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# Domoic Acid and Amnesic Shellfish Poisoning - A Review

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**ABSTRACT**

A new type of seafood toxicity, called amnesic shellfish poisoning, was described from 107 human cases after individuals consumed mussels containing domoic acid harvested from Prince Edward Island, Canada, in 1987. Most of these cases experienced gastroenteritis, and many older persons or others with underlying chronic illnesses developed neurologic symptoms including memory loss. Standard treatment procedures for the neurologic condition were not effective and three patients died. Domoic acid is a known neurototoxin, and it is believed that in these cases enough toxin was absorbed through the gastrointestinal system to cause lesions in the central nervous system. The most severely affected cases still have significant memory loss 5 years after the incident. The source of the domoic acid was identified as the pennate diatom, *Nitzschia pungens* f. *multiseries*, which was ingested by the mussels during normal filter feeding. A possible biosynthetic pathway for the toxin has recently been determined. Certain marine macroalgae also contain this toxin but have no association with human illness. Domoic acid, produced by *N. pseudodelicatissima*, has been found in shellfish in other eastern Canadian locations. In addition, domoic acid was identified in anchovies and pelicans in Monterey Bay, California, the source of which was *Pseudonitzschia australis*. In November, 1991, domoic acid was found in razor clams and crabs harvested in Washington and Oregon states and may have caused human illness from ingestion of the clams. Control mechanisms have been put in place in Canada to prevent harvesting of the shellfish at ≥20 μg/g, and no further human illness has been reported since the 1987 episode.

Seafood toxins are becoming increasingly important as etiologic agents of foodborne disease around the world. This is partly because of more awareness of the potential problems, e.g., paralytic shellfish poisoning (PSP) in tropical as well as temperate countries (*127*), development of standardized methods for some of these toxins, e.g., mouse bioassay for PSP toxins (*50*) and histamine analysis for fish suspected of causing scombroid poisoning (*114*), and the increase in importance of international trade of marine products. It has also been postulated, however, that changes to the environment have increased the possibility of more toxic phytoplankton blooms caused by more phytoplankton species. These may be natural, such as unusual warm currents (*118*), or man-made, such as eutrophication of coastal waters (*101*) and the accidental spread of phytoplankton, e.g., through ballast water, to new locations (*44*). In 1987, several unusual events occurred worldwide that might have been coincidental or had some as yet undetected environmental link: mass fish mortality in Pakistan because of PSP (*94*); the first red tide containing neurotoxic shellfish poison produced by *Ptychodiscus brevis* to kill shellfish and poison humans in North Carolina (*118*) and possibly to cause over 700 mortalities in bluenose dolphins off the eastern shore of the United States (*35,66*); the deaths of 14 humpback whales off Cape Cod probably caused by mackerel containing PSP which they had consumed (*34,36,66*); and the first recorded episode of amnesic shellfish poisoning (ASP), the subject of this review. In the 4 years following the Canadian incident, it was assumed that ASP was a local Canadian problem that would probably not reappear. However, in the fall of 1991, there were reports of anchovies containing domoic acid and pelican deaths in California associated with *Nitzschia pseudoseriata* (now called *Pseudonitzschia australis*) producing domoic acid (*17,32,130*). Low amounts of domoic acid in shellfish have been reported from southern California to Alaska during 1991 and 1992 (Susan Loscutoff, Food and Drug Branch, California Dept. of Health Services, personal communication). Human illnesses have also been attributed to consumption of razor clams containing domoic acid in Washington State (John Kobayashi, State Epidemiologist, Washington State Department of Health, personal communication). This new information indicates that this compound may be much more widespread than previously thought, at least in North American waters, and testing for it may become as frequent as for PSP toxins.

## CHRONOLOGY OF THE 1987 OUTBREAK

The following is an outline of the initial investigation of the outbreak (*120*). On November 22, 1987, two persons in Moncton, New Brunswick, were hospitalized after suf-

fering from gastroenteritis and confusion. Although there was no association between the cases, the symptoms of gastroenteritis and neurologic disturbance seemed similar enough to question them about their food consumption; it was found that they had both eaten mussels (*Mytilus edulis*) bought from two different retail stores. On November 24, reports of two cases were received by health authorities in the Montreal, Quebec area. These were two relatively elderly men who had vomited and become confused with memory loss 4 to 5 h after consuming mussels. This information was transmitted to federal officials and samples of mussels associated with the cases were obtained. On November 27, the mussels were extracted by the Association of Official Analytical Chemists (AOAC) hot acid PSP toxin procedure and injected intraperitoneally (IP) into mice (*50*) in both Department of Fisheries and Oceans (DFO) and Health Protection Branch laboratories. All the mice died within 30 min after being in an excitable state with a hind leg scratching reaction that had not previously been seen by the analysts. Also, that day, there were reports of two possible cases in Charlottetown, Prince Edward Island (PEI). These were two elderly persons who had purchased mussels from a retail store on November 25 and had eaten them steamed and sprinkled with vinegar. The female vomited a few hours later, but the male did not and collapsed with prostration accompanied by head shaking. He remained ill and was hospitalized on November 28. Samples of mussels from their home and from retail stores were collected along with the samples from various leases on eastern PEI estuaries, including the Cardigan and Brudenell rivers. The case-associated mussels caused mouse deaths within 30 min, preceded by typical scratching. The samples from mussel leases gave varying results that ranged from no symptoms to scratching with or without deaths.

Once it was confirmed that the mussels linked to illness came from eastern PEI, shipping was suspended from the Island on November 29. When several more cases were reported from Montreal on December 1, a decision was taken on the same date to advise the public across Canada not to consume mussels from PEI distributed after November 1, 1987, and mussels were taken off the shelves in retail stores and removed from restaurants. All provincial deputy ministers of health were notified by telex on December 1.

On December 3, an Analytical Working Group was organized to coordinate the various research efforts being started in government and university departments. This Group discussed progress every few days by conference calls from December 3, 1987, until January 12, 1988, when the domoic acid was confirmed in shellfish and plankton.

It was decided to extend the testing of shellfish to other parts of the east coast of Canada, and because some extracts from Magdalen Islands mussels and New Brunswick oysters caused mouse deaths (see section Other Issues Associated with Extended Sampling), the sale of all live clams, oysters, mussels, and quahogs from Atlantic Canada was prevented. Once the nature and distribution of the toxin were known, commercial harvesting was permitted in most areas by February, 1988. Mussels from eastern PEI and oysters from New Brunswick were released for sale in April.

## DESCRIPTION OF CASES

Federal, provincial, and local departments of health, in conjunction with hospitals, attempted to identify ill persons who had consumed mussels. A case was defined by Perl et al. (*86,87*) as any person who consumed mussels from PEI on or after November 1, 1987, and developed one or more of the following gastro intestinal symptoms within 24 h: vomiting, diarrhea, or abdominal cramps; or at least one of the following neurologic symptoms or signs within 48 h: confusion, memory loss, disorientation, or other serious neurologic signs such as seizures or coma.

Over 250 reports of illness were documented by various health authorities (*86,87*). Other illnesses occurred but were rejected as cases because their symptoms were either too mild to seek medical help, or they were not properly recorded. Some of these were persons with memory loss who could not adequately describe their symptoms, and without witnesses to their conditions, had to be excluded as cases. If these were elderly people, it was sometimes assumed that they had had a cardiovascular event, e.g., a stroke, unrelated to mussel consumption. From the 250 reports as many as 145 illnesses were originally considered as cases (*87*), but only 107 fitted the strict case definition given above (*86*). For the following discussion cases refer to this definition. For the 107 illnesses that occurred between November 4 and December 5, 1987, the first symptoms were experienced 15 min to 38 h (median 5.5 h) after consumption of mussels. The most common symptoms were nausea (77%), vomiting (76%), abdominal cramps (51%), headache (43%), diarrhea (42%), and memory loss (25%). There was a close association between memory loss and age; those under 40 were more likely to have diarrhea and those over 50 to have memory loss. Other symptoms were not related to age. Memory loss was predominately short-term (i.e., affecting events subsequent to the ingestion of mussels), with some patients disoriented to their surroundings and families, confused, and unable to carry out normal daily activities. The most severely ill were hospitalized (*86*), of which 12 were treated in intensive care units (ICU). Eight of these were ≥65 years old and the other four had pre-existing illnesses - diabetes, chronic renal failure, or hypertension. The ICU patients demonstrated confusion, coma, mutism, seizures, chewing motions, grimacing, hiccups, lack of response to painful stimuli, uncontrolled crying or aggressiveness, profuse respiratory secretion (some cases requiring intubation), and unstable blood pressure or cardiac arrhythmias (*86*).

Neurologic deficits in 14 cases were studied for over a year (*115-117,134*). Eye problems were noted in several of these, including disconjugate gaze, diplopia and ophthalmoplegea, but these resolved within 10 d (up to 10 weeks in one case of diplopia). Electroencephalographic (EEG) studies of seven patients showed moderate to severe generalized disturbance of activity, but these were either normal or improved on examination 4 months later. Acute

denervative changes were apparent on serial electromyographic studies conducted at 4 to 12 months after ingestion. The majority of patients continued to show evidence of selective memory loss, particularly short-term memory, such as difficulty with delayed recall of visuospatial objects. General intellectual ability and language function, however, were normal. The condition observed was similar to one observed in a patient who, some years earlier, had undergone bilateral excision of the amygdala and hippocampus (*75*).

## DEATHS

Three cases (aged 71-84 years) died 11 to 24 d after ingestion of the mussels (*86,116*). The cause of death was listed as septic shock in two and pneumonia in one. A fourth case died 3 months after the incident because of a myocardial infarction, unrelated to the intoxication. However, the patient still exhibited short-term memory loss at the time of his death. All the cases who died were critically ill with severe neurologic deficits and were under intensive care. The death of another patient, not fitting the case definition because of a lack of precise information about the onset of symptoms, was also linked to consumption of mussels. In the brains of these patients, there was severe damage to the hippocampus and amygdaloid nucleus, and there were lesions in the anterior claustrum, nucleus accumbens, and thalamus (*18*). The lesions in the hippocampus could explain the memory loss in severely affected cases.

## MOUSE BIOASSAY

The standard PSP mouse bioassay (*50*) was used to test hot acid extracts of mussels associated with cases and shellfish samples from PEI estuaries and other parts of Atlantic Canada. Because the toxic extracts gave a reaction different from PSP, the procedure was modified to inject IP a minimum of three mice, instead of two, per extract, and to extend the time of observation from 15 min to 4 h, after which the mice were periodically examined and kept overnight for a total of 18-24 h (*3,121*). A positive result was indicated by a reaction not typical of PSP and by the distinctive scratching syndrome within 4 h of injection; death usually occurred within 3.5 h. Typically, the mice showed an involuntary scratching of both shoulders with their hind legs 7-21 min after intraperitoneal injection of extracts of mussels associated with cases. Movements became increasingly uncoordinated and seizures developed until the mice fell on their sides, rolled over several times, and died. Tasker et al. (*113*) subsequently developed a behavioral rating scale from 0 (normal) to 7 (death), which they claimed to be consistently reproducible in mice injected IP. All the extracts from the mussels associated with human cases caused mouse deaths, as did many of the extracts prepared from mussels grown in the implicated estuaries. Other environmental samples were also tested by mouse bioassay, eg., wild shellfish, plankton (both sometimes positive), river mud (negative). Table 1 shows results of some tests conducted on extracts of mussels taken from the Cardigan and Brudenell estuaries, and from water extracts of plankton found offshore or in the Cardigan estuary where most of the toxic mussels originated.

## ISOLATION AND IDENTIFICATION OF THE TOXIN

It was recognized early in the outbreak that the toxin was not one normally associated with shellfish, because of a) the unusual symptoms of the cases; b) the distinctive mouse bioassay result; and c) the waters around PEI had

TABLE 1. *Mouse bioassay results and domoic acid levels from some environmental samples*[a].

| Date sampled | River estuary | No. samples tested | No. mice injected | No. mice with scratching | Time to scratching (min) | | No. mice died | Time to death (min) | | Domoic acid (μg/g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Range | Mean | | Range | Mean | Range | Mean |
| *Mussels* | | | | | | | | | | | |
| Nov. 28, '87 | Brudenell | 7 | 21 | 20 | 17-35 | 27 | 9 | 50-120 | 93 | 90-170 | 140 |
| Nov. 30, '87 | Cardigan | 5 | 15 | 14 | 17-31 | 23 | 9 | 34-140 | 64 | 140-340 | 210 |
| Dec. 4, '87 | Cardigan | 8 | 24 | 24 | 16-69 | 33 | 7 | 38-150 | 73 | 140-330 | 230 |
| Dec. 8,'87 | Cardigan | 1 | 3 | 3 | 24-34 | 29 | 3 | 48-50 | 49 | 180 | 180 |
| Dec. 9, '87 | Cardigan | 1 | 3 | 3 | 23-36 | 27 | 2 | 56-67 | 62 | ND[b] | ND |
| Dec. 10,'87 | Cardigan | 3 | 9 | 7 | 26-89 | 45 | 6 | 79-90 | 85 | 100-160 | 150 |
| Dec. 11,'87 | Cardigan | 1 | 3 | 3 | 46-56 | 51 | 0 | NA[c] | NA | ND | ND |
| *Plankton* | | | | | | | | | | | |
| Dec. 20, '87 | Cardigan | 2 | 5 | 5 | 38-86 | 50 | 0 | NA | NA | 250-530 | 390 |
| Dec. 20, '87 | open sea off Cardigan | 1 | 2 | 2 | 11-13 | 12 | 2 | 21-24 | 23 | 1470 | 1470 |
| Dec. 26, '87 | Murray | 2 | ND | | | | | | | 20-50 | 40 |
| Jan 1, '88 | Cardigan | 1 | ND | | | | | | | 690 | 690 |

[a]Health Protection Branch and Department of Fisheries and Oceans data, unpublished.
[b]ND = Not done.
[c]NA = Not applicable.

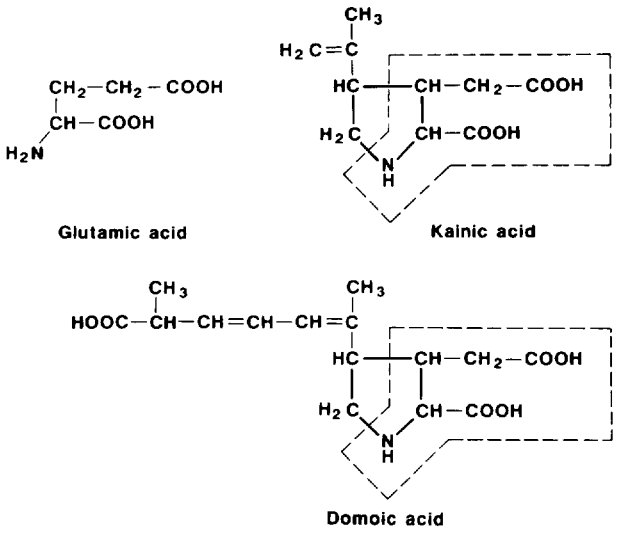


Figure 1. *The structure of domoic acid and its analogs kainic acid and glutamic acid.*

had no problems with PSP, microbial hazards, or chemical pollutants. In addition, contacts around the world with knowledge of seafood toxins were not familiar with this kind of problem. The Analytical Working Group requested analyses from government and university laboratories. No significant levels of heavy metals, pesticides, PCBs, PAHs, or any pathogenic bacteria were present (*132*). Mussel digestive glands and plankton were examined for dinoflagellates; *Protoperidinium*, *Ceratium*, *Prorocentrum*, and *Dinophysis* were identified by electron microscopy but only in very low numbers amongst the dominant phytoplankton, the pennate diatom *Nitzschia pungens* f. *multiseries*. Therefore, no identifiable agent could readily be determined. The toxin itself, whatever its origin, was shown to be water and methanol soluble, and on December 13, 1987, an intense effort was made by the Atlantic Research Laboratory (now the Institute for Marine Biosciences) of the National Research Council of Canada (NRCC) in Halifax to isolate the unknown toxin from toxic mussels, with nontoxic mussels being used as controls (*8,132*). The mouse bioassay was used to monitor progress of the isolation procedures and toxicity was related back to a known weight of mussel tissue. On December 19, the toxin was determined to be domoic acid, an amino acid with a molecular weight of 311 (*132*). Domoic acid is an analog of glutamic acid (Fig. 1), a neurotransmitter in the brain. Some minor associated peaks present in high-pressure liquid chromatography (HPLC) chromatograms of mussels and phytoplankton were found to be isomers of domoic acid and these exhibited less toxicity (*131*). An HPLC method for detecting domoic acid was quickly developed using aqueous methanol or water extraction and detection by a diode array UV (242 nm) system (*92*). Another method used the PSP acid extracts so that both PSP and domoic acid analysis could be conducted on the same sample (*57,60*).

## TESTING OF SHELLFISH AND CLINICAL SPECIMENS FOR DOMOIC ACID

Although the mouse bioassay remained the definitive method for monitoring the shellfish collected from various parts of Atlantic Canada during the crisis, only levels of domoic acid >40 µg/g wet weight of mussel meat caused mouse symptoms (*55*). The Lawrence et al. HPLC method (*60*) using PSP acid extracts detects as low as 0.5-1 µg/g, and this was used in parallel with the mouse bioassay, once it became available in January, 1988. An AOAC collaborative study of the HPLC method showed that 10 different laboratories could obtain good correlation with each other over a variety of different domoic acid concentrations between 0 and 250 µg/g in mussels (75% recovery, *61*). In late 1988 and subsequent years, the HPLC method became the method of choice for analyzing shellfish samples for domoic acid by DFO. Samples of shellfish already tested by mouse bioassay were confirmed by HPLC analysis (Table 1). A water-methanol extraction procedure was found by Lawrence and Ménard (*62*) to be simpler, and yielded higher recoveries of domoic acid, than the hot acid extraction method. However, unlike the older method (*60*), the same extracts could not be used for PSP toxin testing. Pocklington et al. (*89*) developed an HPLC method based on the fluorenylmethoxy-carbonyl derivative of domoic acid for detection of domoic acid in seawater and plankton; it is currently being adapted for shellfish tissue extracts (*93*). Since the domoic acid is unstable in acid, levels diminish over a period of time, and a new AOAC collaborative study is planned based on a methanol extraction-anion exchange clean-up - HPLC procedure for domoic acid (*93*) in mussels, razor clams, and cooked crab viscera (John Wekell, National Marine Fisheries Service, Seattle, WA, personal communication).

Although no feces or urine specimens from patients were available, one cerebral spinal fluid sample and 17 sera, obtained 1-2 weeks after admission, were frozen until the HPLC method was developed. Domoic acid was not found in any of these, but this may have been because of the delay in sampling, and sufficiently sensitive methods were not immediately available (*59,60*). Later immunochemical procedures for detection of domoic acid in serum and urine were more sensitive (*76*).

## DETERMINATION OF AMOUNT OF DOMOIC ACID INGESTED DURING THE OUTBREAK

Only one case was documented for every 500 well persons who ate toxic mussels, although we do not know the precise amounts of toxin ingested by most consumers. For nine elderly cases and one person who ate toxic mussels but did not become ill, however, the amount of mussels consumed was determined and the leftover material was analyzed for domoic acid (*86*). From these limited data the amount of domoic acid ingested was calculated, and there was a clear relationship between dose and symptomatology (Table 2). Amounts of domoic acid ingested ranged from 15-20 mg for an unaffected person to 295 mg for a case with serious neurologic deficits. On the assumption that the average body weights are 50 and 70 kg for elderly females and males, respectively, this translates into 0.2-0.3 mg/kg for the unaffected male. Some persons had mild symptoms (mainly gastrointestinal) after consuming 60-110 mg, equivalent to a dose of 0.9-2.0 mg/kg body

TABLE 2. *Symptoms and amount of domoic acid consumed by cases where information is available.*[a]

| Case | Sex | Age | Amount of mussel meat consumed (g)[b] | Domoic acid concentration in mussels (μg/g) | Domoic acid consumed (mg) | Domoic acid consumed (mg/kg body weight) | Period from consumption to vomiting (h) | Symptoms observed[c] | Memory loss | Hospitalization | ICU[d] | Permanent neurological symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| not ill | M | 60 | 30-40 | 520 | 15-20 | 0.2-0.3 | - | none | - | - | - | - |
| 1 | F | 70 | 120 | 520 | 60 | 1.2 | 3 | NV | - | - | - | - |
| 2 | M | 72 | 120-180 | 520 | 60-90 | 0.9-1.3, average 1.1 | 2 | NV, chills, confusion | - | - | - | - |
| 3 | F | 62 | 120-200 | 450 | 55-90 | 1.1-1.8, average 1.5 | 6 | VCH, dizziness, confusion | + | - | - | - |
| 4 | M | 61 | 360 | 310 | 110 | 1.6 | -[e] | ND, dizziness | - | - | - | - |
| 5 | F | 61 | 300 | 310 | 90 | 1.8 | 9 | NV, dizziness | - | - | - | - |
| 6 | F | 67 | 150 | 680 | 100 | 2.0 | 5 | VPW, loss of appetite | - | - | - | - |
| 7 | M | 74 | 200-400 | 680 | 135-270 | 1.9-3.9, average 2.9 | >24[f] | NVD, confusion, disorientation, head shaking, hallucinations | + | - | - | - |
| 8 | M | 84 | 375[g] | 760 | 285 | 4.1 | 4.5 | NV, disorientation | + | + | + | + |
| 9 | M | 68 | 230 | 1280 | 295 | 4.2 | 6 | NV, disorientation, confusion | + | + | + | + |

[a]Data partially from Perl et al. (*86*).
[b]No. of mussels eaten × 10 g (average contents of a mussel harvested during November, 1987).
[c]N=Nausea, V=vomiting, C=abdominal cramps, D=diarrhea, P=prostration, W=weakness.
[d]ICU=treatment in an intensive care unit.
[e]Patient did not vomit, possibly allowing more domoic acid to be available for absorption than the other cases.
[f]This case did not vomit for over a day, possibly allowing more domoic acid to be available for absorption than in the other cases.
[g]Estimate made of an average restaurant serving (375 g of meat) when number of mussels eaten was not known; cooking would reduce the amount of meat but not the level of domoic acid consumed.

weight. The most serious cases (all males) ingested 135-295 mg for a dose of 1.9-4.2 mg/kg. The largest dose for a female was 2.0 mg/kg. Larger amounts could have been consumed based on the maximum amount of domoic acid found in PEI mussels served in a restaurant (375 g of meat containing 1280 μg/g, equivalent to 6 mg/kg body weight) (*56*), but there is no evidence that this actually occurred. Mice and rats can tolerate oral doses of 30-50 mg/kg without observable adverse effects (*55,56*). These levels are much higher than the 0.5-5 mg/kg for monkeys (*126*) and 0.2-0.3 mg/kg for a person who consumed the mussels and remained well (*86*). Because man and monkeys have proportionally longer gastrointestinal systems than these rodents, absorption could be an important factor in toxicity. Vomiting early after ingestion would have reduced the amount of domoic acid in the gastrointestinal tract and, secondarily, in the blood (Table 2). The only previous data on human ingestion of domoic acid were when 20 mg extracted from the seaweed *Chondria armata* was given to each of three Japanese children (0.4-0.8 mg/kg body weight) without any apparent adverse effects (*22*). However, the precise amount of domoic acid in the seaweed extracts was not known, although intestinal parasites were killed. In the Canadian outbreak, the most seriously ill were aged males with renal damage or some other dysfunction (*86,116*). It is probable, therefore, that apart from dose, the state of health including age, genetic predisposition, and what other foods were consumed with the toxic mussels affect absorption and clearance of the toxin, and also its transmission across the blood-brain barrier to receptor sites (*52,56*). From illnesses reported in Washington State in 1991, the doses appeared to be 0.05 to 0.39 (mean 0.16) mg/kg for cases with mild gastroenteritis and 0.0 to 0.28 (mean 0.08) mg/kg for well persons who also ate razor clams (Robert Quick, Enteric Diseases Branch, Centers for Disease Control, Atlanta, GA, personal communication). These are about one order of magnitude less than those determined for the mild cases of gastroenteritis in Canada. The reason for this difference is not yet known.

## EXPERIMENTAL TOXICITY IN RODENTS AND PRIMATES

Animal dosing studies were conducted as soon as contaminated mussels became available. Mussel extracts, and later domoic acid, were given orally and by injection to mice, rats, and monkeys. Domoic acid in saline and domoic acid added to nontoxic mussel extracts gave identical reactions in mice after IP injection (scratching, rolling, tremors, seizures, death). An $LD_{50}$ (IP) of 3.6 mg domoic acid in

mussel extracts per kg mouse was calculated by Grimmelt et al. (*43*). According to Iverson et al. (*55*), mouse deaths occurred at or above a dose of 100 µg in mussel extracts (5 mg/kg). They also found that the no-effect level was observed from injection of extracts containing 12 µg domoic acid (0.6 mg/kg), equivalent to 24 µg/g mussels because 1.0 ml of extract =0.5 g mussel meat. Tasker et al. (*113*) could reliably detect domoic acid at concentrations as low as 0.8 mg/kg mouse. Oral doses required more than ten times as much toxin to achieve the same effect as IP. Rats given toxic mussel extracts orally developed mastication and seizures when the domoic acid concentration was ≥70 mg/kg body weight and caused death when the level was ≥80 mg/kg. Most of the domoic acid given orally was excreted in the feces of both mice and rats (*55*).

Rats injected IP with 2.0-7.5 mg/kg showed scratching, crab-like walking, "praying", loss of balance, and seizures. The threshold dose for symptoms was 1-2 mg/kg. Lesions were apparent in the higher dosed rats in the amygdala, cortex, hippocampus, hypothalamus, olfactory system, and retina (*123,124*).

Cynomolgus monkeys given extracts of toxic mussels containing 5.63 to 6.62 domoic acid mg/kg body weight demonstrated gastrointestinal reactions, such as anorexia, salivation, retching, vomiting, and diarrhea, although the extent of the symptoms, time of onset, and duration varied from monkey to monkey (*125,126*). Dimethylsulfoxide and monosodium glutamate, which could have enhanced the toxicity through better absorption in the gut or through agonistic effects, made no apparent change in the toxicity (*125,126*). Crude domoic acid at 5.21 mg/kg had an identical effect on monkeys as the toxic mussel extract (5.89-6.62 mg/kg). Large amounts of domoic acid were detected in the feces and lesser amounts in the urine of all orally dosed animals. Neurologic signs included disorientation, trembling, glassy-eye stares, and withdrawal, with considerable variation in the appearance of these for each animal. Pure domoic acid at 0.5-10 mg/kg in four monkeys was used to establish a dose response; vomiting, mastication, and tremors were seen at 5 (onset 12 h) and 10 mg/kg (onset 1 h) (*56,125*). Six other monkeys were injected intravenously (IV) with amounts of domoic acid ranging from 0.00625 to 0.5 mg/kg (*56*). Gagging in all but the lowest dose was apparent with the duration directly proportional to the dose level. One animal injected IP with 4.0 mg/kg showed vomiting, severe neurologic disturbances including tremors and lack of coordination, and death 3.5 h after dosing. Lesions were found in the brain (hippocampus, hypothalamus, medulla oblongata) and also in the retina (*123,125*). The orally dosed animals had minimal or equivocal histopathological changes to brain tissue. Domoic acid studies with retinas from chick embryos in vitro also showed that these were damaged by the toxin (*107*), confirming the work of Coyle et al. (*20*) who found that other excitatory amino acids cause degeneration of the inner layers of the retina.

These results showed that domoic acid has gastrointestinal and neurologic effects identical to those produced by the toxic mussel extracts. Orally dosed animals needed much more toxin to have the same effect as IV or IP dosed ones, and the symptoms were more variable, indicating that domoic acid is poorly absorbed from the gastrointestinal tract and most is excreted unchanged in the feces. Kainic acid, another glutamic acid analog (Fig. 1), gives a reaction similar to that of domoic acid in animals, but has a 3- to 4-fold lower binding capacity to the kainic acid receptors (*14,19,23,24,133*). Tasker et al. (*113*) and Strain and Tasker (*108*) claim that systemically administered domoic acid is 8-11 times more potent than kainic acid; in contrast, with direct injections into the hippocampus, domoic acid is three times as active as kainic acid.

## MECHANISM OF TOXICITY IN THE BRAIN

Systemically administered high doses of L-glutamate and other glutamate analogs destroy cells in the retina and hypothalamus in immature animals, and glutamate is known to gain access to the brain through the periventricular organs (*81*). Because these organs possess an incomplete blood-brain barrier, this is the area in the brain that domoic acid and other excitotoxic amino acids most easily penetrate.

Glutamate and analogs like kainic acid have an excitatory effect to stimulate the neurons through release of endogenous glutamate (*83*). However, excessive amounts of these kinds of amino acids can cause neurotoxicity. The glutamate analogs mimic glutamate but with differing potencies, e.g., domoic acid is 2-3 times more potent than kainic acid and up to 100 times more potent than glutamic acid (*14,15,19,23,81*). Glutamate affects receptors in the dorsal horn of the spinal cord, and if this action is blocked by domoic acid, it would explain why so many patients lost deep pain sensation (*116*). There may also be a synergistic effect between domoic acid and other neurotoxic amino acids normally present in mussels (*79,113*). Strain and Tasker (*108*) also noted that extracts of mussels contaminated with the domoic acid from the outbreak were more toxic to mice than pure domoic acid, domoic acid added to mussels harvested from the Cardigan area, and extracts of the toxic *Nitzschia*, all with the same concentration. Two explanations postulated by the authors were that (a) additional isomers were present in the mussels, as indicated by Wright et al. (*131*), but not present in the spiked samples or the plankton; and (b) the mussels contained amines such as glutamate or aspartate, that have a synergistic effect with the domoic acid. Further work will have to be done to show which, if either, is correct.

From studies on rats subcutaneously injected with domoic acid, Stewart et al. (*107*) contend that most of the brain damage is caused by seizures resulting from excessive release of glutamate or a similar endogenous compound which exerts an excitotoxic action at receptor sites. The ingestion of glutamate itself, as in monosodium glutamate, may be hazardous to humans (*81*), but this is disputed by other researchers (*30,97*). The onset of neuronal necrosis in infant mice has been reported at an oral dose of glutamate of 500-700 mg/kg (*21,80,82,96*), but not at an oral dose of 4 g/kg either in dogs (*49*) or in infant monkeys (*48,106*). The conclusion of a recent workshop is that the infant monkey (the closest test animal to children) is rela-

tively insensitive to glutamate-induced hypothalamic neuronal necrosis (*30*).

## LINKS BETWEEN ANIMAL STUDIES AND HUMAN INTOXICATION

Some of the symptoms and brain lesions observed in animals were seen in the more severe human cases of intoxication caused by the mussels containing domoic acid. Domoic acid is an emetic, causing gagging and vomiting, likely through its effect on the vomit center in the area postrema of the brain, as seen both in human cases and animal studies (*56,86*). Gastrointestinal bleeding was noted in three cases and may have come from ulcers through the action of the domoic acid (*88*). Shellfish extracts containing domoic acid caused gastric and duodenal ulcers in rats and mice (*41,42*); infant mice were more sensitive than adult animals (*40*). Kainic acid also causes gastrointestinal ulcers and necrosis of urinary bladder mucosa in rats (*81*). The convulsions noted in several patients (*86,116*) correlated with histopathologic lesions seen in the hippocampus and other limbic brain areas. Memory is associated with the CA1 and CA3 regions of the hippocampus and mediodorsal nucleus of the thalamus, and all three areas were damaged in autopsied brain tissue; these same areas were also damaged in rats given kainic acid systemically (*18*). In experiments by Sutherland et al. (*111*), rats injected with domoic acid (25 ng/0.5 μl saline) into the hippocampus directly also showed degeneration of the CA1 and CA3 pyramidal cells and dentate gyrus granule cells. These same animals had demonstrated long-lasting anterograde amnesia through their inability to remember tasks, thus confirming that domoic acid itself causes a memory loss. Strain and Tasker (*108*) confirmed that the CA3 region of the hippocampus is most affected by domoic acid.

To indicate neuron activity in various parts of the brain, glucose metabolism was measured by Gjedde and Evans (*39*) using positron emission tomography (PET) and magnetic resonance imaging (MRI). Four patients who had experienced varying degrees of mussel poisoning, including memory loss, were examined 2-4 months after onset of symptoms along with four nonaffected persons of similar ages. The results of the analysis showed that there was a severe decline in the cases of glucose metabolism in the amygdala and hippocampus. The inner layer of the retinas of animals were damaged, probably obscuring vision (*107,125*), and some cases had eye problems for several days following the outbreak (*116*), but retinas of the deceased were not examined. Fortunately, no human genotoxic effects are to be expected, based on domoic acid research with hamster lung fibroblasts (*98*).

It is known that the sensitivity of receptors is affected by age of test animals. This could explain why older persons had more severe neurologic symptoms (*16,81*).

## TREATMENT OF CASES

Kainic acid-affected animals can have damage prevented by phenobarbital and diazepam (*81*). However, these and other standard anticonvulsants did not stop seizures in the severely affected cases who had eaten the toxic mussels, although the condition may have worsened if they had not been treated (*116*). Seizures may have caused most of the brain lesions (*107*). Olney (*81*) suggested using thiobarbituates and procyclidine singly or in combination. Generalized treatment could include the use of valium and hypothermia of the patient (*84*). Calcium ion channel blockers might also be considered since calcium ions are thought to allow consumption of ATP and affect receptor-binding activity of kainic acid and domoic acid (*19,68*).

## DEFINITION AND NAME OF THE CLINICAL SYNDROME

Because this type of outbreak has occurred only once, and the number of patients studied for complete symptomology has been limited to 20 or so of those most seriously ill, the precise definition of the syndrome is not yet clear. The following symptoms are a composite of suggestions presented at a Symposium on Domoic Acid (*88*) and other comments (T. M. Perl, Department of Internal Medicine, University of Iowa, Iowa City, personal communication):

a) One or more gastrointestinal symptoms, such as nausea, vomiting, anorexia, abdominal cramps, diarrhea, gastric bleeding with onset within hours to 1 d.
b) There may be persistent, severe headache, dizziness, visual disturbances, weakness, lethargy, mild disorientation, difficulty walking, cranial nerve abnormalities, somnolence in some cases.
c) More severely affected cases will have
   i) acute confusion within 3 d with disorientation, paucity of speech, lack of response to pain;
   ii) domoic acid possibly detectable in extracts of stools in the first few days following consumption;
   iii) autonomic nervous system dysfunction lasting days to a few weeks (arrhythmias, blood pressure swings, hiccups, excessive bronchial secretion requiring intubation, sweating, low grade fever);
   iv) seizures, abnormal ocular movements, grimacing, posturing, myoclonus, loss of reflexes, coma, loss of deep pain sensation;
   v) prominent late sequelae confined to loss of intermediate visual-spatial recall and mononeuropathies, but no dementia;
   vi) no specific laboratory findings in the acute stages, although leucocytosis, nonspecific EEG abnormalities and increased creatine phosphokinase (CPK) have been found; abnormal MRI, PET and electromyogram findings at 2 to 3 months; and
   vii) death in severely affected persons under intensive care.

The name of the syndrome has variously been described as neurovisceral toxic syndrome (*42,88*), amnesic shellfish poisoning (*86,121*), excitotoxic amnesic shellfish

poisoning (*126*), or more generally, acute encephalopathy caused by mussels. The name with widest acceptance currently is amnesic shellfish poisoning (ASP) which parallels the names of other seafood intoxications, e.g., paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP). However, if it can be shown that illnesses can occur from ingestion of domoic acid in fish, the word shellfish will have to be dropped from the name of the syndrome. Since it is almost universally accepted that domoic acid is the toxic agent, the name could simply be domoic acid intoxication.

## THE MUSSEL INDUSTRY

The development of the mussel industry in PEI has been reviewed by Judson (*58*) and Johnson et al. (*57*). Although mussel cultivation has been practiced in Japan and Europe for several decades, it was only in 1975 that the first government experiments were begun in Atlantic Canada. In 1980, the mussel industry had developed, first in PEI, then in other provinces to export the product to various parts of North America. PEI is particularly suitable because of the many estuaries giving shallow (7-12 m in depth), protected water with a relatively good source of nutrients, and sufficient tidal flushing. Most of the Island's production (85%) comes from the east coast, which includes St. Mary's Bay, the Brudenell, Cardigan and Murray Rivers, where the water temperature ranges from -2°C in January to 23°C in July and August (*58*). It is now the Island's biggest fishing industry after harvesting of lobsters.

Mussels are cultured in nylon socks suspended from long lines. Spat (young seed mussels 15-20 mm in length) are collected from ropes and placed inside the 3-m long socks (600 mussels per m). These soon work their way to the outside of the socks and remain firmly attached by their byssus threads. In about 12-24 months, depending on the productivity of the estuaries, they are ready for harvesting. Much of this is done in the fall and winter months, because optimum nutritive taste and value are obtained from mussels grown in cold water (*57*), they are more economic to harvest through the ice, and because there is more demand at the Christmas season. To minimize ice damage, the long lines are sunk before freeze-up, and mussels are retrieved either by divers or by cutting holes in the ice and dragging the socks out. Mussels are then stripped from the socks, debyssed, cleaned, graded, and packed in 12.5-kg polypropylene bags and shipped to markets in all parts of North America, arriving refrigerated 2-6 d later. There is more demand for mussels in Quebec, where there are specific restaurants serving only mussels, than in other parts of Canada. The Cardigan River estuary, which supplied 98% of the mussels causing human illness, was closed to harvesting from December to April, and after the illness became publicized, the demand for cultured mussels in general dropped for several months before picking up again. However, by the end of 1988, sales had more than recovered, with a 140% increase over those in 1987 (*57*). In 1991, cultured mussel production reached 3.4 million kg, valued at $3,370,000 (Statistics Branch, DFO, Charlottetown, PEI), equivalent to $1.00/kg at the wholesale level.

## SOURCE OF THE TOXIN

Mussels ingest food particles of any type 2-90 μm in size suspended in water with the rate of ingestion dependent on temperature and environment. Under ideal conditions they can siphon 2-5 L/h and extract up to 98% of the available algae (*11*). Because mussels are not selective in their feeding, any plankton or algal fragments are ingested. The source of the toxin was believed to be in the food supply because plankton collected by nets was toxic to mice and was later shown to contain domoic acid (*9*). This plankton came from waters in the Cardigan and neighboring estuaries and the nearby open sea, but not from waters in other parts of PEI (*9*). But what components of the plankton could be the toxic source? This material was examined by electron microscopy for known toxic dinoflagellates. Certain of these were potential candidates, e.g., *Prorocentrum* spp. and *Dinophysis* spp., which have been associated with outbreaks of DSP in other countries, but the mussels and plankton from the Cardigan area did not test positive for DSP toxins. Once domoic acid was determined to be the most likely candidate for the toxin (*131*), the literature was searched for the first report of the amino acid. In the 1950s, Japanese researchers examined *Chondria armata*, a seaweed capable of having anthelminthic and insecticidal properties (*22*). A new compound, domoic acid, was isolated from the *Chondria* (*22,112*). Two decades later Italian workers found the same compound in *Alsidium corallinum*, a Mediterranean species (*54*). Both of these were red macroalgae belonging to the family Rhodophyceae. The question was then asked if there were any of these types of algae in eastern PEI. During the winter when the investigation was proceeding, it was impossible to find mature fronds of red algae (they grow only in warmer conditions), but particles could have been present in the plankton. Fishermen, however, did not recall seeing any red algae growing on their gear or the mussels, and no extensive fragments of red algae were observed under the microscope. Herbarium specimens of red algae collected from PEI, however, were extracted and tested for domoic acid. One of these, *Chondria baileyana*, contained domoic acid at a level about 0.2% dry weight (*12,103*). Socks and shells of mussels were examined for the basal discs of perennial algae, but no evidence of *Chondria* spp. was found (*9*). Even if *Chondria* or similar species had been growing there in the summer, it is unlikely that sufficient domoic acid could have been produced from this source. It was calculated that there was about 6 kg of domoic acid in the 63,000 kg of mussels growing in the Cardigan River area (*132*). This amount probably represents only 1% or less of the total domoic acid produced in the estuaries which could be as much as 1000 kg (*9*). This toxin had probably accumulated in the mussels from the late summer through December, and the only practical source of the domoic acid was the main planktonic component, the pennate diatom *Nitzschia*. A positive relationship was found between the relative abundance of *N. pungens* f. *multiseries* in the plankton recovered from estuaries in eastern PEI and the presence of domoic acid (*9*). Whereas there is a direct correlation between the amounts of *Nitzschia* and domoic

acid in the plankton, the level of the toxin per unit *Nitzschia* cell is relatively constant. Laboratory cultures of *N. pungens* f. *multiseries* and *N. seriata* isolated from the Cardigan estuary were grown and examined for production of domoic acid. Both organisms grew to over $10^5$ cells per ml within 10-15 d, and the cell number thereafter remained constant during the stationary phase. Domoic acid began to be produced by *N. pungens* after 10 d and reached its maximum at 42 d when the experiment was terminated. Domoic acid was only synthesized in *N. pungens* f. *multiseries* cultures after the stationary phase had been reached, with 0.3-2.0 pg per cell per day produced (*2,109,110*). However, cells could accumulate up to 6-10 pg per cell with the surplus being released into the medium (*10*). No domoic acid was found in *N. seriata* cells. Since cellular domoic acid levels increase with the age of the culture, and cellular carbon, nitrogen, and phosphorus decrease, it was inferred by Pan et al. (*85*) that domoic acid is produced under stress conditions. This is the first time a diatom has been documented to produce a neurotoxic amino acid. A biosynthetic pathway for all the kainoid group of excitatory amino acids, including domoic, has been proposed by Douglas et al. (*29,95*) using axenic cultures of *N. pungens* f. *multiseries* fed $^{13}C$ and $^{2}H$-labeled precursors. The biosynthesis indicates that two separate units originate from acetate.

## BLOOMS IN EASTERN PEI IN 1988 AND 1989

In 1988 and 1989, the *Nitzschia* was sampled and analyzed for domoic acid. Smith et al. (*102,103*) studied the progress of blooms of *N. pungens* in the fall of 1988, using the HPLC method of Pocklington et al. (*89*) to measure the amount of domoic acid produced. These blooms occurred from November 1 to December 31, with a maximum domoic acid concentration in eastern PEI of about 350 μg/g mussels in the Brudenell River (*37,38*) in early December. Much of the domoic acid was present in the water column either in the picoplankton (<1 μm in size) or released from *Nitzschia* cells. The peak amount of domoic acid in mussels lagged behind that in the *Nitzschia* by about 9 d (*102,103*). A phytoplankton monitoring program, therefore, is able to give advance warning of any increase of domoic acid in mussels. Blooms of *Nitzschia* appear to be dependent on available nitrate which increases in the fall after rain and strong winds. Local conditions in the inner estuaries are important and agricultural runoff rather than upwelling of nutrients is the probable source of the nitrate (*105*). Turbulence is more important for offshore blooms (*105*). Bates et al. (*10*) and de Freitas et al. (*25*) determined that domoic acid depended not only on the cessation of all cell division (e.g., due to silicate limitation) and on nitrate availability but also the presence of light. Cells in the stationary phase remain viable for weeks and continue to release domoic acid into the water, although less may be produced at 5°C than at warmer temperatures (*64*). Usually grazing by herbivores, such as copepods, shorten the lifespan of a bloom, but this did not seem to occur in 1987 or 1988, and it was postulated that domoic acid or some other agent produced by the *Nitzschia* has an antifeeding property (*13*). This is supported by the fact that domoic acid is insecticidal (*68,69*).

Smith et al. (*104*) also examined plankton a year later. In August, 1989, a bloom of *N. pungens* f. *pungens* reached 1 million cells per L in northern PEI but did not contain any domoic acid. Late August showed a smaller bloom in the Brudenell River (up to 150,000 cells per L). In succeeding weeks, as the portion of *N. pungens* f. *multiseries* rose from 30 to 90% (relative to *N. pungens* f. *pungens*), domoic acid was detected in the plankton, thus confirming laboratory studies that forma *multiseries* is the variety that produces the toxin, and that forma *pungens* is the nontoxic form (*102,103*). Later in the fall *Nitzschia* blooms began in the Cardigan River in November as in the two previous years but was much reduced. The domoic acid concentration was only 40 μg/g mussels at its maximum on November 12, when the *N. pungens* level was 239,200 cells per L (*102*). The early ice cover that year in the river probably prevented photosynthesis and was responsible for the diminished bloom. However, there is no explanation for the even lower levels in 1990 (0.6 μg/g on November 16) unless the proportion of f. *multiseries* was lower relative to f. *pungens*. In October 1991, there were small blooms producing domoic acid in bays of northern PEI recorded for the first time (J. C. Smith, Gulf Fisheries Centre, DFO, Moncton, New Brunswick, personal communication). Therefore, the production of domoic acid by the *Nitzschia* has been variable and generally much lower each year since the 1987 maxima, with the local nitrate concentration and the initial concentration of f. *multiseries* being important.

It is not known whether blooms of the toxic species or illnesses associated with domoic acid occurred earlier than 1987, but prior to 1980 the mussel industry was in its infancy with little product being sold. Interestingly enough, however, there was one incident in 1984 in Calgary, Alberta, in which 12 persons consumed PEI mussels and developed vomiting, diarrhea, and blurred vision 1.5 to 3 h later; the symptoms lasted from 1 to 7 d (*119*). No etiologic agent was identified, and there is a possibility this was caused by low levels of domoic acid, for which at that time there were no detection methods available.

## OTHER SOURCES OF DOMOIC ACID

Domoic acid has been found in areas other than eastern PEI. Up to 30 μg/g were recovered from the digestive glands of clams, mussels, and scallops collected from various locations on the Scotian Shelf, George's Bank and Gulf of Maine in the spring and summer of 1988 (*1*). Levels of 0.1-595 μg/g were found in the digestive glands of scallops along the Maine coast (*100*).

Also, in 1988, Gilgan et al. (*37,38*) examined shellfish in the Bay of Fundy for domoic acid content and found up to 8 μg/g in *Volsella* (horse mussels) on the southeast shore (Nova Scotia) in late July, and nearly 50 μg/g in *Mya arenaria* (soft shell clams) in mid-August in the Passamaquoddy Bay area of the west shore (New Brunswick). The New Brunswick area had to be closed because shellfish exceeded the 20 μg/g limit. The origin of the domoic acid was not determined, although Martin and

Wildish (*73*) and Martin et al. (*72*) argued that *N. pseudodelicatissima* was the major source; *N. pungens* was <1% of the total phytoplankton abundance. Some molluscs (horse mussels, scallops) appear to retain the domoic acid longer than the blue mussel (*Mytilus edulis*). Domoic acid was concentrated in the digestive gland (96.5%) and gills (2.4%) with no toxin in the mantle, gonads, or foot of mussels cultured in Passamaquoddy Bay (*46,47*). When domoic acid was found in shellfish it was also present in plankton tows (0.8-3.5 μg/g wet weight) in 1988 and 1990 but not 1989 (*47,71*).

In 1988, Bates et al. (*9*) examined 28 different plankton strains including 19 strains of *Nitzschia* and two of *Amphora coffeaeformis*. Only the three strains of *Nitzschia* f. *multiseries* isolated from eastern PEI produced domoic acid. Maranda et al. (*70*), however, cultured *A. coffeaeformis* isolated from the Cardigan estuary and obtained 0.2 pg domoic acid per cell after 7 d but recorded only 0.02 pg per cell from *N. pungens* f. *multiseries*. These differences could be explained by some symbiotic relationship with microorganisms being present in some strains and not others. MacPhee et al. (*67*), however, found no evidence for bacteria, viruses, or other agents in the toxic *Nitzschia* cells by scanning and transmission electron microscopy and concluded that production of domoic acid is probably genetically controlled. This was confirmed by axenic culture studies of Douglas and colleagues (*27,28*). Zooplankton may be affected by domoic acid, as it was found that a *Pseudocalanus* copepod under experimental conditions was killed by the compound at concentrations of 52-84 μg/ml seawater (*128*).

Apart from records in eastern Canada and Maine, *N. pungens* f. *pungens* and f. *multiseries* have been isolated from the Texas coast and found to produce 1.8-8.0 pg per cell (*33*) in culture. The diatom blooms were in the fall-winter months. The same strain has been located off British Columbia's Brooks Peninsula, but no domoic acid has been detected in plankton samples nor has any culture work been attempted (*31*). It has also been looked for but not seen in New Zealand waters (*65*).

In September 1991, domoic acid was found in anchovies in Monterey Bay, California (*32,130*). These fish had been consuming *Nitzschia pseudoseriata*, now renamed *Pseudonitzschia australis*. The concentration of the *Pseudonitzschia* in the bay in October and November was $10^5$ per L, with 3-31 pg domoic acid per cell (*17*). The anchovies, in turn, were eaten by brown pelicans and cormorants which showed unusual behavior before dying, including a scratching motion of the wing against the side of the head (T. M. Work, California Dept. of Fish and Game, Rancho Cordova, CA, personal communication). Autopsies of the dead pelicans showed very high numbers of *P. australis* in their stomachs. The anchovies contained domoic acid apparently in the flesh (>50 ppm, *17*) as well as the intestines, but this could have arisen from the dissolution of the intestines between retrieval from the water or from the pelicans and their analysis in the laboratory. Only analysis of freshly caught anchovies will determine whether the flesh itself is contaminated with the toxin. Since anchovies may be eaten whole, fresh, or salted, it is important to know the levels present in whole fish. A method has been developed to detect domoic acid in presence of salt (*26*), which is currently not possible with the procedure of Quillam et al. (*93*). Levels up to 180 μg/g have been found in whole anchovies and 2,300 μg/g in their viscera (Susan Loscutoff, Food and Drug Branch, California Dept. of Health Services, personal communication). Although reports of illnesses from Californian anchovies have been made from Europe (*4*), these have not been confirmed on analysis of the fish. This episode is important because it shows that a) a different species of *Nitzschia* produces domoic acid in sufficient quantity to cause intoxications, b) fin fish act as a vector for the toxin, c) poisonings occurred in a location other than eastern Canada, and d) there is a potential hazard for domoic acid in anchovies that could be used as human food or animal feed. Domoic acid has also been recovered from crustacea in California ≤47 μg/g in mussels and ≤17.5 μg/g in stone and rock crab viscera during the winter of 1991/92 (Susan Loscutoff, Food and Drug Branch, California Dept. of Health Services, personal communication). Although most samples have low levels of domoic acid, a health advisory has been released by the California Department of Health Services indicating that consumption of crab viscera should be avoided.

The recreational fishery in both Washington and Oregon states was closed in November, 1991, after levels of domoic acid in razor clams exceeded 20 μg/g. Up to 200 μg/g has been found in the meat of these clams and also up to 110 μg/g in the uncooked viscera of Dungeness crabs (John Wekell, National Marine Fisheries Service, Seattle, WA, personal communication). From clams taken from one beach in Washington over a 7-month period, the level of domoic acid was 140 μg/g in November, 1991, and this gradually decreased to 14 μg/g in June, 1992; the viscera and meat of Dungeness crabs in the same area in December, 1991 contained 24 and 4-6 μg/g, respectively (Ann Drum, Battelle Northwest Laboratories, Sequim, WA, personal communication). Long-term management strategies and research programs for the West coast are now being considered (*129*). There were also reports of 24 persons in Washington ill from eating the clams (*5-7*; John Kobayashi, State Epidemiologist, Washington State Dept. of Health, Seattle, WA, and Robert Quick, Enteric Diseases Branch, Centers for Disease Control, Atlanta, GA, personal communications). These suffered gastroenteritis, and two of them also developed a mild neurologic condition. The doses for these cases are presented in the section Determination of Amount of Domoic Acid Ingested During the Outbreak. The source of the domoic acid is not known but assumed to be a *Nitzschia* or *Pseudonitzschia* species, as these occur in west coast waters.

In the late summer and fall of 1992, domoic acid was found in the hepatopancreas (up to 150 μg per g) and viscera of Dungeness crabs and in razor clams in certain areas of British Columbia, and appropriate action was taken to prevent further harvesting (Rod Forbes, Institute of Ocean Sciences, DFO, Sidney, B.C., and Stephen Stephens, Inspection Services Branch, DFO, Ottawa, personal communications).

## UPTAKE AND DEPURATION OF DOMOIC ACID BY SHELLFISH

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 and (130 to 20 μg/g at 15°C in 4-6 d in the Cardigan River) (*99*). Complete depuration, however, in the natural habitat may take longer. Domoic acid concentrations in mussels in the Cardigan Bay area, eastern PEI, declined to negligible levels in 40-50 d (November 9, 1987 - March 22, 1988) (*43*). Mussels exposed to radiolabeled domoic acid in seawater at 5°C could only take up <1% of the compound in 24 h, mainly in the mantle, gills, and kidneys (*78*). For domoic acid encapsulated in liposomes up to 6% of the toxin was incorporated into the mussel tissues, mainly in the digestive gland and kidneys. When mussels were no longer exposed to the toxin, it was gradually released from the tissues in soluble form or in the feces (*77,78*).

On the west coast depuration studies of Dungeness crabs and razor clams have been initiated (John Wekell, National Marine Fisheries Service, Seattle, WA, personal communication). Work so far with the crabs indicates that if they are placed in filtered sea water, domoic acid levels drop rapidly within a few weeks, but in harbor water in cages (without access to contaminated shellfish) levels fluctuate but do not go down. One possible explanation is that *Nitzschia* type phytoplankton containing domoic acid are being trapped in the gills and then taken into the mouth. More depuration studies are planned.

## OTHER ISSUES ASSOCIATED WITH EXTENDED SAMPLING

As more and more shellfish and plankton samples from Atlantic Canada were submitted for mouse bioassay and other analyses during 1987 and 1988, some unexpected results were observed. An illness complaint was received in Quebec concerning memory loss associated with mussels harvested in the Magdalen Islands, an area 100 km northeast of PEI with no known history of toxic shellfish. Although this link between illness and mussels was not confirmed, some mice injected with extracts of Magdalen Island mussels caused nonspecific mouse deaths at times greater than 15 min, the maximum time for PSP death according to AOAC (*50*), up to overnight. Even though the symptoms were not typical of PSP or domoic acid, extracts were analyzed by HPLC for both of these toxins. No domoic acid was found, but low levels of PSP were identified in some of the extracts (on average <50 μg/100 g). The conclusion drawn was that small numbers of the toxic dinoflagellate *Alexandrium* had been in the Magdalen Islands' waters but not enough to cause human illness.

Acid extracts of oysters from a few New Brunswick and Nova Scotia bays also caused nonspecific mouse deaths after several hours to overnight. The mice showed a temperature drop, weakness and cyanosis before death. After extensive examination by NRCC scientists, the oysters were found to contain 230-1650 μg zinc per g; amounts higher than 500 μg/g are sufficient to kill mice without any effect on man (*74*). The origin of the zinc could be from natural runoff or industrial pollution; the Nova Scotia contaminated oysters had been relaid from another location within the province. The build-up of zinc could have occurred during many months of exposure, because the metal is not readily released by the oysters. The possibility of zinc in shellfish is now taken into account in the monitoring program.

Plankton taken from around PEI contained no domoic acid nor caused mouse deaths except in the Cardigan River area. However, dinoflagellates (*Dinophysis* spp.) were seen in some nontoxic samples, and there was tentative evidence that some diarrhetic shellfish poison, i.e., okadaic acid and related compounds, was also present, indicating the potential for DSP if conditions for rapid growth of the *Dinophysis* occur (*51*). These concerns were justified when the first DSP episode in North America was recorded two years later within 200 km of eastern PEI. Sixteen persons suffered gastroenteritis after eating mussels containing dinophysis toxin 1 (DTX1) from Mahone Bay, Nova Scotia, in August, 1990 (*90,91,122*).

## THE NEW PSP - DOMOIC ACID MONITORING PROGRAM

Over 15,000 samples from both east and west Canadian coasts are analyzed annually for PSP toxins and domoic acid (A. Gervais, Inspection Services Directorate, Department of Fisheries and Oceans, personal communication). Shellfish are extracted according to the AOAC procedure, one portion for mouse bioassay and one for chemical analysis for domoic acid. The observation time both for PSP and domoic acid is 4 h, although the normal PSP toxins death time is less than 15 min. However, the mice may be kept overnight (18-24 h) if they appear affected (*121*). Confirmatory HPLC is done if deaths occur after 15 min, if toxicity occurs at a key station not expected to have PSP toxins, or if epidemiological information implicates such samples in illness. Concentrations of zinc higher than 275 ppm, which occasionally occur, can cause nonspecific mouse deaths after many hours, and samples may be tested for zinc if such a concentration is suspected, before proceeding with reinjection.

## ACCEPTABLE LEVELS OF DOMOIC ACID IN SHELLFISH

The no-effect level observed in mice is 24 mg/kg mouse body weight as measured by IP injection, and 20 μg/g mussel tissue has been established by Health and Welfare Canada as the upper limit for consumption of shellfish (*121*). The level of domoic acid in shellfish in the absence of *Nitzschia* is ≤1 μg/g. Therefore, areas with shellfish containing ≥5 μg/g are sampled more intensely, but if subsequent three samplings from the same area reveal levels consistently below 20 μg/g, the area can remain open with continued monitoring. However, harvesting will cease as soon as levels approach 20 μg/g, and a public recall will be initiated if shellfish with this quantity or greater have reached the retail market. A minimum concentration of about $2\text{-}4 \times 10^5$ *Nitzschia* cells per L over at least 3-4 weeks is necessary to produce 20 μg domoic acid per g mussels (*45*). No substantiated reports of ASP have been docu-

mented in Canada since these control measures were introduced in 1988.

## FUTURE RESEARCH

Although considerable progress has been made to elucidate the problem caused by contaminated mussels, further research is indicated. Some areas have already been suggested (*53*) and are summarized below.

a. An adequate source of pure domoic acid for absorption, metabolism, and excretion studies.
b. Continued development of analytical methods for detection of domoic acid at levels below 1 μg/g for monitoring the toxin in blood and tissue cells.
c. The pursuit of animal models to examine conditions that affect gut absorption, blood-brain barrier permeability, and damage to specific organs.
d. Animal studies for exposure to low doses of domoic acid to determine the potential for teratogenicity and other long-term effects
e. Continuance of neurobehavioral studies on the cases with memory loss and other long term symptoms.
f. Continuance of studies on the effect of domoic acid on receptors in different parts of the brain to determine the precise mechanism of the toxicity.
g. Continuance of studies on the factors that control the synthesis of domoic acid by *N. pungens* f. *multiseries* and other *Nitzschia* and *Pseudonitzschia* sp., including biosynthetic pathways, using axenic culture studies.
h. Studies of the ecology of toxic strains of *Nitzschia* and *Pseudonitzschia* to determine their growth characteristics, the environmental conditions affecting blooms, and the eventual fate of domoic acid in seawater.
i. Analysis of different planktonic organisms for their potential to produce domoic and other excitotoxic amino acids, and if so, what their mechanisms are for production of the toxin(s), including the genetic sequence that controls these mechanisms.
j. Continuance of uptake and depuration studies in shellfish and crustaceans in culture and in marine environments.
k. Significance of domoic acid in fish, e.g., anchovies, and shellfish for human food and animal feed, and the effects of domoic acid on these fish and shellfish.
l. Development of kits to detect domoic acid for the shellfish industry.
m. Monitoring of fish and shellfish for new toxic agents.

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