The Spanish Food Safety and Nutrition Agency (AESAN)

10 Diciembre 2018, Rome

Risk assessment & scientific cooperation with EFSA:

EuroCigua: Risk Characterization of Ciguatera Food Poisoning

Ana Canals Caballero
International and Scientific Advisor

SPANISH FOOD SAFETY AND NUTRITION AGENCY

Mission

Promotion of food safety

Information for consumers & stakeholders

Develop strategies and actions for health promotion in the field of nutrition

POLITICAL ORGANIZATION OF SPAIN: THE STATE OF AUTONOMIES

State

General State Administration

Regional

Autonomous Communities

Local

Local Authorities

COMPETENT AUTHORITIES DISTRIBUTION ON FOOD SAFETY

FOOD SAFETY SECTORIAL ORGANIZATION

General State Administration

Ministry of Agriculture, Fisheries and Food

AESAN

Ministry of Health, consumer affairs and social welfare

FOOD SAFETY TERRITORIAL ORGANIZATION

Autonomous Communities

Regional Ministry of Agriculture

Regional Ministry of Health

MINISTRY OF HEALTH, CONSUMER AFFAIRS AND SOCIAL WELFARE

GENERAL SECRETARIAT OF HEALTH
AESAN PRESIDENCY

SPANISH FOOD SAFETY AND NUTRITION AGENCY

EXECUTIVE DIRECTORATE

- COMMUNICATION AND INSTITUTIONAL COORDINATION
- NAOS STRATEGY/ OBSERVATORY OF THE OBESITY
- INTERNATIONAL AFFAIRS AND SCIENTIFIC COORDINATION

GENERAL SECRETARIAT

DEPUTY-DIRECTORATE GENERAL FOR FOOD SAFETY PROMOTION

DEPUTY-DIRECTORATE GENERAL FOR ALERT COORDINATION AND OFFICIAL CONTROL PROGRAMMING

NRL

EURLMB

Risk Analysis

The Spanish Food Safety and Nutrition Agency (AESAN)

Risk Assessment

- Scientific committee
- Expert groups
- Data bases
- Food consumption
- Food composition

Risk Management

- Legislation
- Food register
- Codex secretariat
- RASFF/SCIRI
- Official control & coordination

Risk Communication

- Webpage
- Press office
- Publications
- Communication strategies

Risk Analysis

efsa European Food Safety Authority

- ✓ EFSA FOCAL POINT
- ✓ RASFF FOCAL POINT
- ✓ INFOSAN

EC European Commission

Risk Assessment

Scientific Committee (SC)

Experts & areas

1. Food Toxicology
2. Microbiology, Virology, Parasitology or Food Zoonoses
3. Human and Animal Epidemiology
4. Biotechnology and Genetic Modification
5. Immunology and allergology
6. Human Nutrition
7. Pharmacology
8. Food Technology Processes
9. Analysis and Instrumentation
10. Epidemiology and Public Health from the point of view of Nutrition
11. Animal feeding

4 plenary sessions/year

NO FUNDING

There is no financial compensation.
Reimbursement of travel expenses

+Meetings by electronic media

Risk Assessment

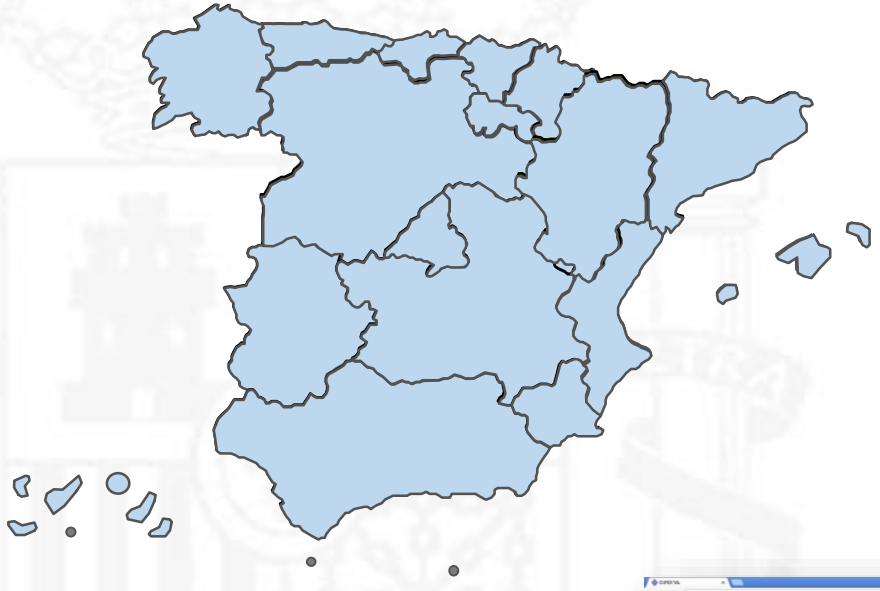
Scientific Committee (SC)

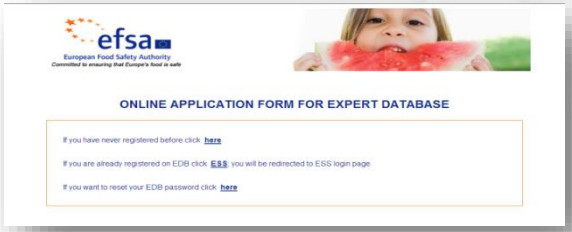
External experts

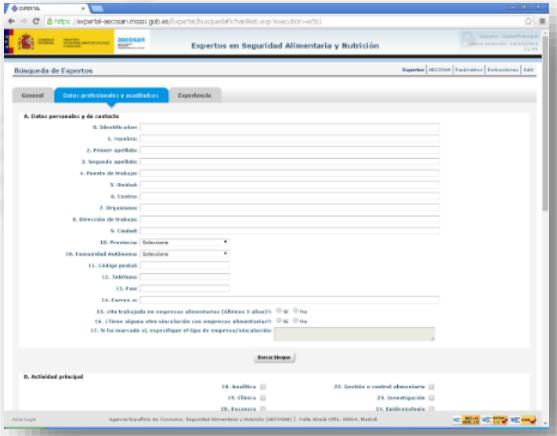
25% of the reports

- **77 Universities**
  - ✓ **56 publics**
  - ✓ **21 privates**
- **Research institutions**

Databases of experts
In food safety and nutrition





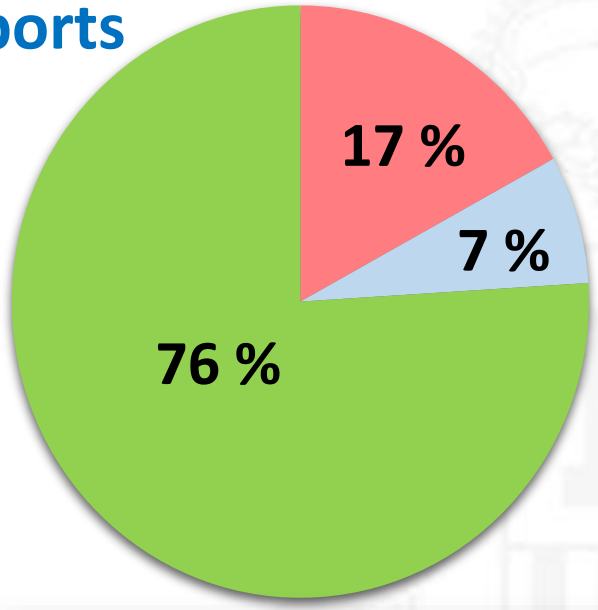


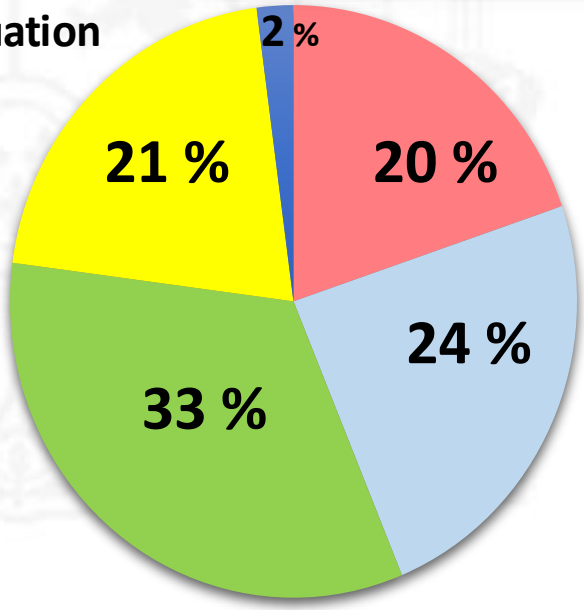
aecosan
agencia española de consumo, seguridad alimentaria y nutrición

Risk Assessment

Scientific Committee (SC)

Reports





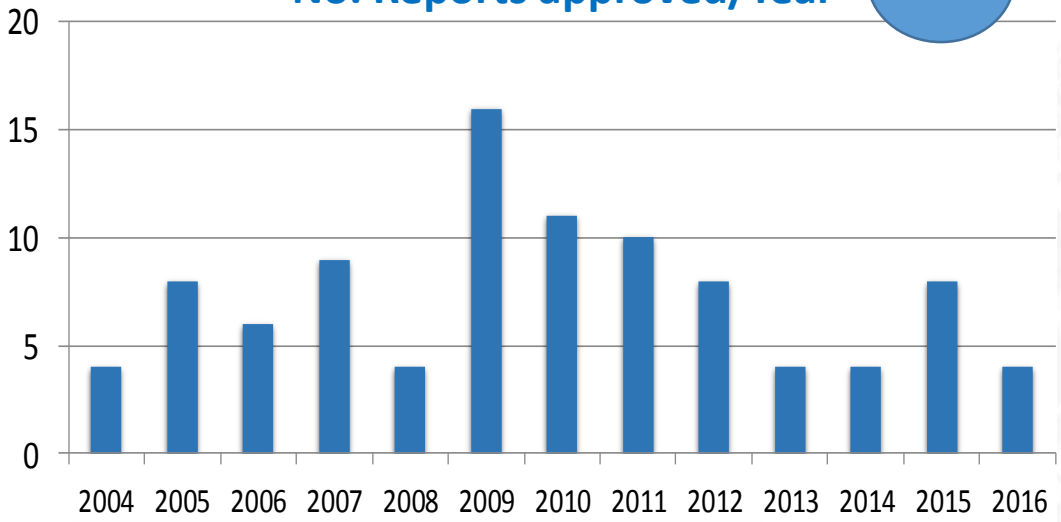
Risk Assessment

Scientific Committee (SC)

Reports

No. Reports approved/Year

96



Requests to the Scientific Committee

- Novel foods
- Technological adjuvants

Organizations

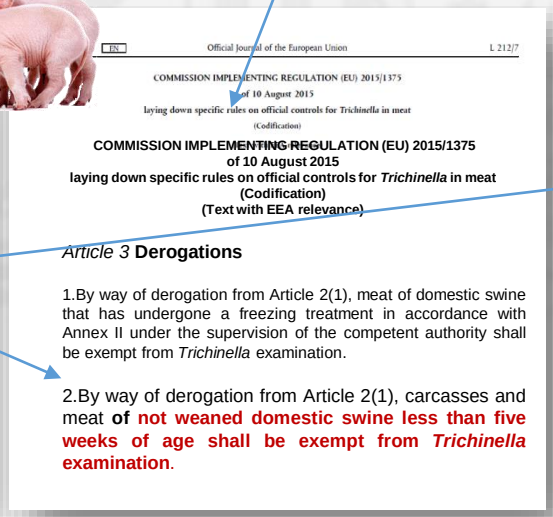
- Professional sectors
- Public administrations
- AESAN

Reports used for risk Management

Informe	Acción
• **Evisceration of lagomorphs**	Royal Decree Evisceration
• ***Anisakis***	Royal Decree fish treatment
• **Nitrates**	Chard consumption recommendation for children
• **Aflatoxins**	Maintained at the national regulation
• ***Trichinella***	Changes in EU control regulations are proposed
• **Food Complements**	Royal Decree autorization
• **Pregnant microbiological risks**	Consumption recommendations (brochure ...)

Trichinella


Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) en relación con el riesgo de triquinosis por consumo de carne de lechón


EN Official Journal of the European Union L 212/7

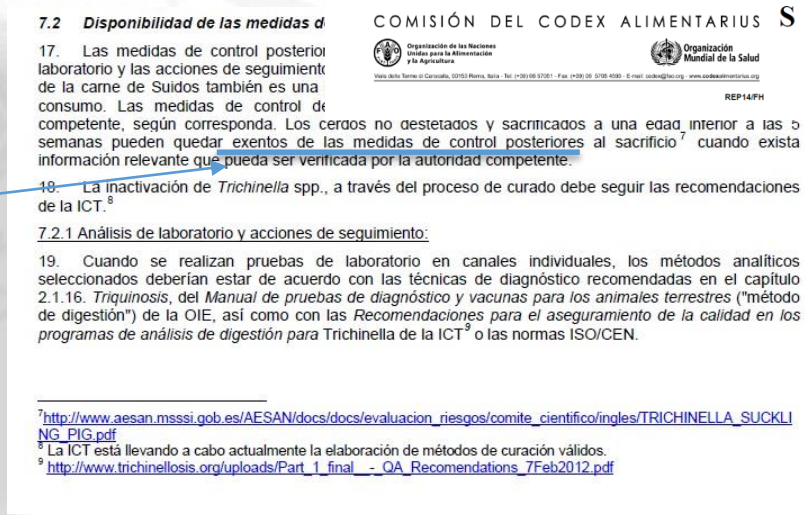
COMMISSION IMPLEMENTING REGULATION (EU) 2015/1375
of 10 August 2015
laying down specific rules on official controls for *Trichinella* in meat
(Codification)

COMMISSION IMPLEMENTING REGULATION (EU) 2015/1375
of 10 August 2015
laying down specific rules on official controls for *Trichinella* in meat
(Codification)
(Text with EEA relevance)

Article 3 **Derogations**

1.By way of derogation from Article 2(1), meat of domestic swine that has undergone a freezing treatment in accordance with Annex II under the supervision of the competent authority shall be exempt from *Trichinella* examination.

2.By way of derogation from Article 2(1), carcasses and meat **of not weaned domestic swine less than five weeks of age shall be exempt from *Trichinella* examination**.


COMISIÓN DEL CODEX ALIMENTARIUS

Organización de las Naciones Unidas para la Alimentación y la Agricultura — Organización Mundial de la Salud

REP14/FH

7.2 Disponibilidad de las medidas d

17. Las medidas de control posterior laboratorio y las acciones de seguimiento de la carne de Suidos también es una consumo. Las medidas de control de competente, según corresponda. Los cerdos no destetados y sacrificados a una edad inferior a las 5 semanas pueden quedar exentos de las medidas de control posteriores al sacrificio[7] cuando exista información relevante que pueda ser verificada por la autoridad competente.

18. La inactivación de *Trichinella* spp., a través del proceso de curado debe seguir las recomendaciones de la ICT.[8]

7.2.1 Análisis de laboratorio y acciones de seguimiento:

19. Cuando se realizan pruebas de laboratorio en canales individuales, los métodos analíticos seleccionados deberían estar de acuerdo con las técnicas de diagnóstico recomendadas en el capítulo 2.1.16. *Triquinosis*, del *Manual de pruebas de diagnóstico y vacunas para los animales terrestres* ("método de digestión") de la OIE, así como con las *Recomendaciones para el aseguramiento de la calidad en los programas de análisis de digestión para* Trichinella de la ICT[9] o las normas ISO/CEN.

[7] http://www.aesan.msssi.gob.es/AESAN/docs/docs/evaluacion_riesgos/comite_cientifico/ingles/TRICHINELLA_SUCKLING_PIG.pdf
[8] La ICT está llevando a cabo actualmente la elaboración de métodos de curación válidos.
[9] http://www.trichinellosis.org/uploads/Part_1_final__-_QA_Recomendations_7Feb2012.pdf

Spain SICENTIFIC COOPERATION WITH EFSA

AF Advisory Forum member

FP Focal Point member

AFWG Focal Point member

1 Number of Scientific Networks representatives from a certain organisation

org Organisations with representatives in Scientific Networks but not being an Article 36 list organisation

org Organisations on Article 36 list

AECOSAN: Focal Point of EFSA

- Advisory Forum representative
- Focal Point representative

SCIENTIFIC NETWORKS

- 5 Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN)
- 3 Ministry of Agriculture, Fisheries and Food (MAPA).
- 2 AECOSAN + MAPA
- 1 Superior Council for Scientific Research (CSIC)
- 1 Technical University of Valencia
- 1 University of Zaragoza
- 1 University of Santiago de Compostela
- 1 University of Córdoba
- 2 National Institute for Agrifood Research and Technology (INIA)

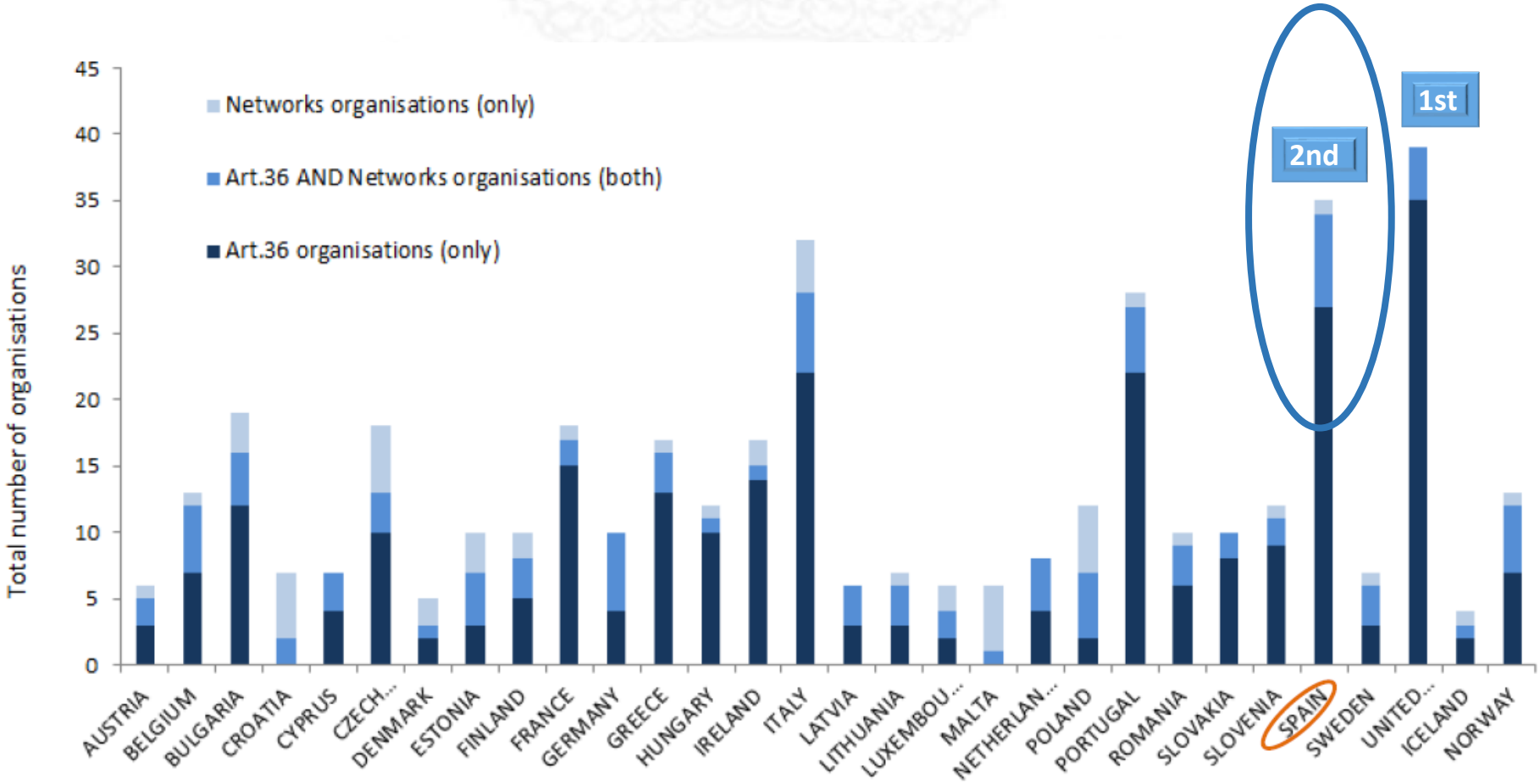
LIST OF COLLABORATING ORGANIZATIONS (ARTICLE 36 REG. 178/2002)

- 36 Spanish organizations

EXPERT PANNELS

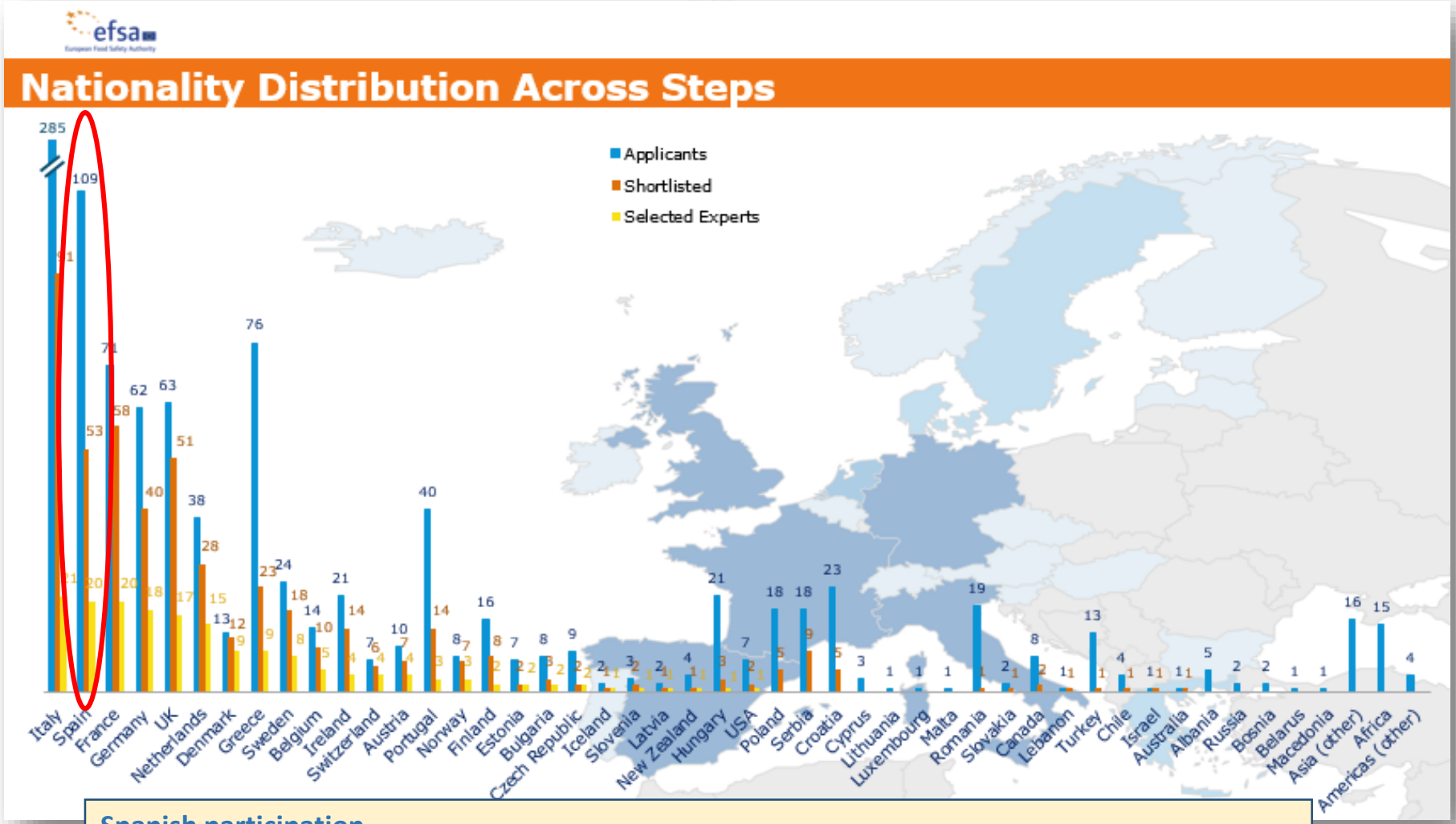
- 20 experts
- 7 Panels with Spanish representation

COLLABORATING ORGANIZATIONS



EXPERT PANELS

Renovation July 2018 2018 – 2021



Spanish participation

- **109 applicants**
- **53 Shortlisted (11% of total shortlisted).**
- **20 Designed: Spain is the second country in number of experts in EFSA panels**

RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE

GP/EFSA/AFSCO/2015/03

EuroCigua

- EuroCigua co-funded by the European Food Safety Authority (EFSA)
- **Framework Partnership Agreement** (FPA).
- EuroCigua started on June 1st 2016 and has a foreseen length of four years.
- Long-term cooperation between EFSA and 14 partners from six Member States with the **common general objective** of characterizing the risk of ciguatera food poisoning in Europe.

GOBIERNO DE ESPAÑA · MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL · aecosan · efsa European Food Safety Authority · Instituto de Salud Carlos III · UNIVERSIDADE DE VIGO · IRTA · Generalitat de Catalunya · Gobierno de Canarias un solo pueblo · Consejería de Agricultura, Ganadería, Pesca y Aguas · Servicio Canario de Salud · IUSA · UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA · ASAE Autoridade de Segurança · ipma · ARISTOTLE UNIVERSITY OF THESSALONIKI · Região Autónoma da Madeira Governo Regional · Instituto Nacional de Saúde Doutor Ricardo Jorge · STATE GENERAL LABORATORY · BfR Bundesinstitut für Risikobewertung · Ifremer · University of Thessaly · Ministry of Health

What is the Ciguatera?

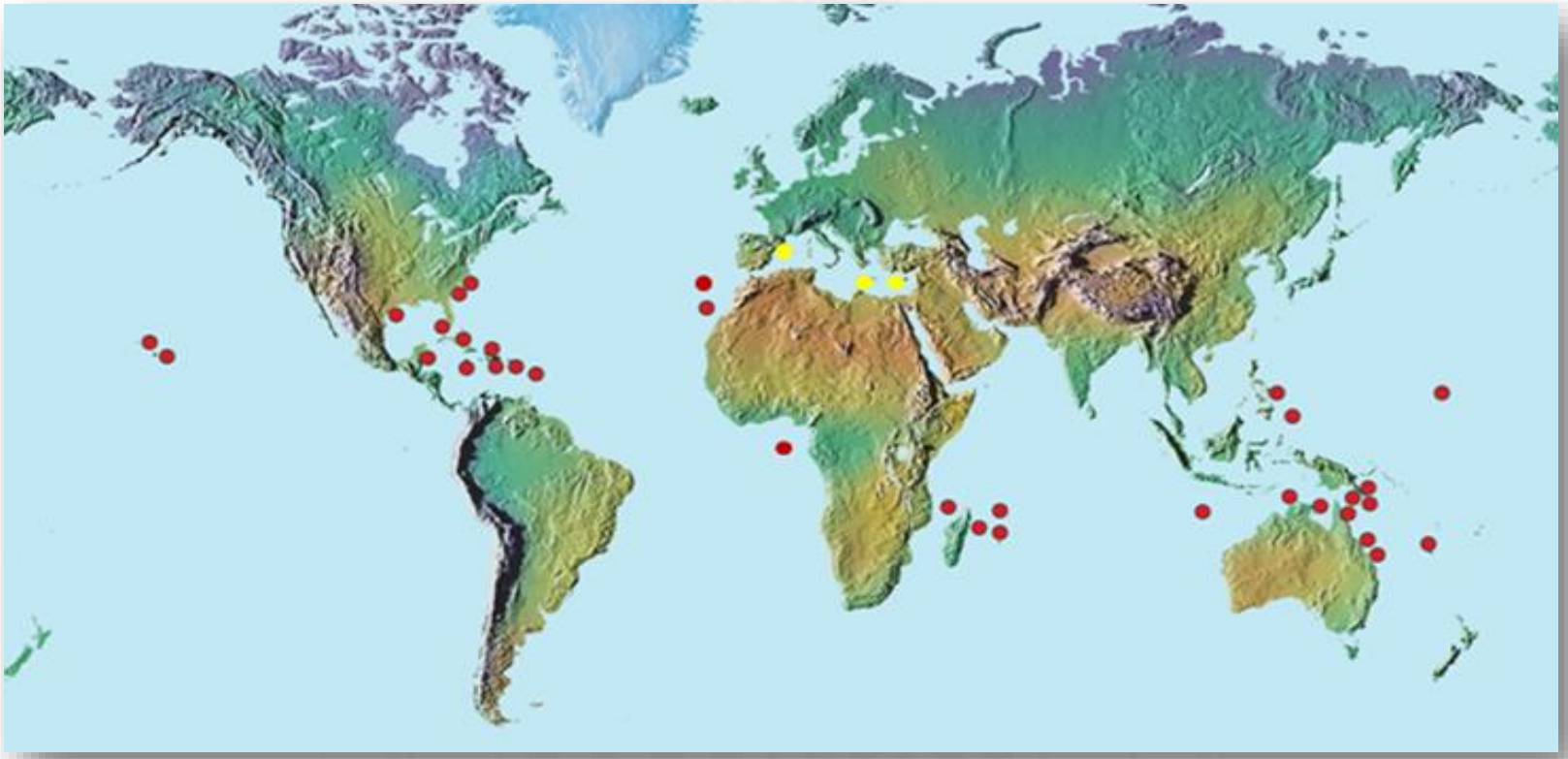
- **Ciguatoxins** are produced by micro algae, or dinoflagellates, called ***Gambierdiscus* spp.** The toxins climb up the food chain until the contaminated fish are caught and served to people.

- These toxins cause **Ciguatera Fish Poisoning (CFP).** It is a complex syndrome: **gastrointestinal, neurological and cardiovascular effects**.

- **CFP** is primarily associated with the consumption of large predator fish that have accumulated the toxins by feeding on smaller contaminated coral reef fish.

- At present, **CFP is the most common type of marine biotoxins food poisoning** worldwide with an estimated number of **10.000 to 50.000** people suffering from the disease annually.

Worldwide distribution of ciguatera



Red dots: ciguatera cases of poisoning. Yellow dots: presence of Gambierdiscus spp.

European Ciguatera

2004 → *Gambierdiscus* spp., responsible for ciguatera in the waters of the Canary Islands and Madeira microalgae, was detected.

2008 → autochthonous ciguatera outbreaks **in Spain (Canary Islands) and in Portugal (Madeira).**

Between **2008 and 2018** → 17 outbreaks recorded in Canary Islands → 111 cases.

Fish *genus* ***Seriola*** was involved in many of the outbreaks.

These new findings suggest the microorganism is becoming an increasing risk for European countries.

Alerts and information about the ciguatera in Europe

Lutjanus spp

						products			
2.	alert	17/03/2017	2017.0345	Germany	ciguatera poisoning suspected to be caused by frozen red snapper fillets (Lutjanus bohar) from Vietnam, via Denmark	fish and fish products	food	serious	Details
3.	information for	22/08/2016	2016.1155	France	ciguatoxins in chilled kingfish (Caranx spp) from India	fish and	food	serious	Details

	Classification	Date of case	Reference	Notifying country	Subject	Product Category	Type	Risk decision	
1.	information for attention	27/07/2017	2017.1112	France	ciguatoxins in frozen red snapper fillets (Lutjanus spp) from India	fish and fish products	food	serious	Details
2.	alert	17/03/2017	2017.0345	Germany	ciguatera poisoning suspected to be caused by frozen red snapper fillets (Lutjanus bohar) from Vietnam, via Denmark snapper (Lutjanus spp.) from India, via the Netherlands	fish and fish fish products	food	serious	Details
7.	information for attention	27/01/2015	2015.0088	France	ciguatoxins in wild-caught fish (Caranx spp and others) from Sri Lanka	fish and fish products	food	serious	Details
8.	alert	16/11/2012	2012.1602	Germany	ciguatera poisoning suspected to be caused by fresh red snapper fillets (Lutjanus spp.) from India	fish and fish products	food	serious	Details

Coordinator and Partners

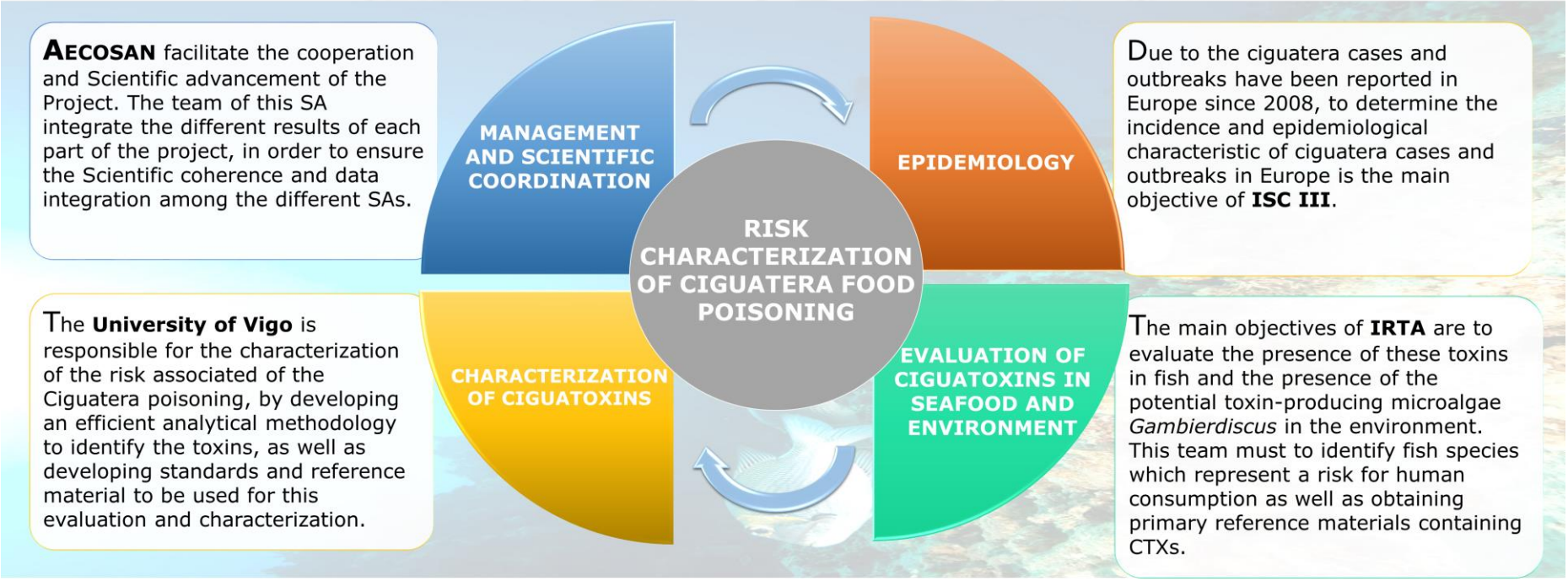
	Organisation	Acronym
Coordinator	Agencia Española de Consumo, Seguridad Alimentaria y Nutrición	AECOSAN
Partner 1	Instituto de Salud Carlos III	ISCIII
Partner 2	Institut de Recerca i Tecnologia Agroalimentaries	IRTA
Partner 3	Universidad de Vigo	UVigo
Partner 4	Portuguese Authority for Food and Economic Safety	ASAE
Partner 5	Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P.	_
Partner 6	University of Thessaly	_
Partner 7	Federal Institute for Risk Assessment	BfR
Partner 8	Canary Health Service (Servicio Canario de la Salud)	SCS
Partner 9	Universidad de Las Palmas de Gran Canaria	ULPGC
Partner 10	Instituto Português do Mar e da Atmosfera	IPMA
Partner 11	State General Laboratory (SGL) / Ministry of Health	SGL
Partner 12	French Research Institute for Exploitation of the Sea	IFREMER
Partner 13	Aristotle University of Thessaloniki	_

Collaborators & AB

Collaborators	Ministry of health, Cyprus
	Regional Ministry of Agriculture, Livestock, Fisheries and Water the Canary Islands Government
	Instituto das Florestas e Conservação da Natureza, IP-RAM/ Governo Regional da Madeira, Secretaria Regional do Ambiente e Recursos Naturais
	Direção de Serviços de Investigação e Desenvolvimento da Pesca, Direção Regional de Pescas, Secretaria Regional de Agricultura e Pesca
	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES)
Advisory Board	Dr. Robert Dickey - University of Texas Marine Science Institute
	Dr. Ronald Manger - Fred Hutchinson Cancer Research Center
	Dr. Takeshi Yasumoto - Japan Food Research Laboratories (JFRL)
	European Food Safety Authority - EFSA
	European Centre for Disease Prevention and Control - ECDC
	European Commission (EC)
	Joint Research Center (JRC)

The project

- It is composed of four independently managed subprojects named Specific Agreements (SA no. 1 - SA no. 4).
- Main task of **AECOSAN** (SA1) → Scientific cooperation and coordination of data integration



MANAGEMENT AND SCIENTIFIC COORDINATION

Main Goal: To provide the mechanisms in order to assure that the objectives of the FPA are accomplished in a global manner according to the main tasks reflected in Annex 1 of the **FPA**.

MINISTERIO DE SANIDAD, SERVICIOS SOCIALES E IGUALDAD

aecosan agencia española de consumo, seguridad alimentaria y nutrición

ASAE Autoridade de Segurança Alimentar e Económica

Main tasks of the SA1

- Scientific cooperation and scientific advancement
- Interchange of information among members of EuroCigua (partners, GB & AB)
- Data integration
- Integrate the results

Meetings

Coordination meetings & audioconferences
8

Meetings with EFSA
5

Meetings among SAs
3

Annual Plenary Meetings
2

Other meetings

JECFA

RASFF

FOREIGN HEALTH

Check Deliverables

SA2

- ✓ Case definition
- ✓ List of possible data sources for ciguatera cases and outbreaks form each EU MS
- ✓ Surveillance protocol for ciguatera cases and outbreaks
- ✓ Database for collecting cases and outbreaks

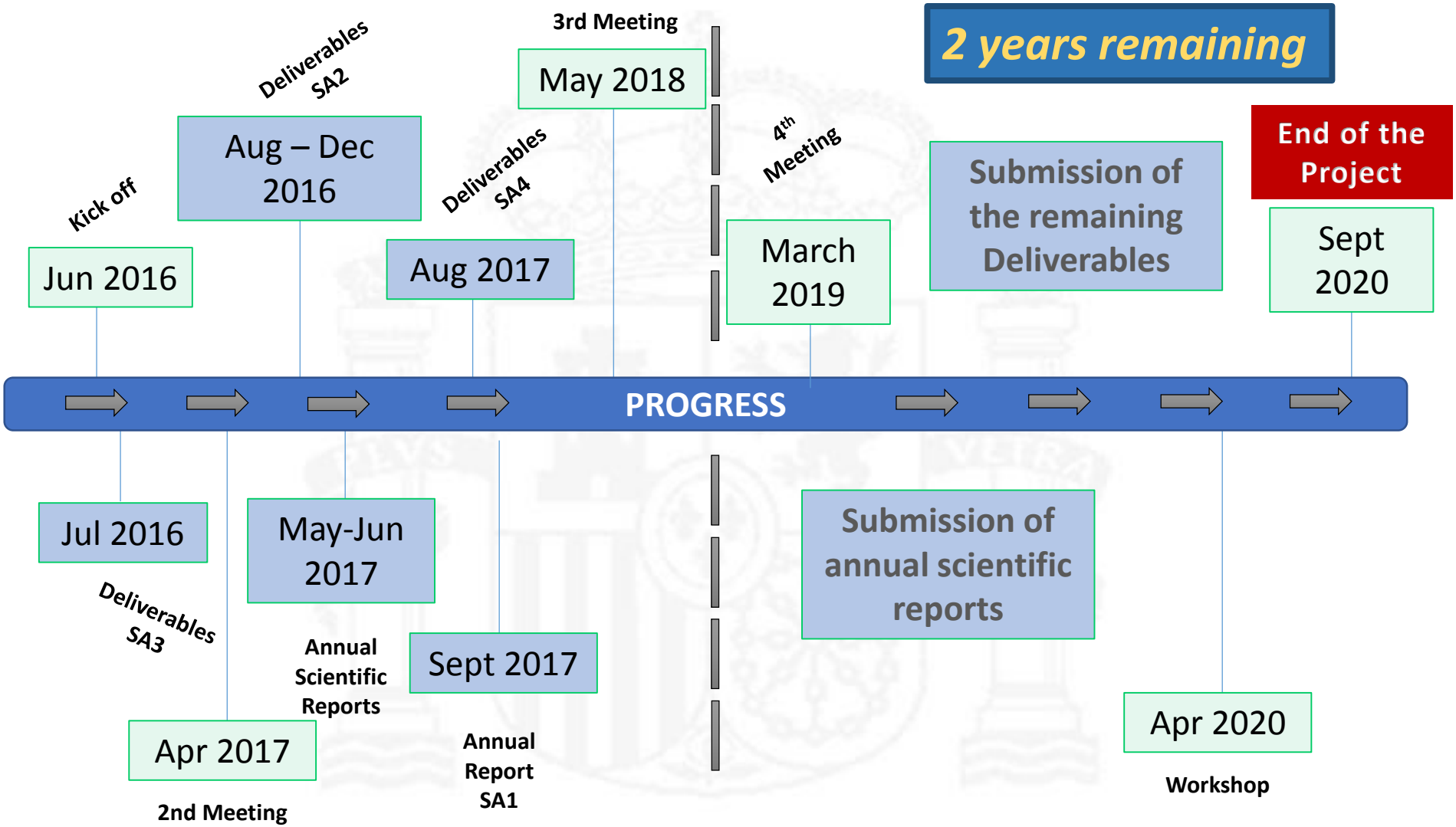
SA3

- ✓ Standarization of the cell-based assay (CBA)
- ✓ Extraction procedures for CTX
- ✓ Description of the protocol literature search and data collection model
- ✓ Sampling strategy Gambierdiscus spp. and fish

SA4

- ✓ Development, optimization and validation of LC-MS/MS and HRMS for identification and confirmation of CTX
- ✓ Development and optimization of LC-MS/MS and LC-HRMS
- ✓ Validation of LC-MS/MS

Progress of EuroCigua

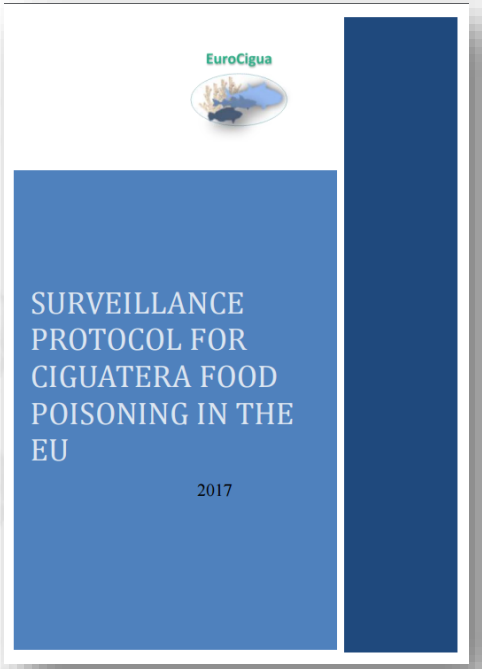


Main Results from Epidemiological area

Epidemiological data of ciguatera outbreaks reported in Europe since 2012 until 2017

EuroCigua

Year	Reporting country	No. of Cases	No. Hospitalized	Type of fish	Origin of fish	CTX detection
2012	Germany	23	4	*Lutjanus* bohar *L.* argentimaculatus	India	Yes
2012	Portugal	12	12	*Seriola* sp. *Lachnolaimus* maximus	Selvagem Island	Unknown
2012	Spain	10	0	*Seriola* sp.	Canary Islands	No
2012	Spain	9	0	*Seriola* sp.	Canary Islands	No
2012	Spain	4	0	*Seriola* sp.	Canary Islands	No
2012	Spain	12	0	*Epinephelus* sp.	Canary Islands	Yes
2013	Spain	16	0	*Epinephelus* sp.	Canary Islands	Yes
2014	Germany	6	1	*Lutjanus* bohar *L.* gentimaculatus *L.* erythropterus *Pinjalo pinjalo*	Indonesia	Yes
2015	Portugal	7	4	*Epinephelus* marginatus	Selvagens Island	Unknown
2015	Spain	3	0	*Mycteroperca* fusca	Canary Islands	No
2015	Spain	2	0	*Pamatomus* saltatriz	Canary Islands	Yes
2015	Spain	3	0	*Mycteroperca* fusca	Canary Islands	No
2015	Germany	16	2	*Lutjanus* bohar	India	Yes
2016	Spain	2	0	*Epinephelus* sp.	Canary Islands	Yes
2016	Spain	3	0	*Seriola* sp.	Canary Islands	Yes
2016	Spain	2	0	*Pagrus pagrus*	Selvagens Island	Yes
2016	Germany	4	2	*Lutjanus* sp	India	Yes
2017	Spain	2	0	*Epinephelus* sp.	Canary Islands	Yes
				Mycteroperca fusca		Yes
2017	Germany	15	2	*Lutjanus* bohar	Vietnam	Yes



From 2012 to 2017, a total of **151 cases** from **19 ciguatera outbreaks** have been notified in Spain, Portugal and Germany.

The collection of epidemiological data from other European countries is still ongoing.

Main Results from Environmental evaluation

Gambierdiscus and/or *Fukuyoa* species have been identified in Macaronesian Islands. Also in Greece and Cyprus.

Gambierdiscus was also identified in Balearic Island for the first time.

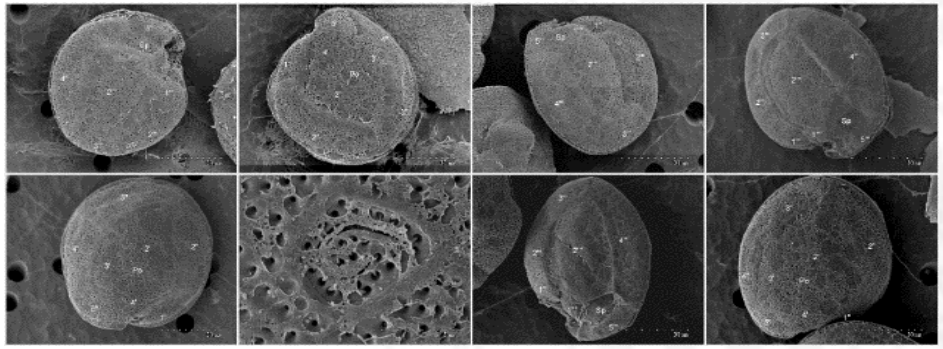


Figure 2. *Gambierdiscus australes from* Menorca, Balearic Island

Harmful Algae News
An IOC Newsletter on toxic algae and algal blooms
No. 59 - February 2018 www.ioc-unesco.org/hab

25 YEARS

Harmful Algae News was first published in early 1992 in response to requests from the participants at a number of IOC meetings and workshops on harmful algae, in particular the IOC-SCOR *ad hoc* Meeting in Rhode Island (USA) 2-3 November 1991, hosted by Ted Smayda where the final draft of the Harmful Algal Bloom Programme was prepared. At this time, e-mail was in its infancy, web sites still very simple but there was demand for a channel to disseminate information about harmful algal events and research, as well as to disseminate information on management/research programmes, conferences, meetings etc. The field of HAB science was new, the community multidisciplinary and at a national level, often scattered between many different institutions. The need for a communication channel to strengthen networks and co-operation was obvious and *Harmful Algae News* became a core element in the IOC Harmful Algal Bloom Programme.

Harmful Algae News started as an annex to the IOC/UNESCO newsletter '*International Marine Science*' (IMS), with the enthusiastic support of IMS editor Gary Wright and assistant Michelle Turner. However, *Harmful Algae News* became independent and eventually survived the IMS which ceased some years later. *Harmful Algae News* has been in publication for longer than any other IOC newsletter and its viability reflects the sustained focus on harmful algae in the IOC programme, as well as the continued interest by governments, institutions, scientists and those im-

United Nations Educational, Scientific and Cultural Organization
Intergovernmental Oceanographic Commission

Content

Vladimir Ryabinin, Exec. Secretary, IOC

"The Decade of Ocean Science will be a unique ten-year, global, large scale cooperative programme to seek urgently-needed scientific solution to support effective ocean management, stewardship and sustainable development" (continue next page)

UNESCO Supplement to Ioc Newsletter, No. 62 1992

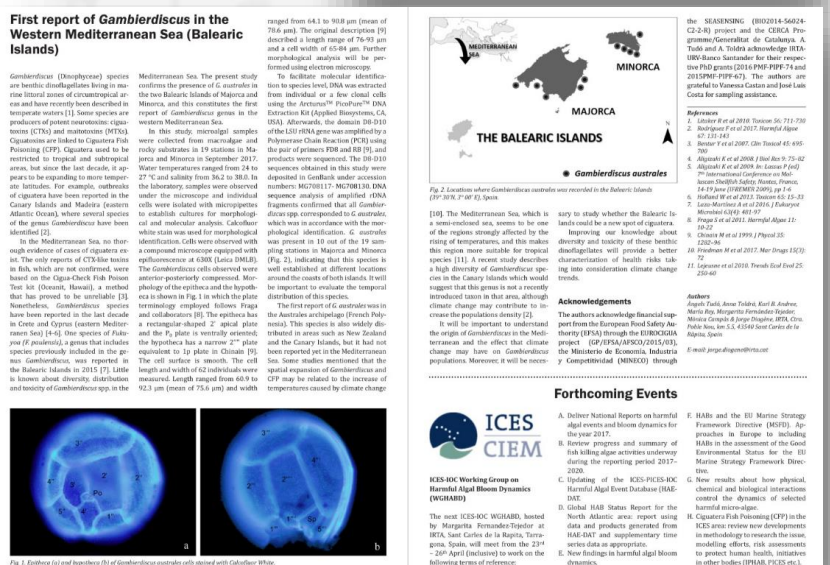
HARMFUL ALGAE NEWS
An IOC Newsletter on toxic algae and algal blooms

Introducing ...
This Newsletter on harmful algae responds to the expressed wishes of participants in several IOC workshops on harmful algal blooms, in particular the

Harmful algal blooms
by Tom Wyatt and Yolanda Pazos

For about a century, studies of phytoplankton and algal blooms have been regarded as fundamental components of fisheries research in the last

of 1898 have only been noted once in the entire recorded history of Nauplius, present Rio "The Chesapeake



First report of *Gambierdiscus* in the Western Mediterranean Sea (Balearic Islands)

Gambierdiscus (Dinophyceae) species are benthic dinoflagellates living in marine littoral zones of circumtropical areas and have recently been described in temperate waters [1]. Some species are producers of potent neurotoxins: ciguatoxins (CTXs) and maitotoxins (MTXs). Ciguatoxins are linked to Ciguatera Fish Poisoning (CFP). Ciguatera used to be restricted to tropical and subtropical areas, but since the last decade, it appears to be expanding to more temperate latitudes. For example, outbreaks of ciguatera have been reported in the Canary Islands and Madeira (eastern Atlantic Ocean), where several species of the genus *Gambierdiscus* have been identified [2].

In the Mediterranean Sea, no thorough evidence of cases of ciguatera exist. The only reports of CTX-like toxins in fish, which are not confirmed, were based on the Cigua-Check Fish Poison Test kit (Oceanit, Hawaii), a method that has proved to be unreliable [3]. Nonetheless, *Gambierdiscus* species have been reported in the last decade in Crete and Cyprus (eastern Mediterranen Sea) [4-6]. One species of *Fukuyoa* (*F. paulensis*), a genus that includes species previously included in the genus *Gambierdiscus*, was reported in the Balearic Islands in 2015 [7]. Little is known about diversity, distribution and toxicity of *Gambierdiscus* spp. in the Mediterranean Sea. The present study confirms the presence of *G. australes* in the two Balearic Islands of Majorca and Minorca, and this constitutes the first report of *Gambierdiscus* genus in the western Mediterranean Sea.

In this study, microalgal samples were collected from macroalgae and rocky substrates in 19 stations in Majorca and Minorca in September 2017. Water temperatures ranged from 24 to 27 °C and salinity from 36.2 to 38.0. In the laboratory, samples were observed under the microscope and individual cells were isolated with micropipettes to establish cultures for morphological and molecular analysis. Calcofluor white stain was used for morphological identification. Cells were observed with a compound microscope equipped with epifluorescence at 630X (Leica DMLB). The *Gambierdiscus* cells observed were anterior-posteriorly compressed. Morphology of the epitheca and the hypotheca is shown in Fig. 1 in which the plate terminology employed follows Fraga and collaborators [8]. The epitheca has a rectangular-shaped 2' apical plate and the P_0 plate is ventrally oriented; the hypotheca has a narrow 2''' plate equivalent to 1p plate in Chinain [9]. The cell surface is smooth. The cell length and width of 62 individuals were measured. Length ranged from 60.9 to 92.3 µm (mean of 75.6 µm) and width ranged from 64.1 to 90.8 µm (mean of 78.6 µm). The original description [9] described a length range of 76-93 µm and a cell width of 65-84 µm. Further morphological analysis will be performed using electron microscopy.

To facilitate molecular identification to species level, DNA was extracted from individual or a few clonal cells using the Arcturus™ PicoPure™ DNA Extraction Kit (Applied Biosystems, CA, USA). Afterwards, the domain D8-D10 of the LSU rRNA gene was amplified by a Polymerase Chain Reaction (PCR) using the pair of primers FD8 and RB [9], and products were sequenced. The D8-D10 sequences obtained in this study were deposited in GenBank under accession numbers: MG708117- MG708130. DNA sequence analysis of amplified rDNA fragments confirmed that all *Gambierdiscus* spp. corresponded to *G. australes*, which was in accordance with the morphological identification. *G. australes* was present in 10 out of the 19 sampling stations in Majorca and Minorca (Fig. 2), indicating that this species is well established at different locations around the coasts of both islands. It will be important to evaluate the temporal distribution of this species.

The first report of *G. australes* was in the Australes archipelago (French Polynesia). This species is also widely distributed in areas such as New Zealand and the Canary Islands, but it had not been reported yet in the Mediterranean Sea. Some studies mentioned that the spatial expansion of *Gambierdiscus* and CFP may be related to the increase of temperatures caused by climate change [10]. The Mediterranean Sea, which is a semi-enclosed sea, seems to be one of the regions strongly affected by the rising of temperatures, and this makes this region more suitable for tropical species [11]. A recent study describes a high diversity of *Gambierdiscus* species in the Canary Islands which would suggest that this genus is not a recently introduced taxon in that area, although climate change may contribute to increase the populations density [2].

It will be important to understand the origin of *Gambierdiscus* in the Mediterranean and the effect that climate change may have on *Gambierdiscus* populations. Moreover, it will be necessary to study whether the Balearic Islands could be a new spot of ciguatera.

Improving our knowledge about diversity and toxicity of these benthic dinoflagellates will provide a better characterization of health risks taking into consideration climate change trends.

Fig. 2. Locations where *Gambierdiscus australes* were recorded in the Balearic Islands (39°-38°N, 3°-00° E), Spain.

Acknowledgements

The authors acknowledge financial support from the European Food Safety Authority (EFSA) through the EUROGIGIJBA project (GP/EFSA/AFSCO/2015/03), the Ministerio de Economía, Industria y Competitividad (MINECO) through the SEASENSING (BIO2014-56024-C2-2-R) project and the CERCA Programme/Generalitat de Catalunya. A. Tudó and A. Toldrá acknowledge IRTA-UBV-Banco Santander for their respective PhD grants (2016 PMF-PIPF-74 and 2015PMF-PIPF-67). The authors are grateful to Vanessa Castan and José Luis Costa for sampling assistance.

References

1. Litaker W et al 2010. Toxicon 56: 711-730
2. Rodríguez F et al 2017. Harmful Algae 67: 131-143
3. Bentur Y et al 2007. Clin Toxicol 45: 695-700
4. Aligizaki K et al 2008. J Biol Res 9: 75-82
5. Aligizaki K et al 2009. In: Lassus P (ed) 7th International Conference on Molluscan Shellfish Safety, Nantes, France, 14-19 June (IFREMER 2009), pp 1-6
6. Hoffand W et al 2013. Toxicon 65: 15-33
7. Laza-Martínez A et al 2016. J Eukaryot Microbiol 63(4): 481-97
8. Fraga S et al 2011. Harmful Algae 11: 10-22
9. Chinain M et al 1999. J Phycol 35: 1202-36
10. Friedman M et al 2017. Mar Drugs 15(2): 72
11. Lejeusne et al 2010. Trends Ecol Evol 25: 250-60

Authors

Angels Tudó, Anna Toldrá, Karl B. Andree, Marta Rey, Margarita Fernández-Tejedor, Mònica Campàs & Jorge Diogène. IRTA, Ctra. Poble Nou, km 5.5, 43540 Sant Carles de la Ràpita, Spain

E-mail: jorge.diogene@irta.cat

Fig. 1. Epitheca (a) and hypotheca (b) of *Gambierdiscus australes* cells stained with Calcofluor White.

22

Forthcoming Events

ICES CIEM

ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

The next ICES-IOC WGHABD, hosted by Margarita Fernandez-Tejedor at IRTA, Sant Carles de la Rapita, Tarragona, Spain, will meet from the 23rd - 26th April (inclusive) to work on the following terms of reference:

A. Deliver National Reports on harmful algal events and bloom dynamics for the year 2017.
B. Review progress and summary of fish killing algae activities underway during the reporting period 2017-2020.
C. Updating of the ICES-PICES-IOC Harmful Algal Event Database (HAEDAT.
D. Global HAB Status Report for the North Atlantic area: report using data and products generated from HAE-DAT and supplementary time series data as appropriate.
E. New findings in harmful algal bloom dynamics.
F. HABs and the EU Marine Strategy Framework Directive (MSFD). Approaches in Europe to including HABs in the assessment of the Good Environmental Status for the EU Marine Strategy Framework Directive.
G. New results about how physical, chemical and biological interactions control the dynamics of selected harmful micro-algae.
H. Ciguatera Fish Poisoning (CFP) in the ICES area: review new developments in methodology to research the issue, modelling efforts, risk assessments to protect human health, initiatives in other bodies (IPHAB, PICES etc.).

23

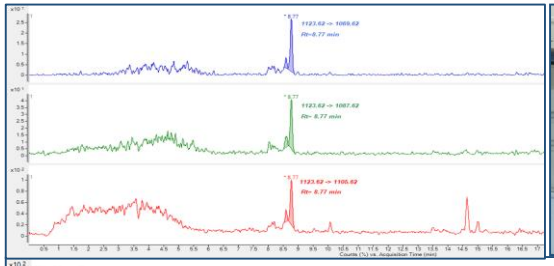
The **N2a Assay** was standardized and implemented on fish samples. Among 349 fish samples from the Canary Islands, **9.7% were CTX-like positive**.

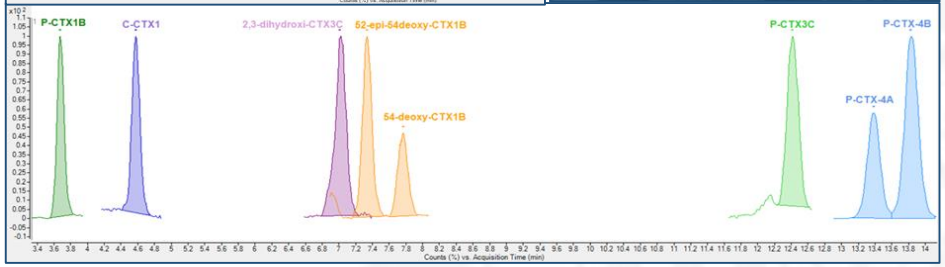
Species	Latin name	No. Samples	Weight (Kg)	*CTX-like toxicity (level)*
Amberjack	***Seriola* spp.**	**10**	**21.00 - 70.00**	**Low /Medium-Low / Medium / High**
Black moray	**Muraena augusti**	**4**	**0.40 - 1.03**	**Medium-Low / Low**
Brown moray	*Gymnothorax unicolor*	1	2.72	High
Common two-banded seabream	*Diplodus vulgaris*	1	0.32	Medium
Dusky grouper	**Epinephelus marginatus spp.**	**3**	**6.00 - 29.00**	**Medium-low / High**
Fangtooth moray	Enchelycore anatina	1	1.44	Medium
Island-grouper	**Mycteroperca fusca**	**4**	**2.50 - 8.00**	**Low / High**
Macaronesian sharpnose-puffers	*Canthigaster capistratas*	1	0.02 - 0.03	High
Mediterranean moray	*Muraena helena*	1	0.82	Medium-low
Parrotfish	**Sparisoma cretense**	**4**	**0.37 - 0.48**	**Low /Medium / High**
Redporgy	**Pagrus pagrus**	**1**	**4.00**	**High**
Wahoo	*Acanthocybium solandri*	1	14.00	Low
White trevally	*Pseudocaranx dentex*	1	0.23	Low
Zebra seabream	*Diplodus cervinus cervinus*	1	0.68	Medium

Positive CTX samples from the Canary Islands (May, 2016 - July, 2018).

Main Results from Characterization area

In fish samples that tested positive by N2a Assay from the Canary Islands, Madeira and Selvagens, CTXs were confirmed using LC-MS/MS.





GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

Detection of ciguatoxins in fish meat: an integrated approach for screening and confirmatory methods

BfR Bundesinstitut für Risikobewertung

A. Preiß-Weigert[1], A. Gago-Martinez[2], M. Nicolas[3], C. Dell'Aversano[4]

Ciguatoxins (CTXs) and Ciguatera Fish Poisoning (CFP)

- secondary metabolites of the algal genus gambierdiscus
- transformed to CTX in the marine food chain via herbivorous and carnivorous fish into fish for human consumption

Fig. 1: Transformation of gambierdiscus to ciguatoxins (FAO/WHO/Codex alimentarius)

- toxicological effects are referred to as ciguatera fish poisoning (CFP)
- symptoms
  - gastrointestinal
  - neurological (tingling of lips, hand and feet, reversal of temperature sensation)
  - cardiovascular symptoms up to fatality

CFP a Topic of Public Concern

- one of the most common food-borne diseases related to fish consumption
- periodic occurence in fish from tropical regions e.g.Carribean and Canary Islands, Pacific and Indian Ocean regions
- CFP cases in fish importing countries due to global trade

International Activities on CTX

- FAO Technical paper 574 (2014) predicted dinoflagellates producing CTX precursors to become one of the increasing food safety threats due to climate change
- Codex Alimentarius CCCF 2017 endorsed CTXs to be included in the „Priority List of Contaminants and naturally occurring Toxicants for evaluation by JECFA"

Main objectives – Validation of Analytical Methods in order to

Proctect consumers against CFP by

- strengthening analysis of CTX in fish
- establishing & disseminating the Neuro-2a for CTX screening
- improving availability of CTX standard substances
- CTX confirmation by LC-MS/MS and LC-HRMS
- collection of occurrence data for risk assessment

Promote animal welfare by

- replacing Mouse Bioassay currently used as a CTX screening-tool

Project Module I – CTX-Screening Implementation of N2a Assay

- call for laboratories interested in implementing Neuroblastoma-2a (N2a) Assay
- development of an expert network and organisation of meetings to compare different method approaches
- transfer of knowledge and expertise from experienced laboratories e.g. the EURL-MB to national laboratories establishing N2a Assay

Fig. 2: chemical structure of P-CTX-1

Project Module II – Standard availability Expert Meeting on „State of the Art"

- inventory of available reference standards (Carribean, Pacific and Indian CTX)
- concept for improvement of standard availability
- support of activities for isolation of CTX from all regions

Project Module III – CTX - Screening by N2a Assay – Collaborative Trial

- collaborative study among the expert network
- preferably with naturally contaminated material depending on availability (otherwise preparation of spiked fish tissue)

Project Module IV – CTX - Confirmation by LC-MS/MS and LC-HRMS

- collection of performance data on methods currently available (expert network):
  - scope regarding CTXs from different regions
  - performance data (e.g. limits of detection and quantification)
- identification of further requirements to assure reliable quantitative data from LC-MS/MS methods and steps needed to achieve them
- method optimization if necessary
- implementation of the method in laboratories of the network
- collaborative study on toxin identification by LC-HRMS

Important Factors for Project's Success

- laboratories willing to establish Neuro-2a
- partners/network who provide/exchange sample material
- reference material (CTX-standards)
- build up on activities of current project EuroCigua

Fig. 3: Lutjanus bohar sample

Project Proponents

1 German Federal Institute for Risk Assessment, Berlin
2 European Reference Laboratory for Marine Biotoxins and University of Vigo/Spain, Department of Analytical and Food Chemistry
3 French agency for food, environmental and occupational health safety, Laboratory for Food safety - site of Maisons-Alfort
4 University of Naples Federico II, Italy, Department of Pharmacy

Bundesinstitut für Risikobewertung • Max-Dohrn-Str. 8-10 • 10589 Berlin • Tel. 0 30 - 184 12 - 0 • Fax 0 30 - 184 12 - 47 41 • bfr@bfr.bund.de • www.bfr.bund.de

Optimization of LC-MS/MS method for the confirmation of the CTX toxicity was performed.

C-CTX1 is the main responsible for the CTX contamination of fish from Canary Islands and Madeira.

RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE

GP/EFSA/AFSCO/2015/03

EuroCigua

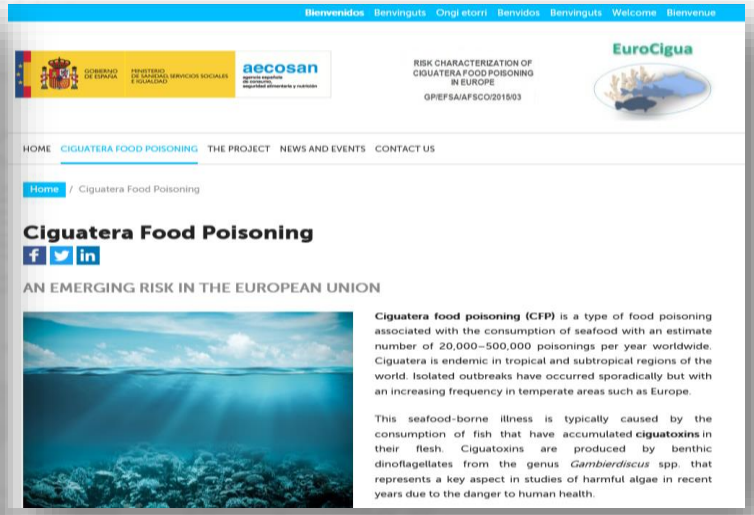
Main Results

- European case definition, comparison with FDA pervious one.
- An epidemiological surveillance protocol for ciguatera in the EU. Included two questionnaires.
- From 2012 to 2017, 151 cases from 19 ciguatera outbreaks have been notified in Spain, Portugal and Germany.
- *Gambierdiscus* and/or *Fukuyoa* species have been identified in Macaronesian Islands. *Gambierdiscus* was also identified in Balearic Island for the first time.
- The N2a Assay was standardized and implemented on fish samples. Among 349 fish samples from the Canary Islands, 9.7% were CTX-like positive.
- Optimization of LC-MS/MS method for the confirmation of the CTX toxicity was performed.
- C-CTX1 is the main responsible for the CTX contamination of fish from Canary Islands and Madeira.

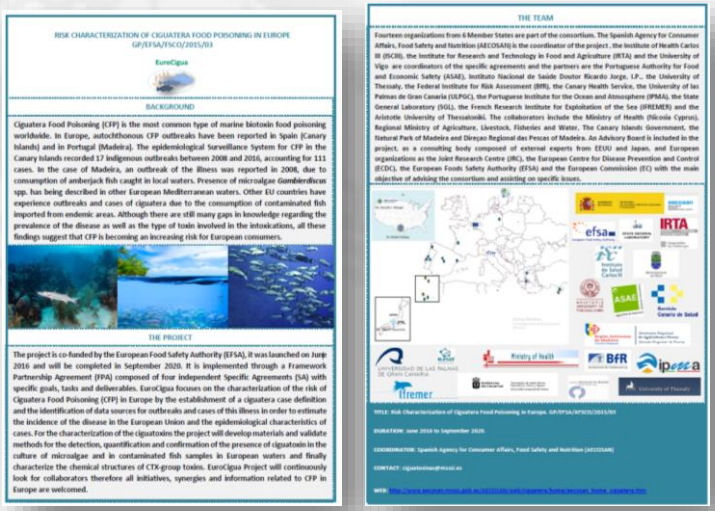
External Communication Plan

Leaflet

EuroCigua website



Factsheet



BUILD UP AWARENESS

English
Portuguese
Spanish

German
French
italian

Ciguatoxins and CODEX

Río de Janeiro, Brazil – 3-7 April 2017

CCCF: Ciguatera is a natural occurring toxin in fish and CCCF will be the relevant subsidiary body of CAC to recommend measures to reduce contamination to safe levels to ensure public health and facilitate trade.

37. The EU informed the Committee of a four-year project co-funded by EFSA and coordinated by Spanish food safety agency (AECOSAN) to determine incidence in Europe of ciguatera fish poisoning and epidemiological incidence cases, assess presence of ciguatoxins in food and environment in Europe, and validate the methods for detection, quantification and confirmation that could contribute to future work on ciguatoxins.

Conclusion

38. The Committee:

- agreed to request scientific advice from FAO/WHO to allow the Committee to develop appropriate risk management options;
- noted that the in-session working group on the priority list of contaminants and naturally occurring toxicants for evaluation by JECFA would consider this matter further (see Agenda Item 14).

Ciguatera Working Group - FAO

Characterization of the risk of ciguatera worldwide

CODEX ALIMENTARIUS COMMISSION **E**

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 3 — CX/CF 17/11/3-Add.2
March 2017

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

Eleventh Session
Rio De Janeiro, Brazil, 3-7 April 2017

MATTERS OF INTEREST ARISING FROM FAO AND WHO (INCLUDING JECFA)

UPDATE ON RELEVANT WORK TO BE CONSIDERED FOR CIGUATOXINS

Background

1. Ciguatera fish poisoning (CFP) is one of the most common food-borne illnesses related to finfish consumption. It has been known for centuries. Its true incidence is not known, but it is estimated that 10 000–50 000 people per year suffer from this illness (FAO 2014), making it one of the most common

Experts from EUROCIGUA together with other experts including the CODEX experts responsible for the request of **risk assessment.**

Developing a document that it will include epidemiological and toxicological information, analytical methods, ecology and phytoplankton.

EuroCigua project is mentioned **as a reference project** for the characterization of ciguatera risk in Europe.

Completed Document → Spring of 2019

Publications

A. Canals[1], C. Varela[2], J. Diogène[3], A. Gago[4]
[1] Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN), [2] Institute of Health Carlos III (ISCIII), [3]Institute for Research and Technology in Food and Agriculture (IRTA), [4]University of Vigo (UVIGO).
ciguatoxinas@msssi.es

EuroCigua

RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE

GP/EFSA/AFSCO/2015/03

BACKGROUND

Ciguatera Food Poisoning (CFP) is the most common type of marine biotoxin food poisoning worlwide. In Europe, autochthonous ciguatera food poisoning (CFP) outbreaks have been reported in Spain (Canary Islands) and in Portugal (Madeira). In Spain an outbreak occurred in 2004 associated with consumption of amberjack fish (*Seriola* spp.) captured in Canarian waters. The epidemiological Surveillance System for Ciguatera poisoning in the Canary Islands recorded 11 indigenous outbreaks of ciguatera food poisoning between 2008 and 2014, accounting for 96 cases. In the case of Madeira, an outbreak of ciguatera was reported in 2008, due to consumption of amberjack fish caught in local waters. Furthermore, some studies have identified the presence of *Gambierdiscus* spp. in waters surrounding the Canary Islands and Madeira, but there are still many gaps in knowledge regarding their prevalence, the type of toxin they produce, among others. These new findings suggest the ciguatera is becoming an increasing risk for European countries.

WHAT IS EUROCIGUA?

EuroCigua Project is implemented through a Framework Partnership Agreement (FPA) and four Specific Agreements (SA), co-funded by the European Food Safety Authority (EFSA). It establishes a long-term cooperation between the European Food Safety Authority (EFSA) and fourteen partners from six Member States with the common general objective of characterizing the risk of ciguatera food poisoning in Europe. It is composed of four independently managed subprojects. The main objectives of this project are to determine the incidence and epidemiological characteristics of ciguatera cases in Europe, to investigate the spatial and temporal distribution of *Gambierdiscus* spp. in European waters, to evaluate the CTX-like toxicity of *Gambierdiscus* spp. populations, to evaluate possible presence of CTXs in fish in EU waters, to establish a reliable methodological approach in order to identify and quantify ciguatoxins (CTXs) in fish and microalgae, as well as to develop standards and reference material. This project started on June 1st 2016 and has a foreseen length of four years.

Specific Agreement no.1. "Management and Scientific Coordination". Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN). The main goals are to facilitate cooperation and scientific advancement of all Specific Agreements, to favor scientific cooperation among partners, and to ensure scientific coherence and data integration in order to provide EFSA with a comprehensive final delivery in terms of the risk characterization of the CFP in the EU.

Specific Agreement no.2 "Epidemiology". Institute of Health Carlos III (ISCIII). The main objective is to determine the incidence and epidemiological characteristics of ciguatera cases in Europe. Specifically, the establishment of a ciguatera case definition, the identification of data sources for outbreaks and cases of this illness, design of a surveillance protocol for the CFP according to the data sources information collected from EU Member States. All the information gathered will be assessed using statistical analysis software in order to generate a report on the ciguatera incidence and the epidemiological characteristics.

Specific Agreement no.3 "Evaluation of ciguatoxins in seafood and environment". Institute for Research and Technology in Food and Agriculture (IRTA) . The main goal is to evaluate the presence of ciguatoxins (CTXs) in fish and also the presence of the potential toxin-producing microalgae of the genus *Gambierdiscus* in the environment, in strategic hotspots in Macaronesian and Mediterranean waters. Specific tasks: Isolation of *Gambierdiscus* spp. from EU waters, species identification, isolation and culturing of microalgae for toxicity evaluation (by cell-based assay, CBA) and large-scale culture of the most interesting toxicogenic strains will be conducted. In addition this sub-project will include the evaluation of CTXs in fish for human consumption, the collection of fish samples from EU waters and from the market (muscle and liver if available), the toxicity evaluation by CBA, the identification of toxin profiles and the quantification of toxins in reference material. Positive samples will be confirmed with LC-MS/MS by Specific Grant no. 4. In addition environmental data literature and data search for the future development of models to understand the ecology of ciguatera will be collected.

Specific Agreement no.4 "Characterization of ciguatoxins". University of Vigo. The mail goal is to characterize the risk associated with Ciguatera poisoning, by developing an efficient analytical methodology with ability to confirm the identity of the toxins involved in the contamination of phytoplankton and fish samples. Also standards and reference materials will be generated. Specific tasks include: development of a Standard Operating Procedure (SOP) for the Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS),analysis of ciguatoxins present in different matrices (phytoplankton and fish), application of the LC-MS/MS to all the samples previously identified as positive in the Specific Grant no.3, and confirmation by High Resolution Mass Spectrometry (HRMS). Performance of interlaboratory testing to evaluate the methodologies developed in this project, and carry out an intercomparative evaluation of the CTXs profiles of the samples included in this study, as well as testing materials (standards and secondary reference materials) developed in the project through the collaboration with the EU Reference laboratory for marine biotoxins.

THE TEAM

PARTNERS: INSTITUTE OF HEALTH CARLOS III (ISCIII) , INSTITUTE FOR RESEARCH AND TECHNOLOGY IN FOOD AND AGRICULTURE (IRTA), UNIVERSITY OF VIGO (UVIGO), PORTUGUESE AUTHORITY FOR FOOD AND ECONOMIC SAFETY (ASAE), INSTITUTO NACIONAL DE SAÚDE DOUTOR RICARDO JORGE, I.P., UNIVERSITY OF THESSALY, FEDERAL INSTITUTE FOR RISK ASSESSMENT (BFR), CANARY HEALTH SERVICE , UNIVERSITY OF LAS PALMAS DE GRAN CANARIA (ULPGC), PORTUGUESE INSTITUTE FOR THE OCEAN AND ATMOSPHERE (IPMA), STATE GENERAL LABORATORY (SGL), FRENCH RESEARCH INSTITUTE FOR EXPLOITATION OF THE SEA(IFREMER), ARISTOTLE UNIVERSITY OF THESSALONIKI.

COLLABORATORS: MINISTRY OF HEALTH, NICOSIA CYPRUS, REGIONAL MINISTRY OF AGRICULTURE, LIVESTOCK, FISHERIES AND WATER THE CANARY ISLANDS GOVERNMENT, NATURAL PARK OF MADEIRA, DIREÇAO REGIONAL DAS PESCAS OF MADEIRA.

THE ADVISORY BOARD: EUROPEAN FOOD SAFETY AUTHORITY (EFSA), EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL (ECDC), EUROPEAN COMMISSION (EC), JOINT RESEARCH CENTRE (JRC), DR. ROBERT DICKEY, DR. RONALD MANGER, DR. TAKESHI YASUMOTO.

PRELIMINARY RESULTS OF RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE

GP/EFSA/AFSCO/2015/03

EuroCigua

L. Cebadera-Miranda[1], A. Canals*[1], F. Melo[2]. E.V. Martínez[2], D. Núñez[2], C. Varela*[2], F. Martín[4], M. Friedemann[5], M.Oleastro[6], I. Boziaris[7], F. Real[8], J.A. Sánchez[9], N. García[8], P. Reis-Costa[8], N. Gouveia[10], G. Papageorgiou[11], A. Aligizaki[12], J. Diogène*[13], D. Castro[14], A. Pequeño[14], P. Estevez[14], O. Vilariño[14], J.M.Leao[14], A. Gago-Martínez*[14]
[1]Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN); [2]Portuguese Authority for Food and Economic Safety (ASAE); [3]Institute of Health Carlos III (ISCIII); [4]Canary Health Service (CHS); [5]German Federal Institute for Risk Assessment (BfR); [6]Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P. (INSA); [7]University of Thessaly; [8]University of las Palmas de Gran Canaria (ULPGC); [9]Instituto Português do Mar e da Atmosfera (IPMA); [10]Fisheries Department (JTO). Direção Regional das Pescas of Madeira; [11]General State Laboratory (GSL); Ministry of Health of Cyprus; [12]Aristotle University of Thessaloniki; [13]Institute for Research and Technology in Food and Agriculture (IRTA); [14]University of Vigo (UVIGO).
*Corresponding authors
ciguatoxinas@mscbs.es

Background

Ciguatera food poisoning is one of the most common food-borne illnesses related to seafood consumption worldwide, mainly in tropical and subtropical areas. However autochthonous outbreaks have been reported in Spain and Portugal, specifically in Macaronesia area.

EuroCigua Project

EuroCigua is a project co-funded by the European Food Safety Authority (EFSA) and it is implemented through a Framework Partnership Agreement (FPA). It is composed of four independently managed subprojects named Specific Agreements (SA). SA1: AECOSAN & ASAE; SA2: ISCIII, CHS, BfR and INSA; SA3: IRTA, UPLGC, U. Thessaly, IPMA, FD, GSL, U. Thessaloniki and finally SA4: UVIGO & IFREMER. EuroCigua started on June 1st 2016 and has a foreseen length of four years.

Figure 1. Organization and specific objectives of EuroCigua project.

Objectives

The EuroCigua project focuses on characterizing the risk of ciguatera food poisoning in Europe. The main objectives are: to estimate the incidence of ciguatera in Europe; to assess the presence of ciguatoxin in food; and to develop and validate methods for the determination of ciguatoxin contaminated specimens. In this poster, an update of results is presented.

Methods & Results

SA No. 2: Epidemiology

An epidemiological surveillance protocol for ciguatera in the EU has been developed by SA2.

Year	Reporting country	No. of Cases	No. Hospitalized	Type of fish	Origin of fish	CTX detection
2012	Germany	23	4	*Lutjanus bohar* *L. argentimaculatus*	India	Yes
2012	Portugal	12	12	*Seriola* sp. *Lachnolaimus maximus*	Selvagem Island	Unknown
2012	Spain	10	0	*Seriola* sp.	Canary Islands	No
2012	Spain	9	0	*Seriola* sp.	Canary Islands	No
2012	Spain	4	0	*Seriola* sp.	Canary Islands	No
2012	Spain	12	0	*Epinephelus* sp.	Canary Islands	Yes
2013	Spain	16	0	*Epinephelus* sp.	Canary Islands	Yes
2014	Germany	6	1	*Lutjanus bohar* *L. gentimaculatus* *L. erythropterus* *Pinjalo pinjalo*	Indonesia	Yes
2015	Portugal	7	4	*Epinephelus marginatus*	Selvagens Island	Unknown
2015	Spain	3	0	*Mycteroperca fusca*	Canary Islands	No
2015	Spain	2	0	*Ramatomus saltatrix*	Canary Islands	Yes
2015	Spain	3	0	*Mycteroperca fusca*	Canary Islands	No
2015	Germany	16	2	*Lutjanus bohar*	India	Yes
2016	Spain	2	0	*Epinephelus* sp.	Canary Islands	Yes
2016	Spain	3	0	*Seriola* sp.	Canary Islands	Yes
2016	Spain	2	0	*Pagrus pagrus*	Selvagens Island	Yes
2016	Germany	4	2	*Lutjanus* sp	India	Yes
2017	Spain	2	0	*Epinephelus* sp. *Mycteroperca fusca*	Canary Islands	Yes
2017	Germany	15	2	*Lutjanus bohar*	Vietnam	Yes

Table 1. Epidemiological data of ciguatera outbreaks reported in Europe since 2012 until 2017

SA No. 3:

Sampling of microalgae for the isolation of ciguatoxin (CTX) producing species of the genera *Gambierdiscus* and *Fukuyoa* has proceeded in Macaronesia (Canary Islands and Madeira) and in the Mediterranean area (Cyprus, Crete and Balearic Islands).

Figure 2. *Gambierdiscus australes* from Menorca, Balearic Island

After taxonomic and morphological evaluation and molecular genetics, *Gambierdiscus* species was found in all islands. Environmental data were recorded and are currently under analysis.

Species	Latin name	No.	Weight (Kg)	CTX-like toxicity (/level)
Amberjack	*Seriola* spp.	10	23.00 - 70.00	Low /Medium-Low / Medium / High
Black moray	*Muraena augusti*	4	0.40 - 1.10	Medium-Low / Low
Brown moray	*Gymnothorax unicolor*	1	2.72	High
Common two-banded seabream	*Diplodus vulgaris*	1	0.82	Medium
Dusky grouper	*Epinephelus marginatus* spp.	3	6.00 - 29.00	Medium-low / High
Fangtooth moray	*Enchelycore anatina*	1	1.44	Medium
Giant grouper	*Mycteroperca fusca*	8	2.00 - 8.00	Low / High
Macaronesian sharpnose-puffer	*Canthigaster capistrata*	1	0.01 - 0.03	High
Mediterranean moray	*Muraena helena*	1	0.82	Medium-low
Porcelain	*Serranus cabrilla*	4	0.37 - 0.48	Low /Medium / High
Sailfin	*Canthidermis sufflamen*	1	8.00	Low
Wahoo	*Acanthocybium solandri*	1	14.00	Low
White trevally	*Pseudocaranx dentex*	1	0.23	Low
Zebra seabream	*Diplodus cervinus cervinus*	1	0.68	Medium

Table 2. Positive CTX samples from the Canary Islands (May, 2016 - July, 2018). Bold green correspond to fish species with the higher number of positive CTX samples. Green shaded lines correspond to samples related to ciguatera cases & outbreaks.

In the Canary Islands, a total of 349 fish samples were analyzed for CTX detection by Neuroblastoma-2a (N2a) Assay, and 9.7 % (34 samples) were positive. The higher number of positive samples was collected during the warm season mainly in Tenerife and El Hierro (23% and 47% respectively). Among positive samples, 35% presented low levels of CTX-like toxicity.

CTX-like TOXICITY	EC_{50} (mg H_2O/ml)	EC_{80} (mg H_2O/ml)
High	<10	
Medium-High	20-40	<80
Medium	20-40	>80
Medium-Low	40-80	
Low	>80	

Table 3. Toxicity ranking according to the tissue equivalents of fish muscle causing 50 or 80 % mortality in cells.

SA No. 4:

In fish samples that tested positive by N2a Assay from the Canary Islands, Madeira and Selvagens, CTXs were confirmed using LC-MS/MS.

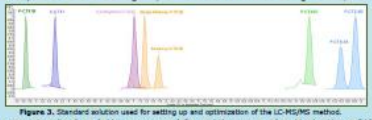


Figure 3. Standard solution used for setting up and optimization of the LC-MS/MS method.

A pure standard solution was used for setting up and optimization of the LC-MS/MS method using $[M+Na]^+$ as precursor and product ion. This standard solution consisted on a mix of Pacific CTXs.

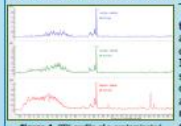


Figure 4. CTX profile of a contaminated fish from Selvagens, containing C-CTX1

The Multiple Reaction Monitoring (MRM) transitions selecting $[M+H-nH2O]^+$ were also used for the analysis of CTXs in contaminated fish samples from Canary Islands, Madeira and Selvagem Islands. A similar CTX profile was observed for the contaminated samples. Other CTX analogues, including C-CTX1 isomers, might be also present (confirmatory experiments are part of the undergoing research).

Additional MRM transitions using C-CTX1 standard and selecting fragments as $[M+H-nH2O]^+$ were also used for confirmation.

Conclusions

From 2012 to 2017, a total of 151 cases from 19 ciguatera outbreaks have been notified in Spain, Portugal and Germany. The collection of epidemiological data from other European countries is still ongoing. *Gambierdiscus* and/or *Fukuyoa* species have been identified in Macaronesian Islands. *Gambierdiscus* was also identified in Balearic Island for the first time. The N2a Assay was standardized and implemented on fish samples. Among 349 fish samples from the Canary Islands, 9.7% were CTX-like positive. Optimization of LC-MS/MS method for the confirmation of the CTX toxicity was performed. C-CTX1 is the main responsible for the CTX contamination of fish from Canary Islands and Madeira.

Advisory Board: Dr. Takeshi Yasumoto, Dr. Robert Dickey, Dr. Ronald Manger

GOBIERNO DE ESPAÑA

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

Publications

aecosan
agencia española de consumo, seguridad alimentaria y nutrición

marine drugs MDPI

Review

An Updated Review of Ciguatera Fish Poisoning: Clinical, Epidemiological, Environmental, and Public Health Management

Melissa A. Friedman [1,*], Mercedes Fernandez [2], Lorraine C. Backer [3], Robert W. Dickey [4], Jeffrey Bernstein [5,6], Kathleen Schrank [5], Steven Kibler [7], Wendy Stephan [6], Matthew O. Gribble [8], Paul Bienfang [9], Robert E. Bowen [10], Stacey Degrasse [11], Harold A. Flores Quintana [12], Christopher R. Loeffler [12], Richard Weisman [6], Donna Blythe [13], Elisa Berdalet [14], Ram Ayyar [15], Danielle Clarkson-Townsend [8], Karen Swajian [11], Ronald Benner [12], Tom Brewer [16] and Lora E. Fleming [17]

SCIENTIFIC REPORTS

OPEN

Incidence and clinical characteristics of ciguatera fish poisoning in Guadeloupe (French West Indies) between 2013 and 2016: a retrospective cases-series

Received: 17 January 2018
Accepted: 2 February 2018
Published online: 15 February 2018

Denis Boucaud-Maitre[1], Jean-Paul Vernoux[2], Stéphane Pelczar[3], Elise Daudens-Vaysse[4], Lydéric Aubert[4], Sylvie Boa[5], Serge Ferracci[6] & Robert Garnier[7]

"Canary Islands (NE Atlantic) as a biodiversity 'hotspot' of *Gambierdiscus*: Implications for future trends of ciguatera in the area"

CrossMark

Francisco Rodríguez[a,*], Santiago Fraga[a], Isabel Ramilo[a], Pilar Rial[a], Rosa Isabel Figueroa[a,b], Pilar Riobó[c], Isabel Bravo[a]

[a] Centro Oceanográfico de Vigo, Instituto Español de Oceanografía (IEO), Subida a Radio Faro 50, 36390 Vigo, Spain
[b] Aquatic Ecology, Biology Building, Lund University, 22362 Lund, Sweden
[c] Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello, 6, 36208 Vigo, Spain

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

ELSEVIER

Research Paper

Human neuronal cell based assay: A new *in vitro* model for toxicity evaluation of ciguatoxin

CrossMark

Teresa Coccini[a,*], Francesca Caloni[b], Uliana De Simone[a]

[a] Laboratory of Clinical and Experimental Toxicology, Poison Control Centre, Toxicology Unit, Maugeri Clinical Scientific Institutes S.p.A.-BS, IRCCS Pavia, Pavia Italy
[b] Department of Veterinary Medicine (DIMEVET), Università degli Studi di Milano, Milano, Italy

Contents lists available at ScienceDirect

Toxicon

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

ELSEVIER

Review

Differential toxin profiles of ciguatoxins in marine organisms: Chemistry, fate and global distribution

Lucía Soliño[a,b,*], Pedro Reis Costa[a,b]

[a] IPMA - Instituto Português do Mar e da Atmosfera, Rua Alfredo Magalhães Ramalho, 6, 1495-006, Lisbon, Portugal
[b] CCMAR – Centre of Marine Sciences, University of Algarve, Campus of Gambelas, 8005-139, Faro, Portugal

Ciguatera fish poisoning outbreaks from 2012 to 2017 in Germany caused by snappers from India, Indonesia, and Vietnam

Miriam Friedemann[1]

Received: 9 January 2018 / Revised: 25 September 2018 / Accepted: 16 October 2018

SCIENTIFIC REPORTS

OPEN

Identification of ciguatoxins in a shark involved in a fatal food poisoning in the Indian Ocean

Received: 10 March 2017
Accepted: 20 July 2017
Published online: 15 August 2017

Jorge Diogène[1], Laia Reverté[1], Maria Rambla-Alegre[1], Vanessa del Río[1], Pablo de la Iglesia[1], Mònica Campàs[1], Oscar Palacios[2], Cintia Flores[2], Josep Caixach[2], Christian Ralijaona[3], Iony Razanajatovo[4], Agathe Pirog[5], Hélène Magalon[5], Nathalie Arnich[6] & Jean Turquet[7]

GOBIERNO DE ESPAÑA | MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL | aecosan agencia española de consumo.

GOBIERNO DE ESPAÑA | MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL | aecosan agencia española de consumo, seguridad alimentaria y nutrición

RISK CHARACTERIZATION OF CIGUATERA FOOD POISONING IN EUROPE

GP/EFSA/ASCO/2015/03

EuroCigua

HOME CIGUATERA FOOD POISONING THE PROJECT NEWS AND EVENTS CONTACT US

CIGUATERA FOOD POISONING

The leading non-bacterial food-borne disease associated with consumption of seafood worldwide.

THE PROJECT

This project focuses on the risk characterization of ciguatera food poisoning in Europe.

THE TEAM

The partners belong to fourteen organizations from six Member States.

LATEST NEWS

THIRD SCIENTIFIC CONFERENCE

Third Scientific Conference "Science, Food, Society"

CALL FOR DATA

CONTACT: ciguatoxinas@mscbs.es

GOBIERNO DE ESPAÑA

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

aecosan
agencia española de consumo, seguridad alimentaria y nutrición

Bienvenidos Benvinguts Ongi etorri Benvidos Benvinguts **Welcome** Bienvenue

GOBIERNO DE ESPAÑA

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

aecosan
agencia española de consumo, seguridad alimentaria y nutrición

Buscar

Contact Links Site map FAQ's

HOME AGENCY CONSUMER AFFAIRS FOOD SAFETY NUTRITION LABORATORIES NEWS AND UPDATES CONSUMER

Spanish Agency for Consumer Affairs, Food Safety and Nutrition

We promote your rights, protect your health and we ensure your safety as a consumer and user.

Presentation of the Agency
Duratin 6 minutes

Quick access

ACTUALIZACIÓN INFORMACIÓN IDental

Virtual office

Pago de Tasas No On Line

Magazine AECOSANews

Galería de medios