

Implementation of analytical approaches for a first evaluation of risk associated to ciguatoxins in New Caledonia

Manoëlla Sibat, Simon Tanniou, Korian Lhaute, Florence Antypas, Philipp Hess, Thierry Jauffrais

Context in New Caledonia



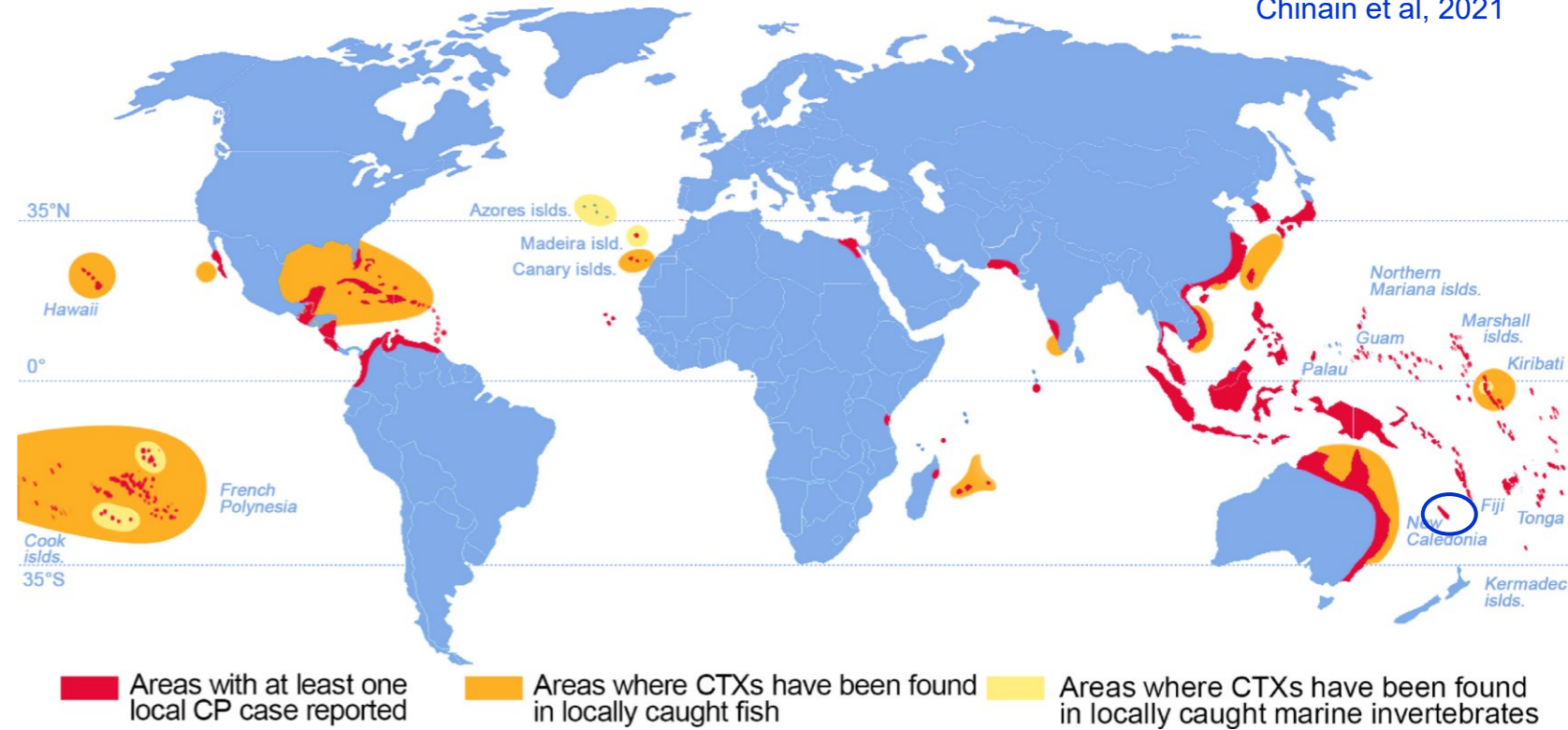
- New Caledonia is a french territory located in the South Pacific
- Major economic activities: Tourism, reef and lagoon fisheries, aquaculture
- Like other islands in the Southwestern Pacific HAB have increasingly been reported in recente decades

- Impact of HAB and associated phycotoxins are poorly documented
- A lack of knowledge regarding potential sanitary and socioeconomic threats



Ciguatera poisoning in NC

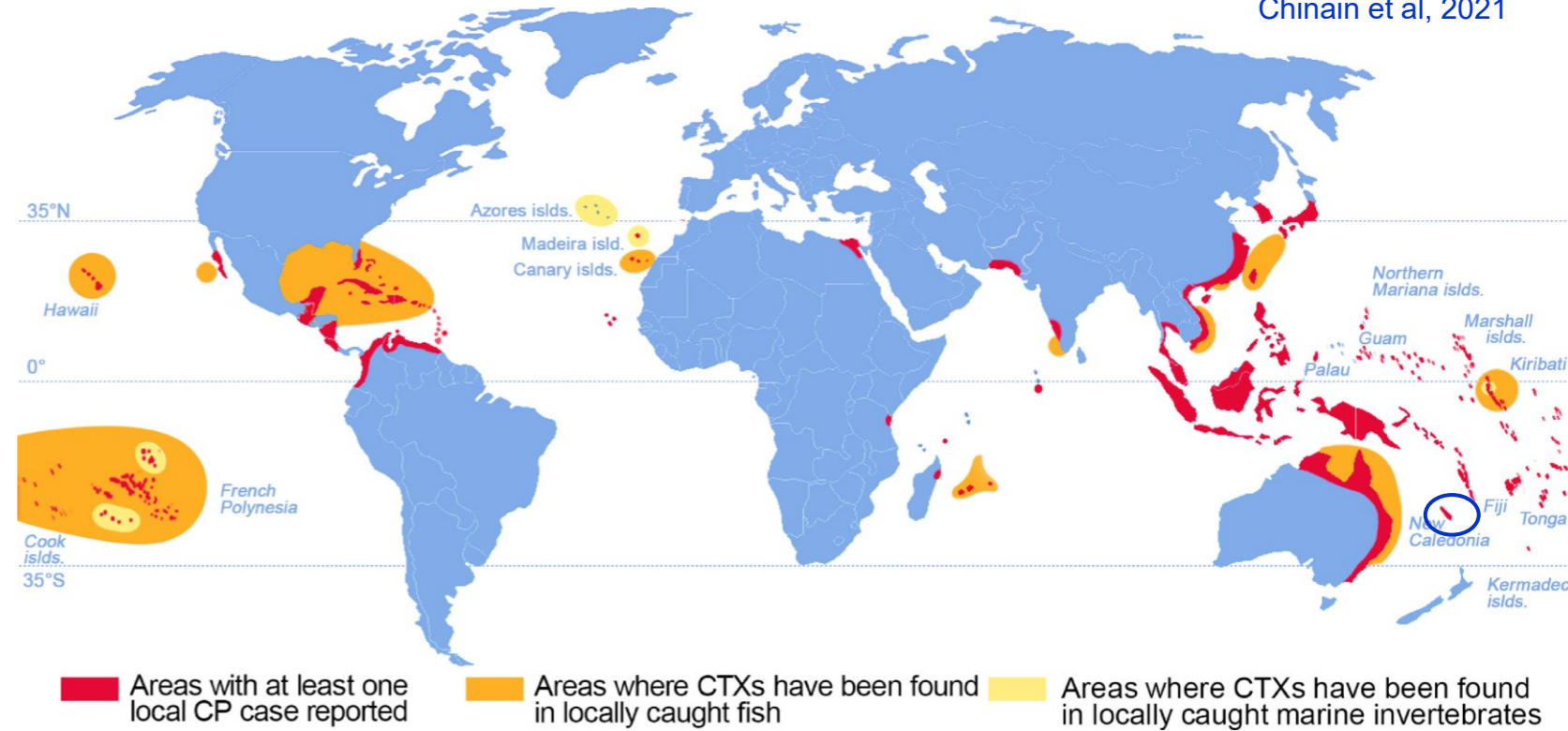
- Causative organisms : unknown
- CTXs compounds responsible : unknown
- Toxin profile : unknown



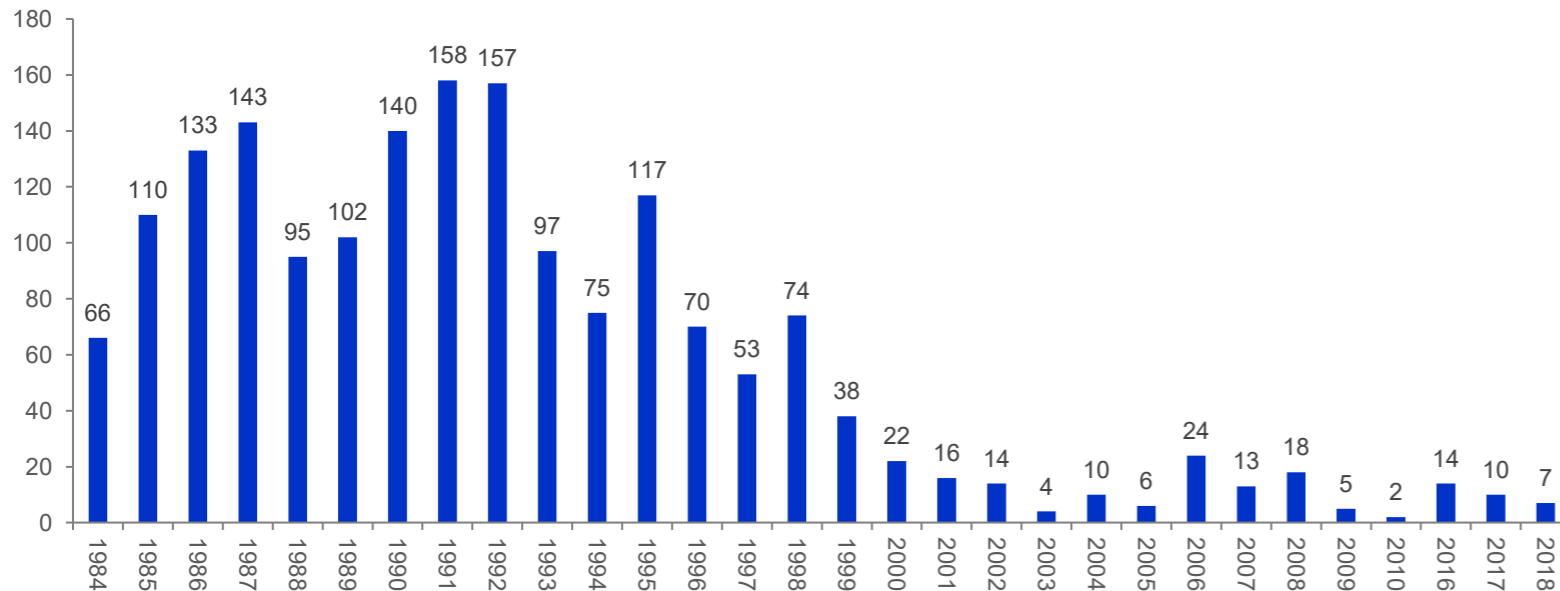
Ciguatera poisoning in NC

Chinain et al, 2021

- Causative organisms : unknown
- CTXs compounds responsible : unknown
- Toxin profile : unknown



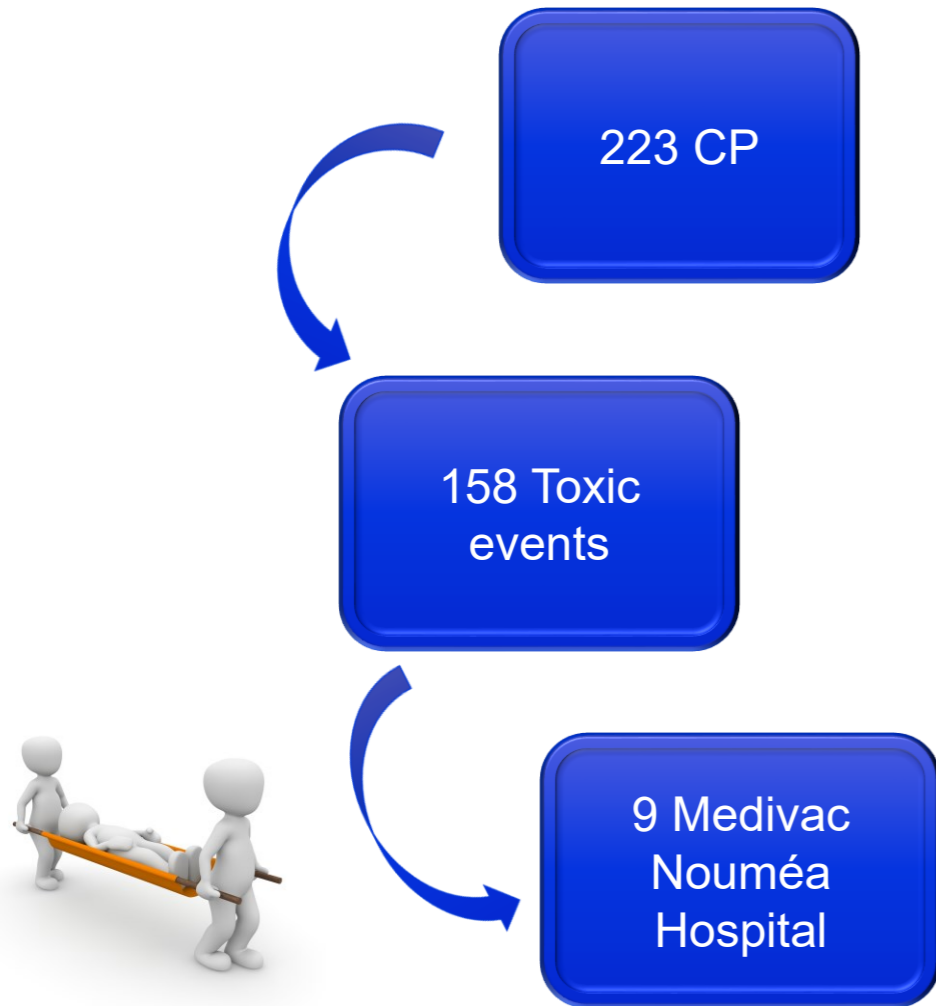
Number of reported cases per year



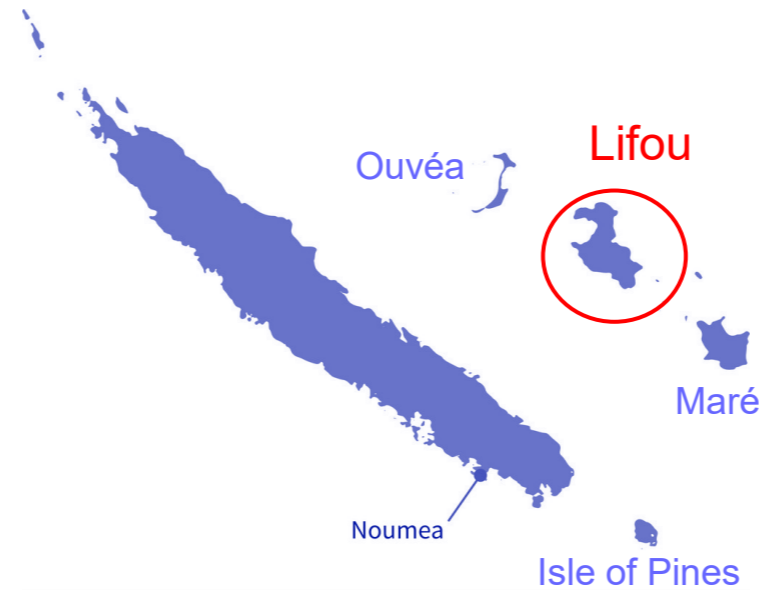
DASS, 2017

- Health authorities no longer alerted
- One major problem: cases are no longer reported to the authorities
- Locals self-medicate (traditional remedies)

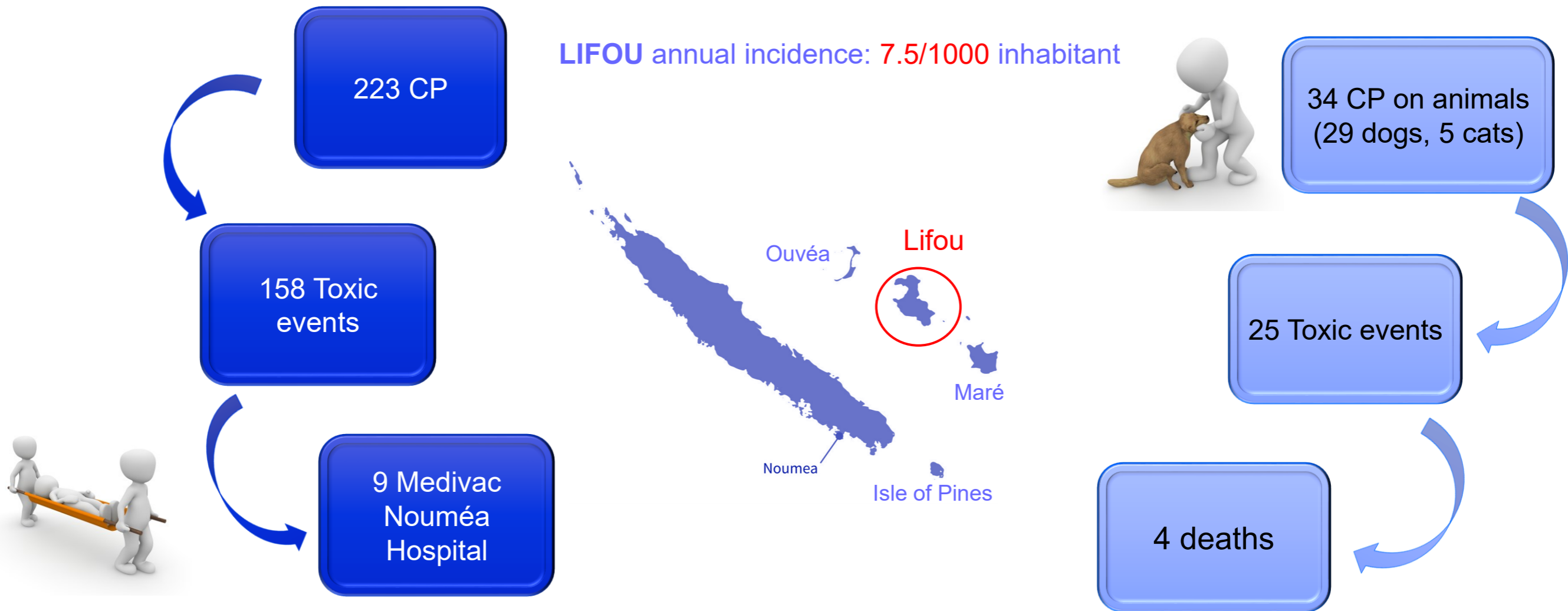
Epidemiological study in Lifou from 2017 to 2020



LIFOU annual incidence: 7.5/1000 inhabitant



Epidemiological study in Lifou from 2017 to 2020



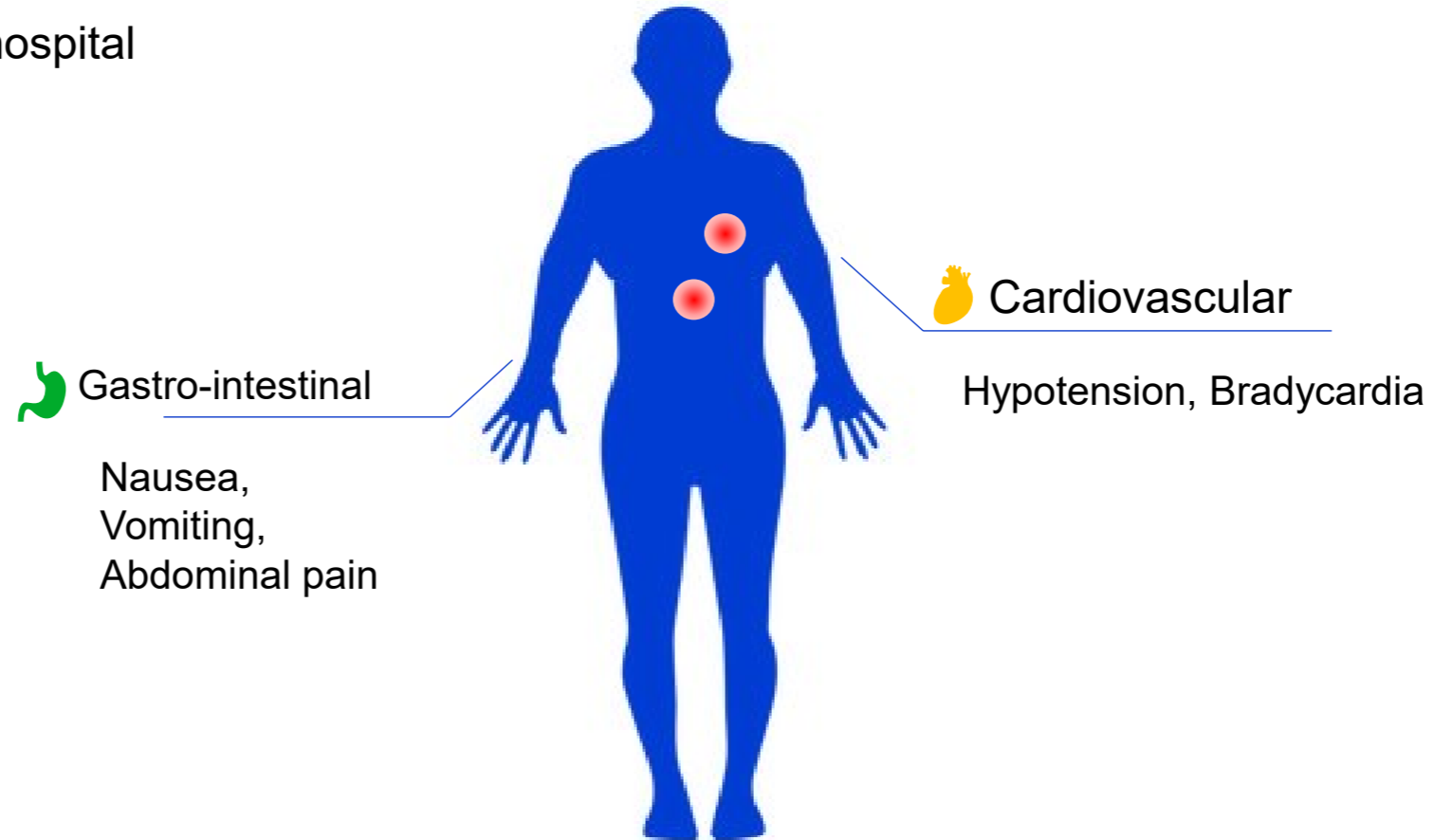
Food poisoning cases reported in Lifou 2021&2022

Dr Y. M. Ducrot (Doctor)
Dr A. Barnaud (Veterinary)

CP 1: Leopard coralgroupers

3 persons 3 Medivac to Nouméa hospital

Symptoms 🍷 +++ 🫀 +++



Food poisoning cases reported in Lifou 2021&2022

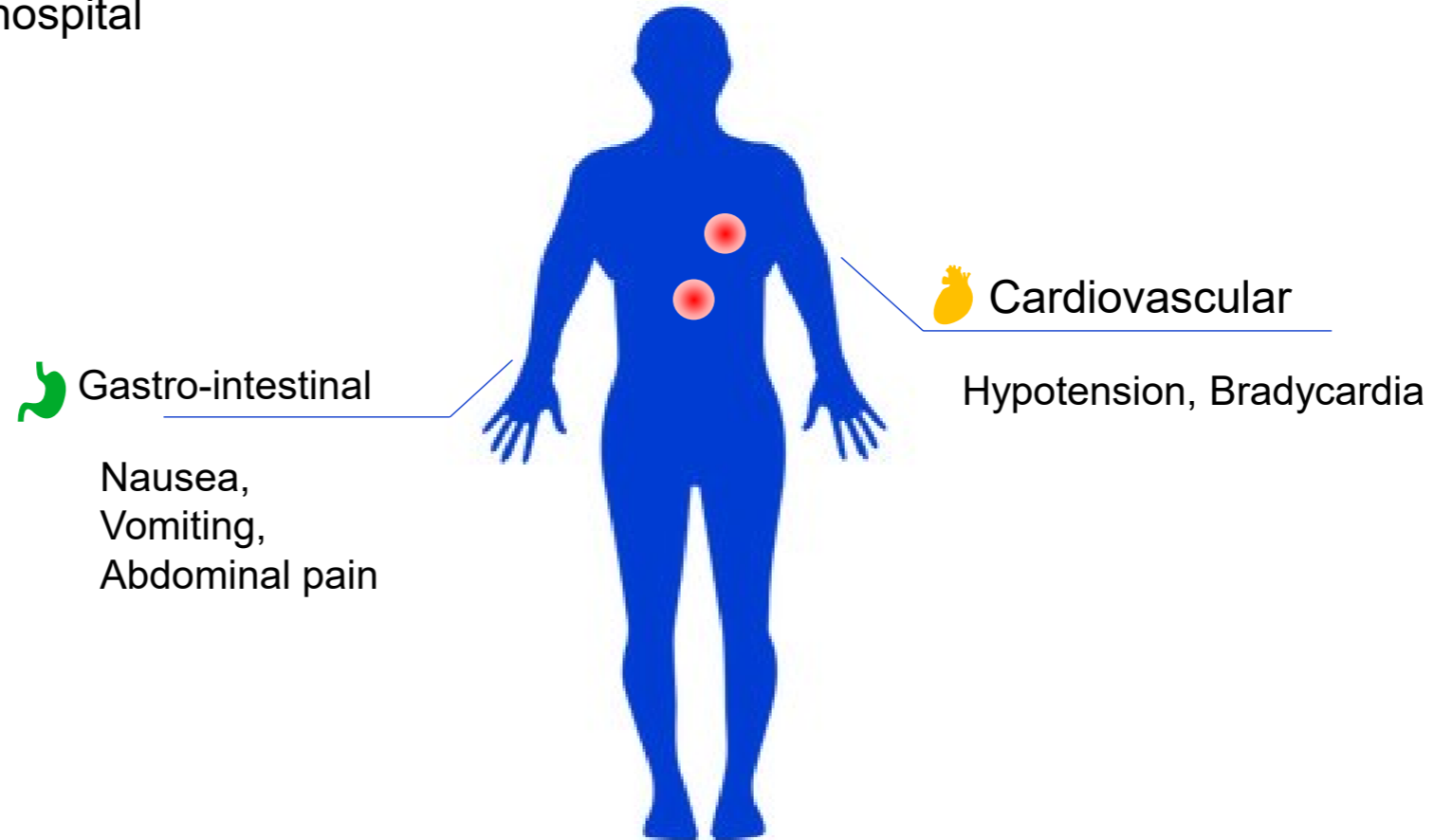
CP 1: Leopard coralgroupier

3 persons 3 medivac to Nouméa hospital

Symptoms 🍷 +++ 🫀 +++

CP 2: Common Silver-biddy

1 person Symptoms 🍷+ 🫀+



Food poisoning cases reported in Lifou 2021&2022

CP 1: Leopard coralgroupier

3 persons 3 medivac to Nouméa hospital

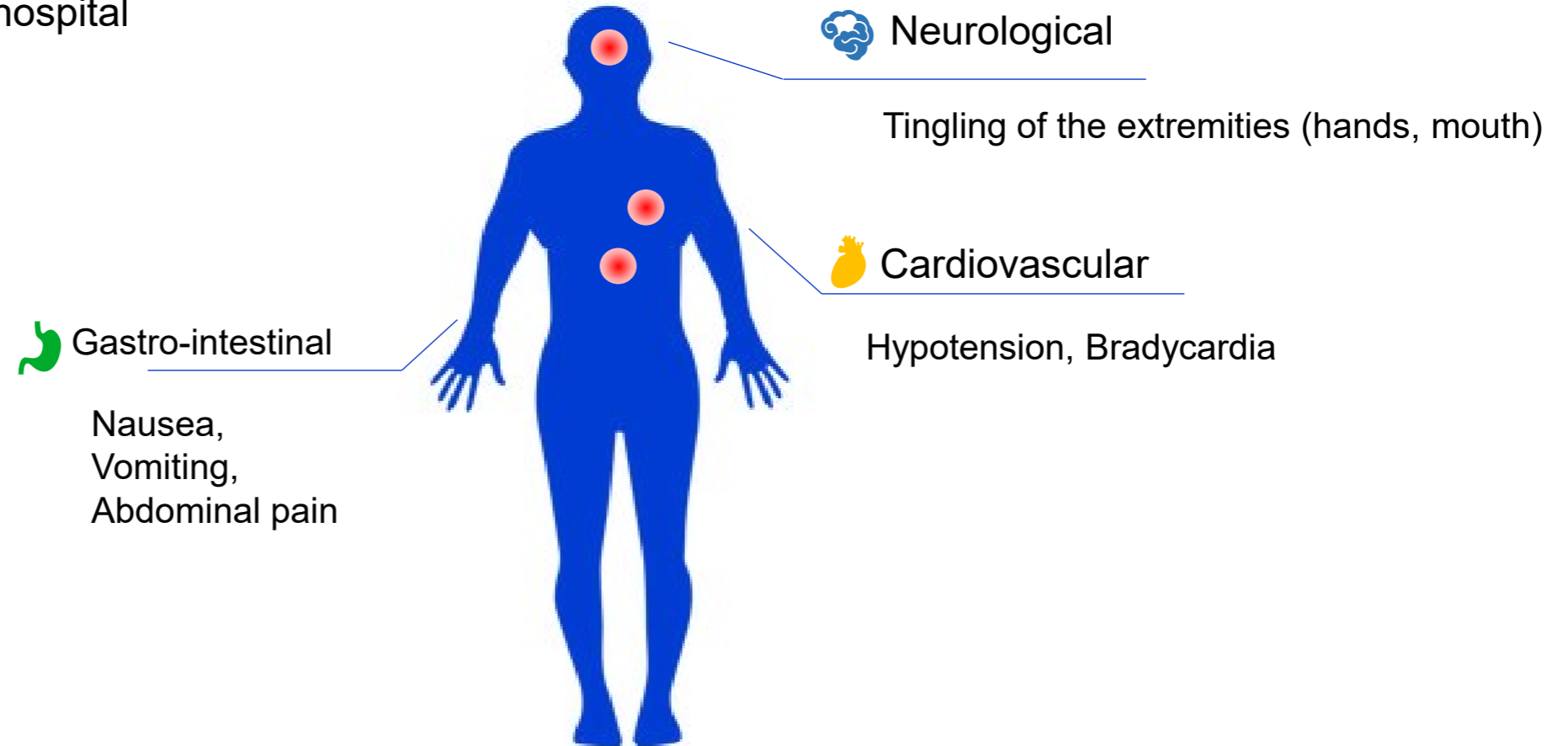
Symptoms 🍷 +++ 🍷 +++

CP 2: Common Silver-biddy

1 person 🍷 + 🍷 +

CP 3: Golden trevally

1 person 🍷 + 🍷 ++ 🧠 ++



Food poisoning cases reported in Lifou 2021&2022

CP 1: Leopard coralgrouper

3 persons 3 medivac to Nouméa hospital

Symptoms 🍷 +++ 🍷 +++

CP 2: Common Silver-biddy

1 person 🍷 + 🍷 +

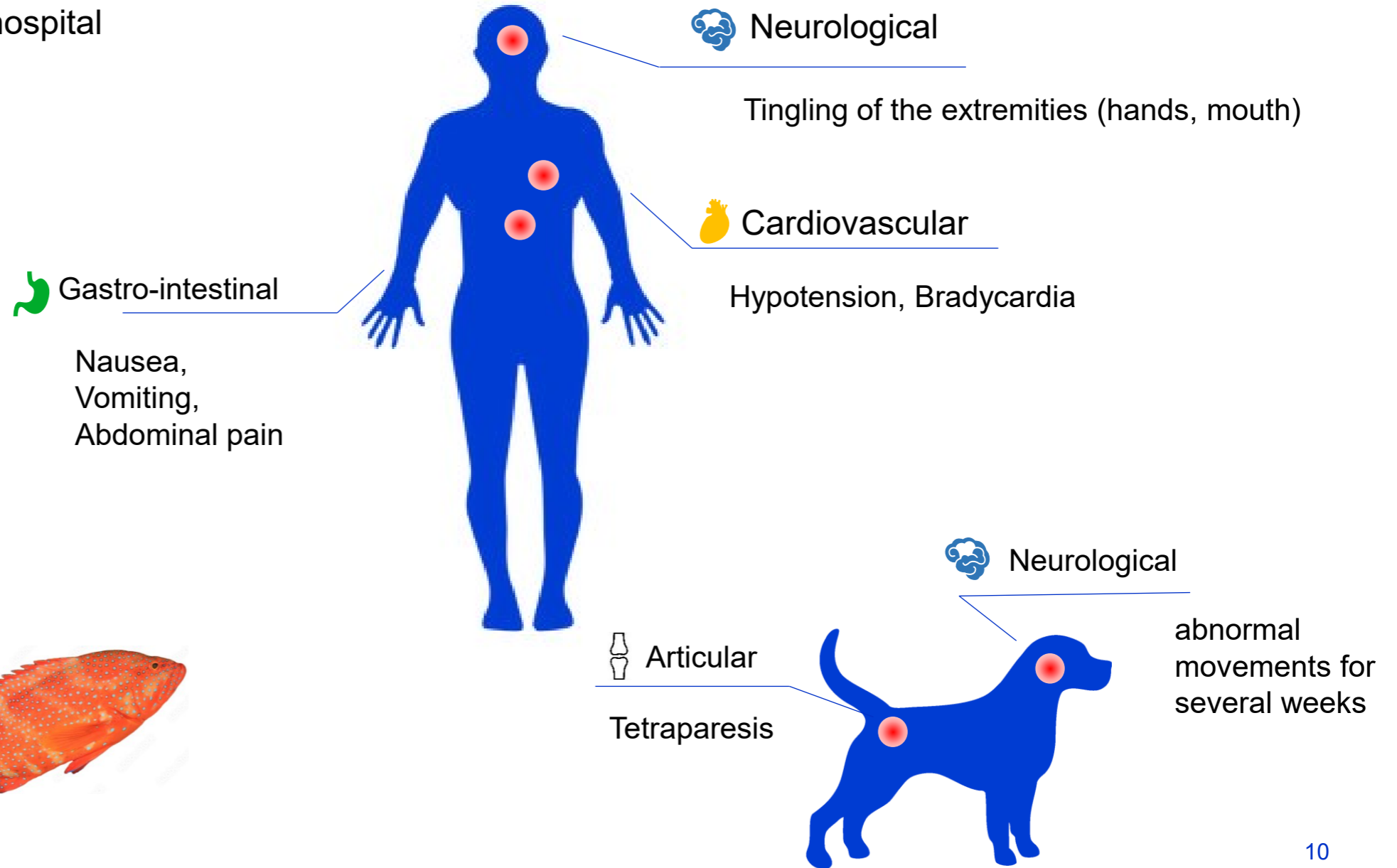
CP 3: Golden trevally

1 person 🍷 + 🍷 ++ 🧠 ++

CP 4: Leopard coralgrouper

2 persons 🍷 ++ 🍷 🧠 ++

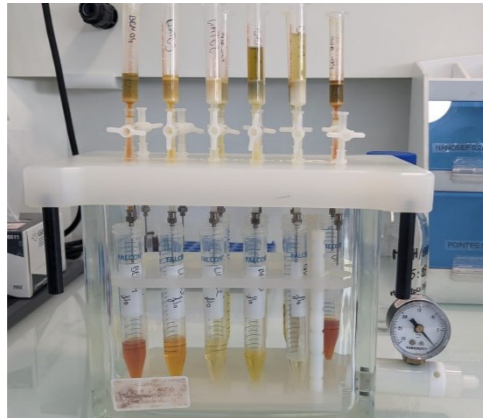
Dog 🦴 ++ 🧠 +++



Method

Extraction

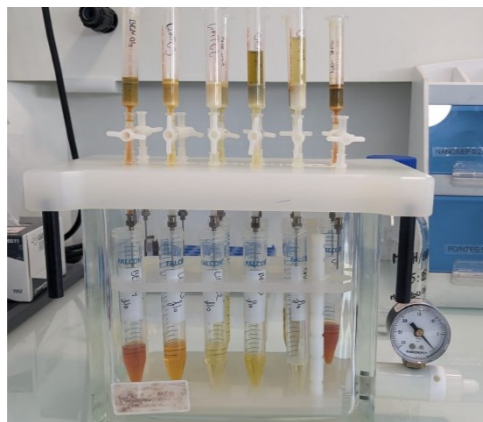
- Freeze dried fish flesh
- MeOH 90%
- Hexane clean up
- L/L partitioning MeOH 60% / DCM
- Two-step SPE purification
 - Florisil Si
 - C18



Method

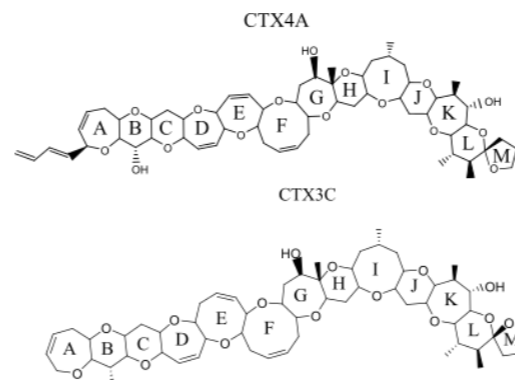
Extraction

- Freeze dried fish flesh
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LC-MS/MS

- API 4000 Qtrap (Sciex)
- CTX3C-type and CTX1B type (> 20 compounds)
- Mode: ESI⁺ MRM (33 pairs)
- 3 MRM transitions per toxin
- LC conditions: Zorbax C18 50*2.1mm (1.8μm)
- Eluent A H₂O } + 2 mM Ammonium formate
- Eluent B MeOH } + 50 mM Formic acid
- Quantification: CTX3C standard (from ILM)



Method

Extraction

- Freeze dried fish flesh
- MeOH 90%
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- L/L partitioning MeOH 60% / DCM
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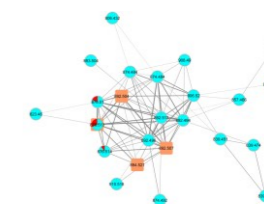
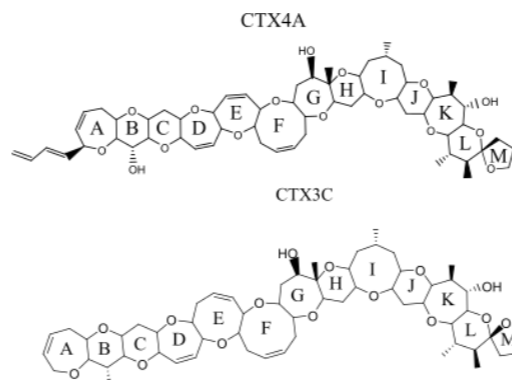
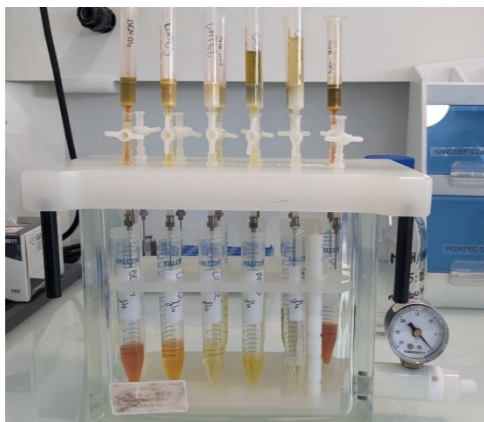
Sibat et al, 2018

LC-MS/MS

- API 4000 Qtrap (Sciex)
- CTX3C-type and CTX1B type (> 20 compounds)
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- Eluent B MeOH } + 50 mM Formic acid
- Quantification: CTX3C standard (ILM)

HRMS/MS

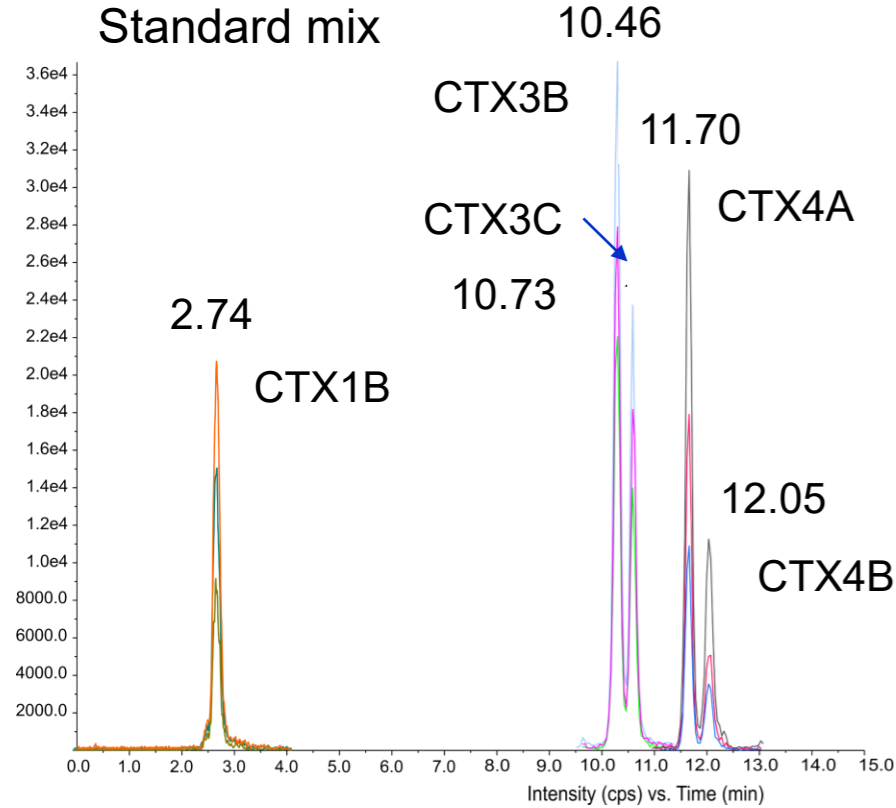
- QTOF 6550 (Agilent technologies)
- Same LC conditions as LRMS
- ESI⁺ full scan mode (*m/z* 100 to 1700)
- ESI⁺ autoMSMS mode
- Data processing using **MZmine 3**
- Generate Molecular Network using **GNPS**
- Visualization with **Cytoscape**



Ifremer

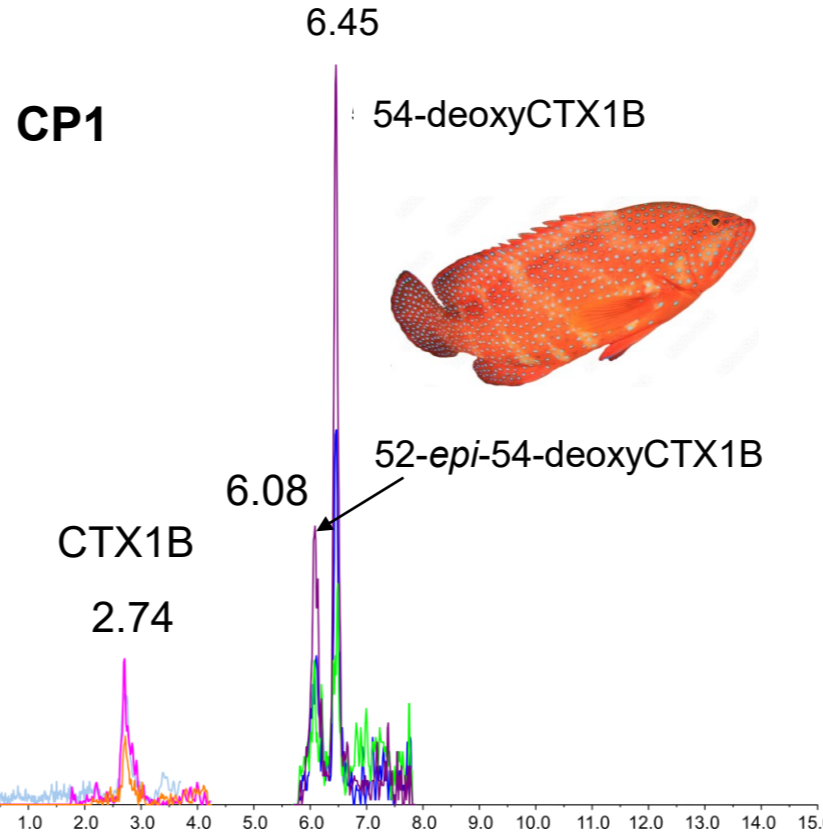
Quantitative Results and LC-MS/MS Chromatograms

Standard mix



CP2 No P-CTXs detected

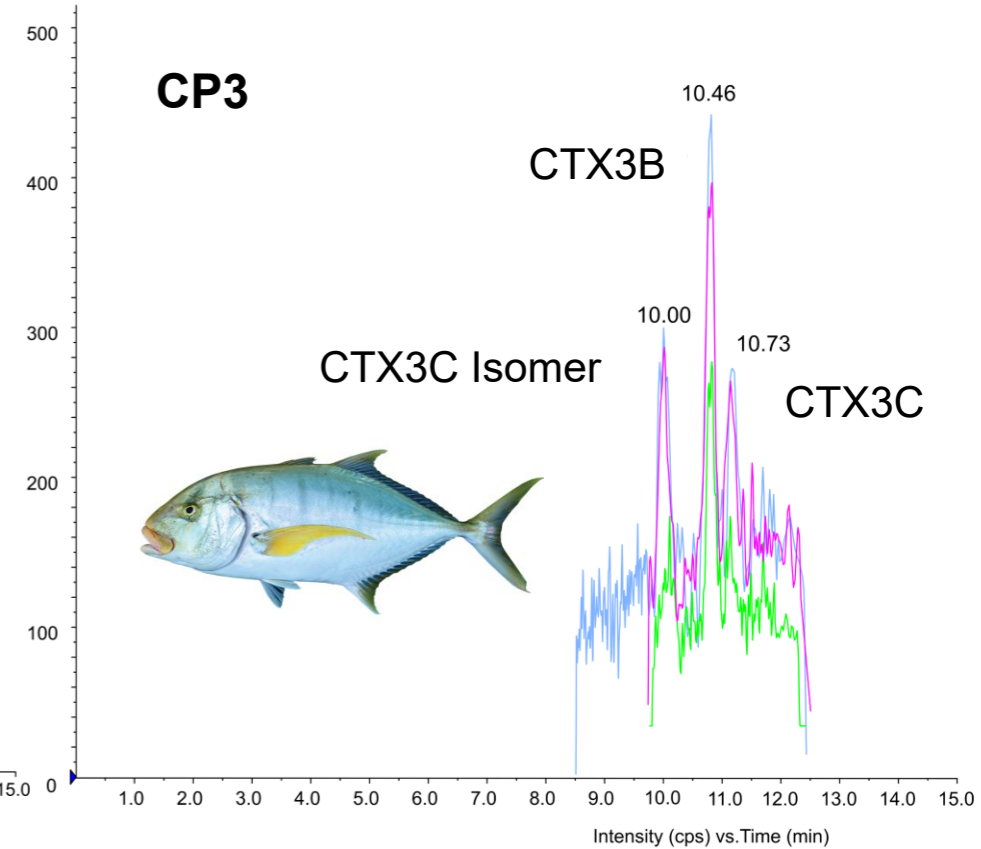
EFSA Threshold > 0.01 $\mu\text{g CTX1B eq. Kg}^{-1}$



$\text{CTX1B} = 1.98 \mu\text{g Kg}^{-1}$
 $52\text{-epi-54-deoxyCTX1B} = 1.52 \mu\text{g Kg}^{-1}$
 $54\text{-deoxyCTX1B} = 2.52 \mu\text{g Kg}^{-1}$

Total 5.32 $\mu\text{g eq CTX3C Kg}^{-1}$
1.06 $\mu\text{g eq CTX1B Kg}^{-1}$

CP3



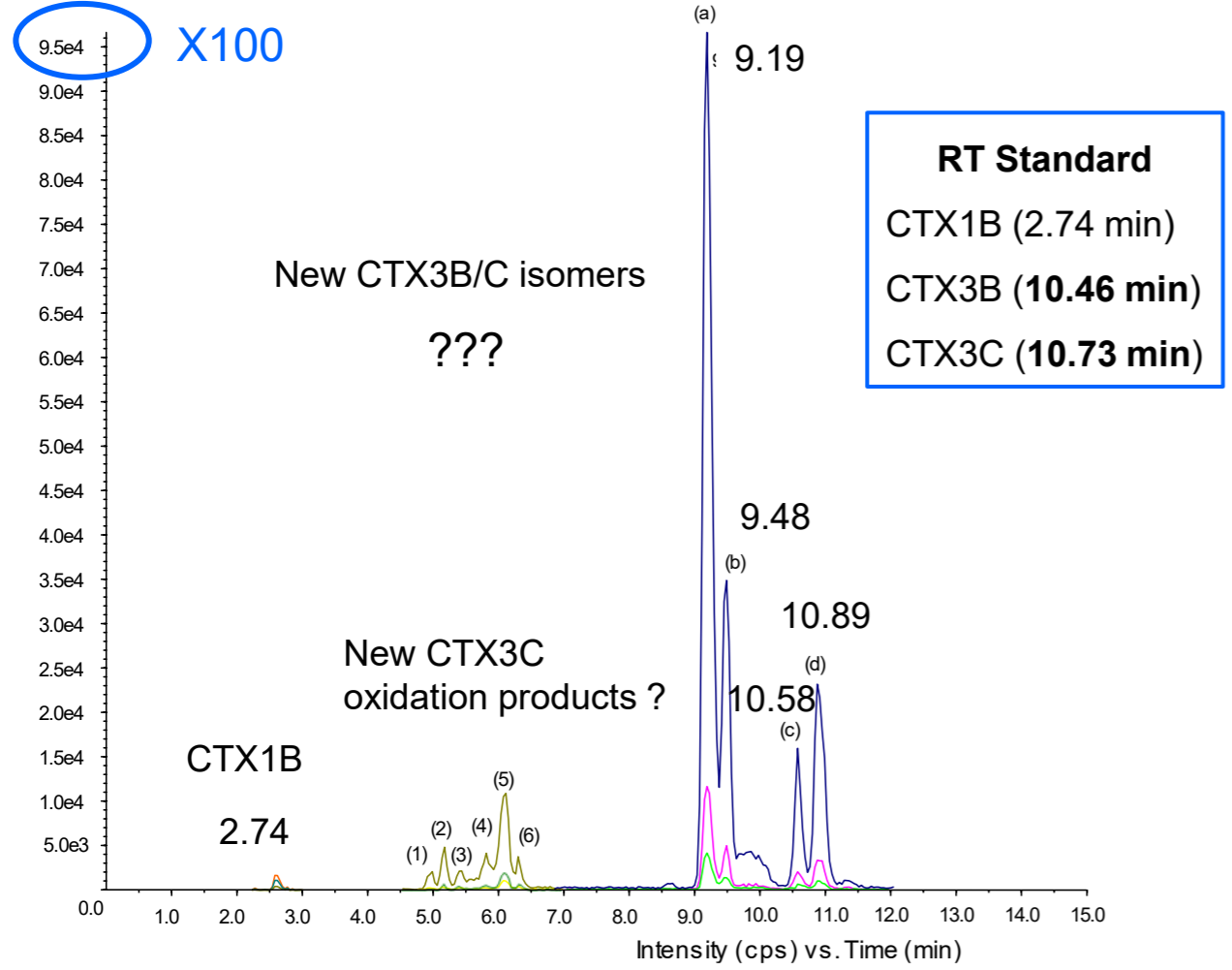
$\text{CTX3B} = 0.16 \mu\text{g Kg}^{-1}$
 $\text{CTX3C} = 0.35 \mu\text{g Kg}^{-1}$
 $\text{CTX3C isomer} = 0.27 \mu\text{g Kg}^{-1}$

Total 0.78 $\mu\text{g CTX3C eq Kg}^{-1}$
0.16 $\mu\text{g CTX1B eq Kg}^{-1}$

LC-MS/MS Chromatograms of CP4



Intense peaks !



- A cluster of 4 intense peaks (9-11min)
- 3 MRM transitions of CTX3B/C
- RT drift 1.15%
- Ion ratios are different

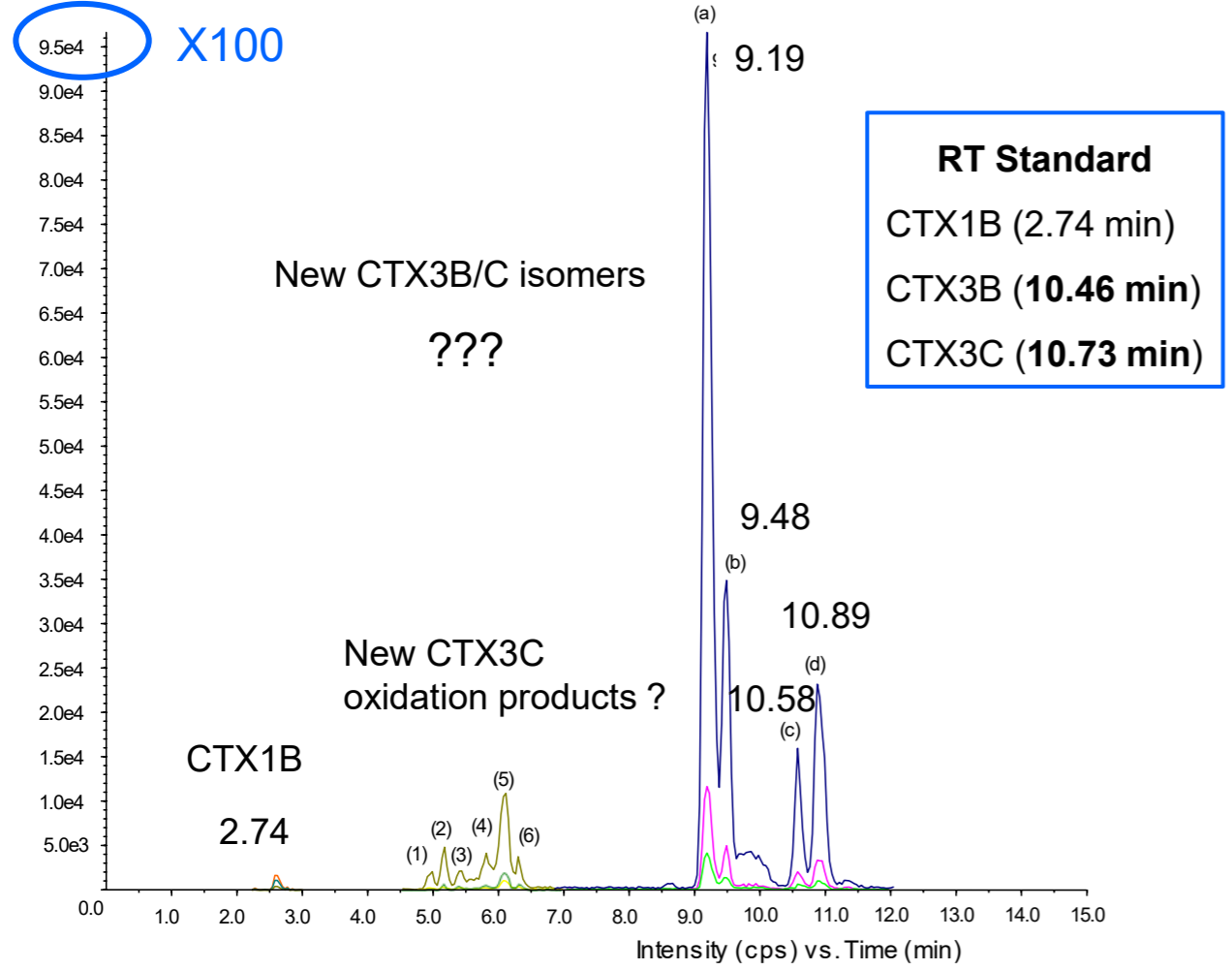
MRM transitions :

—	1023.5/1005.6	} CTX3B/C
—	1040.5/1005.6	
—	1023.5/125.1	
—	1057.6/1039.6	} 2,3-diOHCTX3C
—	1074.5/1039.6	
—	1039.6/125.1	

LC-MS/MS Chromatograms of CP4



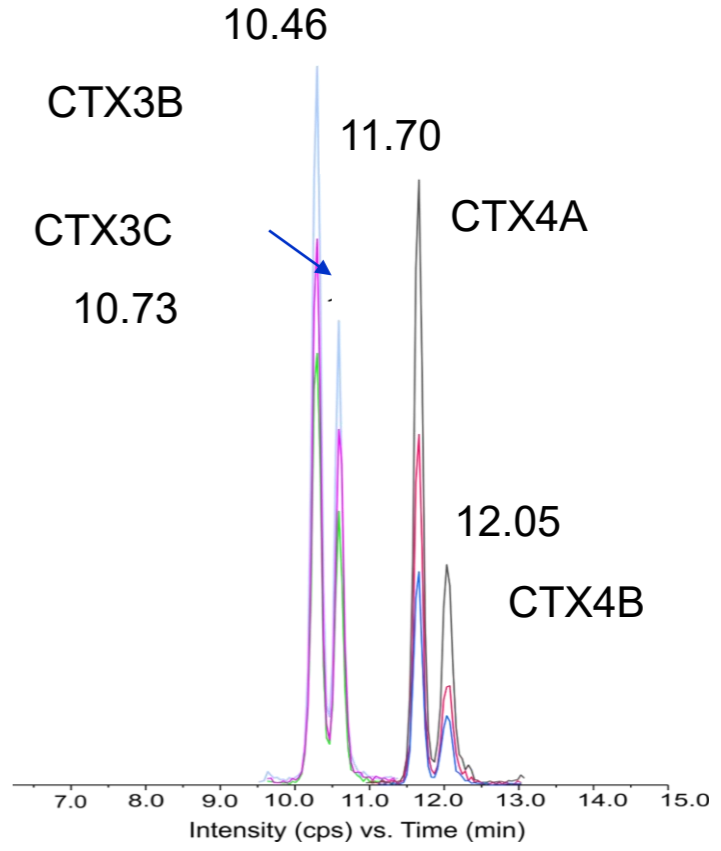
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Standard mix

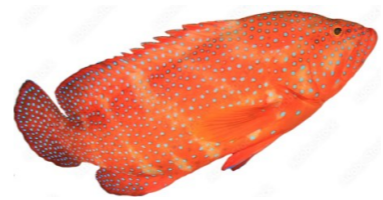
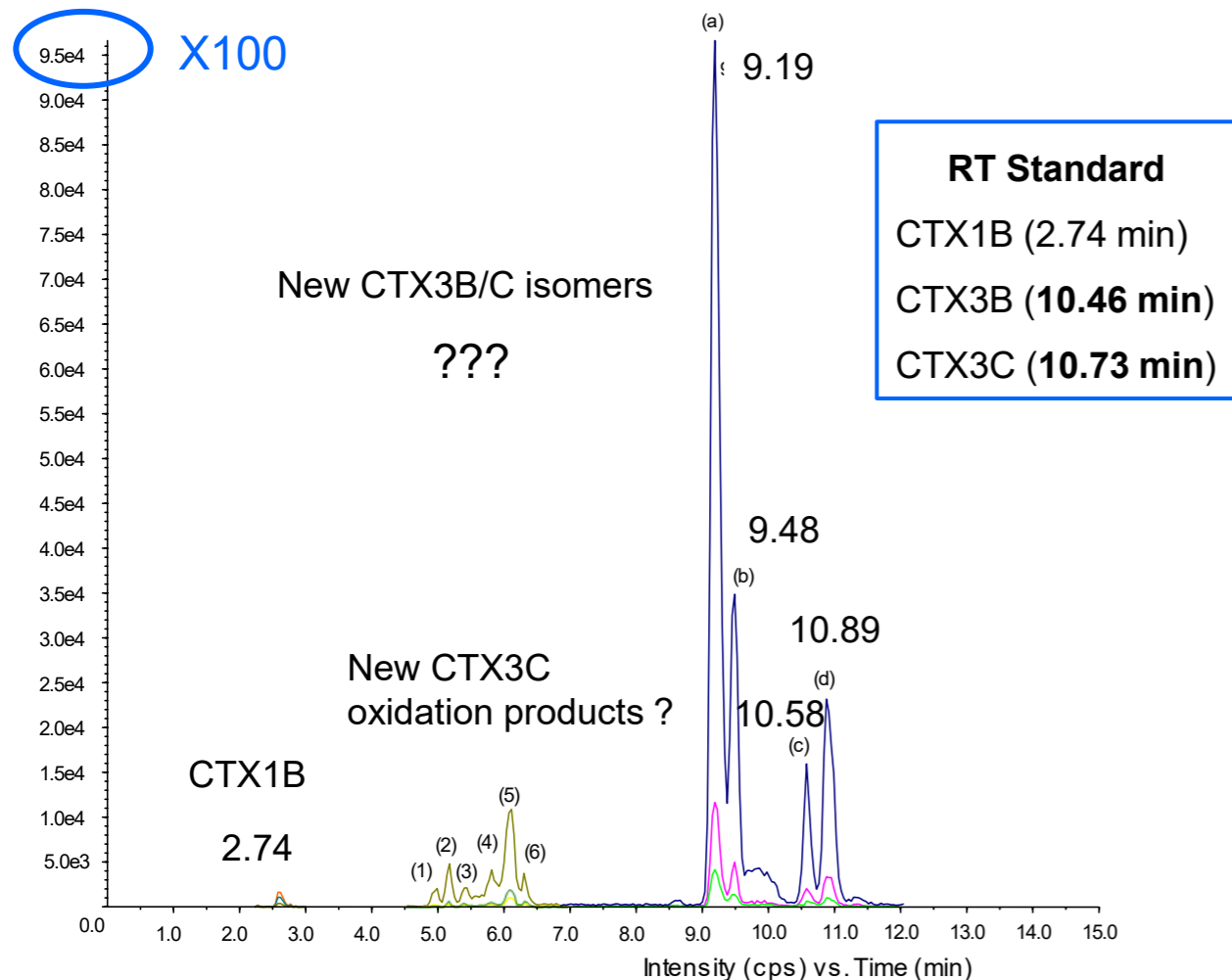


MRM transitions :

—	1023.5/1005.6	} CTX3B/C
—	1040.5/1005.6	
—	1023.5/125.1	} 2,3-diOHCTX3C
—	1057.6/1039.6	
—	1074.5/1039.6	
—	1039.6/125.1	

LC-MS/MS Chromatograms of CP4

Intense peaks !

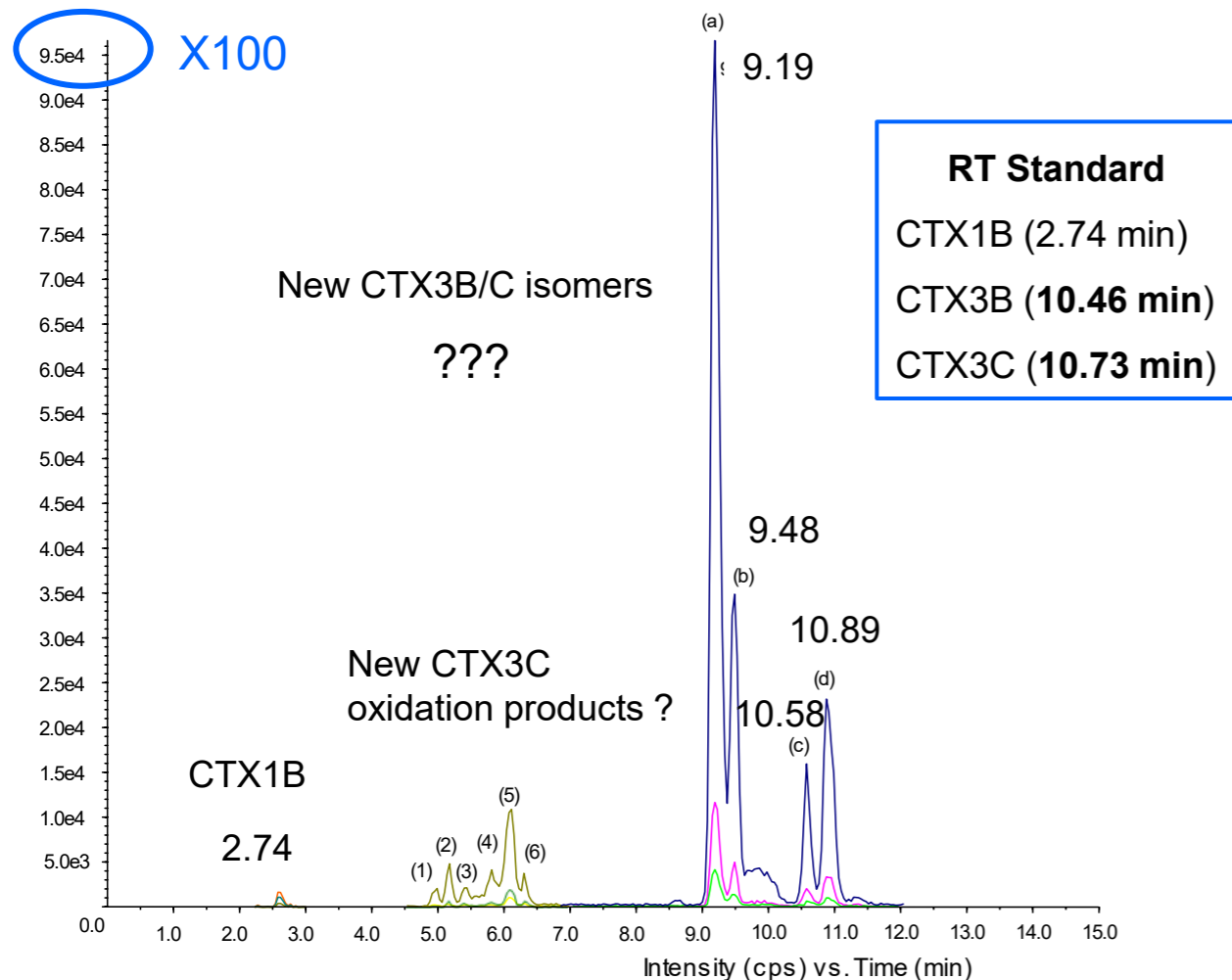


- A cluster of 4 intense peaks (9-11min)
- 3 MRM transitions of CTX3B/C
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- Ion ratios are different

- Cluster of 6 peaks (5-7 min)
- 3 MRM transitions of 2,3-diOH-CTX3C
- RT corresponding to CTX3C oxidation products

LC-MS/MS Chromatograms of CP4

Intense peaks !



MRM transitions :

—	1023.5/1005.6	} CTX3B/C
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—	1023.5/125.1	} 2,3-diOHCTX3C
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—	1074.5/1039.6	
—	1039.6/125.1	



- A cluster of 4 intense peaks (9-11min)
- 3 MRM transitions of CTX3B/C
- RT drift 1.15%
- Ion ratios are different

- Cluster of 6 peaks (5-7 min)
- 3 MRM transitions of 2,3-diOH-CTX3C
- RT corresponding to CTX3C oxidation products

- Only CTX1B confirmed
- CTX1B = 0.256 µg eq. CTX1B Kg⁻¹

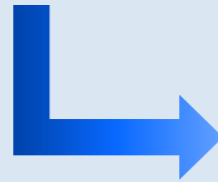
Analytical approach for P-CTXs

LRMS
API4000QTrap

MRM mode

- 3 MRM transitions per toxin
- RT same as the standard
- Compare Ion ratios

Targeted approach



EPI mode

- To have a MS/MS spectra
- To compare with standard or literature



Analytical approach for P-CTXs

LRMS
API4000QTrap

MRM mode

- 3 MRM transitions/toxins
- RT same as the standard
- Compare Ion ratios

Targeted approach

EPI mode

- To have a MS/MS spectra
- To compare with standard or litterature

HRMS
QTOF 6550

Full scan
mode

- To have a HRMS spectra
- To find Exact mass

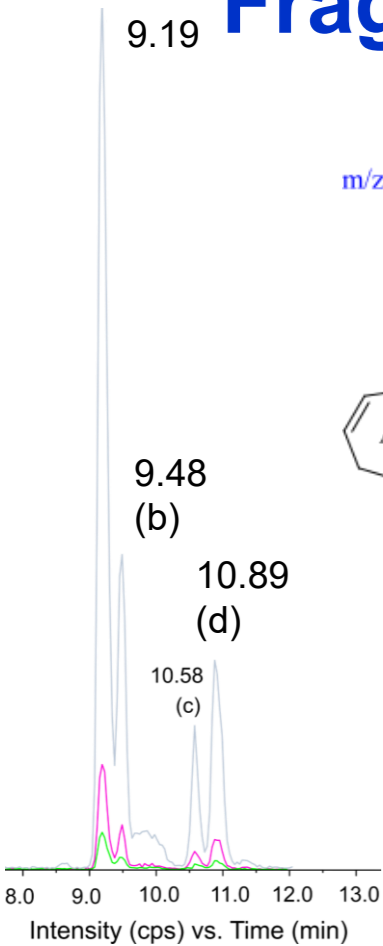
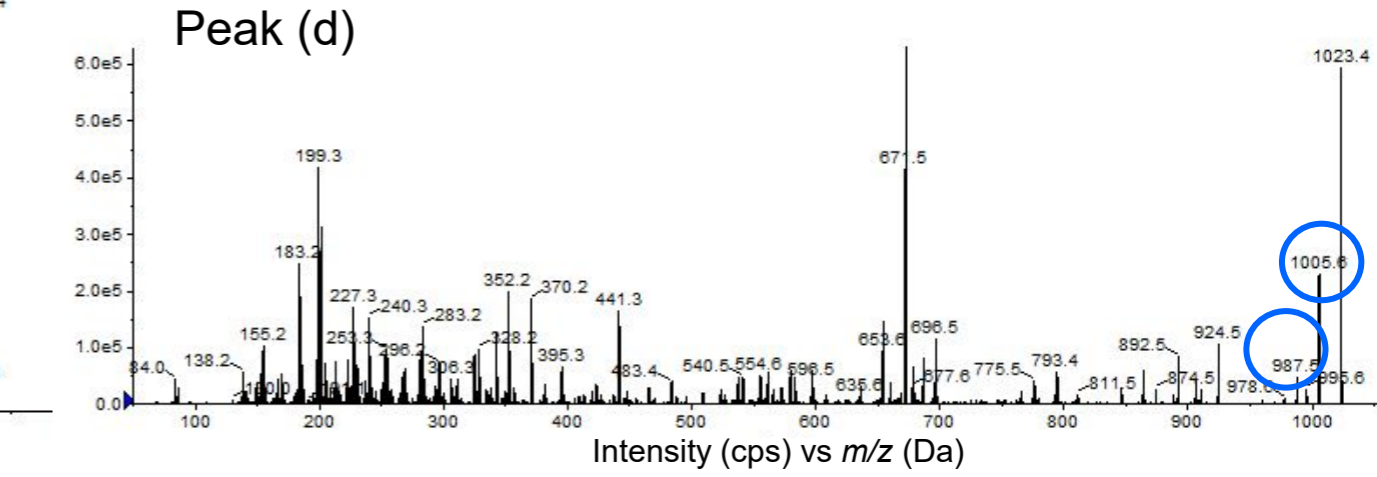
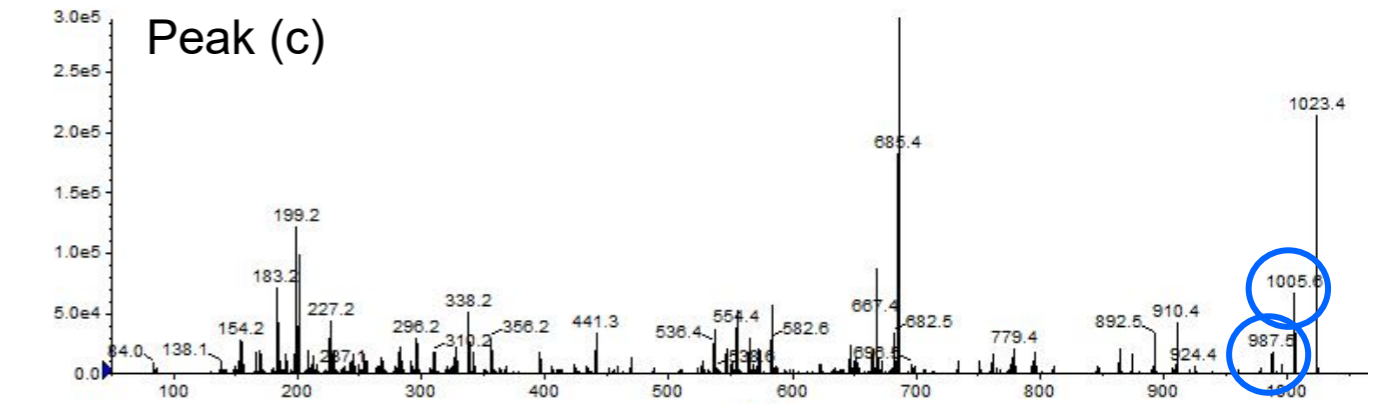
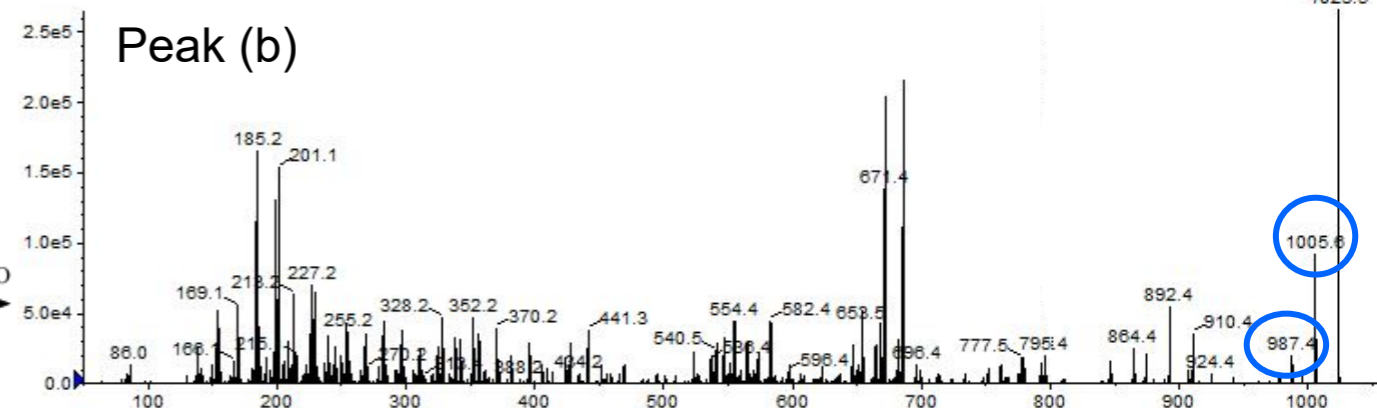
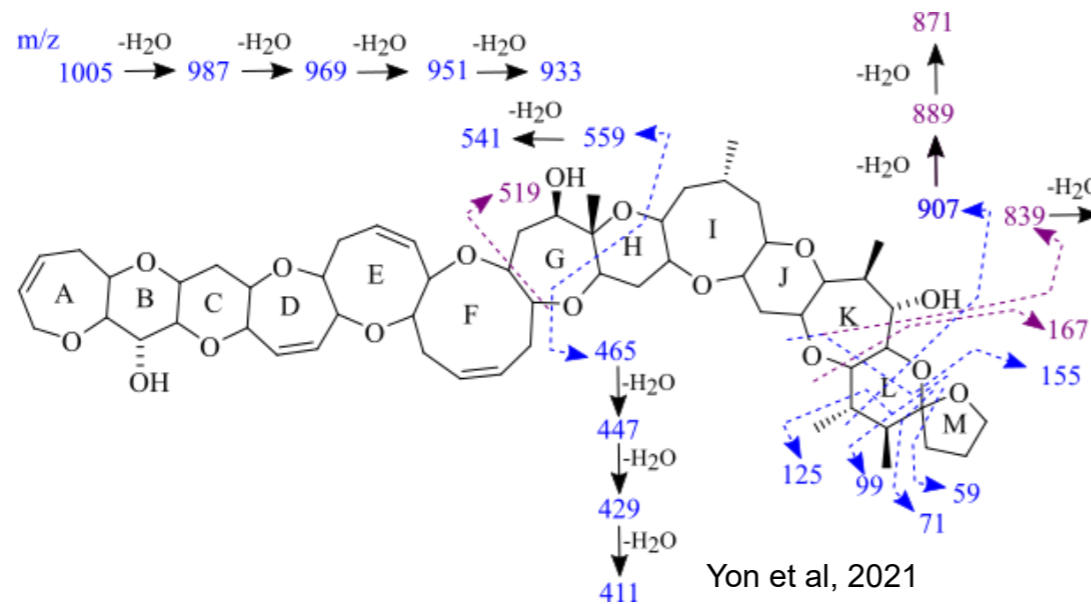
Untargeted approach

AutoMSMS
mode

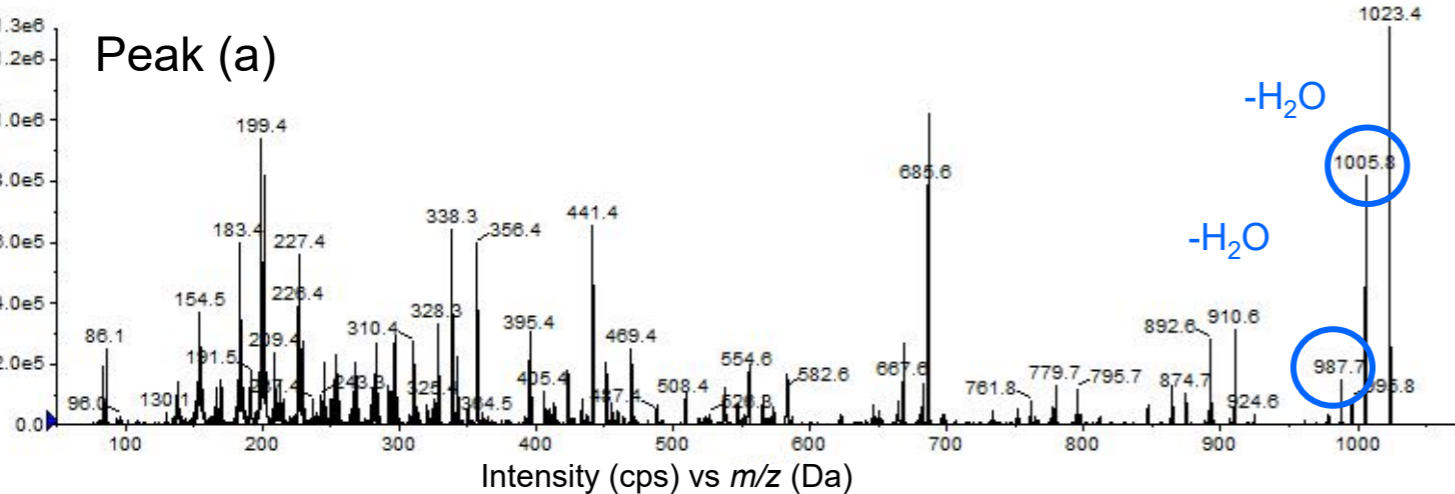
- HRMS/MS spectra
- Metabolomic workflow to explore chemical diversity



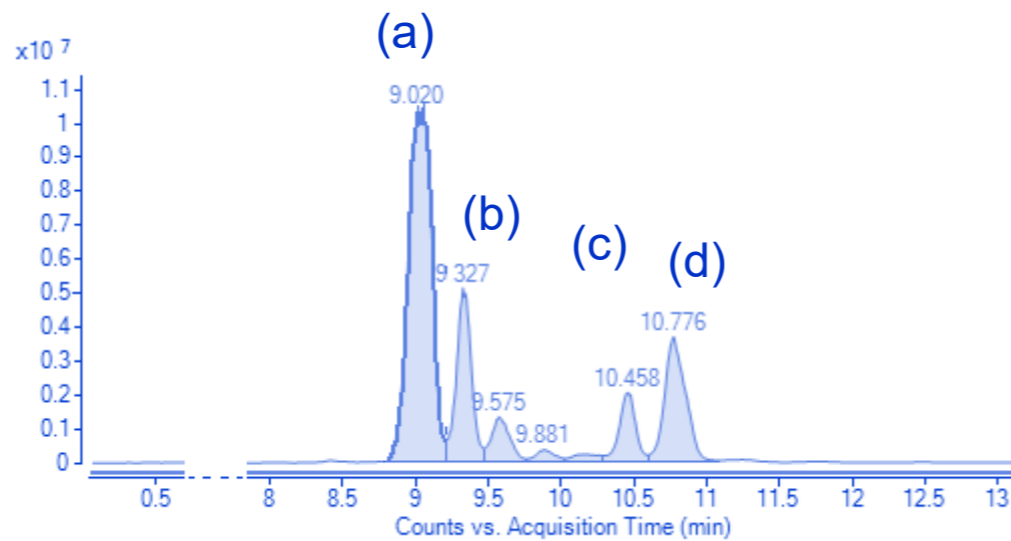
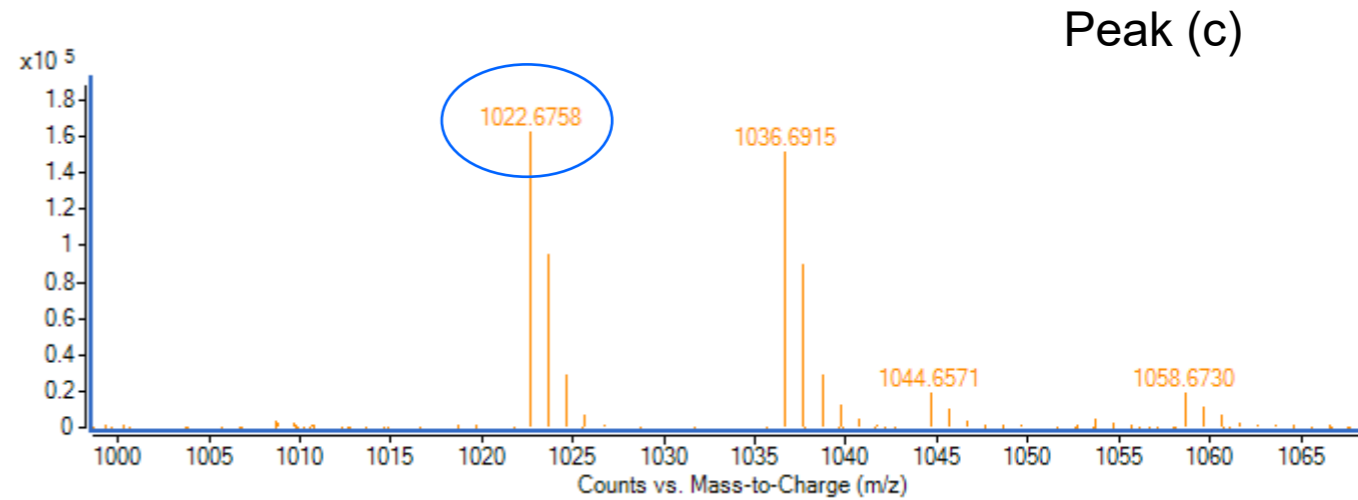
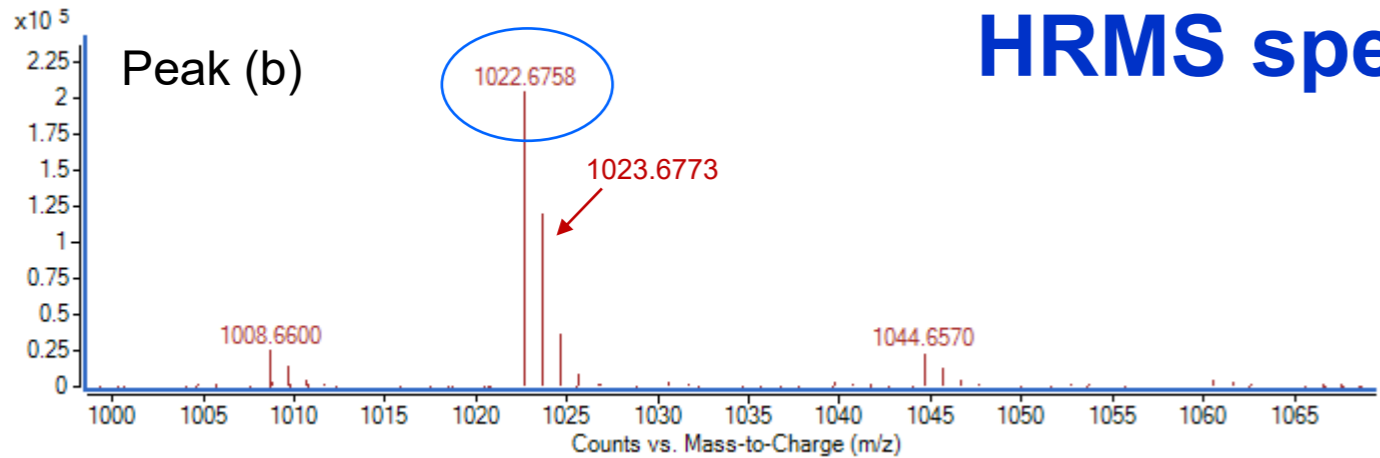
(a) Fragmentation pathways of CTX3C



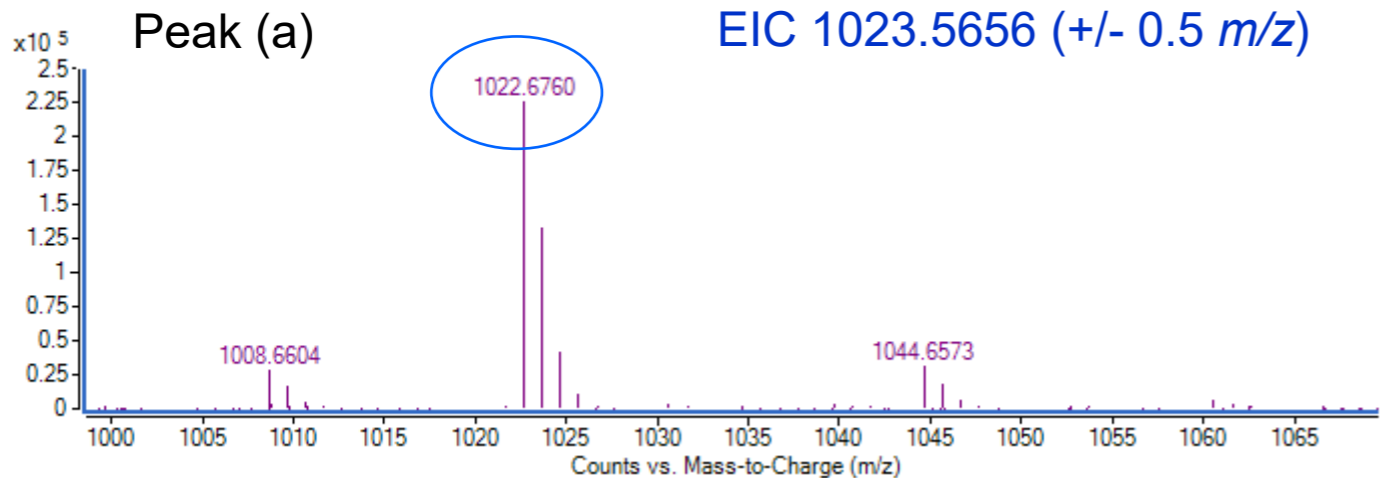
➤ EPI on m/z 1023.5
➤ CE 60 eV, CES 20 eV



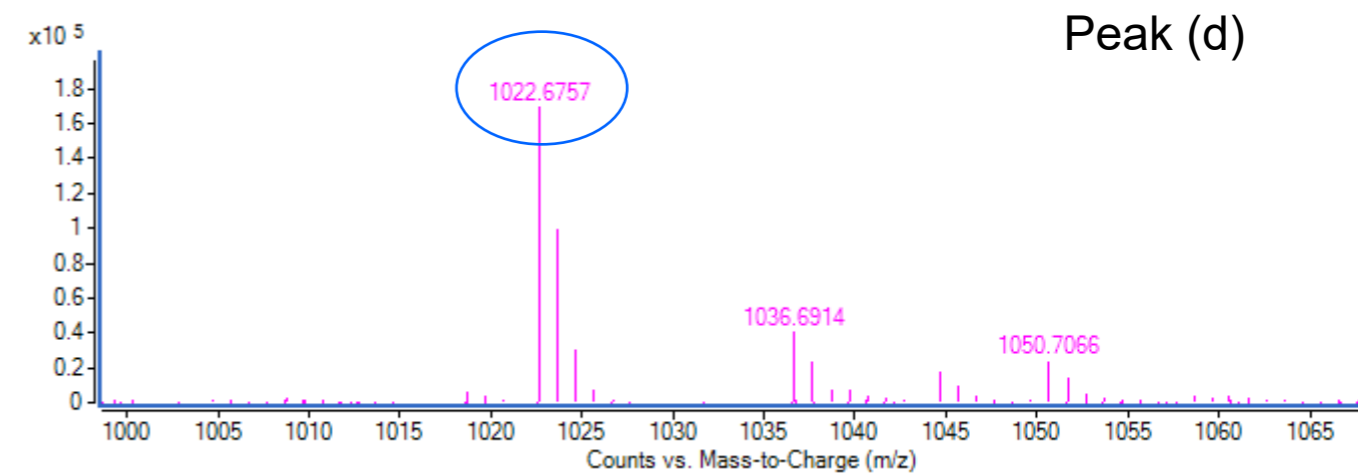
HRMS spectra



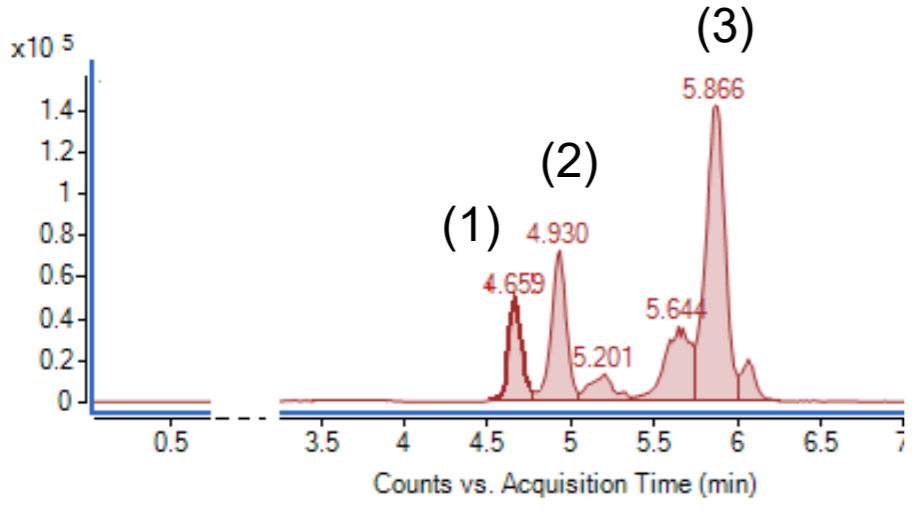
The monoisotopic m/z is 1022.675
Not 1023.5656 !



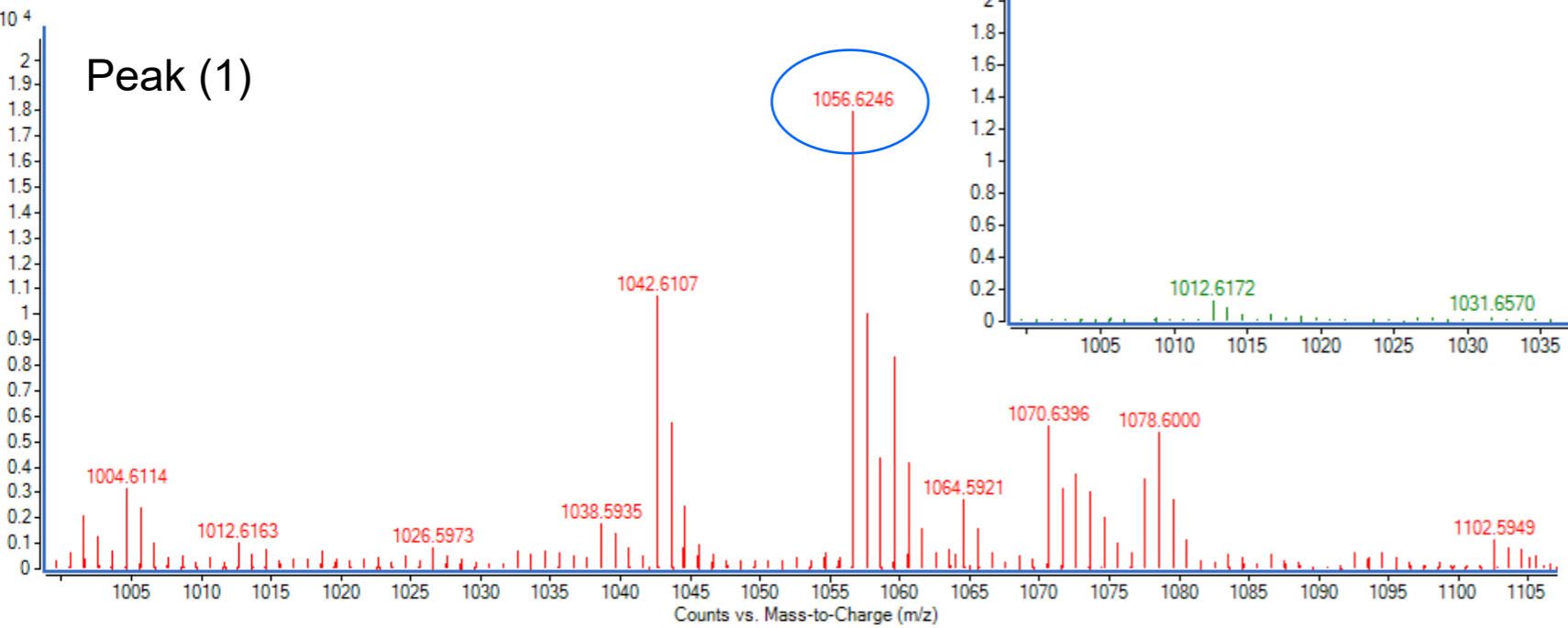
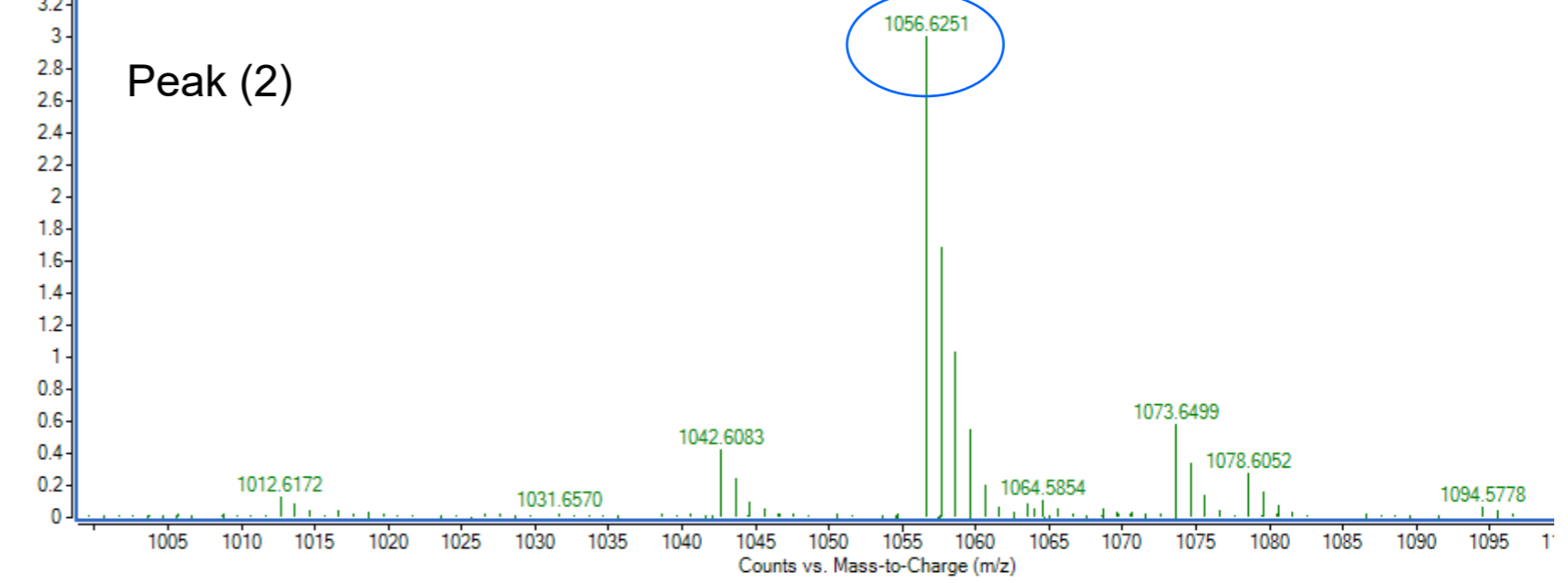
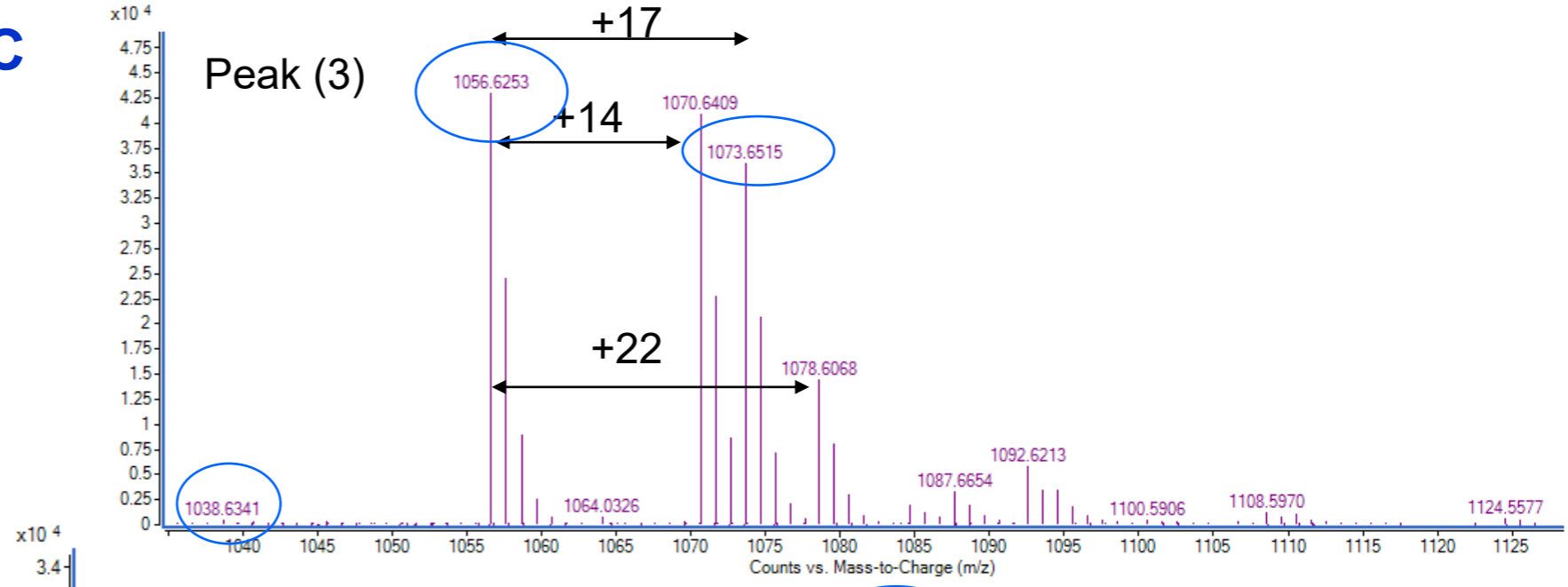
EIC 1023.5656 ($\pm 0.5 m/z$)



HRMS of putative 2,3-diOH-CTX3C isomers



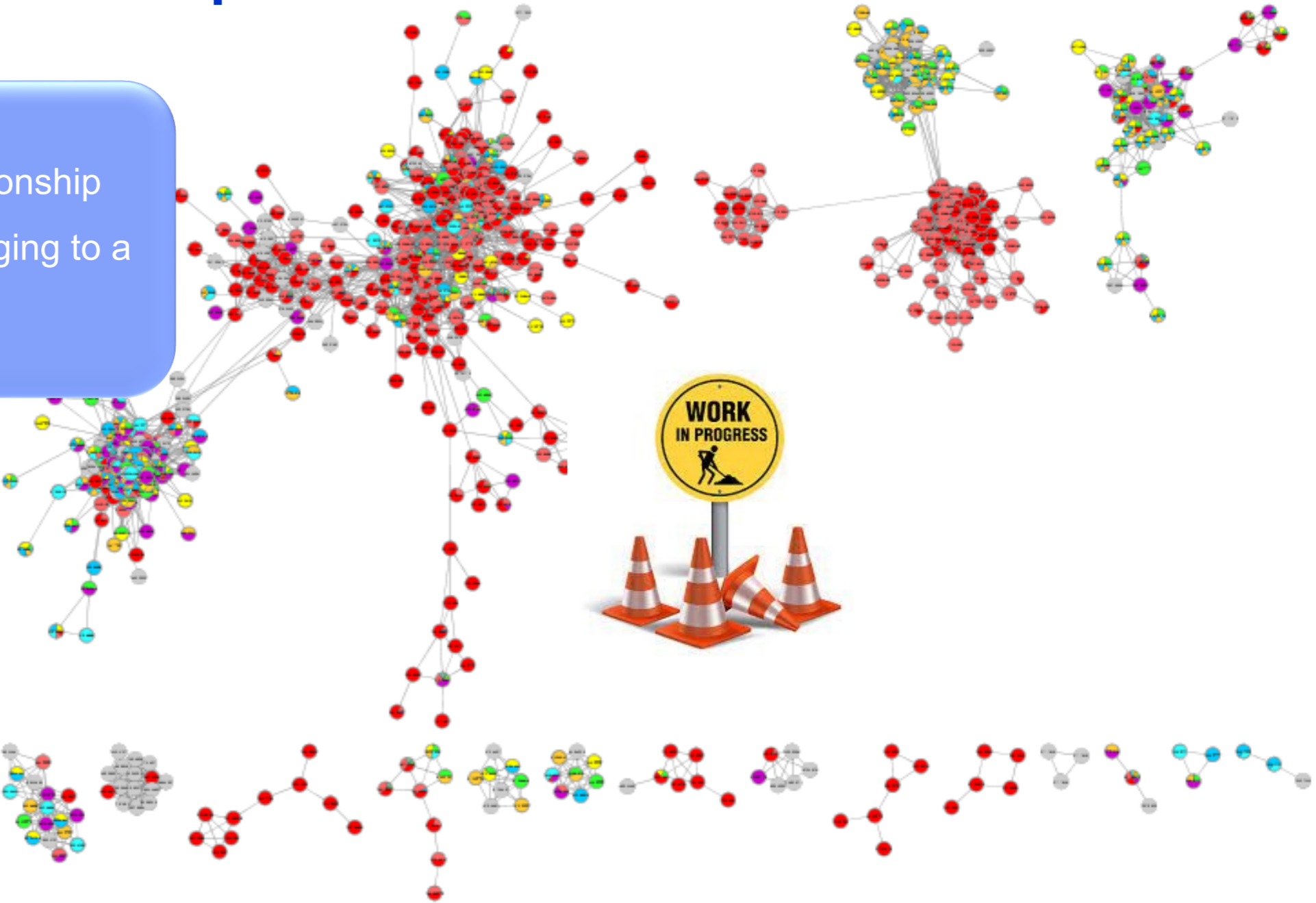
EIC 1057.5730 (+/-) 0.5 m/z



The monoisotopic *m/z* is 1056.625
Not 2,3-diOHCTX3C isomers

Molecular network on samples

➤ To visualize structural relationship between compounds belonging to a same molecular family



- Fish 3
- Fish 4
- *G. belizeanus*
- *G. carpenteri*
- *G. polynesiensis*
- Standards



Conclusion

- ✓ CTXs were detected in fish flesh from Lifou
- ✓ Toxicity > 0.01 μg CTX1B eq. Kg^{-1} (EFSA threshold)
- ✓ 3/4 fishes linked to seafood intoxication are Ciguatera poisoning



Golden trevally



Toxin profile : CTX3C type



Leopard coralgroupers



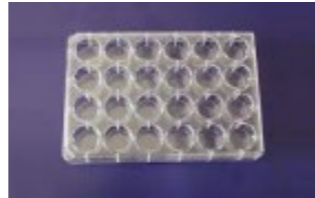
Toxin profile: CTX1B type

- ✓ Further investigations are required to tentatively identify the intense peaks in the leopard coralgroupers (CP4)

Ongoing project in New Caledonia:

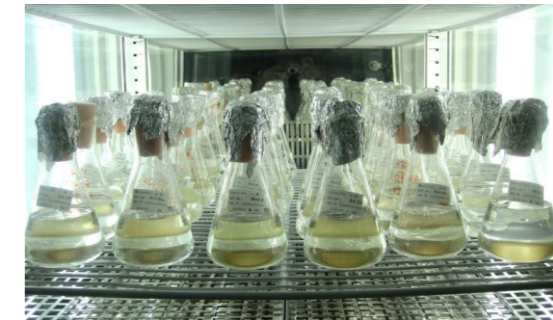
To assess the presence of any health risks associated with phycotoxins

- Characterize species diversity and distribution



- Establish a strain library of benthic species of NC

➤ ~40 strains: *Gambierdiscus* spp., *Ostreopsis* spp., *Coolia* spp. *Prorocentrum* sp. ...



- highlight the bioaccumulation of phycotoxins in marine animals

➤ 100 fishes from NC and from Lifou bivalves, sea urchin and giant clams (to analyse)



Thanks to my collaborators:

- Ifremer LEAD NC (F. Antypas, [Thierry Jauffrais](#))
- Ifremer LER-BO (G. Bilien, N. Chomerat)
- Ifremer PHYTOX-Metalg (S. Tanniou, K. Lhaute, D. Réveillon, P. Hess)
- Lifou Dispensaries (Dr. Y.M Ducrot, Dr. M. Bahu)
- Lifou Veterinary (Dr. A. Barnaud)
- Loyalty province of Lifou (G. Kakue, J.M. Ita)
- Louis Malardé Institute (M. Chinain, T. Darius)

Thank you for your attention

