Analytical Challenges in the transition from biological to chemical methods for the control of marine biotoxins in seafood

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OUTLINE

- EURLMB
- Introduction to Marine biotoxins and their control in the EU
- Transition from animal tests to chemistry
- An update on the present situation
- Future perspectives, challenges, and needs
EURL FOR MARINE BIOTOXINS

EUROPEAN REFERENCE appointed by EU Commision (DGSANTE)

SPANISH REFERENCE appointed by Spanish Competent Authority (AECOSAN)
Harmful algal blooms and marine biotoxins

Natural contamination: Proliferation of toxic phytoplankton

BIOLOGICAL RISK
Shellfish Toxins

Azaspiracid

Okadaic Acid

Pectenotoxin

Saxitoxin

Domoic Acid

Yessotoxin

Adriatoxin (ATX)
The Mouse Bioassay

- the long-standing reference method for shellfish toxins

Disadvantages

- Poor precision (± 20-30%)
- Poor accuracy, especially near regulatory levels
- Subject to false positives
- Subject to false negatives due to either poor detection limit or unsuitability for some toxins
- Animal rights concerns
Alternative methods
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 Analytical Challenges

- To provide monitoring of a large number of regulated toxins in shellfish.

- To detect and identify toxins at low levels in shellfish before they cause problems – earliest possible warning.

- To monitor comprehensively for a wide range of emerging toxin groups and various structural analogues, even if they are not regulated.
Current Situation in the EU Legislation

- **ip. injection**

- **HPLC injection**

- **HPLC /FLD**
  Official method for PSP

- **HPLC /UV**
  Ref. method for ASP

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

- EU Ref. Method SOON!!

- Absence of CODEX Criteria (Type IV)

- CODEX criteria Type III (Reference)

- Complex analyte and complex matrix
Analytical Performance need to be tested

**Proficiency Testing:** Tool to determine laboratory testing performance by interlaboratory comparisons

Follow up

- Good NRL performance crucial for proper implementation of official controls
- Appropriate actions should be taken by EURL if results of PTs reveal underperformance

Expression of the results, (recovery correction or not, LOD and LOQs) Protocols used, lack of experience, etc
Analytical Issues

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

Harmonised Criteria

AUDITS: Harmonisation is needed

From LC-MS/MS to HRMS
Dealing with Uncertainty: a priority issue

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

Competent Authorities

Risk Assessment
# Toxicological Issues

<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, dinophysisotoxins 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>

OA equivalents??

TEF
Toxicity Equivalent Factor
IP/ORAL

MBA inheritance

Are the TEFs adequate?

How to express results?
Challenging Issues

Complex matrices

Interferences removal

raw/processed

RAW/ COOKED

Possible Biotransformations
The Challenge of 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.
Method development for identification and confirmation of Emerging toxins in the EU

- Development of standards and reference materials
- Development of sample pretreatment protocols
- Development of screening and confirmation methods

Ciguatoxin

Cell Assay → LC-MS/MS and HRMS
Harmonization of phytoplanton control
SUMMARY

New and Emerging toxins

ciguatoxin

tetrodotoxin

Reference materials

phytoplankton

TEFs

Harmonization

Mass Spectrometry MS/MS, HRMS

Screening methods
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