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

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Ciguatoxins (CTXs) are potent marine neurotoxins that cause ciguatera fish poisoning (CP). CP occurs mainly in the tropical and subtropical Indo-Pacific region and Caribbean Sea. However, their presence, toxicity and toxin profile have been poorly described in the Indian Ocean.

In this study, the liver, muscle, and viscera of four fish specimens of three different species (one *Lutjanus bohar*, two *Variola louti* and one *Carcharinus leucas*) caught in the waters of La Réunion Island were analysed. One of the *V. louti* was obtained after a CP incident. Toxicity was analysed by screening CTX-like toxicity with the neuro-2acell-based assay. Their toxicity values ranged between 0,013 to 1,26 µg equiv CTX1B/kg in flesh, 2,55 to 16,40 equiv CTX1B/kg in liver and 0,51 to 3,12 equiv CTX1B/kg in viscera. In general, liver and viscera showed higher concentrations of CTX-like compounds than flesh.

The three fish tissue extract clean-up were evaluated using the combination of Florisil and C18 SPE cartridges, recoveries of CTX1B were between 48,6 and 89.7% depending on the tissue. Additionally, a new screening method using a UPLC coupled with a Xevo TQXS for CTXs was optimized at 11 minutes per sample and in which a total of fourteen analogues, with three transitions each one, were included. This method together with three other methods including more than 30 known CTX congeners into the LC-MS/MS were used.

The toxin profile for the four fish specimens were evaluated using liquid chromatography coupled to mass spectrometry (LC-MS/MS) and to high resolution mass spectrometry (LC-HRMS). Several potential CTX congeners were identified: the flesh of the *V. louti* after a CFP incident confirmed the presence of CTX1B, (0.12 µg equiv CTX1B/kg), 52-epi-54-doxyCTX1B and 54-deoxyCTX1B. In the second *V. louti* individual and the *L. bohar*, CTX1B was identified in the three tissues. The *C. leucas* samples revealed the presence of a complex CTX contaminant profile showing congeners of the CTX3C group.

Keywords: marine toxins, ciguatoxin, ciguatera, chromatography, clean-up, CBA, liver, flesh, viscera, La Réunion Island