Algal toxin discovery, management and regulation over the last 25 years

Algal toxins in the dark ages (pre-1992)

From a historic perspective, knowledge about algal toxins can be divided into truly prehistoric occurrences such as known from paleontological studies [1-2] and more recent historic records. In these historic records, there are descriptions of poisoning incidents that clearly point towards the occurrence of the algal toxins centuries ago, such as the description of Captain George Vancouver, whose crew suffered from paralytic shellfish poisoning during the exploration of the Pacific Northwest in 1793 [3]. Other examples include ciguatera [4] and paralytic poisoning [5-6]. During the 19th century, modern taxonomy emerged as a science with developments in microscopy; by 1900, rather systematic studies of phytoplankton communities are common [7-10] and provide the basis for the biogeography of many toxic genera. In the early to mid-20th century the links between algae and toxins, or at least the toxic effects of algae, are being made [11-15].

By the beginning of the 1990s, many major algal toxins that cause acute poisoning had already been discovered, including brevetoxins [16-19], ciguatoxins [20-27], domoic acid [28-36], okadaic acid and analogues [37-40], prorocentrolide [41], and saxitoxins (STXs) [42-46]. Quite a few analogues of the main toxins had already been discovered [47-50], as well as several groups of compounds produced by dinoflagellates that provoke death in mice used for the mouse bioassay (MBA) but are not necessarily related to human poisoning events, such as pectenotoxins [50] and yessotoxins [51].

Discovery of toxins over the last 25 years

While discovery was mostly driven by human poisoning prior to the 1990s, afterwards the discovery of further compound groups that are produced by dinoflagellates and their metabolites in shellfish was facilitated by the introduction of the MBA for lipophilic toxins in routine shellfish safety testing in European legislation [52-53], as well as by several technological advances. One of the main technological drivers in discovery was certainly the onset of the use of liquid chromatography coupled to mass spectrometry (LC-MS) [54-56], which became quite widespread by the beginning of the 2000s [57-61].

The number of toxin groups that were discovered over the period from 1966 to 1990, (fifteen), was not much less than those discovered over the last 25 years, (nineteen) (Fig. 1). However, the number of analogues in each group has rapidly increased. A good example of this is the STX-group where a review in 1990 counted nineteen observed analogues with a further five predicted from plausible metabolization or chemical transformation pathways [62]. In 2010 a review reported over 50 observed analogues [63]. A simi-

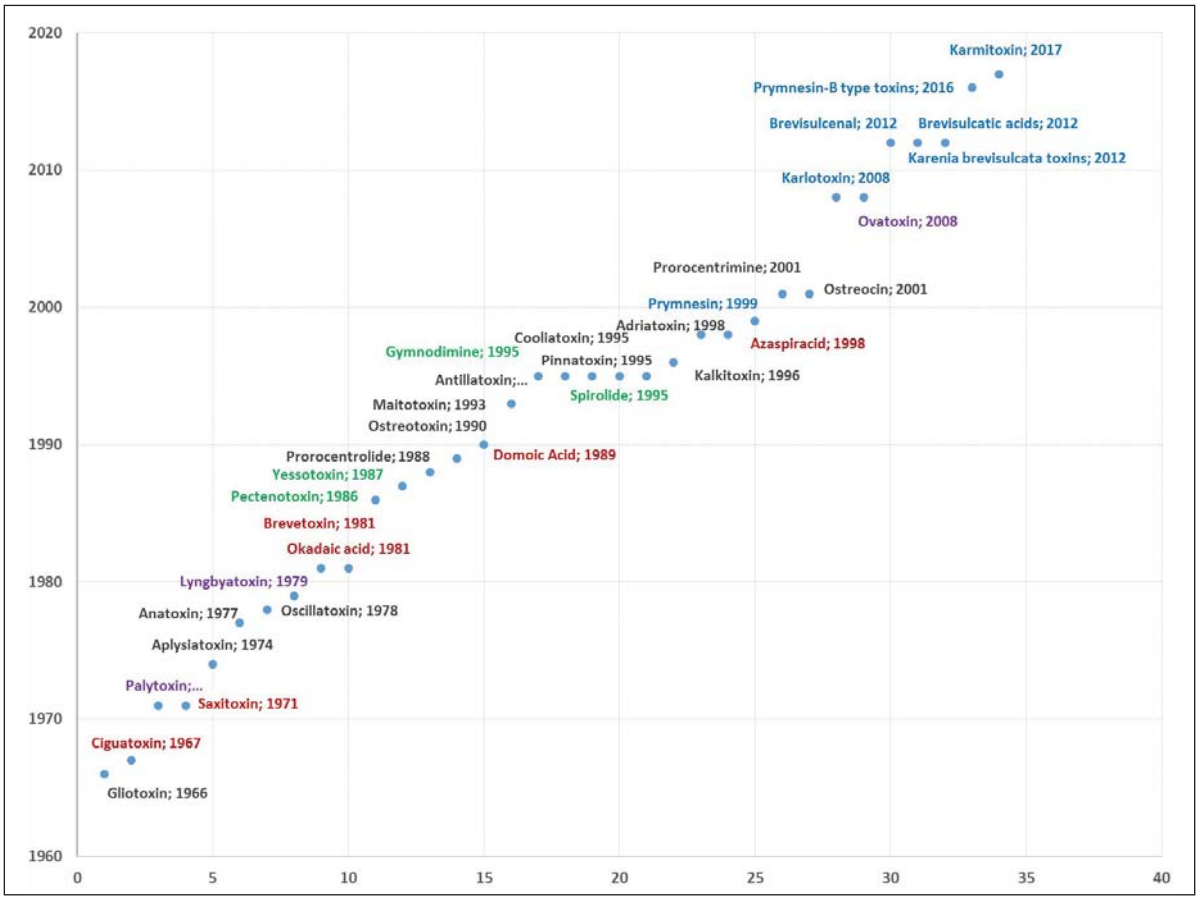

Fig. 1. Discovery or description of the structure of the first analogue of 34 toxin groups (1966 – 2017). Colour code: blue: toxins involved in fish kills; red: toxins involved in human poisoning, violet: toxins causing skin irritation or respiratory problems (BTXs should be red, blue and violet), green: toxins known for 20-30 years and not proven to have negative effects on humans or aquatic organisms, black: toxic compounds yet to be related to effects in humans or aquatic compounds. Nota bene: not many toxin groups relevant to human poisoning are being discovered while more and more toxins related to fish kills are (toxins of Karenia brevisulcata may be related to the Wellington Harbour syndrome)[79-108]

lar rapid increase in known analogues has been observed for the azaspiracid (AZA) group, with the first analogue described in 1998 [64] and a review in 2014 reporting 30 analogues [65]. Only three years later, over 50 analogues are known for this group, including novel phosphate derivatives [66-71]. This discovery rate could have been even more rapid if the causative organisms of AZAs had been elucidated earlier. However, the delay from the first poisoning report in 1995 [72] to the discovery of the culprit organism [73] in this case was likely due to: i) the initial misidentification of the heterotrophic dinoflagellate *Protoperidinium crassipes* (a vector of AZAs upon its feeding on *Azadinium*) as the causative agent, and ii) the difficulties in identification of such a small organism (<15 μm) by optical microscopy in water samples fixed with acidic Lugol's solution, the most common way to preserve samples in routine plankton monitoring.

Another phenomenon that has appeared repeatedly over the past 25 years is the discovery of slightly modified base skeletons for toxin groups. The ciguatoxin (CTX) or CTX1B (= P-CTX-1B) had been reported relatively early on and had been isolated from the moray eel [74]. The algal precursor CTX4A was only described in 1997 [75], yet a slightly modified base skeleton had been reported a few years earlier from *Gambierdiscus*, i.e. CTX3C [76]. Prymnesins are another example of such skeleton variation which is indeed very labour-intensive in natural product discovery as basically the full discovery pipeline has to be completed: bioguided fractionation and isolation of the compound, purification and structural characterisation including mass spectrometry, nuclear magnetic resonance (NMR), UV, infrared and potentially many other studies [77-78].

Finally, it should be noted that only a few compound groups discovered since 1992 have been clearly related to human health issues. These include AZAs (diarrhoea), ovatoxins and to a lesser extent the toxins of *Karenia brevisulcata* (aerosol and direct contact exposure).

There is a significant increase of compounds that appear related to fish kills, e.g. karlotoxins, karmitoxins, prymnesins (A, B and C-Type) and *K. brevisulcata* toxins. The need to clarify the agents involved in fish kills has also been highlighted by a recent systematic review of toxic and harmful algae [109], as well as by the Intergovernmental Oceanographic Commission of UNESCO (IOC) Intergovernmental Panel on Harmful Algal Blooms (IOC-IPHAB), that included the topic in its list of Task Teams.

The systematic inventory of toxins has also been updated [78-108] by the IOC-IPHAB Task Team on Biotoxins, Management and Regulation over the past few years and international databases, e.g. the Harmful Algal Event Database (HAEDAT) updated accordingly. This same panel also contributes to other IPHAB activities whenever chemical expertise is required (e.g. fish kills, HABs and desalination etc.).

Drivers of change in management and regulation

There have been many drivers of change in management practises (e.g. detection methodology) and regulation. These include (i) increased awareness by governments of poisoning events and fish and shellfish mortalities through IPHAB communication with member states (ii) increased pressure from shellfish industry against the MBA for lipophilic toxins due to its qualitative character, false positive results and delays in reporting (iii) technological advances. The conflicts caused by the disadvantages of the animal assays (mouse and rat) for lipophilic toxins has been subject to much debate [110] and decade-long efforts to produce the necessary standards and reference materials for the validation of alternative methods, which have been aided by researchers in Canada (e.g. Michael Quilliam), Ireland, Japan (e.g. Takeshi Yasumoto), New Zealand and many other countries [111-122]. Again the IPHAB panel played a pivotal role in pushing this issue at European and international levels for several years with the help of Phil Busby† (New Zealand Food Safety Authority), a long battle for which the international community will remember him.

Monitoring systems, management practises and legislative changes have been recently reviewed for different trade blocks [123-125]. A major step has been made with the switch from the mouse bioassay to chemical testing by LC-MS/MS for lipophilic toxins, first in New Zealand, then Europe [126] and most recently Japan.

Outlook

Several points can be raised looking foreward from the historic perspective. Climate change is one of the most striking challenges that has been raised with regards to prediction of harmful algal blooms (HABs), and while certain trends appear to manifest themselves [127], much more research is needed to fully anticipate the impacts of climate change on our ecosystems, HABs and their impacts on society [128]. As mentioned above, the need to improve our understanding of the impacts of micro-algae on other aquatic organisms, in particular those that serve as major food resources, i.e. fish and shellfish, has been recognised and requires major international efforts. The multiplicity of compounds in the marine environment only emerges with the recent onset of the omics and while recent studies have shown the feasibility to explore this chemical diversity in the marine environment with techniques such as metabolomics [129-131], more systematic studies will be required to effectively monitor our coastal waters to protect our resources and consumers. Finally, it should be noted that one of the longest-known groups of toxin, i.e. the ciguatoxins, still continue to cause the highest number of seafood poisoning globally [132] and thus deserves the attention of the scientific community over the next few decades.

References:

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2. *http://www.phycotox.fr/decouvrir/chimie/toxines-d-algues-et-classification.html?lng=en*

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