The Hunt for Red Tide Toxins

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National Research Council Canada | Conseil national de recherches Canada | Canada

A National Crisis

Nov. 22-25, 1987

- **Hundreds of people turn up at emergency wards in Montreal**

  - symptoms of vomiting, diarrhea, confusion, facial ticks, memory loss

- **Three victims go into comas and die.**
- **A mysterious poison is suspected.**

Quilliam, M. A. and Wright, J. L. C.
Anal. Chem. 1989; 61: 1053A-60A.

The Amnesic Shellfish Poisoning Mystery

1987

- Nov. 22-25 Poisonings reported
- **Nov. 29**
  - **Health Canada:**
    - **Illnesses correlated with mussels eaten at restaurants**
    - **Neurotoxic symptoms when aqueous extracts injected into mice**
  - **Fisheries & Oceans Canada:**
    - **Mussels traced to Cardigan Bay, PEI**

The Amnesic Shellfish Poisoning Mystery

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- Nov. 22-25 Poisonings reported
- Nov. 29 Toxic mussels implicated
- **Dec. 11 Atlantic shellfishery closed**
  - **Economic loss: millions of dollars**
  - **Political pressure to solve problem**
  - **A national emergency is declared and all federal regulatory labs mobilized**

The Amnesic Shellfish Poisoning Mystery

- **An extensive survey for environmental contaminants conducted by the regulatory labs (Health, Fisheries, Environment):**
  - **Heavy metals**
  - **Pecticides**
  - **Organophosphates**

  – all negative

- **NRC formulated an approach and asked to be involved**

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- **Dec. 12 NRC begins investigation**

  Mouse bioassay technician from DFO and team of 40 assembled in Halifax laboratory
- **Dec. 16 Toxin identified**

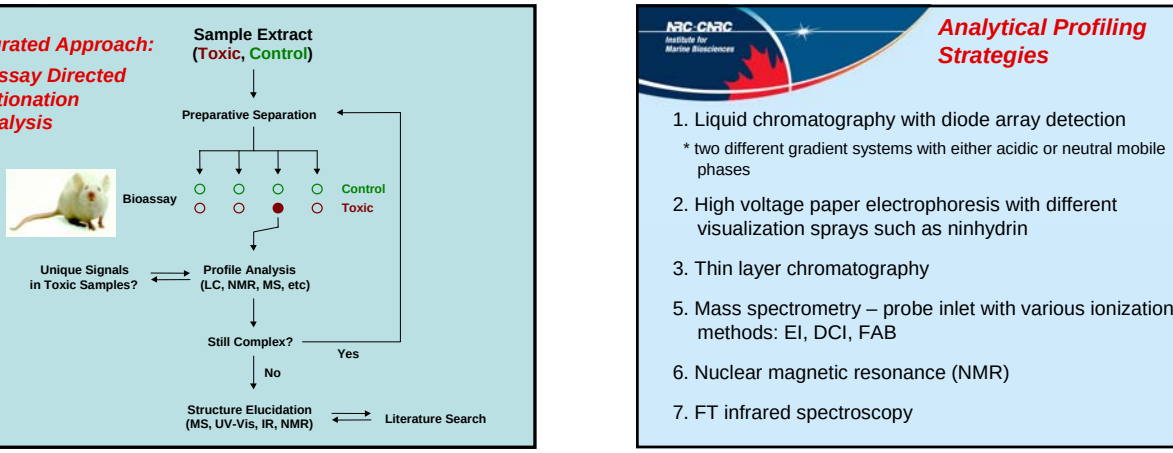
The start of the investigation at NRC – shucking mussels

A key step was to secure a large quantity of toxic mussel reference material, as well as negative control material.

The Crisis Team

Coping strategies

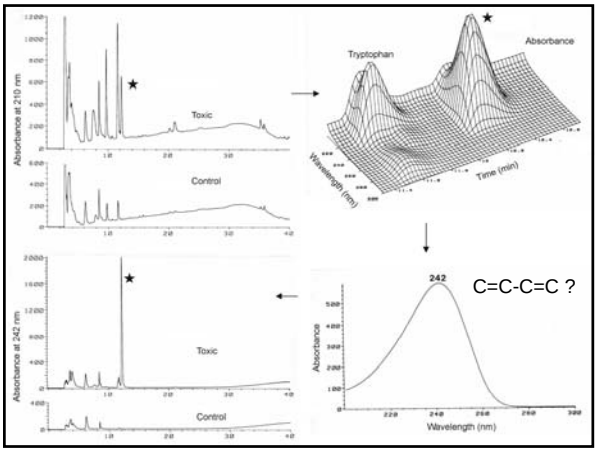
Integrated Approach: Bioassay Directed Fractionation + Analysis



Analytical Profiling Strategies

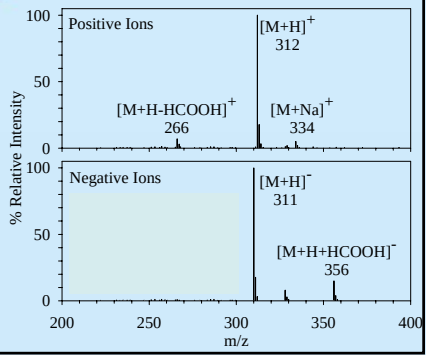
1. Liquid chromatography with diode array detection
   * two different gradient systems with either acidic or neutral mobile phases
2. High voltage paper electrophoresis with different visualization sprays such as ninhydrin
3. Thin layer chromatography
5. Mass spectrometry – probe inlet with various ionization methods: EI, DCI, FAB
6. Nuclear magnetic resonance (NMR)
7. FT infrared spectroscopy

LC-DAD Instrument



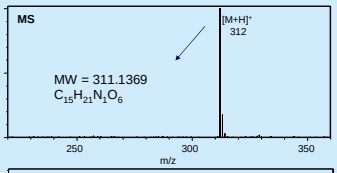
Mass Spectra of Suspect

Fast atom bombardment (FAB) ionization on a Micromass ZAB (magnetic sector)

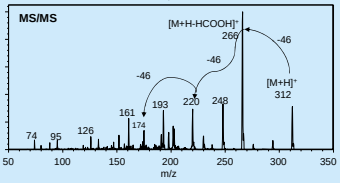


Mass Spectra of Suspect

High resolution FAB-MS revealed the elemental composition as:
$C_{15}H_{21}N_1O_6$



Tandem MS revealed the probable presence of 3 carboxyl functions (46 = HCOOH)



Literature Search

An on-line chemical abstracts search revealed a possible hit!

Daigo, K.
Constituents of *Chondria armata*. IV. Determination of domoic acid.
Yakugaku Zasshi. 1959; 79: 360-4.

Biscoe, T. J. et al.
Structure-activity relations of excitatory amino acids on frog and rat spinal neurons.
Br. J. Pharmacol. 1976; 58: 373-82.

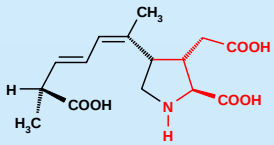
Nagao, S.
Chemical destruction of brain tissues with kainic acid.
Seitai No Kagaku 1981; 32:162-70.

Ohfune, Y. et al.
Total synthesis of (-)-domoic acid. A revision of the original structure.
J. Am. Chem. Soc. 1982; 104: 3511-13.

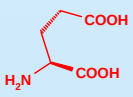
Domoic Acid

… a very potent neurotoxin

... "Glutamic Acid Impersonator"



Domoic Acid

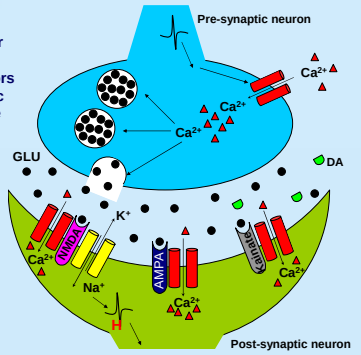


Glutamic Acid

Mechanism of Action

Domoic acid is an excitatory or excitotoxic amino acid which can activate glutamate receptors on the surface of post-synaptic neurons resulting in excessive accumulation of Ca^{2+} and Na^{+}

- Glutamic Acid (GLU)
- Domoic Acid (DA)



^{1}H-NMR Spectrum

Toxicity Balance

1. A pure standard of domoic acid was obtained for testing.
2. The isolated compound, the standard, and the original mussel tissue sample were tested for toxicity and domoic acid concentration.

* more than 95% of the toxicity was accounted for by domoic acid

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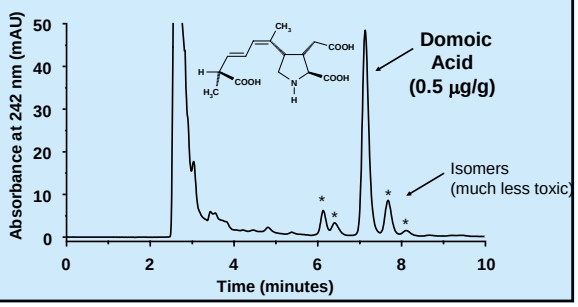
- **Dec. 20 Analytical method for toxin developed**

LC-UVD Method for Domoic Acid

Shellfish → 50% CH_3OH Extraction → LC-UVD



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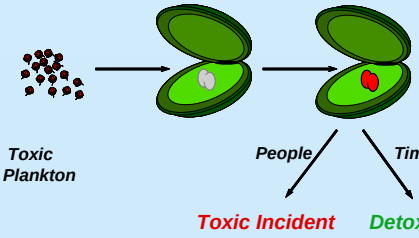
- **Jan. 7, '88 Shellfishery re-opened**
- **Apr. 18, '88 Source of toxin identified**

Red Tides (Harmful Algal Blooms)

Shellfish Poisoning



Identifying the Toxin Source

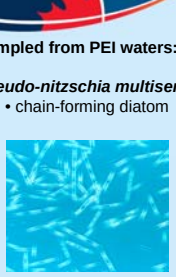
Two approaches:

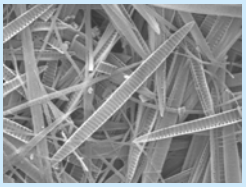
- **Plankton monitoring and correlation with appearance of toxin.**
- **Production of toxin by a uni-algal culture.**

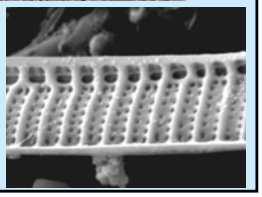
Identifying the Toxin Source

Sampled from PEI waters:

Pseudo-nitzschia multiseries
• chain-forming diatom





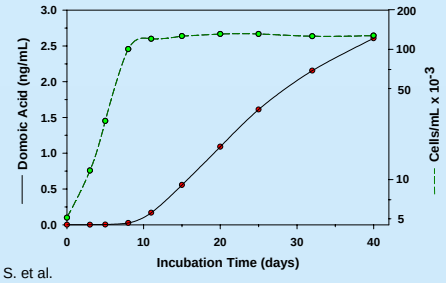


Identifying the Toxin Source

***Pseudo-nitzschia* culture**

Identifying the Toxin Source

Production of domoic acid by *Pseudo-Nitschia* in a uni-algal culture



Bates, S. S. et al.
Can. J. Fish. Aquat. Sci. 1989; 46:1203-1215.

Certified Reference Materials (CRMs)

The lack of accurate calibration standards has impeded the development and routine implementation of new analytical methods for seafood toxins.

We were determined that this would not be the case for domoic acid.

First CRMs for shellfish toxins were produced by NRC in 1989:

- domoic acid calibration solution CRM
- mussel tissue CRM

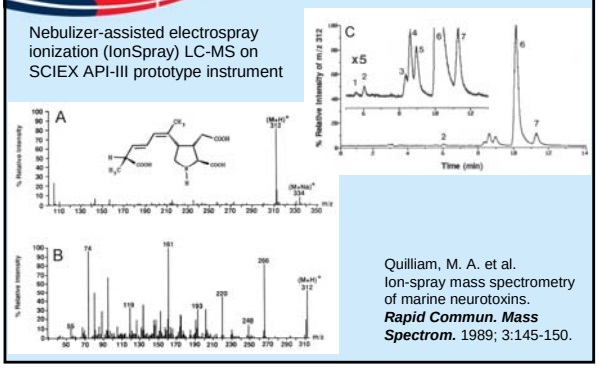
Hardstaff, W. R.
Fresenius. J. Anal. Chem. 1990; 338: 520-5.

First Commercial API-LC-MS System, 1989

SCIEX API-III (#1)

Atmospheric Pressure Ionization LC-MS, 1989

Nebulizer-assisted electrospray ionization (IonSpray) LC-MS on SCIEX API-III prototype instrument

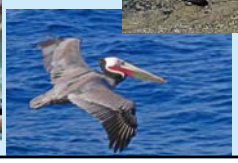


Quilliam, M. A. et al.
Ion-spray mass spectrometry of marine neurotoxins.
Rapid Commun. Mass Spectrom. 1989; 3:145-150.

Case #2

September 1991 Monterey Bay, California

- Hundreds of dead pelicans and cormorants, but no evidence of pollution linked to deaths



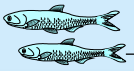
September 1991 Monterey Bay, California

- Thierry Work, CA State Vet, sends a package of samples to Canada
- LC-MS at NRC revealed **domoic acid** in pelicans' stomachs, in anchovy (100 μg/g) and in the plankton
- Birds had been eating anchovies
- *Pseudonitzschia australis* in waters

Fritz, L. et al
J. Phycol. 1992; 28: 439-442.

September 1991
Monterey Bay, California

Pseudo-nitzschia australis



Anchovies

Pelicans

Marine mammals

The US-FDA was alerted to presence of domoic in anchovy and a large shipment of anchovy to Spain was re-called

Possibly Drunk Pelican Hits Windshield

The Associated Press
Saturday, June 24, 2006; 11:26 PM

LAGUNA BEACH, Calif. -- The driver was sober. The bird that crashed through the windshield of his car might have been flying under the influence.

A California brown pelican probably was intoxicated by a naturally occurring toxin found in algae blooms when she hit the car on the Pacific Coast Highway in Orange County Thursday, wildlife officials said.

October 1991,
Oregon & Washington

Pseudo-nitzschia australis

Methods and reference materials were provided to US-FDA and NOAA for a new monitoring program which later revealed domoic in clams and crabs

Razor Clams

+

Dungeness Crabs

People

Case #3

New Jersey Poisonings, 2002

March 18	New Jersey Poison Center identified several poisoning cases, which followed consumption of pufferfish caught in Titusville, Florida (18 cases turned up eventually) Symptoms: paralysis
March 26	Pufferfish tissue samples are shipped by NJ Health Authority to NRC-IMB
April 2	Samples arrived in Halifax
April 4	Toxin identified and FDA informed of results

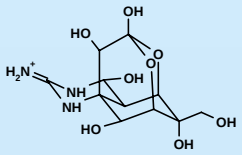
Pufferfish in Florida

- There are over 100 species of pufferfish worldwide
- Nine species occur in Florida

Photo/Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission

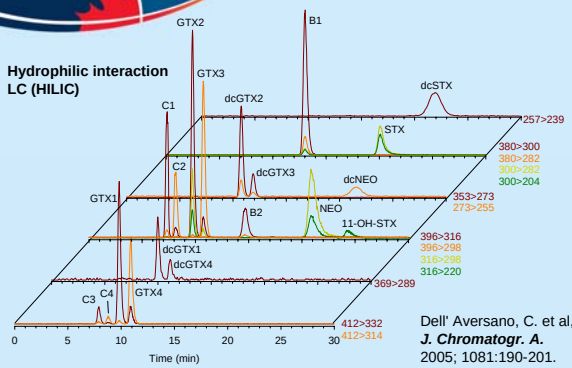
Cause of Poisoning?

- In Japan, pufferfish toxicity is usually related to the presence of tetrodotoxin (TTX) -- the symptoms do match the New Jersey cases



- However, in 2000, approximately 41 tons of pufferfish were sold in the USA with no reported toxic effects
- Was TTX the cause? ... or was another toxin present?

HILIC-MS Method for Polar Toxins

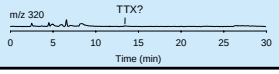


Dell' Aversano, C. et al, ***J. Chromatogr. A.*** 2005; 1081:190-201.

HILIC-MS Method for Polar Toxins

Multi-toxin screening method:

- Hydrophilic interaction LC on Amide-80 column
- CH_3CN/H_2O (65:35) with $HCOOH/HCOONH_4$
- Electrospray ionization
- Selected ion monitoring on API165 MS
- Sample was first extracted with a literature method for TTX -- using aqueous methanol



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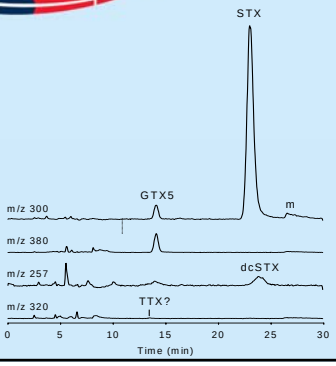


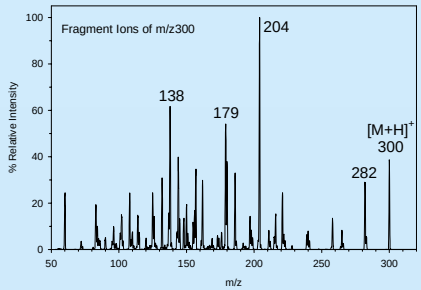
STX
Saxitoxin

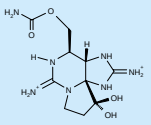
dcSTX
Decarbamoyl-saxitoxin

GTX5
N-sulfocarbamoyl-gonyautoxin

Toxin profile very similar to that from ***Pyrodinium bahamense***

MS/MS Confirmation





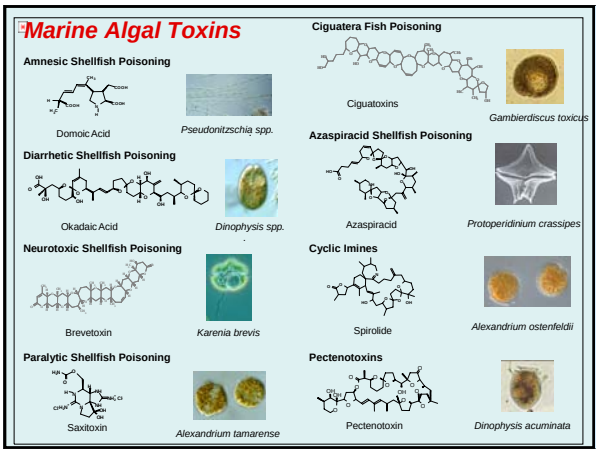
Saxitoxin (STX)

The Complete Story

- What is the origin of the saxitoxins in the pufferfish?

Algae (*Pyrodinium bahamense*)
→ shellfish → puffers

- Puffer fish seem to have a taste for saxitoxins and can accumulate them without paralysis symptoms; they may use this as a chemical defence
- The public was notified and areas posted – no further incidents


Marine Algal Toxins
Amnesic Shellfish Poisoning
Domoic Acid
Pseudonitzschia spp.
Diarrhetic Shellfish Poisoning
Okadaic Acid
Dinophysis spp.
Neurotoxic Shellfish Poisoning
Brevetoxin
Karenia brevis
Paralytic Shellfish Poisoning
Saxitoxin
Alexandrium tamarense
Ciguatera Fish Poisoning
Ciguatoxins
Gambierdiscus toxicus
Azaspiracid Shellfish Poisoning
Azaspiracid
Protoperidinium crassipes
Cyclic Imines
Spirolide
Alexandrium ostenfeldii
Pectenotoxins
Pectenotoxin
Dinophysis acuminata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Thanks!


Thanks for your attention!