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Editorial

**21st Meeting of the French Society of Toxinology (SFET)
Special Issue on “Freshwater and Marine Toxins”**

This Special Issue of *Toxicon* is published on the occasion of the 21st “Rencontres en Toxinologie (RT)” meeting held at the Pasteur Institute in Paris on December 9-10, 2013. More than 120 participants, from Algeria, Australia, Belgium, Brazil, Canada, Denmark, Finland, France, Germany, Ireland, Israel, Italy, Morocco, Norway, Portugal, Spain, Switzerland, Tunisia, and the United States of America, attended this meeting organized by the French Society of Toxinology (SFET, www.sfet.asso.fr). Should a reminder be appropriate, please note that the SFET missions aim at (i) promoting research in all domains concerning the venoms and toxins from animal, vegetal or microbial origins, *i.e.* the chemical, immunological, environmental, pharmacological, physiological domains, along with mode of action, structure-function relationships, drug-design and applicability questionings; (ii) favouring the meeting of colleagues in the field, communication to other scientists, education and training at all levels, and dissemination to more general audiences; (iii) improving fundamental research on toxinology and enhance its relevance and applications.

We warmly thank all those who made this meeting achievable, and we particularly acknowledge the cogent contribution of Professor Alan Harvey, Editor-in-chief of *Toxicon*, who made the publication of the RT21 proceedings possible as a Special Issue focused on Freshwater and Marine toxins. This Special Issue gathers 16 original peer-reviewed articles and reviews, and 48 abstracts, all reflecting those works and data that were presented during the SFET meeting.

For decades, scientists have been working on terrestrial toxins. The main reason is that the accessibility to the producers – venomous animals, pathogenic bacteria or poisonous plants – and the related human health issues in endemic areas have motivated pharmacologists and physiologists to study the mode of action of natural toxic molecules found just outside of laboratories. Moreover, natural products have long been used in traditional medicine as drugs to treat a variety of human diseases. A well-known example is morphine, which keeps being the most widely used molecule to alleviate pain. However, the exploration of shorelines and deep oceans with modern equipment, the recreational use of seas and the emergence of eutrophication of freshwaters (a process where water bodies receive excess nutrients that stimulate excessive phytoplankton and bacteria growth) in occidental and developing countries have contributed to the molecular identification of toxic cocktails from aquatic producers. The human colonization of coastal waters and the growing activity along the rivers have increased the probability of human interactions with toxin producers. Nowadays, sea snakes, cone snails, cnidarians, toxic microalgae, parasitic bacteria are common features of the global concern in modern toxicology. Moreover, should one take into account the recent approval of marine toxins as chemical templates to design new therapeutic agents directed toward major human pathologies – *i.e.* pain and cancer (Prialt® and Yondelis®, respectively) – one would be convinced that pharmacological active molecules found underwater may represent an endless pipeline for the next generation of natural drugs.

From this point of view, the underwater world has long been unexplored. Indeed, the study of marine and freshwater substances began truly around 1950 only. It led to describe some 3,000 to 4,000 new molecules produced by microorganisms, algae and invertebrates, characterize nearly 500 biologically active molecules (with antitumor, antiviral, immunomodulatory, antibacterial, antifungal or anti-inflammatory properties), and put into market new

drugs such as cephalosporins (antibiotics from a marine fungus), vidarabine and cytarabine (antiviral and chemotherapy agents, respectively, isolated from sponges). However, because of environmental constraints, it is extremely rare to obtain products from a marine or freshwater organism in amounts sufficient for industrial exploitation. Therefore, chemical synthesis is favored, and this approach has the potential to improve product performance by designing derivatives that are less toxic and sometimes more powerful than the original, natural substances.

During the RT21 meeting, freshwater toxins were largely represented by cyanobacterial toxins because of their increasing ecotoxicological risk for human and animal health. Hence, a large range of organisms seem to be affected by cyanotoxins in the aquatic media, such as bacteria, phytoplankton, zooplankton, larger invertebrates, vertebrates and aquatic plants so as their terrestrial counterparts *via* irrigation procedures (Vasconcelos, *abstract#2*). Cyanobacteria have adopted multiple parallel strategies for producing potent toxins but they also are a prolific source of bioactive compounds (Sivonen, *unpublished*). The potential production of toxins and other secondary metabolites in the cyanobacterial phylum was also approached through the first phylum-wide genome investigation, using large-scale bioinformatics analyses (Gugger, *unpublished*).

Increasing knowledge on more specific well-known cyanobacterial toxins was achieved regarding (i) the detection of microcystins using recombinant immunoconjugates (Alvarenga et al., *unpublished*), the proteomic response of freshwater mussels, filter feeders that are exposed to these toxins (Malécot et al., *unpublished*), the biosynthesis of secondary metabolites from *Microcystis*, a widespread toxic bloom-forming cyanobacterium, at genomic and analytical levels (Pancrace et al., *unpublished*), the intestinal bidirectional transport of microcystins using Caco-2 monolayer, an *in vitro* human intestinal epithelium model, and a mathematical model to identify the limiting factors involved in the low transport of these toxins (Henri et al., *article*); and (ii) the biosynthesis of homoanatoxin-a and/or anatoxin-a and analogues at the genomic, enzymatic and analytical levels, in relation to their production by cyanobacteria (Méjean et al., *abstract#6* and *article*; Paci et al., *abstract#42*). Moreover, information was provided on the human intestinal transport of cylindrospermopsin, another well-known cyanobacterial toxin, by performing *in vitro* permeation studies across the Caco-2 cell monolayer. The results indicated a limited passage of the toxin through the intact intestinal epithelium without inducing any cellular effects, in contrast to hepatic Clone 9 cells exposed to cylindrospermopsin where a noticeable cytotoxicity was observed (Fernández et al., *article*). Cylindrospermopsin metabolites were identified by using various *in vitro* metabolic systems (such as cellular and enzymatic models) and applying a combination of linear trap quadrupole and high-resolution mass spectrometry (Kittler et al., *abstract#1*). The last step of cylindrospermopsin and its isomer biosynthesis was determined in relation to their production by cyanobacteria in the environment (Gautier et al., *abstract#33*). To prevent intoxication, a reliable LC-MS/MS quantification method based on stable isotope dilution assay was developed for directly measuring cylindrospermopsin in water and foodstuff (Maul et al., *abstract#11*). Toward the same goal, an ultra-performance liquid chromatography tandem mass spectrometry method was set up to simultaneously identify and quantify cylindrospermopsin, microcystins and some anatoxin-a analogues (Rodriguez et al., *article*). Finally, optimizing the identification and quantification of beta-N-methylamino-L-alanine and at least three of its isomers in biological samples led to precise the extent to which they are represented in freshwater and marine cyanobacteria and are accumulated in shellfish (mussels and oysters) intended for human consumption (Réveillon et al., *abstract#9*).

Among the toxins from the marine environment, the macrocyclic imine phycotoxins from dinoflagellates, associated with marine algal blooms and shellfish toxicity, aroused the interest

of researchers over the latest fifteen years. Since pinnatoxins can accumulate in shellfish and reach humans through oral intake, their hazard for human health was investigated by studying their transport across Caco-2 monolayer, showing that the ability of these toxins to cross the human intestinal epithelium is higher for the crude extract of toxin-containing dinoflagellates than for the toxins alone (Huguet et al., *abstract#35*). Moreover, pinnatoxins were reported to efficiently block synaptic transmission at the skeletal neuromuscular junction of vertebrates and to drastically affect chick embryo development (Coesnon et al., *abstract#28*), in agreement with their ability, as well as that of most other cyclic imine toxins, to target particular nicotinic acetylcholine receptor subtypes with high affinity. From a structural point of view, the cyclic imine toxins have a common imine functional group engaged in a heterocyclic carbon skeleton. Crystal structures of the *Aplysia* pentameric acetylcholine-binding protein, a soluble surrogate of the extracellular ligand-binding domain of nicotinic acetylcholine receptors, in complexes with some of these phycotoxins showed toxins neatly imbedded within the aromatic nest where agonists and competitive antagonists bind (Bourne et al., *abstract#18*). A better understanding of the conformational flexibility of cyclic imine toxins in solution was obtained from long-timescale all-atom molecular dynamics simulations in OPLS-AA force field, providing additional information regarding the thermodynamics of the interaction between these toxins and their main target, which ultimately leads to the design of new derivatives with improved biological activities, subtype selectivity and pharmacological properties (Aráoz et al., *abstract#36*). Because of the affinity of cyclic imine toxins for nicotinic acetylcholine receptors, a colorimetric microtiter plate-based receptor-binding assay was developed and validated for the detection and quantification of these toxins and the freshwater anatoxin-a (Rubio et al., *abstract#12* and *article*). Finally, an extended evaluation of mixed-mode and lipophilic sorbents for passive sampling of cyclic imines toxins, as well as palytoxins, okadaic acid and azaspiracids, demonstrated for the first time the usefulness of mixed-mode sorbents in mesocosm and field studies for these marine biotoxins (Zendong et al., *abstract#10* and *article*).

Azaspiracids, which are polyether marine toxins produced by dinoflagellates, may also accumulate in edible shellfish and render them improper for human consumption since they induce severe gastro-intestinal symptoms. The identification of unknown analogues and the isolation of some known minor analogues of these toxins, as well as their full structural elucidation and toxicological assessment using the Jurkat T lymphocyte cell assay, were reported, highlighting those analogues that are relevant for public health protection (Kilcoyne et al., *abstract#19*). Moreover, *in vitro* chronic effects of azaspiracids on a hERG Chinese hamster ovary recombinant cell line pointed out that these toxins may have chronic cardiotoxicity related to hERG channel trafficking (Ferreiro et al., *article*). The pharmacokinetics (absorption, distribution, metabolism and excretion) evaluation of azaspiracids in mini-pigs and chemical analysis of tissue samples indicated that the absorption and metabolism of toxins was quite rapid (*i.e.* 24 hours after feeding trial period of animals) and that they were widely distributed throughout the animal body (Geraghty et al., *abstract#20*). Detailed pathological changes were described in mini-pigs fed spiked with a semi-purified extract from mussels contaminated with a mixture of azaspiracids, with toxin effects more similar to those observed in mice than in human (Aasen Bunæs et al., *abstract#3*). Hence, an acute oral toxicity of three azaspiracid analogues was obtained in gavaged mice, providing information about the signs of animal toxicity and lethality and the macroscopic alterations of tissues (Tubaro et al., *abstract#45*).

The current knowledge on ciguatoxins, which are still other polyether marine toxins produced by dinoflagellates, was reviewed (Mattei, *abstract#17*). These sodium channel-activating toxins are responsible for ciguatera, a human poisoning acquired through the consumption of contaminated fish classically in tropical and sub-tropical areas and more

recently Europe (Mattei et al., *article*). This poisoning is mainly characterized by gastrointestinal and neurological disorders. The neurological disorders include the pathognomonic symptom of cold allodynia, an intense stabbing and burning pain in response to mild cooling of the skin whose relation with sodium channels, and indirectly with transient receptor potential A1 channels, was detailed in mice, following intraplantar injection of ciguatoxins (Zimmermann, *abstract#16*). It is worth noting that the voltage-dependent sodium channel, due to its important role in action potential initiation and propagation, is one of the preferential targets of marine toxins (Mattei and Legros, *article*), including tetrodotoxin that is widely distributed in marine animals and was recently reported as the causative agent of an octopus food poisoning event (Wu et al., *article*).

Increasing knowledge was also provided on *in vitro* cytotoxic and antitumor activity of substances produced by marine prokaryotes, showing that cultivation conditions can influence production of the secondary metabolites of these prokaryotes (Romano et al., *abstract#43*); on the histopathological modifications arising from subchronic toxicity trials by mice gavage with C17-sphinganine analog mycotoxin, a marine fungal toxin (Marrouchi et al., *abstract#37*); and on the mode of action of marine sponge xestospongins, showing for the first time that the muscle-type nicotinic acetylcholine receptor is a secondary molecular target for these toxins (Aráoz et al., *abstract#25*).

The structural characterization and pharmacology of venomous toxins were also largely represented during the SFET RT21 meeting because of the high diversity of these proteins and reticulated peptides, which makes them top candidates for the discovery of novel therapeutic agents (Verdenaud et al., *abstract#47*). From this point of view, sea anemones and cone snails are true pharmacological treasures of potential lead compounds for drug development with biological active compounds acting upon diverse biological targets, such as voltage-dependent sodium and potassium channels (Tytgat, *abstract#13*; Peigneur and Tytgat, *abstract#14*). As potassium channels are key players in a number of physiological processes, no wonder then that venoms from marine (sea anemones, cone snails ...) as well as terrestrial organisms (scorpions, snakes, spiders ...) are well known to be rich sources of potassium channel modulators whose functional divergence and evolutionary convergence were exemplified (Rodríguez de la Vega et al., *abstract#5*). Toxins blocking potassium channels and cytolysins dominate the sea anemone and Hydrozoan secreted venoms, respectively, whereas jellyfish venoms contain both groups (Rachamim et al., *unpublished*; Glasser et al., *article*) along with bioactive compounds with cytotoxic or enzymatic activities (Badré, *article*). Similarly, a cytotoxic activity of crown-of-thorns starfish venom was reported specifically against human melanoma cells, which were found to be more sensitive to the venom than human normal cells (Lee et al., *article*).

Venomous organisms have evolved to acquire a vast arsenal of proteins and peptides used for defense and predation. However, whereas cnidarian venom toxins have evolved through dynamic gene recruitment and multiplication and/or gene loss or “derecruitment” (Rachamim et al., *unpublished*), the evolutionary innovation of cone snail venoms originates in the hypermutation events seen at the mRNA level along with variable peptide processing such as differential post-translational modifications (Dutertre, *abstract#7*). To this purpose, a novel conotoxin from *Conus bandanus* venom containing bromotryptophan gamma-carboxyglutamic acid was reported as being the first III-cysteine framework *Conus* peptide containing bromotryptophan in the M-superfamily (Nguyen et al., *abstract#41*). Interestingly, the above mentioned evolutionary innovations of cone snail venoms were also taken into account to explain the differences of venom composition not only between those injected and in dissected venom glands, but also between those used for prey capture and defense (Dutertre et al., *article*).

The enormous structural diversity of venomous toxins is mainly based on various disulfide-linked molecular scaffolds. Cysteine pairing is not easy to identify but it is primordial to insure the specific folding that dictates the biological activity of peptides and proteins, and the synthesis of chemical homologues displaying the same bioactivity than the natural compound. The case of small peptides that contain two disulfide bonds was investigated by partial oxidation/reduction of peptides, followed by the separation of the generated species by ion-mobility mass spectrometry and their characterization by tandem mass spectrometry (Quinton et al., *abstract#8*). Once cysteine connectivity has been attributed, efficient synthesis and refolding are a real challenge to obtain peptides and proteins in sufficient amounts, and various strategies with an easy purification of peptides containing two disulfide bridges, with different pairing and arrangements, were proposed (Upert et al., *abstract#46*). There is no doubt that the structural and pharmacological diversity of venomous toxins will yet be increasing, at least for conotoxins as exemplified by the discovery of still a new alpha-conotoxin subclass in the venom of *Conus australis*, a yet unexplored cone snail species (Lebbe et al., *article*), by elucidation of the structure-function relationships of a new alpha-conotoxin from *Conus longurionis*, an unknown *Conus* species (Lebbe et al., *abstract#15*), and by the isolation, purification and functional characterization of alpha-conotoxins from *Conus bandanus* (Nguyen et al., *article*) and novel conotoxins from *Conus amadis* targeting the voltage-dependent sodium channel (Wijesekara et al., *abstract#48*).

Hence, we hope that this Special Issue of *Toxicon* will be an attractive and comprehensive source of information not only for “toxinologists”, but also for all researchers and students that are fascinated by the “freshwater and marine toxins” world.



Société Française pour l'Etude des Toxines
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