ISOLATION OF OVATOXIN-A.
A KEY STEP FOR HAZARD CHARACTERIZATION AND RISK MANAGEMENT OF OVATOXINS

Carmela Dell'Aversano[1,2], *Michela Varra*[1], *Fabio Varriale*[1], *Valentina Miele*[1], *Luciana Tartaglione*[1], *Antonella Miglione,*[1] *Caterina Fattorusso*[1], *Marco Persico*[1], *Oleh Tkachuk*[1], *Mark Poli*[3], *Keersten Ricks* [3], *Pearse Mc Carron*[4], *Elizabeth M. Mudge*[4], *Christopher O. Miles*[4], *David Kulis*[5], *and Don Anderson*[5]

[1] *UNINA-DF, University of Naples Federico II, School of Medicine and Surgery, Department of Pharmacy, Napoli, Italy.* dellaver@unina.it

[2] *NBFC, National Biodiversity Future Center, Palermo, Italy*

[3] *USAMRIID, U.S. Army Medical Research Institute of Infectious Diseases, Diagnostic Systems Division, Ft Detrick, MD, USA*

[4] *NRCC, National Research Council, Biotoxin Metrology, Halifax, NS, Canada*

[5] *WHOI, Woods Hole Oceanographic Institution, Woods Hole, MA, USA*

World Seafood Congress 2023
in association with International Conference on Molluscan Shellfish Safety
25-27 September 2023 Peniche-PORTUGAL

Risk assessment

A specialised field of applied science that involves reviewing scientific data and studies in order to evaluate risks associated with certain hazards. It involves four steps:

STEP 1 >

HAZARD IDENTIFICATION: The first step in risk assessment, this involves the **identification of** biological, chemical, and physical **agents capable of causing adverse health effects**

STEP 2 >

HAZARD CHARACTERIZATION: The second step in risk assessment, this involves **defining the nature of the adverse health effects associated with** biological, chemical and physical **agents** which may be **present in food**. The process should, if possible, involve an **understanding of the doses involved and related responses**.

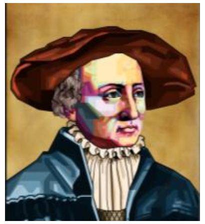
STEP 3 >

EXPOSURE ASSESSMENT: One of the key steps in risk assessment, this relates to a thorough **evaluation of who, or what, has been exposed to a hazard and a quantification of the amounts involved**.

STEP 4 >

RISK CHARACTERIZATION: The final stage of risk assessment, in which **the likelihood that a particular substance will cause harm is calculated** in the light of the nature of the hazard and the extent to which people, animals, plants and/or the environment are exposed to it.

"The dose makes the poison"



Paracelsus (1493-1541)

Concentration levels, FATE, Bio-accumulation, Trophic transfer

https://www.efsa.europa.eu/en/glossary/risk-assessment

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THE CHALLENGE OF NATURAL TOXINS

- HABs are sporadic/periodic events
- Toxic outbreaks occur under particular circumstances
- Many congeners, difficult to synthesize
- **Well characterized and quantified reference material is the priority**

https://www.efsa.europa.eu/en/glossary/risk-assessment

Risk assessment of ovatoxins

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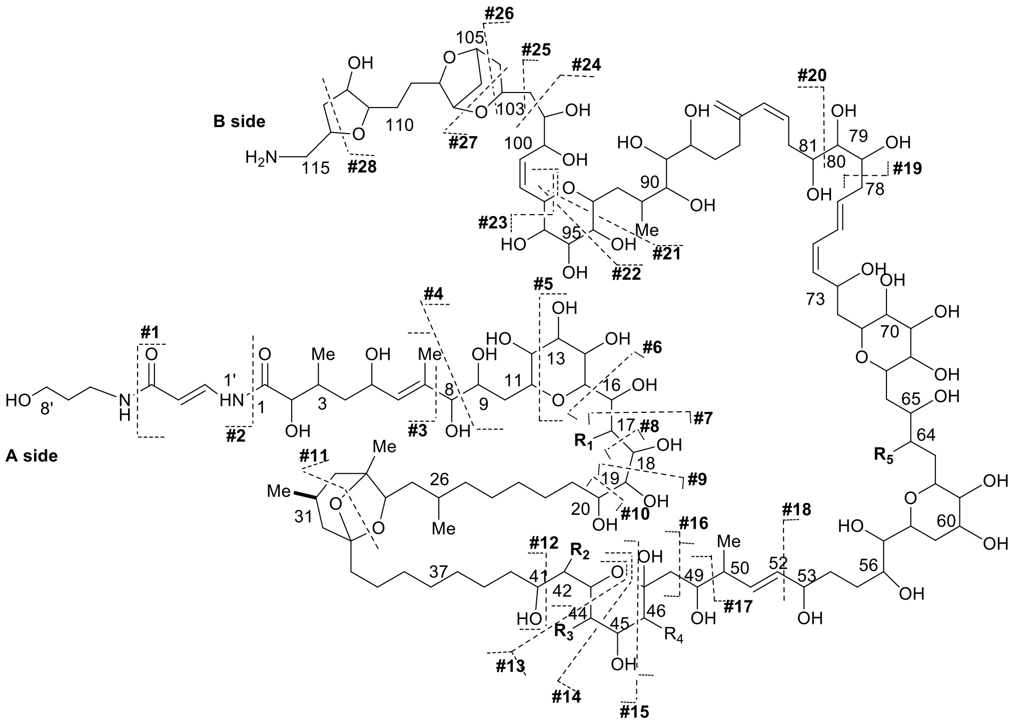

Toxin	R1	R2	R3	R4	R5	Other
PLTX	OH	H	OH	OH	OH	
OVTX-a	H	OH	H	OH	H	
OVTX-b	H	OH	H	OH	H	+ C_2H_4O in region N-C8’
OVTX-c	H	OH	OH	OH	H	+ C_2H_4O in region N-C8’
OVTX-d	H	OH	OH	OH	H	
OVTX-e	H	OH	H	OH	H	+ O in region C8-C8’
OVTX-f	H	OH	H	OH	H	+ C_2H_4 in region C95-C102
OVTX-g	H	OH	H	OH	H	
OVTX-h	H	OH	H	H	H	Open ring in region C42-C49
OVTX-i	H	OH	H	OH	H	+ $C_2H_2O_2$ – 1 unsaturation in region C49-C52 - O in region C53-C78
OVTX-j1	H	OH	OH	OH	H	+ $C_2H_2O_2$ – 1 unsaturation in region C49-C52 - O in region C53-C78
OVTX-j2	H	OH	H	OH	H	+ $C_2H_2O_2$ – 1 unsaturation in region C49-C52
OVTX-k	H	OH	OH	OH	H	+ $C_2H_2O_2$ – 1 unsaturation in region C49-C52
Isobaric PLTX	H	OH	OH	OH	H	+ O in region C8-C8’

Environmental Science and Technology (2017) *51, 13920* https://doi.org/10.1021/acs.est.7b03827

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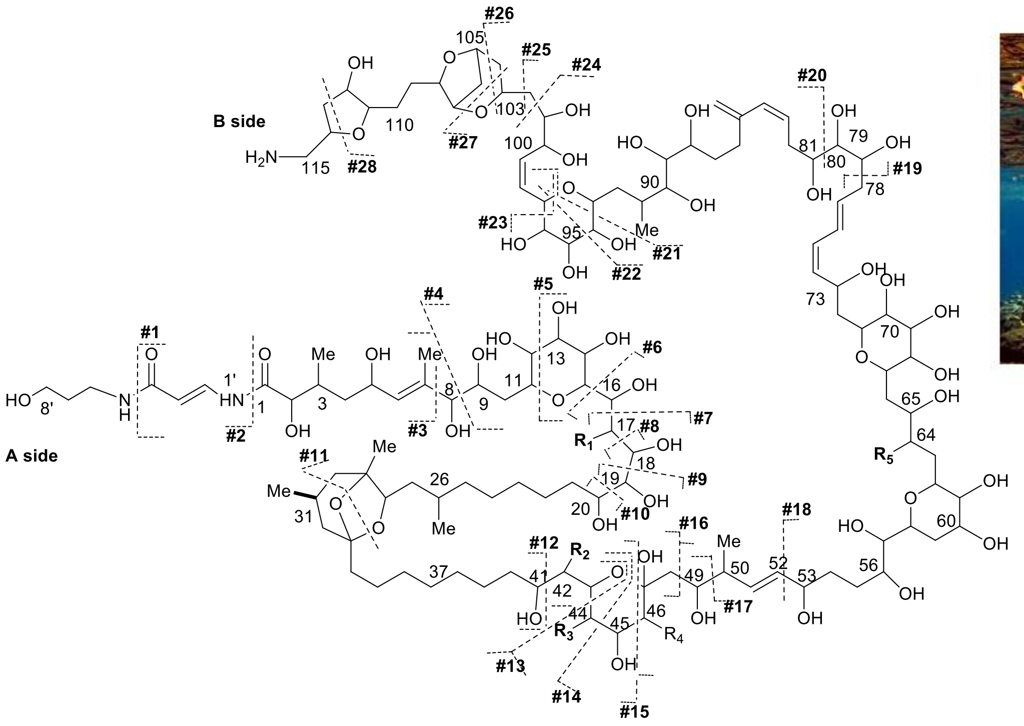

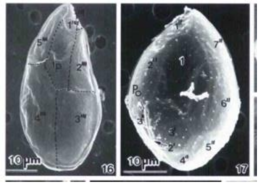

Ostreopsis ovata
From Faust et al 1996

Environmental Science and Technology (2017) *51, 13920* https://doi.org/10.1021/acs.est.7b03827 *Environmental Pollution (2019) 244, 617* https://doi.org/10.1016/j.envpol.2018.09.110

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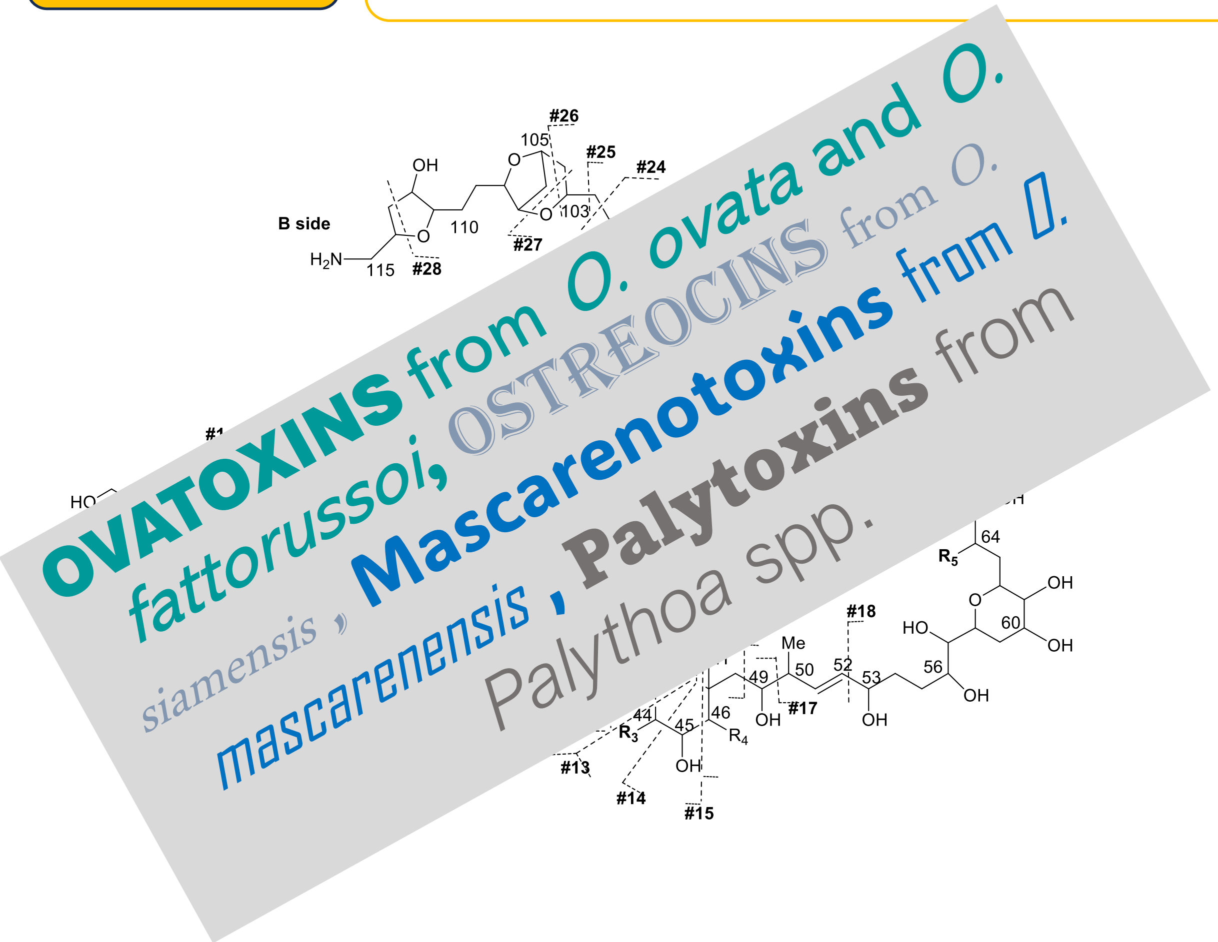

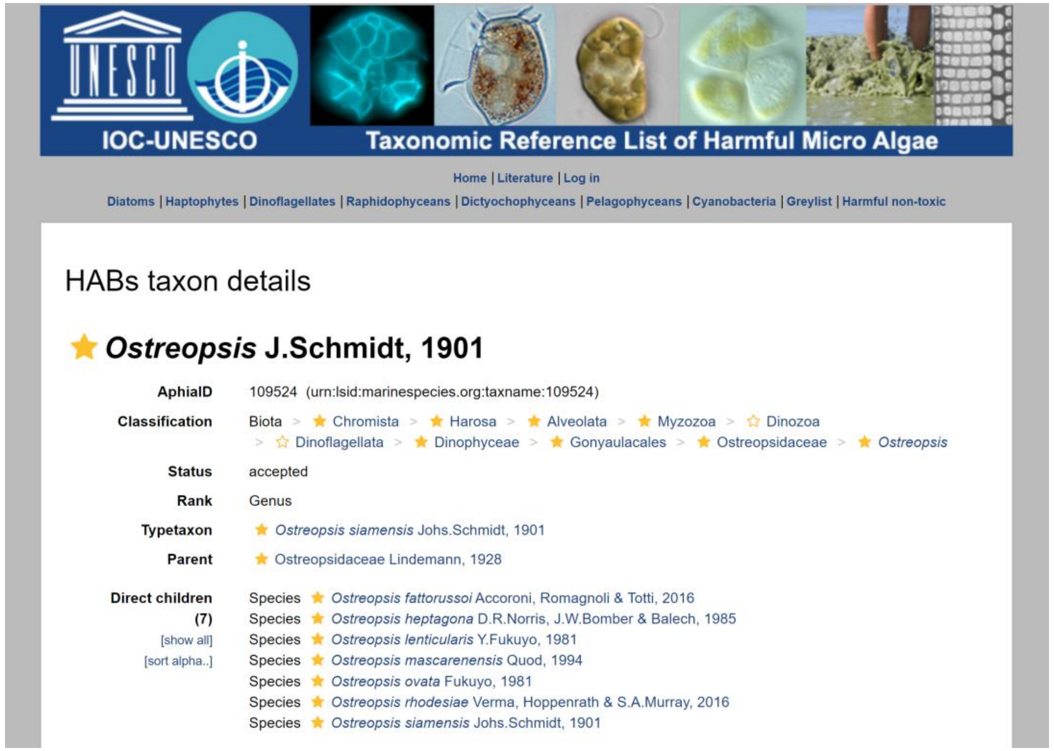

https://www.marinespecies.org/hab/aphia.php?p=taxdetails&id=109524

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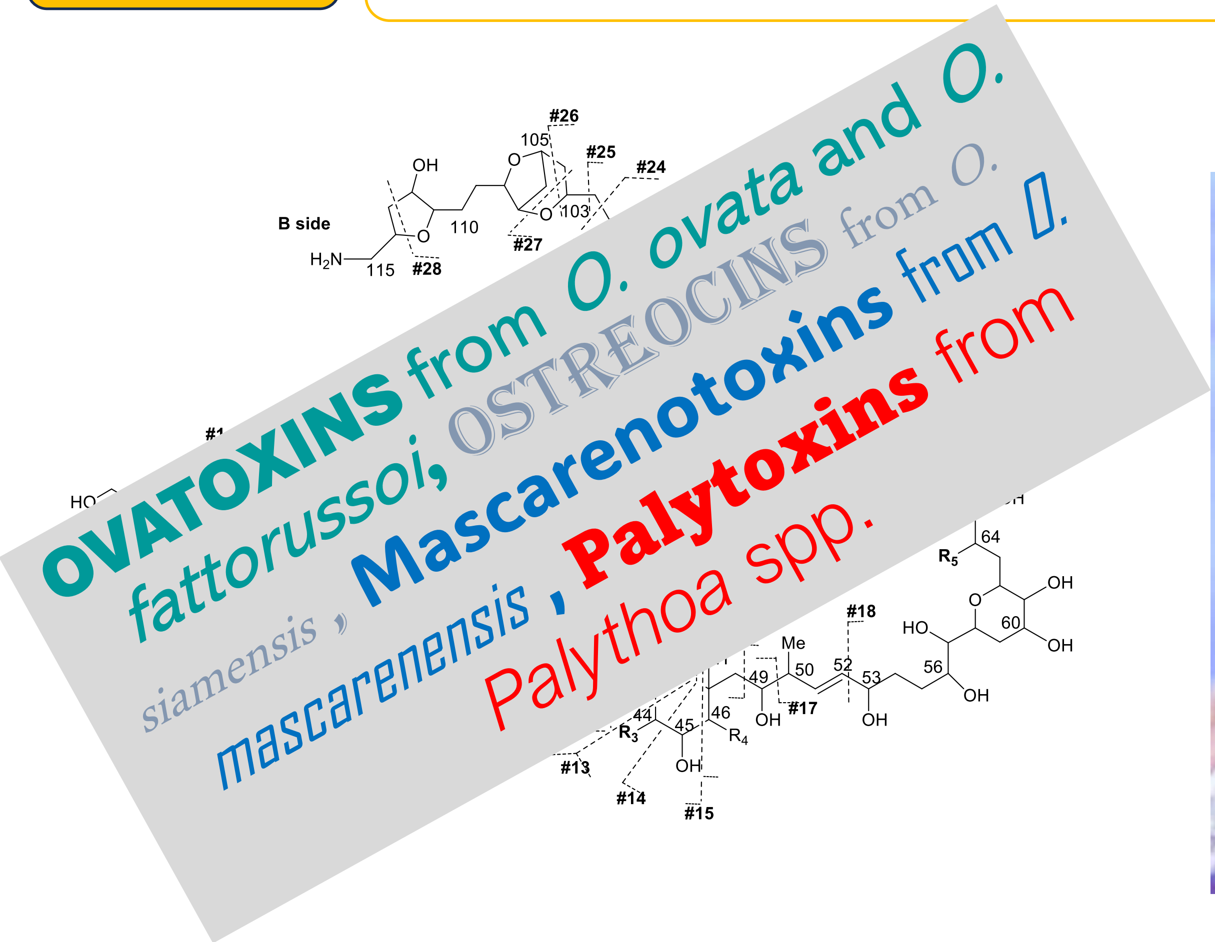

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Ostreopsis-related poisonings

O U T D O O R

Palythoa-related poisonings

I N D O O R

PLoS One. 2011 Apr 4;6(4):e18235. doi: 10.1371/journal.pone.0018235. *Environmental Science and Technology (2016) 50, 1023-30* DOI: 10.1021/acs.est.5b05469

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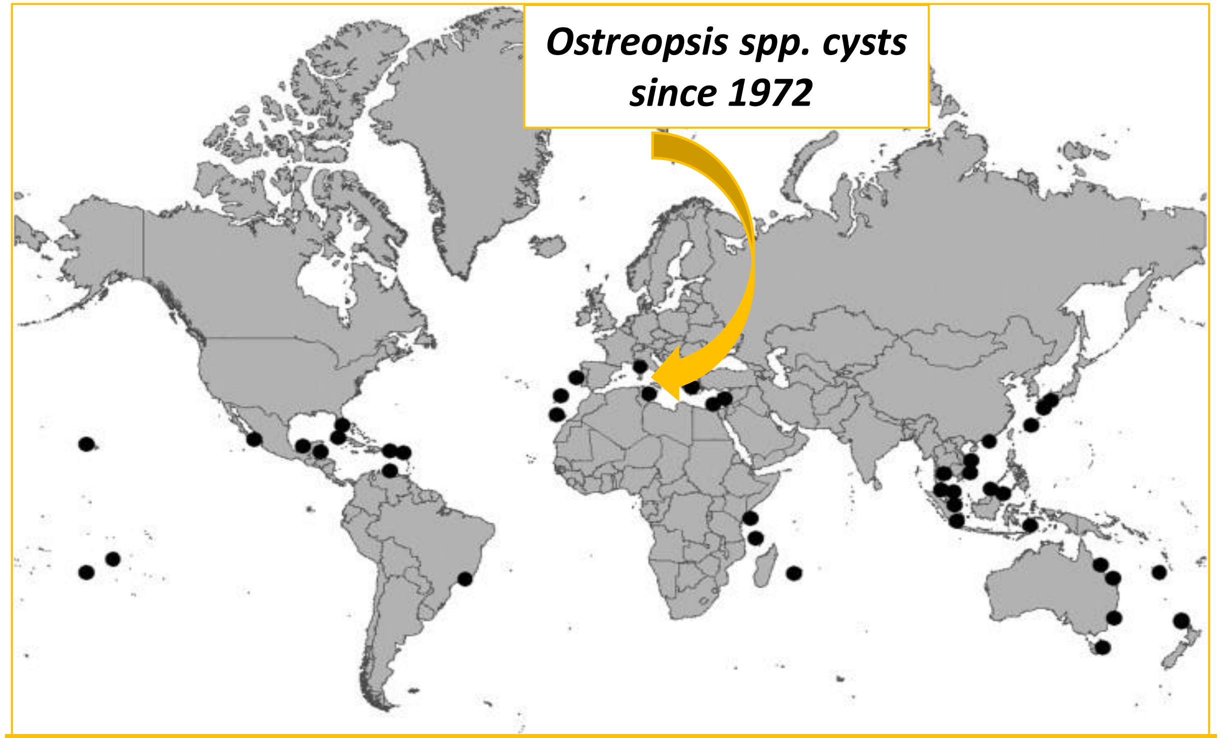

Extracted from Toxicon (2011) 57, 400

MID '90s

BENTHIC HARFUL ALGAL BLOOMS (BHAB) of *Ostreopsis* spp. in the Mediterranean area as the result of:

- Nutrient enrichment along coastal waters
- Global warming
- Dispersal and redistribution of algal cells through ship ballast waters and plastic debris

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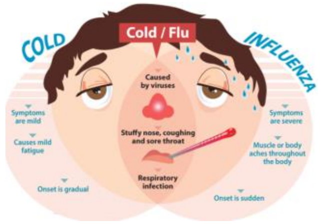
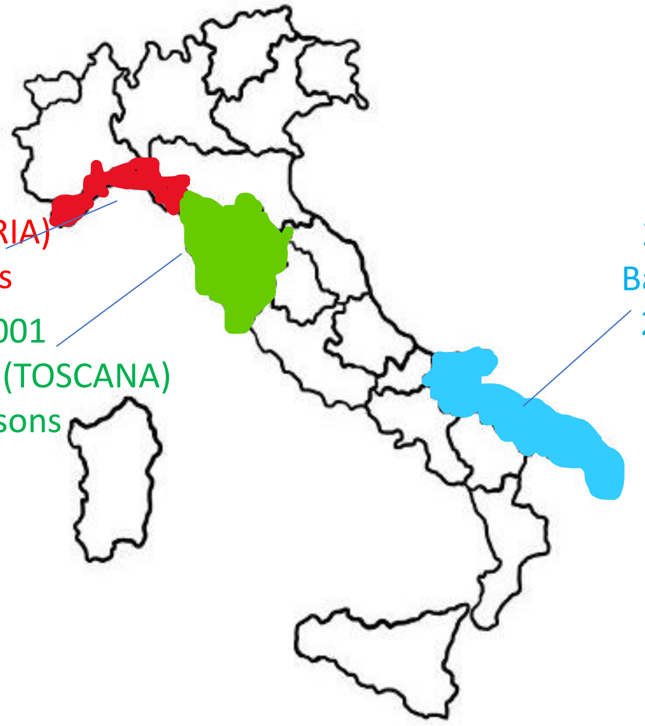


	Ostreopsis-related poisonings					
n° of cases	228	28	100	4	43	57
fever (≥38°C)	x	x	x	x	x	x
Difficulty breathing/dyspnea and/or bronchoconstriction	x	x		x	x	x
Cough/dry or mildly productive cough	x	x	x			x
Arthralgia/joint pain/myalgia			x		x	x
Weakness and discomfort of the extremities/fatigue/ malaise					x	x
Headache	x				x	x
Dysguesia/nausea and/or vomiting/diarrhea	x			x	x	
Dizziness/lightheadedness/vertigo					x	x
Mucous hypersecretion/rhinorrhea	x	x			x	x
Dermatitis/skin irritation/ pruritus/erythema/swelling	x				x	x
Chest Pain						x
Wheezes		x				
Conjunctivitis/lacrimation/tearing	x	x			x	x
Mucosal irritation (eye, nose, lip, tongue)/sneezing			x	x	x	x
Dry throat/sore throat/ pharyngeal pain/pharyngitis/odynophagia	x			x		x
Chills/shivering						
Numbness/paresthesia/glassy eyes/ speech disturbance/collapse						
Rhabdomyolysis						
Tachycardia						



Environmental Science and Technology (2016) 50, 1023 https://doi.org/10.1021/acs.est.5b05469
Environmental Science and Technology (2014) 48(6), 3532 https://doi.org/10.1021/es405617d

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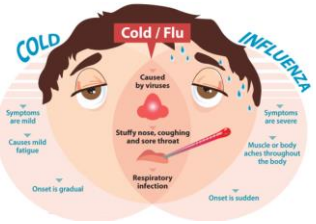

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Cough/dry or mildly productive cough	x	x	x			x
Arthralgia/joint pain/myalgia			x		x	x
Weakness and discomfort of the extremities/fatigue/ malaise					x	x
Headache	x				x	x
Dysguesia/nausea and/or vomiting/diarrhea	x			x	x	
Dizziness/lightheadedness/vertigo					x	x
Mucous hypersecretion/rhinorrhea	x	x			x	x
Dermatitis/skin irritation/ pruritus/erythema/swelling	x				x	x
Chest Pain						x
Wheezes		x				
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Mucosal irritation (eye, nose, lip, tongue)/sneezing			x	x	x	x
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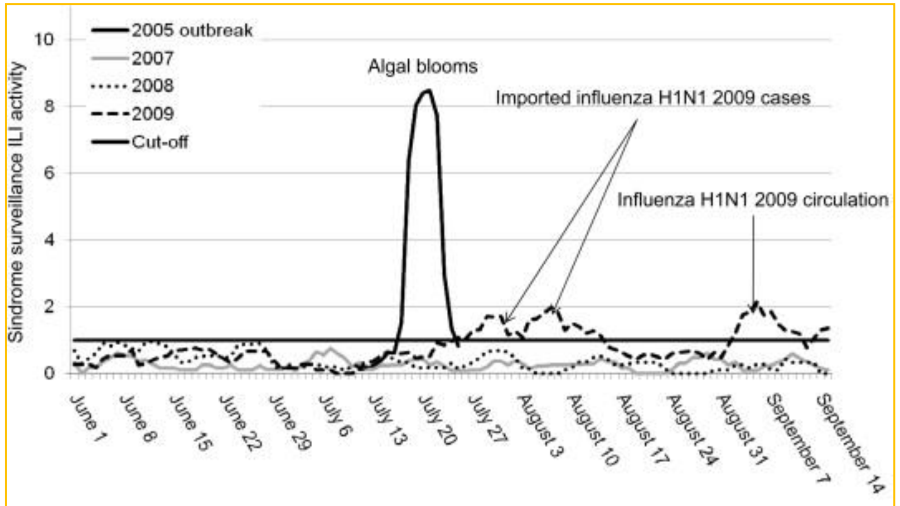

For Influenza Like Illness (ILI), epidemiological events was defined as ≥ 10 accesses to ED in a 3 days period, corresponding to a threshold value for the syndrome equal to a 5-day moving average of 1.6 cases per day (thick horizontal line). This value allows the activation of the alert state with a specificity of 90.3% and a sensitivity of 72.9% in the case of an ILI epidemic event.

Extracted from Toxicon (2011) 57, 478-95

Environmental Science and Technology (2016) 50, 1023 https://doi.org/10.1021/acs.est.5b05469

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Università degli Studi di Napoli Federico II | Dipartimento di Farmacia

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Anal. Chem. 2006, *78*, 6153–6159

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Patrizia Ciminiello, Carmela Dell'Aversano, Ernesto Fattorusso, Martino Forino, G. Silvana Magno, Luciana Tartaglione, Claudio Grillo, and Nunzia Melchiorre

2006

Putative Palytoxin and Its New Analogue, Ovatoxin-a, in *Ostreopsis ovata* Collected Along the Ligurian Coasts During the 2006 Toxic Outbreak

Patrizia Ciminiello, Carmela Dell'Aversano, Ernesto Fattorusso, Martino Forino, Luciana Tartaglione, Claudio Grillo, and Nunzia Melchiorre

2008

RAPID COMMUNICATIONS IN MASS SPECTROMETRY — RCM

Rapid Commun. Mass Spectrom. 2010; 24: 2735–2744

Complex palytoxin-like profile of *Ostreopsis ovata*. Identification of four new ovatoxins by high-resolution liquid chromatography/mass spectrometry

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2009-10

Environmental Science & Technology — Article

First Finding of *Ostreopsis* cf. *ovata* Toxins in Marine Aerosols

Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Luciana Tartaglione, Gioia Benedettini, Marzia Onorari, Fabrizio Serena, Cecilia Battocchi, Silvia Casabianca, and Antonella Penna

2011

Anal Bioanal Chem (2011) 401:1043–1050 — ORIGINAL PAPER

Palytoxin in seafood by liquid chromatography tandem mass spectrometry: investigation of extraction efficiency and matrix effect

Patrizia Ciminiello · Carmela Dell'Aversano · Emma Dello Iacovo · Ernesto Fattorusso · Martino Forino · Luciana Tartaglione · Rachele Rossi · Vittorio Soprano · Daniela Capozzo · Luigi Serpe

JACS — Journal of the American Chemical Society — Article

Isolation and Structure Elucidation of Ovatoxin-a, the Major Toxin Produced by *Ostreopsis ovata*

Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Laura Grauso, and Luciana Tartaglione

2012

J. Am. Soc. Mass Spectrom. (2012) — RESEARCH ARTICLE

High Resolution LC-MS^n Fragmentation Pattern of Palytoxin as Template to Gain New Insights into Ovatoxin-a Structure. The Key Role of Calcium in MS Behavior of Palytoxins

Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Laura Grauso, Luciana Tartaglione

Chemical Research in Toxicology — Article

Unique Toxin Profile of a Mediterranean *Ostreopsis* cf. *ovata* Strain: HR LC-MS^n Characterization of Ovatoxin-f, a New Palytoxin Congener

Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Luciana Tartaglione, Cecilia Battocchi, Rita Crinelli, Elisa Carloni, Mauro Magnani, and Antonella Penna

2013

Environmental Science & Technology — Article

Variability in Toxin Profiles of the Mediterranean *Ostreopsis* cf. *ovata* and in Structural Features of the Produced Ovatoxins

Luciana Tartaglione, Emma Dello Iacovo, Antonia Mazzeo, Silvia Casabianca, Patrizia Ciminiello, Antonella Penna, and Carmela Dell'Aversano

Harmful Algae

Investigation of toxin profile of Mediterranean and Atlantic strains of *Ostreopsis* cf. *siamensis* (Dinophyceae) by liquid chromatography-high resolution mass spectrometry

Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Luciana Tartaglione, Takeshi Yasumoto, Cecilia Battocchi, Mariagrazia Giacobbe, Anna Amorim, Antonella Penna

RESEARCH PAPER

The novel ovatoxin-g and isobaric palytoxin (so far referred to as putative palytoxin) from *Ostreopsis* cf. *ovata* (NW Mediterranean Sea): structural insights by LC-high resolution MS^n

Maria García-Altares · Luciana Tartaglione · Carmela Dell'Aversano · Olga Carnicer · Pablo de la Iglesia · Martino Forino · Jorge Diogène · Patrizia Ciminiello

2014

Environmental Science & Technology — Article

Determination of Palytoxins in Soft Coral and Seawater from a Home Aquarium. Comparison between *Palythoa*- and *Ostreopsis*-Related Inhalatory Poisonings

Luciana Tartaglione, Carmela Dell'Aversano, Antonia Mazzeo, Martino Forino, Andre Wieringa, and Patrizia Ciminiello

2015

RESEARCH PAPER

Liquid chromatography–high-resolution mass spectrometry for palytoxins in mussels

Patrizia Ciminiello · Carmela Dell'Aversano · Emma Dello Iacovo · Martino Forino · Luciana Tartaglione

OSTREOPSIS FATTORUSSOI SP. NOV. (DINOPHYCEAE), A NEW BENTHIC TOXIC *OSTREOPSIS* SPECIES FROM THE EASTERN MEDITERRANEAN SEA

Stefano Accoroni, Tiziana Romagnoli, Antonella Penna, Samuela Capellacci, Patrizia Ciminiello, Carmela Dell'Aversano, Luciana Tartaglione, Marie Abboud-Abi Saab, Valentina Giussani, Valentina Asnaghi, Mariachiara Chiantore, and Cecilia Totti

Risk assessment of ovatoxins

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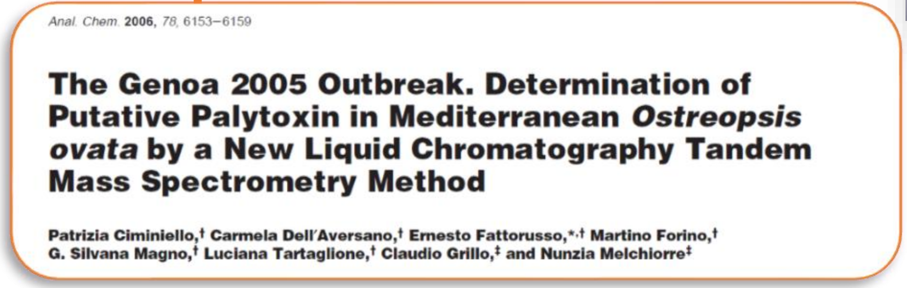
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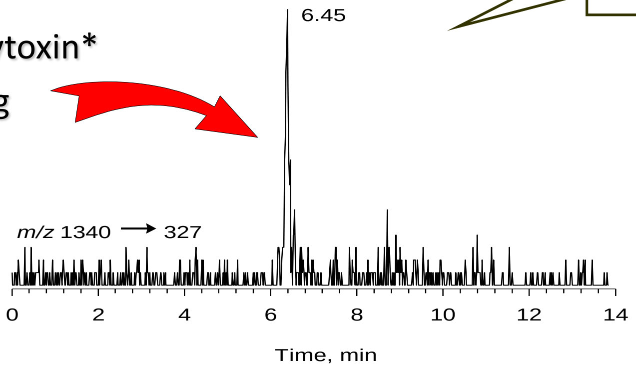
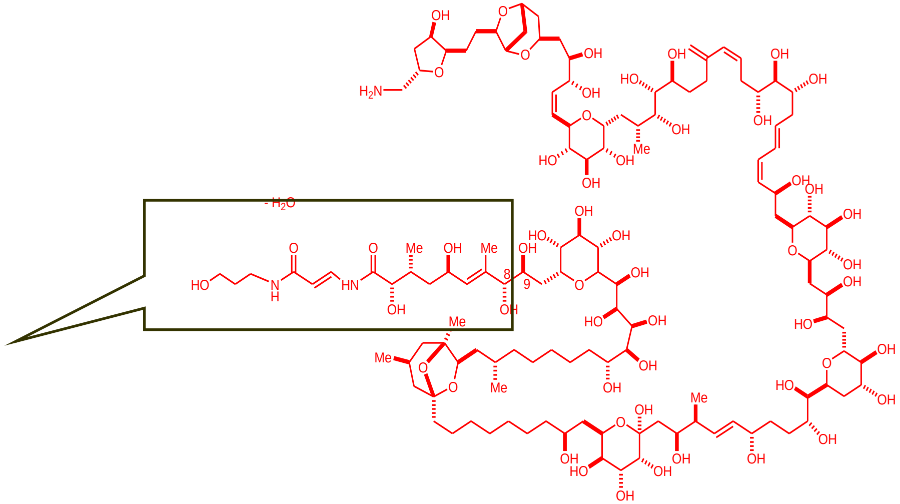
Putative palytoxin*
1.35 μg





UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II | Dipartimento di Farmacia

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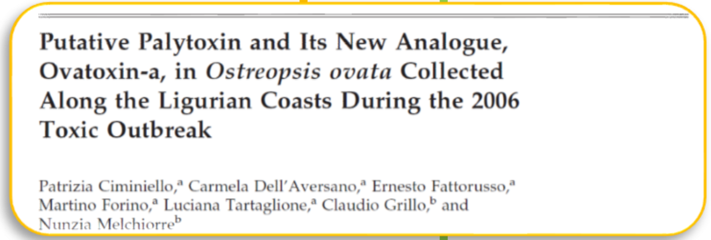
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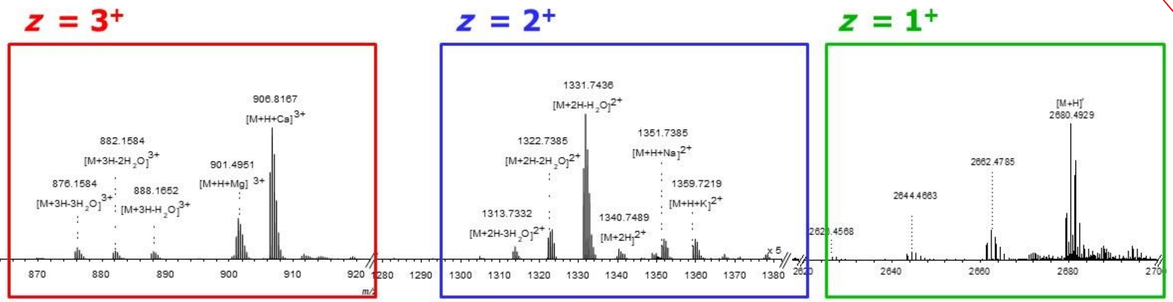
Ovatoxin-a **54%**

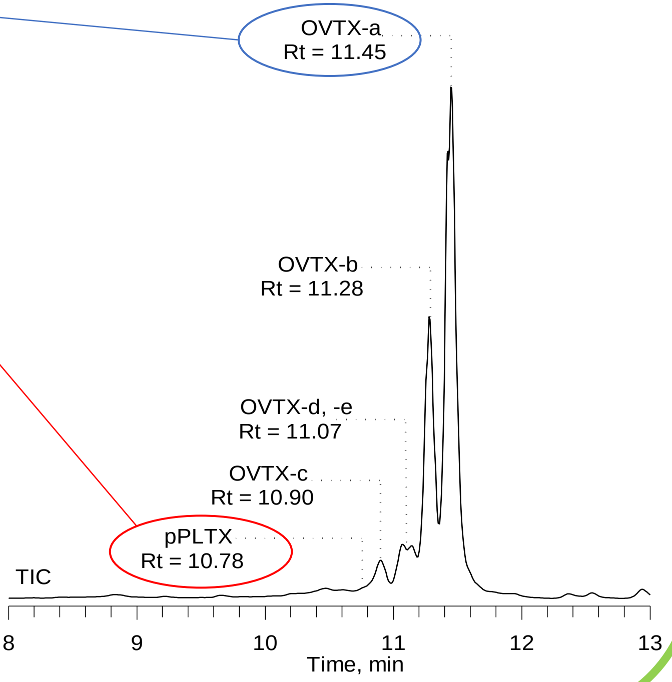
Ovatoxin-b

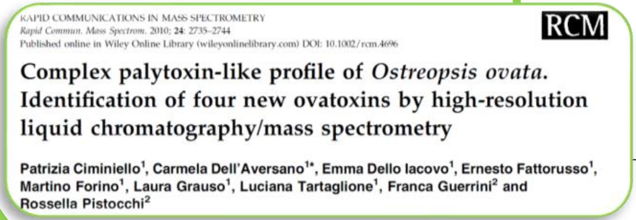
Ovatoxin-d/e

Ovatoxin-c

Putative palytoxin **0.6%**



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Rapid Commun. Mass Spectrom. 2010; 24: 2735–2744
Published online in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/rcm.4696

RCM

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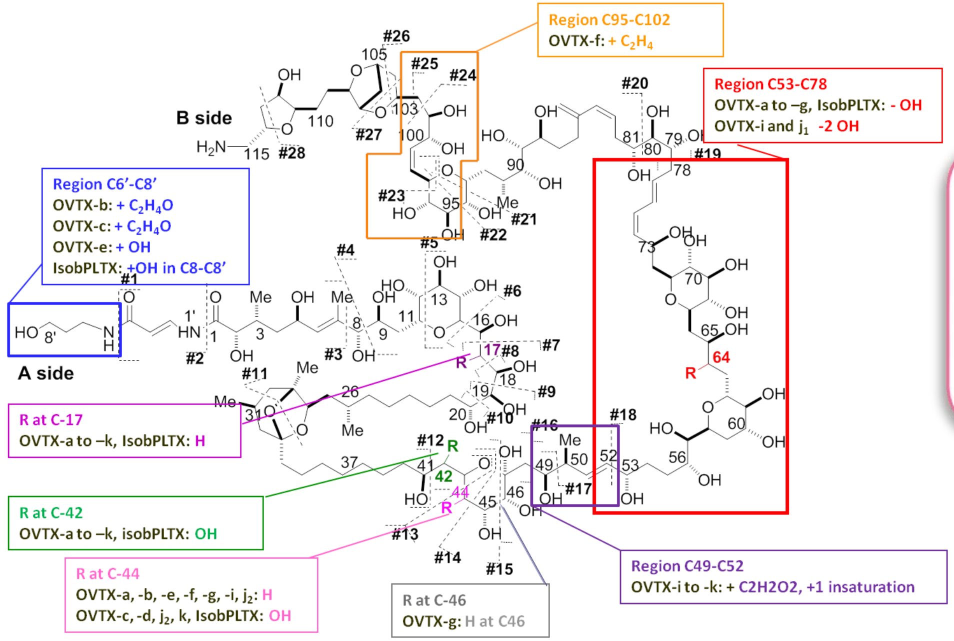

© American Society for Mass Spectrometry, 2012 — J. Am. Soc. Mass Spectrom. (2012) 23:952-963 — DOI: 10.1007/s13361-012-0345-7

RESEARCH ARTICLE

High Resolution LC-MS^n Fragmentation Pattern of Palytoxin as Template to Gain New Insights into Ovatoxin-a Structure. The Key Role of Calcium in MS Behavior of Palytoxins

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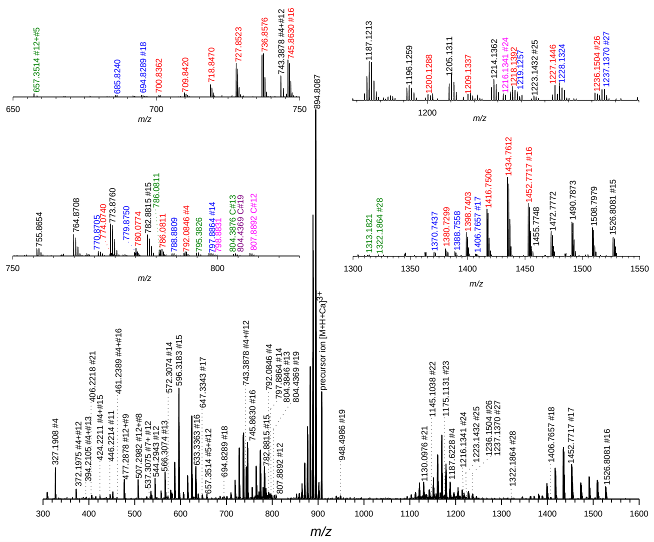


Chemical Research in Toxicology — Article — pubs.acs.org/crt

Unique Toxin Profile of a Mediterranean *Ostreopsis* cf. *ovata* Strain: HR LC-MS^n Characterization of Ovatoxin-f, a New Palytoxin Congener

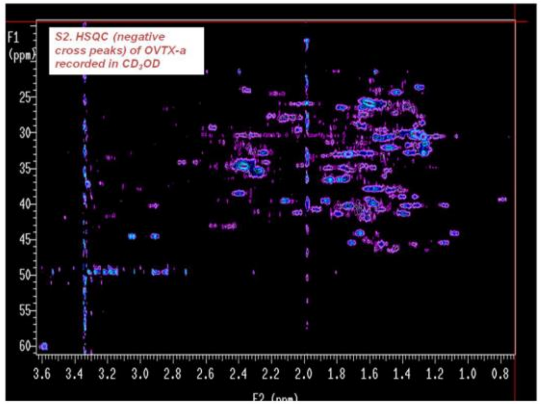
Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Luciana Tartaglione, Cecilia Battocchi, Rita Crinelli, Elisa Carloni, Mauro Magnani, and Antonella Penna

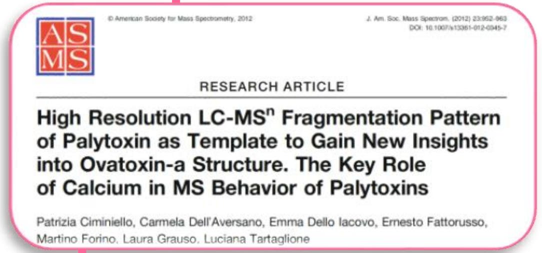
Risk assessment of ovatoxins

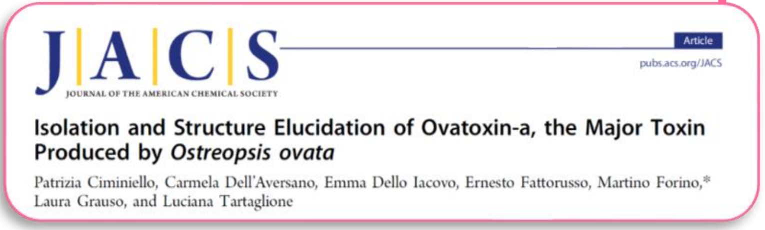
STEP 1 >

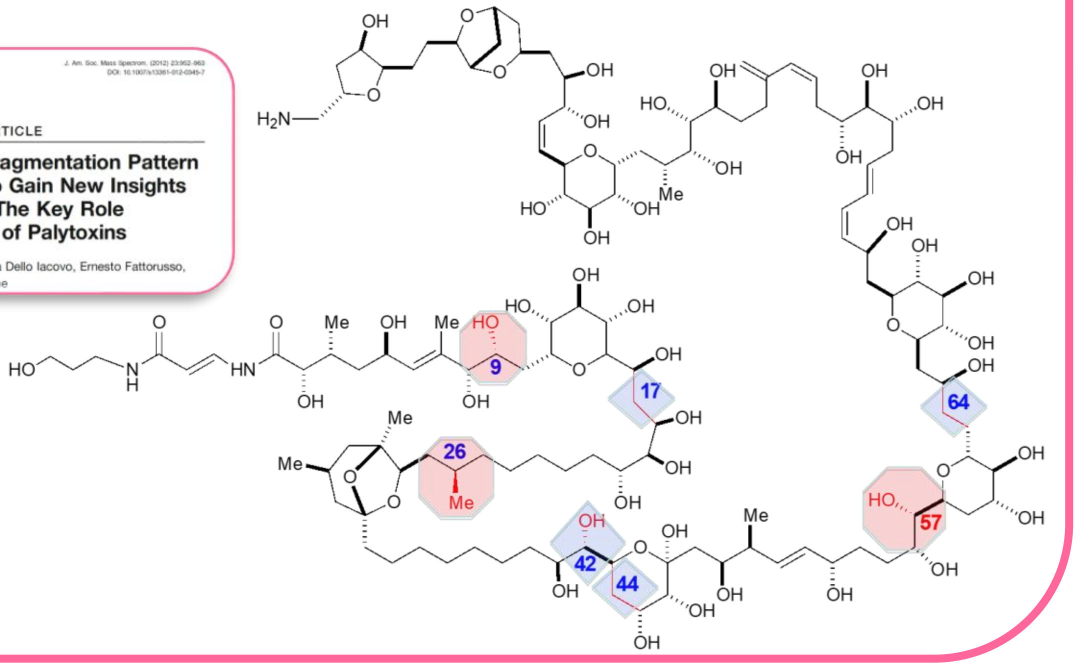
HAZARD IDENTIFICATION: The first step in risk assessment, this involves the **identification of** biological, chemical, and physical **agents capable of causing adverse health effects**

2005 | 2006 | 2008 | 2009-10 | 2011 | 2012 | 2013 | 2014 | 2015




© American Society for Mass Spectrometry, 2012
RESEARCH ARTICLE
High Resolution LC-MS^n Fragmentation Pattern of Palytoxin as Template to Gain New Insights into Ovatoxin-a Structure. The Key Role of Calcium in MS Behavior of Palytoxins
Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Laura Grauso, Luciana Tartaglione


JACS Journal of the American Chemical Society
Article
pubs.acs.org/JACS
Isolation and Structure Elucidation of Ovatoxin-a, the Major Toxin Produced by *Ostreopsis ovata*
Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino,* Laura Grauso, and Luciana Tartaglione



Risk assessment of ovatoxins

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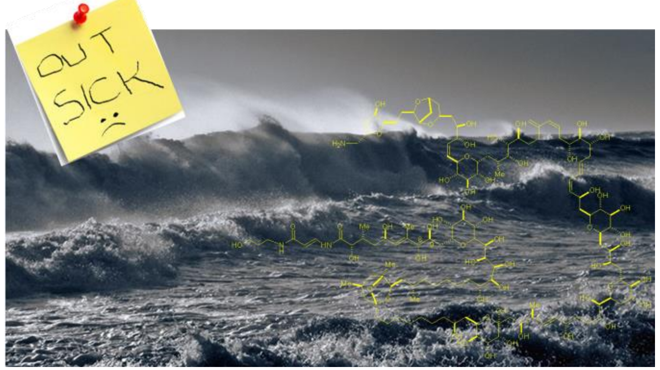
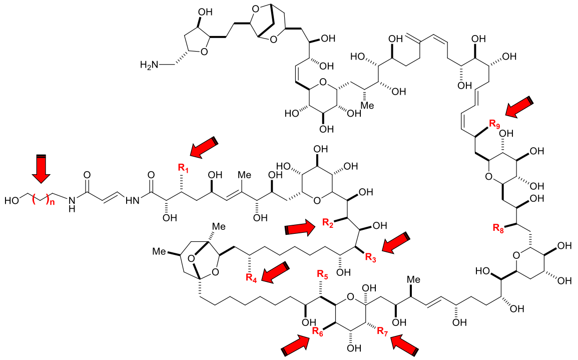




Environmental Science & Technology — Article — pubs.acs.org/est

First Finding of *Ostreopsis* cf. *ovata* Toxins in Marine Aerosols

Patrizia Ciminiello,† Carmela Dell'Aversano,*,† Emma Dello Iacovo,† Ernesto Fattorusso,† Martino Forino,† Luciana Tartaglione,† Gioia Benedettini,‡ Marzia Onorari,‡ Fabrizio Serena,‡ Cecilia Battocchi,§ Silvia Casabianca,§ and Antonella Penna§,⊥

Environmental Science & Technology — Article — pubs.acs.org/est

Determination of Palytoxins in Soft Coral and Seawater from a Home Aquarium. Comparison between *Palythoa*- and *Ostreopsis*-Related Inhalatory Poisonings

Luciana Tartaglione,† Carmela Dell'Aversano,*,† Antonia Mazzeo,† Martino Forino,† Andre Wieringa,‡ and Patrizia Ciminiello†

	Ostreopsis-related poisonings						*Palythoa-related poisonings*							
n° of cases	228	28	100	4	43	57	2	4	1	6	11	3	1	4
fever (≥38°C)	x	x	x	x	x	x		x		x	x	x		x
Difficulty breathing/dyspnea and/or bronchoconstriction	x	x		x	x	x	x	x	x	x	x	x	x	
Cough/dry or mildly productive cough	x	x	x			x	x	x		x	x	x		
Arthralgia/joint pain/myalgia			x		x	x			x	x				x
Weakness and discomfort of the extremities/fatigue/ malaise					x	x	x		x	x				x
Headache	x				x	x					x	x		x
Dysguesia/nausea and/or vomiting/diarrhea	x			x	x			x		x	x	x		x
Dizziness/lightheadedness/vertigo					x	x	x		x					
Mucous hypersecretion/rhinorrhea	x	x			x	x	x							
Dermatitis/skin irritation/ pruritus/erythema/swelling	x				x	x			x		x			x
Chest Pain						x	x	x	x	x	x		x	
Wheezes		x								x			x	
Conjunctivitis/lacrimation/tearing	x	x			x	x								
Mucosal irritation (eye, nose, lip, tongue)/sneezing			x	x	x	x								
Dry throat/sore throat/ pharyngeal pain/pharyngitis/odynophagia	x			x		x								
Chills/shivering									x	x		x		x
Numbness/paresthesia/glassy eyes/ speech disturbance/collapse							x		x	x				x
Rhabdomyolysis							x							
Tachycardia							x	x		x	x	x		x

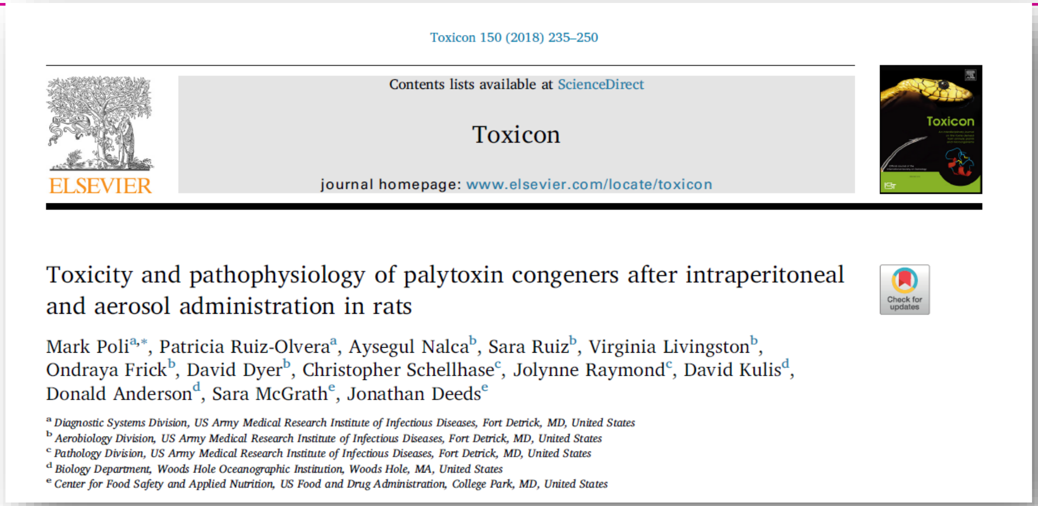
FOR IMMEDIATE RELEASE
ACS News Service Weekly PressPac: Wed Jan 13 13:27:00 EST 2016
Toxins related to 'red tides' found in home aquarium

Risk assessment of ovatoxins by inhalation

STEP 2 >

HAZARD CHARACTERIZATION: The second step in risk assessment, this involves **defining the nature of the adverse health effects associated with** biological, chemical and physical **agents** which may be **present in food**. The process should, if possible, involve an **understanding of the doses involved and related responses**.

Intra-peritoneal



Toxicon 150 (2018) 235–250

Contents lists available at ScienceDirect

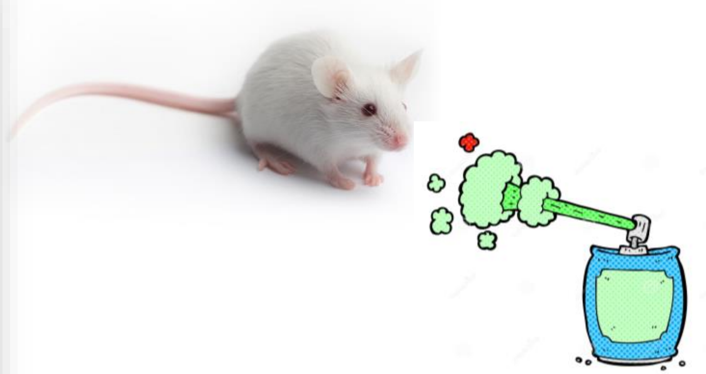
Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Toxicity and pathophysiology of palytoxin congeners after intraperitoneal and aerosol administration in rats

Mark Poli[a,*], Patricia Ruiz-Olvera[a], Aysegul Nalca[b], Sara Ruiz[b], Virginia Livingston[b], Ondraya Frick[b], David Dyer[b], Christopher Schellhase[c], Jolynne Raymond[c], David Kulis[d], Donald Anderson[d], Sara McGrath[e], Jonathan Deeds[e]

[a] Diagnostic Systems Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States
[b] Aerobiology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States
[c] Pathology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States
[d] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, United States
[e] Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, MD, United States



Aerosol

Toxin Prep	Source	LD50 (ug/kg)
50:50 mix	Hawaiian *P. tuberculosa*	0.92 (0.54 – 1.54)
42-OH-PLTX	Hawaiian *P. toxica*	1.93 (1.07 – 4.65)
PLTX	Japanese *P. tuberculosa*	1.81 (1.11 – 3.30)
Ovatoxin-a	*Ostreopsis ovata* culture	3.26 (2.04 – 5.66)

Toxin Prep	Source	LD50 (ug/kg)
50:50 mix	Hawaiian *P. tuberculosa*	0.063 (0.053 – 0.078)
42-OH-PLTX	Hawaiian *P. toxica*	0.045 (0.037 – 0.055)
PLTX	Japanese *P. tuberculosa*	0.041 (0.032 – 0.052)
Ovatoxin-a	*Ostreopsis ovata* culture	0.031 (0.025 – 0.039)

Risk assessment of ovatoxins

STEP 2 >

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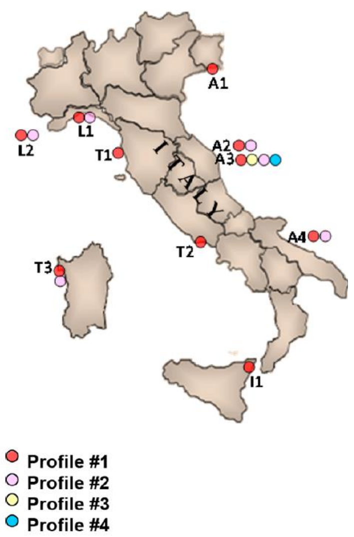
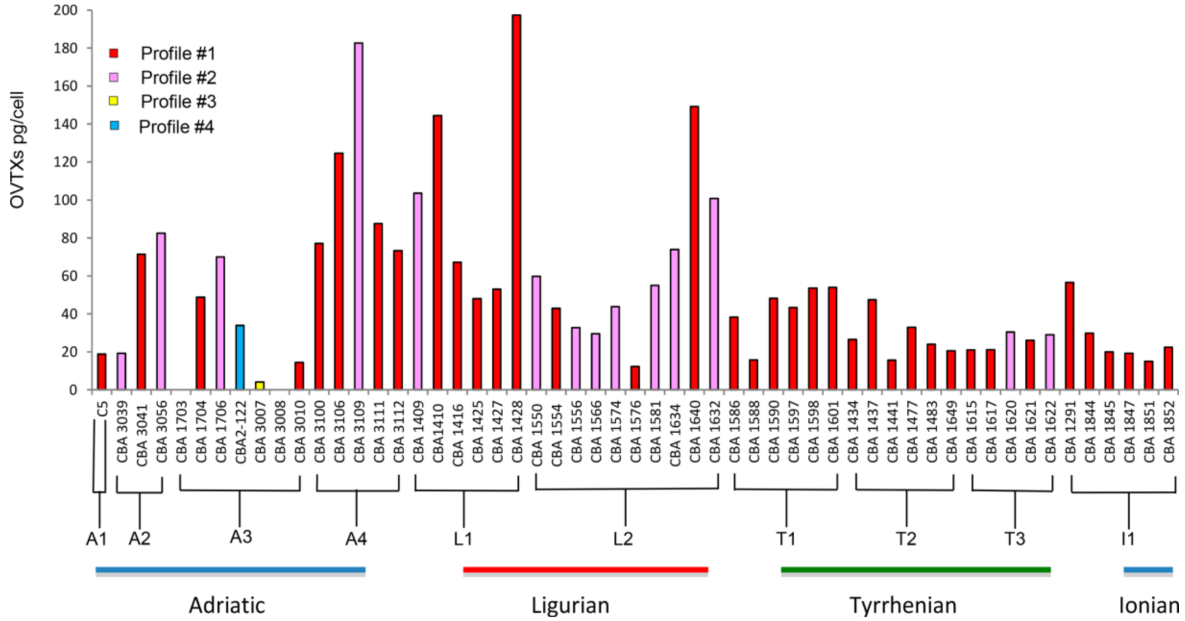





Environmental Science & Technology

Article

Variability in Toxin Profiles of the Mediterranean *Ostreopsis* cf. *ovata* and in Structural Features of the Produced Ovatoxins

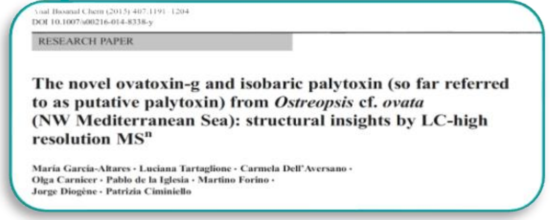
Luciana Tartaglione, Emma Dello Iacovo, Antonia Mazzeo, Silvia Casabianca, Patrizia Ciminiello, Antonella Penna, and Carmela Dell'Aversano


Harmful Algae

ELSEVIER

Investigation of toxin profile of Mediterranean and Atlantic strains of *Ostreopsis* cf. *siamensis* (Dinophyceae) by liquid chromatography–high resolution mass spectrometry

Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Luciana Tartaglione, Takeshi Yasumoto, Cecilia Battocchi, Mariagrazia Giacobbe, Ana Amorim, Antonella Penna


RESEARCH PAPER

The novel ovatoxin-g and isobaric palytoxin (so far referred to as putative palytoxin) from *Ostreopsis* cf. *ovata* (NW Mediterranean Sea): structural insights by LC-high resolution MS

María García-Altares · Luciana Tartaglione · Carmela Dell'Aversano · Olga Carnicer · Pablo de la Iglesia · Martino Forino · Jorge Diogène · Patrizia Ciminiello

Risk assessment of ovatoxins

STEP 2 >

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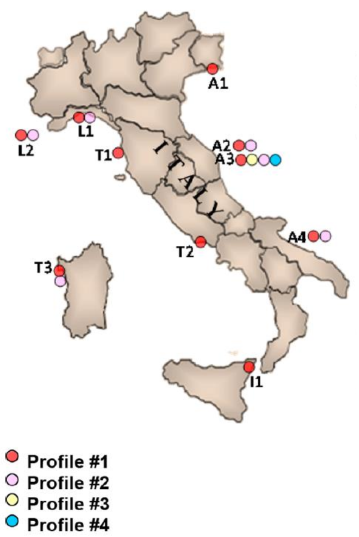

	Profile #1	Profile #2	Profile #3	Profile #4
OVTX-a	56% ± 10.1	76.8% ± 4.2		23.6%
OVTX-b	26.1% ± 7.0		87.8%	17.7%
OVTX-c	3.8% ± 1.4		11.3%	2.4%
OVTX-d	6.9% ± 2.3	12.4% ± 3.9		2.9%
OVTX-e	4.1% ± 2.0	6.3% ± 3.1		2.9%
OVTX-f				50.1%
OVTX-g	0.6% ± 0.29	0.9% ± 0.5		
isob PLTX	0.34% ± 0.18	0.8% ± 0.3		0.29%


Environmental Science & Technology

Variability in Toxin Profiles of the Mediterranean *Ostreopsis* cf. *ovata* and in Structural Features of the Produced Ovatoxins

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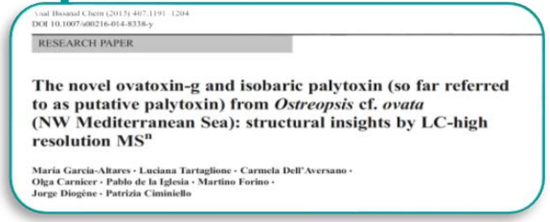
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Università degli Studi di Napoli Federico II | Dipartimento di Farmacia

Risk assessment of ovatoxins

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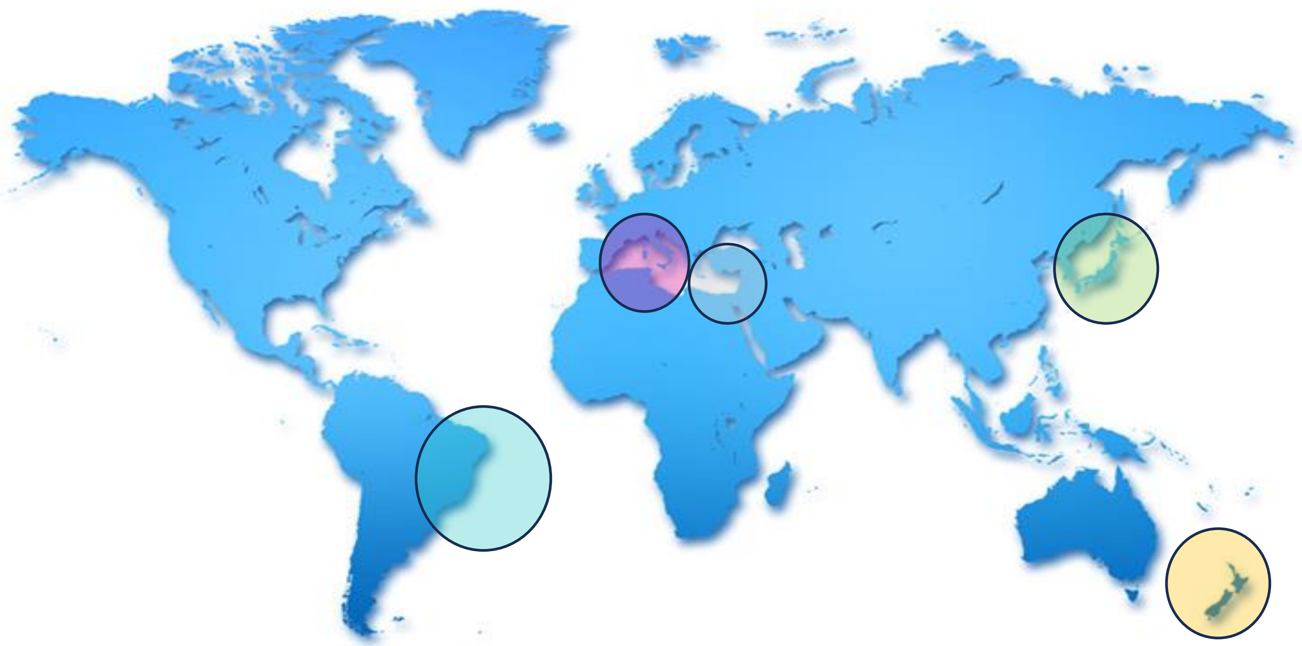

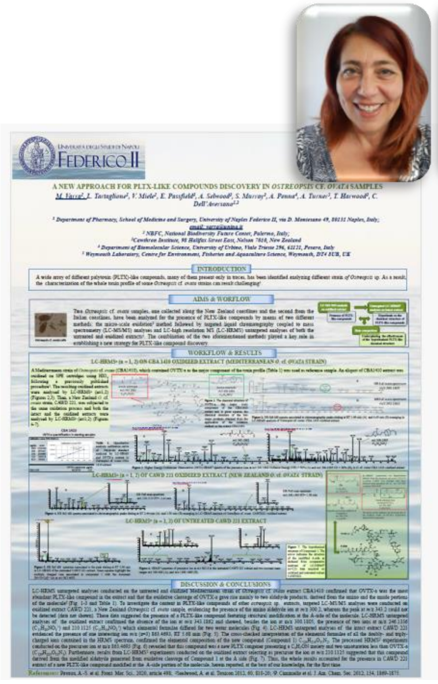

Brazil, Portugal, Spain, France, Italy, Croatia, Greece, Cipro, Lebanon,, Vietnam, Japan, New Zealand

Risk assessment of ovatoxins

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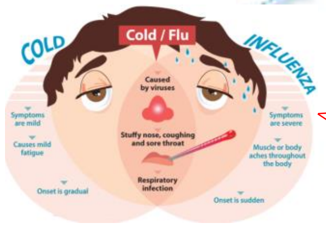
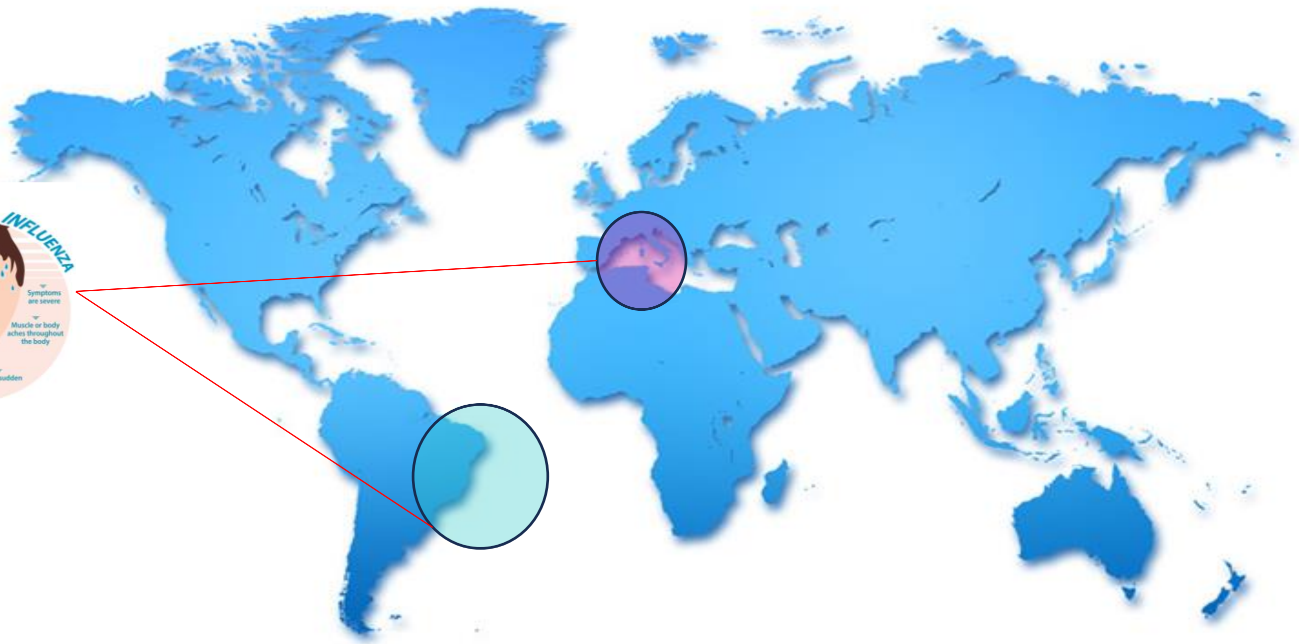




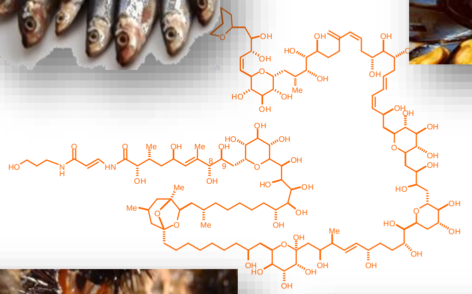
Brazil, Portugal, **Spain**, France, **Italy**, *Croatia*, Greece, CIPRO, Lebanon,, Vietnam, Japan, *New Zealand*

Risk assessment of ovatoxins by ingestion

STEP 3 >

EXPOSURE ASSESSMENT: One of the key steps in risk assessment, this relates to a thorough **evaluation of who, or what, has been exposed to a hazard and a quantification of the amounts involved**.

2005	2006	2008	2009-10	2011	2012	2013	2014	2015

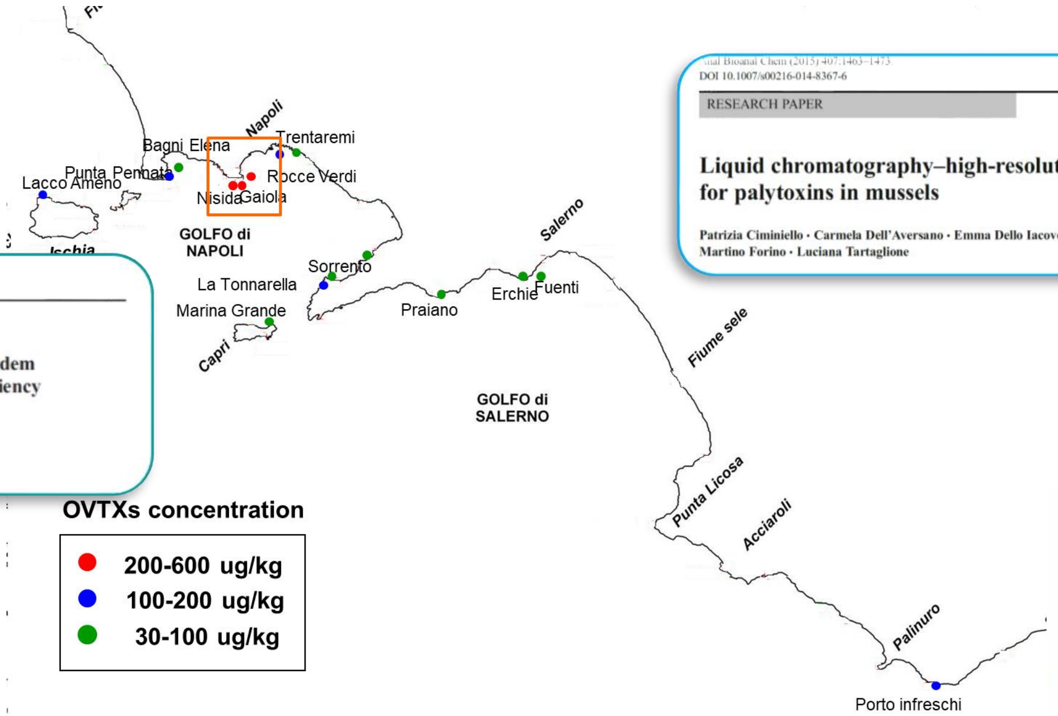


Anal Bioanal Chem (2011) 401:1043–1050
DOI 10.1007/s00216-011-5135-8

ORIGINAL PAPER

Palytoxin in seafood by liquid chromatography tandem mass spectrometry: investigation of extraction efficiency and matrix effect

Patrizia Ciminiello · Carmela Dell'Aversano · Emma Dello Iacovo · Ernesto Fattorusso · Martino Forino · Luciana Tartaglione · Rachele Rossi · Vittorio Soprano · Daniela Capozzo · Luigi Serpe



Anal Bioanal Chem (2015) 407:1463–1473
DOI 10.1007/s00216-014-8367-6

RESEARCH PAPER

Liquid chromatography–high-resolution mass spectrometry for palytoxins in mussels

Patrizia Ciminiello · Carmela Dell'Aversano · Emma Dello Iacovo · Martino Forino · Luciana Tartaglione

REGIONE CAMPANIA

Risk assessment of ovatoxins by ingestion

STEP 3 >

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Haff Disease in Salvador, Brazil, 2016-2021: Attack rate and detection of toxin in fish samples collected during outbreaks and disease surveillance

Cristiane Wanderley Cardoso,[a] Monaise Madalena Oliveira e Silva,[b] Antônio Carlos Bandeira,[c] Renan Bispo Silva,[b] Ana Paula Pitanga Barbuda Prates,[a] Ênio Silva Soares,[a] José Jorge Moreno Silva,[a] Lázaro José Rodrigues de Souza,[a] Mirela Maisa da Silva Souza,[a] Marcela Almeida Muhana,[a] Rosildete Silva Santos Pires,[a] José Fernando Araujo Neto,[c] Manuela Sampaio Souza Santos,[c] Luiz Laureno Mafra Junior,[d] Thiago Pereira Alves,[e] Mathias Alberto Schramm,[e] and Guilherme Sousa Ribeiro,[b,f,]*

[a]Secretaria Municipal de Saúde de Salvador, Salvador, Brazil
[b]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil
[c]Secretaria de Saúde do Estado da Bahia, Salvador, Brazil
[d]Centro de Estudos do Mar, Universidade Federal do Paraná, Pontal do Paraná, Brazil
[e]Instituto Federal de Santa Catarina, Campus Itajaí, Itajaí, Brazil
[f]Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil

Summary

Background From late 2016 to early 2021, cases of Haff disease, a rare cause of rhabdomyolysis, possibly due to poisoning by palytoxin-like compounds in seafood, were detected in Salvador, Brazil. Surveillance was established to detect additional cases aiming at describing the clinical characteristics of the cases, identifying associated factors, estimating disease attack rate, and investigating the presence of biotoxins and trace metals in selected fish specimens obtained from cases.

Characteristics	Subjects with laboratory-confirmed rhabdomyolysis (N = 43)	Subjects without laboratory-confirmed rhabdomyolysis (N = 22)
	Number/response (%) or median [IQR]	
Demographics		
Age, years	42 [30-62]	42 [32-51]
Female	22/43 (51)	16/22 (73)
Skin color		
White	16/41 (39)	9/20 (45)
Mixed	14/41 (34)	6/19 (32)
Black	11/41 (27)	6/19 (32)
Clinical manifestations		
Muscle pain	43/43 (100)	22/22 (100)
First affected region/ muscle		
Trapezius	22/41 (54)	14/22 (64)
Lower limbs	6/41 (15)	2/22 (9)
Latissimus dorsal	2/41 (5)	0/20 (0)
Upper limbs	2/41 (5)	3/22 (14)
Abdomen	1/41(2)	1/22 (5)
Others	8/38 (21)	2/22 (9)
Dark urine	20/41 (49)	7/22 (32)
Muscle pain at touch	19/42 (45)	10/22 (46)
Dry mouth	13/42 (31)	8/22 (36)
Arthralgia	12/42 (29)	12/22 (55)
Headache	12/43 (28)	13/22 (59)
Dyspnea	11/42 (26)	7/22 (32)
Fever	5/42(12)	2/22 (9)
Vomit	5/42 (12)	3/22 (14)
Cough	5/42 (12)	4/22 (18)
Pruritus	4/42 (10)	8/22(36)
Retro-orbital pain	4/42 (10)	4/22 (18)
Diarrhea	4/42 (10)	7/20 (35)
Conjunctivitis	3/42 (7)	3/22 (14)
Adenopathy	3/42 (7)	2/22 (9)
Exanthema	2/42 (5)	7/22 (32)
Disease outcomes		
Number of days of illness	3 [2-5]	3 [2-7]
Search for medical care [a]	42/43 (98)	15/22 (68)
Hospitalization	38/43 (88)	3/20 (15)
Intensive care unit admission	11/43 (26)	0/21 (0)
Dialysis	3/42 (7)	0/22 (0)

***Table 1*: Demographic and clinical characteristics of suspected Haff disease cases during an outbreak in Salvador, Brazil, according to laboratory confirmation of rhabdomyolysis, 2016-2017 (N=65)**

[a] One laboratory-confirmed rhabdomyolysis case did not seek for medical care but performed CPK testing.

Risk assessment of ovatoxins by ingestion

STEP 3 >

EXPOSURE ASSESSMENT: One of the key steps in risk assessment, this relates to a thorough **evaluation of who, or what, has been exposed to a hazard and a quantification of the amounts involved**.


Boletim Epidemiológico | Secretaria de Vigilância em Saúde e Ambiente | Ministério da Saúde Volume 54 | N.º 9 | 5 jun. 2023

Situação epidemiológica da doença de Haff no Brasil, 2021 e 2022

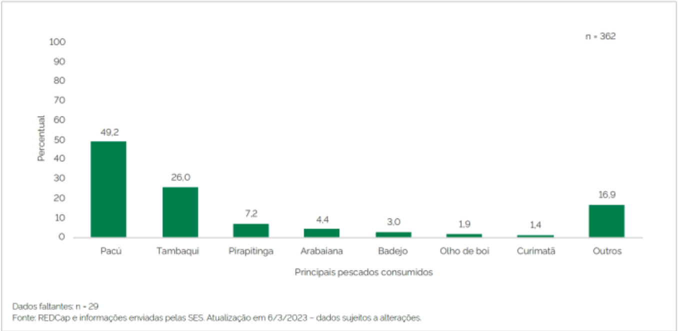

FIGURA 6 Distribuição dos casos compatíveis com a doença de Haff por pescado consumido, Brasil, 2021 a 2022*

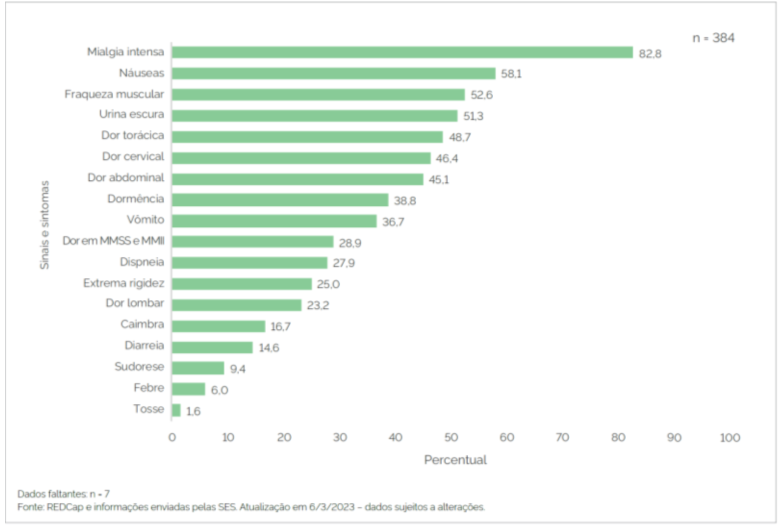

FIGURA 5 Distribuição da frequência dos sinais e sintomas dos casos compatíveis com a doença de Haff, Brasil, 2021 a 2022

Thanks to Rodrigo Barcellos Hoff and collaborators. Advanced Laboratorial Section - SLAV/SC Federal Laboratory of Animal and Plant Health and Inspection - LFDA/RS Ministry of Agriculture and Livestock

Risk assessment of ovatoxins by ingestion

STEP 2 >

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efsa European Food Safety Authority

EFSA Journal 2009; 7(12):1393

SCIENTIFIC OPINION

Scientific Opinion on marine biotoxins in shellfish – Palytoxin group[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

"... Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods can be valuable tools for the determination, but method optimisation and validation as well as the development of certified reference materials and standards are necessary..."

ISOLATION SCHEME

Università degli Studi di Napoli Federico II | Dipartimento di Farmacia

Woods Hole Oceanographic Institution

USAMRIID — United States Army Medical Research Institute of Infectious Diseases — Biodefense solutions to protect our nation

2021

15 mg OVTX-a
OVTX-d/e pPLTX

2.5 mg OVTX-a
94% purity
25% Recovery

2012

JACS — Isolation and Structure Elucidation of Ovatoxin-a, the Major Toxin Produced by *Ostreopsis ovata*
Patrizia Ciminiello, Carmela Dell'Aversano, Emma Dello Iacovo, Ernesto Fattorusso, Martino Forino, Laura Grauso, and Luciana Tartaglione

0.7 mg OVTX-a
50% purity
10% Recovery

Starting Material
Crude extract containing ~ 30 mg of OVTX-a

→ 15 mg crude OVTX-a used for **MPLC#3**

→ *15 mg crude OVTX-a used for* ***MPLC#4*** *(purification ongoing)*

From MPLC#3:

- **Fr 5** — 4.8 mg of semi-purified OVTX-a
- ***Fr 6-8*** — *1.1 mg of semi-purified OVTX-a (purification ongoing)*

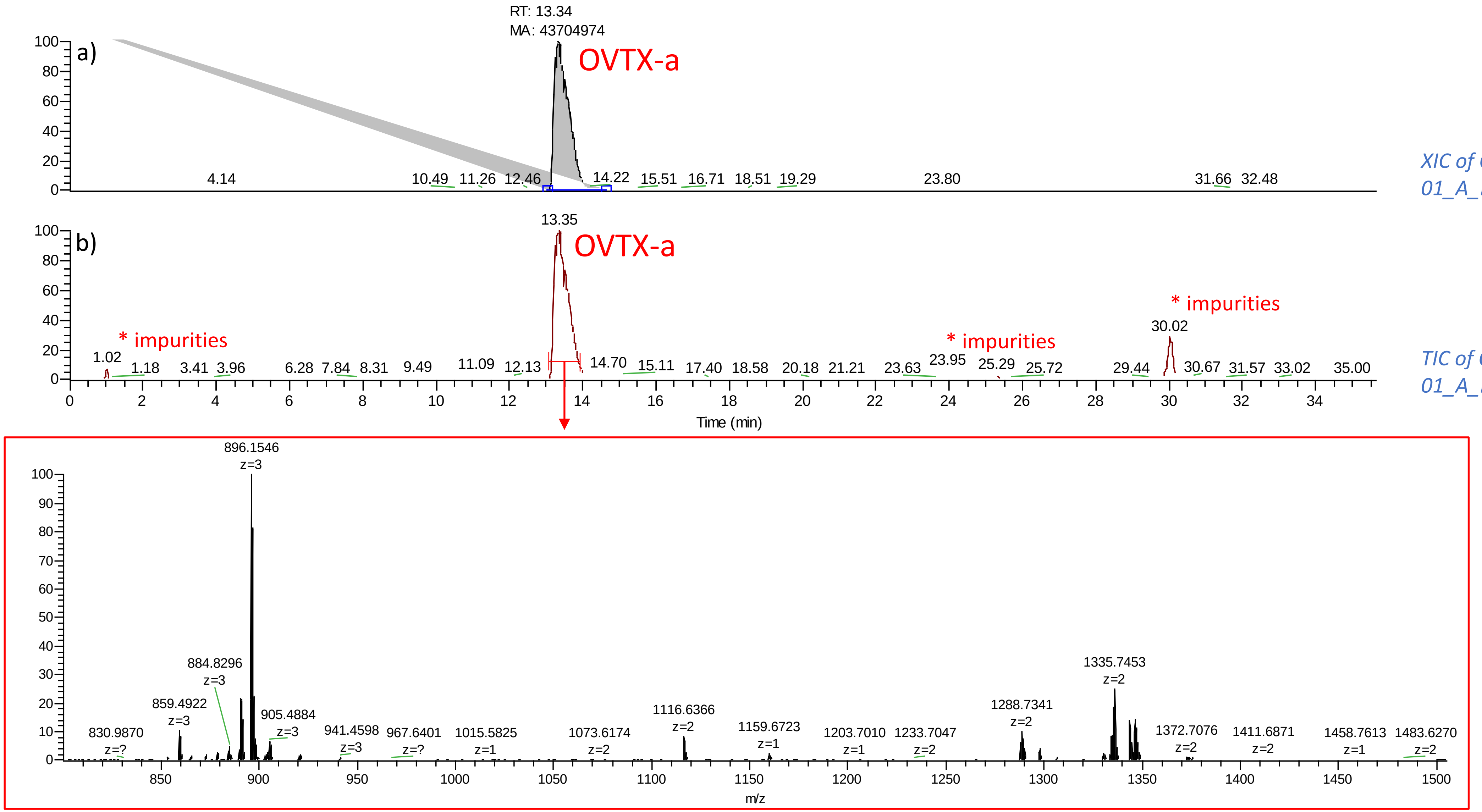
HPLC step 1
5.5 mg of semi-purified OVTX-a (Recovery 104.6%)

HPLC step 2
2.2 mg of pure OVTX-a (Recovery 61.9%)

→ **Divided in 8 aliquots => Lyophilization**

OVTX-a N°01	**OVTX-a N°02**	**OVTX-a N°03**	**OVTX-a N°04**	**OVTX-a N°05**	**OVTX-a N°06**	**OVTX-a N°07**	**OVTX-a N°08**
in 15 mL size-glass vial	in 15 mL size-glass vial	in 15 mL size-glass vial	in 15 mL size-glass vial	in 15 mL size-glass vial	in 15 mL size-glass vial	in 15 mL size-glass vial	in 50 mL size-PP tube
177.6 ± 7.8 µg OVTX-a	177.6 ± 7.8 µg OVTX-a	177.6 ± 7.8 µg OVTX-a	177.6 ± 7.8 µg OVTX-a	177.6 ± 7.8 µg OVTX-a	177.6 ± 7.8 µg OVTX-a	177.6 ± 7.8 µg OVTX-a	1243.2 ± 54.6 µg OVTX-a

LC-HRMS of QC sample OVTX-a N° 07. Lot 01_A_MPLC#03_5. TIC, XIC and related Full Scan HRMS of OVATOXIN-a

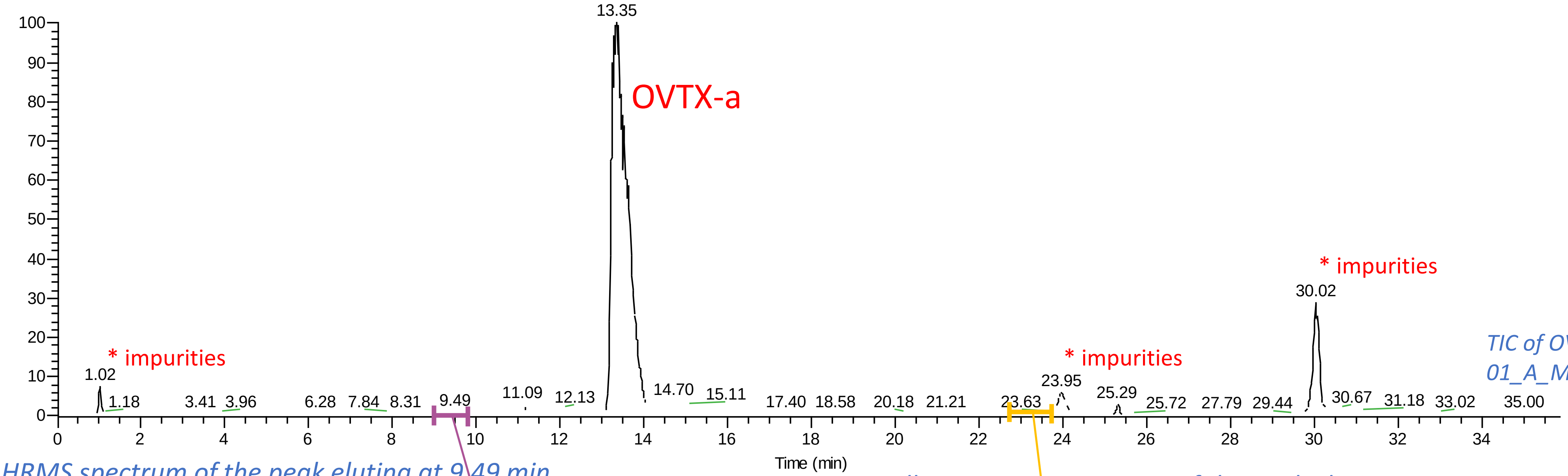


XIC of OVTX-a N° 07_Lot 01_A_MPLC#03_5 at m/z 895.8195

TIC of OVTX-a N° 07_Lot 01_A_MPLC#03_5

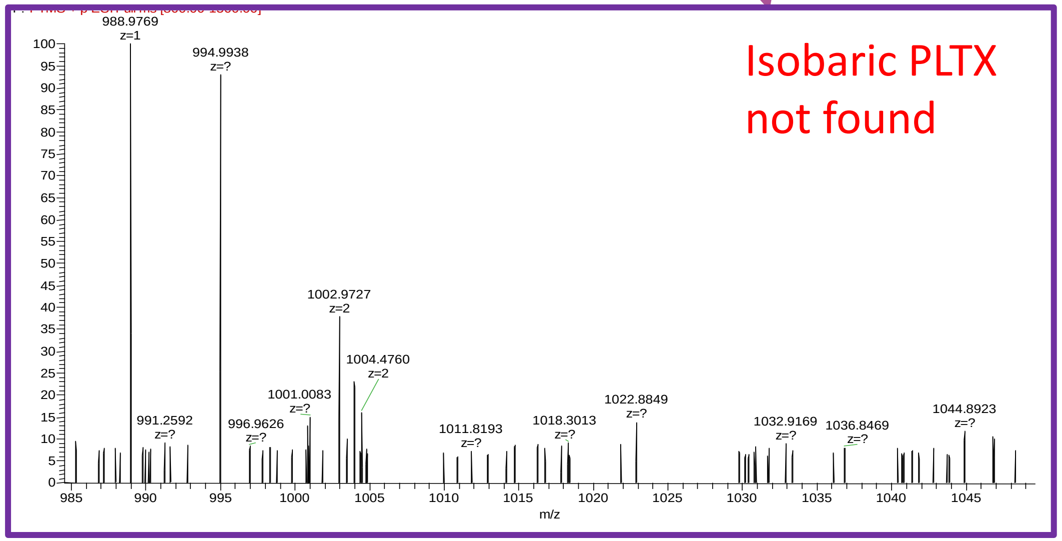
Full scan HRMS spectrum of the OVTX-a peak

Enlargements of the peaks eluting in the nearby of OVTX-a peak : other OVTXs



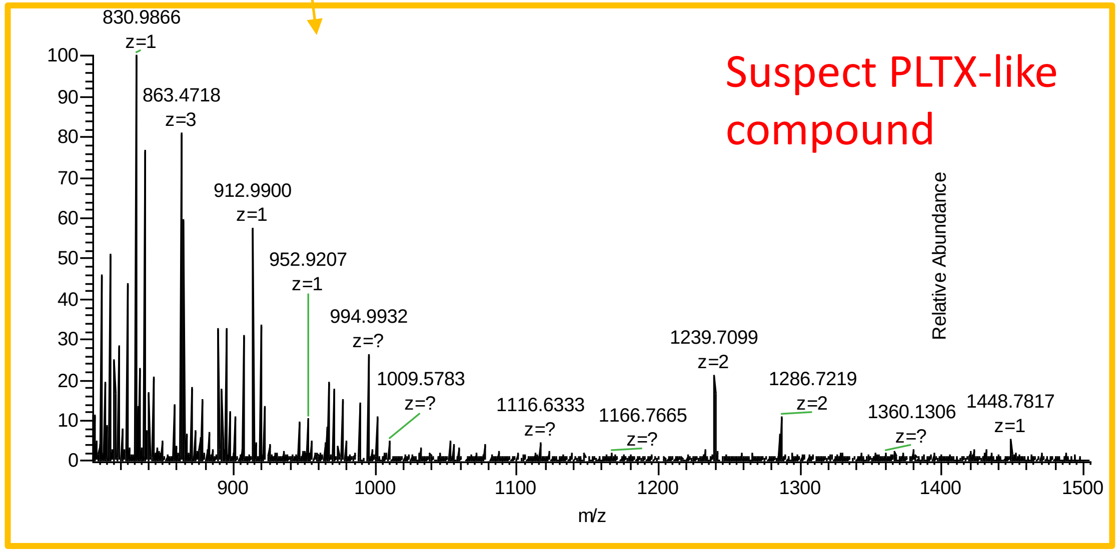
TIC of OVTX-a N° 07_Lot 01_A_MPLC#03_5

Full scan HRMS spectrum of the peak eluting at 9.49 min



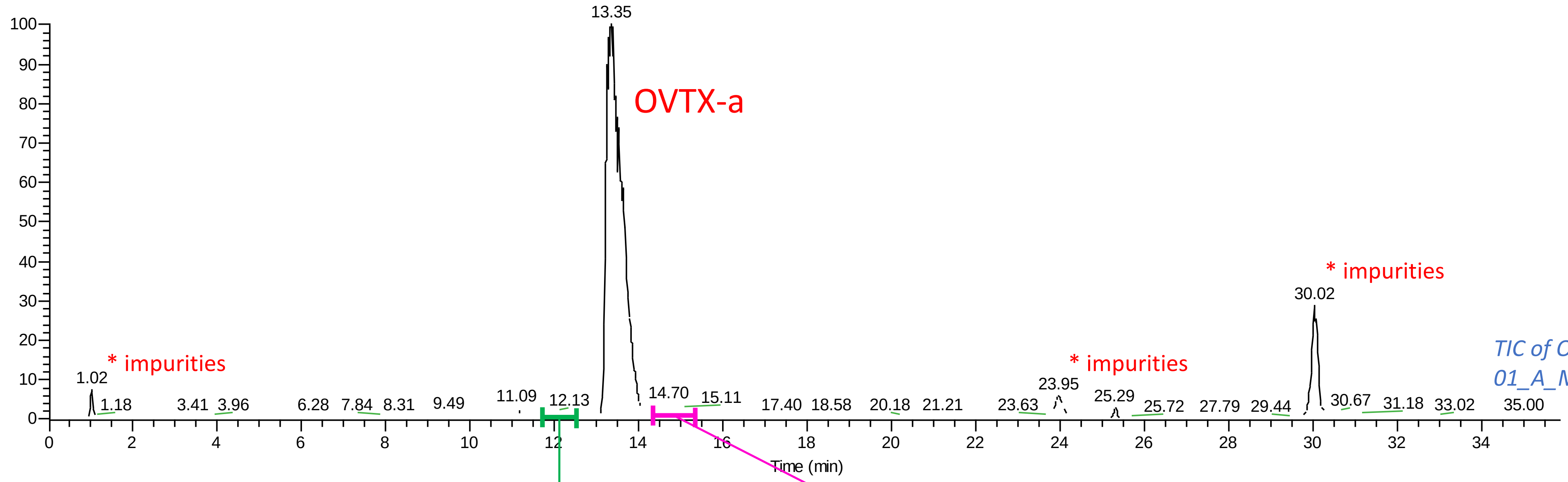
In this Rt region normally elutes Isobaric PLTX, but in the HRMS spectrum associated to this region no ions relevant to isobaric PLTX were detected.

Full scan HRMS spectrum of the peak eluting at 23.23 min



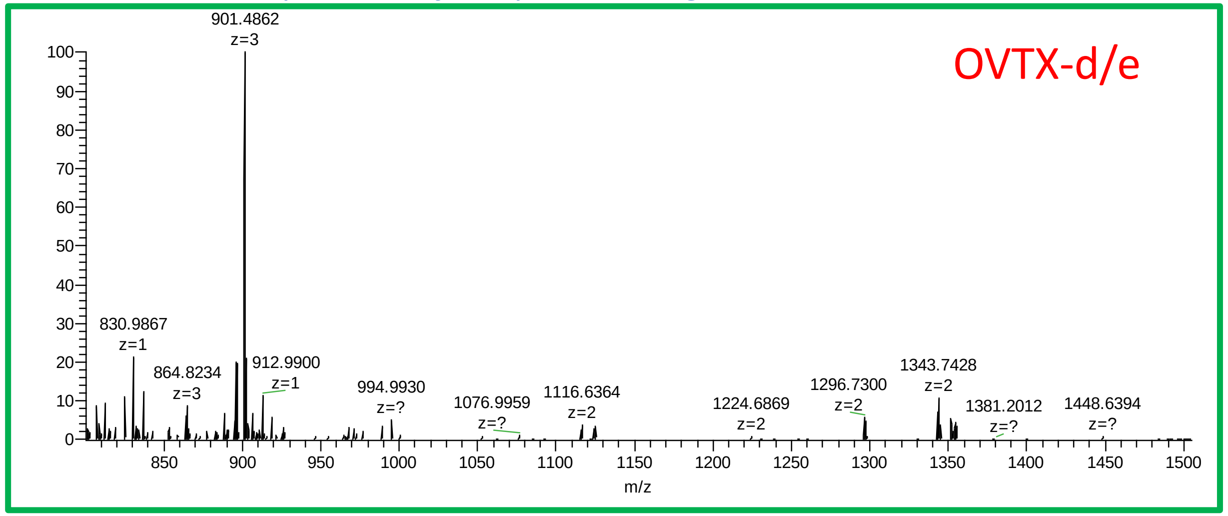
This molecule is a OVTX-like compounds and thus was considered in the assessment of the purity grade

Enlargements of the peaks eluting in the nearby of OVTX-a peak : other OVTXs

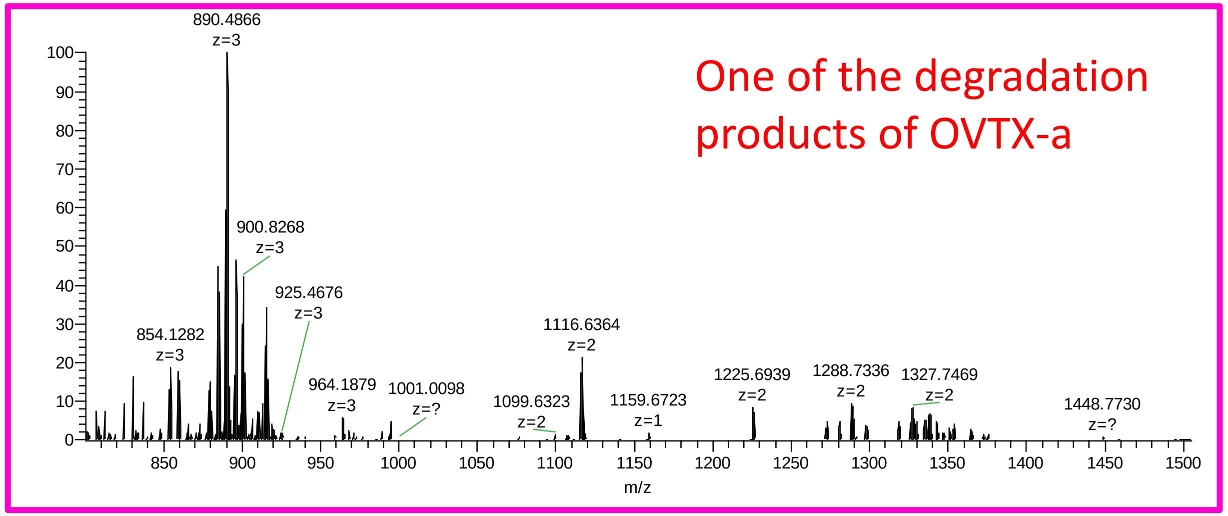


TIC of OVTX-a N° 07_Lot 01_A_MPLC#03_5

Full scan HRMS spectrum of the peak eluting at 12.13 min



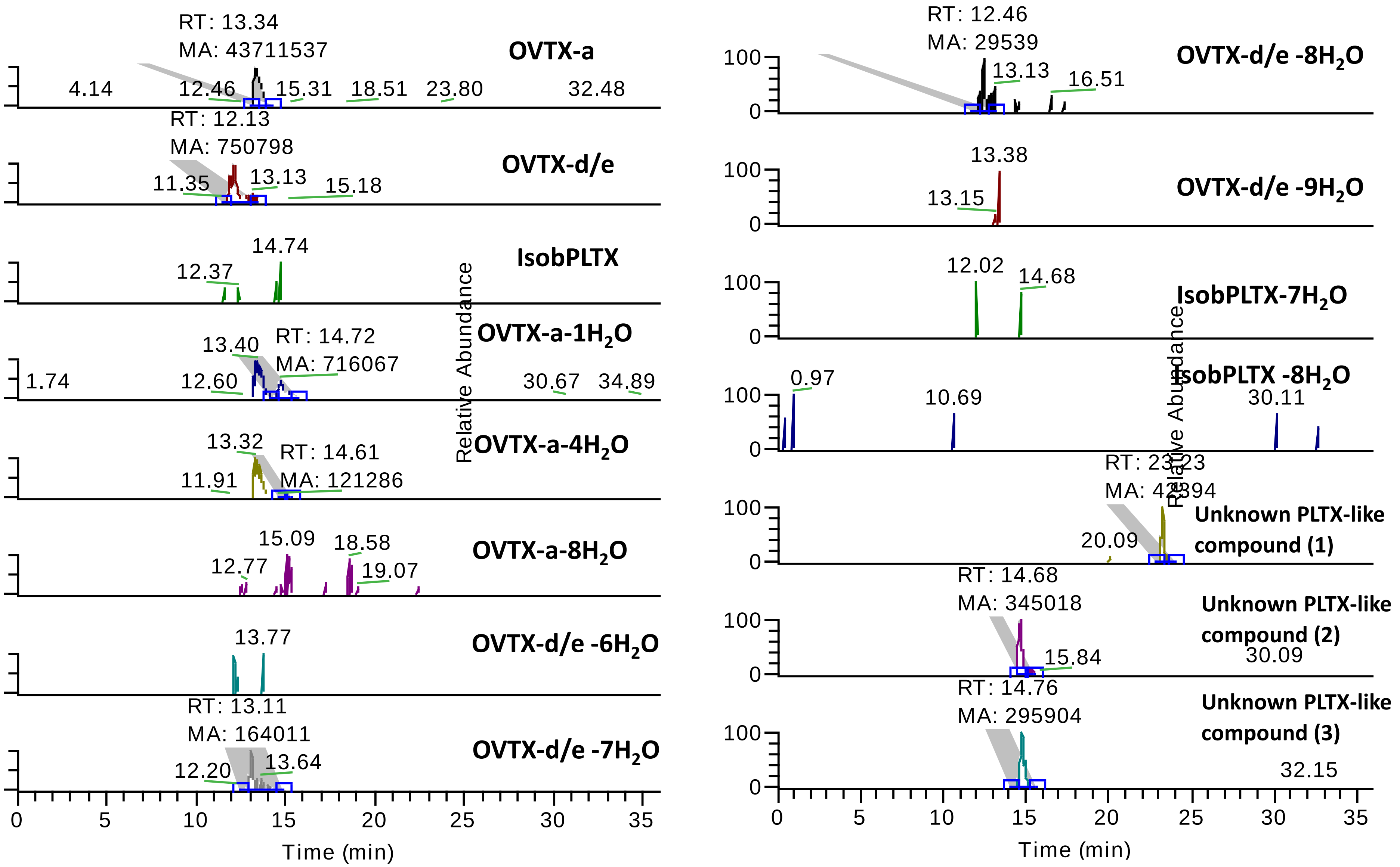
Full scan HRMS spectrum of the peak eluting at 14.70 min



These molecules (OVTX d/e and degradation products) are PLTX-like compounds and thus were considered in the assessment of the purity grade

Extracted ion chromatograms (XIC) of OVTX-a and other PLTX-like compounds with their degradation products including the new suspected PLTX-like compounds

m/z	Toxins	%	Rt, min
895.8195	OVTX-a	94.3	13.34
901.1511	OVTX-d/e	1.9	12.13
906.4828	isobaric PLTX	nd	nd
889.8158	OVTX-a -H2O	1.8	14.72
859.1576	OVTX-a -4H2O	0.3	14.61
847.7913	OVTX-a -8 H_20	nd	nd
865.1288	OVTX-d/e -6 H_20	nd	nd
859.1245	OVTX-d/e -7 H_20	0.3	13.11
853.1210	OVTX-d/e -8 H_20	nd	nd
847.1177	OVTX-d/e -9 H_20	nd	nd
864.4587	Isob PLTX -7 H_20	nd	nd
858.4531	Isob PLTX -8 H_20	nd	nd
863.1378	Unknown PLTX-like compound (1)	0.1	23.23
900.4922	Unknown PLTX-like compound (2)	0.7	14.68
915.1671	Unknown PLTX-like compound (3)	0.6	14.76

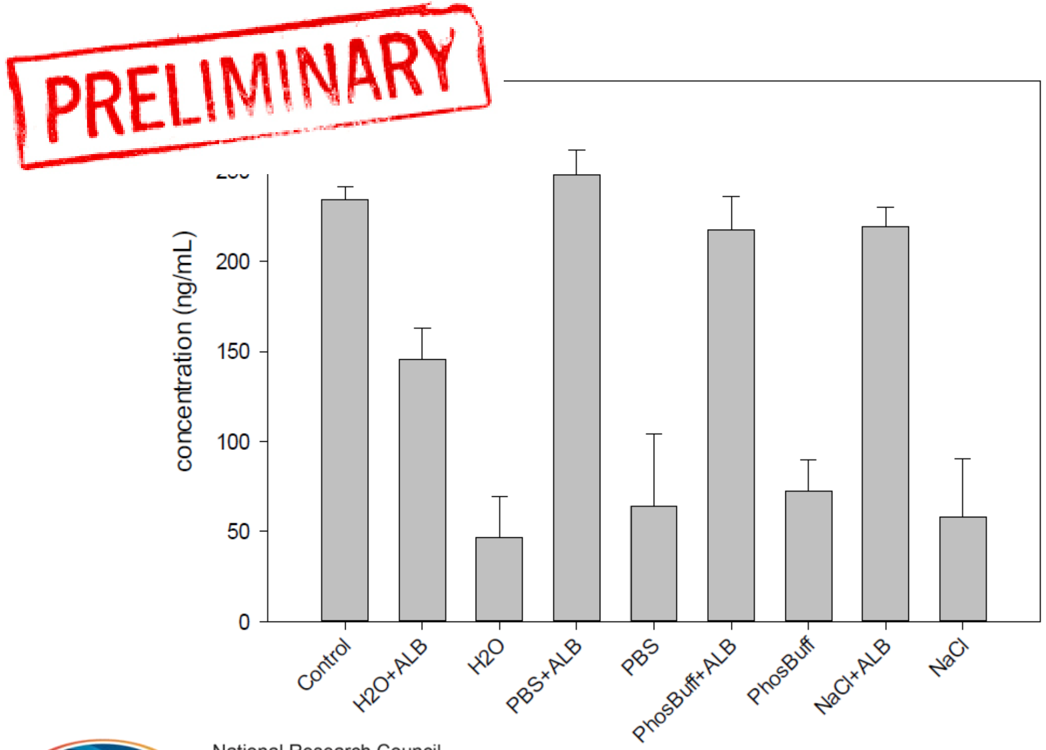


Based on the new suspect PLTX-like compoundsthe purity grade of OVTX-a samples was re-calculated and resulted to be 94%

Risk assessment of ovatoxins

STEP 2 >

HAZARD CHARACTERIZATION: The second step in risk assessment, this involves **defining the nature of the adverse health effects associated with** biological, chemical and physical **agents** which may be **present in food**. The process should, if possible, involve an **understanding of the doses involved and related responses**.

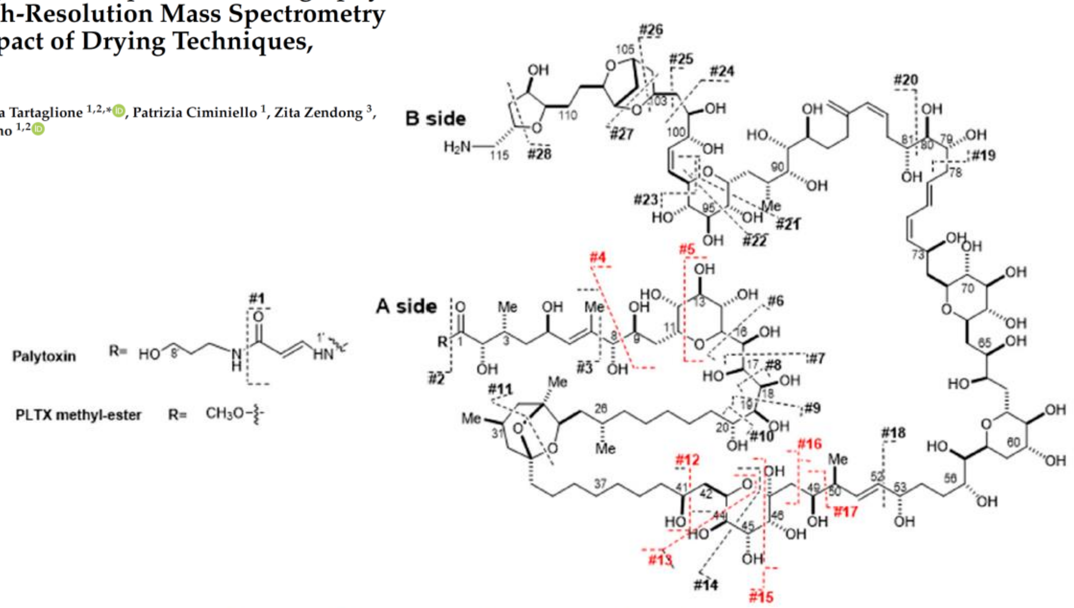


toxins

Article

Toward Isolation of Palytoxins: Liquid Chromatography Coupled to Low- or High-Resolution Mass Spectrometry for the Study on the Impact of Drying Techniques, Solvents and Materials

Antonia Mazzeo [1], Michela Varra [1], Luciana Tartaglione [1,2,*], Patrizia Ciminiello [1], Zita Zendong [3], Philipp Hess [3] and Carmela Dell'Aversano [1,2]



Tentative Mechanism of formation of the PLTX methyl ester

National Research Council
NRC·CNRC
Canada

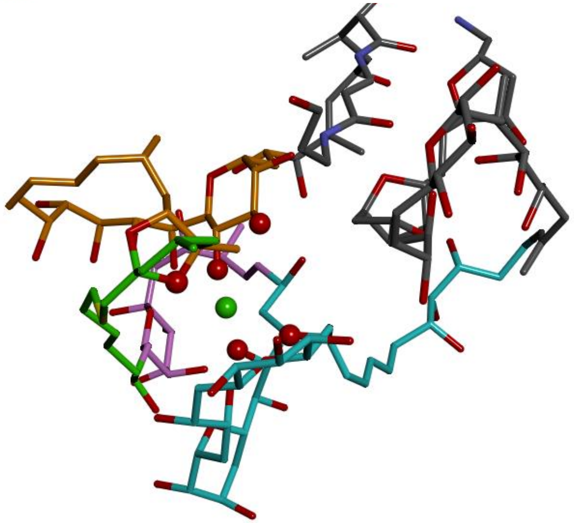
Pearse McCarron, Elizabeth Mudge, Chris Miles

Risk assessment of ovatoxins

STEP 2 >

HAZARD CHARACTERIZATION: The second step in risk assessment, this involves **defining the nature of the adverse health effects associated with** biological, chemical and physical **agents** which may be **present in food**. The process should, if possible, involve an **understanding of the doses involved and related responses**.

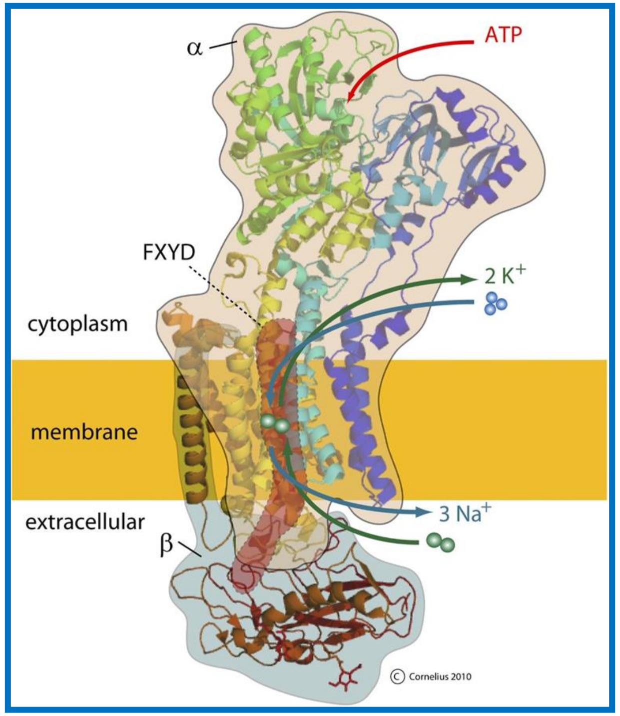
PRELIMINARY



In-silico studies demonstrated that PLTX adopts a folded conformation characterized by several turns that is able to bind calcium ions.

On-going studies are investigating how the peculiar shape and charge distribution on the calculated PLTX structure are related to its ability to

i) adapt to different chemical environment and bind other metal ions,
ii) change the function of Na+/K+-ATPase into that of an ion channel and to form itself ion channels on cell membranes.



Università degli Studi di Napoli Federico II
Dipartimento di Farmacia
Caterina Fattorusso, Marco Persico, Oleh Tkachuk

Summing up

Isolation of mg amounts of well characterized OVTX-a is paving the way:

- To measure dose/response relationship by oral exposure
- To prepare Certified Reference Material
- To validate analytical methods for reliable detection and accurate quantification of OVTXs in seafood and in the environment
- To calculate the extent to which *Ostreopsis ovata* and ovatoxins produce seafood contamination and/or environmental sufferings

STEP 4 >

RISK CHARACTERIZATION: The final stage of risk assessment, in which **the likelihood that a particular substance will cause harm is calculated** in the light of the nature of the hazard and the extent to which people, animals, plants and/or the environment are exposed to it.

Funding

USAMRIID Service Contract W81XWH20C0135
"Isolation and Purification of Ovatoxin-a from O. ovata cell pellets over one 12-months base period and two 12-months option periods" (12M+ 12M 9/2020-9/2022)

Catalyst New Zealand Project 21-CAW-002-CSG
"Developing capability to identify and monitor marine toxins produced by Ostreopsis species in microalgal cultures and seafood" (24M, 2/2022-2/2024)

HORIZON-MSCA-2021-SE-01-01 BlueShellfish
"Solutions to prevent and mitigate the impacts of HABs in Aquaculture and Fisheries, in the context of global Warming" (48 M since 01/2023)

NBFC - Mapping and monitoring actions to preserve marine ecosystem biodiversity and functioning
"New early warning systems, LC-HRMS methods and databases for the comprehensive determination of marine and freshwater toxins in the environment and in the trophic chain"(48 M since 09/2022)

Università degli Studi di Napoli Federico II — Dipartimento di Farmacia

International Agreements with
A. Turner, Cefas, UK
P. McCarron, NRC Canada
D. Bodi, BfR Germany
T. Harwood, Cawthron Institute, New Zealand
P. Hess, Ifremer, France
A. Hjiskia, NCSR Demokritos, Greece
B. E. Janssen, EWAG, Switzerland

Cooperation Agreements with
A, Penna, Univ. Urbino
A. Tubaro, Univ. Trieste

NATIONAL BIODIVERSITY FUTURE CENTER

The MarBioTox team

Analytical Chemistry / Environmental Chemistry and Toxicology

Development and validation of targeted and untargeted LC- tandem MS and/or HRMS methods for the identification, structural characterization and quantification of marine and freshwater toxins in environmental, food and biological matrices. Isolation and LC-MS based purification of natural toxins from aquatic sources

Keywords:

- LC-HRMS, LC-MS/MS, Solid Phase Adsorption Toxin Tracking (SPATT), Biosensors
- Harmful algal bloom (HAB), *Ostreopsis*, *Ciguatera Fish Poisoning* (CFP)
- Emerging toxins (palytoxins, ciguatoxins, cyanotoxins, etc) and pollutants (drugs, plastics)