



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

Ana Gago-Martínez^{1,2}, José M. Leao¹

1.- University of Vigo, Faculty of Chemistry, Dept. of Analytical and Food Chemistry, Campus Universitario de Vigo, 36310-Vigo, Spain

2.- EU Reference Laboratory for Marine Biotoxins, Campus Universitario de Vigo, 36310-Vigo, Spain

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)**



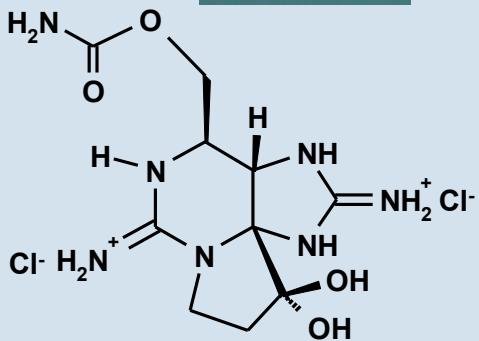
Harmful algal blooms and marine biotoxins



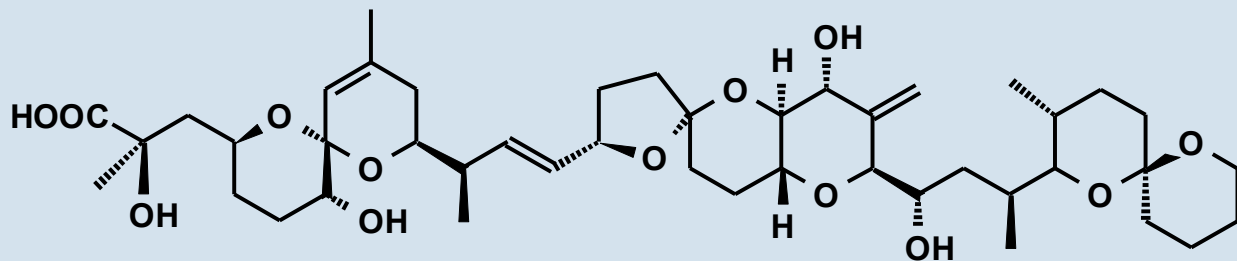
Natural contamination : Proliferation of toxic phytoplankton

BIOLOGICAL RISK

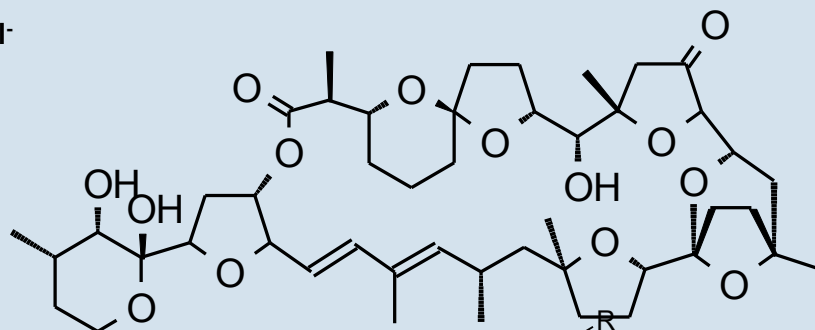
Shellfish Toxins



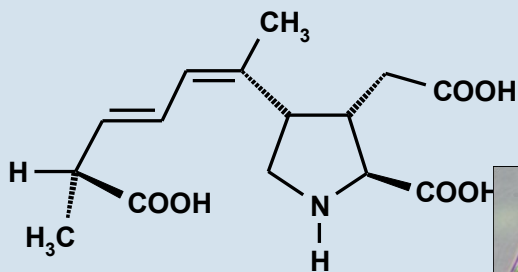
Saxitoxin



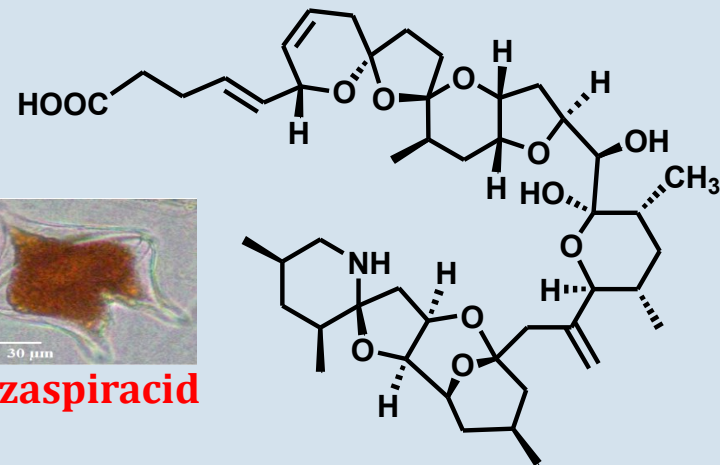
Okadaic Acid



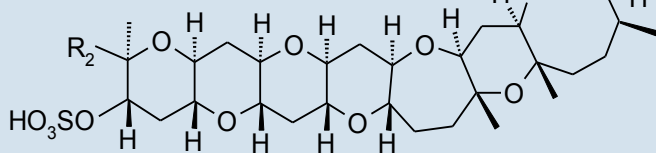
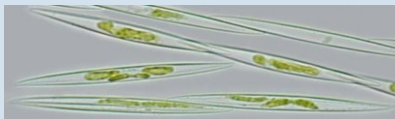
Pectenotoxin



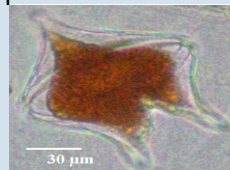
Domoic Acid



Azaspiracid



Yessotoxin



The Mouse Bioassay

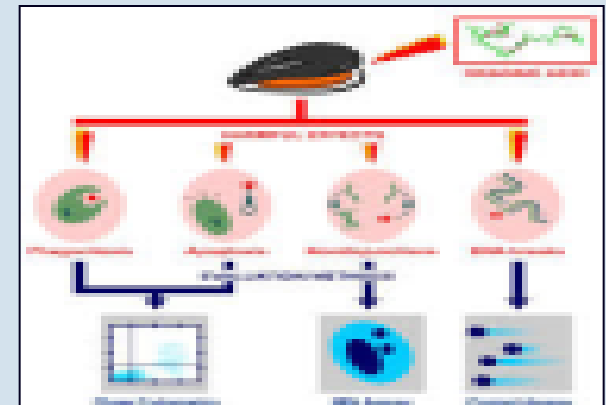
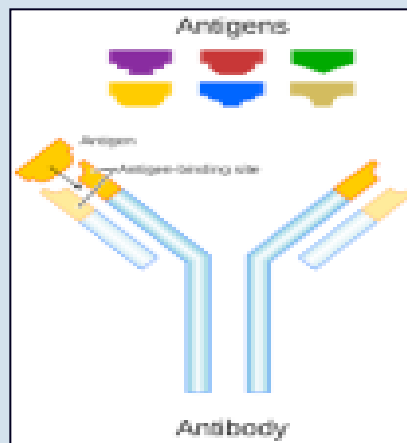
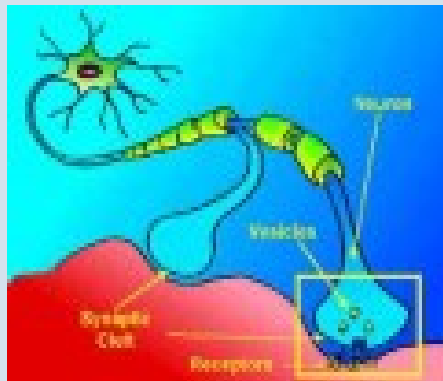
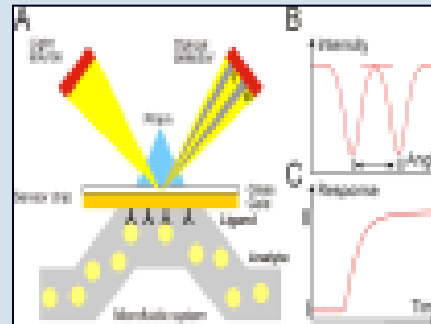
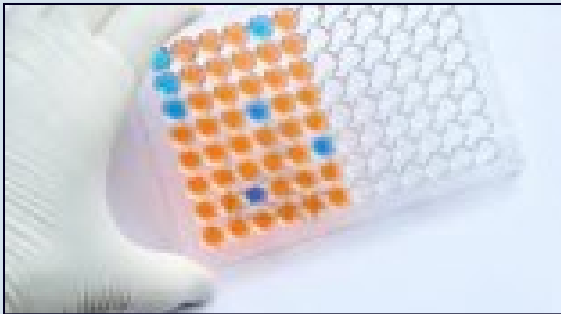
- the long-standing reference method for shellfish toxins

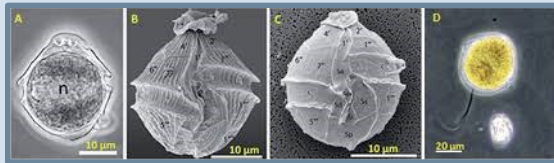
Disadvantages

- Poor precision ($\pm 20\text{-}30\%$)
- 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

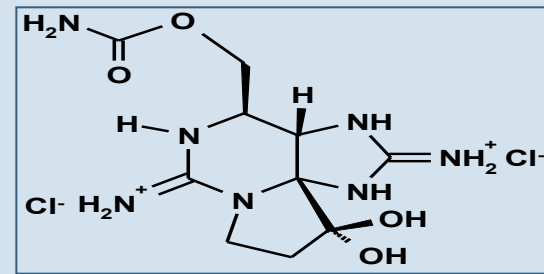
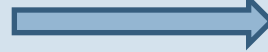


Alternative methods





Biological risk

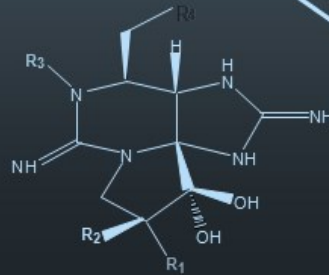


Chemical Risk

Transition from mouse to chemistry



Multiple toxins with multiple toxicities



Toxicity of Analogues Relative to STX

Toxin	Relative Toxicity (MU ₅₀ μM) ^{1,2}	Relative Toxicity ² (%)
B1	0.422	5.08
B2	0.273	3.29
C1	0.032	0.38
C2	0.503	6.06
C3	0.087	1.05
C4	0.291	3.51
spGTX2	4.59	55.3
spGTX3	5.31	64.1
spSTX	4.97	59.9
GTX1	6.00	72.3
GTX2	2.28	27.2
GTX3	4.01	48.3
GTX4	4.38	52.8
NEO	7.27	87.7

¹Delina et al., 1995, ²corrected for molecular weight

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.

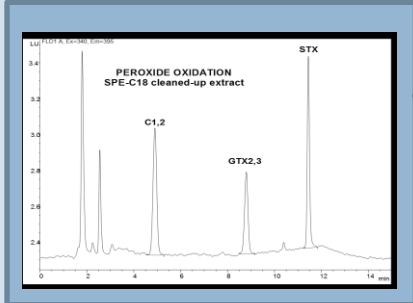
Current Situation in the EU Legislation

ip.
injection

HPLC
injection

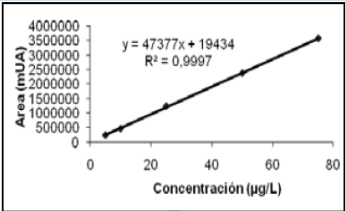
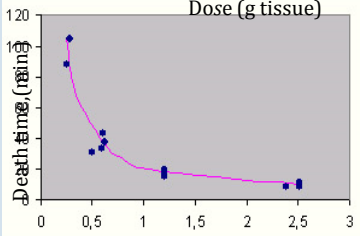
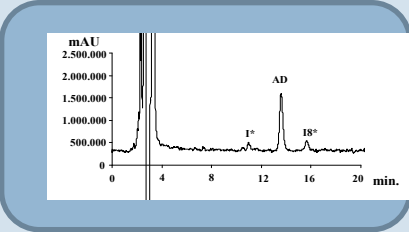


HPLC /FLD
Official method for PSP



EU Ref.
Method
SOON!!

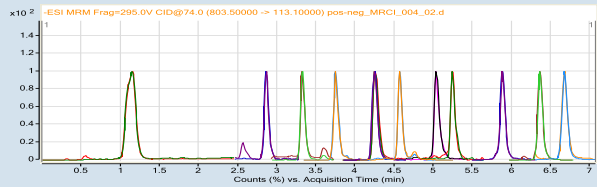
HPLC /UV
Ref. method for ASP



Absence of CODEX
Criteria (Type IV)

CODEX criteria
Type III (Reference)

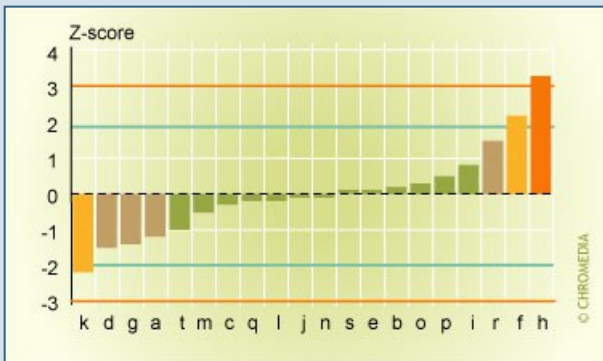
LC-MS/MS
Reference method for LPTs
(Dec. 2014)



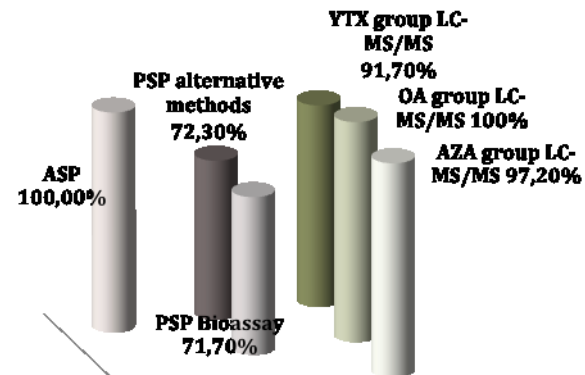
Complex analyte and complex matrix

Analytical Performance need to be tested

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



EURLMB PTs
NRLs % with satisfactory results in PTs by toxins group and method

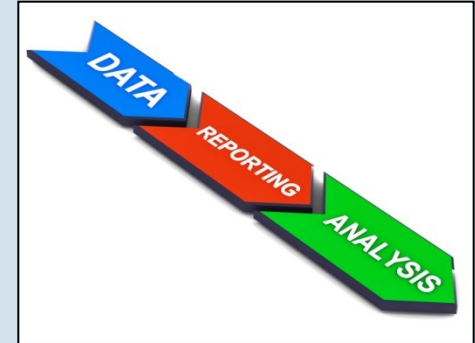
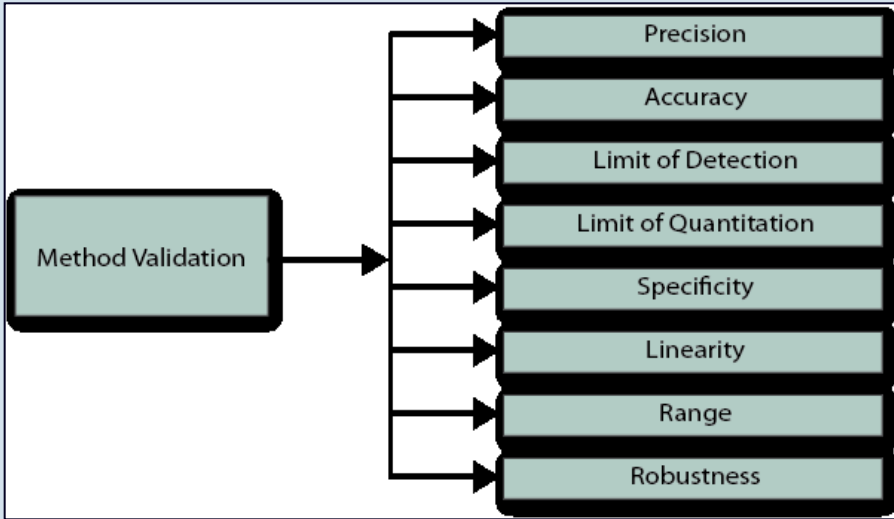


Follow up

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

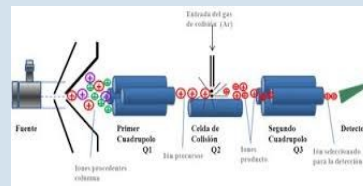
Expression of the results, (recovery correction or not, LOD and LOQs)
Protocols used, lack of experience , etc

Analytical Issues

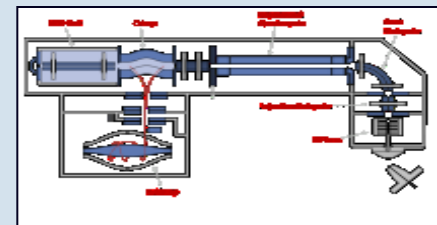


Harmonised Criteria

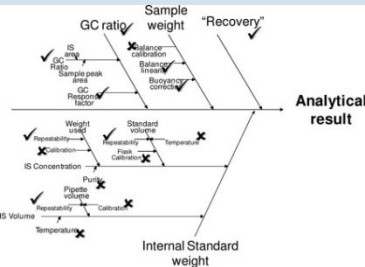
AUDITS: Harmonisation is needed



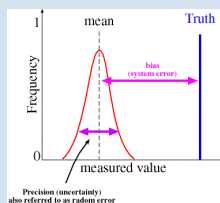
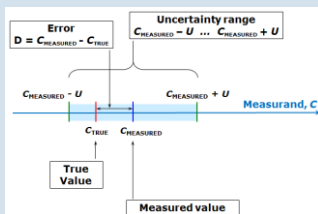
From LC-MS/MS to HRMS



Dealing with Uncertainty : a priority issue



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



Toxicological Issues



EU LEGISLATION	MARINE BIOTOXIN	REGULATORY LIMIT
REGULATION (EC) No 853/2004	Okadaic Acid, dinophysistoxins and pectenotoxins together	160 micrograms of okadaic acid equivalents per kilogram
	Azaspiracids	160 micrograms of azaspiracid equivalents per kilogram
REGULATION (EU) No 786/2013	Yessotoxins	3,75 milligram of yessotoxin equivalent per kilogram

OA equivalents??



MBA inheritance

TEF
Toxicity Equivalent Factor
IP/ORAL



Figure 1 - Administration of DMBA by gavage.

Are the TEFs adequate?

How to express results?

Challenging Issues

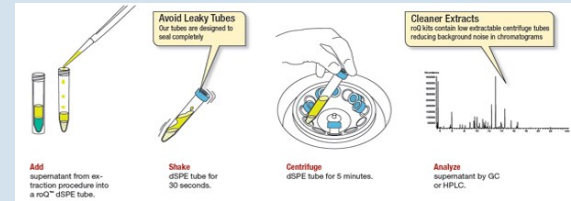
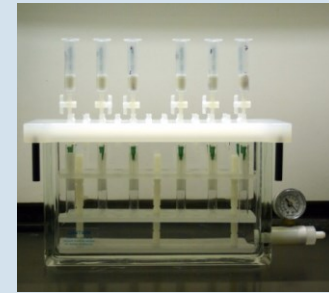
Complex matrices



raw/processed

RAW/ COOKED

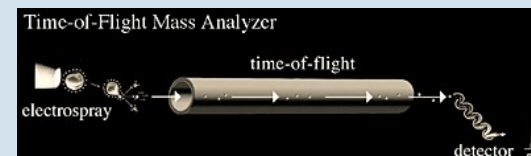
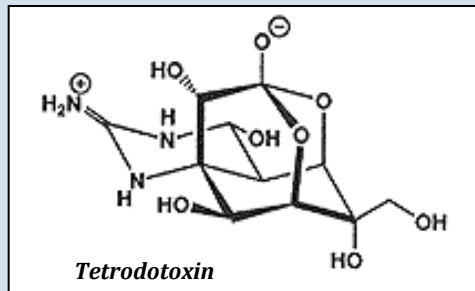
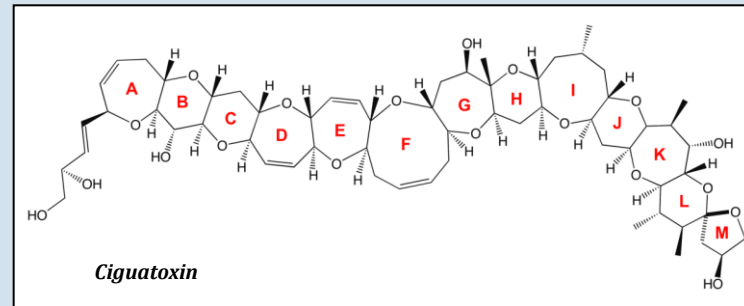
Interferences removal



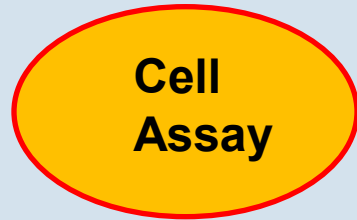
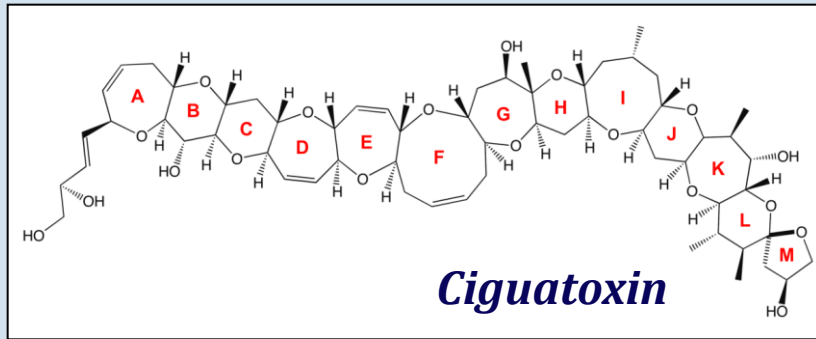
Possible Biotransformations

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

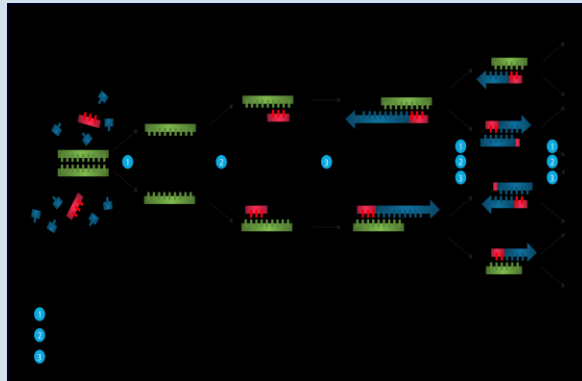
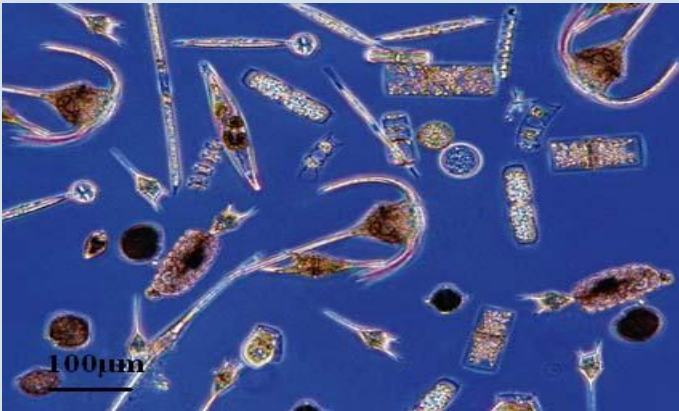


Method development for identification and confirmation of Emerging toxins in the EU



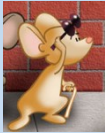
- ✓ Development of standards and reference materials
- ✓ Development of sample pretreatment protocols
- ✓ Development of screening and confirmation methods

Harmonization of phytoplankton control



SUMMARY

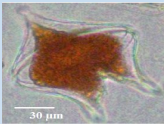
New and Emerging toxins



Reference materials



phytoplankton



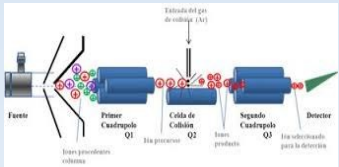
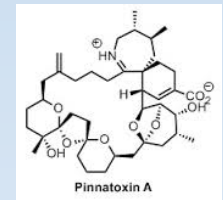
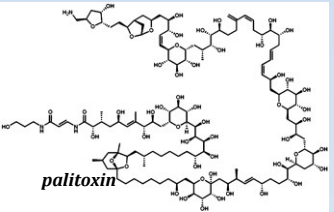
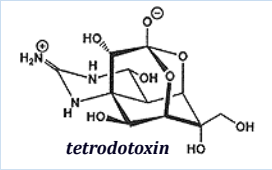
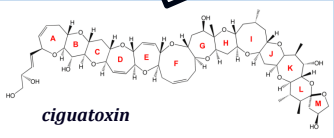
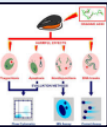
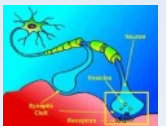
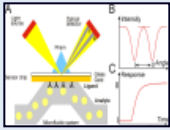
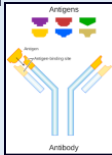
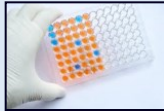
TEFs



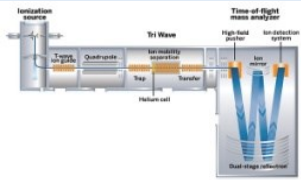
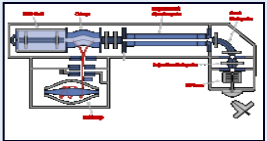
Harmonization



screening methods



Mass Spectrometry MS/MS- HRMS





Acknowledgments



UNIVERSIDAD
DE VIGO

❖ EURLMB staff

B. Ben; O. Vilariño; S. Otero; M. Barreiro; J. Iturbe,
M. Quintans

❖ UVIGO Staff

J.M. Leao; S. Rivera; J. Prado; N. Garcia; G. Moreiras; J.
Giráldez; S. Rodríguez