

Toxicity and toxin profile on Indian Ocean fish containing CTX-like compounds

Lourdes Barreiro¹; Sandra Gimeno¹; Andres Sanchez-Henao¹; Jean Turquet²; Fanny Maillot²; Cintia Flores³; Jorge Diogène¹ and Maria Rambla-Alegre¹

¹IRTA, Ctra Poble Nou km 5.5, 43540, La Ràpita, Spain

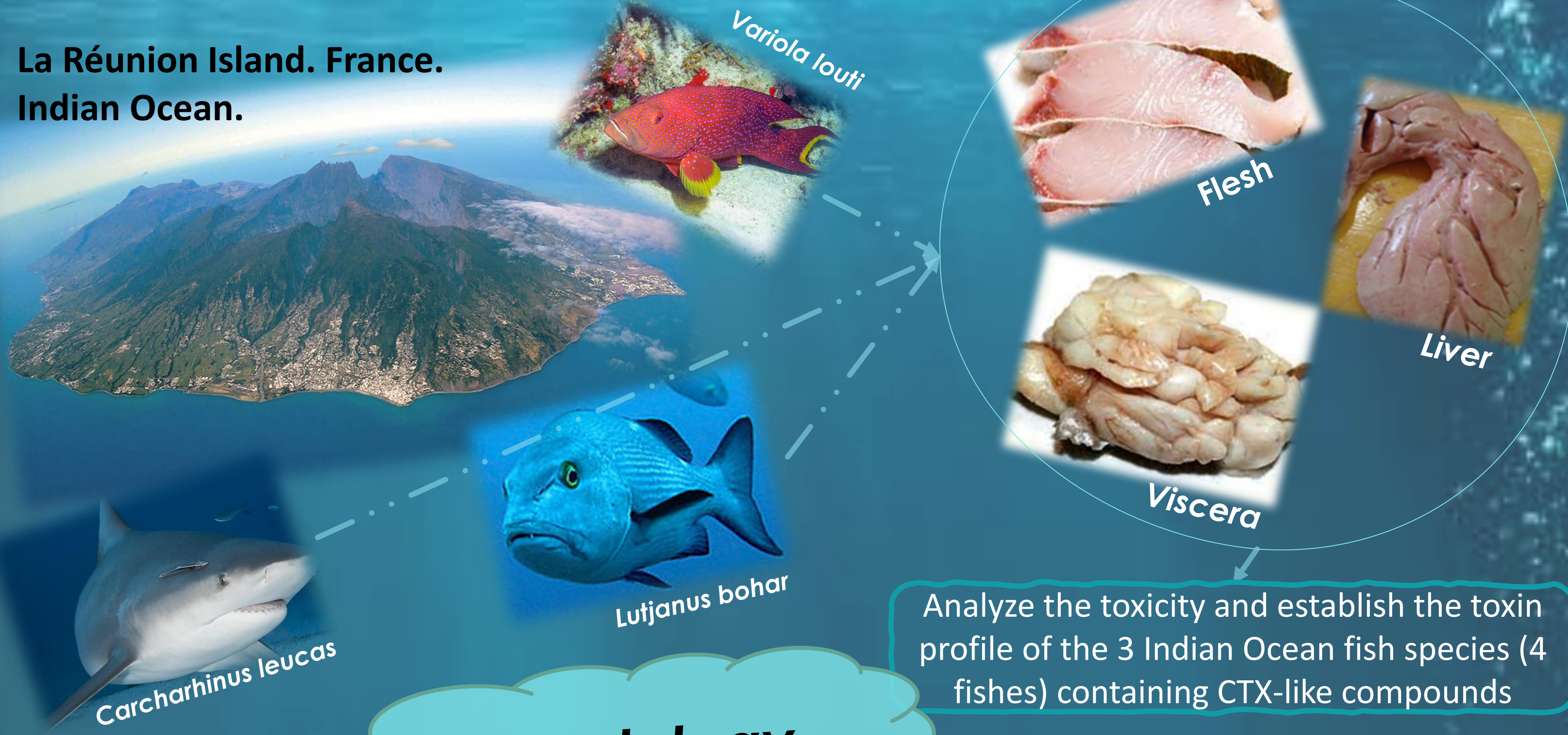
²Centre Technique de Recherche et de Valorisation des Milieux Aquatiques (CITEB), La Réunion, France

³Mass Spectrometry Laboratory, Organic Pollutants, IDAEA-CSIC, Jordi Girona 18, 08034, Barcelona, Spain

What are CTXs?

Ciguatoxins (CTX) are potent marine neurotoxins produced by dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa* (Roeder et al. 2010), that cause ciguatera poisoning (CP) (Spielmeyer et al., 2022). CFP occurs mainly in the tropical and subtropical Indo-Pacific region and Caribbean Sea and is characterized by severe neurological, gastrointestinal, and cardiovascular disorders affecting approximately between 50,000 and 500,000 consumers annually (Pearn, J. 2001). CP is one of the most relevant seafood-borne diseases worldwide.

La Réunion Island. France.
Indian Ocean.



Analyze the toxicity and establish the toxin profile of the 3 Indian Ocean fish species (4 fishes) containing CTX-like compounds

Methodology

1. Extraction

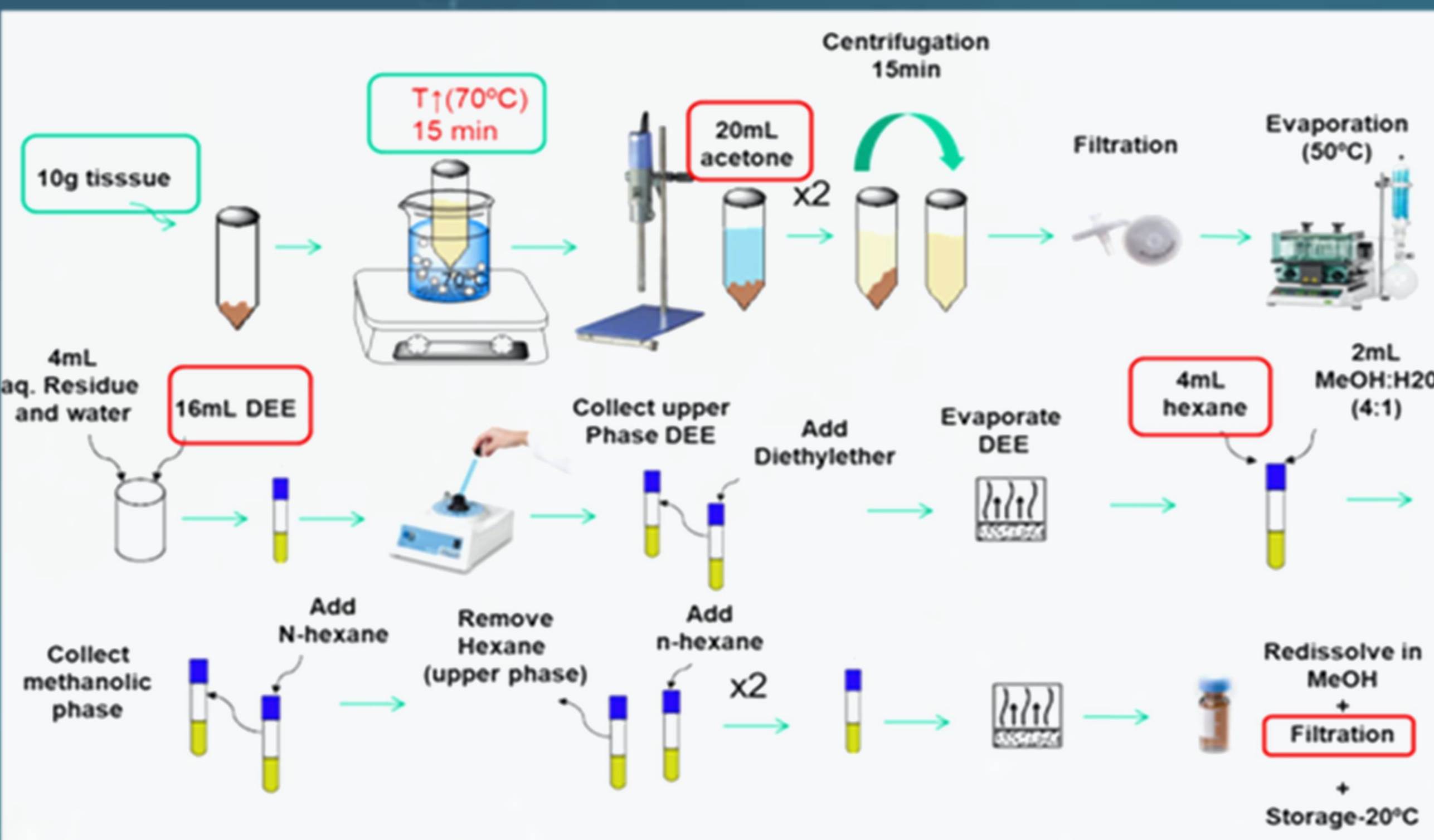
(Lewis R.J., 2003)

2. Clean UP

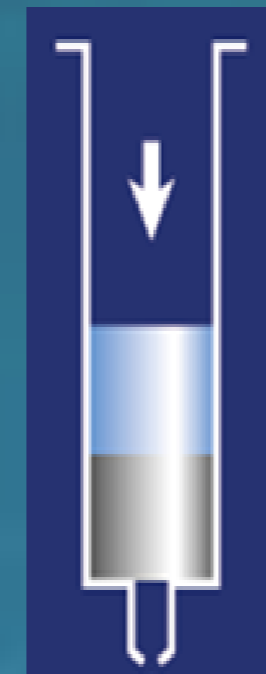
(Estevez et al., 2019)

3. LC-MS/MS + LC-HRMS

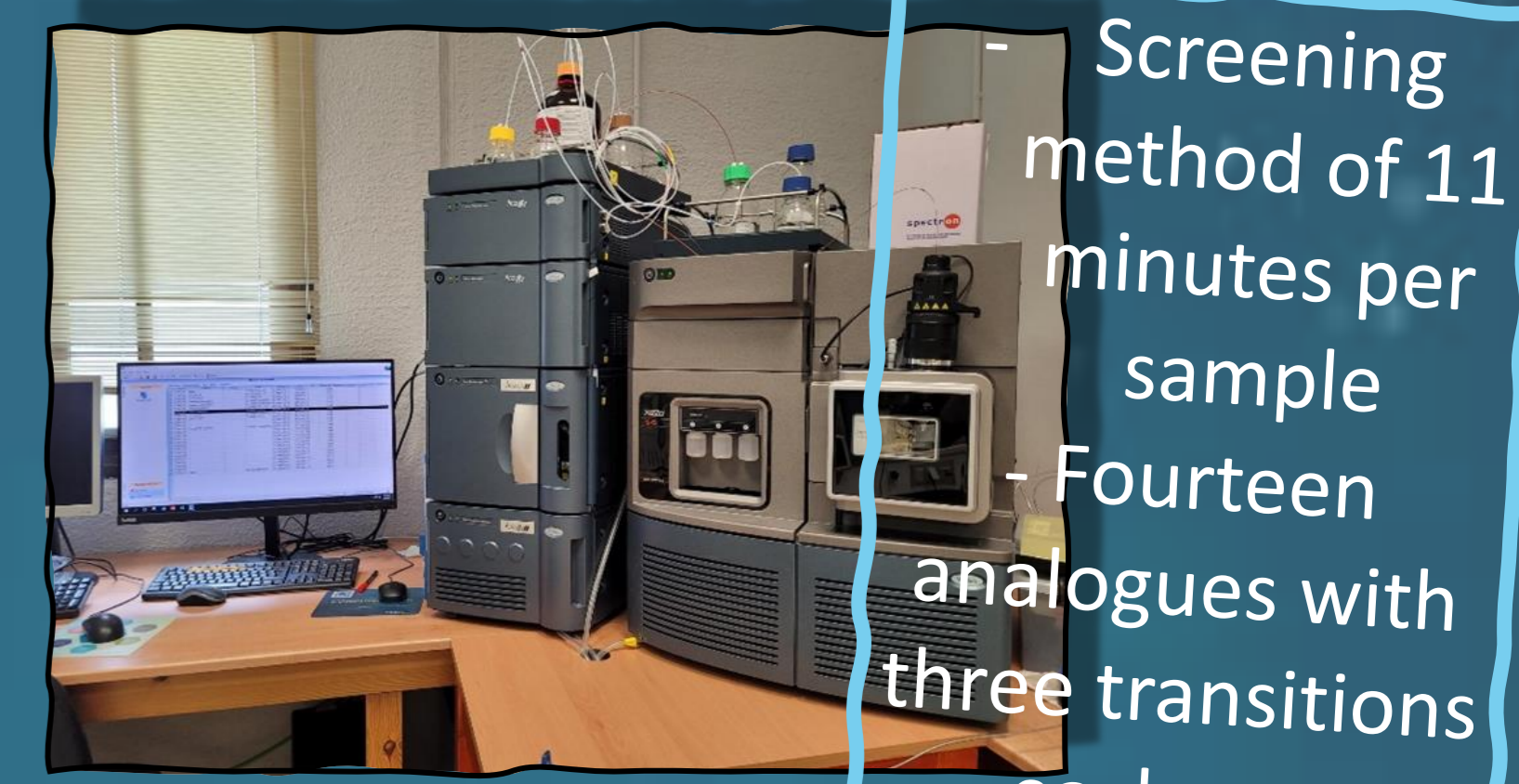
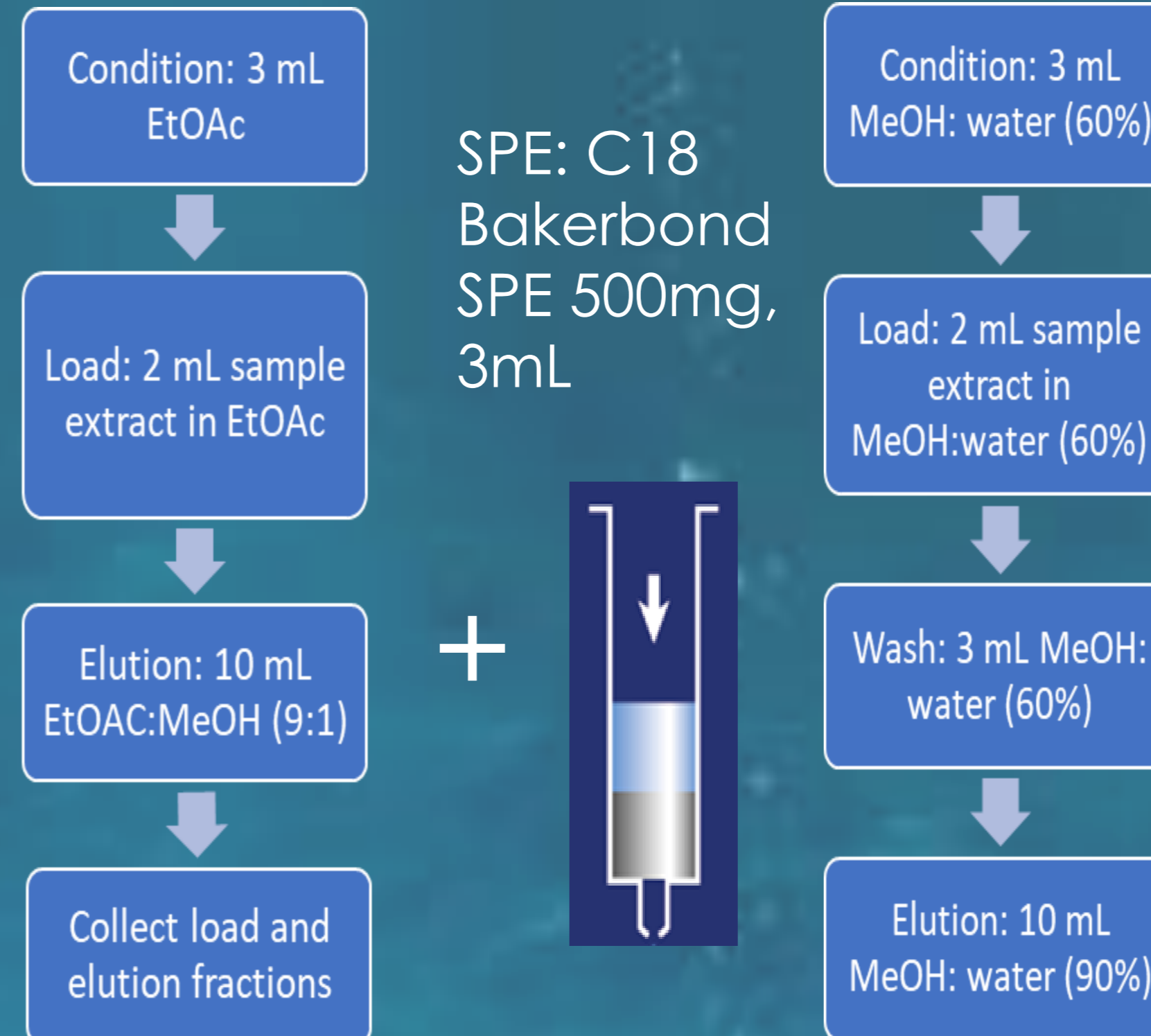
Screening method of 11 minutes per sample
Fourteen analogues with three transitions each one



SPE: Florisil Bakerbond SPE 500mg, 3mL



SPE: C18 Bakerbond SPE 500mg, 3mL



What was obtained?

Flesh of the *V. louti* after a CP incident in La Réunion Island confirmed the presence of CTX 1B, (0.12 μg equiv CTX 1B/kg), 52-epi-54-deoxy CTX 1B (CTX3) and 54-deoxy CTX 1B (CTX2).

In the second *V. louti* individual and the *L. bohar*,

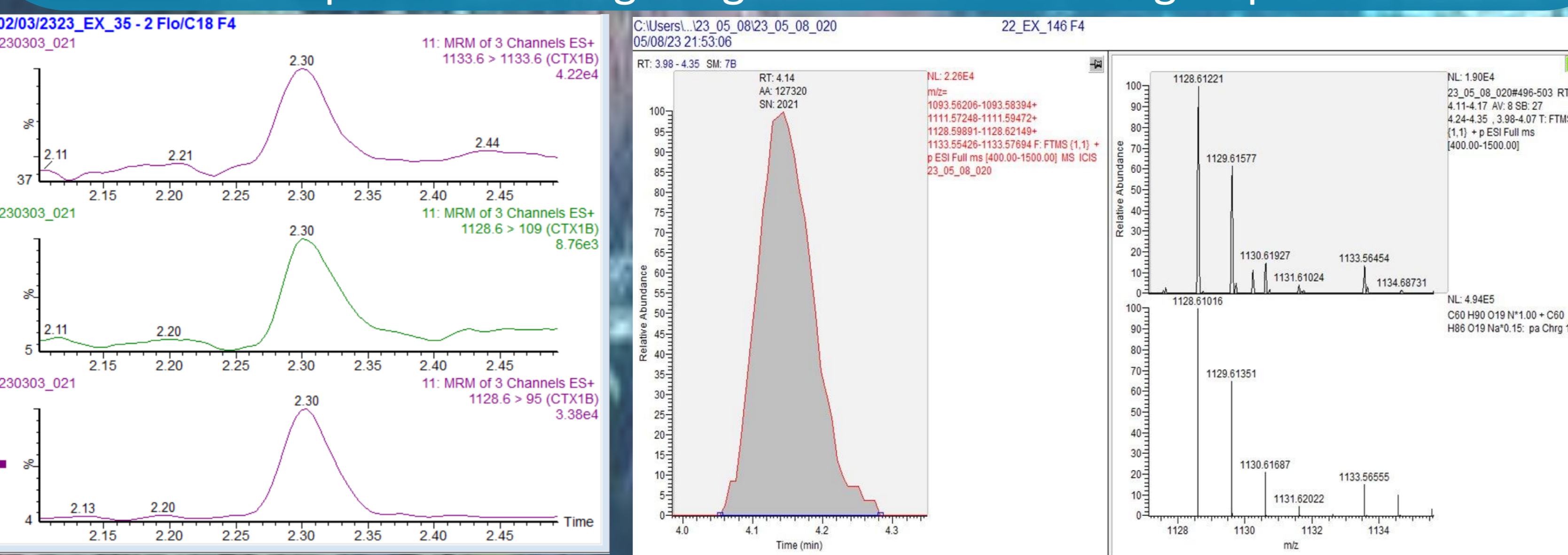
CTX 1B was identified in the three tissues (0.01 – 0,07 μg equiv CTX 1B/kg) and CTX2 was identified for flesh of *V. louti* (0.05 μg /kg) and CTX 4A in viscera of *L. bohar*.

The *C. leucas* samples revealed the presence of a complex CTX contaminant profile showing congeners of the CTX 3C group.

Conclusion

- Four fishes from the La Reunion Island presented CTX-like toxicities.
- Toxicities were higher in liver followed by viscera and flesh in all fishes except for *V. louti* 1. Cell-based assay (Neuro-2a) was performed in two laboratories and results were consistent between both.
- CTX 1B was found in all tissues for three fishes (*L. bohar* and two *V. louti*); CTX2 and CTX3 was founded in *V. louti* flesh and congeners of the CTX 3C group were found in *C. leucas* flesh and viscera
- Combined use of SPE Florisil + C18 is more effective minimizing the matrix effect
- Liquid chromatography coupled to high-resolution mass spectrometry is an emerging and very attractive approach that combines qualitative and quantitative analyses and reducing the inaccuracies
- The combined use of Neuro-2a CBA + LC-HRMS + HRMS can be a good method for detection and evaluation of CTX and determination of toxin profiles.

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LC-MS/MS (CTX1B) Flesh *L. bohar*

LC-HRMS full MS Liver *L. bohar*