Marine and Freshwater Toxins

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Learning Outcomes

- **Develop an ability to define and classify biotoxins**
- **Understand the current food safety hazards arising from biotoxins and their implications to human health**
- **Identify emerging issues in food security from biotoxins**
- **Knowledge of control measures that are implemented to prevent biotoxins entering into the food chain**
- **Recognise bioanalytical approaches used to monitor the presence of biotoxins in order to protect human health.**

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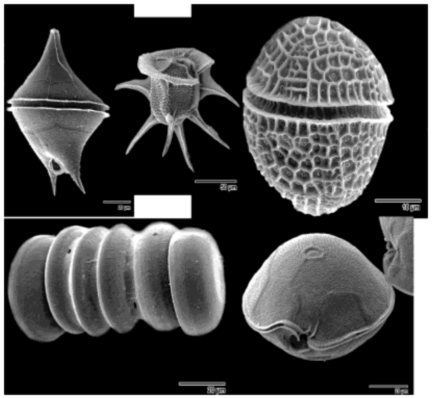
Common Feature?





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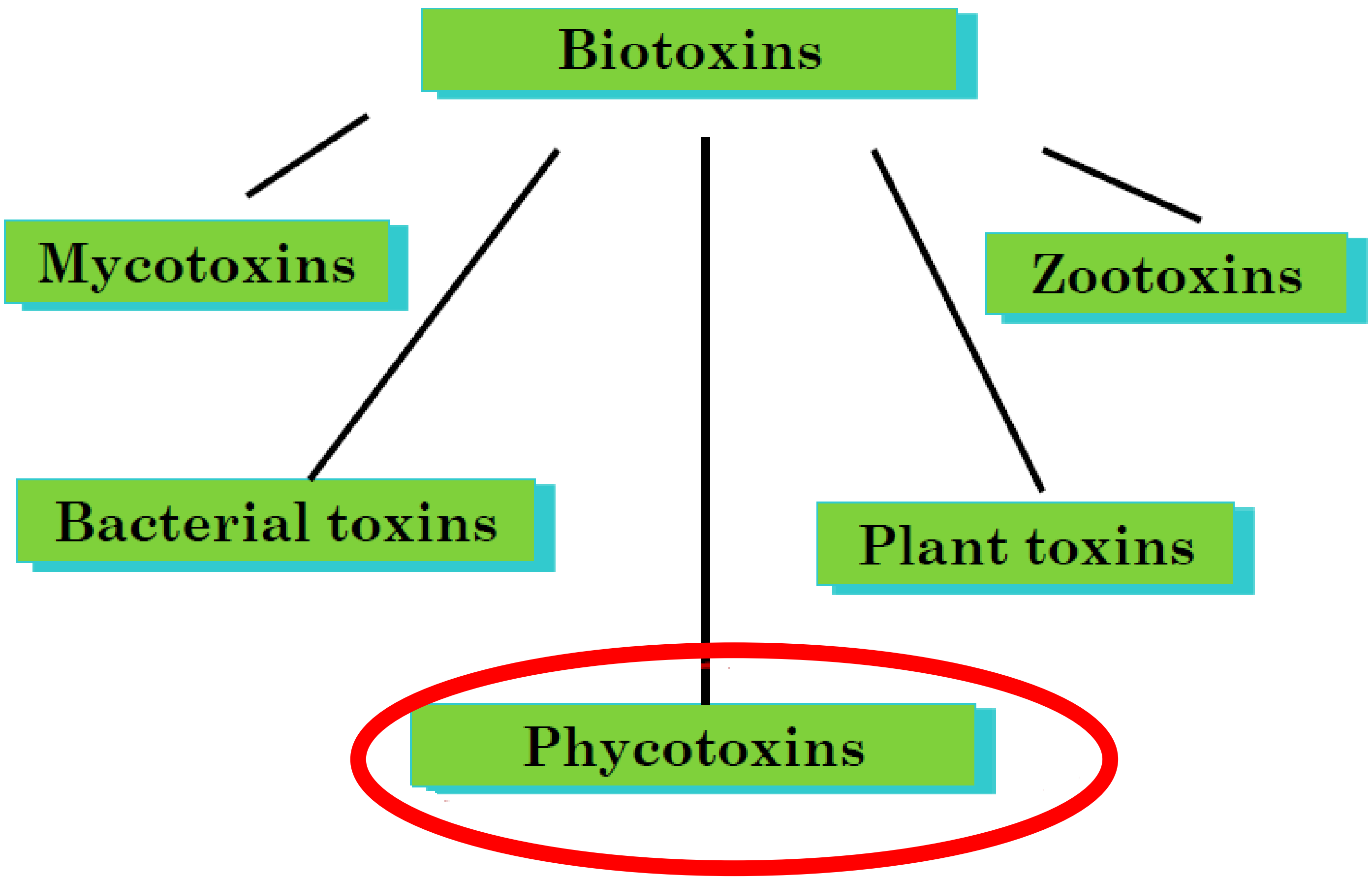
Classification of Biotoxins

Biotoxins/natural toxins can be divided into 5 main categories from their origin



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Definition

- **A biotoxin is a poisonous substance that is a specific product of the metabolic activities of a living organism**
- **The living organism can be**
  - **algae,**
  - **bacteria (cyanobacteria)**
  - **fungus**
  - **plant,**
  - **animal.**
- **Biotoxins do not replicate themselves and are more analogous to chemical toxins.**
- **Biotoxins are naturally occurring in our environment and can enter the food chain through direct contamination in animal feed and food**
- **Natural potent toxins with severe adverse effects on human and animal health**

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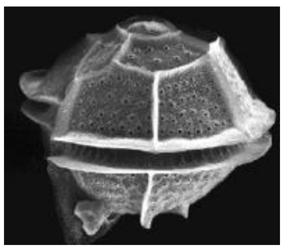
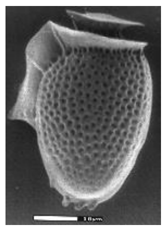
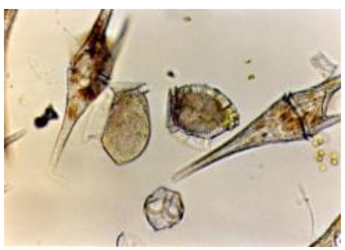
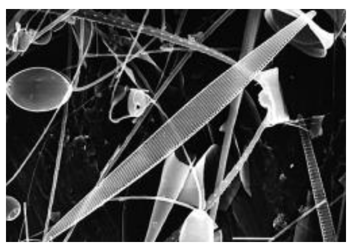
Phycotoxins

- ***Phycotoxins: toxins produced by algae (eg dinoflagellates, diatoms)***

- Phycotoxins (phyco = seaweed) are a diverse group of poisonous substances produced by various aquatic species in marine and fresh waters throughout the world.

Clovelly Beach, Australia, 2012


Floirda, 2016

- Phycotoxins produced by harmful algae and when in high density are known as harmful algal blooms

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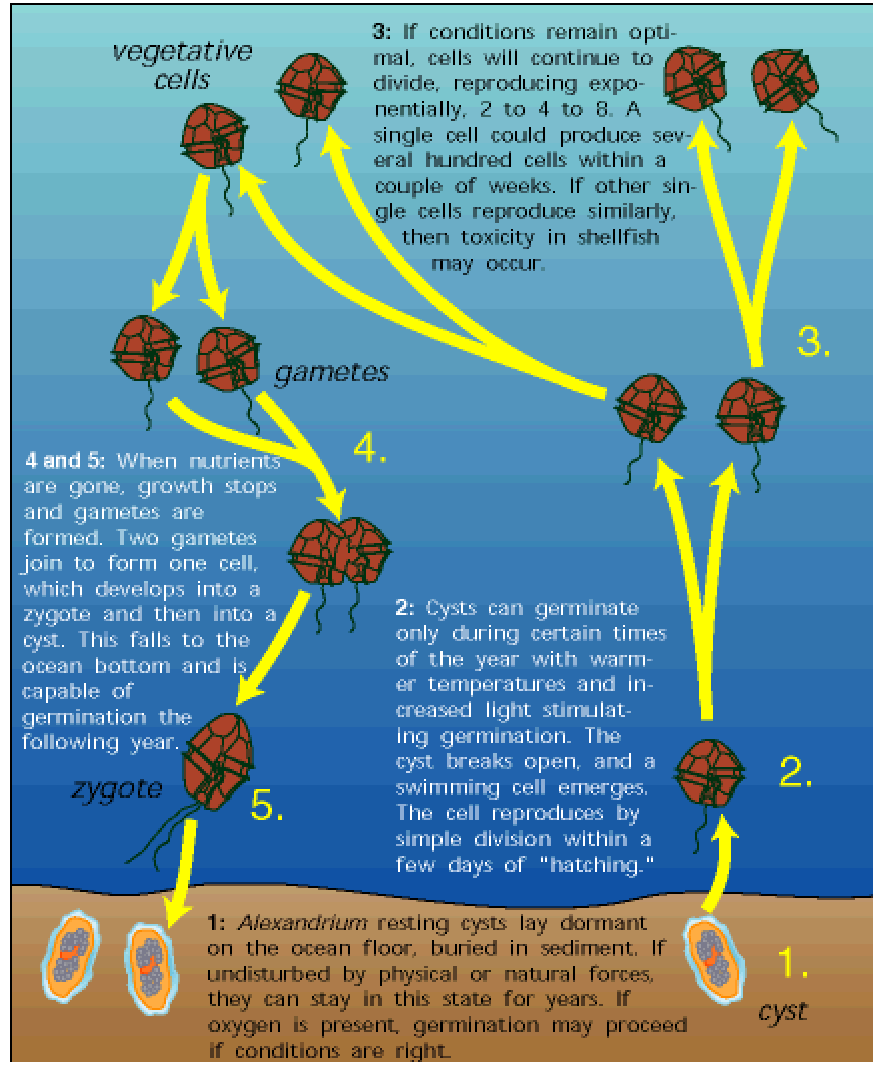
Algal Bloom

- Algae –usually autotrophic organisms, unicellular or multi-cellular
  - Largest –seaweed
  - Only organism to readily make PUFAs
- Plankton –mass of micro-organisms , drift or float
- Phytoplankton –diatoms, dinoflagellates
- How a toxic algal bloom occurs



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Algal Bloom

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- Algae populations can increase rapidly to form dense concentrations of cells that may be visible.
  - "Red Tides"
  - Blooms are not always visible
- Discolouration varies with species of phytoplankton, size and concentration, and depth
- Phytoplankton generally proliferate during the summer months when the water is calm and warmer
- Conditions favouring this growth include water temperature, sunlight, competing micro-organisms, nutrients (phosphorus, nitrogen, and microelements), wind and the directions of currents.
- Algal blooms can be non-toxic providing energy to fuel food webs.

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Harmful Algal Bloom

- HAB also include blooms of non-toxic species
  - Masses of algae die and decompose
  - Depletion of oxygen content in water
  - Oxygen levels become so low, animals die or leave

- Small percentage produce powerful toxins
- Toxin release mechanism is poorly understood but may be a defence mechanism against other organisms or harmful environmental conditions

- Lead to
  - Fish kills
  - Death of mammals and birds
  - Illness in humans

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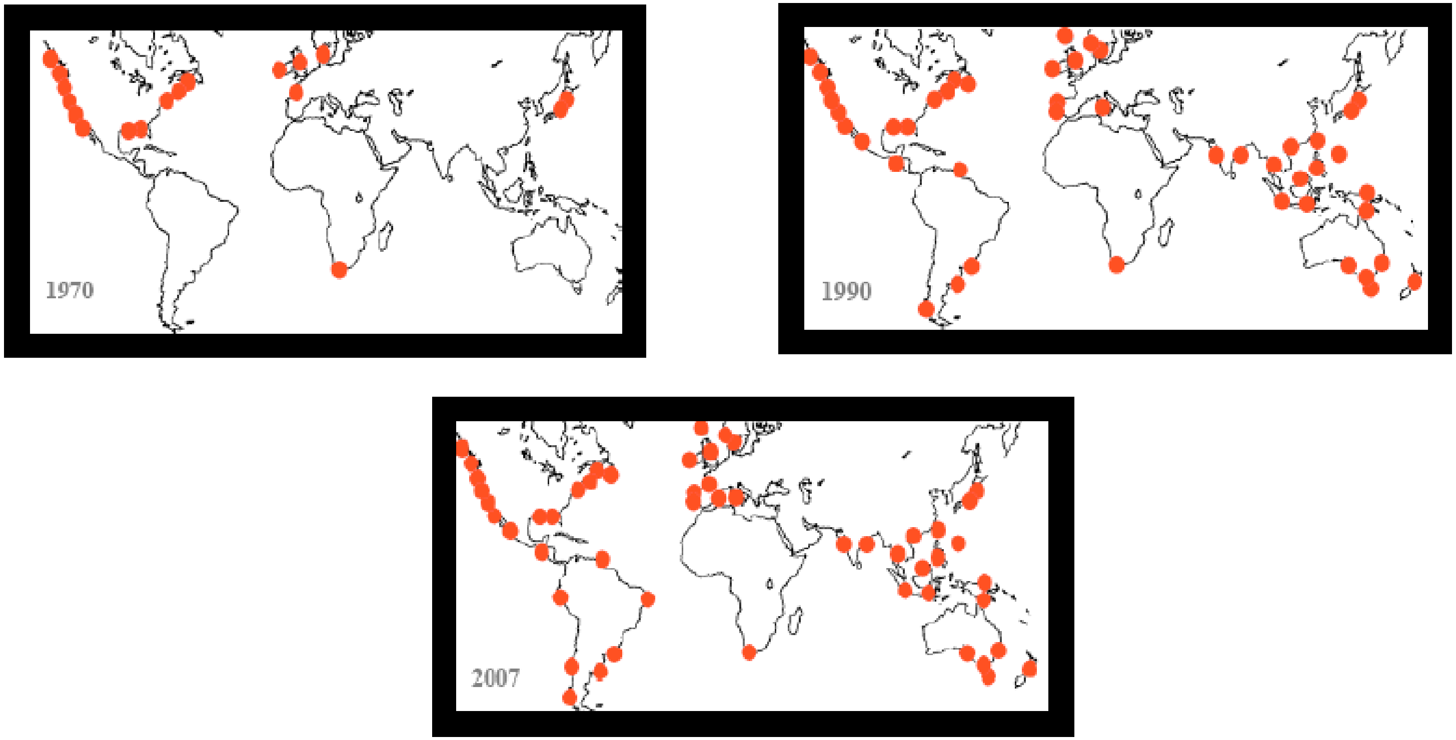
Increased Incidence of blooms



Adapted from Hallegraeph, 1995 IOC HAB Manual

TASK 1: On examining these three diagrams in which regions have the incidences increased over the time period and why might this be?

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Increase of HABs

- Factors affecting HAB Growth are not well understood
- Explanations for possible increase
  - Increased scientific awareness
  - Increased utilisation of coastal waters for aquaculture
  - Eutrophication
  - Climate change
  - Increased global marine traffic

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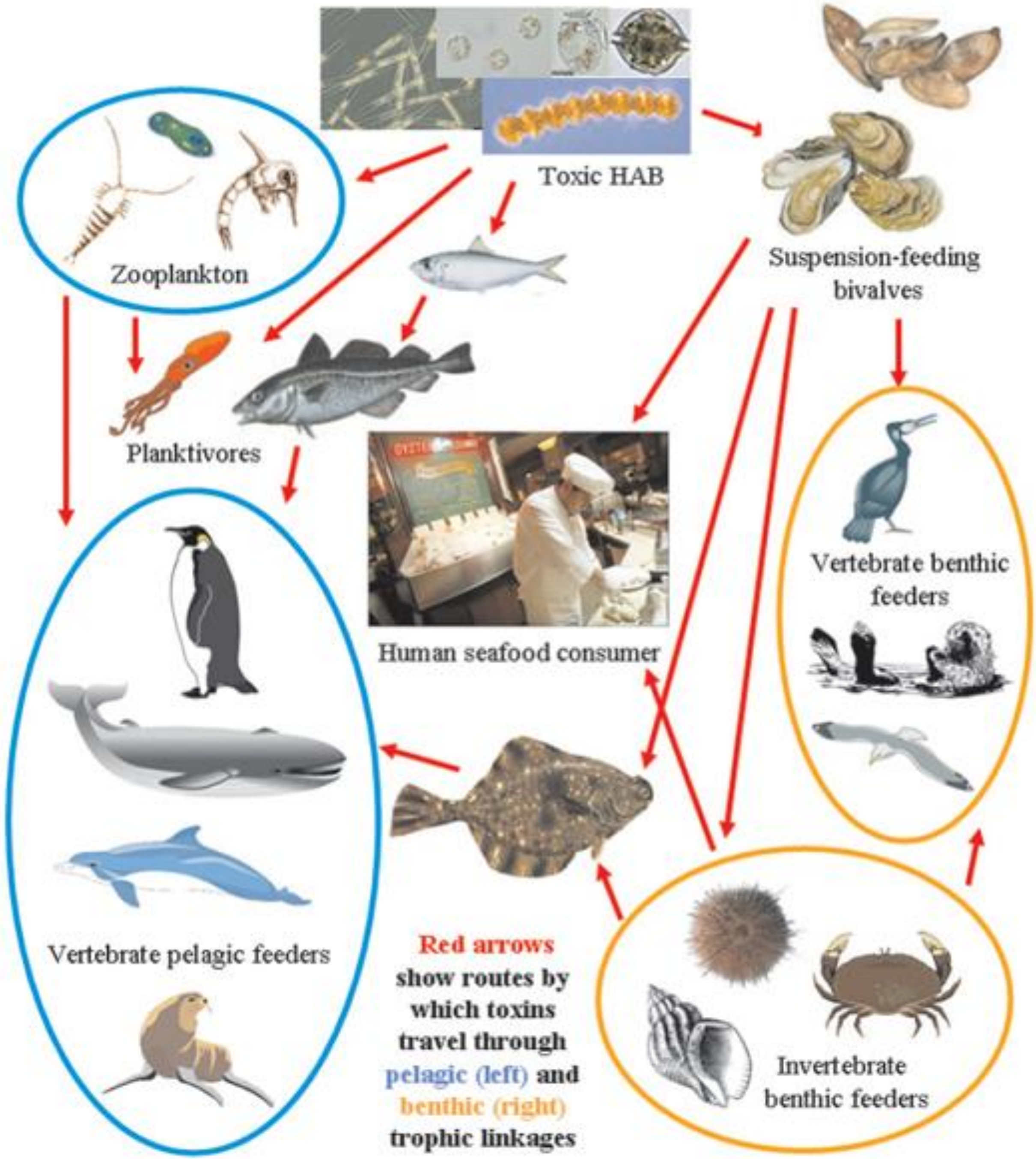
Complex Marine Biotoxin Food Web

Bioaccumulation through the chain

Bivalve molluscs (mussels, clams, scallops, oysters and cockles) common vector for human poisoning

Shellfish filter large volumes of water containing Phytoplankton

Phytoplankton that accumulates in the digestive gland may be toxic or non-toxic



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Marine Toxin Syndromes

- Diarrhetic Shellfish Poisoning (DSP)
  - Lipophilic Shellfish Poisoning
  - Azaspiracid Shellfish Poisoning (AZP)
- Amnesic Shellfish Poisoning (ASP)
- Paralytic Shellfish Poisoning (PSP)
- Neurotoxic Shellfish Poisoning (NSP)
- Ciguatera Fish Poisoning Syndrome (CFP)

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Marine Toxin Syndromes

- Toxic episodes in shellfish can last for months or years producing toxins at lethal levels
- Economic damage due to shutting down of shellfish harvesting and marketing
- Not in Europe, but every year dozens of people die
- At the peak of a toxic PSP bloom, 1 mussel can have up to 1.5 mg STX (LD 0.3 - 1 mg)
- At the peak of a toxic bloom, this is a Family Lethal dose

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NEWS LIVE BBC NEWS CHANNEL

Page last updated at 15:19 GMT, Saturday, 22 August 2009 16:19 UK

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Algae 'causes lower crab catches'

An algae which has killed thousands of fish off the coast of Cornwall has now begun to affect crab catches in Looe, according to the town's harbour master.

The Red Tide algal bloom produces toxins lethal to fish and shellfish and also depletes oxygen levels which makes it difficult for marine life to breath.

It was blamed for the death of fish in St Austell Bay and temporarily led to four shellfishing sites being shut.

Harbour master Geoff Penhaligon said the crabs vanished when the algae came.

Catches reduced

"When it came up the river it was black - we had two days when the river was totally black," he said.

"It's killed a lot of lug worm up the river, a lot of cockles and winkles have died off and the crabs have disappeared.

"For two days nobody caught a crab and for the last two days I've looked in the buckets here and there's only been the odd one or two - not the great numbers we usually have."

According to marine experts, the toxin from the algae can become concentrated in the flesh of shellfish grown in contaminated waters.

However, the Shellfish Association of Great Britain said last week that it did not believe the local industry would be too badly affected

NEWS NE SCOTLAND, ORKNEY & SHETLAND

25 July 2013 Last updated at 19:02

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Shetland shellfish sites close after high toxin levels found

All shellfish harvesting sites in Shetland have closed and mussels from the islands withdrawn from sale after unusually high levels of toxins were detected.

A total of 11 shellfish harvesting areas in waters to the north and west of Shetland have been shut

The Food Standards Agency said the naturally-occurring toxins can cause acute food poisoning.

The move came after about 70 people in south-east England reported symptoms of diarrhetic shellfish poisoning.

The cases have been linked to the eating of mussels from Shetland.

It was after the harvest of these mussels that the FSA recorded the high levels of toxins during its weekly monitoring programme.

According to the FSA, the toxins are produced by marine phytoplankton and levels are typically higher in summer.

A total of 11 harvesting areas in waters to the north and west of the islands have been shut by Shetland Islands Council and businesses operating in

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Dorset shellfish 'have dangerous toxins'

16 August 2014 Last updated at 00:25 BST

There are concerns that certain types of shellfish being caught off the Dorset coast may contain toxin levels above those safe for human consumption.

People who gather clams, oysters, mussels and scallops are being warned of the dangers.

Amnesic shellfish poisoning (ASP) can cause neurological symptoms such as dizziness and confusion, and in very rare cases, death.

NEWS IN BRIEF

Marks & Spencer's recall mussels linked to illnesses

By Joe Whitworth+

04-Dec-2013

Mussels have been recorded by Marks and Spencer

Related tags: Investigation illness, Marks & Spencer, Mussels, FSA, Recall, Withdrawal

Related topics: Food Outbreaks, Meat, fish and poultry, Data management

Marks & Spencer has recalled three branded mussel-based products as part of investigations of illnesses in the UK.

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Friday, July 10, 1998 Published at 01:36 GMT 02:36 UK

Health

Rare food poisoning strikes London's mussel fans

Molly Malone: mussel purveyor extraordinaire

Scientists fear diners who fell ill after eating mussels suffered a type of food poisoning not seen in Britain for 30 years.

A total of 49 people who ate at two London restaurants suffered acute nausea, vomiting, diarrhoea, abdominal pain, and feverishness lasting eight hours.

All had been served dishes of mussels originating from the UK, including one who was made ill by mussel soup.

Food safety inspectors shut down Netherlands mussel trader

1

Posted on February 8, 2014 by Doug Powell

Food safety inspectors have stopped a shellfish trader in Zeeland from exporting 47 tonnes of mussels after it failed to take action over two food poisoning incidents in England and Switzerland.

A small quantity of the mussels, which were exported to Ireland, are also being recalled, the food safety body NVWA says.

In November, the same company was at the centre of another recall after several people became ill in England after eating mussels containing biotoxins, a poison common in shellfish. Those mussels came from Ireland but had been sold by the Dutch company.

The company was aware of the problem but did not register it with the authorities in time, food safety inspectors say. In December there was a second incident involving mussels in Switzerland.
Those mussels, said at the time to be of Danish origin, turned out to be from the same Irish consignment as in the English food poisoning case.

Food inspectors have now effectively closed down the trader pending a full investigation and possible criminal charges.

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Volume 19, Number 8—August 2013

Dispatch

Diarrhetic Shellfish Poisoning, Washington, USA, 2011

Jennifer K. Lloyd , Jeffrey S. Duchin, Jerry Borchert, Harold Flores Quintana, and Alison Robertson
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Suggested citation for this article

Abstract

Diarrhetic shellfish poisoning is a gastrointestinal illness caused by consumption of bivalves contaminated with dinophysistoxins. We report an illness cluster in the United States in which toxins were confirmed in shellfish from a commercial harvest area, leading to product recall. Ongoing surveillance is needed to prevent similar illness outbreaks.

Diarrhetic shellfish poisoning (DSP) is an acute gastrointestinal illness caused by consumption of bivalve mollusks that have accumulated okadaic acid (OA) or related dinophysistoxins through filter feeding. DSP toxins

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RESEARCH ARTICLE

Food-Borne Disease Outbreak of Diarrhetic Shellfish Poisoning Due to Toxic Mussel Consumption: The First Recorded Outbreak in China

Tingrui Chen, Xuqing Xu, Jinjiao Wei, Jiang Chen, Renchao Miu, Liming Huang, Xiaoxiao Zhou, Yun Fu, Rui Yan, Zhen Wang, Biyao Liu, Fan He

Published: May 21, 2013 • DOI: 10.1371/journal.pone.0065049

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Abstract

Objectives

This investigation was undertaken in response to an outbreak of suspected shellfish poisoning in Zhejiang Province, China. The objectives of this project were to confirm the outbreak and to identify the aetiology, source and mode of transmission.

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Microreview

Marine Toxins in Italy: The More You Look, the More You Find†

Patrizia Ciminiello, Carmela Dell'Aversano, Martino Forino, Luciana Tartaglione

First published: 05 December 2013
https://doi.org/10.1002/ejoc.201300991

† Dedicated to the memory of Professor Ernesto Fattorusso

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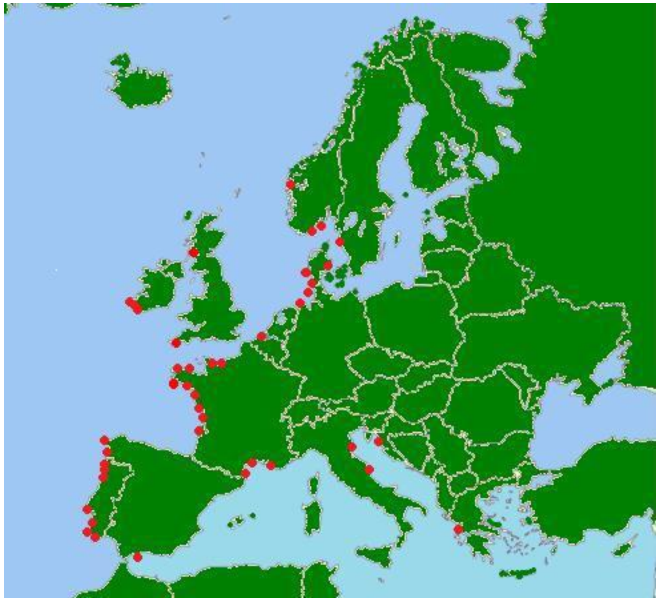
Diarrhetic Shellfish Poisoning

- History:
- First documented incidence of Diarrhetic Shellfish poisoning, occurred in 1976 in North-eastern Japan.
- 164 people affected
- Diarrhoea, nausea, vomiting and abdominal pain
- Caused by ingestion of mussels and scallops
- The dinoflagellate, *Dinophysis fortii was identified as the source of the toxin*
- Toxin responsible was named Dinophysistoxin (DTX)
- Legend in the region warned that mussels become toxic during the month of the paulownia flowers (June)



http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/589.pdf

Diarrhetic Shellfish Poisoning

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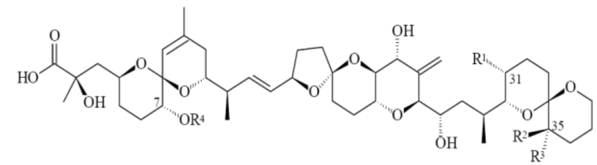
- Categorised into three structural groups:
  - Okadaic acid (OA) and dinophysistoxins (DTX)
  - DTX 2 –an isomer of okadaic acid, isolated from Irish mussels
  - DTX 3 –isolated from the digestive gland of the scallop *Patinopecten yessoensis*
  - Pectenotoxin (PTX)
  - Yessotoxin (YTX)

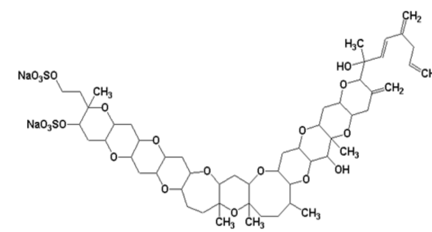
Toxins removed from DSP classification:

- Yessotoxins – sulphated polyether compounds, were isolated from scallops along with DTX1 and DTX3
- Pectenotoxins – polyether macrolides, isolated from toxic scallops
- Do not cause diarrhoea
- Produce a toxic response when injected i.p into mice
- No case of human poisoning has been reported

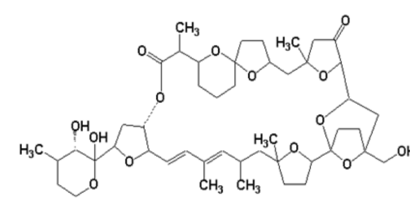


	R^1	R^2	R^3	R^4
OA	CH_3	H	H	H
DTX1	CH_3	CH_3	H	H
DTX2	H	H	CH_3	H
DTX3 (acylated forms of OA, DTX1 and DTX2)	H/CH_3	H/CH_3	H/CH_3	fatty acid

Yessotoxin



Pectenotoxin



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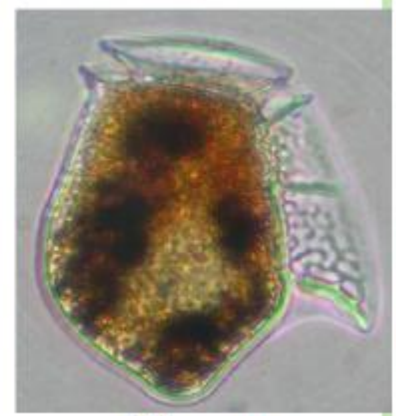
Diarrhetic Shellfish Poisoning

- Produced by the dinoflagellates Dinophysis spp. and Prorocentrum spp.
- Toxin profile varies within a single species
- Europe –OA and DTX2
- Japan –OA and DTX1
- Regulatory limit for these toxins worldwide is 160 µg/kg shellfish meat


Prorocentrum Lima

D. fortii


D. acuminata


D. acuta

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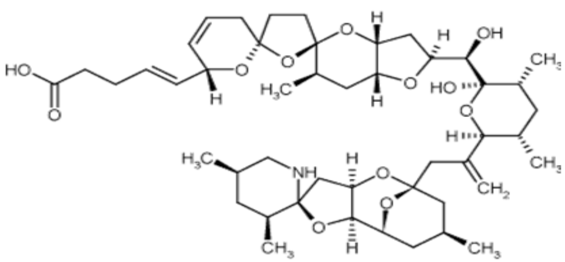
Azaspiracid Shellfish Poisoning

- Most recently discovered marine toxin
- Several analogues have been identified
- First confirmed incidence of Azaspiracid Shellfish poisoning occurred in 1995 in Netherlands.
- Caused by consumption of mussels (*M. edulis)*
- Cultivated in Killary Harbour, in the west of Ireland
- At least eight individuals were affected
- Abdominal pain, nausea, vomiting, diarrhoea –similar to DSP
- Azaspiracid 1 (AZA1) isolated from these mussels –its structure identified

Azaspiracid



http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/723.pdf

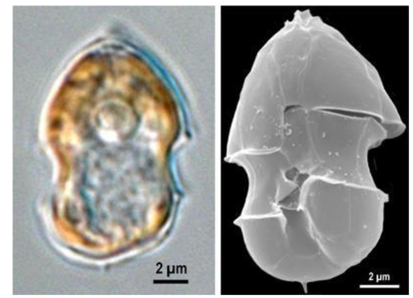
Azaspiracid Shellfish Poisoning

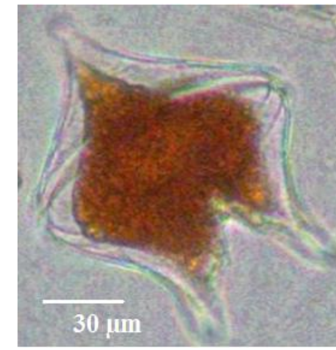
There are greater than 20 known analogues of azaspiracids

- Symptoms:
  - Diarrhoea, nausea, vomiting and abdominal cramps
  - Recovery within 3 days
- Toxicological studies
  - Induce widespread organ damage in mice
  - More dangerous than other classes of shellfish toxins
  - Target organs –liver, spleen, the small intestine
  - Carcinogenic
  - Chronic exposure –tumour formation in the lungs and malignant lymphomas
  - Interstitial pneumonia
  - Shortened small intestinal villi

Azaspiracid Shellfish Poisoning

- *Protoperidinium crassipes was initially thought to be the causative agent*
  - Cannot produce their own food by photosynthesis
  - Predators of dinoflagellates
  - Do not proliferate into large blooms
  - Vector of AZA toxins



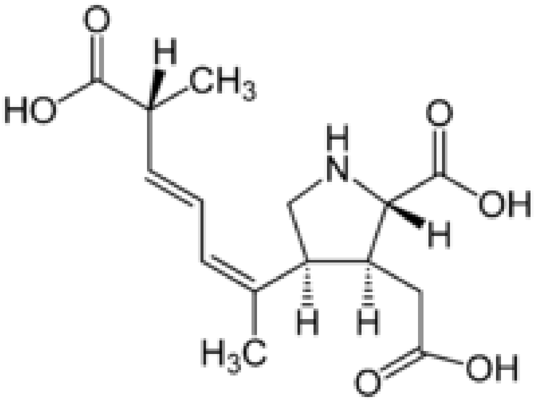


- *Azadium spinosum*
  - Dinoflagellate
  - Identified as the producer of AZA toxins

- AZA 2 recently found in a sponge (*Echinoclathria sp.) –Japan*
- First report of AZA in Asia
- Global outbreaks: UK, Norway, France, Italy, Spain, Denmark, N. Africa
- Human intoxications due to AZP
- Shellfish passed as safe for human consumption using DSP mouse bioassays.
- DSP mouse bioassay protocols result in the extraction of only 5-40% of total azaspiracids present in mussels.

Amnesic Shellfish Poisoning

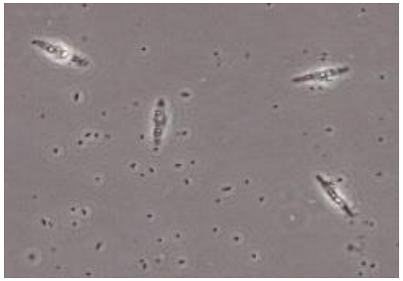
History:



- 1987 in Canada
- 107 cases of shellfish poisoning
- Vector responsible were mussels (*M. edulis)*
- Cultivated on Prince Edward Island
- Victims suffered gastrointestinal disturbances as well as unusual neurological symptoms –memory impairment
- 3 people died within 18 days of admission
- Neurological symptoms –headaches, confusion, disorientation, seizures and coma
- Permanent short term memory loss –Amnesic shellfish poisoning
- Domoic acid was identified as the toxin responsible
- Regulatory limit for these toxins in Europe is 20mg/kg shellfish meat

https://www.afbini.gov.uk/publications/efsa-domoic-acid

Amnesic Shellfish Poisoning



Pseudo-nitzschia pseudodelicatissima

- Epidemiological studies
  - Age-dependant response
  - Less than 40 years old –gastrointestinal problems
  - Greater than 50 years old –suffer from memory loss
- Onset of symptoms:
  - Gastrointestinal –within 24hrs
  - Neurological -within 48hrs
  - Symptoms can last for a few days -possibility of permanent memory loss
- Diatom of the *Pseudonitzschia spp.*
- In Japan, domoic acid previously known as a natural product
  - Anthelminthic and insecticidal properties
  - Originally discovered in a seaweed

Amnesic Shellfish Poisoning

- Marine animals
  - 1998 -70 sea lions washed up onto beaches in California
  - Suffering from neurological problems
  - 47 animals died
  - DA identified in faecal samples and in nearby anchovies
- Birds
  - 1991 –DA poisoning report in Monterey Bay, California
  - Pelicans and cormorants behaving strangely
  - Vomiting, unusual head movements, scratching , many deaths
  - Vector –northern anchovy
  - Similar event in 1961 in Santa Cruz, prompted production “The Birds”

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https://youtu.be/0fJh2gIBOto

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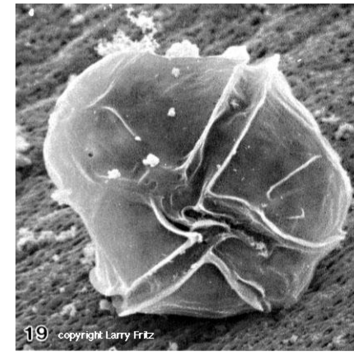
Paralytic Shellfish Poisoning

PSP toxins are collectively called saxitoxins (STXs)

- Reported >57 analogues of these cyclic guanidines
- Saxitoxin the most common
- Dinoflagellates responsible:
- *Alexandrium, Gymnodinium, Pyrodinium species*



- Symptoms:
  - Mild –tingling sensation or numbness of the lips, face and neck. Prickly sensation in fingertips and toes
  - Severe –headache, nausea, vomiting, diarrhoea, muscular paralysis and respiratory difficulty
  - High risk of **death in the absence of artificial respiration**
  - Onset of symptoms occurs rapidly
  - Lethal dose is 1-4 mg STX or equivalents
  - Saxitoxins clears from the blood within 24hrs
  - No organ damage or long term effects
- **Schedule 1 list of the Chemical Weapons Convention**
- Regulatory limit for these toxins in Europe is 800 µg/kg shellfish meat

Paralysis

https://www.afbini.gov.uk/publications/efsa-saxitoxin

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Paralytic Shellfish Poisoning

- History:
  - 1927 –Northern California
  - 102 people poisoned from eating mussels
  - 6 deaths
- PSP outbreaks have occurred on both the eastern and western coastlines of N. America
  - Alaska –badly affected
  - PSP events for almost 100 years
- 1987 –Cape Cod Bay
  - 14 humpback whales
  - Mackerel
- Have been detected in European waters, human intoxications are rare
  - 1970‟s –PSP intoxications from mussels cultivated in Spain, Portugal and U.K
  - 80-120 individuals
- Repeated outbreaks in Chile and Argentina
  - 21 PSP deaths reported in Chile since 1991
  - Rare identification of toxins in body fluids of victims
- Philippines
  - 2000 cases of PSP 1983-1998 and 115 deaths

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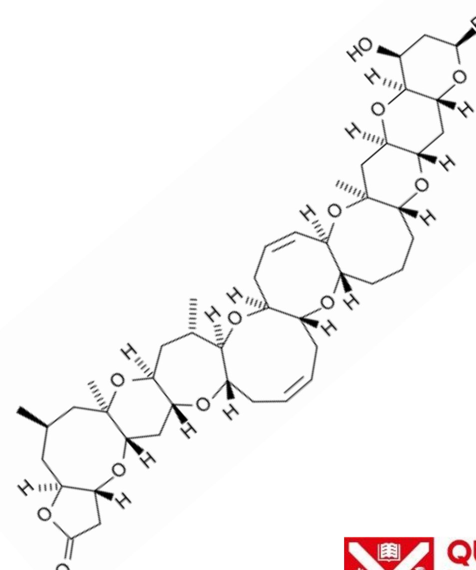
Neurotoxic Shellfish Poisoning

- Marine dinoflagellate – Karenia Brevis
- Produce neurotoxins called Brevetoxins
- Naked dinoflagellate as no protective layer
- Affects finfish, aquatic mammals and birds
- Death of large manatees and bottlenose dolphins
- Brevetoxin producing HABS have cause problems in the Gulf of Mexico for decades since records began in 1947
- Though npt geographically widespread with some reports in New Zealand
- A regulatory level of 800 µg brevetoxin/kg shellfish flesh

Karenia brevis



https://www.afbini.gov.uk/publications/efsa-brevetoxins

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Neurotoxic Shellfish Poisoning

- **Symptoms:**
  - **Act in three ways depending on route of exposure –onset 30mins to 3hrs**
  - **Symptoms persist for a few days**
- **Oral -gastroenteritis, chills, sweats, reduction in core temperature, hypotension, arrhythmias, numbness, peripheral tingling and in severe cases broncho-constriction, paralysis, seizures and coma.**
- **Inhalation–sea spray contains brevetoxins due to delicate nature of Karenia brevis. Causes irritation to the eyes and nasal passages, respiratory problems**
- **Skin–eye and nasal irritation**

- **1987, the Gulf stream carried a Karenia brevis bloom from Florida to North Carolina**
  - **48 people with neurological symptoms 1 person hospitalised**
  - **Vector –oysters**

- **1993, New Zealand**
  - **186 individuals affected with gastrointestinal symptoms and respiratory problems**
  - **Vectors –green mussels, cockles and oysters**
  - **New brevetoxin analogues were detected**

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Emerging toxins

- Described as emerging toxins as limited regulation governing them
- New toxins in old areas
- New toxins in new areas

- Palytoxin

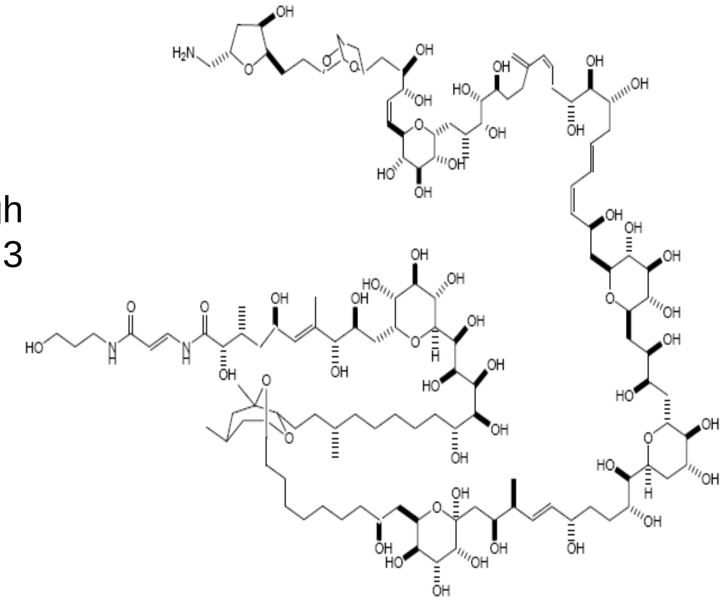
- Palytoxin is from a toxin group mainly detected in marine zoanthids (genus *Palythoa*) and benthic dinoflagellates (genus *Ostreopsis*)

- First reports in Hawaii (1971) and Japan (1981)

- Very potent toxin – Na^+/K^+-ATPase (or sodium pump) high affinity cellular receptor – toxic dose to human between 2.3 and 31.5 µg/kg

- Recent blooms of *Ostreopsis* spp. Reported in Mediterranean sea and 4 EU countries: France, Greece, Italy and Spain with reports of respiratory irritations affecting sunbathers in coastal waters

- Victims (>100) require medical assistance. Events related to toxic aerosols derived from epibenthic microalgae of the genus Karenia and Ostreopsis that live in the wáter column



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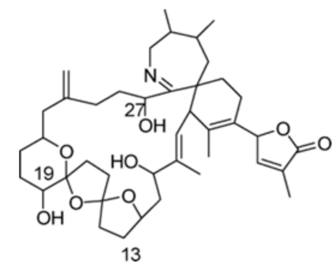
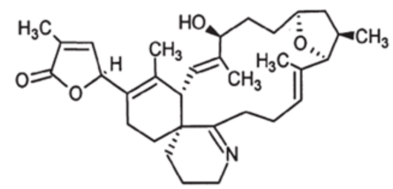
Emerging toxins

- Cyclic imines:
  - Spirolides
  - Gymnodimine
  - Pinnatoxins
  - Pteriatoxins
  - Prorocentrolide
  - Spiroprorocetrimine

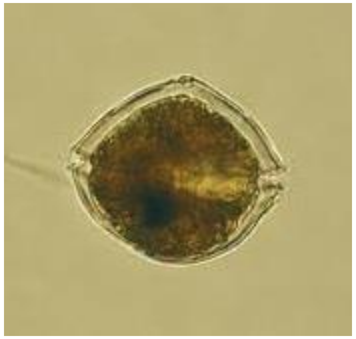
Spirolides



Gymnodimine



Spirolides are neurotoxins that antagonise the nicotinic receptors
First reported in 1990s (Canada)
Unknown toxicity and NOT regulated
Toxic → MBA → False positives



A. ostenfeldii

A. peruvianum

13-Desmethyl Spirolide C

i.p. LD_{50} → 40 µg/kg

Oral LD_{50} → 1 mg/kg

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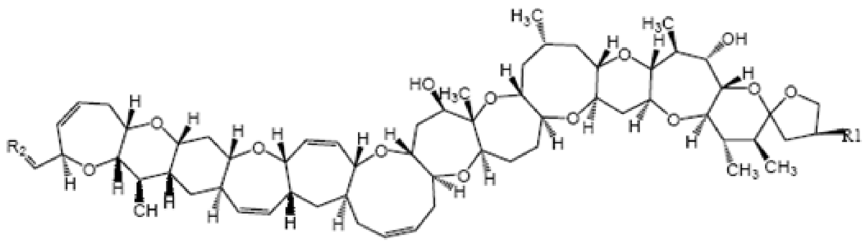
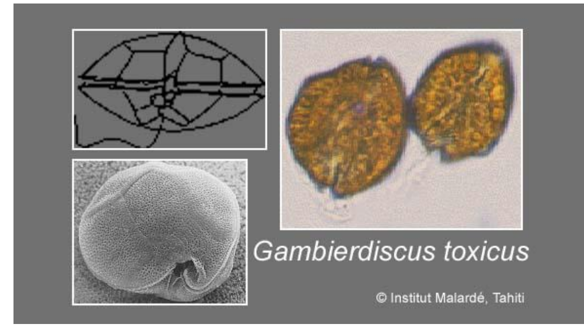
Ciguatera Fish Poisoning

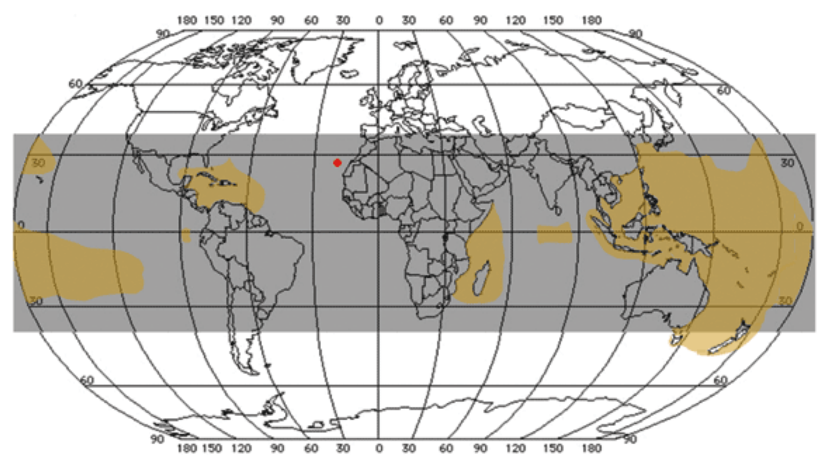
- Two Groups
    - ciguatoxins (CTXs) and gambierol (lipid soluble)
    - maitotoxin (MTX) (water soluble).





- Ciguatoxin is the main agent responsible for ciguatera fish poisoning.
- Ciguatoxin has a higher affinity for site 5 of voltage dependent sodium channels than brevetoxin.

- Worldwide distribution of ciguatera (*Gambierdiscus*).
    - Gray indicates coral reef regions located between 35° north and 35° south latitudes;
    - Brown indicates disease-endemic areas of ciguatera;
    - Red circle indicates Canary Islands (latitude 28°06´ north, longitude 15°24´ west.
    - Ciguatera in Canary Islands
    - 2 outbreaks in 2008 & 2009
    - Location: fish near Canary Islands
    - People affected: 23 people 2008.
    - No available data in 2009



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Ciguatera Fish Poisoning

- Symptoms appear within 30 hours
  - gastrointestinal disorders (vomiting, diarrhoea, abdominal pain),
  - neurological symptoms (headaches and nausea, ataxia, sensibility disorders, muscular and joint pains),
  - itching of the skin, feet and lips,
  - cardiovascular symptoms (arrhythmia, bradycardia or tachycardia, low blood pressure),
  - hallucinations and coma.

- Pacific and Caribbean Toxins differing in structure

- Despite the high toxicity of CFP toxins, neither tolerance limits nor official methods of analysis have been set due to the extremely low detection limits required to ensure consumer safety. Nevertheless, a “safe” level of 0.01µg/kg of fish flesh, a real challenge for the analyst, has been proposed.

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Summary of Toxin Syndromes

Poisoning syndrome	Toxin	Phytoplankton species (Action Limit cells/L)	Vector (EU Action Limit)	Short term Health Consequences	Long term Health consequences
Amnesic Shellfish Poisoning	Domoic acid	Pseudo-nitzscia sp (150,000)	Shellfish (20mg/kg)	Vomiting, diarrhea, liver inflammation, abdominal pain, confusion disorientation, memory loss	Anterograde memory deficit, seizures leading to coma and death
Diarrhetic Shellfish Poisoning	Okadaic acid and dinophysistoxins	Dinophysis sp Prorocenturum lima (100)	Shellfish (160µg/kg)	Nausea, vomiting, diarrhea, abdominal pain accompanied by chills, headache, fever	Gastrointestinal tumour promoter in laboratory animals
	Azaspiracids	Azadinium sp	Shellfish (160µg/kg)	Causes diarrhea and neurotoxic effects	Unknown
	Yessotoxin	Protoceratium reticulatum Lingulodinium polyedrum	Shellfish (1mg/kg)	Unknown	Unknown
	Pectenotoxin	Dinophysis fortii	Shellfish (160µg/kg		
Paralytic shellfish poisoning	Saxitoxins	Alexandrium sp (Present ~20)	Shellfish Crustaceans (800µg/kg)	Tingling, burning, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death	Unknown
	Tetrodotoxin	Shewanella alga sp Pseudomonas sp	Gastropods, fish		
Neurotoxic shellfish poisoning	Brevetoxin	Karenia sp	Shellfish	Numbness of lips, tongue and throat, muscular aches, fever chills, muscle pains, abdominal cramping, nausea, diarrhea, vomiting, reduced heart rate, pupil dilation	Unknown
	Palytoxin ostreocin Maitotoxin	Palythea Ostreopsis sp	Fish Crustacean Shellfish	Causes vomiting, diarrhea, respiratory distress and death	Unknown
	Gymnodimine Spirolides Pinnatoxins	Gymnodinium sp Karenia sp Alexandrium ostenfeldii	Shellfish	Unknown. Causes similar effects in mice to DSP toxins	Unknown
Ciguatera Fish Poisoning	Ciguatoxin	Gambierdiscus sp Amphidinium sp	Reef Fish	Abdominal pain, nausea, vomiting, diarrhea, paresthesias, temperature dysthesia, pain, weakness, bradycardia, hypotension	Recurrent symptoms from months to years of chronic depression

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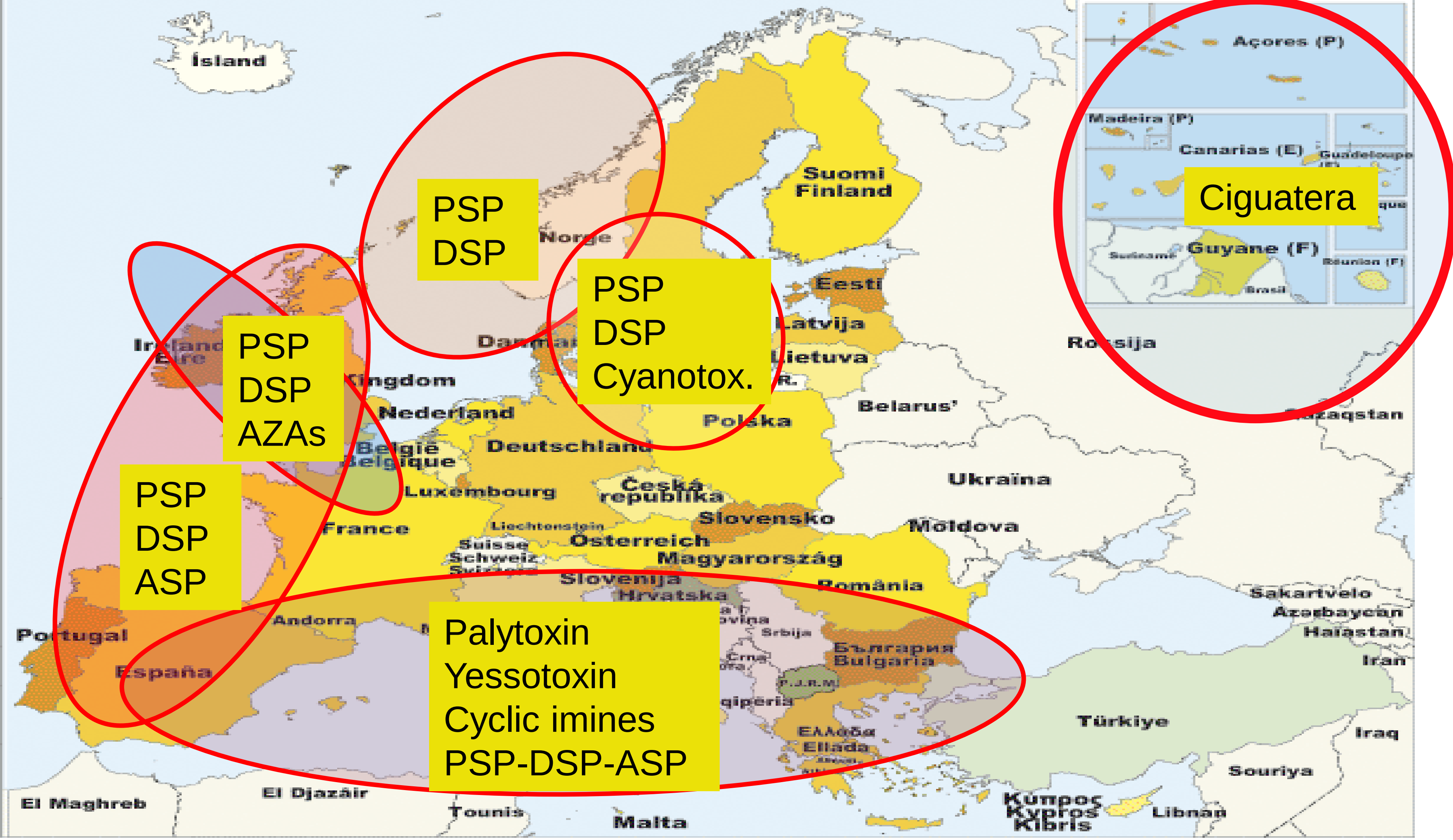

Different ecological and climatic conditions determine the different toxin profiles

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Reasons to expect new toxins

Changes in the ecological conditions of the sea may produce alien species to survive or native species to change metabolic routes:

- Global warming
- Eutrophication of waters
- Enhanced surface stratification
- Alteration of ocean currents
- Intensification /weakening of local nutrient upwelling
- Stimulation of photosynthesis by elevated CO_2
- Reduced calcification through ocean acidification
- Heavy precipitation and storm events causing changes in land runoff and micronutrient availability

References

Diarrheic Shellfish Poisoning
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Azaspiracid Shellfish Poisoning
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/723.pdf

Amnesic Shellfish Poisoning
https://www.afbini.gov.uk/publications/efsa-domoic-acid

Paralytic shellfish Poisoning
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Neurotoxic Shellfish Poisoning
Brevetoxin
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Cyclic Imines
http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1628/epdf

Palytoxin
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Ciguatera fish poisoning
https://www.afbini.gov.uk/sites/afbini.gov.uk/files/publications/EFSA%20ciguatoxin%20group.pdf

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If you were Robinson Crusoe on a desert island and the only source of food you could eat to survive was toxin contaminated shellfish growing suspended on ropes. Which toxin would you hope was present in the shellfish and why?

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STRATEGIES OF MANAGEMENT

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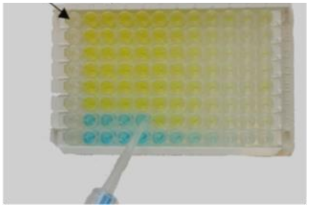
Mitigation

Dealing with an existing bloom and taking the steps to reduce negative impacts.

- The routine monitoring programs
- Detection of toxins in shellfish that leads to harvesting restrictions
- Towing of fish nets away from the sites of HABs



Prevention

Actions taken to keep HABs from happening or impacting a particular resource:

- Control of sewage or waste discharges
- Control of Nitrogen compounds, chemical fertilizers or fossil fuel.

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STRATEGIES OF MANAGEMENT

Control

Actions taken to destroy HABs - to directly intervene in the bloom process:

- Mechanical: removal of HAB cells from the water by dispersing clay over the water surface

- Biological: organisms that could control HABs. Opposition to the concept of releasing one organism to control another.

- Chemical: such as copper sulfate, difficult to find an acceptable chemical that would target a particular HAB species but not cause widespread mortality of other organisms

- Genetic: genetic engineering species that are purposely introduced to alter the reproduction or other processes in the undesirable species.

- Environmental: the manipulation of nutrient levels in coastal waters, dredging or opening of channels, aeration or other methods to disrupt stratification.

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CONTROL: EC REGULATIONS

Regulation (EC) No. 853/2004 (OJ L139, 30.4.2004, page 55) lays down specific hygiene rules for food of animal origin specifying the required health standards for live bivalve molluscs

Annex III. Section VII. Chapter V. Point 2 specifies the limits:

Bivalve molluscs must not contain marine biotoxins in total quantities (measured in the whole body or any part edible separately) that exceed the following limits:

- Paralytic shellfish poison (PSP): 800μg saxitoxin equivalents per Kg
- Amnesic shellfish poison (ASP): 20mg domoic acid per Kg
- Lipophilic toxins
  - Okadaic Acid, dinophysistoxins (DTXs) and pectenotoxins: 160ug of okadaic acid equivalents per Kg
  - Yessotoxin: 1mg of yessotoxin equivalents per Kg
  - Azaspiracids: 160μg of azaspiracid equivalents per Kg

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Methods of Analysis

- Two types of Mouse Bioassay
  - Inject replicate mice with extract of sample
    - Extract for lipophilic first set of mice set
    - Extract of hydrophilic second set of mice
  - Observe symptoms exhibited in mice
  - Measure time it takes for the mice to die – overall toxicity
  - Primitive and non-selective
  - Results dependent on mouse strain, gender, age and weight
  - Results difficult to produce by other labs

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EC REGULATION - No. 2074/2005

Alternative detection methods:

- should not be less effective than the biological methods and that their implementation will provide an equivalent level of public health protection
- If new analogues of public health significance are discovered, they should be included in the analysis.
- Standards must be available before chemical analysis is possible.
- Total toxicity shall be calculated using conversion factors based on the toxicity data available for each toxin.
- The performance characteristics of these methods shall be defined after validation following an internationally agreed protocol.
- Work to replace biological method

If results are challenged, the reference method will be the biological method

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Methods of Analysis

- **Functional assays**
  - Functional assays measure total toxicity – cell based or enzyme based
- **Immunoassays – ELISA, LFD, Biosensors**
  - Immunological methods used as rapid tests for on-site detection
  - High throughput rapid turnaround
- **HPLC with fluorimetric detection**
  - High performance liquid chromatography (HPLC) - reference method for ASP toxins analysis; HPLC considered as official method for PSP toxins.
- **LC-MS / MS**
  - In respect of chemical methods, liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) as it is unequivocal detection. This technique has being implemented for DSP and AZP toxins.

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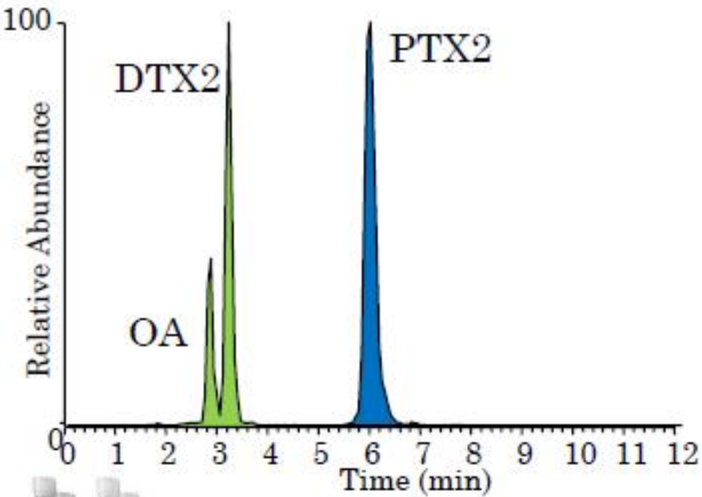
Methods of analysis

Analytical techniques

- ➢ Can be validated
- ➢ Robust
- ➢ Does no harm animals
- ➢ Sensitive and selective
- ➢ Toxin identification

Meet Regulatory Limits
AZP and DSP 160µg/kg
ASP – 20mg/kg
NSP - 800µg/kg
PSP - 800µg/kg



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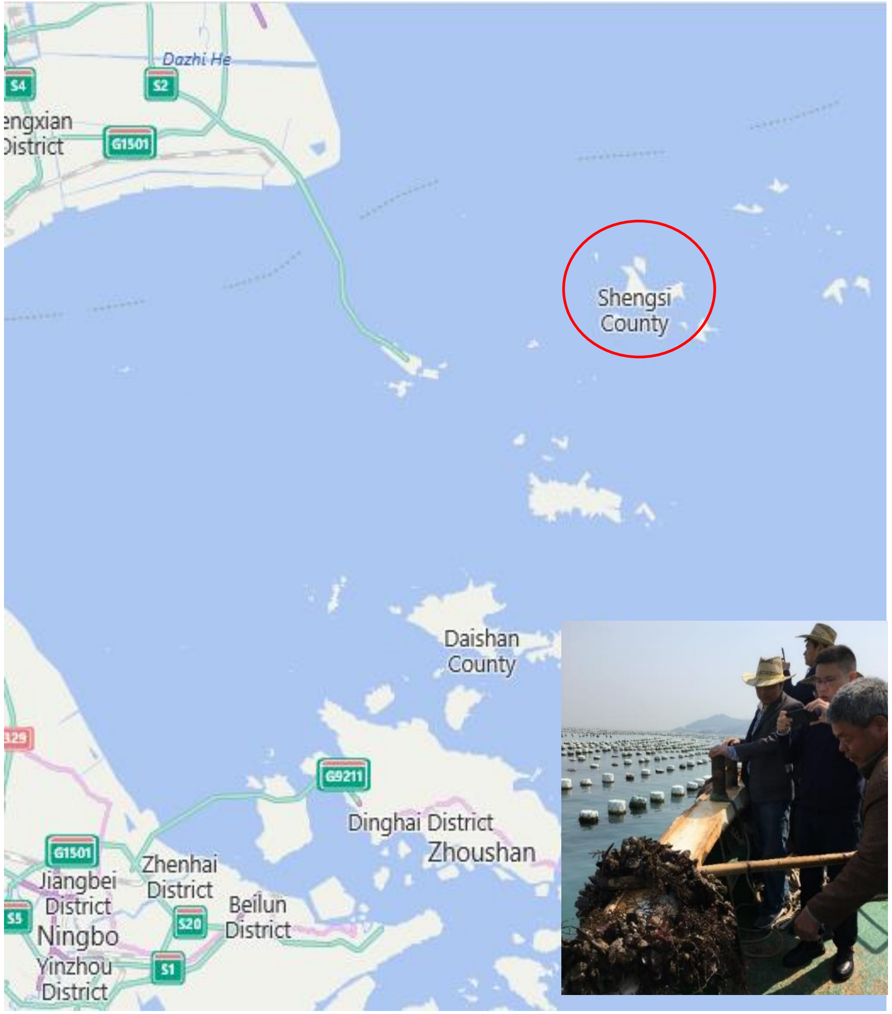
Remote analysis

Australia

China



Methods of Analysis

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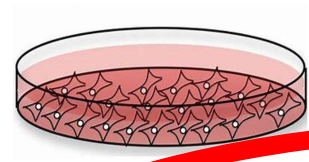
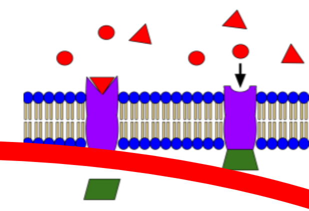
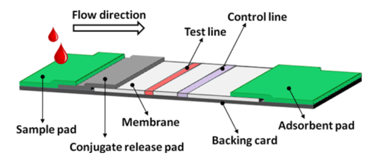
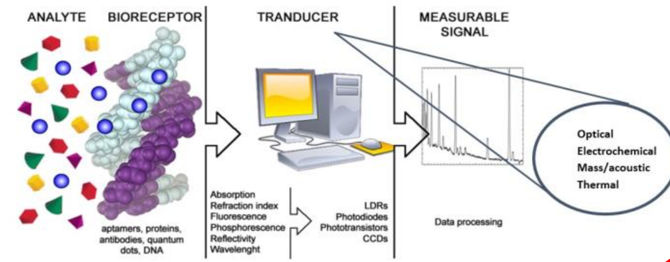
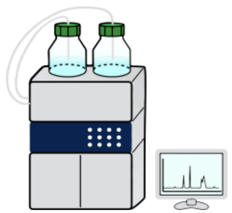
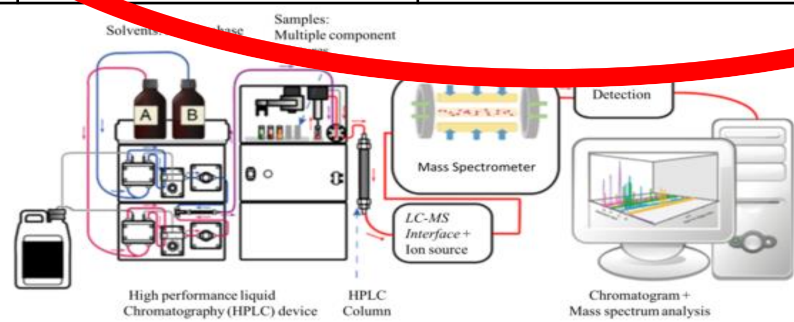
BIOLOGICAL METHODS	 Mouse bioassay	 Cell based Assays	 Receptor based Assays
BIOCHEMICAL METHODS	 ELISA	 Lateral flow devices	 Biosensor Methods
CHEMICAL METHODS	 HPLC-UV HPLC-FLD	 LC-MS/MS	

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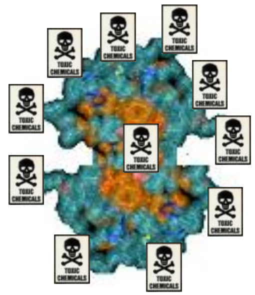
Antibody Production for small molecules

Toxin

Protein Conjugation

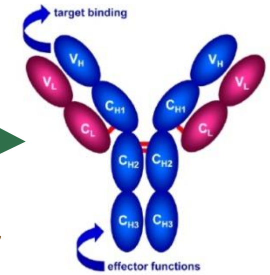


Protein carrier

Immunisation Programme

Polyclonal antibody production

Titre Specificity

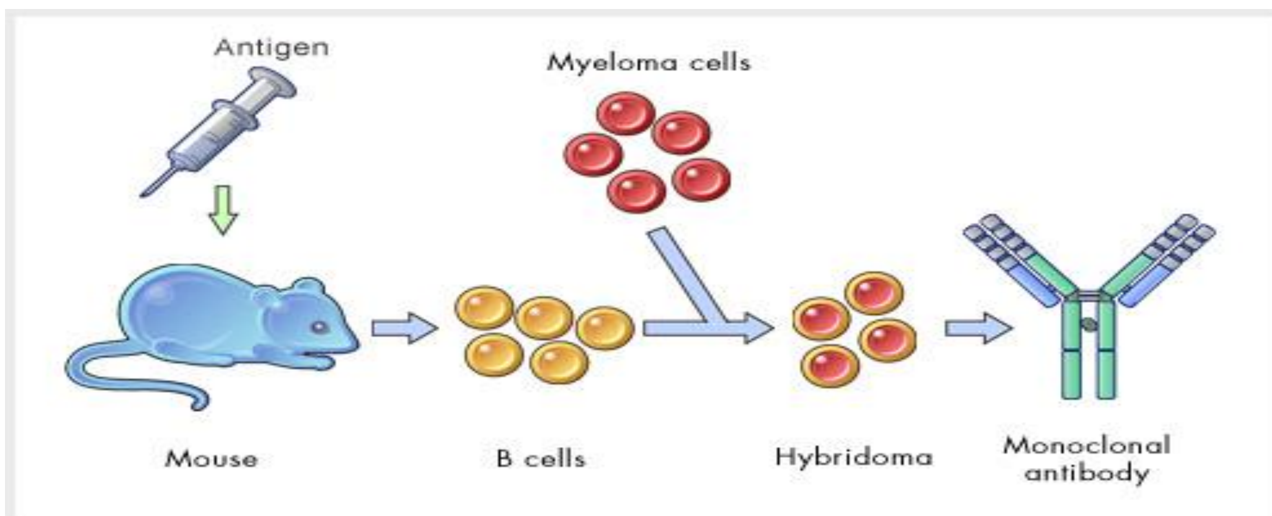


Major Problems:

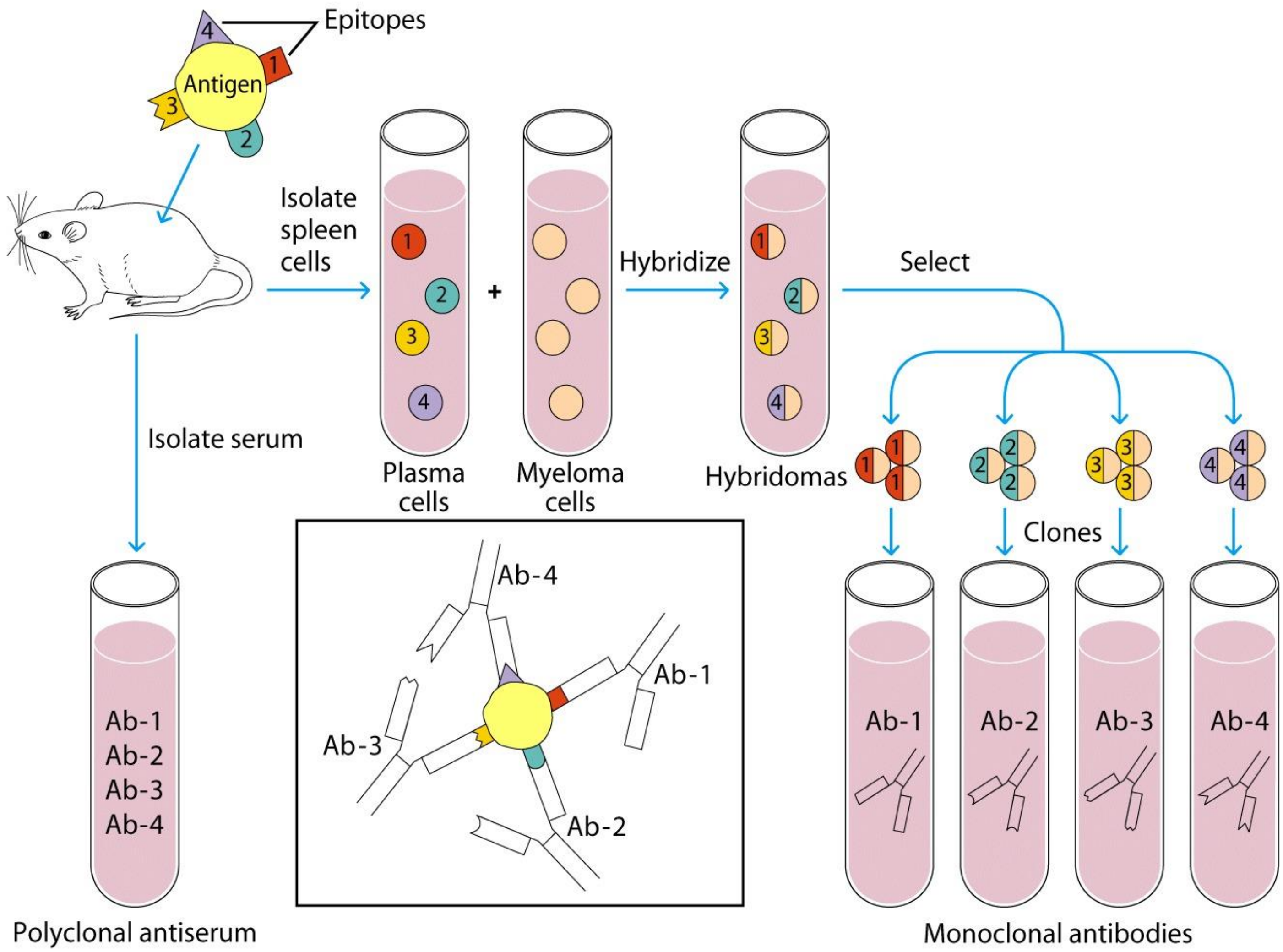
Toxicity

Source of toxin

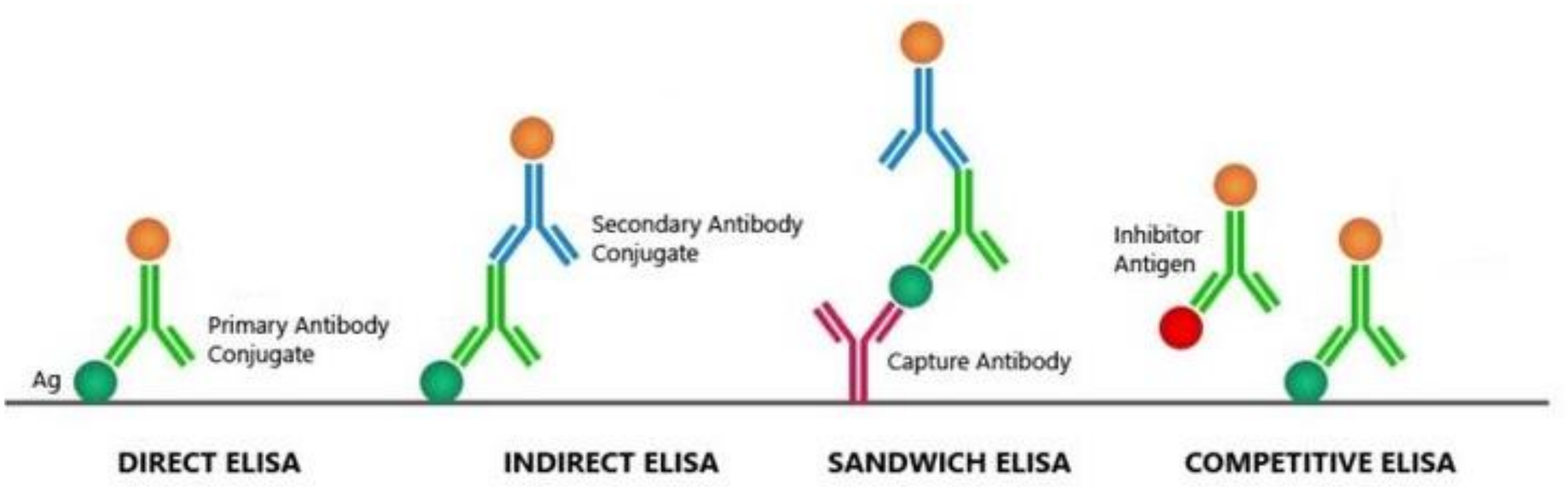
Specificity



Monoclonal Antibody Production



Immunological Assay – SPR Characterisation - LFD



- Based on the principle of immunoassays
- SPR – low response limited or no binding

- LFD - the presence/absence of test line will indicate whether the target antigen is present in the sample
- Real time interpretation of results - individual can see by eye if test line has developed (qualitative analysis)
- Reader systems allow semi-quantitative determination of the amount of antigen present in a sample

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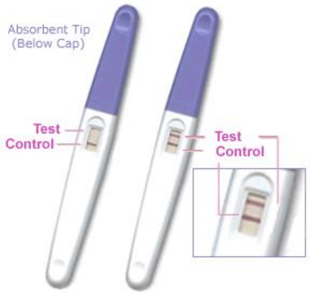
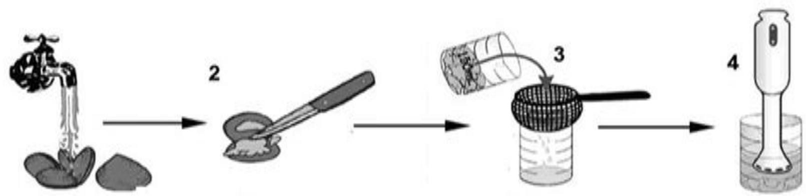
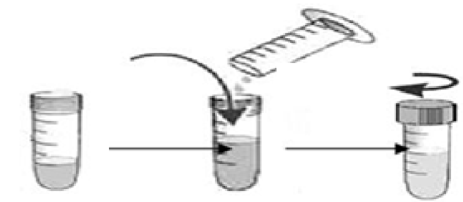
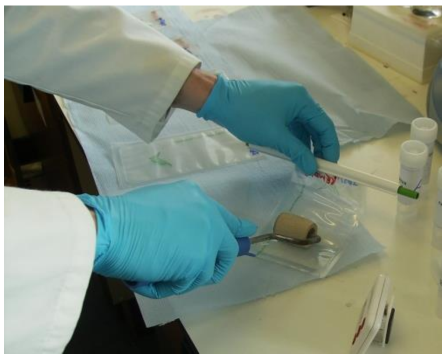
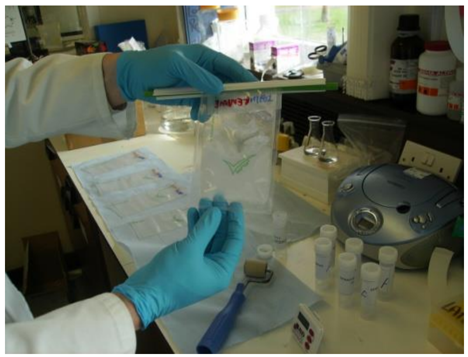
On site end product testing

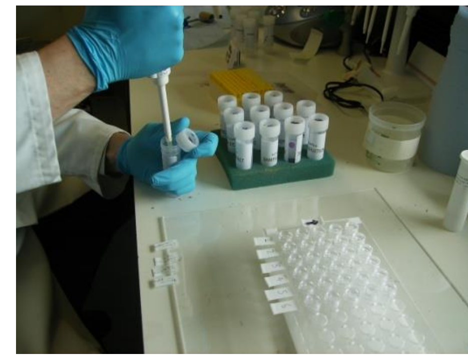


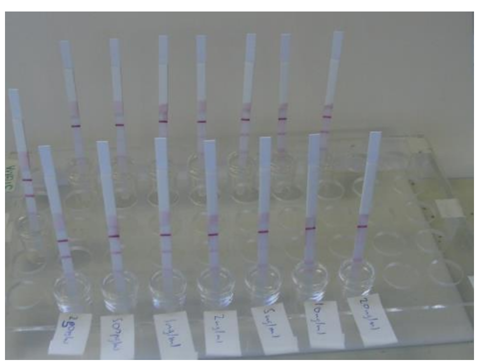


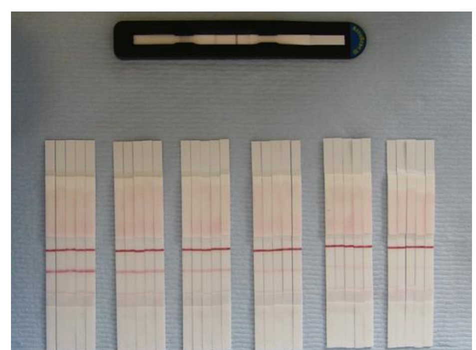












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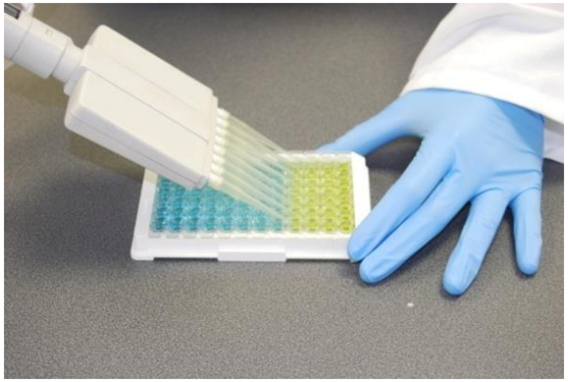
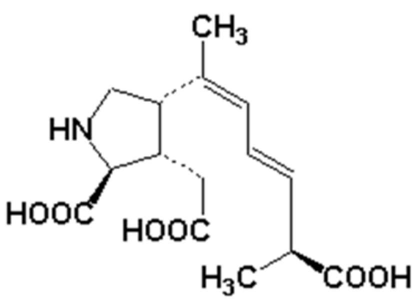
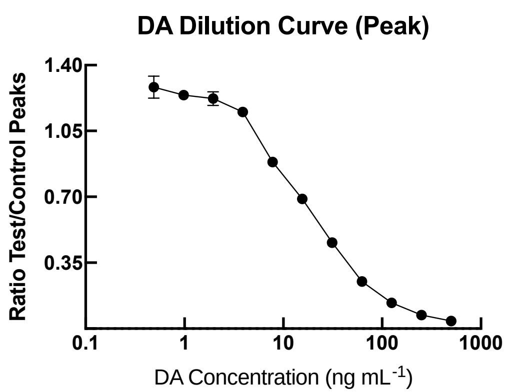
ASP Tests for the industry





Domoic Acid



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Development and validation of the first high performance-lateral flow immunoassay (HP-LFIA) for the rapid screening of domoic acid from shellfish extracts

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[c] Neogen Corporation, 620 Lesher Place, Lansing, MI 48912, USA

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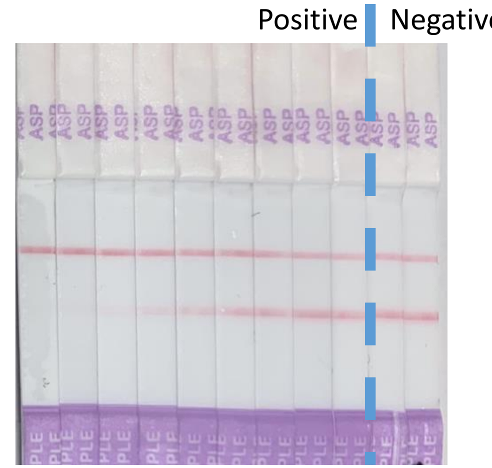
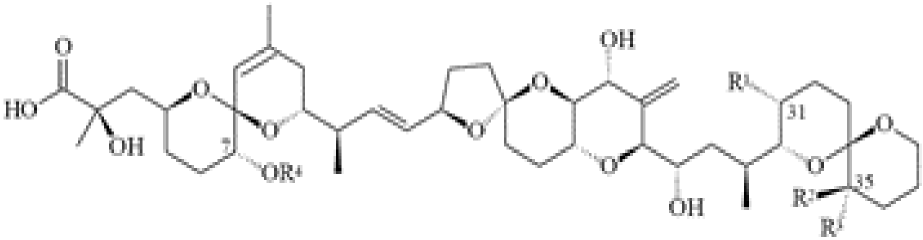
DSP Tests for the industry

Okadaic Acid and DTXs





	R^1	R^2	R^3	R^4
OA	CH_3	H	H	H
DTX1	CH_3	CH_3	H	H
DTX2	H	H	CH_3	H
DTX3 (acylated forms of OA, DTX1 and DTX2)	H/CH_3	H/CH_3	H/CH_3	fatty acid

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Development and Validation of a Lateral Flow Immunoassay for the Rapid Screening of Okadaic Acid and All Dinophysis Toxins from Shellfish Extracts

Waqass Jawaid,[*,†,‡] Julie P. Meneely,[‡] Katrina Campbell,[‡] Karrie Melville,[†] Stephen J. Holmes,[†] Jennifer Rice,[§] and Christopher T. Elliott[‡]

[†]Neogen Europe Limited, The Dairy School, Auchincruive, Ayr, KA6 5HU, Scotland, U.K.

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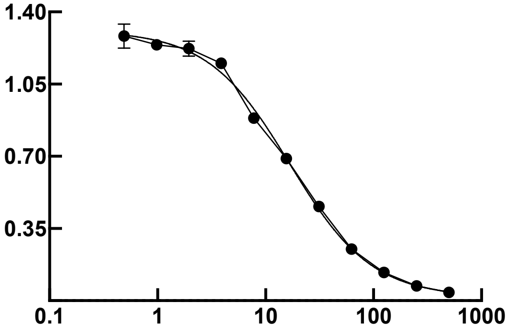
Quantification of Toxin Detection



For example, cut off for non-compliant results:
~120 µg OA / kg (reg. limit 160 µg)

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PSP Toxins

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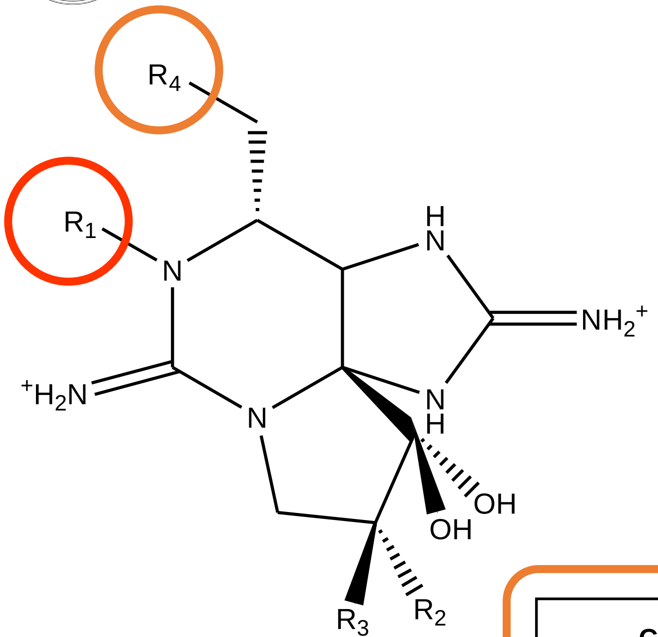

ANTIBODIES RAISED EITHER TO

STX – Saxitoxin

R_1 – Non hydroxylated toxins

NEO – Neosaxitoxin

R_1 – Hydroxylated toxins

			Carbamate Toxins	***N*-Sulfocarbamoyl Toxins**	**Decarbamoyl Toxins**	**Deoxydecarbamoyl Toxins**
R_1	**R_2**	**R_3**	**R_4: $OCONH_2$**	**R_4: $OCONHSO_3^-$**	**R_4: OH**	**R_4: H**
H	H	H	STX	B1 (GTX 5)	dc-STX	do-STX
H	H	OSO_3^-	GTX 2	C1	dc-GTX 2	do-GTX 2
H	OSO_3^-	H	GTX 3	C2	dc-GTX 3	do-GTX 3
OH	H	H	NEO	B2 (GTX 6)	dc-NEO	
OH	H	OSO_3^-	GTX 1	C3	dc-GTX 1	
OH	OSO_3^-	H	GTX 4	C4	dc-GTX 4	

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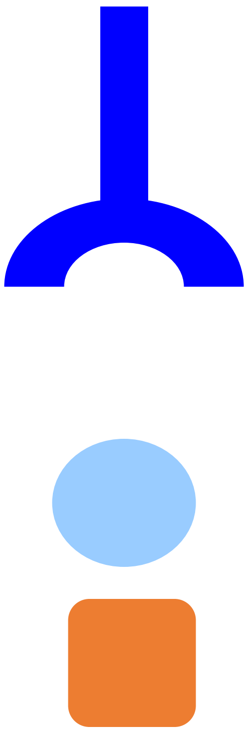
Cross Reactivity – SPR characterisation

- Saxitoxin Dihydrochloride
- Neosaxitoxin
- Gonyautoxin 2/3
- Gonyautoxin 1/4
- Decarbamoyl Saxitoxin
- Decarbamoyl Neosaxitoxin
- Decarbamoyl Gonyautoxin 2/3
- Gonyautoxin 5
- C1/C2
- C3/C4



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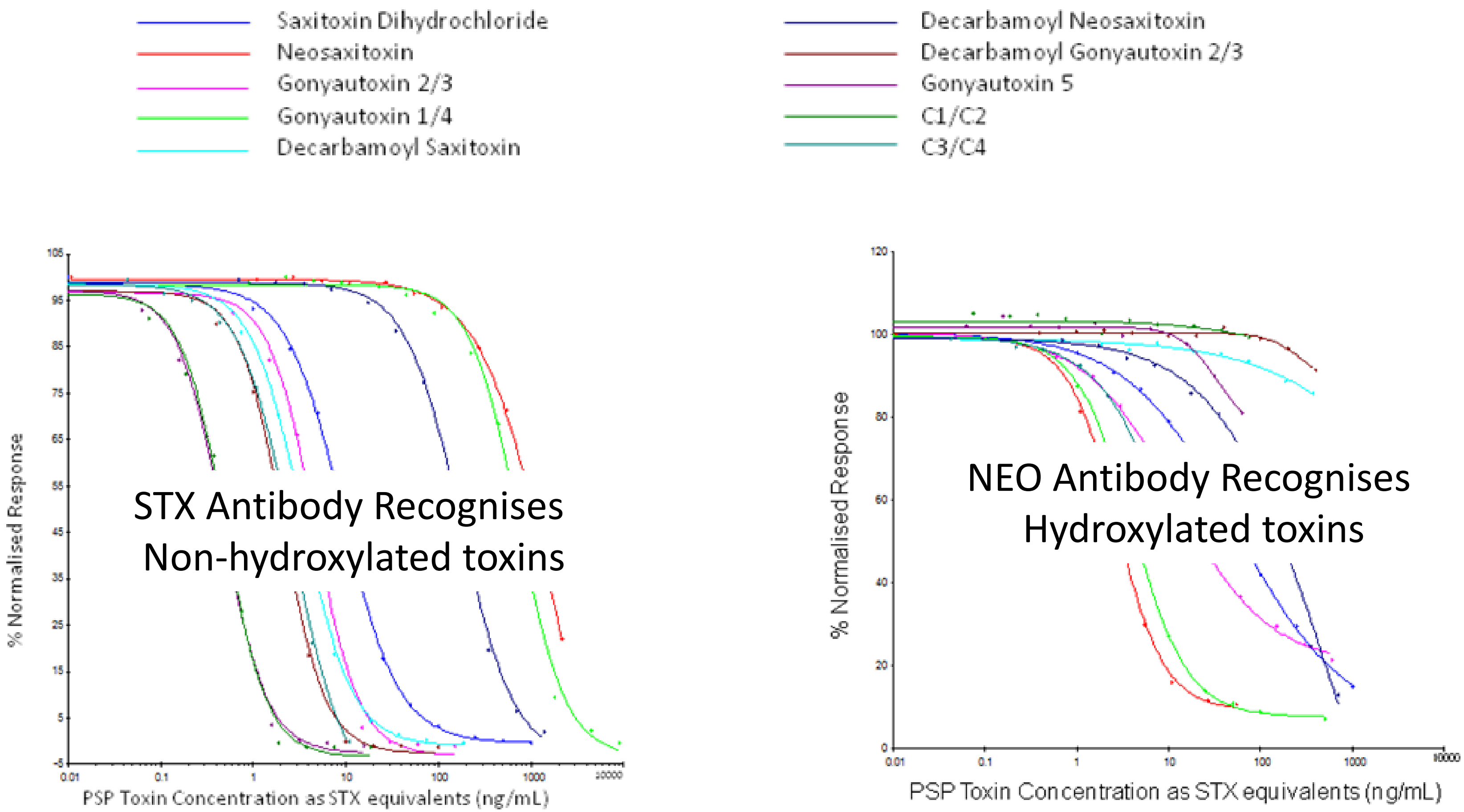
Cross Reactivity



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Progress for use of tests by industry

analytical chemistry

Article

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Development and Vali
(LFIA) for the Rapid Sc
from Shellfish Extracts

Waqass Jawaid,*[†,‡] Katrina Campl
and Christopher T. Elliott[‡]

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Supporting Information

Toxicon 125 (2017) 110–119

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Comparative performance o
detection of Paralytic Shellfi

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Christopher T. Elliott [b], D. Tim Harw
Katrina Wilson [f], Megan Burgoyne [f],

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New Town, Tasmania 7008, Australia

FOOD CHEMICAL CONTAMINANTS

Single-Laboratory Validation of the Neogen Qualitative Lateral Flow Immunoassay for the Detection of Paralytic Shellfish Toxins in Mussels and Oysters

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South Australian Research and Development Institute, 2b Hartl
SARAH C. UGALDE and GUSTAAF M. HALLEGRAEFF
University of Tasmania, Institute for Marine and Antarctic Stuc
KATRINA CAMPBELL
Queen's University Belfast, School of Biological Sciences, Instit
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University of Tasmania, Institute for Marine and Antarctic Stuc

FOOD CHEMICAL CONTAMINANTS

Detection of Paralytic Shellfish Toxins in Mussels and Oysters Using the Qualitative Neogen Lateral-Flow Immunoassay: An Interlaboratory Study

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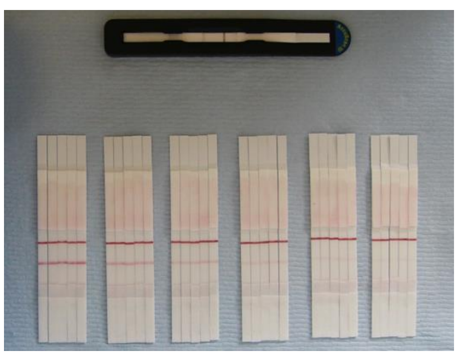
JILL K. BARTLETT



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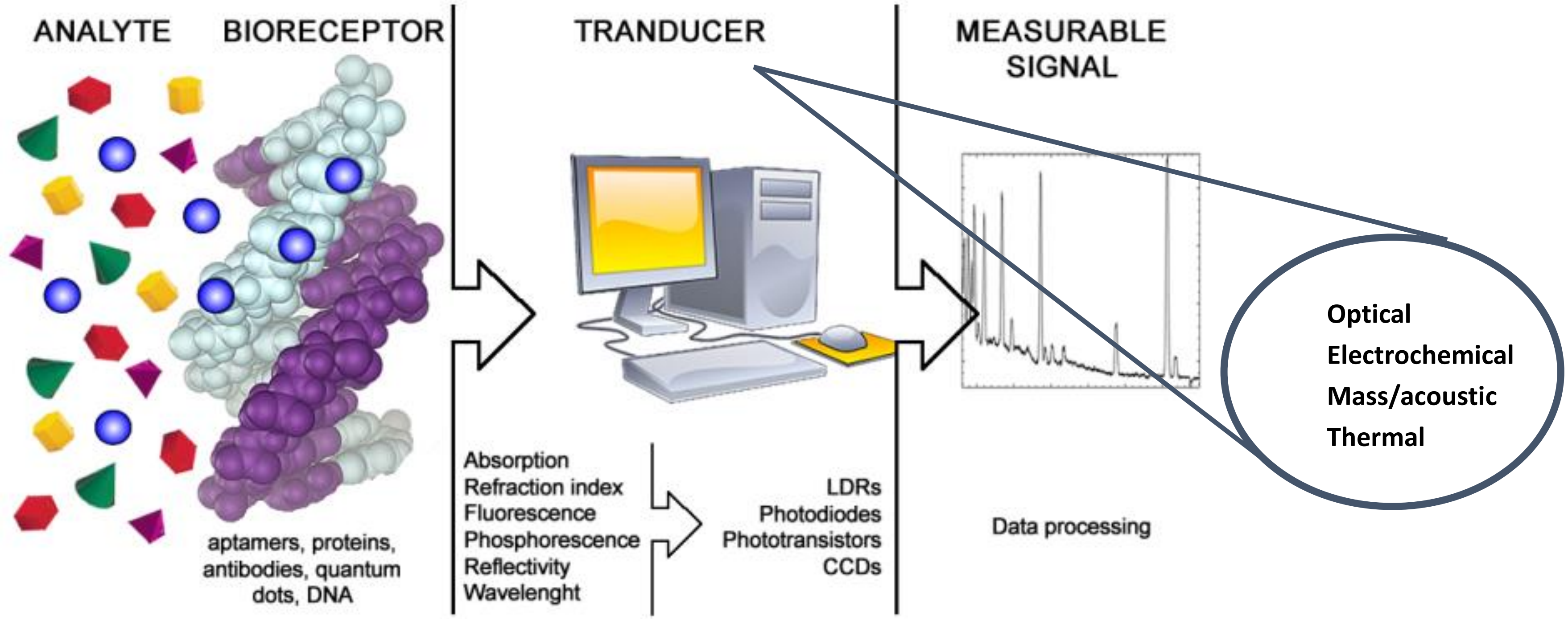
Biosensors - Nanosensors



"A biosensor is an analytical device incorporating a biological or biologically derived sensing element either intimately associated with or integrated within a physicochemical transducer. The usual aim is to produce a digital electronic signal which is proportional to the concentration of a specific analyte or group of analytes"

Turner, A.P.F., Karube, I. and Wilson, G.S. (1987). *Biosensors: Fundamentals and Applications. Oxford University Press, Oxford. 770p.*

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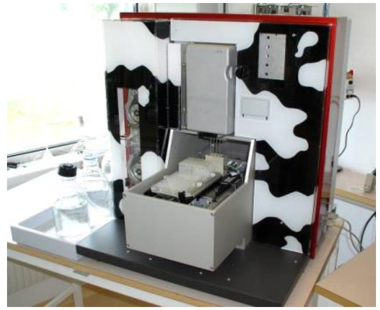
Commercial Biosensor technology



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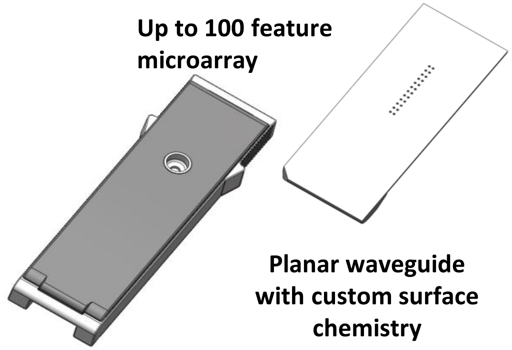
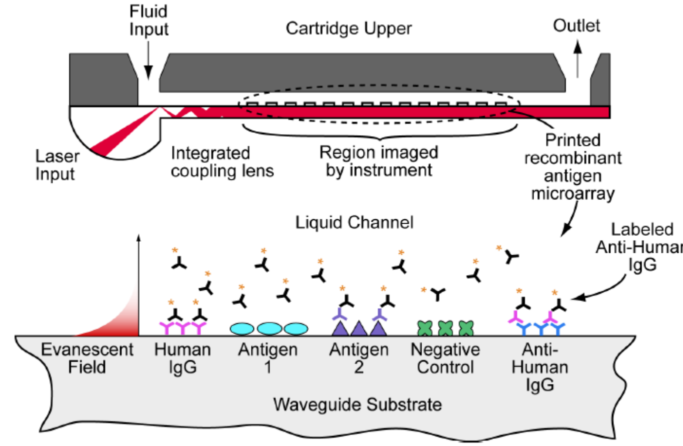
Rapid Multiplex Portable Diagnostics

MBio Diagnostics



Scientific know how



Aim to produce a lower cost platform offering

- Low cost analysis
- Simplicity in use
- Highly specific single target analysis
- Multiplexing – multiple target analysis
- Bespoke sensitivity
- Robust – high performance
- Field deployable

Suitable for source to product testing

- **Molecular level – DNA / RNA for pathogen and speciation testing**
- **Protein Level – Allergen testing eg milk, nuts, eggs, seafood**
- **Residual level – Low molecular weight toxins / antibiotics / contaminants**

SMART Diagnostics:
Specific multiplex accurate real time analysis

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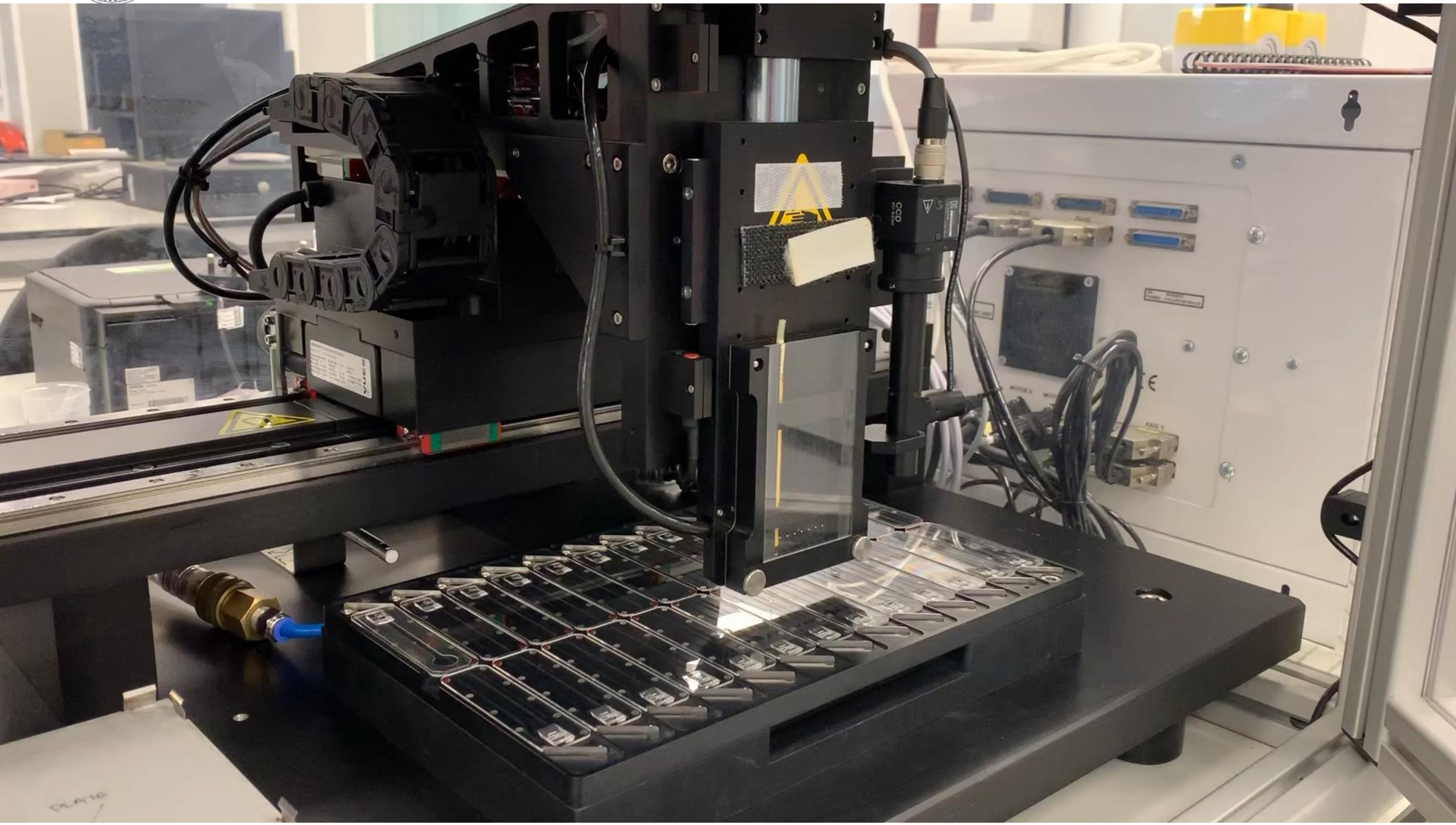

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Simplicity in Use

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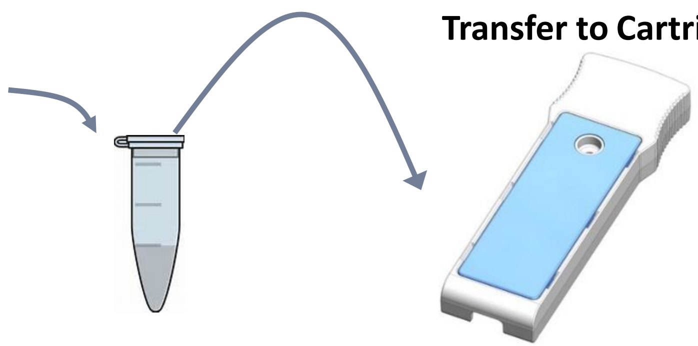
Important to implement simple testing regimes to allow FBOs to perform testing

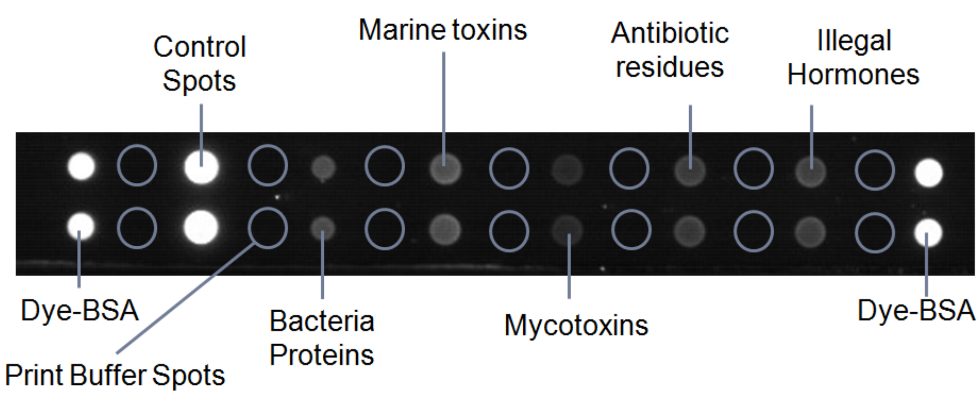
Offer a simple device requiring minimal sample preparation through either simple fluid application (blood, milk, juice) or dissolution of solid foods in buffering reagents

Add Sample to Assay Buffer
Dye-labeled Ab

Transfer to Cartridge



Read at 5-10 min



Depending on the complexity required
Qualitative – YES/NO Answer
Quantitative – Provide a concentration

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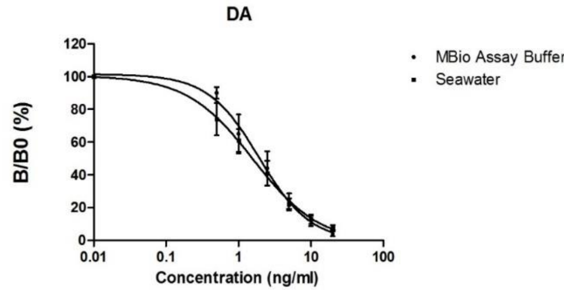
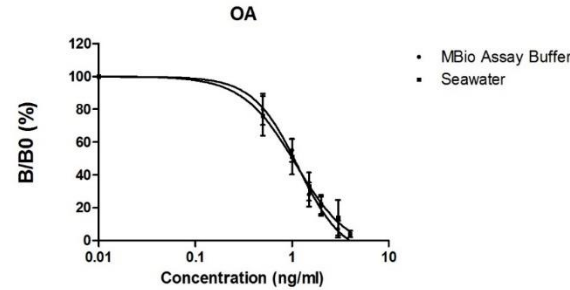
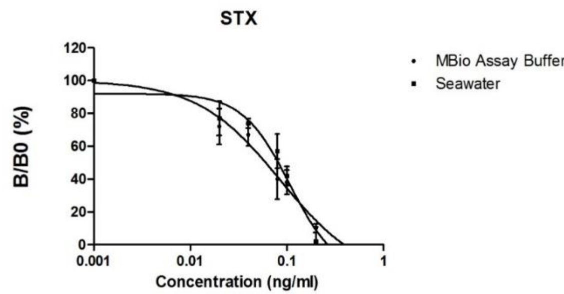
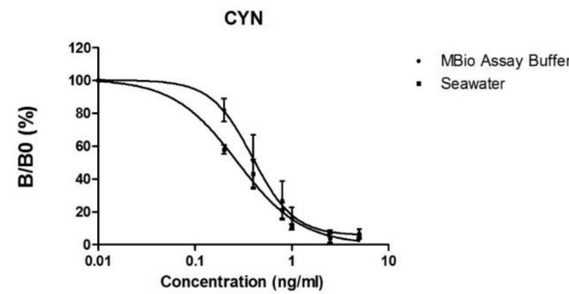
Multiplexing Natural Toxin Detection in Harmful Algae

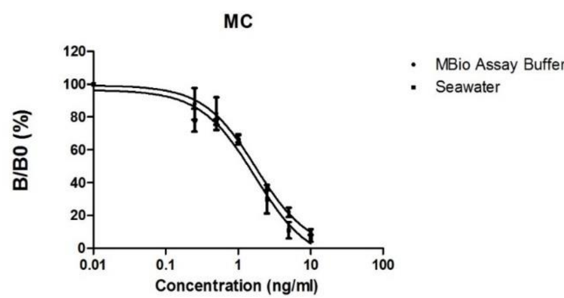
Biotoxin	Mid Point IC_{50} (ng/ml)	Dynamic Range IC_{20} - IC_{80} (ng/ml)
DA	1.37	0.37 - 4.43
OA	0.96	0.44 - 1.94
STX	0.09	0.05 - 0.15
CYN	0.26	0.08 - 0.72
MC	1.31	0.40 - 3.35












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

Development of a Planar Waveguide Microarray for the Monitoring and Early Detection of Five Harmful Algal Toxins in Water and Cultures

Sara E. McNamee,[†] Christopher T. Elliott,[†] Brett Greer,[†] Michael Lochhead,[‡] and Katrina Campbell*[,†]

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[‡]MBio Diagnostics Inc., 5603 Arapahoe Avenue, Boulder, Colorado 80303, United States

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Conclusions

- Marine toxins are naturally occurring
- Certain toxins can be lethal
- Climate change appears to be causing an increase in HABs worldwide
- New toxins in old and new areas
- Old toxins in new areas
- Prevention and Mitigation measures are not complete
- Future outlook – these toxins may become an increasing problem

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Freshwater toxins

Katrina Campbell
Prof in Food Security & Diagnostics
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Freshwater Toxins

- Blue green algae (cyanobacteria) produced phycotoxins in freshwater
- There are distinct cyanotoxin producing and non-cyanotoxin producing organisms
- Organisms involved in toxin production include an estimated 40 genera
- Cyanobacteria can produce a wide array of cyanotoxins which fall into three broad groups: cyclic peptides, alkaloids and lipopolysaccharides
- Most cyanobacterial toxins are intracellular. Toxins released as cells die or break open on treatment with chemicals eg copper sulphate
- Toxin content is highest in cells at temperatures between 18-25 ^{0}C; low (10 ^{0}C) or very high temperatures (30 ^{0}C) decreased toxin content.
- Cells found to have higher toxin levels when grown at high and low pH
- Toxins content can be higher in nutrient rich waters with increased phosphorus or nitrogen

Blue Green Algae in the St. Lucie River in Florida.

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Health Effects & Possible Exposure Pathways

- These toxins can act as neurotoxins, liver toxins, cell toxins and skin irritants

- Toxins may result in animal deaths with large losses among wildlife and domestic animals

- Human poisoning cases have occurred globally - Children more susceptible to toxins due to smaller body size, potential for more incidental ingestion and response to symptoms

    - Chronic ingestion through contaminated drinking water

    - Ingestion of water, inhalation of droplets or contact with nasal mucous membranes through recreational contact

    - Dermal contact with toxins through bathing or recreational activities such as wading, swimming, skiing and canoeing

    - Consumption of fish and shellfish from contaminated waters

    - Water used as recreational bathing waters and human consumption should be regularly monitored for cyanotoxins

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Overview Freshwater Toxins
Producer, toxin and health effects

Chemical structure	Toxin	Genera	Vector	Short Term Health Effects	Long Term Health Effects
Cyclic Peptides	Microcystins	Anabaena, Aphanocapsa, Hapalosphon, Microcystis, Nostoc, Oscillatoria, Planktothrix	Drinking water Irrigation water 1µg/L MC-LR	Gastrointestinal, liver inflammation, and hemorrhage and liver failure leading to death, pneumonia, dermatitis	Tumor promoter, liver failure leading to death
	Nodularins	Nodularia spumigena	Drinking water Irrigation water	Similar to Microcystins	Similar to Microcystins
Alkaloids	Saxitoxins	Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya	Drinking water Irrigation water 3µg/L	Tingling, burning, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death	Unknown
	Anatoxins	Anabaena, Aphanizomenon, Oscillatoria, Planktothrix	Drinking water Irrigation water	Tingling, burning, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death	Cardiac arrhythmia leading to death
	Cylindrospermopsin	Aphanizomenon, Cylindrospermopsis, Umezakia	Drinking water Irrigation water	Gastrointestinal, liver inflammation and hemorrhage, pneumonia, dermatitis	Malaise, anorexia, liver failure leading to death
	Lipopolysaccharide	Aphanizomenon, Oscillatoria	Drinking water Irrigation water	Gastrointestinal, dermatitis	Unknown
	Lyngbyatoxins	Lyngbya	Drinking water Irrigation water	Dermatitis	Skin tumours unknown
Poyketides	Aplysiatoxins	Lyngbya, Schizothrix, Planktothrix (Oscillatoria)	Drinking water Irrigation water	Dermatitis	Carcinogen; activator of protein kinase C tumour promoting effect
Amino acid	BMAA	Anabaena, Cylindrospermopsin, Microcystis, Nostoc, Planktothrix	Drinking water Irrigation water		Potential link to neurodegenerative diseases

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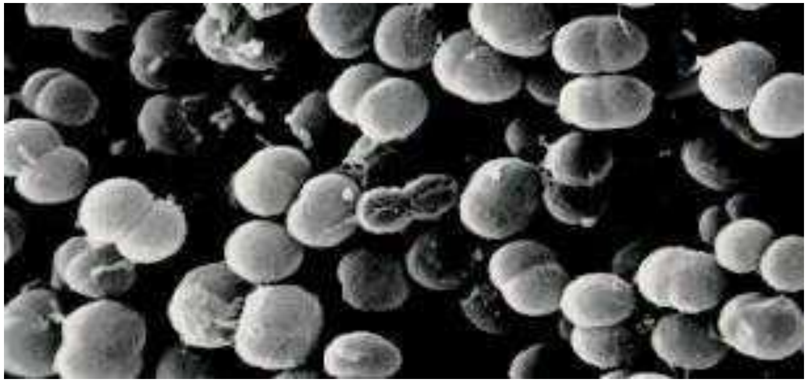
Microcystins

- Globally, microcystins are the most commmon detected cyanobacterial toxin in fresh & brackish waters.
- The toxins are water soluble and most microcystins cannot penetrate directly through plant or animal cell membranes.
- Uptake into cells occurs through transmembrane organic anion transporting polypetptides (OATPs).
- The liver is the ultimate target organ for toxic effects.
- Microcystins are highly toxic with low doses required for lethal effects.
- There is some evidence that microcystins can act as tumor promoters as well.
- Some studies have found microcystin in fish fillet (range: 16- 300 ppb microcystin in fillet).



Microcystis aeruginosa

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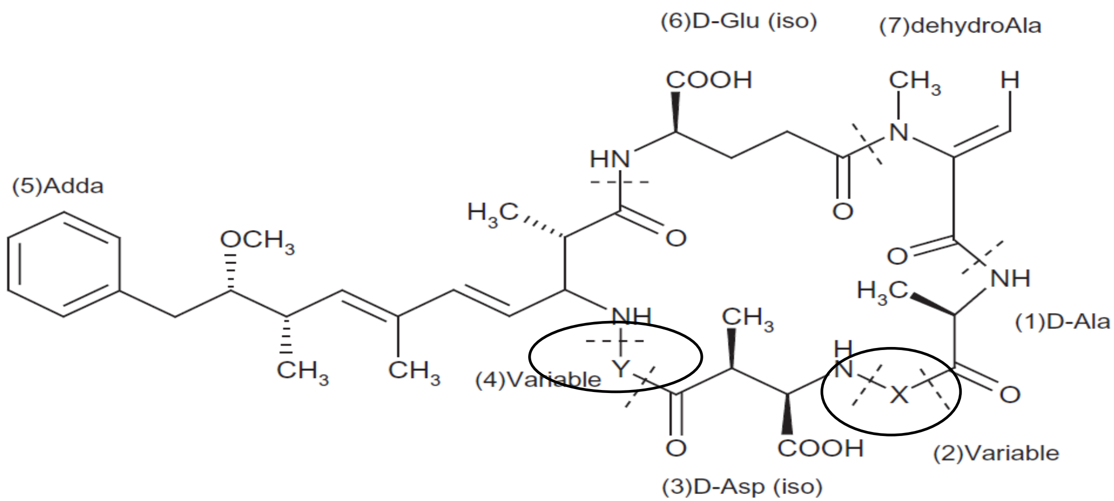
Microcystins

Microcystin variant	LD50 I.P. mice (µg/kg)	Relative Molecular Weight	X (variable amino acid residue at 2)	Y (variable amino acid residue at 4)
MC-LR	50	995.2	Leucine (Leu, L)	Arginine (Arg, R)
MC-RR	600	1038.2	Arginine (Arg, R)	Arginine (Arg, R)
MC-YR	70	1045.2	Tyrosine (Tyr, Y)	Arginine (Arg, R)
MC-LA	50	910.1	Leucine (Leu, L)	Alanine (Ala, A)
MC-LW	Not Determined	1025.2	Leucine (Leu, L)	Tryptophan (Trp, W)
MC-LF	Not determined	986.2	Leucine (Leu, L)	Phenylalanine (Phe, F)
Nodularin	60	825.0	2-methylamino-2-dehydrobutyric acid (MDhb)*	Arginine (Arg, R)

*MDhb replaces 1,2 and 7 on the parent structure



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Microcystin Exposure Occurrences

- 1988 (Brazil): Severe gastro-enteritis epidemic developed with over 2000 cases reported (88 deaths). Results pointed to a massive bloom of *Anabaena and Microcystis* in reservoir.
- Epidemiological link to liver cancer in rural Chinese populations infected with Hepatitis-B and drinking water contaminated with microcystins.
- In Brazil, 117 patients developed liver disease (50+ deaths) attributed to dialysis with microcystin-contaminated water (also occurred in the U.S. in 1975)
- Atlantic salmon reared in B.C. and Washington dying of progressive liver disease from an unidentified organism producing microcystins; severe economic losses
- A record outbreak of blooming microcystis occurred in Lake Erie 2011, in part related to the wettest spring on record, and expanded lake bottom dead zones, reduced fish populations, fouled beaches, and the local tourism industry that generates more than $10 billion in revenue annually
- August 2014, the City of Toledo, Ohio detected higher levels of microcystin than deemed safe in its water supply due to harmful algal blooms (HABs) in Lake Erie,

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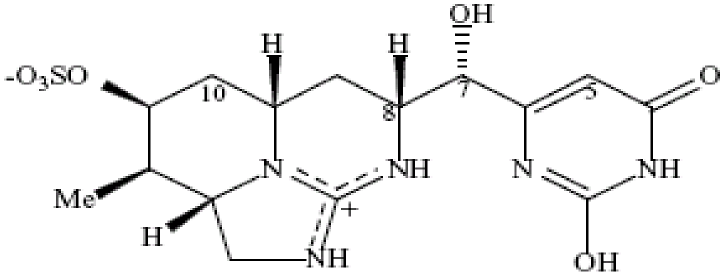
Cylindrospermopsin

- As more bodies of water are analyzed for the presence of cylindrospermopsin, these toxins are found on a global scale.
- Certain strains of *Cylindrospermopsi, Anabaena, Aphanizomenon* and others have been determined capable of producing this toxin.
- Main target of toxicity is the liver. Other organs such as the thymus, kidneys, lungs, intestinal tract and heart may be affected.
- Cylindrospermopsin was reported to be a possible tumor initiator
- Symptoms of cylindrospermopsin toxicity in cattle have been reported as weakness, anorexia, palor of mucous membranes and diarrhea. Several incidences of death in livestock have been attributed to this toxin.

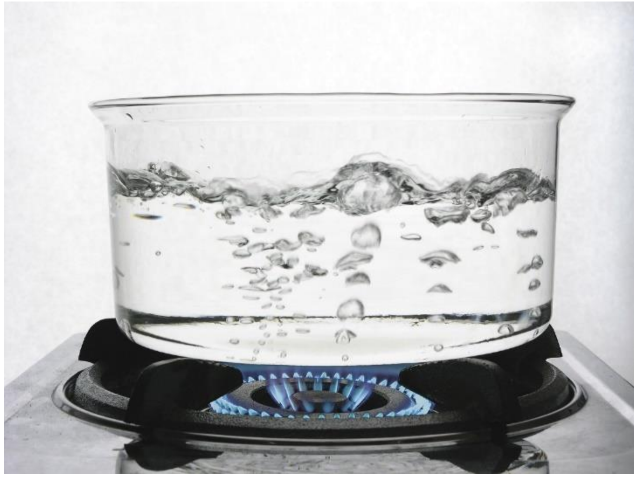
Cylindrospermopsis raciborskii



Cylindrospermopsin

Characteristics of Cylindrospermopsin Toxin

- Highly water soluble
- Stable in extreme temperatures and pH
- No degradation of toxin after 15 minutes of boiling
- Toxin will degrade more rapidly under natural light compared to shortwave UV light
- Often, the level of cylindrospermopsin produced extracellularly will exceed the level of toxin inside the cells
- **Cylindrospermopsin:** distribution of toxin in crayfish was 5:1 between viscera:muscle.
- No cylindrospermopsin was detected in the muscle of rainbow fish, but was detected in the liver.



Cylindrospermopsin Exposure
Palm Island Mystery

- In 1979, a major bloom occurred in a reservoir of Palm Island, Australia (water was chlorinated but unfiltered).

- Residents complained of a bad taste and smell of drinking water, so the water was treated with copper sulfate to kill the bloom.

- Shortly after, 139 children and 10 adults complained of hepatitis-like symptoms including malaise, anorexia, vomiting, headache and stomach pain. Kidney malfunction, bloody diarrhea and urine were also reported. In a few cases, the loss of electrolytes was so severe that patients suffered from hypovolemic shock.

- A culture of the reservoir water revealed the presence of *Cylindrospermopsis raciborskii.*

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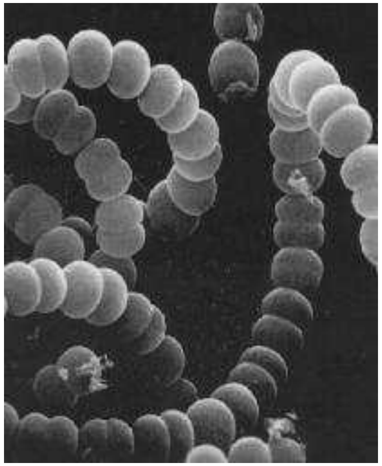
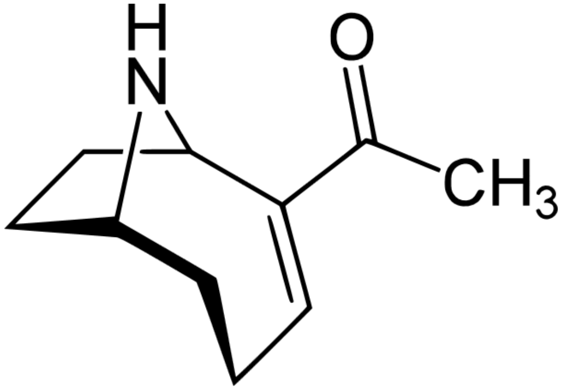
Anatoxins

- Anatoxins are neurotoxic agents produced by a variety of cyanobacterial organisms.
- Alkaloids, which are capable of transformations into toxic by-products.
- Anatoxins are stable in dark waters, but are susceptible to photo-chemical degradation. Breakdown is further accelerated by alkaline conditions.
- Anatoxins have been implicated in numerous animal and wildfowl poisonings. Symptoms, including death, can occur in minutes.
- No information could be found on chronic exposure to anatoxins



Anabaena flos-aquae



Anatoxin A

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Anatoxins

Anatoxin-a: sufficient exposure can lead to paralysis, asphyxiation and death; oral LD_{50} (mice) ~ 5 ppm; repeated exposures to toxin caused fetal malformations and stunted growth in hamster litters; no maternal toxicity noted

Anatoxin-a(S): potent organophosphate produced by A. flos-aquae; this toxin blocks acetylcholinesterase activity; no oral toxicity studies could be found; symptoms include muscle weakness, respiratory distress and convulsions

Case history of anatoxin-a exposure:

- In July 2002, five teenagers went swimming in a pond at a golf course in Dane County, Wisconsin.
- The pond was described as “scummy” and “dirty.” The boys splashed around and two had their head submerged underwater.
- Of the two who went underwater, one boy died of acute heart failure 48 hours later and the other became ill with acute diarrhea and abdominal pain.
- Blood tests on the boys confirmed the presence of *Anabeana flos aquae* and anatoxin-a.
- An algal toxin expert was quoted as saying the toxin was present in amounts that could cause symptoms & death based on animal studies, but was puzzled by the amount of time that had elapsed prior to death (Milwaukee Journal Sentinel, Sept. 5, 2003).
- The Dane County coroner’s report lists incidental ingestion of algae contaminated water as the causative agent for this death.

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Risk Levels & Standards

Drinking water (provisional): 1 ug/L microcystin-LR (WHO)
3 ug/L anatoxin-a (Australia)

BGA dietary supplements: 1 ppm microcystin (Optimum Daily Allowance)

Tolerable Daily Intake (provisional): 0.04 ug/kg/day (WHO)

Recreational Bathing Waters (WHO):

Relatively **low-risk** of adverse effects: 20,000 cells/ml
(4 ug/L microcystin)
Moderate probability of adverse effects: 100,000 cells/ml
(20 ug/L microcystin)
High probability of adverse effects: scums

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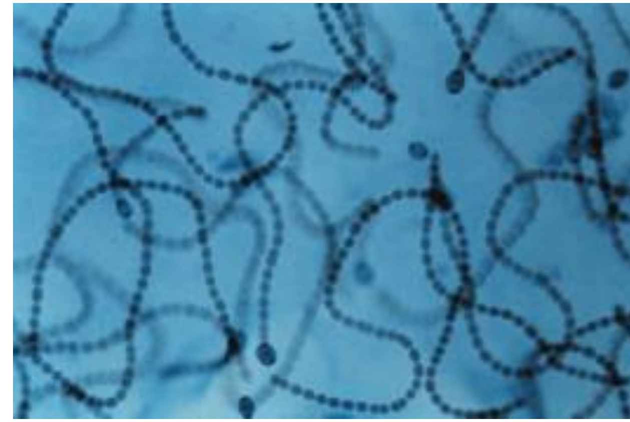
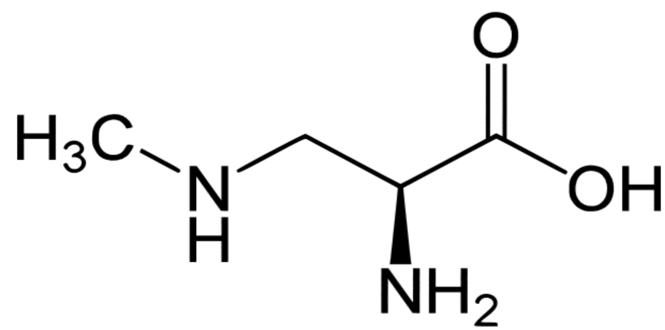
BMAA: β-N-methylamino-L-alanine

- BMAA is a neurotoxic amino acid first identified in 1967 in Guam to be produced by the Nostoc spp.
- In 2005 it was reported that over 95% of all analyzed cyanobacterial strains produce BMAA
- Believed to bioaccumulate through the food chain
- Climate change, particularly temperatures increase have already led to rising problem in the frequency and severity of toxic events in freshwaters, some of which have been due to cyanobacteria blooms.
- BMAA is believed by some researchers to be implicated in a range of neurological disorders such as Alzheimer disease
- However, a matter of great debate in the literature is in BMAA detection and in the relation to the fact that the amount of BMAA consumed is insufficient to cause neurodegenerative diseases such as Alzheimer's and Parkinson's.



Nostoc spp.



β-N-methylamino-L-alanine

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Sustainability

If the frequency of HABs are increasing how can we ensure seafood security ?

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Learning Outcomes

- **Develop an ability to define and classify biotoxins**
- **Understand the current food safety hazards arising from biotoxins and their implications to human health**
- **Identify emerging issues in food security from biotoxins**
- **Knowledge of control measures that are implemented to prevent biotoxins entering into the food chain**
- **Recognise bioanalytical approaches used to monitor the presence of biotoxins in order to protect human health.**

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Marine and Freshwater Toxins

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