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Amnesic shellfish poisoning: A review

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Abstract

This review contains information on the amnesic shellfish poisoning (ASP) syndrome and the provoking ASP toxins, of which domoic acid is the major component. Data on chemical structures and detection methods of ASP toxins, sources for ASP toxins, marine organisms associated with ASP, toxicity of ASP toxins for animals and man, possible preventive measures for ASP, case reports/outbreaks of ASP and regulations and monitoring of ASP toxins are included. Finally some recommendations are given for a better control of the ASP problem in the future.

Contents

SAMENVATTING 5

SUMMARY 7

1. INTRODUCTION 9

2. THE ASP TOXINS 10

2.1 Chemical structures and properties 10

2.2 Determination of ASP toxins 12
2.2.1 Bioassays 12
2.2.2 Biochemical assays 12
2.2.3 Chemical assays 14
2.2.4 Reference materials 17

3. SOURCES OF ASP TOXINS 18

3.1 Global increase of harmful algal blooms 18

3.2 Source organisms for ASP toxins 18

3.3 Distribution of source organisms 19

3.4 Habitat of Pseudonitzschia species producing domoic acid 20

4. MARINE ORGANISMS ASSOCIATED WITH ASP 23

4.1 Uptake and elimination of ASP toxins in marine organisms 23

4.2 Shellfish containing ASP toxins 25

4.3 Other marine organisms containing ASP toxins 25

5. TOXICITY OF ASP TOXINS TO ANIMALS AND MAN 27

5.1 Mechanism of action 27

5.2 Toxicity to laboratory animals 27
5.2.1 Acute toxicity 27
5.2.2 Repeated dose toxicity 28
5.2.3 Teratogenicity 29

5.3 Mutagenicity 29

5.4 Toxicity to man 30

5.5 Toxicity to aquatic organisms 30

5.6 Toxicity to water fowl 31

6. PREVENTION OF ASP INTOXICATION 32

6.1 Depuration 32

6.2 Preventive measures 33

7. CASE REPORTS/OUTBREAKS AND SURVEYS 34

7.1 North America 34
7.1.1 Canada 34
7.1.2 USA 35
7.1.3 Mexico 37

7.2 Europe 37
7.2.1 Denmark 37
7.2.2 The Netherlands 37
7.2.3 Portugal 37
7.2.4 Spain 38
7.2.5 United Kingdom 38

7.3 Asia 38
7.3.1 Japan 38

7.4 Oceania 38
7.4.1 Australia, Tasmania and New Zealand 38

8. REGULATIONS AND MONITORING 40

8.1 North-America 40
8.1.1 Canada 40
8.1.2 USA 40

8.2 Europe 40
8.2.1 Denmark 40

8.3 Oceania 40
8.3.1 Australia 40
8.3.2 New Zealand 41

9. RISK ASSESSMENT 42

10. DISCUSSION AND RECOMMENDATIONS 43

REFERENCES 45

APPENDIX 1 MAILING LIST 53

Samenvatting

"Amnesic shellfish poisoning" (ASP) werd voor de eerste maal gerapporteerd vanuit Canada, Prince Edward Island in 1987. Deze uitbraak van ASP werd toegeschreven aan de consumptie van (gekweekte) blauwe mosselen (*Mytilus edilus*) en de veroorzakende component werd geidentificeerd als "domoic acid" (DA) afkomstig van het mariene diatomee *Pseudonitzschia pungens* f. *multiseries* (ook genoemd *Pseudonitzschia multiseries*).
Naast *P. multiseries* zijn tenminste 4 andere *Pseudonitzschia* soorten gerapporteerd die DA produceren; *P. australis*, *P. pseudodelicatissima*, *P. delicatissima* en *P. seriata*. *Pseudonitzschia* soorten komen voor in alle kustwateren van de Atlantische, de Stille en de Indische oceaan. Het is niet duidelijk onder welke hydrografische en milieu condities schadelijke, DA producerende, *Pseudonitzschia* "blooms" ontstaan. Diverse mogelijke oorzaken voor schadelijke "blooms" van *Pseudonitzschia* soorten worden genoemd in dit rapport, zoals temperatuur, lichtsterkte, zoutgehalte, "upwelling", hoeveelheid phytoplankton en het gehalte aan voedingsstoffen (stikstof, silicaat, fosfaat). De groeifase van de betreffende *Pseudonitzschia* soort is ook een kritische factor.
Schelpdieren accumuleren DA door filtratie van planktoncellen of door directe consumptie van gecontamineerde organismen. De accumulatiesnelheid van toxische algen (en het toxine) in schelpdieren is soort-afhankelijk en is, in de meeste gevallen direct gerelateerd aan het aantal planktoncellen dat beschikbaar is voor het schelpdier. De accumulatiesnelheid van toxische algen in de afzonderlijke schelpdieren in een bepaald gebied is zeer variabel. De eliminatiesnelheid van het toxine varieert met het seizoen en lage watertemperaturen vertragen de afname van het toxinegehalte. Verder is eliminatie in grote mate afhankelijk van de plaats in het schelpdier waar het toxine is opgeslagen; toxines in het maagdarmkanaal worden veel sneller verwijderd dan toxines gebonden aan bepaalde weefsels. DA gehaltes in blauwe mosselen dalen vrij snel. In mariene kammosselen (*Placopecten magellanicus*) en rode mosselen (*Modiolus modiolus*) wordt DA voor langere periodes vastgehouden. De daling van het DA gehalte in de "razor clam" (*Siliqua patula*) is een lange termijn proces.
Accumulatie van DA is gerapporteerd in de blauwe mossel (*Mytilus edilus*), rode mossel (*Modiolus modiolus*), "Californian" mossel (*Mytilus californianus*),"soft-shell clam" (*Mya arenaria*), "razor clam" (*Siliqua patula*), "bay scallop "(*Argopecten iradians*), "sea scallop" (*Placopecten magellanicus*), oester (*Crassostrea virginica*), "Pacific" oester (*Crassostrea gigas*), "Dungeness" krab (*Cancer magister*), makreel (*Scomber japonicus*) en ansjovis (*Engraulis mordax*).
Naast DA zijn nog een aantal geometrische isomeren van DA geidentificeerd, maar alleen "isodomoic acids" D, E en F en de C5' diastereomeer van DA zijn aangetroffen in plankton en/of schelpdieren. De geometrische isomeren van DA zijn geen *de novo* producten van het plankton, maar worden beschouwd als fotoisomeren. Sommige van deze fotoisomeren zijn minder toxisch dan DA zelf.
Er zijn diverse detectiemethodes beschreven voor DA i.e: een biologische bepalingsmethode (intraperitoneaal: "paralytic shellfish poisoning" (PSP) bepaling in muizen met een verlengde observatietijd), biochemische bepalingsmethodes (receptor-binding test, immunologische bepalingsmethodes, *in vitro* neurofysiologische bepaling) en chemische bepalingsmethodes (TLC, aminozuur bepaling, capillaire electroforese, GC-MS en HPLC-MS, HPLC-UV). De, de in officiele regelgeving gehanteerde, bepalingsmethode voor de detectie van DA is HPLC met UV detectie.

DA behoort tot de klasse van "kanoid" verbindingen en is sterk neurotoxisch. Het meest karakteristieke symptoom na intraperitoneale toediening aan muizen en ratten, is krabben aan de schouders met de achterpoot gevolgd door stuiptrekkingen en vaak de dood. Bij orale toediening zijn voor het opwekken van dezelfde symptomen, tot 10 maal zo hoge doses nodig als na intraperioneale toediening.
Studies gericht op de neurotoxische effecten van DA, vertoonden verlies van neuronen in het CA1, CA3 en CA4 gebied van de hippocampus, oedeem en degeneratie van neuronen in de "arcuate nucleus" en "vacuolated" en pyknotische cellen in de binnenste laag van de retina.
Blootstelling van mensen aan DA, via de consumptie van schelpdieren, veroorzaakt buikkrampen, misselijkheid, overgeven, hoofdpijn, desoriëntatie, geheugenverlies (amnesie) en coma. Er is geen onderzoek beschikbaar naar effecten ten gevolge van chronische blootstelling aan lage concentraties, maar kortdurende studies in proefdieren met herhaalde blootstelling wijzen niet op veranderde DA klaring uit het serum of ernstiger neurotoxische effecten dan na éénmalige blootstelling.

Summary

Amnesic shellfish poisoning (ASP) was described for the first time in Canada, Prince Edward Island, in 1987. This outbreak of ASP was ascribed to the consumption of cultured blue mussels (*Mytilus edilus*) and the causative agent was identified as domoic acid (DA) originating from the marine diatom *Pseudonitzschia pungens* f. *multiseries* (also called *Pseudonitzschia multiseries*).
Besides *P. multiseries* at least 4 other *Pseudonitzschia* species were reported to produce domoic acid; *P. australis*, *P. pseudodelicatissima*, *P. delicatissima* and *P. seriata*. *Pseudonitzschia* species are widely distributed in the coastal waters of the Atlantic, Pacific and Indian oceans. The hydrographic and environmental conditions that contribute to harmful blooms of *Pseudonitzschia* species producing domoic acid are not clear. Several possible causes for harmful blooms of *Pseudonitzschia* species are listed in this report such as temperature, irradiance, salinity, upwelling, phytoplankton abundance and nutrient concentration (a.o. nitrogen, silicate, phosphate). The growth phase of the *Pseudonitzschia* species appears to be also a critical factor.
Shellfish accumulate domoic acid either by direct filtration of the plankton cells or by feeding directly on contaminated organisms. Rate of accumulation of toxic algae (and the toxin) in filter-feeding shellfish is species-specific and is, in most cases, directly related to the number of plankton cells available to the animals. The rate of accumulation of toxic algae in individual shellfish in any given area is highly variable. The rate of elimination of the toxin varies with season and low water temperatures retard toxin loss; however, the degree to which temperature affects the uptake and release of toxins is not clearly understood. Furthermore, the rate of elimination is highly dependent on the site of toxin storage within the animal i.e. toxins in the gastrointestinal tract are eliminated much more readily than toxins bound in tissues. Domoic acid was shown to depurate from blue mussels (*Mytilus edilus*) fairly rapidly. Sea scallops (*Placopecten magellanicus*) and red mussels (*Modiolus modiolus*) were reported to retain domoic acid for lengthy periods. Depuration of domoic acid by razor clams (*Siliqua patula*) appeared to be a long-term process.
Blue mussels (*Mytilus edilus*), red mussels (*Modiolus modiolus*), Californian mussels (*Mytilus californianus*), soft-shell clams (*Mya arenaria*), razor clams (*Siliqua patula*),
bay scallops (*Argopecten iradians*), sea scallops (*Placopecten magellanicus*), oysters (*Crassostrea virginica*), "Pacific" oysters (*Crassostrea gigas*), Dungeness crabs (*Cancer magister*), mackerel (*Scomber japonicus*) and anchovies (*Engraulis mordax*) were reported to accumulate the ASP toxin domoic acid.
Besides DA, also a number of geometric isomers of domoic acid have been identified. However, only isodomoic acids D, E and F and the C5' diastereomer were isolated from (both) plankton and shellfish tissue. The geometrical isomers are not *de novo* products of the plankton, but are considered to be photoisomers. Some of these photoisomers are less toxic than domoic acid itself.
Several detection methods for domoic acid are described i.e. a bioassay (mouse i.p. PSP assay with increased observation time), biochemical assays (receptor binding assay, immunoassays, *in vitro* neurophysiological assay) and chemical assays (TLC, amino acid analysis, capillary electrophoresis, GC-MS and HPLC-MS, HPLC -UV). HPLC with UV detection is the method for determination of domoic acid, indicated in official regulations.
Domoic acid belongs to the kanoid class of compounds and is a potent neurotoxin. Toxic effects of domoic acid have been studied in mice, rats and cynomolgus monkeys. The most characteristic symptoms in mice and rats after i.p. (intraperitoneal) dosing include a unique

scratching of the shoulders by the hind leg, followed by convulsions and often death. Oral doses required more than 10 times as much toxin to achieve the same effects as i.p. doses. Studies on the neurotoxic effects showed a loss of neurons in the CA1, CA3 and CA4 area of the hippocampus, oedema and neuronal degeneration in the arcuate nucleus and vacuolated and pyknoted cells in the inner layer of the retina. Exposure of man to domoic acid, via consumption of shellfish, causes abdominal cramps, nausea, vomiting, headache, disorientation, memory loss (amnesia) and coma. There is no knowledge of the effects of long-term exposure to low levels of domoic acid. However, short-term animal studies with repeated exposure do not point to altered domoic acid clearance from serum or greater neurotoxic responses than after single exposures.

1. Introduction

The microscopic planktonic algae of the world's oceans are critical food for filter-feeding bivalve shellfish (oysters, mussels, scallops, clams) as well as the larvae of commercially important crustaceans and finfish. In most cases, the proliferation of plankton algae (so-called "algal blooms"; up to millions of cells per liter) therefore is beneficial for aquaculture and wild fisheries operations. However, in some situations, algal blooms can have negative effect, causing severe economic losses to aquaculture, fisheries and tourism operations and having major environmental and human health impacts.
There are algal species which produce basically harmless water discolourations; however, under exceptional conditions in sheltered bays, blooms can grow so dense that they cause indiscriminate kills of fish and invertebrates due to oxygen depletion.
There are also algal species which produce toxins that can find their way through the food chain to humans, causing a variety of gastrointestinal and neurological illnesses, such as paralytic shellfish poisoning (PSP), diarrhoeic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), ciguatera fish poisoning (CFP) and cyanobacterial fish poisoning.
Furthermore, there are also algal species, which are non-toxic to humans, but harmful to fish and invertebrates (especially in intensive aquaculture systems) by damaging or clogging their gills.
Among the 5000 species of extant marine phytoplankton, some 300 species can at times occur in such high numbers that they obviously discolour the surface of the sea (so-called "red tides"), while only 40 or so species have the capacity to produce potent toxins that can find their way through fish and shellfish to humans. In the past two decades the public health and economic impacts of algal blooms appeared to have increased in frequency, intensity and geographic distribution (as cited in Hallegraeff, 1995).
In this report a review is given of the knowledge of amnesic shellfish poisoning (ASP, also called domoic acid poisoning (DAP) because amnesia is not always present) toxins with respect to origin, occurrence, chemical structure, analysis, toxicity and existing regulations.
ASP was first recognised in 1987 in Prince Edward Island, Canada, where it caused 3 deaths and 105 cases of acute human poisoning following the consumption of blue mussels. The symptoms included abdominal cramps, vomiting, disorientation and memory loss (amnesia). The causative toxin (the excitatory amino acid domoic acid) was produced by the diatom species *Pseudonitzschia pungens* f. *multiseries* (=*Nitzschia pungens* f. *multiseries*) (as cited in Hallegraeff, 1995). In September 1991 the unexplained deaths of pelicans and cormorants in Monterey Bay, California were attributed to an outbreak of domoic acid poisoning produced by a related diatom *Pseudonitzschia australis*. This diatom was consumed by anchovies that in turn were eaten by the birds. In October 1991 extracts of razor clams from the coast of Oregon were found to induce domoic acid-like symptoms in mice. These incidents prompted the regulatory authorities in the USA to conduct a massive survey of many marine species for the presence of domoic acid. The toxin was found widely spread from California to Washington and was also found unexpectedly in crabs, the first time this toxin was demonstrated in a crustacean. Since these incidents, global awareness to domoic acid and its producing sources has been raised (as cited in Wright and Quilliam, 1995).

2. The ASP Toxins

2.1 Chemical structures and properties

The major component of ASP toxins is domoic acid (see figure 1 on page 10), a naturally occurring compound belonging to the kainoid class of compounds that have been isolated from a variety of marine sources including macro- and microalgae (Wright and Quilliam, 1995). Domoic acid is a crystalline water-soluble acidic amino acid. It can be purified by a variety of chromatographic methods and contains a strong chromophore that facilitates detection by UV spectroscopy. Domoic acid was originally discovered as a product of a red macroalga *Chondria armata* and was later isolated from several other red macroalgae. However, these seaweeds were not the source of domoic acid in the first reported ASP incident on Prince Edward Island in Canada in 1987. The source of domoic acid in this outbreak of ASP was found to be the diatom *Pseudonitzschia* (formerly *Nitzschia*) *pungens* forma *multiseries*. Domoic acid is a potent neurotoxin and the kainoid class of compounds to which domoic acid belongs, is a class of excitatory neurotransmitters that bind to specific receptor proteins in neuronal cells causing continual depolarisation of the neuronal cell until cell rupture occurs (Wright, 1995).
Investigation of the kainoids present in *Chondria armata* resulted in the discovery, in minor amounts, of the geometrical isomers isodomoic acid A, B and C (see figure 1 on page 10) as well as domoilactones. None of these isomers found in seaweed, were detected in extracts of plankton or shellfish tissue. However, three other geometrical isomers (isodomoic acids D, E and F) and the C5' diastereomer (see figure 1 on page 10) were isolated from both plankton cells and shellfish tissue (Wright and Quilliam, 1995; Ravn, 1995). The geometrical isomers can be prepared in the laboratory by brief exposure of dilute solutions of domoic acid to UV light, and are therefore not considered to be *de novo* products of the plankton. Pharmacological studies indicate that these photoisomers bind less strongly to the kainate receptor proteins than domoic acid itself suggesting that they are not as toxic as the parent amino acid. Formation of the C5' diastereomer is accelerated with warming. This C5' diastereomer shows almost the same binding efficacy to the kainate receptor as domoic acid itself (Wright and Quilliam, 1995). Zaman *et al.* (1997) reported the isolation of two new isomers of domoic acid from the red alga *Chondria armata*, i.e isodomoic acid G and H, (see figure 1 on page 10).

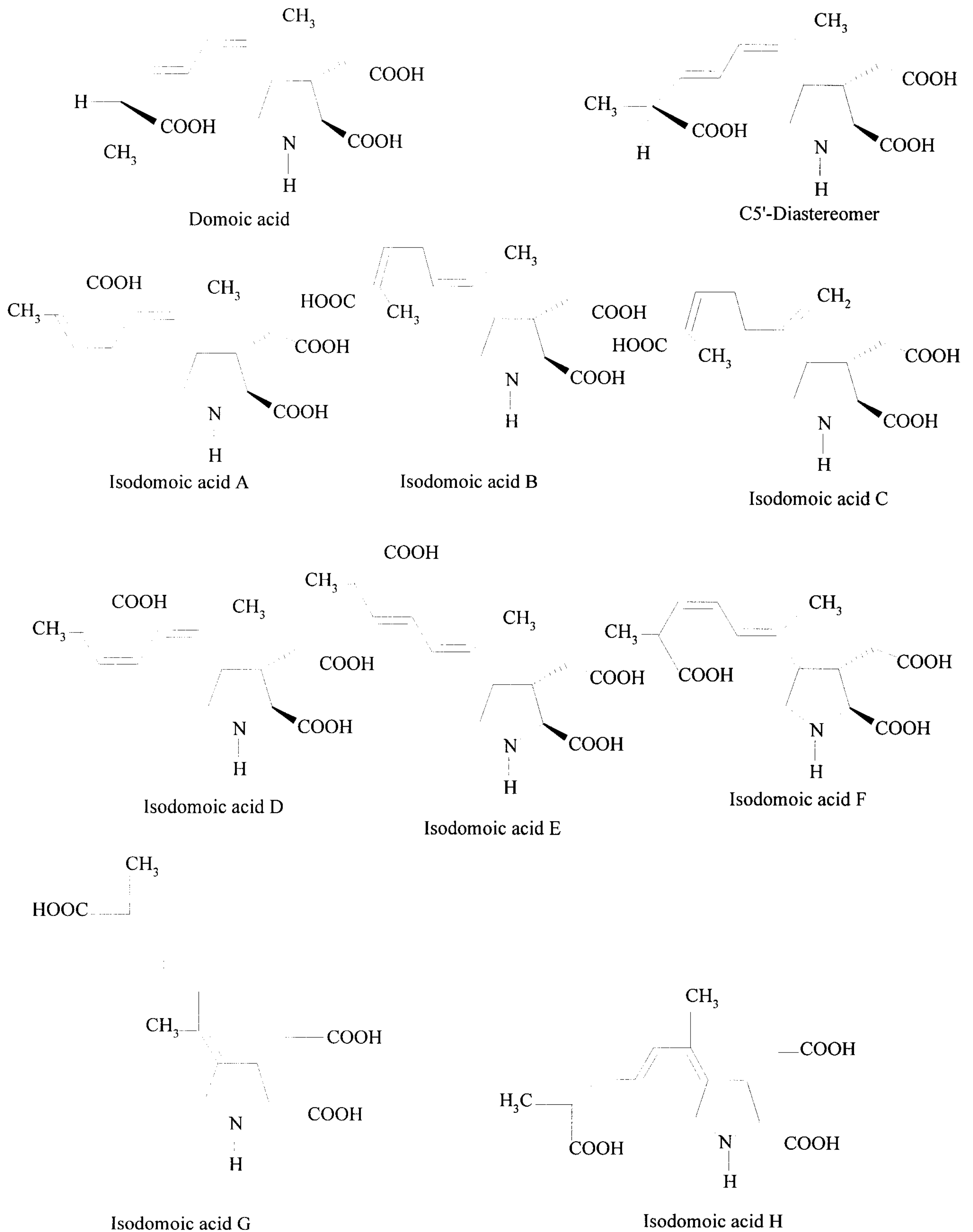

Figure 1 Chemical structures of domoic acid and its isomers

2.2 Determination of ASP toxins

2.2.1 Bioassays

The Association of Official Analytical Chemists (AOAC) mouse bioassay for PSP toxins (AOAC, 1990) can also detect domoic acid at concentrations of approximately 40 μg/g tissue. The PSP mouse bioassay involves acidic aqueous extraction of the tissue (whole animal or selected organs) followed by intraperitoneal injection of 1 ml of the extract into mice. The time from initial injection to mouse death (usually <15 minutes) is recorded. This procedure was used when ASP toxicity was first identified in Canada in shellfish extracts. The first indications of toxicity associated with the ASP syndrome were revealed in the course of routine AOAC mouse bioassays for PSP toxicity . Skilled bioassay technicians noted that the aberrant symptoms of ASP were distinguishable from the classic symptomology of PSP intoxication and consequent death. The success of the approach during the first ASP incident was due in part to the high levels of toxin present in contaminated shellfish from the eastern Prince Edward Island. The typical sign of the presence of domoic acid is a unique scratching syndrome of the shoulders by the hind leg, followed by convulsions. The time of observation must be extended from 15 minutes to 4 hours. Although the AOAC extraction procedure can yield substantial recovery of domoic acid, the limit of detection of the AOAC bioassay procedure is not low enough to be used with confidence for regulatory purposes to quantify this toxin. The guideline value in mussels established in Canada and subsequently adopted by certain other countries is 20 μg/g of mussel tissue. For the routine detection of ASP toxins, the AOAC mouse bioassay has been superseded by HPLC methods using diode-array/UV or fluorometric detection which have been proven to be more sensitive and reliable tools (as cited in Fernandez and Cembella, 1995).
Tasker *et al.* (1991) pretended to have developed a behavioural rating scale from 0 (normal) to 7 (death), which these authors claimed to be consistently reproducible in mice injected intraperitoneally. These authors further claimed that the rating scale could be used to reliably quantitate domoic acid concentrations as low as 20 μg/mouse (~0.8 mg/kg b.w.).

2.2.2 Biochemical assays

Receptor binding assay
A competitive microplate receptor binding assay for domoic acid using frog (*Rana pipiens*) brain synaptosomes was developed. The analysis of domoic acid was based upon binding competition with radiolabelled-[^{3}H]-kainic acid for the kainate/quisqualate glutamate receptor. The method appeared to be sensitive (IC_{50} 0.89 nM ~ 0.3 μg) and showed high promises as a rapid automated assay for domoic acid in contaminated seafood and toxic phytoplankton samples. Preliminary results with extracts of *Pseudonitzschia pungens* f. *multiseries* indicated good qualitative correlation with the fluorenylmethoxycarbonyl-HPLC method (Van Dolah *et al.*, 1994). In 1995 the method was reported to be still in the latter stages of pharmacological trials (Wright and Quilliam, 1995). In 1997, Van Dolah *et al.* reported the further development of the receptor assay by replacement of frog brain by a cloned rat GLUR6 glutamate receptor to eliminate animals from the testing procedure. The limit of detection and selectivity of the assay were optimized through inclusion of the glutamate decarboxylase pretreatment step to eliminate potential interference due to high concentrations of endogenous glutamate in shellfish.

Immunoassays

An enzyme-linked immunoassay (ELISA) for the presence of domoic acid in mammalian serum and urine was developed, using polyclonal antibodies produced in rabbits. The method was effective in determining domoic acid concentrations in rat urine, with a reported lower quantification limit of 40 ng/ml. Domoic acid levels in rat and monkey plasma could not be determined accurately using this antiserum in ELISA. This detection system had not been subjected to extensive collaborative testing for use as a routine technique (as cited in Cembella *et al.*, 1995 and Smith and Kitts, 1994). The method above involved several steps and lacked the desired limit of detection. In addition the method depended on the physical immobilization of domoic acid on the microplate via a carrier protein. At present, CovaLink NH® microplates have been developed in which a projecting secondary amino group has been applied in ELISAs for coupling peptides, steroids, oligonucleotides, and DNA to the microplate well surface, directly and chemically. The use of these CovaLink microplates for simplification and improvement of the previous ELISA method, has resulted in two different versions of the ELISA, which are based on a physical and a chemical immobilization, respectively, of domoic acid (Osada *et al.*, 1995).

Enzyme-linked immunoassays (ELISA) for domoic acid determination in human body fluids and in mussel extracts were developed by Smith and Kitts (1994) and Smith and Kitts (1995), respectively. The assays employed a polyclonal antiserum raised in mice against an ovalbumin-domoic acid conjugate. The assay was used to quantify domoic acid concentrations in human body fluids spiked with pure domoate. The lower quantitation limits were 0.2 μg/ml in urine, 0.25 μg/ml in plasma and 10 μg/ml in milk. The relative high quantitation limit in milk was probably due to the high fat content of the milk. The authors suggested that human milk samples may require extraction prior to analysis (Smith and Kitts, 1994). Recovery experiments in both aqueous and acid extracts of mussel tissue demonstrated that the domoic acid concentration could be accurately measured to within 8% of the actual value. The limit of detection was 0.25 μg/ml of extract. This value represents 0.5 μg domoic acid/g of mussel tissue when acid (AOAC) extracts are analyzed. (Smith and Kitts, 1995). Direct comparison of domoic acid determinations with HPLC and ELISA analyses correlated well ($r = 0.96$), although the ELISA method resulted in higher values in most samples. It was suggested that this was partially attributed to a loss of domoic acid in the solid phase extraction prior to HPLC or to the possible presence of domoic acid isomers. Domoic acid isomers that do not coelute with domoic acid, are not determined in routine HPLC analyses. However, the ELISA method measures total domoic acid content including a diastereoisomer and at least two *cis-trans* isomers (Smith and Kitts, 1995) .

In vitro neurophysiological assay

The effectiveness of *in vitro* hippocampal slice preparations was examined as a means of rapidly and specifically detecting domoic acid. Extracellular neuronal responses were recorded from region CA1 of fully submerged, perfused adult Sprague-Dawley rat hippocampal slices, using reference standards in 50 to 1000 nM range. Domoic acid produced a rapid and reversible increase in amplitude of the orthodromic population spike and a decrease in field EPSP. The results of this experiment indicated that the hippocampal slice preparation is a viable tool for detecting domoic acid (Saba *et al.*, 1997).

2.2.3 Chemical assays

Thin Layer Chromatography

Domoic acid can be determined by thin layer chromatography as a weak UV-quenching spot that stains yellow after spraying with a 1% solution of ninhydrin. Normal amino acids that are present in crude extracts will interfere and must be separated from domoic acid. This can be accomplished for plankton samples by two dimensional TLC. Crude extracts of shellfish tissues cannot be analyzed directly, as they are too complex. A cleanup procedure (strong anion change solid phase extraction [SAX-SPE] with minor modification) yields fractions that can be used directly or concentrated in vacuo before applying to a silica gel plate. Only one-dimensional TLC is required when this cleanup is used as almost all interfering amino acids are removed. The detection limit for domoic acid is about 0.5 μg by this method, which permits detection in shellfish tissues at about 10 μg/g. It is also possible to detect domoic acid on the TLC plate using some other spray reagents. For example, after spraying a TLC plate with vanillin, a yellow colour with domoic (or kainic) acid forms first and changes to pink on standing (as cited in Wright and Quilliam, 1995).

Amino Acid Analysis

Crude aqueous extracts of plankton can be analyzed directly by an amino acid analyzer system. Using the buffer solutions and ion-exchange column normally used for the analysis of protein hydrolysates, domoic acid elutes close to methionine. Absorbance measurement at 440 nm provides detection of amino acids with primary amine groups, while absorbance at 570 nm selectively detects imino acids such as proline and domoic acid. The detection limit of this method for domoic acid is about 1 μg/ml, with about 50 μl of extract injected on-column. Although the limit of detection of the amino acid analysis method is close to that of HPLC-UV methods, it is not as effective for samples containing a high concentration of free amino acids and the analysis time is much longer. Shellfish extracts can be analyzed with this approach after the necessary cleanup and concentration of material (as cited in Wright and Quilliam, 1995).

High Performance Liquid Chromatography (HPLC)

Domoic acid can be analyzed, as well as preparatively isolated, by either HPLC or ion exchange chromatography using UV absorbance detection. Reversed-phase HPLC-UV gives the fastest and most efficient separations. Use of an acidic mobile phase to suppress ionization of the carboxyl functions is recommended, and selective separation of domoic acid and its isomers is best achieved with "polymeric-like" octadecylsilica phases. HPLC-UV is the preferred analytical technique for the determination of domoic acid in shellfish. The detection of domoic acid is facilitated by its strong absorbance at 242 nm. The HPLC-UV detection limit for domoic acid is about 10-80 ng/ml, depending on the sensitivity of the UV detector that is used. The detection limit in tissue is dependent upon the method of extraction and cleanup. If crude extracts (either acidic or aqueous methanol) are analyzed without cleanup, the practical limit for quantitation is about 1 μg/g (Lawrence *et al.*, 1989a). This is suitable for most regulatory laboratories concerned with detecting contamination levels greater than 20 μg/g. However, interferences are commonly encountered that can give false positives with crude extracts. For example, it has been shown that tryptophan and some of its derivatives are often present in substantial concentrations in shellfish and finfish tissues and that these compounds elute close to domoic acid. A photo-diode array detector can be used to examine UV spectra in order to confirm domoic acid. An alternative approach is to prepare a chemical derivative and to analyze the sample with comparison to a known standard carried

through the same procedure. Derivatization techniques involving reaction with phenyl- or butyl-isothiocyanate or esterification with isopropanol have been developed for this purpose (Lawrence *et al.*, 1989b; Lawrence and Ménard, 1991). A more sophisticated technique of confirmation is to replace UV detection by electrospray mass spectrometry with selected ion monitoring (Lawrence *et al.*, 1994). Application of this technique offers the possibility of confirming the presence of domoic acid down to levels of 0.1 μg/g without additional sample treatment.
An improved HPLC-UV analysis procedure was developed by Quilliam (1995). In this procedure an aqueous methanol extraction is applied in combination with SAX (strong anion exchange)-SPE (solid phase extraction) cleanup, leading to chromatograms free from interferences. Other advantages of the method of Quilliam (1995) over the method of Lawrence *et al.* (1989a) seem more stable extracts, higher recoveries and a lower limit of detection (20-30 ng/g). The method of Quilliam (1995) is planned to be studied in an AOAC collaborative study, so as to derive performance characteristics. A slight modification to the SPE (solid phase extraction) clean-up step in the method of Quilliam (1995) was given by Hatfield *et al.* (1994). The standard 10% acetonitrile wash and 0.5 M ammoniumcitrate buffer in 10% acetonitrile eluting solution have been replaced with a 0.1 M sodium chloride in 10% acetonitrile wash and a 0.5 M sodium chloride in 10% acetonitrile eluting solution. This modified method permits the analyses of samples with complex matrices, such as crab viscera. Additionally domoic acid appeared more stable in the elutes from the SAX SPE cartridges, permitting storage of the samples if analyses cannot be made immediately.

Thus far, HPLC methods of analysis for ASP, validated in formal collaborative studies, are scarce. The method of Lawrence *et al.* (1989a) is the only method in that respect (by the AOAC, 1995). In the method of Lawrence *et al.* (1989a) domoic acid is extracted from homogenized mussel tissue by boiling 5 minutes with 0.1 M HCl, similar as in the AOAC's PSP mouse assay extraction procedure. The mixture is cooled and centrifuged, and an aliquot of supernate is diluted, filtered and analyzed by isocratic LC with UV detection at 242 nm.

The method of Lawrence *et al.* (1989a) has been modified by the working group on biotoxins of the Eurpean Committee for Standardization (CEN) (i.e. extraction with high purity water instead of HCl and use of hepatopancreas instead of whole shellfish tissue). If the modifications lead to improvement of the method which has to be demonstrated by laboratory research, the modified procedure will be standardized and eventually appear as a CEN standard.

A very sensitive procedure, based on reaction with 9-fluorenylmethylchloroformate to form the fluorenylmethoxycarbonyl (FMOC) derivative and HPLC analysis with fluorescence detection, has been developed for monitoring of domoic acid in marine matrices such as seawater and phytoplankton. The detection limit is as low as 15 pg/ml for domoic acid in seawater (Pocklington *et al.*, 1990). This procedure has also been adapted to shellfish extracts (as cited in Wright and Quilliam, 1995).

A rapid and sensitive automatic method for the determination of domoic acid using HPLC with a column-switching system and UV-detection was reported. Interfering peaks resulting from matrix protein components are excluded by use of an especially designed reversed-phase HPLC column for pre-separation. The method is suitable for extracts from both mussels and algae. Sample material is extracted with pure water and the crude extract is injected directly. Application of a column-switching system eliminated the need for any further sample clean-up after extraction (Hummert *et al.*, 1997).

Capillary Electrophoresis (CE)

This relatively simple method allows rapid, high resolution separations of complex polar compounds. A narrow bore fused silica capillary tube filled with buffer is connected between two liquid reservoirs. After a small volume of sample extract (typically 1-10 nL) is injected into the capillary, a differential voltage of 20-30 kV is applied at the ends of the capillary. Ionic substances migrate as narrow bands down the capillary, eventually passing by a detector (UV absorbance, fluorescence) (as cited in Wright and Quilliam, 1995). Nguyen *et al.* (1990) reported a detection limit of 2 µg/g (signal-to-noise ratio 5:1) of a treated extract of mussel tissue. During the extraction and extract treatment procedure, if care was taken to limit the amount of liquid introduced, the treated extract was 5 fold less concentrated than the tissues. The detection limit in wet tissues therefore was 10 µg/g. Domoic acid was readily separated from components of the mussel sample matrix in 10 min. With excellent mass detection limits, the CE method requires only 3-15 nl samples and will find applications where sample size is severely limited. Zhao *et al.* (1997) studied methods based upon capillary electrophoresis combined with UV absorbance detection. Domoic acid could be analyzed using bare fused-silica capillaries in either the cationic or anionic mode with acidic or basic buffer systems, respectively. Highest performance, in terms of both separation efficiency and analysis time, was achieved with phosphate or borate buffers at a pH of approximately 9. The addition of β-cyclodextrin to the borate buffer permitted a separation of domoic acid and several of its isomers (isodomoic acids) that was superior to that achieved with liquid chromatography. In addition, an extraction and clean-up procedure was developed and tested with mussels, clams and anchovies. A mass detection limit of 3 pg of domoic acid injected and a method detection limit of 150 ng/g in tissues could be achieved. Comparison with HPLC showed that comparable precision and accuracy could be attained by the two techniques.

Gaschromatography-Mass Spectrometry (GC-MS) and HPLC-MS

Fast atom bombardment (FAB) mass spectrometry using a direct probe inlet was found to be useful for the qualitative confirmation of domoic acid in concentrated LC isolates. The development of an analytical procedure based on combined gaschromatography-mass spectrometry (GC-MS) for the analysis of domoic acid and related compounds in shellfish tissue samples has been reported (Pleasance *et al.*, 1990). Although this method is applicable to concentrations of domoic acid in contaminated shellfish ranging from 1 to 500 µg/g wet tissue, its implementation required the development of an extensive cleanup procedure to facilitate derivatization to the *N*-trifluoroacetyl-*O*-silyl derivatives. Since HPLC-UV is the method most commonly used for routine regulatory purposes, a combined liquid chromatography-mass spectrometry (HPLC-MS) method would be most desirable for confirmation of peak identity. Various HPLC-MS interfaces for the analysis of ASP toxins have been investigated; these include continous-flow FAB thermospray, and ion-spray interfaces. In a prelimary communication it was shown that ion-spray HPLC-MS is a very promising method for the analysis of ASP toxins, as well as of other marine toxins. Ion-spray HPLC-MS has a detection limit similar to that of HPLC-UV (as cited in Wright and Quilliam, 1995).

2.2.4 Reference materials

Helpful tools for analytical quality assurance are the certified materials that have been developed for ASP and that have been made available through the Marine Analytical Chemistry Standards Program of the National Research Council, Canada (NRC, 1999). NRC provides domoic acid certified calibration solution (DACS-1C) and mussel tissue reference material for domoic acid (MUS-1B). DACS-1C is a certified instrument calibration solution prepared to aid the analyst in the determination of domoic acid. It is available as a set of 4 ampoules, with each ampoule containing 0.5 mL of a solution of domoic acid dissolved in acetonitrile/water (1:9 v/v) at a concentration of 100 µg/mL. This concentration is suitable for calibration of liquid chromatography experiments and for spiking shellfish control samples for recovery experiments. MUS-1B is an homogenized slurry of mussel tissue (*Mytilus edilus*) containing 36 ± 1 µg/g domoic acid, as well as several related compounds. MUS-1B is distributed as a set of 4 bottles, each containing 8.1 ± 0.1 g of mussel homogenate.

3. Sources of ASP toxins

3.1 Global increase of harmful algal blooms

The past two decades public health and economic impacts of harmful algal blooms appear to have increased in frequency, intensity and geographic distribution. A number of explanations can be given for these phenomena (Hallegraeff, 1995)

- The increased scientific awareness of toxic species.
  Reports of human illnesses or damage to aquaculture operations are receiving increased attention in newspapers, the electronic media and the scientific literature. Consequently more and more surveys in local waters take place to detect the causative organisms.
- Increased utilization of coastal waters for aquaculture.
  More and more countries are looking towards aquaculture as an alternative for increased problems of overfishing of coastal waters. Some fisheries scientists predict that within the next 10-20 years, the increasing production of world aquaculture may well approach the decreasing total catch of wild fish and shellfish. Aquaculture operations act as sensitive 'bioassay systems' for harmful algal species and can trace the presence in water bodies of problem organisms not known to exist there before. The increase in shellfish farming worldwide is leading to more reports of amnesic shellfish poisoning.
- Stimulation of plankton blooms by cultural eutrophication and/or unusual climatological conditions.
  There is accumulating evidence that 'cultural eutrophication' from domestic, industrial and agricultural wastes can stimulate harmful algal blooms. The nutrient composition of treated wastewater is never the same as that of the coastal waters in which it is being discharged. There is considerable concern that such altered nutrient ratios in coastal waters may favor algal blooms. Algal species which are normally not toxic (*P.pungens* f. *multiseries*), may be rendered toxic when exposed to atypical nutrient regimes (e.g. phosphate deficiency) resulting from cultural eutrophication. Changed patterns of land use, such as deforestation, can also cause shifts in phytoplankton species composition by increasing the concentrations of humic substances in land run-off. Acid precipitation can further increase the mobility of humic substances and trace metals in soils. Experimental evidence from Sweden indicates that river water draining from agricultural soils (rich in N and P) stimulates diatom blooms.
  Furthermore global climate change studies need to consider possible impacts on algal bloom events (Hallegraeff, 1995). In fall 1991 a domoic acid incident occurred at the USA west coast during an El Niño year, but the cause/effect relationship is not clear (Horner and Postel, 1993).

3.2 Source organisms for ASP toxins

ASP was reported for the first time in 1987 at Prince Edward Island in Canada and the toxin responsible for this syndrome has been identified as domoic acid (Bates *et al.*, 1989).
Domoic acid was originally isolated in the 1950s from the red macroalga *Chondria armata* (Ravn, 1995). Two decades later domoic acid was detected in the Mediterranean red macroalga *Alsidium corallinum* (Todd, 1993). There is a single report that another diatom, *Amphora coffaeformis*, produces also domoic acid (as cited in Lundholm *et al.*, 1994).

The origin of domoic acid in the first ASP incident in 1987 was postulated to be the red alga *Chondria baileyana*, a species found in Prince Edward Island waters. However the diatom *Pseudonitzschia pungens* f. *multiseries* pointed out as the producer of domoic acid. The outbreak of food poisoning in humans was traced to cultured blue mussels (*Mytilus edilus*) contaminated with identifiable fragments of *Pseudonitzschia pungens* f. *multiseries* (Bates *et al*, 1989).
Domoic acid is produced by *Pseudonitzschia pungens* f. *multiseries* in culture as well as under field conditions (Hasle and Fryxell, 1995).
In the soutwestern Bay of Fundy in Canada *Pseudonitzschia pseudodelicatissima* appeared to be the organism producing domoic acid which led to inacceptable levels of domoic acid in shellfish destined for human consumption (Martin *et al.*, 1993).
ASP occurred also on the West Coast of the USA causing the death of pelicans and cormorants after eating anchovies contaminated with domoic acid. In that region the pennate diatom *Pseudonitzschia australis* appeared to be the source organism (Wright and Quilliam, 1995). *Pseudonitzschia australis* produced domoic acid in culture as well as under field conditions (Hasle and Fryxell, 1995).
Both *Pseudonitzschia delicatissima* (syn. *Nitzschia actydrophila*) and *Pseudonitzschia seriata* were found to produce domoic acid under culture conditions (Hasle and Fryxell, 1995). *Pseudonitzschia pungens* and *Pseudonitzschia multiseries* (syn. *P. pungens* f. *multiseries*) are now considered separate species because of morphological and genetic differences (Wright and Quilliam, 1995).

3.3 Distribution of source organisms

Pseudonitzschia species are widely distributed diatoms and according to Hasle and Fryxell (1995) the different species mentioned in paragraph 3.2, are found in the following waters;

P. multiseries:

Atlantic: waters of North America, Europe, and South America.
Pacific: waters of North America and Northeast Asia.

P. pseudodelicatissima:

Atlantic: waters of Europe and Africa (Denmark Strait to Northwest Africa, including Norwegian and Danish coastal waters, Skagerrak, Kiel Bay), Canada and USA (Arctic to Gulf of Mexico).

Pacific: California and British Columbia waters.

P. australis

Atlantic: coastal waters of Spain, Portugal, Southwest Africa and Argentina.

Pacific: coastal waters of Peru, Chile, New Zealand, west coast of USA from San Diego , California to Puget Sound, Washington, British Columbia.

P. delicatissima

Atlantic: Norwegian coastal waters, Danish waters, Skagerrak, Northwest Africa, Rhode Island.

Pacific: California.

P. seriata

Barents Sea (ca. 80°N), Norwegian Sea, North Sea, Norwegian coastal waters, Skagerrak, Kiel Bay, English Channel, Greenland to New Foundland (45°N), Alaska, British Columbia.

3.4 Habitat of Pseudonitzschia species producing domoic acid

Toxic *Pseudonitzschia* blooms may become a recurring phenomenon, and it is important to determine if there is any seasonal or spatial predictability.
Since the first ASP incident in Canada in 1987, the autoecology of *P. pungens* f. *multiseries* has been intensively studied. Although it has been reported to dominate at colder temperatures, it is able to survive up to 30°C (Villac *et al*., 1993b). Pan *et al.* (1993) concluded that optimal growth and photosynthesis of *P. pungens* f. *multiseries* occurred in the temperature range of 15-20 °C. However, monospecific blooms of *P. pungens* f *multiseries* have been seen in late fall or winter when the prevailing water temperature was low (-1 to 3 °C). Since the temperature for optimal growth is much higher than this, it is evident that factors other than temperature must have initiated the development of blooms.
In studies of Lewis *et al.* (1993) the highest growth rates for *P.pungens* f. *multiseries* were observed at 20° and 25°C. The highest stationary phase cell concentrations occurred at 5° to 15°C and decreased at 20° to 25°C.
Upwelling of cold water with high nitrogen concentrations (such as found in Monterey Bay, California, USA) might have stimulated the increase of *P. pungens* f. *multiseries* populations (Villac *et al*., 1993a). A direct evidence of increased cell numbers with even small amounts of upwelling were reported by Fryxell *et al.* (1997). During the 1991-1994 period at the west coast of the USA *P. australis* blooms were most common and persisted longer during late summer and autumn when hydrographic conditions were associated with the end of the upwelling season and were usually characterized by higher sea-surface temperatures, thermal stratification, and lower concentrations of nutrients (Horner *et al.,* 1997). Nutrients might be even more important to phytoplankton growth than direct effects of seasonal temperature changes (Fryxell *et al*., 1997). *P. seriata*, another domoic acid producing *Pseudonitzschia* species, was found from the Barents Sea near Spitsbergen in the north to Germany in the south (Kiel Bay in Baltic) and in the western Atlantic from the west and east coasts of Greenland to 45°N in Canada. *P. seriata* was apparently restricted to cold water and reached

low latitudes only in winter. It is known in the Pacific from Alaska to British Columbia/Washington and it may be expected to occur in northern Japan also (Lundholm *et al.*, 1994).
Within the genus *Pseudonitzschia* , production of domoic acid varies greatly from one species to another, and it is vital to be able to distinguish species (Fryxell *et al.*, 1997).
The relationship between domoic acid production by *Pseudonitzschia* spp. and environmental conditions is not yet clear.
Garrison *et al.* (1992) noted that conditions in shallow regions with restricted circulation may provide the condition of stress with an excess of inorganic nitrogen needed to initiate domoic acid production.
The peak of the *P. pungens* f. *multiseries* bloom in Canada in 1987 took place after an unusually dry spell in late summer, followed by severe rainstorm in early September. A relationship between pulses of nitrate availability and *P. pungens* f. *multiseries* peaks was found which was attributed mostly to freshwater runoff following the rains (Villac *et al.*, 1993a; Villac *et al.*, 1993b). Studies of population densities in Cardigan River Estuary (Prince Edward Island, Canada) indicated cell densities of approximately 3 x 10^5 per liter might be needed before shellfish exceed the 20 mg/kg domoic acid tolerance level for human consumption (as cited in Dickey *et al.*, 1992). The domoic acid incident in fall 1991 at the Washington waters on the USA west coast occurred also after a record hot, dry period lasting 45 days, followed by rain in mid October (Horner and Postel, 1993).
In batch culture studies domoic acid production by *P. multiseries* occurred only in the stationary phase and was not evident during exponential growth (Bates *et al.*, 1991). Lewis *et al.* (1993) demonstrated that the rate of domoic acid production by *P. pungens* f. *multiseries* in the stationary growth phase could be greatly reduced by a small decrease in temperature. However, even though the concentrations and rates of domoic acid production were low at low temperatures, high cell yields could allow sufficient domoic acid production to toxify molluscan shellfish. The studies of Lewis *et al.* (1993) further demonstrated that experimental photon flux densities had no apparent effect on the initial rate of domoic acid production or on growth rate of *P. pungens* f. *multiseries*.
Bates *et al.* (1991) stated that the availability of extracellular nitrate or other nitrogen source and the presence of light were required in order to produce domoic acid during the stationary phase. Nutrient stress has also been implicated as a causative factor in eliciting domoic acid production in *Pseudonitzschia pungens* f. *multiseries*.
Pan *et al.* (1996c) demonstrated that the production of domoic acid was greatly enhanced when a severe stress was applied to the algal population after a period of active growth. The production was accelerated by a factor of ca.3 during the transition period from steady state to batch culture when growth was slowed and uptake of silicate or phosphate was diminished.
The *Pseudonitzschia* species identified in the 1991 domoic acid incident in Monterey Bay (California) at the USA west coast, *P. australis*, appeared to be a common inhabitant of these waters and nutrient depletion was most common in late summer and autumn following the period of seasonal upwelling. At the time of the 1991 *P. australis* bloom, Monterey Bay area was completing its annual dry season; the waters were moderately stratified, the surface temperature was 13-14 °C, salinity was 30-33 ‰ and nutrients were relatively depleted (Villac *et al.*, 1993b). The relatively confined area of Monterey Bay has been characterized as a persistent "upwelling shadow" zone; a region in which water is trapped by a front along the coast restricting its offshore flow (Work *et al.*, 1993).
Walz *et al.* (1994) suggested that cellular levels of domoic acid in *P. australis* were correlated with silicate concentrations. In batch culture studies, domoic acid production by *P. multiseries* was restricted to the stationary growth phase in silicate limited conditions (Bates *et al.*,1991). Pan *et al.* (1996a; 1996b; 1996c) studied the effects of silicate and phosphate

limitation on the production of domoic acid by *P. multiseries* in batch culture studies and continuous culture studies. Higher domoic acid production was demonstrated under lower supplies of silicate even at the same growth rate. It is very likely that the natural blooms of *P. multiseries* producing high amounts of domoic acid were severely silicate stressed. A preceding bloom of another diatom or a prolonged bloom of *P. multiseries* can deplete the silicate in the seawater. When the toxicogenic bloom of *P. multiseries* was at his height in Cardigan Bay, Canada on December, 1987, silicate concentration was as low as 0.62 μM. Domoic acid peaked 10 days later. In further studies of Pan *et al.* (1996c) it was demonstrated that also phosphate limitation in the culture medium enhanced domoic acid production. The concurrence of high rates of domoic acid production at steady state with low rates of nutrient uptake and with high levels of adenosine triphosphate (ATP) further suggests that synthesis of domoic acid required a substantial amount of ATP as a source of biogenic energy. Subba Rao *et al.* (1998) reported that cultures of *P. multiseries* produced substantially higher levels of domoic acid (230 fg/cell) upon enrichment with lithium (385.6 μM) than control cultures (135 fg/cell). Nitrogen, phosphate or silicate were not limiting in the cultures.

Garrison *et al.* (1992) speculated that domoic acid events might be limited to seasons when stratification and nutrient depletion occurred or to nearshore regions where developing blooms depleted the dissolved nutrients. However field observations inMonterey Bay (Walz *et al.*, 1994) showed that domoic acid was produced by the large-celled *P. australis* at low cell densities and at moderate nutrient concentrations. Results from culture studies by Garrison *et al.* (1992) indicated that *P. australis* was able to produce domoic acid regardless of the growth phase. These findings suggested a pattern for *P. australis* which was different from that of *P. multiseries*.

However, results from batch culture experiments cannot be extrapolated to natural populations.

The culture studies by Pan *et al.* (1996c) also showed that cessation of growth of *P. multiseries* was not essential to domoic acid production, contrary to the conclusion of Bates *et al.* (1991). Domoic acid was indeed produced by slowly dividing populations of *P. multiseries* in steady-state chemostat cultures under either phosphate or silicate limitations.

Bates *et al.* (1995) reported that axenic cultures of *Pseudonitzschia multiseries* produced less domoic acid than the original bacteria-containing cultures. Bacterial strains isolated from two nonaxenic *P. multiseries* clones were reintroduced individually into cultures of three axenic *P. multiseries* strains. The bacteria did not substantially affect division rates or cell yields. However, they did cause a 2 to 95 fold enhancement of domoic acid production (per cell basis) relative to the axenic culture, depending on the *P. multiseries* and bacterial strain used.

In agreement with the studies on *P. pungens* f. *multiseries* and *P. australis*, cultures of *P. seriata* (isolated from Danish waters) produced domoic acid mainly during the stationary growth phase. Only traces of domoic acid were detectable during the exponential growth phase. The domoic acid level in whole cultures appeared to equal that of *P. pungens* f. *multiseries* from Prince Edward Island, Canada, which the Danish isolates of *P. seriata* parallel in cell volume. Domoic acid production by *P seriata* also appeared to be temperature-dependent, with higher amounts produced at 4°C than at 15°C (Lundholm *et al.*, 1994).

4. Marine organisms associated with ASP

4.1 Uptake and elimination of ASP toxins in marine organisms

Shellfish (bivalve molluscs, gastropods, crabs, lobsters and others) accumulate phycotoxins either by direct filtration of the plankton cells or by feeding directly on contaminated organisms (e.g. carnivores and scavengers). Generalizations regarding the uptake and retention of phycotoxins by shellfish should be avoided. Rate of accumulation of toxic algae (or toxin) by filter-feeding shellfish is species-specific and are, in most cases, directly related to the number of cells available to the animals. The rate of accumulation of toxic algae in individual shellfish in any given area is highly variable. The rate of elimination of the toxin varies with season and low water temperatures retard toxin loss; however, the degree to which temperature affects the uptake and release of toxins is not clearly understood. Further, the rate of elimination is highly dependent on the site of toxin storage within the animal i.e. toxins in the gastrointestinal tract are eliminated much more readily than toxins bound in tissues. In the blue mussel (*Mytilus edilus*) and the oyster (*Crassostrea virginica*) the bulk of domoic acid is resided in the gut.
The majority of information available concerns bivalve molluscs. Domoic acid was shown to depurate from mussels fairly rapidly (as cited in Villac *et al.*, 1993a). The level of domoic acid in mussels in the absence of *Pseudonitzschia* is <1 µg/g. A minimum concentration of 2-4 x 10^5 *Pseudonitzschia* cells per L over a period of at least 3-4 weeks was needed to produce 20 µg domoic acid per g mussels in eastern Prince Edward Island, Canada (as cited in Todd, 1993). When blue mussels (*Mytilus edilus*) were presented domoic acid for 24 h in dissolved form (125 nM; at 5°C; salinity 28 ‰) or as food encapsulated in liposomes, <1% of dissolved domoic acid and up to 6% of food-borne domoic acid was incorporated in mussel tissues. Domoic acid absorbed from solution was most concentrated in gills and kidneys, whereas domoic acid ingested as food was most concentrated in digestive gland and kidneys. Gonad, muscle, foot and connective tissues retained the lowest concentrations of toxin. Domoic acid levels in mussel tissues did not decrease consistently over a depuration period of 48 h, nor did domoic acid appear to be translocated to any tissue for storage. Small amounts of domoic acid were eliminated in faeces and larger amounts in dissolved form (Novaczek *et al.*, 1991). Preliminary feeding studies with the New Zealand greenshell mussel (*Perna canaliculus*) fed *P. pungens* f. *multiseries* showed that the mussels were contaminated with domoic acid but that the toxin was rapidly eliminated after feeding ceased. Under some conditions the rate of excretion was equivalent to the rate of ingestion and accumulation in tissues did not take place (MacKenzie *et al.*, 1993).
When Pacific oysters (*Crassostrea gigas*) and Californian mussels (*Mytilus californianus*) were exposed continuously to the domoic acid producing diatom *P. pungens* f. *multiseries*, for 48 h followed by a 120 h clearance period, body burden was the greatest in the Pacific oyster after 4 h of exposure (36.3 µg/g; highest level in soft tissue 32.9 µg/g). However, after 4 h of exposure the Pacific oyster showed closure of their shell and body burden of domoic acid decreased. At 120 h of clearance the gill, muscle and soft tissue still revealed detectable levels of domoic acid. In the Californian mussel body burden of domoic acid reached a maximum also after 4 h of exposure (3.6 µg/g; highest level in the gill tissue 2.5 µg/g) No

detectable levels in the mantle, gill or soft tissue were observed after 24 h of clearance. In muscle tissue only trace levels were found after 24 h of depuration (Jones *et al.*, 1995).
Depuration from razor clams is not very rapid. In razor clams, higher domoic acid levels were concentrated in the edible muscular tissues and lower levels in the non-edible tissue parts. When depuration rates are low, low values of domoic acid can be intoxicating, as is the case for razor clams. Therefore, the constant presence of domoic acid producing diatoms at low densities might result in long-term high concentrations in the clam.
In anchovies domoic acid was found not only in the viscera but also in the fish muscle (as cited in Villac *et al.*, 1993a).
The bay scallop *Argopecten iradians* was reported to take up domoic acid up to levels of 60 µg/g in the digestive gland after approximately 84 h of exposure to toxic *P. multiseries* Domoic acid levels decreased to 5 µg/g after 48 h of depuration (as cited in Douglas *et al.*, 1997).
When sea scallops (*Placopecten magellanicus*) were fed *P .multiseries* cells with a high domoic acid content (4-6.6 pg/cell) for 22 days, followed by 14 days of feeding with nontoxic microalgae, domoic acid was incorporated within 24 h , with increased uptake after 6 days. Domoic acid was concentrated in tissues in the following order: digestive gland >> remaining soft tissue >> adductor muscle. A maximum of 3108 µg/g was recorded in the digestive gland; however, only trace amounts (0.7-1.5 µg/g) were found in the adductor muscle. At the end of the exposure period, 50.9% of the supplied domoic acid had been incorporated into the tissues. Domoic acid level in the digestive gland 14 days after termination of the toxic diet, remained high, 752 µg/g. Throughout the experiment, there were no signs of illness or mortality of the sea scallops attributable to high domoic acid loading. However, the destructive sampling of the scallops did not allow assessment of long-term effects (Douglas *et al.*, 1997). Also the red mussel (*Modiolus modiolus*) retained domoic acid for lengthy periods (as cited in Stewart et al., 1998).
Stewart *et al.* (1998) suggested the strong possibility that autochthonous bacteria might be a significant factor in the elimination of domoic acid from molluscan species that eliminate domoic acid readily. This was demonstated in blue mussels *Mytilus edilus* and soft shell clams *Mya arenaria*. Stewart *et al.* (1998) suggested different mechanisms used by different shellfish in dealing with domoic acid, i.e., freely available in blue mussels and soft shell clams but likely sequestered in the digestive glands of sea scallops and red mussels and thus, largely unavailable for bacterial utilization.
Few data are available for retention times of toxins in crabs and carnivorous gastropods; the general trend in these organisms appears to be towards long-term retention.
A retention time longer than 2 years was reported for *Siliqua patula* with a not defined *Pseudonitzschia* species as toxin source (as cited in Shumway *et al.*, 1995).
A decrease in domoic acid content from 50 µg/g to 5 µg/g within 72 h was observed in blue mussels derived from the 1987 Canada incident (toxin source *P. pungens* f. *multiseries*), whereas in razor clams derived from the USA West California Monterey Bay 1991 incident (toxin source *P. australis*) a decrease from 47.9 µg/g to 44.3 µg/g lasted over 3 months (Villac *et al.*, 1993a). Dungeness crabs (*Cancer magister*) accumulated the toxin mostly in the viscera, although it can enter meat during cooking if the crabs were not eviscerated previously (Villac *et al.*, 1993a). When Dungeness crabs were fed domoic acid via contaminated razor clam meats, for 6 or 9 days, analyses of the raw crabs indicated that domoic acid was rapidly accumulated and was confined to the viscera, principally in hepatopancreas (22 µg/g). No domoic acid was detected in either body or leg meats of the raw crabs (Hatfield *et al.*, 1995). Also studies of Lund *et al.* (1997) showed that Dungeness crabs absorbed domoic acid (via contaminated clam meat) rapidly and accumulated domoic acid only in the hepatopancreas. Domoic acid was effectively depurated from the

hepatopancreas (via feces) over a 3-week period once the toxic feeding ceased. Depuration proceeded at a faster rate when crabs were fed toxin-free feed than when they were starved.

4.2 Shellfish containing ASP toxins

Cultured mussels (*Mytilus edilus*) sampled during the first outbreak of ASP poisoning in Canada (eastern Prince Edward Island) during autumn 1987 contained up to 790 μg domoic acid/g wet tissue (whole mussel) (up to 1280 and 1500 μg/g in soft tissue and digestive gland, respectively) (Bates *et al.*, 1989; Todd, 1997). During August-October 1988 domoic acid was detected also in blue mussels and furthermore in soft-shell clams (*Mya arenaria*) from the southwestern Bay of Fundy, Canada (Martin *et al.*, 1993).
In October 1991 , domoic acid was detected in razor clams (*Siliqua patula*) from Oregon and Washington states in the USA. Levels peaked in the first week of December 1991 (maximum level in edible portion was 147 μg/g , average level was 106 μg/g for all Washington state beaches). The domoic acid levels in the clams remained above the regulatory closure level of 20 μg/g for at least 6 months. Domoic acid levels were declined to <10 μg/g by late spring of 1992. From the spring of 1992 until the spring of 1993, levels of domoic acid were < 5 μg/g for most of the coastal sampling areas (Wekell *et al.*, 1994a, b). Domoic acid appeared to distribute itself throughout the various body parts of the razor clam. The highest level was found in the foot or"digger", followed by the body, viscera and siphon (or neck). The domoic acid level in the razor clam foot reached 230 μg/g (Wekell *et al.*, 1994b).
The bay scallops *Argopecten iradians* was reported to take up domoic acid up to levels of 60 μg/g in the digestive gland after approximately 84 h of exposure to toxic *P. multiseries* Domoic acid levels decreased to 5 μg/g after 48 h of depuration (as cited in Douglas *et al.*, 1997). In autumn 1993 an unexplained mortality among sea scallops (*Placopecten magellanicus*) occurred in the Bay of Fundy, Canada. The digestive gland of the scallops appeared to contain 93.4 μg domoic acid/g. Although some bivalve molluscs have been reported to contain high levels of domoic acid without showing any symptoms, the spiny scallop (*Chlamys hastata*) died rapidly (within 12 h) after exposure to cultures of toxic *P. multiseries*. To get an explanation for the mortality of the sea scallops *Placopecten magellanicus*, a feeding experiment with *P. multiseries* with a high content of domoic acid was carried out (see under 4.1) (Douglas *et al.*, 1997). Substantial amounts of domoic acid are found routinely in the digestive glands but not in the adductor muscles of offshore sea scallops from Georges Bank or Browns Bank and frequently in Bay of Fundy sea scallops (as cited in Stewart *et al.*, 1998).

4.3 Other marine organisms containing ASP toxins

In Dungeness crabs (*Cancer magister*) from Washington and Oregon states of the USA domoic acid was detected, but only in the viscera. Domoic acid levels in raw viscera of individual crabs from Washington state in December 1991 averaged 13 μg/g and ranged from 0.8 to 90 μg/g. The highest average levels of domoic acid in Washington state crabs were found in Grays Harbor and Willapa Bay samples, 32 and 31 μg/g, respectively. By 1992 domoic acid level averages were <5 μg/g in preseason samples of Dungeness crabs taken along the Oregon and Washington coasts, ranging from 0 to 71 μg/g. The highest levels of domoic acid in 1992 (36-71 μg/g) were recorded in samples taken in January through April (Wekell *et al.*, 1994a, b). The immediate source of domoic acid for Dungeness crabs was

unclear. These crabs were considered opportunistic predator-scavengers in the marine benthos. It is possible that Dungeness crabs prey on toxic subtidal razor clams, although some razor clams live in the "surf" zone and others persist in the subtidal regions. On the other hand, high domoic acid levels were also observed in crabs taken from areas where few, if any, razor clams were found. Therefore other benthic sources of domoic acid must also be considered (Wekell *et al.*, 1994a).

Domoic acid was also found in benthic crustaceans, but the sources and pathways transferring domoic acid to the benthic community have not been established and no studies were performed to determine how accumulated toxin might affect secondary consumers (Horner *et al.*, 1997).

In September 1991 water fowl (brown pelicans (*Pelecanus occidentalis*) and cormorants (*Phalacrocorax penicillatus*)) died in Monterey Bay, California, after eating anchovies (*Engraulis mordax*) contaminated with domoic acid . Up to 485 µg/g domoic acid was detected in the viscera of the anchovies. Frozen samples of anchovies which were harvested in April 1991 appeared to contain 270 µg/g domoic acid in their viscera. By May, domoic acid levels in frozen anchovy samples from the same area were less than 1 µg/g. Anchovies are primarily carnivorous but they will consume phytoplankton if other food sources are not available (Wekell *et al.*, 1994a). McGinness *et al.* (1995) showed that the stomach of northern anchovies (*Engraulis mordax*) from Monterey Bay, Calfornia, (August 1992), contained nine different *Pseudonitzschia* species, including four that have produced domoic acid either under natural or under laboratory conditions. The study demonstrated that northern anchovies are able to filter pennate diatoms from the near-surface seawater.

In January 1996 the death of brown pelicans (*Pelecanus occidentalis*) at Cabo San Lucas on the tip of the Baja California Peninsula (Mexico) was ascribed to the feeding of mackerel (*Scomber japonicus*) contaminated by domoic acid-producing *Pseudonitzschia* spp. (Sierra-Beltrán *et al.*, 1997). These two incidents showed that also finfish is able to accumulate domoic acid.

5. Toxicity of ASP toxins to animals and man

5.1 Mechanism of action

The mechanism of action of domoic acid is known on excitatory amino acid receptors and on synaptic transmission. Excitatory amino acids, most notably L-glutamate and L-aspartate, have long been considered to be the most likely neurotransmitters. These amino acids are known to act on several receptor types, the best characterized of which are named after the selective exogenous excitants N-methyl-D-aspartate (NMDA), kainate and quisqualate.
Domoic acid is a glutamate analogue and binds with high affinity to glutamate receptors of the quisqualate type. Glutamate and also NMDA subclass act to open membrane channels permeable to Na^+, leading to Na^+ influx and membrane depolarization. Only the channel opened by NMDA receptor accessible to kainate, quisqualate and to domoic acid is, in addition, highly permeable to Ca^{2+} and induced lethal cellular Ca^{2+} entry. Actions at NMDA receptors can be selectively antagonized by micromolecular concentrations of magnesium ions, organic antagonists such as D-2-amino-5-phosphonovalerate (APV) and dissociative anaesthetics, such as phencyclidine (as cited in Viviani, 1992). In mice kynurenic acid, administered intraperitoneally, protected powerfully and significantly against mussel-extract induced neurotoxicity. (Pinsky *et al.*, 1989). Since drugs blocking the domoate-sensitive receptor are known, their use as antidotes for domoate poisoning has been considered (Laverty, 1993). Domoic acid is a 2-3 times more potent neuroexcitator than the structurally related kainic acid, and is up to 100 times more potent than glutamic acid. A synergistic effect between domoic acid and other neurotoxic amino acids normally present in mussels, is possible (as cited in Ravn, 1995). Domoic acid has two primary targets in the central nervous system; the hippocampal formation and its associated regions which are involved in processing memory, and the brain stem region of the area postrema and nucleus of the solitary tract associated with visceral function (Peng *et al.*, 1994).

5.2 Toxicity to laboratory animals

5.2.1 Acute toxicity

The toxic effects of domoic acid have been studied using mice, rats and cynomolgus monkeys. The toxin induces very characteristic symptomology in mice and rats following intraperitoneal injection. The most characteristic symptoms include a unique scratching of the shoulders by the hind leg, followed by convulsions and often death. More subtle effects include hypoactivity, sedation-akinesia, rigidity, sterotypy, loss of posteral control and tremors (as cited in Wright and Quilliam, 1995).
A series of publications on the neurotoxic effects caused by domoic acid, showed that the agonist produced a loss of neurons in the CA1, CA3 and CA4 area of the hippocampus, oedema and neuronal degeneration in the arcuate nucleus and vacuolated and pyknoted cells in the inner layer of the retina. The hippocampal lesions were identical to those found in human ASP victims (as cited in Iverson and Truelove, 1994).
An intraperitoneal LD_{50} value in the mouse of 3.6 mg domoic acid (via mussel extracts)/kg b.w. was reported. Intraperitoneal doses of 100 μg or above in mussel extracts (equivalent to

5 mg domoic acid /kg b.w.) were reported to cause deaths (as cited in Todd, 1993). The lowest level of domoic acid causing behavioural effects in mice was 23 μg given i.p., which is equivalent to 46 mg domoic acid/kg of mussels. No behavioural changes were noted at 20 mg/kg of mussels and this was considered to be the no-effect level (as cited in Iverson and Truelove, 1994).
Rats showed scratching, crab-like walking, "praying", loss of balance and seizures after i.p. doses of 2.0-7.5 mg/kg b.w. The threshold i.p. dose for symptoms in rats was 1-2 mg/kg b.w. Lesions in the higher dosed rats were apparent in the amygdala, cortex, hippocampus, hypothalamus, olfactory system and retina (as cited in Todd, 1993). Sobotka *et al.* (1996) reported that i.p. doses less than 1 mg domoic acid/kg b.w. produced measurable behavioural effects in adult rats without apparent signs of neurological dysfunction or neuropathology. Slightly higher doses of 1.32 mg/kg b.w. and 2.25 mg/kg b.w. produced not only behavioural effects, clinical signs of neurotoxicity and occasional morbidity, but also hippocampal damage.
Oral doses required more than 10 times as much toxin to achieve the same effects as i.p. doses. Mice and rats tolerated, orally, 30-50 mg domoic acid/kg b.w. without observable effects (as cited in Todd, 1993).
Rats given toxic mussel extracts orally, developed mastication and seizures when domoic acid level was ≥70 mg/kg b.w. and died when the level was ≥80 mg/kg b.w. (as cited in Todd, 1993). The inability of the rat to vomit precluded what appeared to be a very sensitive clinical sign of domoic acid intoxication in humans. The absorption of domoic acid after oral administration to rats is poor as was demonstrated by almost complete recovery in the feces. Domoic acid is cleared from plasma by the kidneys and, more specifically, by the process of glomerular filtration. The plasma half-life in rats was 21.6 min. (as cited in Iverson and Truelove, 1994). There was no evidence of any biotransformation of domoic acid by the rodent or primate as it has always been recovered unchanged (as cited in Todd, 1993).
Xi *et al.* (1997) reported that neonatal rats were approximately 80-fold more sensitive to domoic acid induced scratching and approximately 40-fold more sensitive to domoic acid induced seizures and lethal effects than adult rats (after intraperitoneal dosing). The i.p. LD_{50} for postnatal day 2 and day 10 rats was 0.25 and 0.7 mg/kg b.w., respectively.
In studies in rodents the effects induced by extracts of contaminated mussels were compared with the effects induced by pure domoic acid and the effects induced by extracts of non-contaminated mussels. The extract of contaminated mussels appeared to be the most potent formulation. This was ascribed to potentiation of the excitatory effect of domoic acid by glutamate and aspartate; both are excitatory amino acids found in mussel extracts (as cited in Mariën, 1996).
In cynomolgus monkeys single oral doses of 1.0 mg domoic acid/kg b.w. caused vomiting, gagging and yawning, but single doses of 0.75 or 0.5 mg/kg b.w. did not result in overt effects. Evidence of neurotoxicity, at the light microscope level, was absent at 5.0 mg/kg b.w. p.o., and present at 10 mg/kg b.w. p.o. Intravenous domoic acid given at doses as low as 12.5 μg/kg b.w. were able to induce readily observable clinical signs (gag response) in the monkeys. On a mg/kg b.w. basis, this level is 1000 fold lower than that observed in the mouse bioassay using the clinical response as endpoint (as cited in Iverson and Truelove, 1994).

5.2.2 Repeated dose toxicity

Mice from two strains (outbred and seizure-sensitive inbred strain, respectively) received single or 4 intraperitoneal injections (every other day for 7 days) with either subsymptomatic (0.5 mg/kg b.w.) or symptomatic sublethal (2.0 mg/kg b.w.) doses of domoic acid in order to

investigate the possibility of enhanced toxicity (observable behavioural responses) after repeated exposure. The serum domoic acid levels did not differ following single or repeated exposures. Both strains showed comparable concentration dependent toxic responses. The study did not provide evidence that short-term repeated exposures altered domoic acid clearance from serum or led to a more sensitive or greater neurotoxic response than single exposure (Peng *et al.*, 1997)

Three groups of 10 male and 10 female rats received daily, orally by gavage, 0, 0.1 or 5.0 mg domoic acid/kg b.w. dissolved in water for 64 days. No clinical abnormalities were observed. Hematology and clinical chemistry did not show abnormalities. Organ weights did not reveal abnormalities. Histopathology of several tissues (including eyes and brain) and immuno-histochemistry of selected sections of hippocampus and retina were unremarkable. Domoic acid determinations in urine and feces revealed that absorption was approximately 1.8% of the administered dose (Truelove *et al.*, 1996).

Three cynomolgus monkeys received daily, orally by gavage, 0.5 mg domoic acid/kg b.w. dissolved in water, for 15 days and then for another 15 days 0.75 mg/kg b.w. After the 30-day treatment period the monkeys were killed. Body weight, food and water consumption were recorded, clinical observations were made, hematology and serum chemistry were performed, histopathology of all major organs (including brain and retina) and glial fibrillary acid protein immunohistochemistry were carried out. All examined parameters remained unremarkable. Domoic acid concentrations in urine and serum were measured at several time points. Absorption in the monkeys appeared to be 4-7% (compared to 1.8% in rats) and the plasma half- life was 114.5 min. (compared to 21.6 min. in rats) (Truelove *et al.*, 1997).

5.2.3 Teratogenicity

Nine to fifteen pregnant rats/group received intraperitoneally during days 7-16 of gestation 0, 0.25, 0.5, 1.0, 1.25, 1.75 or 2.0 mg domoic acid/kg b.w., respectively. On day 22 of gestation the dams were killed and fetuses were examined for developmental changes and for visceral anomalies. No signs of maternal toxicity were observed up to doses of 1.25 mg/kg b.w. At the dose of 2 mg/kg b.w. 6 out of 9 dams died after two doses. The remaining 3 dams on this dose-level aborted after 3 doses. At 1.75 mg/kg b.w. 6 out of 12 dams aborted prior to Caesarian section. At 1.0 and 0.5 mg/kg b.w. a reduction in live fetuses/litter were seen. However, this effect was neither dose-related, nor associated with an increased incidence of resorptions plus dead fetuses. A statistically significant increased incidence of retarded ossification of the sternebrae was observed at 1.25 mg/kg b.w., but this effect was not seen at any other dose level. At the lowest dose level of 0.25 mg/kg b.w. no maternal or fetal toxicity was seen. No teratogenic effects were observed in this study (Khera *et al.*, 1994).

5.3 Mutagenicity

Rogers and Boyes (1989) investigated the mutagenicity of domoic acid in a hepatocyte mediated assay with V79 Chinese hamster lung fibroblasts. The genetic endpoints measured were: mutation to 6-thioguanine resistance at the HPRT locus; mutation to ouabain resistance at the Na^+, K^+-ATPase locus; sister chromatid exchanges and micronuclei frequency. No significant cytotoxicity was seen. None of the genetic endpoints was significantly affected by exposure to domoic acid at dose levels of 27.2 and 54.4 μg/ml with or without metabolic activation by freshly isolated rat hepatocytes.

5.4 Toxicity to man

Anecdotal evidence indicated that Japanese islanders once prized seaweed extracts containing domoic acid as a very useful tonic. Trials were apparently undertaken to test the anthelmintic properties of domoic acid and single 20 mg doses of unknown purity were administered to adults and children without harmful effect (as cited in Iverson and Truelove, 1994).
However domoic acid is toxic to both the central and peripheral nervous systems of man. Domoic acid is an emetic, causing gagging and vomiting, likely through its effect on the vomit center in the area postrema of the brain. It produces a syndrome of axonal sensorimotor neuropathy, amnesia, seizures, coma and death. Because of its impact on memory, among other ill effects, domoic acid intoxication was named amnesic shellfish poisoning (ASP) (as cited in Todd, 1993 and Watters, 1995).
In the first ASP outbreak in 1987 at Prince Edward Island in Canada, 107 cases were reported. The first symptoms were experienced 15 min to 38 h (median 5.5 h) after mussel consumption. The most common symptoms were nausea (77%), vomiting (76%), abdominal cramps (51%), headache (43%), diarrhea (42%), and memory loss (25%). There was a close correlation between memory loss and age; those under 40 were more likely to have diarrhea and those over 50 to have memory loss. Memory loss was predominantly short-term. The most severely ill were hospitalized, of which 12 were treated in intensive care units (ICU). Eight of these were ≥65 years old and the other 4 had pre-existing illnesses (diabetes, chronic renal feature or hypertension). The ICU patients demonstrated confusion, coma, mutism, seizures, chewing motions, grimacing, hiccups, lack of response to painful stimuli, uncontrolled crying or agressiveness, profuse respiratory secretion, and unstable blood pressure or cardiac arrhytmias. Fourteen patients showed persistent neurological defects. Eye problems were noted in several of these, including disconjugate gaze, diplopia and ophthalmoplegea, but these resolved within 10 days (as cited in Todd, 1993). In addition they manifested seizures, myoclonus, anterograde memory deficits, decreased glucose metabolism in the medial temporal lobes on positrone-emission tomography (PET) scanning, and EMG changes of puremotor or sensorimotor axonopathy. Four out of the 14 patients remained in coma and died. Post-mortem examination revealed necrosis and neuronal loss predominantly in the hippocampus and amygdala (Teitelbaum et al., 1990). Amounts of domoic acid consumed, ranged from 15-20 mg/person for an unaffected person to 295 mg/person for a case with severe neurological symptoms. Assuming that average body weight is 50-70 kg the unaffected male ingested 0.2-0.3 mg domoic acid/kg b.w. Some persons showed mild symptoms (mainly gastrointestinal) after consuming 60-110 mg domoic acid, equivalent to 0.9-2.0 mg/kg b.w. The most serious cases (severe neurological deficits) consumed 135-295 mg, equivalent to 1.9-4.2 mg domoic acid/kg b.w. (as cited in Todd, 1993).

Long-term effects on human health of low concentrations of domoic acid in mussels or fish are not known (as cited in Lundholm et al., 1994).

5.5 Toxicity to aquatic organisms

In a laboratory study domoic acid appeared to be toxic to the marine copepod *Tigriopus californicus* at low concentrations. LC_{50} (24 h) was found to be 8.62 μM (Shaw *et al.*, 1997).
In autumn 1993 an unexplained mortality among sea scallops (*Placopecten magellanicus*) occurred in the Bay of Fundy, Canada. The digestive gland of the scallops appeared to

contain 93.4 µg domoic acid/g. Although some bivalve molluscs have been reported to contain high levels of domoic acid without showing any symptoms, the spiny scallop (*Chlamys hastata*) died rapidly (within 12 h) after exposure to cultures of toxic *P. multiseries* (Douglas *et al.*, 1997). *Chlamys hastata* might be the source of crab toxicity. The swimming scallops *Chlamys hastata*, when exposed to domoic acid by feeding on *Pseudonitzschia multiseries*, lost motor or escape responses and would fall easy to prey bottom dwelling scavengers such as crabs (as cited in Whyte *et al.*, 1997). In a laboratory study sea scallops (*Placopecten magellanicus*) were fed *P. multiseries* with a high content of domoic acid (4-6.6 pg/cell) for 22 days, followed by 14 days of feeding with nontoxic microalgae. No signs of illness or mortality were observed during this study despite the high domoic acid loading (see under 4.1). However, the destructive sampling of the scallops did not allow assessment of long-term effects (Douglas *et al.*, 1997).

Jones *et al.* (1995) investigated the physiological effects of domoic acid on the marine invertebrates Pacific oyster (*Crassostrea gigas*) and California mussel (*Mytilus californianus*) which were known to accumulate this neurotoxin. The oysters and the mussels were exposed continuously to the domoic acid producing diatom *P. pungens* f. *multiseries*, for 48 h followed by a 120 h clearance period. The Pacific oyster rapidly accumulated significant soft tissue burdens of domoic acid resulting in a generalized stress response characterized by shell closure 4h after introduction of the algae, haemolymph acidosis and an acute transient hypoxia. The Californian mussel appeared to increase its ventilatory flow resulting in a mild non-compensated respiratory alkalosis.

Domoic acid in *Pseudonitzschia multiseries* caused feeding inhibition of the rotifer, *Brachionus plicatilis*, with subsequent reduced nutritional condition and loss of fecundity (as cited in Whyte *et al.*, 1997).

5.6 Toxicity to water fowl

In September 1991 water fowl (brown pelicans (*Pelecanus occidentalis*) and cormorants (*Phalacrocorax penicillatus*)) died in Monterey Bay, California, after eating anchovies (*Engraulis mordax*) contaminated with domoic acid (Wekell *et al.*, 1994a). In January 1996 brown pelicans (*Pelecanus occidentalis*) died at Cabo San Lucas on the tip of the Baja California Peninsula, Mexico. The death of these birds was the result of feeding on mackerel (*Scomber japonicus*) contaminated by domoic acid-producing *Pseudonitzschia* spp. (Sierra-Beltrán *et al.*, 1997).

6. Prevention of ASP intoxication

6.1 Depuration

To date there have been no useful methods devised for effectively reducing phycotoxins in contaminated shellfish. All methods tested have been either unsafe, too slow, economically unfeasible or yielded products unacceptable in appearance and taste (as cited in Shumway *et al.*, 1995). Mussels were reported to take up domoic acid rapidly but also depurated rapidly, while other bivalves retained domoic acid for longer periods. Depuration of domoic acid by razor clams is a long-term process (as cited in Horner *et al.*, 1997). Depuration of domoic acid from starved mussels and clams was relatively rapid (43 to 15 μg/g at 13 °C in 24 h with traces remaining for up to 6 d in Passamaquaddy Bay, Canada, and 130 to 20 μg/g at 15 °C in 4-6 d in the Cardigan River, Canada). Complete depuration, however, in the natural habitat may take longer. Domoic acid concentrations in Cardigan Bay area, eastern Prince Edward Island, Canada, declined to neglegible levels in 40-50 d (as cited in Todd, 1993).
Whole scallops (*Pecten maximus*) flesh contaminated with domoic acid, showed a 43% decrease (mostly in hepatopancreas) in domoic acid content after 180 days of frozen storage (-20 °C). During frozen storage, there was a transfer of domoic acid from the hepatopancreas to the rest of the body, with a net average decrease in the whole product. Subsequently pickling of the scallops flesh or packing with brine and canning after frozen storage did not cause a further decrease of the domoic acid content. During canning there was a notable transfer of toxin from the scallops to brine or the pickling medium (>30% of total domoic acid content in canned product) (Leira *et al.*, 1998). Sea scallops *Placopecten magellanicus* and red mussels *Modiolus modiolus* were reported to retain domoic acid for lengthy periods (as cited in Stewart *et al.*, 1998). The domoic acid level in the digestive gland of sea scallops was found to be only slightly lower at the end of a 19-month depuration study than it was at the beginning (Stewart *et al.*, 1998). Dungeness crabs (*Cancer magister*) accumulated the toxin mostly in the viscera, although it can enter meat during cooking if the crabs were not eviscerated previously (Villac *et al.*, 1993a). When contaminated whole crabs (22 μg domoic acid/g confined to hepatopancreas) were cooked in fresh or salted water, visceral domoic acid was reduced by 67-71%. After cooking, domoic acid was detected not only in hepatopancreas (6.4 μg/g) but also in the body (1.9 μg/g) and leg (1.1 μg/g) meats. However, the majority of the domoic acid was extracted out and diluted into the cook water. When cooked crabs were held 1 or 6 days at 1 °C, domoic acid was detected in hepatopancreas (6.1-8.2 μg/g) and body meats, but not in leg meats. Body meats proximal to the viscera contained higher domoic acid levels (1.5-2.1 μg/g) than those distal (0.57-0.92). When cooked crabs were held for 90 days at -23 °C, domoic acid was detected in the viscera (7.6 μg/g), body (0.67-0.79 μg/g) and leg (0.38 μg/g) meats. The storage conditions of cooked crabs had some effect on domoic acid distribution, but no effect on the total domoic acid content in each crab (Hatfield *et al.*, 1995).
In laboratory studies Lund *et al.* (1997) showed that domoic acid was effectively depurated from the hepatopancreas of Dungeness crabs over a 3-week period once the toxic feeding of domoic acid via contaminated clam meat ceased. Depuration proceeded at a faster rate when crabs were fed toxin-free clam meat than when they were starved.
Depuration studies on the west coast of Canada with Dungeness crabs indicated that, if the crabs were placed in filtered sea water, domoic acid levels dropped rapidly within a few

weeks, but in harbor water in cages (without access to contaminated shellfish), domoic acid levels fluctuated but did not go down (as cited in Todd, 1993).

6.2 Preventive measures

Commercial harvest areas and aquaculture facilities are adversely and often unpredictably affected by toxic blooms. A problem is that certain algal species, which have never occurred in a certain area, may suddenly appear and then rapidly cause problems. Therefore preventive measures can hardly be taken. Extensive monitoring of the marine environment and the possibly contaminated fishery products together with regulations (see chapter 8) will be required to prevent (shell)fish poisoning incidents. Data on the occurrence of toxic algal species may indicate which toxins may be expected during periods of algal blooms and which fishery products should be considered for analytical monitoring. In the case of domestic produce, several countries stopped the harvest of fishery products if levels of the toxin exceeded the limits and a waiting period was established until the concentrations of the toxin are below the acceptable limit(as cited in Shumway *et al.*, 1995). Within the genus *Pseudo-nitzschia* domoic acid production can vary greatly with the species and it is vital to be able to distinguish species (Fryxell *et al.*, 1997). Toxin concentrations in the fishery products can also vary with the species of fishery product involved and with the area of harvest. Harvested fishery products containing too much toxin were usually destroyed. Toxic doses are often estimated from left-over toxic seafood, but these may not be always representative of the ingested food (as cited in Shumway *et al.*, 1995).
Direct analysis of phytoplankton samples for domoic acid will probably remain the fastest and most reliable method to confirm the presence of domoic acid (Waltz *et al.*, 1994). Although the need for routine phytoplankton monitoring often has been stressed, phytoplankton monitoring has been implementedonly in a few areas (Horner *et al.*, 1997). Monitoring of intertidal shellfish may be not a particularly sensitive method for detecting of low but significant levels of domoic acid in plankton, missing all but the most extreme events (Waltz *et al.*, 1994). This is caused by the differences in uptake and depuration in the shellfish species. Mussels, for instance, were reported to take up domoic acid rapidly but also depurated rapidly, while other bivalves retained domoic acid for longer periods. Depuration of domoic acid by razor clams is a long-term process (as cited in Horner *et al.*, 1997). However, Whyte *et al.* (1997) claimed that studies of the uptake and depuration of domoic acid by the mussel *Mytilus californianus* had shown that this species was adequate as a sentinel organism in a weekly testing program, despite the relatively rapid elimination of domoic acid by this species.

7. Case reports/outbreaks and surveys

7.1 North America

7.1.1 Canada

The first report of ASP was coming from Canada in 1987. An outbreak of food poisoning during November and December 1987 was traced to cultured blue mussels (*Mytilus edulis*) from the Cardian Bay region of eastern Prince Edward Island. Three deaths and 105 confirmed cases of acute intoxication following consumption of mussels from this area, were recorded. The toxin was identified as domoic acid and the source organism appeared to be the pennate diatom *Pseudonitzschia pungens* f. *multiseries*. A plankton bloom at the time of the outbreak consisted almost entirely of this diatom and a positive correlation was found between the number of *P. pungens* f. *multiseries* cells and domoic acid concentration in the plankton. This toxic shellfish incident was the first known outbreak of human intoxication due to ingestion of domoic acid (Bates *et al.*, 1989). It is not known whether ASP occurred earlier than 1987, but prior to 1980 the mussel industry was in its infancy. However, there was one incident in 1984 in Calgary, Alberta, in which 12 persons consumed Prince Edward Island mussels and developed vomiting, diarrhea and blurred vision 1.5 to 3 h later; the symptoms lasted from 1 to 7 days. No etiologic agent was identified (as cited in Todd, 1993). In the years after the 1987 incident, blooms have occurred, but these were less extensive. Only in the autumn domoic acid levels in shellfish sometimes reached the guideline value at which harvesting areas had to be closed for a short period. In October 1991 a limited bloom was recorded for the first time from northern Prince Edward Island (Todd, 1997).

During August-October 1988 domoic acid levels greater than the acceptable levels for human consumption (20 µg/g) were detected in soft-shell clams (*Mya arenaria*) and blue mussels (*Mytilus edulis*) from the southwestern Bay of Fundy, New Brunswick, resulting in the closure of some shellfish harvesting areas. *P. pseudodelicatissima* was found to be the source of domoic acid and was detected in all plankton tows (collected since 1987) where domoic acid was found. *P. pseudodelicatissima* was detected throughout the year with higher concentrations in June/July followed by the highest concentrations in September when water temperatures were elevated. The highest concentration (1.2×10^6 cells/l) of *P. pseudo-delicatissima* was measured during 1988 and persisted throughout the water column for a longer period than during 1987, 1989 and 1990. Analysis of nutrients (chlorophyll a, salinity, nitrate, phosphate, silicate at surface and 10 and 1 m above bottom; measured, however, during 1989 and 1990 and not in 1988) did not reveal an obvious correlation between *P. pseudodelicatissima* and nutrient concentrations (Martin *et al.*, 1993).

In adductor muscles of offshore sea scallops from Georges Bank or Browns Bank and in Bay of Fundy sea scallops no domoic acid was found, but substantial amounts (10-200 µg/g) were routinely found in the digestive glands. Only the adductor muscles were available for sale because the digestive glands usually contain paralytic shellfish poisons (PSP). In April-May 1995 sea scallops on Georges Bank showed domoic acid levels in their digestive glands in excess of 1300 µg/g and up to 150 µg/g in the roe, while Brown Bank scallops had >2500 µg/g in their digestive glands. The single highest individual value recorded for Brown Banks

was 4300 μg/g of scallop digestive gland. The source of domoic acid in this 1995 episode was not discovered (as cited from Stewart et al., 1998).

7.1.2 USA

Alaska

In Alaska no severe problems with domoic acid existed although potentially toxic *Pseudonitzschia* spp. had been identified in Alaskan waters. Approximately 3000 samples, primary commercially valuable shellfish and finfish, had been tested since 1992 . The highest domoic acid value was 11.1 μg/g (for a razor clam) with only 17 values above 2 μg/g (Horner *et al.*, 1997)

West coast

In early September 1991 (18-27 September 1991) more than 100 brown pelicans (*Pelecanus occidentalis*) and cormorants (*Phalacrocorax penicillatus*) in Monterey Bay, central California died or suffered from unusual neurological symptoms which were attributed to the neurotoxin domoic acid. The source was identified as a bloom of the pennate diatom *P. australis* (Horner et al., 1997). At the peak of this incident, domoic acid levels in coastal waters were 10 μg/l and abundances of *P. australis* exceeded 10^6/l (Waltz *et al.*, 1994). Approximately 100 μg domoic acid/g (wet weight) was found in
P. australis (Fritz *et al.*, 1992). Remnants of *P. australis* frustules and high levels of domoic acid were found in the stomach contents (40-50 μg/g) of affected birds. Domoic acid was detected in viscera (up to 190 μg/g) and flesh (up to 40 μg/g) of local anchovies, a principal food source of seabirds (Work *et al.*, 1993). Wekell *et al.* (1994a) reported domoic acid levels in viscera of anchovies up to even 485 μg/g.

In late October and November 1991, razor clams (*Siliqua patula*) living in the surf zone on Pacific coast beaches in Washington and Oregon contained domoic acid at levels in the edible parts (i.e., foot, siphon, and mantle) as high as 154 μg/g (wet weight). Therefore recreational and commercial harvest of the clams was closed (Horner and Postel, 1993). Only a dozen cases of illnesses of humans were reported with mild gastrointestinal symptoms and one complaint of memory deficit, but the occurrence of ASP was never confirmed (Villac *et al.*, 1993b). Domoic acid levels were still above the harvest closure measure of 20 μg/g at least until May 1992. Other molluscan shellfish, including oysters grown commercially in coastal embayments, and mussels, never became toxic (Horner *et al.*, 1997).
Subsequently, domoic acid was found in the viscera of Dungeness crabs (*Cancer magister*) in coastal waters of California, Oregon and Washington. As a consequence, this important commercial fishery was closed for several weeks until investigators determined that proper cleaning of the crabs before cooking kept domoic acid out of the edible meat (Horner and Postel, 1993). The source of domoic acid in razor clams and Dungeness crabs during these incidents was not determined (Horner *et al.*, 1997).

During the fall of 1991, besides *P. australis* at the Monterey Bay, California, other *Pseudonitzschia* spp. were also present at several sites (as mentioned above) on the USA west coast from Southern California to the mouth of the Columbia River (Newport, Coos bay, Ilwaco). In the fall of 1992, besides *P.australis*, other potentially domoic acid producing *Pseudonitzschia* spp. were present in Monterey Bay (*P. delicatissima*, *P. pungens* f. *multiseries* and *P. pseudodelica-tissima*), but no report of a domoic acid outbreak was

reported. There is a strong evidence from the literature that the *Pseudonitzschia* species found in 1991 and 1992, except
P. australis, have been part of the diatom community of the USA west coast at least since the 1940's (Villac *et al.*, 1993a).

East coast
The domoic acid-producing diatom *P. pungens* f. *multiseries* was isolated from Massachusetts Bay near Boston and produced domoic acid levels ranging from undetectable to 0.21 pg/cell. *P. pseudodelicatissima* was also isolated but did not produce detectable levels of domoic acid. These findings provided at least one probable source for domoic acid accumulation in mussels from Nantucket in January-February 1991 (Villareal *et al.*, 1994).

In fall 1994, in Hood Canal in western Washington, a bloom of *P. pungens*, *P. multiseries* and *P. australis* persisted for more than 6 weeks. Mussels, the sentinel organism in this state to test for algal toxins, contained ~10 µg domoic acid/g (wet weight) and the phytoplankton ~14 µg/g wet weight (Horner *et al.*, 1997).

Gulf of Mexico
Extracts from shellfish and phytoplankton from the Gulf of Mexico indicated the presence of domoic acid in phytoplankton (2.1 pg/cell). The marine diatom *Pseudonitzschia pungens* f. *multiseries* was first observed as the dominant species in a scanning electron microscopy study of plankton from Offats Bayou, Galveston Bay, TX, at 25 february 1989. In the waters around Galveston Bay *P. pungens* f. *pungens* appears to be the most abundant during the warmer months, to be gradually replaced by *P. pungens* f. *multiseries* when fall and winter storms occur. However viable cultures of both forms have been established from water as warm as 29.5 °C (Dickey *et al.*, 1992).
Direct evidence for the accumulation of ASP toxins in Gulf shellfish has not been obtained. *Pseudonitzschia pungens* f. *multiseries* has been observed only in low densities in Galveston Bay. Domoic acid production from the Galveston Bay isolate (cell vs. whole culture) of *Pseudonitzschia pungens* f. *multiseries* is equivalent to that reported from Canadian isolates. All of the culture clones of this form isolated from Galveston Bay have produced domoic acid in the stationary and senescent growth phases. The concentrations of ASP toxins in the Gulf of Mexico phytoplankton were not considered to be a public health hazard (Dickey *et al.*, 1992).

Pseudonitzschia spp. were extremely abundant (up to 10^8 cells/L; present in 67% of 2159 samples) from 1990 to 1994 on the Louisiana and Texas , USA continental shelves and moderately abundant (up to 10^5 cell/L; present in 18% of 192 samples) over oyster beds in Terrebonne Bay estuary in Louisiana in 1993 and 1994. On the shelf there was a strong seasonal cycle with maxima every spring for 5 yr and sometimes in the fall, which were probably related to river flow, water column stability, and nutrient availablity. In contrast, in the estuary no apparent seasonal cycle in abundance was observed. The *Pseudonitzschia* spp. were not routinely identified during this study. However, toxin producing *P. multiseries* has been identified previously from Galveston Bay, Texas (see foregoing paragraph), and cells from a bloom on the shelf in June 1993 were identified by scanning electron microscopy as *P. pseudodelicatissima*, which is sometimes toxic. There have been no known outbreaks of ASP in this area (Dortch *et al.*, 1997).

7.1.3 Mexico

In January 1996 150 dead brown pelicans (*Pelecanus occidentalis*) were found within a period of 5 days at Cabo San Lucas on the tip of the Baja California Peninsula. The death of these birds was the result of feeding on mackerel (*Scomber japonicus*) contaminated by domoic acid-producing *Pseudonitzschia* spp. (Sierra-Beltrán *et al.*, 1997).

Ochoa *et al.* (1997) reported that the Baja Californa Peninsula has witnessed several toxic algal blooms during 1991-1996 among which *Pseudonitzschia* spp. Bahia Magdalena was considered as an ideal site for aquaculture exploitation and huge projects are underway. At Bahia Magdalena the presence of domoic acid in shellfish was suggested during winter 1994 and 1995. The domoic acid levels were well below the guideline value, but continuously monitoring was recommended. In February 1996 also a bloom of *Pseudonitzschia* spp. was observed, but no toxin was detected.

7.2 Europe

7.2.1 Denmark

Investigations in Danish waters have shown that *P. seriata*, a widely distributed species in colder areas of the Northern hemisphere, produced domoic acid in concentrations similar to those found in *Pseudonitzschia pungens* f. *multiseries* in Canada (1-20 pg/cell; see Bates *et al.*, 1989) (Lundholm *et al.*, 1994). During the survey five species and one subspecies of *Pseudonitzschia* were found in Danish waters: *P delicatissima*, *P. fraudulenta*, *P. pseudodelicatissima*, *P. pungens*, *P. pungens* f. *multiseries* and *P. seriata*. Isolates of *P. seriata* appeared to contain domoic acid. In isolates of 3 other *Pseudonitzschia* species detected during this survey, *P. pseudodelicatissima*, *P. delicatissima* and *P. pungens*, no domoic acid was present. *P. seriata* is one of the most common species of *Pseudonitzschia* in the North Atlantic. These findings support the idea that toxic and non-toxic strains occur within the same species of the diatom (Lundholm *et al.*, 1994).

7.2.2 The Netherlands

In the Dutch Wadden Sea *Pseudonitzschia*-like pennate chain-forming diatom species with cell numbers ranging from 10^2 to 10^5 per litre were detected between November 1993 and July 1994. Elecron microscopy of cultured isolates and field samples revealed the majority to be *Pseudonitzschia pungens*. At the beginning of June 1994, *P. fraudulenta* was also present and occasionally *P. delicatissima* was detected. One isolate showed the characteristic morphology of *P. multiseries*. The isolate of *P. multiseries* produced domoic acid; after 55 days of growth about 19 pg/cell was measured. The *Pseudonitzschia* species found in Dutch coastal waters have not yet been implicated in shellfish poisoning (Vrieling *et al.*, 1996).

7.2.3 Portugal

In 1996 domoic acid was detected in very small amounts (<20 μg/g) in almost every bivalve species all around the Portuguese coast for short periods scattered in time and coincident with the occurrence of *Pseudonitzschia* spp., mainly *P. australis* in concentrations below 10^5

cells/L. The first detected occurrence of domoic acid in bivalves over 20 μg/g was in smooth callista (*Callista chione*) in 1995 (CRL, 1998).

7.2.4 Spain

Domoic acid was detected in mussels in April 1995 (Galician Rias) coincident with the occurrence of *Pseudonitzschia australis*, in August and October 1995 (Galician Rias). and in September and November 1996 (Galician Rias) coincident with the occurrence of *Pseudonitzschia* spp. Quick detoxification of the mussels occurred in 1996.
Domoic acid was detected in scallops in September-December 1995 and all 1996 (Galician Rias) coincident with the occurrence of *Pseudonitzschia* spp. Slow detoxification occurred.
It was reported that a few the events were highly virulent, produced toxin levels in mussels and other shellfish near the guideline value, and usually occurred in restricted areas (CRL, 1998).

7.2.5 United Kingdom

In 1997 traces of domoic acid were detected for the first time in Scotland (Shetland). No details were available (CRL, 1998). In July 1999 a scallop fishing area of 8000 square miles on the west coast of Scotland was closed following the discovery of ASP toxins (as cited by Wyatt, 1999).

7.3 Asia

7.3.1 Japan

Since 1991 ASP screening of cultured bivalves and of diatoms has been carried out in Japan, domoic acid has not been detected in industrially important shellfish from 1991 to 1994, nor in diatoms, except for a *Pseudonitzschia pungens* sample (0.01 pg of domoic acid per cell) collected from a red tide which occurred in Hiroshima Bay in August 1994. On the other hand, large amounts of domoic acid were detected in the red alga *Chondria armata* occurring in Kagoshima Prefecture, Southern Japan. In this area the xanthid crab *Atergatis floridus* contained 10 mg domoic acid/kg. Since the crab feeds on seaweeds, it is suggested that the domoic acid may have originated from the food web (Noguchi and Arakawa, 1996).

7.4 Oceania

7.4.1 Australia, Tasmania and New Zealand

An Australian-wide taxonomic survey for species of the potentially toxic diatom genus *Pseudonitzschia* was carried out. The dominant bloom-forming *Pseudonitzschia* species in Australian coastal waters were *P. fraudulenta* (New South Wales), *P.pungens* f.*pungens* and *P. pseudodelicatissima* (Tasmanian and Victorian waters). *P. pungens* f. *multiseries* was detected on only one occasion and only as a minor component (5% of total biomass) of a dense *P. pungens* f. *pungens* bloom in a New South Wales estuary. *P. australis* was never detected in Australian waters. Cultured diatom populations of *P .pseudodelicatissima* from Tasmanian and Victorian coastal waters were consistently non-toxic. Cultures of *P. pungens*

f. *pungens* from Australia (Hallegraeff, 1994) and Tasmania (as cited in Hallegraeff, 1994) were also non-toxic. *P. fraudulenta* has proved non-toxic in North America (as cited in Hallegraeff, 1994). Traces of domoic acid have been detected in some scallop viscera by both HPLC and mass spectrometry, but the concentrations in edible shellfish products were all well below 20 μg/g of shellfish meat (Hallegraeff, 1994). In New Zealand domoic acid was not identified in 150 greenshell mussel (*Perna canaliculus*) samples and in plankton samples taken during *Pseudonitzschia* bloom periods (MacKenzie *et al.*, 1993). During the summer of 1992/1993 domoic acid has been detected in the marine biotoxin program of New Zealand at low levels in phytoplankton samples from Otago to Northland. *P. pungens* has been found in low numbers (up to 3000 cells per liter) in phytoplankton samples from the Bay of Islands, the Hauraki Gulf and Bay of Plenty (Smith *et al.*, 1993). Both *P. pungens* and *P. pseudoseriata* have been detected in New Zealand waters, but ASP has never been clearly associated with shellfish from the Pacific Ocean. Chemical analysis of shellfish samples has identified low levels of domoic acid. The highest level (16.5 μg/g) came from Manukau Harbour. Other detectable levels were well below 20 μg/g (Bates *et al.*, 1993)

8. Regulations and monitoring

8.1 North-America

8.1.1 Canada

In Canada a regulation came into force in 1988 including a guideline value of 20 μg domoic acid/g of mussel. Fishery product harvesting areas are closed when toxin levels in shellfish exceed the guideline value. The analytical method to be used involves HPLC. Monitoring for *Pseudonitzschia pungens* takes place. (Shumway *et al.*, 1995).

8.1.2 USA

In the USA a not-official guideline value of 20 μg domoic acid/g for bivalves exists. The analytical method to be used involves HPLC. For cooked crab (viscera+hepatopancreas) a guideline of 30 μg domoic acid/g is valid. The analytical method to be used involves HPLC (Shumway *et al.*, 1995). The Department of Marine Resources conducted a limited sampling program for domoic acid. Information from adjacent Canada is available on a up-to-date basis. Closures will be made whenever domoic acid levels reach 20 μg/g. In order to sell shellfish to EU countries shellfish must be accompanied by a Health Certificate (Shumway *et al.*, 1995).

8.2 Europe

In Member States of the European Union a guideline value of 20 μg/g is valid for domoic acid in bivalved molluscs (in the whole animal or in each individual edible part of the animal). The analytical method to be used involves HPLC. Unfortunately, the regulation is unclear as to indicate which method should be used or what its performance characteristics should be (Council of the European Union, 1997).

8.2.1 Denmark

Monitoring of shellfish by regulations since 1993 (Ravn, 1995). Monitoring for *Pseudonitzschia pungens* takes place. At approximately 5×10^5 cells/L fishery product harvesting areas are closed (Shumway *et al.*, 1995).

8.3 Oceania

8.3.1 Australia

Monitoring by regulations since 1993 for mussels and algae (Ravn, 1995).

8.3.2 New Zealand

Monitoring by regulations since 1993 for shellfish. The analytical method to be used involves HPLC (Ravn, 1995).

9. Risk assessment

The guideline value for domoic acid in mussels of 20 µg/kg is equal to an intake of 0.1 mg domoic acid/kg b.w. per human being assuming that body weigt is 60 kg and that consumption of mussels is 300 g. Mild gastrointestinal effects in man are seen at 1 mg domoic acid/kg b.w. The guideline value of 20 µg/kg of mussels, that is usually applied in regulations, was based originally on epidemiological data from the Prince Edward Island incident and was taken on by several other countries. Afterwards this guideline value was supported by acute studies in animals. However, when doses required to cause overt toxicity in animal species were compared, mice and rats appeared to be relatively insensitive compared with monkeys and oral dosing required more toxin (more than 10 times in rodents) to achieve the same effects as i.p. dosing. Rats showed overt effects of domoic acid poisoning at single oral doses of about 80 mg/kg b.w., whereas monkeys showed vomiting, gagging and yawning already at 1 mg/kg b.w. A single oral dose of 0.75 mg domoic acid/kg b.w. in monkeys did not induce overt effects. This apparent decreased sensitivity in rodents may be the result of their inability to vomit and/or the finding that the plasma half-life of domoic acid in the rat is about 6 times less than that of the monkey. Comparing the guideline value of 20 µg domoic acid/kg of mussel tissue (~ 0.1 mg/kg b.w. for man) with the no-effect dose (0.75 mg/kg b.w.) in acute oral studies in monkeys, a factor smaller than 10 is between these figures. There is no knowledge of the effects of long-term exposure to low levels of domoic acid. However, short-term animal studies with repeated exposure do not point to altered domoic acid clearance from serum or greater neurotoxic responses than after single exposures.

10. Discussion and recommendations

Compared to the paralytic and diarrhoeic shellfish poisons, problems with amnesic shellfish poisons seem to be of a lesser magnitude. Only one confirmed outbreak of ASP causing severe illness in exposed people, was reported world-wide, namely in Canada, Prince Edward Island, in 1987. The outbreak of ASP was ascribed to the consumption of contaminated blue mussels. Since that period toxic *Pseudonitzschia* blooms have been documented from other areas in Canada, USA, Mexico, Europe, Japan and Oceania. After the first outbreak in Canada only in one outbreak human illnesses (mild and short lived) were observed namely after consumption of contaminated razor clams (from the West coast of USA). However health authorities were not able to confirm that the illnesses were caused by domoic acid. In two outbreaks the death of cormorants and/or brown pelicans due to the consumption of contaminated anchovies or mackerel was reported indicating that herbivorous fish can act as vectors for domoic acid. It is suggested that some species as the scallop *Chlamys hastata* may be the source of contamination of Dungeness crabs with domoic acid. These scallops, when exposed to domoic acid by feeding on toxic *Pseudonitzschia* species, lose motor or escape responses and would fall easy to prey to bottom dwelling scavengers such as crabs. Other molluscan species, including oysters, mussels and little neck clams, do not lose motor response when intoxicated with domoic acid. Concerning these observations the role of domoic acid into the marine food web and its possible effects on marine mammals may require further investigation.

Methods of analysis are rather straightforward, and less complex than those that exist for paralytic and diarrhoeic shellfish poisons. One chemical method for domoic acid in mussels (HPLC with UV detection; Lawrence *et al.*, 1989a) has been successfully validated in a formal collaborative study, whereas another (improved) method (Quilliam, 1995) is in the planning stage of such a collaborative study. Certified reference materials and calibrants are readily available.

ASP may spread to new areas and (shell)fish species, and cause economic losses and health hazards. The growing harvest of non-traditional shellfish, such as moon snails, whelks, barnacles, etc., will increase human health problems and management responsibilities. Most monitoring and regulatory programs often are not adequate to meet the expanding threat of new harmful algal blooms. As a result, when new outbreaks occur, the response is often uncoordinated and slow.
Because harmful algal blooms cannot be predicted, there is little information on bloom initiation; investigators usually find out about a bloom when the bloom is already present (or past), or only suspect a bloom occurred because shellfish became toxic. One of the most serious problems is the lack of information on the biology of harmful algae. For example, little is known about the abundance, distribution, population dynamics and physiology of most of the harmful species, both in local waters and elsewhere. Long-term, routine monitoring of phytoplankton and the environment is essential to obtain data necessary to determine even the most elementary ecology of harmful species. Moreover, because bloom dynamics are complex, the factors that determine bloom dynamics of a species in one geographic area may not affect that species in another area, even though the areas are not widely separated (as cited in Taylor and Horner, 1994).

The guideline value for domoic acid in mussels of 20 μg/kg is equal to an intake of 0.1 mg domoic acid/kg b.w. per human being assuming that body weigt is 60 kg and that consumption of mussels is 300 g. Comparing this intake with the no-effect dose (0.75 mg/kg b.w.) in acute oral studies in monkeys, a factor smaller than 10 is between these figures. There is no knowledge of the effects of long-term exposure to low levels of domoic acid. However, short-term animal studies with repeated exposure do not point to altered domoic acid clearance from serum or greater neurotoxic responses than after single exposures.

Based on the discussion above it would be recommendable to:

- further standardize the analytical methodology and to develop performance criteria to which methods of analysis, intended for official purposes, should fulfill.
- set up and maintain monitoring programs for relevant algae and (shell)fish in production areas.
- investigate the role of domoic acid in the marine food web and its possible effects on marine mammals.

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