ELSEVIER

Trends in Food Science & Technology 11 (2000) 235–244

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Keeping shellfish safe to eat: a brief review of shellfish toxins, and methods for their detection

Ian Garthwaite

Toxinology, Food Science, AgResearch, Ruakura Research Centre, Private Bag 3123, East St Hamilton, New Zealand (Tel: +64-838-5147; Fax: +64-7-838-5189; e-mail: ian.garthwaite@agresearch.co.nz)

Poisoning can result from the ingestion of shellfish contaminated with phycotoxins. Various types of poisoning may occur, each of which is caused by a toxin (or group of toxins) from a particular alga. Classically, the mouse bioassay has been used to detect shellfish toxins, but there is pressure, both ethical and regulatory, to move away from this. A number of techniques have been developed to replace the bioassay, including immunoassay, chromatography, pharmacological assay and tissue-culture tests. All have advantages and limitations. These methods and their potential are reviewed. © 2001 Published by Elsevier Science Ltd. All rights reserved.

Shellfish are a delicious and nutritional food. They can, however, accumulate toxins which cause food poisoning. One health risk posed by the consumption of shellfish is due to the accumulation of algal biotoxins. Sporadic algal blooms in areas where shellfish are traditionally gathered or commercially farmed bring a requirement for monitoring of shellfish to ensure consumer safety.

The classical means of monitoring toxicity is the mouse bioassay, in which an extract of the shellfish is injected into mice. If the mice die, the shellfish are toxic [1,2]. Modern analytical techniques, such as HPLC, LC–MS, immunoassay, cellular bioassays and molecular probes, have an increasing role to play in both the monitoring process and in the identification of the environmental conditions and organisms responsible for toxin production. These new methods are not only humane, but more rapid and specific than the mouse assay. I will discuss the advances that these techniques have permitted toward our understanding of toxic bloom incidents, and present the further development of methodologies for the monitoring of shellfish toxins.

'Shellfish toxins' (more accurately known as phycotoxins) are produced by free-living micro-algae, upon which the shellfish feed. Shellfish concentrate the toxins and act as a vector transferring these toxic compounds further up the food chain as the shellfish are ingested by carnivores, including fish and crabs, which in-turn transfer the toxin to organisms predating on them. During an algal bloom, the concentration of toxin can rapidly increase within healthy shellfish to a level that is harmful to people. Sensitive analytical techniques that provide low (parts per billion) detection limits are required to ensure the safety of the product. These techniques must be able to deal with complicated sample matrices, and must be able to detect all toxins within a given toxin group, and differentiate these from related non-toxic compounds and from toxins of a different toxin group.

'Shellfish toxins' are non-proteinacious compounds of low molecular weight (Mr 250–3500 daltons). They have vastly differing chemical structures (Fig. 1), solubilities, and modes of action, and hence produce quite different effects when consumed. The toxins may be polar or lipophilic and may or may not be thermally labile; some are sensitive to pH, oxygen and light while others are highly stable.

There are approximately 20 species of dinoflagellates, and a smaller number of diatoms, that are currently known to produce phycotoxins (less than 2% of all algal species). Filter-feeding shellfish concentrate the toxins in their flesh, often with little or no ill-effects; toxins are passed into the food chain when these shellfish are gathered for eating, and poisoning occurs when a sufficient quantity of the contaminated shellfish is consumed. Five major classes of shellfish poisoning have been identified:

- Neurotoxic Shellfish Poisoning (NSP);
- Diarrhetic Shellfish Poisoning (DSP);

0924-2244/00/$ - see front matter Copyright © 2001 Published by Elsevier Science Ltd. All rights reserved.
PII: S0924-2244(01)00006-1

- Paralytic Shellfish Poisoning (PSP);
- Amnesic Shellfish Poisoning (ASP);
- Ciguaterra Fish Poisoning (CFP).

These occur world-wide, and are distinguished by the symptoms and the solubility of the toxins causing the poisoning.

NSP

The condition known as Neurotoxic Shellfish Poisoning (NSP) manifests with incoordination, paralysis and convulsions, and is caused by the lipid soluble brevetoxins (10 currently identified) [3]. These polyether toxins are produced by the organism *Gymnodinium breve*, and have been shown to be chemically modified by shellfish [4]. Seaspray contaminated with the organism irritates the eyes and nasal passages, leading to coughing and asthma-like symptoms [5]. *G. breve* blooms occur almost annually in the Gulf of Florida, where they are responsible for widespread fish kills, and have caused the death of manatees [6]. No human deaths have been recorded from NSP, although the condition is severely debilitating [7]. Regulatory authorities have set the maximum permitted level (MPL) of toxin as 80 μg brevetoxin/100 g flesh, equivalent to 20 standard mouse units (MU)/100 g flesh.

DSP

Diarrhetic Shellfish Poisoning (DSP) is caused by okadaic acid and analogues, including the dinophysis toxins DTX-1, 2, and 3, which are also lipid soluble. The major symptom is diarrhoea, and the illness is self-limiting. Unlike bacterial diarrhoea, symptoms usually begin within 30 min to a few hours after eating contaminated shellfish. Okadaic acid and the DTX-toxins have been shown to cause injury to the intestinal mucosa, and are implicated in tumor promotion [8].

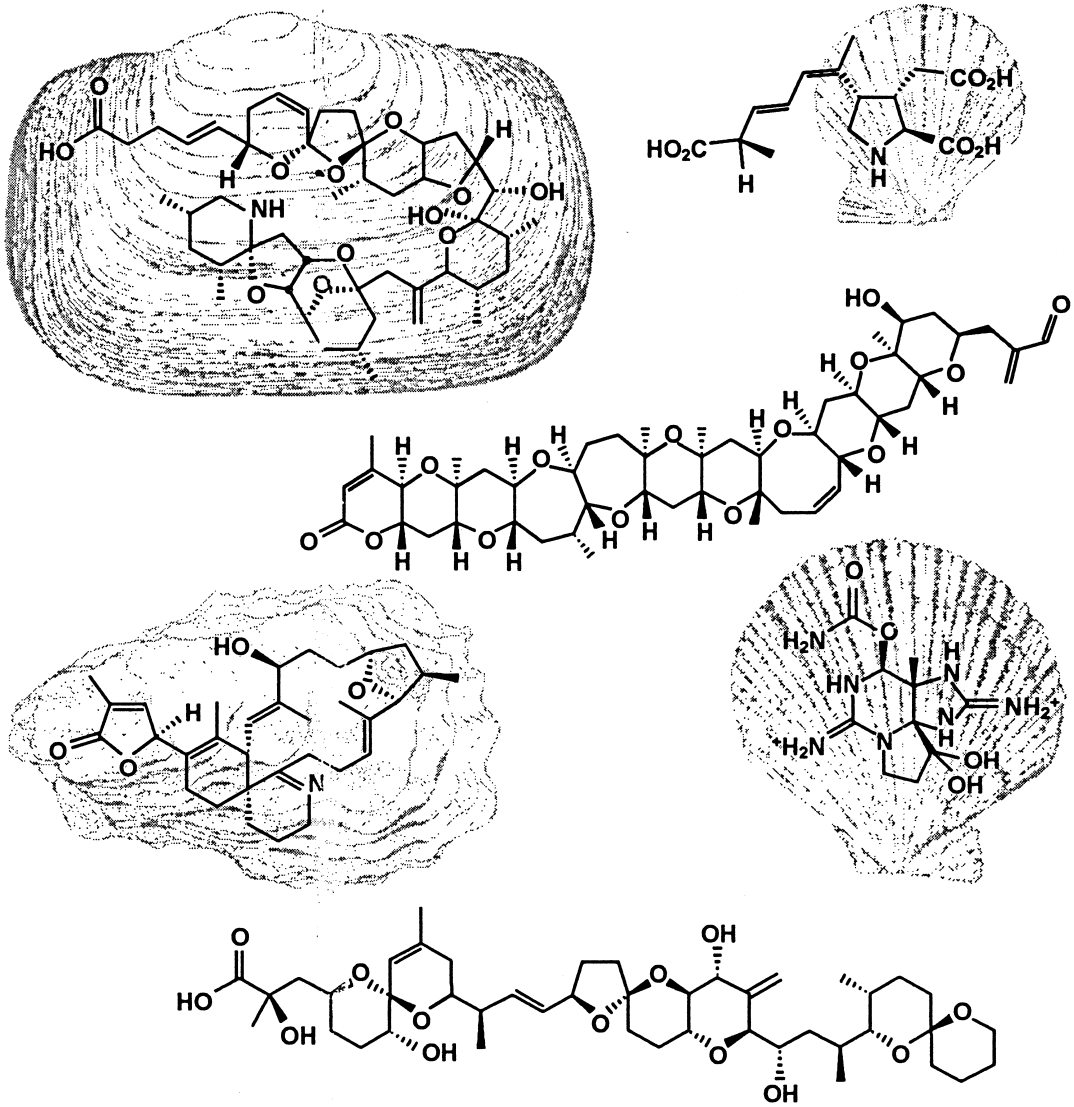

Fig. 1. Chemical structures of some of the phycotoxins (shellfish toxins). Clockwise from top left: azaspiracid, domoic acid (ASP), brevetoxin-2 (NSP), saxitoxin (PSP), okadaic acid (DSP), gymnodimine.

Regulations for this group limit the toxin content to below 16–20 μg 'okadaic acid equivalents' per 100 g flesh, and also include the structurally unrelated pectenotoxins and yessotoxins, the toxicities of which are currently under investigation [9].

PSP

The saxitoxins, a group of more than 20 water-soluble compounds of widely varying toxicity, are responsible for Paralytic Shellfish Poisoning (PSP), a serious, life threatening syndrome. At the onset, symptoms are neurological and include numbness, tingling and burning of the lips and skin, giddiness, ataxia and fever. Severe poisoning may lead to general muscular incoordination, respiratory distress and death from respiratory failure [10]. No antidote is available, and treatment is symptomatic. It is therefore important to prevent intoxication by monitoring shellfish for the presence of these toxins. Regulations restrict sale or harvest of shellfish containing PSP levels above 80 μg 'saxitoxin equivalents'/100 g shellfish flesh.

ASP

Amnesic Shellfish Poisoning (ASP) is caused by another water-soluble toxin, domoic acid. Symptoms include loss of balance, nausea, headache, disorientation, and vomiting. The condition results in the permanent loss of short-term memory. Action limits for this toxin were established following an outbreak of poisoning in Canada, when more than 150 people became ill after eating shellfish. Four people died and toxin levels over 900 ppm were detected in shellfish [11,12]. An MPL of 20 ppm domoic acid in whole shellfish has been adopted by many countries. ASP affects the whole marine food chain, and has been recently shown to cause deaths in Californian sealions which had eaten anchovies which had fed upon the diatom *Pseudo-nitzschia pungens* [13].

CFP

Ciguaterra poisoning (CFP), due to a phycotoxin produced by the benthic alga *Gambierdiscus toxicus*, is generally contracted from eating subtropical and tropical fin-fish, clams and marine snails may also be contaminated with the toxin. The lipophilic ciguatoxins (CTX) are highly potent, and produce symptoms similar to the NSP toxins, although CFP cases are typically more severe and include vomiting and diarrhoea. The condition can be fatal. CFP is the most frequently reported phycotoxin problem, with an estimated 10–50,000 cases per year. The high toxicity (0.25 μg/Kg *I.P.* in mice), and therefore extremely low detection limit required to ensure consumer safety (MPL 3.5 μg/100 g fish flesh, poses a challenge to the analyst [14,15].

More recently, other algal toxins, including azaspiracid, and the 'fast acting toxins' (gymnodimine, the spirolides, and pinnatoxins), have been added to the list. Azaspiracid causes symptoms reminiscent of DSP in humans, although in animals the effects are distinctly different from DSP [16]; and there is additional concern that azaspiracid is a potent hepatotoxin also. The fast acting toxins are so called because these bioactives cause rapid death in mice when injected intraperitoneally (i.p.) confounding the mouse bioassay. This is preceded by neurological symptoms including convulsions. There are no known cases of human intoxication by gymnodimine. Recent studies on the spirolides and pinnatoxins, which affect specific receptors in mammalian systems, may warrant concern for human consumers of shellfish [17–19].

Many countries have established shellfish screening programs, and regulatory maximum permitted levels (MPL) for human exposure have been set worldwide [20–23]. The toxicity of the phycotoxins is summarised in Table 1, for more details see [23–28].

It is beyond the scope of this review to document the distribution, regulation, and economic cost of phycotoxin incidents, however, at the time of writing, there is a widespread problem with ASP toxicity in Northern Europe, and PSP incidents in New Zealand and Scotland. Algal blooms cost millions of dollars per year in closures of harvestable shellfish, lost production and monitoring costs. Other major incidents of note are the 26 deaths from PSP in Guatemala [10], and PSP incidents in the USA [29].

The mouse bioassay procedures [1,2,12] for toxin detection date back to incidents of PSP and NSP toxicity in the 1940s in the USA; the extraction procedures have been modified over the intervening years (e.g. [30]), although the basic procedure has changed little. Water-soluble PSP and ASP toxins are simply extracted in boiling acid and the extract injected intraperitoneally into mice. However, the extraction procedures for lipid-soluble NSP and DSP toxins are more complex, and unsuited to large-scale screening. Acetone extracts are prepared, the acetone is removed, and the extract partitioned against dichloromethane. The dichloromethane layer is evaporated to produce a 'lipid extract', which is further processed for injection. The mouse bioassay does not distinguish between NSP and DSP toxins, so positive samples must be re-extracted and tested by an alternate method, e.g. DSP ELISA.

Over the past decade, improved technologies and animal ethics concerns have led to calls to replace the mouse assay. Indeed, this assay has been banned in a number of countries, including Germany, and is scheduled for removal from other European Union countries.

Methods for the analysis of shellfish toxins fall into six main categories: 1. Chromatographic analysis 2. Immunological analysis 3. Receptor binding assays 4. Cytotoxicity/Cell culture assays 5. Algal monitoring 6. Invertebrate bioasay

Each method has its merits and drawbacks, and ultimately it will be the responsibility of regulators and analysts to decide upon the most appropriate technology for their particular situation.

Chromatographic analysis

The first alternative methodologies to be developed were chromatographic procedures. Each class of toxin requires an individually tailored procedure, and the lack of chromophores on the majority of toxins means that a derivatisation reaction is required for toxin detection. Chromatographic procedures with UV or fluorescence detection are now available for the majority of shellfish toxins (Table 2). The increased availability of analytical standards has played a key role in the recent development of these methods although there is still a great need for reference standards.

The very recent application of HPLC–MS analysis to shellfish toxins has led to rapid advances in our understanding of the toxins, their production, accumulation, depuration, and chemistry. LC–MS relies upon an interface technology to deliver a small fraction of the HPLC flow into the mass spectrometer. Two interface technologies are available, ion-spray/electrospray (ESI) and atmospheric pressure chemical ionisation (APCI). Both have been applied to the analysis of shellfish toxins. The combination of LC to a double (or higher) quadropole MS is invaluable for the legal confirmation of the presence of toxin and its quantitation; unfortunately, the technology carries a very high price tag. LC–MS is able to resolve the majority of shellfish toxins, with the PSP group being the major exception.

LC–MS is reviewed by Quilliam [31], who is also developing procedures for the multiple determination of toxins in shellfish, a procedure that may permit the use of LC–MS as a first action screen [32].

Immunological analysis

Antibodies were first developed against the PSP toxins in 1964 [33], although it is only in recent years that immunoassays have been developed which show promise for the routine detection and quantification of shellfish toxins. The most common procedure is the enzyme

Table 1. Toxin properties: areas of common occurrence, toxicity, and regulatory action levels

Toxin	Most common geographical areas	LD_{50} (i.p. mouse μg/kg)	MPL (μg/100 g flesh)	MPL (mouse units)
ASP (domoic acid)	Canada, Northern USA, North Sea	120	2000	Sensitivity very low
PSP (saxitoxins)	North East USA, Asia, South America, Europe	10	80[a]	400
NSP (brevetoxins)	Florida, Mexico, New Zealand	200	80[b]	20
DSP (okadaic acid)	Japan, Europe	200	16[c] or 20	5
CTX (ciguatoxin)	Tropical areas—reef fish	0.25–0.9	3.5 (fish flesh)	Sensitivity too low

[a] As 'saxitoxin equivalents'.
[b] MPL assuming 1 MU = 4 μg PbTx-2 (pers. commun Professor T. Yasumoto).
[c] MPL (Europe) (pers. commun. M.L. Fernandez, EU Reference Laboratory, Vigo, Spain).

Table 2. Chromatographic detection procedures for shellfish toxins

Toxin class	Detection method	Derivatization	Detection limit in shellfish[a]	Reference and comments
ASP	UV 242 nm	None	400 ng/g	[79,80]
		SAX cleanup	20–30 ng/g	
	Fluorescence	FMOC	(15 pg/ml)	[81]
	LC–MS		10 ng/g	[31]
PSP	Fluorescence	Pre-column oxidation	(1–2 pg/injection)	[82] SPE cleanup- correlated with mouse at 40 μg/100g
	Fluorescence	Post-column oxidation	(5–30 pg/injection)	[83] Limit in flesh not given
	CE–MS (ESI)		(30 pg/injection)	[84] C-toxin fragmentation is problematic
DSP	UV 205 nm	None	(10 μg/ml)	[85] Extensive sample clean-up required
	Fluorescence	ADAM	10–20 ng/g[b]	[85,86] Matrix problems
			100 ng/g[c]	
	LC–MS (APCI)		8 ng/g	[32,87]
NSP	UV 215 nm	None	(500 ng/injection)	[7], Baden (pers. commun.)
	LC–MS (ESI)		(10 pg/injection)	[88]
CTX	Fluorescence			Insufficient for clinical relevance
	LC–MS		P-CTX-1 0.04 ng/g[d]	[89] Clinically relevant
			C-CTX-2 0.2 ng/g[d]	

[a] Detection limit of instrumental method is given (pg/injection) where limit in shellfish following extraction is not given by author and unknown.
[b] Hepatopancreas only.
[c] Whole shellfish.
[d] Fish flesh.

linked immunosorbent assay (ELISA). ELISAs are relatively cheap and quick, and are therefore suited to handling large numbers of samples. They do not require sophisticated and expensive facilities, and can be automated [34]. They also have the potential to be further developed for accurate quantitation of toxin concentration.

Immunoassays are developed using antibodies which recognise specific toxin structures. The choice of antibody is crucial for determining the usefulness of the assay—too specific and the assay will fail to recognise all members of a toxin family and underestimate the total toxin content (false negatives), not specific enough and it may detect non-toxic analogues and lead to the reporting of false positives. This is now well-recognised by the developers of immunoassays. The situation is highlighted in the case of the PSP toxins, where saxitoxin antibodies have poor cross-reactivity with neo-saxitoxin and vice versa.

ELISAs or commercial ELISA test kits are available for all four toxin families.

Antibodies for the detection of PSP toxins have been raised by a number of groups. Chu *et al.* demonstrated the utility of combining the results of saxitoxin and neosaxitoxin ELISA to cover the PSP toxin family, finding a very high correlation between the summed results and the mouse bioassay [35]. The state of the art of PSP immunoassay has been recently reviewed [36]. There is a commercial test kit available (Ridascreen, R-Biopharm, Darmstadt, Germany).

For ASP toxins, several groups have developed immunological assays for domoic acid [37–39]. Smith and Kitts assayed shellfish flesh and their ELISA results correlated well with results obtained by HPLC, but were approximately 9% higher, perhaps because the ELISA recognized the presence of DA isomers not determined by HPLC [40]. Unfortunately, their assay relied upon the limited resource of a serum from a single mouse, and will have limited use in shellfish testing. More recently, we have employed novel hapten chemistries to develop a highly sensitive antibody specific for domoic acid, and used this to develop an ELISA [41]. The ELISA can detect toxin levels more than 500 times below the MPL. Furthermore, the ELISA is highly specific, and analogous compounds that are sometimes found in algae, such as kainic acid, do not interfere with the assay. The assay is robust, and has been used successfully on a number of shellfish species and also on sea water samples and algal cultures; we have been able to identify and classify *Pseudo-nitzschia* species as toxigenic or non-toxigenic [42].

Brevetoxin-specific antibodies were first developed in the early 1990s [43–45]. These were used for the development of RIAs, and a cell based ELISA [46]. Using antibodies provided by Dr Poli, we have developed an ELISA system for NSP toxins, and compared the results with those obtained in the neuroblastoma assay and the mouse bioassay [47]. This assay was not cost effective, as it required a costly amplification step. We have subsequently developed another ELISA using our own antibodies.

This assay has a limit of quantitation for brevetoxin-3 of 0.53 ng /ml. The assay is now being developed for use with shellfish extracts, and looks very promising. The assay has already been applied to the direct analysis of seawater and has great potential as a tool for studying bloom dynamics and for identification of toxic dinoflagellates [48].

Poli and co-workers have used RIA and the receptor binding assay to study extracts of shellfish and urine from patients diagnosed with NSP [7]. Affinity-purification of the shellfish extracts using specific anti-brevetoxin antibodies yielded four major peaks of activity. One peak was identified by HPLC–MS as brevetoxin-3, while the other peaks contained compounds of higher mass, possibly conjugated metabolites. The latter substances were recognised by both RIA and the receptor binding assay, although they quantified differently. This finding suggests that these compounds react differently in the two assays, a result that may have important implications for seafood safety and regulation. The authors suggest that these metabolites are the true cause of NSP, and should be taken into account during regulatory testing. It should be noted that some assays, such as LC–MS, would miss these compounds.

Commercial test kits for DSP detection are available from the following suppliers: (i) SCETI Laboratories LTD, 2-2-8 Minami-Aoyama, Minato-ku, Tokyo, Japan, (ii) Rougier Biotech, Montreal, Canada, (iii) Iatron Laboratories, Chiyoda-ku, Tokyo, Japan. These test kits have been successfully used for the detection of okadaic acid and DTX toxins in shellfish hepatopancreas [49–52]. They have limited sensitivity when applied to whole shellfish, and have only limited cross-reactivity with some of the DTX toxins, notably DTX-2 and DTX-3. Quantitation of DTX-2 requires a specific assay, while DTX-3 may be measured after conversion to DTX-1 [53], which is detected with good sensitivity. An antibody that detects the DTX-toxins equally would be ideal.

Antibodies against yessotoxin have been raised [54], and both ELISA and affinity matrices are currently being developed. The ELISA is able to detect yessotoxin in shellfish with a sensitivity below 2.5 μg/100 g. These antibodies are being used in the isolation and identification of a number of new yessotoxin analogues from both algae and shellfish. Antibodies against the pectinotoxins are required to fully cover the toxins of the extended DSP group.

Antibodies against the ciguatoxins were first developed by Hokama, and used to develop an RIA [55]. This assay has provided a cost-effective method for the detection of CFP. The assay has been re-formatted into a ‘stick test’, in which toxin is captured onto a ‘liquid

paper' coated bamboo skewer, which is poked into the suspect tissue, and the toxin disclosed using specific labelled antibody [56]. This method is currently being evaluated by the AOAC. Pauillac *et al.* have also recently developed antibodies against ciguatoxin for ELISA development, by using a four-ring fragment of the toxin as immunogen [57].

To further increase the utility of the ELISA technique, and reduce the costs of regulatory testing, our laboratory has been developing a simple screening system. We have developed ELISAs for ASP, NSP, and DSP toxins including yessotoxin, and have combined these, along with a commercially available PSP ELISA, into a screening battery [58]. By employing a simple extraction protocol, we are able to detect each toxin group with a sensitivity below the MPL. The ease of performing ELISAs allows for the ready expansion of the system to screen for other toxins and bioactive compounds if these come under regulatory requirements in the future. We are now working on the validation of this screen.

Other immunological procedures have been established for the qualitative detection of shellfish toxins using dipstick, flow through and immuno-chromatographic techniques [15,56,59]. These are potentially extremely useful tools for shellfish producers as they are simple to use, require no laboratory equipment, and may be applied in the field. Antibodies developed for use in ELISA can also be incorporated into biosensor chips [60,61]. An immunosensor for DSP detection has been developed [62]. These immuno-biosensors have an advantage over the simpler 'kit-set' techniques, in that they are semi-quantitative. This would give better management information on whether toxin levels are low, close to, or above the MPL, for example. Producers could use them to determine whether toxin levels are rising or falling.

Receptor binding assays

Many of the toxins exert their biological activity at the cellular level, through the binding of receptors. The ASP toxins disrupt binding of glutamate to the AMDA glutamine receptors of the brain; DSP toxins bind to type 2a serine/threonine protein phosphatase enzymes, and the NSP and PSP toxins bind to receptors within the cellular sodium channel. Radio-ligand displacement assays have been established using isolated receptors for ASP [63], PSP [64] and NSP [65] toxins. Enzyme inhibition assays have been developed for the DSP toxins using chromogenic [66] and fluorescent [67] endpoint determination.

These assays have been used to good effect in the analysis of algal extracts and, in some cases, of shellfish flesh. With the development of suitable extraction methods, they have high potential for use in the screening of shellfish. The major drawback of radio-ligand displacement assays is the limited availability of radiolabelled standards and the often high regulatory (and personal) barriers to the use of radio-isotopes. For the PSP toxins, the adoption of the chemical weapons convention (saxitoxin is classed as a Schedule 1 chemical warfare agent) has had a major impact, severely restricting the movement of saxitoxin between countries and thus the availability of ^{3}H-saxitoxin.

Cytotoxicity/cell culture assays

Neuroblastoma cells, which contain many sodium channels within the cell wall, have been used to develop assays for PSP, CTX, and NSP toxins. Ouabain, a compound that inhibits the intracellular Na^+/K^+ pump (therefore blocking sodium release from the cell), and veratridine, which slightly opens the Na^+ channel, are added to a monolayer of cells. NSP and CTX toxins, which further open the channel, increase sodium influx, leading to cellular swelling and cell death during the 6–24 hour incubation. The higher the toxin concentration, the greater the mortality. The PSP toxins, however, block the Na^+ channel, reversing the effect of the veratridine, and the presence of these toxins is indicated by an increase in cell survival. Cell death has been monitored visually [68], by dye exclusion [69,70], and by the MTT technique, which detects live cells by measuring the metabolic cleavage of MTT [47,71]. This assay can be rapidly evaluated using a 96-well plate reader.

Other cell-based assays include a tissue biosensor for PSP, consisting of a Na^+ electrode covered with a frog bladder membrane containing sodium channels, integrated within a flow cell. [72], and a classical cytotoxicity assay for the DSP toxins, employing kidney cell monolayers [73,74].

These tissue culture-based procedures, while useful for research, are difficult to maintain in an analytical laboratory, and require specialised technical staff and facilities. To overcome these limitations, Jellet *et al.* have attempted to produce and distribute a commercially available assay (the MIST assay for PSP detection), based upon this principle [75]. Whilst this has largely been successful, it does rely on tight controls of transportation and temperature and a major effort has been made to develop packaging that will permit distribution world-wide. This method is currently undergoing AOAC evaluation. It remains to be seen if the logistics of delivery can be maintained to exacting requirements at a sufficiently low cost.

Algal monitoring

The monitoring of the coastline around shellfish beds by microscopic evaluation of water samples for toxigenic algae has been successfully used in a number of countries as a first step/early warning system. Trigger levels are used to signal closure of the shellfish beds and initiate the testing of shellfish for toxin. This procedure relies upon a good understanding of the toxigenic species

present in the environment, and upon the availability of highly trained taxonomists. Molecular probes, both immunological-based e.g. [76], and nucleic acid-based e.g. [42,77], have been developed to simplify screening in this manner. These procedures rely on a good correlation between the presence and number of a particular species and the presence of toxin in the flesh. This is often not the case, so cell counts tend to over-estimate the risk, which, while acceptable to the consumer, is costly to producers.

Invertebrate bioassays

Invertebrate bioassays using, for example, *Daphnia magna* [78] are cheap and qualitative. These assays lack sensitivity. When cost is a major issue, however, their use can save lives by at least detecting the high toxin levels that could lead to deaths.

The future?

In the immediate future the procedures which have been developed must be validated. The accuracy and reliability of the assays must be demonstrated using already proven methods as the yardstick, and this must be performed to the satisfaction of the regulatory agencies across a number of international markets. The assays will then be available to reduce our reliance upon the mouse bioassay.

The goal is to replace the bioassay with these more rapid, accurate and specific assays. My pick of the techniques would be the screening of shellfish flesh by immunoassays such as ELISA, as these are easily automated, and can be set-up to follow the same format regardless of toxin class, followed by confirmation using LC–MS. This combination would permit the rapid screening of large numbers of samples, with confirmation of toxin identity and quantification by MS analysis where necessary.

The antibody reagents employed in these ELISAs would then be available for development of more automated immuno-sensor assays. In the longer term, we will see the adoption of early warning systems in the more advanced shellfish growing regions. Scholin of MBARI is already working on the development of a prototype offshore buoy, upon which will be mounted immunological and molecular probe technologies. The buoy will be self-contained, and will send a signal when it detects levels of algae or toxin in the water that may constitute a risk.

A number of questions remain. *How* are the organisms spread—have they been here all the time? *Why* are the algae triggered to bloom; *why* do they produce the toxins? And, *what* can we do to understand and predict future incidents in order to protect both consumers and producers? The methodologies that are described here will play a key role in helping provide the answers to these questions.

References

1 McFarren, E.F., Tanabe, H., Silva, F.J., Wilson, W.B., Campbell, J.E. and Lewis, K.H. (1965) 'The Occurrence of a Ciguatera-Like Poison in Oysters, Clams, and *Gymnodinium breve* Cultures' in *Toxicon* 3, 111–123

2 AOAC. (1995) 'Official Method 959.08' in *Official Methods Of Analysis*, AOAC International, Gaithersburg, MD

3 Baden, D.G. (1989) 'Brevetoxins: Unique Polyether Dinoflagellate Toxins' in *The FASEB Journal* 3, 1807–1817

4 Ishida, H., Nozawa, A., Totoribe, K., Muramatsu, N., Nukaya, H., Tsuji, K., Yamaguchi, K., Takeshi, Y., Kaspar, H., Berkett, N. and Kosuge, T. (1995) 'Brevetoxin B_1, a New Polyether Marine Toxin from The New Zealand Shellfish, *Austrovenus stutchburyi*' in *Tetrahedron Letters* 36, 725–728

5 Baden, D.G., Mende, T.J., Bikhazi, G. and Leung, I. (1982) 'Bronchoconstriction Caused by Florida Red Tide Toxins' in *Toxicon* 20, 929–932

6 Bossart, G.D., Baden, D.G., Ewing, R.Y., Roberts, B. and Wright, S.D. (1998) 'Brevetoxicosis in Manatees (*Trichechus manatus latirostris*) from the 1996 Epizootic: Gross, Histologic, and Immunohistochemical Features' in *Toxicologic Pathology* 26, 276–282

7 Poli, M.A., Musser, S.M., Dickey, R.W., Eilers, P.P. and Hall, S. (2000) 'Neurotoxic Shellfish Poisoning and Brevetoxin Metabolites: A Case Study from Florida' in *Toxicon* 38, 981–993

8 Fujiki, H., Suganuma, M., Suguri, H., Yoshizawa, S., Takagi, K., Uda, N., Wakamatsu, K., Yamada, K., Murata, M. and Yasumoto, T., *et al.* (1988) 'Diarrhetic Shellfish Toxin, Dinophysistoxin-1, is a Potent Tumor Promoter on Mouse Skin' in *Japanese Journal of Cancer Research* 79, 1089–1093

9 Aune, T., Stabell, O.B., Nordstoga, K. and Tjotta, K. (1998) 'Oral Toxicity in Mice of Algal Toxins from the Diarrheic Shellfish Toxin (DST) Complex and Associated Toxins' in *Journal of Natural Toxins* 7, 141–158

10 Rodrigue, D.C., Etzel, R.A., Hall, S., de Porras, E., Velasquez, O.H., Tauxe, R.V., Kilbourne, E.M. and Blake, P.A. (1990) 'Lethal Paralytic Shellfish Poisoning in Guatemala' in *American Journal of Tropical Medicine and Hygene* 42, 267–271

11 Wright, J.L.C., Boyd, R.K., de Freitas, A.S.W., Falk, M., Foxall, R.A., Jamieson, W.D., Laycock, M.V., McCulloch, A.W., McInnes, A.G., Odense, P., Pathak, V., Quilliam, M.A., Ragan, M.A., Sim, P.G., Thibault, P., Walter, J.A., Gilgan, M., Richard, D.J.A. and Dewar, D. (1989) 'Identification of Domoic Acid, a Neuroexcitatory Amino Acid, in Toxic Mussels from Eastern Prince Edward Island' in *Canadian Journal of Chemistry* 67, 481–490

12 Iverson, F. and Truelove, J.F. (1994) 'Toxicology and Seafood Toxins: Domoic Acid' in *Natural Toxins* 2, 334–339

13 Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordaro, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K., Lipscomb, T., Loscutoff, S., Lowenstine, L.J., Marin, R., Miller, P.E., McLellan, W.A., Moeller, P.D.R., Powell, C.L., Rowles, T., Silvagni, P., Silver, M., Spraker, T. and Trainer, V., *et al.* (2000) 'Mortality of Sea Lions along the Central California Coast Linked to a Toxic Diatom Bloom' in *Nature* 403, 80–84

14 Hokama, Y. (1993) 'Recent Methods for Detection of Seafood Toxins: Recent Immunological Methods for Ciguatoxin and Related Polyethers' in *Food Additives and Contaminants* 10, 71–82

15 Park, D.L. (1994) 'Evolution of Methods for Assessing Ciguatera Toxins in Fish' in *Reviews of Environmental Contamination and Toxicology*, pp. 1–21, Springer-Verlag, New York.

16 Ito, E., Satake, M., Ofuji, K., Kurita, N., McMahon, T., James, K. and Yasumoto, T. (2000) 'Multiple Organ Damage Caused by a New Toxin Azaspiracid, Isolated From Mussels Produced in Ireland' in *Toxicon* 38, 917–930

17 Yasumoto, T. and Satake, M. (1998) 'New Toxins and Their Toxicological Evaluations' in *Harmful Algae* (Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., eds), pp. 461–464, Xunta de Galicia and IOC/UNESCO, Vigo

18 Stewart, M., Blunt, J.W., Munro, M.H.G., Robinson, W.T. and Hannah, D.J. (1997) 'The Absolute Stereochemistry of the New Zealand Shellfish Toxin Gymnodimine' in *Tetrahedron Letters* 38, 4889–4890

19 Uemura, D., Chou, T., Haino, T., Nagatsu, A., Fukuzawa, S., Zheng, S. and Chen, H. (1995) 'Pinnatoxin A: A Toxic Amphoteric Macrocycle from the Okinawan Bivalve *Pinna muricata*' in *Journal of the American Chemical Society* 117, 1155–1156

20 van Egmond, H.P., Aune, T., Lassus, P., Speijers, G.J.A. and Waldock, M. (1993) 'Paralytic and Diarrhoeic Shellfish Poisons: Occurrence in Europe, Toxicity, Analysis and Regulation' in *Journal of Natural Toxins* 2, 41–83

21 Park, D.L. (1995) 'Surveillance Programmes for Managing Risks from Naturally Occurring Toxicants' in *Food Additives and Contaminants* 12, 361–371

22 Park, D.L., Guzman-Perez, S.E. and Lopez-Garcia, R. (1999) 'Aquatic Biotoxins: Design and Implementation of Seafood Safety Monitoring Programs' in *Reviews of Environmental Contamination and Toxicology* 161, 157–200

23 Quilliam, M.A. (1999) 'Phycotoxins' in *Journal of AOAC International* 82, 773–781

24 Morse, E.V. (1977) 'Paralytic Shellfish Poisoning: A Review' in *Journal of the American Veterinary Medical Association* 171, 1178–1180

25 Lagos, N. (1998) 'Microalgal Blooms: A Global Issue with Negative Impact in Chile' in *Biological Research* 31, 375–386

26 Clark, R.F., Williams, S.R., Nordt, S.P. and Manoguerra, A.S. (1999) 'A Review of Selected Seafood Poisonings' in *Undersea and Hyperbaric Medicine* 26, 175–184

27 Van Dolah, F.M. (2000) 'Marine Algal Toxins: Origins, Health Effects, and their Increased Occurrence' in *Environmental Health Perspectives* 108(Suppl 1), 133–141

28 Shimizu, Y. (1996) 'Microalgal Metabolites: A New Perspective' in *Annual Review of Microbiology* 50, 431–465

29 Gessner, B.D. and Middaugh, J.P. (1995) 'Paralytic Shellfish Poisoning in Alaska: A 20-year Retrospective Analysis' in *American Journal of Epidemiology* 141, 766–770

30 Hannah, D.J., Till, D.G., Deverall, T., Jones, P.D. and Fry, J.M. (1995) 'Extraction of Lipid-Soluble Marine Biotoxins' in *Journal of AOAC International* 78, 480–483

31 Quilliam, M.A. (1996) 'Liquid Chromatography–Mass Spectrometry of Seafood Toxins' in *Applications of LC–MS in Environmental Chemistry*, (Barcelo, D., ed), pp. 415–444, Elsevier, Amsterdam

32 Quilliam, M.A. (1998) 'Liquid Chromatography–Mass Spectrometry: a Universal Method for the Analysis of Toxins?' in *Harmful Algae*, (Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., eds), pp. 509–514, Xunta de Galicia and IOC/UNESCO, Vigo

33 Johnson, H.M., Frey, P.A., Angelotti, R., Campbell, J.E. and Lewis, K.H. (1964) 'Haptenic Properties of Paralytic Shellfish Poison Conjugated to Proteins by Formaldehyde Treatment' in *Proceedings of Society of Experimental Biology and Medicine* 117, 425–430

34 Wilkinson, A.P., Ward, C.M. and Morgan, M.R.A. (1991) 'Immunological Analysis of Mycotoxins' in *Plant Toxin Analysis* (Linskens, H.F., Jackson, J.F., eds), pp. 185–225, Springer-Verlag, Berlin

35 Chu, F.S., Hsu, K.H., Huang, X., Barrett, R. and Allison, C. (1996) 'Screening of Paralytic Shellfish Poisoning Toxins in Naturally Occurring Samples with Three Different Direct Competitive Enzyme-Linked Immunosorbent Assays' in *Journal of Agricultural and Food Chemistry* 44, 4043–4047

36 Usleber, E., Dietrich, R., Burke, C., Schneider, E. and Maetlbauer, E. (in press) 'Immunoassay Methods for Paralytic Shellfish Poisoning (PSP) Toxins' in *J. AOAC International*

37 Newsome, H., Truelove, J., Hierlihy, L. and Collins, P. (1991) 'Determination of Domoic Acid in Serum and Urine by Immunochemical Analysis' in *Bulletin of Environmental Contamination and Toxicology* 47, 329–334

38 Osada, M., Marks, L.J. and Stewart, J.E. (1995) 'Determination of Domoic Acid by Two Different Versions of a Competitive Enzyme-Linked Immunosorbent Assay (ELISA)' in *Bulletin of Environmental Contamination and Toxicology* 54, 797–804

39 Kawatsu, K., Hamano, Y. and Noguchi, T. (1999) 'Production and Characterization of a Monoclonal Antibody Against Domoic Acid and its Application to Enzyme Immunoassay' in *Toxicon* 37, 1579–1589

40 Smith, D.S. and Kitts, D.D. (1995) 'Enzyme Immunoassay for the Determination of Domoic Acid in Mussel Extracts' in *Journal of Agricultural and Food Chemistry* 43, 367–371

41 Garthwaite, I., Ross, K.M., Miles, C.O., Hansen, R.P., Foster, D., Wilkins, A.L. and Towers, N.R. (1998) 'Polyclonal Antibodies to Domoic Acid, and their Use in Immunoassays for Domoic Acid in Sea Water and Shellfish' in *Natural Toxins* 6, 93–104

42 Rhodes, L., Scholin, C. and Garthwaite, I. (1998) '*Pseudo-nitzschia* in New Zealand and the Role of DNA Probes and Immunoassays in Refining Marine Biotoxin Monitoring Programmes' in *Natural Toxins* 6, 105–111

43 Trainer, V.L. and Baden, D.G. (1991) 'An Enzyme Immunoassay for the Detection of Florida Red Tide Brevetoxins' in *Toxicon* 29, 1387–1394

44 Levine, L. and Shimizu, Y. (1992) 'Antibodies to Brevetoxin B: Serologic Differentiation of Brevetoxin B and Brevetoxin A' in *Toxicon* 30, 411–418

45 Poli, M.A., Rein, K.S. and Baden, D.G. (1995) 'Radioimmunoassay for PbTx-2-type Brevetoxins: Epitope Specificity of Two Anti-PbTx Sera' in *Journal of AOAC International* 78, 538–542

46 Baden, D.G., Melinek, R., Sechet, V., Trainer, V.L., Schultz, D.R., Rein, K.S., Tomas, C.R., Delgado, J. and Hale, L. (1995) 'Modified Immunoassays for Polyether Toxins: Implications of Biological Matrixes, Metabolic States, and Epitope Recognition' in *Journal of AOAC International* 78, 499–508

47 Garthwaite, I., Ross, K., Poli, M. and Towers, N.R. (1996) 'Comparison of Immunoassay, Cellular, and Classical Mouse Bioassay Methods for Detection of Neurotoxic Shellfish Toxins' in *Immunoassays for Residue Analysis. American Chemical Society Symposium series 621 (Beier, R.C., Stanker, L.H., eds)*. pp. 404–412 (Chapter 32)

48 Garthwaite, I., Ross, K.M. and Haywood, A. (1998) 'Detection of NSP (Brevetoxins) in Algal Cultures and Seawater by ELISA' in *Proceedings of the 10th Biotoxin Science Workshop*, pp. 51–54, MAF New Zealand, Wellington, NZ

49 Chin, J.D., Quilliam, M.A., Fremy, J.M., Mohapatra, S.K. and Sikorska, H.M. (1995) 'Screening for Okadaic Acid by Immunoassay' in *Journal of AOAC International* 78, 508–513

50 Carmody, E.P., James, K.J. and Kelly, S.S. (1995) 'Diarrhetic Shellfish Poisoning: Evaluation of Enzyme-Linked Immunosorbent Assay Methods for Determination of Dinophysistoxin-2' in *Journal of AOAC International* 78, 1403–1408

51 Tubaro, A., Sosa, S., Bruno, M., Gucci, P.M.B., Volterra, L. and Loggia, R.D. (1992) 'Diarrhoeic Shellfish Toxins in Adriatic Sea Mussels Evaluated by an ELISA Method' in *Toxicon* 30, 673–676

52 Morton, S.L. and Tindall, D.R. (1996) 'Determination of Okadaic Acid Content of Dinoflagellate Cells: A Comparison of the HPLC-Fluorescent Method and Two Monoclonal Antibody ELISA Test Kits' in *Toxicon* 34, 947–954

53 Suzuki, T., Ota, H. and Yamasaki, M. (1998) 'Direct Evidence of Transformation of Dinophysistoxin-1 to 7-*O*-Acyl-dinophysistoxin-1 (dinophysistoxin-3) in the Scallop *Patinopecten yessoensis*' in *Toxicon* 37, 187–198

54 Briggs, L., Towers, N.R., Miles, C.O., Garthwaite, I. and Ross, K.M. (1999) 'Progress Towards ELISAs for Okadaic Acid and Yessotoxin' in *Proceedings of the 11th Biotoxin Science Workshop*, pp. 98–102, MAF New Zealand, Wellington, NZ

55 Hokama, Y., Banner, A.H. and Boylan, D.B. (1977) 'A Radioimmunoassay for the Detection of Ciguatoxin' in *Toxicon* 15, 317–325

56 Hokama, Y., Takenaka, W.E., Nishimura, K.L. and Ebesu, J.S.M. (1998) 'A Simple Membrane Immunobead Assay for Detecting Ciguatoxin and Related Polyethers from Human Ciguatera Intoxication and Natural Reef Fishes' in *Journal of AOAC International* 81, 727–735

57 Pauillac, S., Sasaki, M., Inoue, M., Naar, J., Branaa, P., Chinain, M., Tachibana, K. and Legrand, A.M. (2000) 'Characterization of Mice Antisera Elicited with a Ciguatoxin Tetracyclic Synthetic Ring Fragment (JKLM) Conjugated to Carrier Proteins' in *Toxicon* 38, 669–685

58 Garthwaite, I., Ross, K.M., Miles, C.O., Briggs, L.R., Towers, N.R., Borrell, T. and Busby, P. (in press) 'An Integrated ELISA Screen for ASP, NSP, DSP and PSP Toxins Found in New Zealand' in *J. AOAC International*

59 Usleber, E., Schneider, E. and Terplan, G. (1991) 'Direct Enzyme Immunoassay in Microtitration Plate and Test Strip Format for the Detection of Saxitoxin in Shellfish' in *Letters in Applied Microbiology* 13, 275–277

60 Cheun, B., Endo, H., Hayashi, T., Nagashima, Y. and Watanabe, E. (1996) 'Development of an Ultra High Sensitive Tissue Biosensor for Determination of Shellfish Poisoning, Tetrodotoxin' in *Biosensors & Bioelectronics* 11, 1185–1191

61 Marquette, C.A., Coulet, P.R. and Blum, L.J. (1999) 'Semi-Automated Membrane Based Chemiluminescent Immunosensor for Flow Injection Analysis of Okadaic Acid in Mussels' in *Analytica Chimica Act* 19931, 1–10

62 Kreuzer, M.P., O'Sullivan, C.K. and Guilbault, G.G. (1999) 'Development of an Ultrasensitive Immunoassay for Rapid Measurement of Okadaic Acid and its Isomers' in *Analytical Chemistry* 71, 4198–4202

63 Van Dolah, F.M., Leighfield, T.A., Haynes, B.L., Hampson, D.R. and Ramsdell, J.S. (1997) 'A Microplate Receptor Assay for the Amnesic Shellfish Poisoning Toxin, Domoic Acid, Utilizing a Cloned Glutamate Receptor' in *Analytical Biochemistry* 245, 102–105

64 Doucette, G.J., Logan, M.M., Ramsdell, J.S. and Van Dolah, F.M. (1997) 'Development and Preliminary Validation of a Microtiter Plate-Based Receptor Binding Assay for Paralytic Shellfish Poisoning Toxins' in *Toxicon* 35, 625–636

65 Trainer, V.L., Baden, D.G. and Catterall, W.A. (1995) 'Detection of Marine Toxins Using Reconstituted Sodium Channels' in *Journal of AOAC International* 78, 570–573

66 Tubaro, A., Florio, C., Luxich, E., Sosa, S., della Loggia, R. and Yasumoto, T. (1996) 'A Protein Phosphatase 2A Inhibition Assay for a Fast and Sensitive Assessment of Okadaic Acid Contamination in Mussels' in *Toxicon* 34, 743–752

67 Mountford, D.O., Kennedey, G., Garthwaite, I., Quilliam, M., Trueman, P. and Hannah, D.J. (1996) 'Evaluation of the Fluorometric Protein Phosphatase Inhibition Assay in the Determination of Okadaic Acid in Mussels' in *Toxicon* 37, 909–922

68 Kogure, K., Tamplin, M.L., Simidu, U. and Colwell, R.R. (1988) 'A Tissue Culture Assay for Tetrodotoxin, Saxitoxin and Related Toxins' in *Toxicon* 26, 191–197

69 Jellett, J.F., Marks, L.J., Stewart, J.E., Dorey, M.L., Watson-Wright, W. and Lawrence, J.F. (1992) 'Paralytic Shellfish Poison (Saxitoxin Family) Bioassays: Automated Endpoint Determination and Standardization of the *In Vitro* Tissue Culture Bioassay, and Comparison with the Standard Mouse Bioassay' in *Toxicon* 30, 1143–1156

70 Gallacher, S. and Birkbeck, T.H. (1992) 'A Tissue Culture Assay for Direct Detection of Sodium Channel Blocking Toxins in Bacterial Culture Supernates' in *FEMS Microbiology Letters* 92, 101–108

71 Manger, R.L., Leja, L.S., Lee, S.Y., Hungerford, J.M. and Wekell, M.M. (1993) 'Tetrazolium-Based Cell Bioassay for Neurotoxins Active on Voltage-Sensitive Sodium Channels: Semi-automated Assay For Saxitoxins, Brevetoxins, and Ciguatoxins' in *Analytical Biochemistry* 214, 190–194

72 Cheun, B.S., Loughran, M., Hayashi, T., Nagashima, Y. and Watanabe, E. (1998) 'Use of a Channel Biosensor for the Assay of Paralytic Shellfish Toxins' in *Toxicon* 36, 1371–1381

73 Tubaro, A., Florio, C., Luxich, E., Vertua, R., Della Loggia, R. and Yasumoto, T. (1996) 'Suitability of the MTT-based Cytotoxicity Assay to Detect Okadaic Acid Contamination of Mussels' in *Toxicon* 34, 965–974

74 Croci, L., Cozzi, L., Stacchini, A., de Medici, D. and Toti, L. (1997) 'A Rapid Tissue Culture Assay for the Detection of Okadaic Acid and Related Compounds in Mussels' in *Toxicon* 35, 223–230

75 Jellett, J.F., Wood, C.R., Belland, E.R. and Doucette, L.I. (1998) 'The MIST Shippable Cell Bioassay Kit for PSP: An Alternative to the Mouse Bioassay' in *Harmful Algae* (Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., eds), pp. 558–257, Xunta de Galicia and IOC/UNESCO, Vigo

76 Vrieling, E.G. and Anderson, D.M. (1996) 'Immunofluorescence in Phytoplankton Research: Applications and Potential' in *J. Phycology* 32, 1–16

77 Scholin, C.A. and D.M. Anderson, D.M. (1998) 'Detection and Quantification of HAB Species Using Antibody and DNA Probes: Progress to Date and Future Research Objectives' in *Harmful Algae* (Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., eds), pp. 253–257, Xunta de Galicia and IOC/UNESCO, Vigo

78 Vernoux, J.-P., Le Baut, C., Masselin, P., Marais, C., Baron, B., Choumiloff, R., Proniewski, F., Nizard, G. and Bohec, M. (1993) 'The Use of *Daphnia magna* for Detection of Okadaic Acid in Mussel Extracts' in *Food Additives and Contaminants* 10, 603–608

79 Quilliam, M.A., Sim, P.G., McCulloch, A.W. and McInnes, A.G. (1989) 'High-Peformance Liquid Chromatography of Domoic Acid, a Marine Neurotoxin, With Application to Shellfish and Plankton' in *International Journal of Environmental Analytical Chemistry* 36, 139–154

80 Lawrence, J.F., Charbonneau, C.F., Ménard, C., Quilliam, M.A. and Sim, P.G. (1989) 'Liquid Chromatographic Determination of Domoic Acid in Shellfish Products Using the Paralytic Shellfish Poison Extraction Procedure of the Association of Official Analytical Chemists' in *Journal of Chromatography* 462, 349–356

81 Pocklington, R., Milley, J.E., Bates, S.S., Bird, C.J., de Freitas, A.S.W. and Quilliam, M.A. (1990) 'Trace Determination of Domoic Acid in Seawater and Phytoplankton by High-Performance Liquid Chromatography of the Fluorenylmethoxycarbonyl (FMOC) Derivative' in *International Journal of Environmental Analytical Chemistry* 38, 351–368

82 Lawrence, J.F., Ménard, C. and Cleroux, C. (1995) 'Evaluation of Prechromatographic Oxidation for Liquid Chromatographic Determination of Paralytic Shellfish Poisons in Shellfish' in *Journal of AOAC International* 78, 514–520

83 Oshima, Y. (1995) 'Post-Column Derivatization HPLC Methods for Paralytic Shellfish Poisons' in *Manual on Harmful Marine Microalgae*, (Hallengraef, G.M., Anderson, D.M., Cembella, A.D., eds), pp. 81–94, UNESCO, Paris

84 Locke, S.J. and Thibault, P. (1994) 'Improvement in Detection Limits for the Determination of Paralytic Shellfish Poisoning Toxins in Shellfish Tissues Using Capillary Electrophoresis/Electrospray Mass Spectrometry and Discontinuous Buffer Systems' in *Analytical Chemistry* 66, 3436–3446

85 Quilliam, M.A. and Wright, J.L.C. (1995) 'Methods For Diarrhetic Shellfish Poisons' in *Manual on Harmful Marine Microalgae* (Hallengraef, G.M., Anderson, D.M., Cembella, A.D., eds), pp. 95–112, UNESCO, Paris

86 Quilliam, M.A. (1995) 'Analysis of Diarrhetic Shellfish Poisoning Toxins in Shellfish Tissue by Liquid Chromatography With Fluorometric and Mass Spectrometric Detection' in *Journal of AOAC International* 78, 555–570

87 James, K.J., Bishop, A.G., Gillman, M., Kelly, S.S., Roden, C., Draisci, R., Lucentini, L., Giannetti, L. and Pierpaolo, B. (1997) 'Liquid Chromatography With Fluorimetric, Mass Spectrometric and Tandem Mass Spectrometric Detection for the Investigation of the Seafood-Toxin-Producing Phytoplankton, *Dinophysis acuta*' in *Journal of Chromatography A* 777, 213–221

88 Hua, Y.S. and Cole, R.B. (2000) 'Electrospray Ionization Tandem Mass Spectrometry for Structural Elucidation of Protonated Brevetoxins in Red Tide Algae' in *Analytical Chemistry* 72, 376–383

89 Lewis, R.J., Jones, A., Vernoux, J.-P. and Marquis, M. (1998) 'Sensitive Detection of Multiple Ciguatoxins by HPLC/MS/MS' in *Harmful Algae* (Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., eds), pp. 254–523, Xunta de Galicia and IOC/UNESCO, Vigo

Any Suggestions?

Articles published in TIFS are usually **specially invited** by the Editors, with assistance from our International Advisory Editorial Board. However, we **welcome ideas** from readers for articles on exciting new and developing areas of food research. A brief synopsis of the proposal should first be sent to the Editors, who can provide detailed guidelines on manuscript preparation.

Mini-reviews focus on promising areas of food research that are advancing rapidly, or are in need of re-review in the light of recent advances or changing priorities within the food industry. Thus they are shorter than conventional reviews, focusing on the latest developments and discussing likely future applications and research needs.

Features are similar in style to mini-reviews, highlighting specific topics of broad appeal to the food science community.

The **Viewpoint** section provides a forum to express personal options, observations or hypotheses, to present new perspectives, and to help advance understanding of controversial issues by provoking debate and comment.

Conference Reports highlight and assess important developments presented at relevent conferences worldwide.

TIFS also welcomes **Letters** to the Editor concerned with issues raised by published articles or by recent developments in the food sciences.

All Review-style articles are subject to editorial and independent **peer review** by international experts in the appropriate field.