARY

New and Emerging toxins

ciguatoxin
tetrodotoxin
palitoxin

Reference materials

phytoplankton

TEFs

Harmonization

Mass Spectrometry MS/MS-HRMS

Screening methods
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SPANISH NRL NETWORK

Collaborators worldwide
Thanks for your attention