ANATOXIN-A IN SEA FIGS ASSOCIATED WITH HUMAN FOOD POISONINGS IN FRANCE

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1 — Symptomatology of intoxication related to the consumption of sea figs

2 — Preliminary investigations
   a. Protocol implemented
   b. Non-targeted analyses (LC-HRMS)

3 — Complementary investigations
   a. Protocol implemented
   b. Targeted analyses (HILIC-MS/MS)
   c. Confirmatory analyses (HILIC-HRMS)

4 — Study of the variability of contamination levels in sea figs
   a. Samples analyzed
   b. Protocol implemented
   c. Targeted analyses (HILIC-MS/MS)

5 — Conclusions and perspectives
1 — Symptomatology of intoxication related to the consumption of sea figs
Poisoning cases involving sea figs

- Sea figs of the genus Microcosmus, fished in the Mediterranean. Products highly prized for their iodized taste
- 20 poisoning cases between January 2011 and January 2020
- 30 people involved
- 20 women and 10 men
- Aged 17 to 80 years

Symptoms:
- Vomiting
- Dizziness
- Tremors
- Paresthesia
- Nausea
- Hypersudation
- Diplopia, visual disturbances
- Difficulty concentrating
- Diarrhea
- Muscle cramps
- Abdominal cramps
- Headaches
- Ringing in the ears
- Ataxia
- Asthenia

Schmitt et al. (2019). Cerebellar syndrome associated with ingestion of Mediterranean Microcosmus: a French case series

Sometimes fortuitous nature of the reports!!

Case of a patient who took an oral medication intended for vaginal administration

Symptoms actually due to consumption of sea figs!!

Attention, les gélules ne sont pas réservées qu'à la voie orale !

Capsules are not only for oral use
2 — Preliminary investigations
Protocol implemented

Analysis of regulated toxins
- Lipophilic toxins
- Domoic acid
- Saxitoxins
- Hemolytic activity

Complementary investigations
- Non-targeted analysis (high resolution mass spectrometry – LC-HRMS)

Contamination of sea figs with ATX

Test portion
Homogenate
Grinding
Extraction
Centrifugation
Extract
Pellet

Extraction
Toxins

Organic / aqueous solvent
Non-targeted analysis - LC-HRMS (I. Dom PhD thesis)

1. Non discriminating extraction (lipophilic)

2. LC-HRMS analysis
   - suspect screening (screening of a list of toxins)
   - looking for unknown compounds (unbiased analysis)

Complementary investigations

LC-MS/MS
high resolution

API 5600 QTof

Contamination of sea figs with ATX
Non-targeted analysis - LC-HRMS (I. Dom PhD thesis)

List of 820 toxins (marine and freshwater toxins)

ền Suspicion of anatoxin-a (ATX) but

- Chromatographic method not adapted (co-elution of ATX-a and Phe, isobaric compounds)
- ATX found in 1 sample but for 1 replicate only (out of 3)
- unexpected result (freshwater cyanotoxin in a marine organism)

Need for additional analyses

 halk Method adapted for hydrophilic toxins (cyanotoxins) → HILIC

Contamination of sea figs with ATX
3 — Complementary investigations
Protocol implemented

Aqueous solvent

Test portion

Homogenate

Grinding

SPE

Extraction

Hydrophilic toxins

Centrifugation

Extract

LCMS analyses in low and high resolution (HILIC-MS)

TSQ Vantage (low resolution)

API 5600 Qtof (high resolution)

Low (LR) versus high resolution (HR)

Contamination of sea figs with ATX

2022/03/20
Targeted analyses: HILIC-MS/MS (low resolution)

Analytical method developed by the national reference laboratory for marine biotoxins as part of the monitoring of emerging compounds in France (EmergTox)

In low resolution, no mass distinction (165.1 Da)!

→ Prerequisite: need for good chromatographic separation ATX / Phe
Targeted analysis - HILIC-MS/MS (low resolution)

Verification of the presence of ATX in the sea fig extracts

- Presence of a single peak at the retention time of ATX
- Increase in ATX peak intensity correlated with the amount of ATX added to the sea fig extract

Contamination of sea figs with ATX
Targeted analysis - HILIC-MS/MS (low resolution)

ATX concentrations found in the sea fig samples

| Samples            | ATX concentration (µg/kg) |
|--------------------|---------------------------|
| FP-1-2011          | 194                       |
| FP-6-2012          | 1240                      |
| FP-17-2018         | 1133                      |
| Sea fig Control    | 22                        |
| Mussel control     | < LOD*                    |

(*) LOD = limit of detection (8 µg/kg)
Confirmatory analyses: HILIC-MS/MS (high resolution)

ATX standard

Sea fig sample

Same retention time

Same MS/MS spectrum

Contamination of sea figs with ATX
Confirmatory analyses: HILIC-MS/MS (high resolution)

List of ATX analogues searched in the sea fig samples

| Toxin                     | Formula   | Mass (Da)  | Extraction Mass [M + H]^+ (Da) |
|---------------------------|-----------|------------|---------------------------------|
| ATX-a                     | C10H15NO  | 165.11536  | 166.12264                       |
| hATX-a                    | C11H17NO  | 179.13101  | 180.13829                       |
| Carboxy ATX-a             | C11H15NO3 | 209.10519  | 210.11247                       |
| Carboxy hATX-a            | C12H17NO3 | 223.12084  | 224.12812                       |
| Carboxy dihydroATX-a      | C11H17NO3 | 211.12084  | 212.12812                       |
| N-methyl ATX a            | C11H17NO  | 179.13101  | 180.13829                       |
| (10S)-ATX alcohol         | C10H17NO  | 167.13101  | 168.13829                       |
| (10R)-ATX alcohol         | C10H17NO  | 167.13101  | 168.13829                       |
| nor ATX-a                 | C9H13NO   | 151.09971  | 152.10699                       |
| Dihydro ATX-a             | C10H17NO  | 167.13101  | 168.13829                       |
| Dihydro hATX-a            | C11H19NO  | 181.14666  | 182.15394                       |
| Epoxy ATX-a               | C10H15NO2 | 181.11028  | 182.11756                       |
| Epoxy hATX-a              | C11H17NO2 | 195.12593  | 196.13321                       |
| ATX-(a)s                  | C7H17N4O4P| 252.09874  | 253.10602                       |
| Phe                       | C9H11NO2  | 165.07898  | 166.08626                       |

None of the ATX analogues were found in the sea fig samples (non-targeted analyses in "suspect screening" mode)
4 — Study of the variability of contamination levels in sea figs
Samples analyzed

- **Microcosmus sulcatus**
  - Area: FAO 37.2.1, Adriatic Sea (Croatia)
  - Sample related to a food poisoning in the Gard department (Jan. 2020) → 3 people (2×W 54 y/o, W 17 y/o). Important quantities of sea figs eaten by the two 54-y/o women.

- **Microcosmus sulcatus**
  - Area: FAO 37.2, Central Mediterranean Sea
  - Sample coming from a store in the Var department (Géant Casino of Hyères), not related to any food poisoning.

- **Microcosmus sp.**
  - Thau lagoon
  - Sample bought from a fisherman, not related to any food poisoning.
Samples analyzed

Sample preparation

- Cleaning
- Shucking
- Weighing of each individual (flesh versus liquid/exudate)

- 9 individuals
  - Total weight of the animals 12.4 – 38.4 g
  - Mean = 24.7 g, SD = 9.0 g
  - Flesh/liquid ratio: 0.5 – 3.3

- 12 individuals
  - Total weight of the animals 20.7 – 86.6 g
  - Mean = 49.0 g, SD = 21.9 g
  - Flesh/liquid ratio: 0.4 – 1.2

- 5 individuals
  - Total weight of the animals 15.3 – 59.0 g
  - Mean = 32.9 g, SD = 17.7 g
  - Flesh/liquid ratio: 0.5 – 0.8

Contamination of sea figs with ATX
Analysis in low resolution (HILIC-MS)

Protocol implemented

Extract

Hydrophilic toxins

Contamination of sea figs with ATX

2022/03/20
Contamination of sea figs with ATX
Conclusion: These preliminary results show that it is not possible to generalize about the existence or not of a correlation between the size of the sea figs and their contamination level. Several factors come into play.
Suspicion of the presence of hATX in 1/5 sea fig of the Thau lagoon (LC-MS low resolution)

- Specific transitions of hATX
- But RT < that of the hATX standard

- Different HRMS2 fragmentation spectra for the hATX standard and the violet
- However, some low mass fragments are common → not hATX but related compound?
Conclusions and perspectives

- Evidence of ATX-a in violets involved in TIAC cases (low and high resolution)
- High variability of contamination of samples (194 - 1240 µg/kg)
- Need for further investigations to know if ATX-a is indeed responsible for the intoxication cases
- What about contamination of other marine organisms?
Results published in May 2020 in an A+ journal (IF 4,379)

Contamination of sea figs with ATX
