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THE ORIGIN OF THE REGULATORY LIMITS FOR PSP AND ASP TOXINS IN SHELLFISH

JOHN C. WEKELL,1 JOHN HURST2 AND KATHI A. LEFEVBRE1

ABSTRACT Understandably, commercial and recreational seafood harvesters are interested in how regulatory limits are set for various toxins in seafood. Here we summarize the origins of the safety levels for paralytic shellfish poisoning (PSP) and amnesic shellfish poisoning (ASP) toxins. PSP toxins consist of a suite of neurotoxins identified as saxitoxin, gonyautoxin, and their derivatives. The regulatory limit for these toxins (80 µg STX equiv. / 100 g shellfish) was established in the 1930s and is based on bioassays measuring toxic activity in mice. Amnesic shellfish poisoning (ASP) is a more recently discovered syndrome caused by one toxin, domoic acid (DA). It was identified in 1987 and the regulatory limit of 20 µg DA/g tissue was established in the following year, based on the estimated DA dosage levels consumed by the first human victims of ASP. This study attempts to preserve the history of the origin of these regulatory limits, both of which have not changed and have effectively protected consumers of commercial seafood since their implementation.

KEY WORDS: paralytic shellfish poisoning, amnesic shellfish poisoning

INTRODUCTION

At seafood safety meetings, scientists and policy makers are frequently asked by recreational and commercial seafood harvesters about the origins of the regulatory limits set for marine biotoxins in particular seafood. In the case of paralytic shellfish poisoning (PSP) toxins, the regulatory limit (80 µg/100 g) was established over 60 years ago and its origin is beginning to be lost to the fog of history. Amnesic shellfish poisoning (ASP) caused by domoic acid (DA), on the other hand, is a relatively new syndrome. The first DA poisonings were observed in 1987 in eastern Canada and the regulatory limit for DA in seafood (20 µg/g) was established by Health Canada in the late 1980s. With the discovery of DA in finfish and shellfish on the west coast of the United States in 1991 (Work et al. 1993), the US Food and Drug Administration also adopted the Canadian value.

The regulatory limits for PSP and ASP toxins evolved quite differently. The chemical structures and toxicology of the PSP toxins were only vaguely understood in the 1930s when the limits were developed. In contrast, the chemical structure of DA had already been long established (Takemoto et al. 1966) before its regulatory limit in shellfish was set by Health Canada in 1987. In fact, extracts of marine algae that contained domoic acid and a related congener, kainic acid, had been used as an antihelminthic for children in Japan (Baslow 1969). However, specific information about the toxicity of DA at higher doses in adults was obtained following the first ASP outbreak in 1987. In an effort to preserve the history of the origin of the regulatory limits for PSP and ASP, we have prepared a brief note describing how these values were first derived based on sound epidemiology and toxicology.

Regulatory Limit for PSP Toxins (80 µg STX Eqv. 100 g-1 Shellfish)

The Toxic Syndrome, PSP

PSP is caused primarily by the consumption of molluscan shellfish that have accumulated PSP toxins as a result of filter-feeding on toxic dinoflagellates (Shumway et al. 1988). In some rare cases fish and crabs have also been implicated as vectors of PSP toxins. Saxitoxin is the most toxic of the PSP toxins (Oshima 1995). At least 16 variants of this basic structure have been identified and the variants range in toxicity from high (saxitoxin) to almost nontoxic (the sulfonated toxins). The composition of the suite of PSP toxins found in shellfish is both dependent on the specific phyttoplankton (Alexandrium sp.) consumed and the metabolic processes within the organism, since shellfish may convert one toxin to another. A full discussion of this molluscan conversion process is beyond the scope of this study, but a detailed description can be found in Sullivan et al. (1983). Thus, PSP is caused by a “suite” of toxins present in shellfish. The syndrome is characterized in its most severe form by paralysis of the breathing muscles, which if left untreated by competent medical intervention, ultimately leads to death. In cases of paralysis, mechanical ventilation is highly effective. However, at lower doses, symptoms can range from mild stomach upset to a tingling sensation in the lips.

Alcoholic Versus Acidic Extraction Methods for PSP Toxins

In 1937, Sommer and colleagues established the connection between toxic shellfish and blooms of the dinoflagellate, Alexandrium catenella (then known as Gonyaulax catenella) along the California coast. Sommer and Meyer (1937), in the course of their monumental study on PSP toxins, developed two extraction methods in their attempts of quantifying the amount of toxin present in shellfish. Their preferred method used acidic alcohol for the extraction of the toxin. Due to the laborious nature of this method, they developed a second more practical "field test" that used diluted hydrochloric acid and boiling for extraction. This field assay also used mice for the quantification of PSP toxins. Whereas the alcoholic extraction has been largely forgotten, the simplified field test (which was only minimally described) is still used today, with minor modifications, as part of the risk management of PSP throughout the world. Because the chemical structure and properties of the shellfish toxins were unknown in the 1930s and the purity of the extracts were also unknown, Sommer and Meyer’s field test required them to define toxicity in terms of a Mouse Unit (MU) to quantify toxic activity. In their original work, Sommer and Meyer defined the mouse unit as the amount of toxin that killed a 20 g mouse within 10 to 20 min.

Sommer and Meyer’s field method of extraction called for the
homogenization of 100 g of shellfish with 100 mL of 0.1 N hydrochloric acid, boiling this mixture, adjusting the final volume to 200 mL, and then filtering the mixture. Testing for toxin required the intraperitoneal injection of 1.0 mL of the filtrate into a mouse and then observing how long it took the mouse to die; therefore, an injection that killed a mouse in 15 min contained, by definition, 1 MU, and the total toxin content of the original 200 mL extract was 1.0 MU/mL x 200 mL of extract or 200 MU. Because this extract was obtained from 100 g of shellfish meats, the shellfish were said to contain 200 MU per 100 g of shellfish.

**Purification of STX From Shellfish**

This unit of measuring PSP toxins was used up until the 1950s when a purified toxin standard became available from the US Food and Drug Administration (FDA). In the late 1940s and 1950s, researchers at Fort Detrick isolated and purified saxitoxin (STX), the principal toxic component of PSP. Whereas initially intended for chemical warfare purposes, the unused portion of STX was eventually released to the FDA and became a shellfish standard. An official method that used this standard as a reference was accepted by the AOAC in 1959 (McFarren 1959). To use this standard as a control in bioassays, it was necessary to establish a relationship with the MU. Through experimentation, it was found that one MU was equal to approximately 0.2 µg of the purified FDA standard, although variability between mouse strains ranged from 0.17 µg to 0.28 µg (J. Wekell unpub data). Because of this variation between strains, laboratories performing the mouse bioassay must "calibrate" their particular mouse strain against this toxin standard.

**Calibration of the Mouse Unit**

When the FDA accepted the use of the standard, they also accepted that 0.2 µg STX equiv. = 1 MU as a reasonable average for a conversion factor (CF). Therefore, 200 MU became equivalent to 40 µg of the purified toxin. Because the 200 MU detection limit was established from a 100 g batch of shellfish, the detection limit was subsequently expressed as 40 µg STX equiv./100 g of shellfish, which remains the value that is still used today. Most likely, if the detection limit were set today, it would be expressed as 0.4 µg/g or 0.4 ppm. However, because virtually all of the literature and databases have used the "µg/100 g" notation for more than 60 years, it continues to be used to this day.

The use of a purified toxin standard also permitted more consistent estimates of PSP in shellfish. Sommer and Meyer developed a curve plotting MU and "death-times" (Fig. 1A). The curve in Figure 1 inset was derived from data in the report by McFarren (1959) and is currently used by regulatory agencies (AOAC 2002). Examining the death time curve, it is clear that very fast death times (e.g., less than ~5 min) can lead to very large errors in obtaining the correct number of MU. For this reason, the current regulatory method requires dilutions of the sample to bring the death times of the mice within 5 to 7 min, an area where the curve has a very minimal slope and time can be measured with reasonable precision and also permit good interpolation from the curve (Fig. 1B). From the curve and assuming a mouse correction factor of 0.2 we can derive the following: mice dying sooner than 5 min would exceed the regulatory limit of 80 µg/100 g. Death times greater than 15 min place the concentration of PSP toxins at 40 µg/100 g, approximately the accepted detection limit of the mouse bioassay. Therefore, using the mouse bioassay a regulatory labo-

![Figure 1. Death times plotted against mouse units (A) and the suggested working range for the mouse bioassay (inset, B). Data from McFarren (1959) and AOAC (2002).](image)

**New Chemical Techniques for Characterizing the Structure of Toxins**

Within the past 30 years new chemical techniques such as high performance liquid chromatography (HPLC), x-ray crystallography, and nuclear magnetic resonance spectroscopy (NMR) have permitted the elucidation of the structure of the PSP toxins. The predominant toxin was named saxitoxin after the origin of the initial toxin source, the Alaska butter clam (Saxidomus giganteus). A trace component in the FDA standard was later found to be a closely chemically related structure and was named neosaxitoxin. Using the techniques listed earlier, further research revealed that PSP poisoned found in shellfish consists not only of saxitoxin and neosaxitoxin but also a suite of closely related toxins that are commonly referred to as "gonyautoxins" (named after the original genus of the dinoflagellate that produces the toxins). Subsequent studies have shown that not all of these related toxins are equally toxic to mice or humans; some are more toxic than others. Because there is only one toxin standard, predominantly saxitoxin, the resulting collective concentration of toxins in shellfish extracts are referred to as "X µg saxitoxin equivalents/100 g shellfish"; sometimes abbreviated as "µg STX equiv/100 g".

**Establishment of the Regulatory Limit (80 µg STX Equiv. 100 g \textsuperscript{-1} Shellfish)**

How the specific 80 µg 100 g \textsuperscript{-1} regulatory level was arrived at is open to some conjecture and the details are now, after over 60 years, probably lost to history. Prior to the establishment of the acid extraction method (only briefly described in Sommer & Meyer 1937) as the standard for regulatory purposes, California instituted quarantine measures when 2 mg of dried alcoholic extract contained 2 MU (Medcalf et al. 1947). Tests at the Laboratory of Hygiene have shown this 2 mg to be equivalent to a toxicity of 400 MU per 100 g of whole meats, which is 80 µg 100 g \textsuperscript{-1} in today's parlance (Medcalf et al. 1947). Canada also adopted the California standard of 400 MU as the quarantine level (Medcalf et al. 1947).

Medcalf et al. (1947) also examined epidemiologic records from
eastern Canada in the mid 1940s and found that in some cases a dose of 1,000 MU produced mild symptoms of PSP. Using the current conversion factor, 1,000 MU equals approximately 200 μg of STX equivalents, because the lowest dose reported for illness is 200 μg, and assuming that 100 g of shellfish meats might represent a reasonable quantity for an adult to consume, the lowest illness-producing concentration in shellfish would be 200 μg per 100 g. Based on these estimates and using a <10 safety margin, the regulatory level could be set at 20 μg/100 g. However, this is well below the mouse bioassay detection limit. Therefore, the 20 μg/100 g level was probably derived as a compromise based on the detection limit of the mouse bioassay (roughly 40 μg/100 g) and yet still safely removed from the minimal toxicity of 200 μg/100 g observed in the early studies.

Prudence and caution by risk management agencies over the years have maintained the regulatory level at 80 μg/100 g. This level seems to have weathered the “test of time” in that after 60 years of use the authors are not aware of a case of PSP reported in properly tested and released shellfish. Virtually all recent illnesses and deaths due to PSP are due to the recreational or subsistence victims ingesting untested shellfish or shellfish taken from quarantined or untested beach areas. In Alaska, because of the remoteness of the beaches and shoreline, the state has mandated that all beaches be quarantined to the taking of all shellfish unless otherwise specified by the state health authorities.

**Regulatory Limit for Domonic Acid (20 μg DA/ g Tissue)**

**The Syndrome, ASP**

Domonic acid (DA), the toxin responsible for amnesic shellfish poisoning (ASP), is a recent arrival to the list of known marine biotoxins and is naturally produced by some diatoms of the genus *Pseudo-nitzschia*. However, not all members of the *Pseudo-nitzschia* genus are highly toxic. The first reported ASP event occurred in Eastern Canada in 1987 when 4 people died and over 100 people suffered varying degrees of intoxication after consuming DA-contaminated mussels taken from Prince Edward Island (Todd 1990). Herbivorous fish were the vectors of DA in more recent intoxication episodes along the west coast of the United States in which dozens of sea birds and marine mammals died or were stricken with neurologic symptoms (Work et al. 1993, Lefebvre et al. 1999, Scholin et al. 2000). Fortunately, due to an extensive monitoring and surveillance program by the western coastal states (California, Oregon, Washington, and Arkansas) and the Province of British Columbia, no human cases of DA poisoning have been reported and officially confirmed since 1987.

Domonic acid is a low molecular weight, neuroexcitatory amino acid that can cross the blood-brain barrier and destroy neural brain cells. It attacks the glutamate signaling nerve system, destroying the cells that release the neurotransmitter glutamic acid (Debomme et al. 1989, Berman & Murray 1997). Because regeneration of most brain cells is a relatively slow process, it appears that for practical purposes this damage is permanent and irreversible. In the known cases so far, elderly people seem to be more at risk than younger people (Todd 1993). In the Canadian outbreak, some people were institutionalized with permanent brain damage, while others have suffered varying degrees of short-term memory loss (Todd 1990, 1993, 1997). Follow up studies of the 1987 victims reported that some lost their businesses and the ability to conduct normal daily personal affairs. There is no known treatment for this intoxication.

**Establishment of the Regulatory Limit for DA (20 μg DA/ g Tissue)**

As in the case of PSP, arriving at a toxic dose of domonic acid in the 1987 episode was difficult because it was dependent largely on indirect measurements on recovered or uneaten products. Two important workshops that strove to set regulatory limits for DA were held immediately after significant DA outbreaks: The first was held in Ottawa, Canada from April 11 and 12, 1989 (Proceedings of a Symposium, Domonic Acid Toxicity, Can. Diseases Weekly Report, Vol. 16S1E, September 1990) and the other was held in San Pedro CA, February 6 to 8, 1992 (Domonic Acid Workshop, San Pedro, California, US Food and Drug Administration, San Francisco, CA, 1992). Lengthy discussions during these workshops illustrate the difficulty of arriving at the most rational regulatory levels that balance the protection of public safety with the economic provision of shellfish resources.

Because no documented and certified human illnesses were ever reported during the 1991 and 1992 United States west coast DA outbreak, all consumption and dose analyses were based on 1987 Canadian data. Recovery of uneaten mussels involved in that outbreak and subsequent analysis indicated that they contained levels ranging from 310 ppm to 1280 ppm domonic acid (Perl et al. 1990). Using these data, it was concluded that victims exhibiting mild and severe symptoms are shellfish containing approximately 500 ppm DA and received a total maximum dose of between 200 to 300 mg (Wright et al. 1990). The lowest total dose that a minimally symptomatic victim received in the Canadian outbreak was reported to be about 50 mg (Iverson et al. 1989, Hynie et al. 1990, Iverson & Truelove 1994). Assuming a human adult of 70 kg, this translates into a toxicity range of 0.7 mg to 4 mg/kg, very close to animal oral toxicity levels in rats, mice, and monkeys (Iverson et al. 1990); however, mice and rats seem to be less sensitive than primates to the effects of DA. Using the lowest total dosage of 50 mg, a consumption of 200 g shellfish meats, and a safety factor of approximately 12, it was concluded that the regulatory limit should be set at 20 μg DA g⁻¹ shellfish.

In 1992, at a Domonic Acid Workshop held in San Pedro, CA sponsored by the FDA, the toxicity data was reexamined by epidemiologists and toxicologists from Canada and the FDA. During the San Pedro workshop, it was suggested that consumption of certain shellfish might be higher than 100–200 g of meat, perhaps as high as 500–1000 g. Nevertheless, after an extended discussion that reviewed the Canadian data, it was concluded that setting the regulatory limit of 20 μg DA g⁻¹ shellfish would provide sufficient safety margins that would account for the variability between individual responses and the amount of shellfish consumed.

**SUMMARY**

The regulatory limits for both ASP (20 μg DA g⁻¹ shellfish) and PSP (80 μg STX equiv. 100 g⁻¹ shellfish) toxins have not changed since they were first implemented. These safety levels seem to be successfully protecting the seafood-consuming public and since their establishment no human cases of ASP or PSP intoxication resulting from regulated commercial seafood have been reported. These levels were driven by the detection capabili-

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1The San Pedro Workshop publication by the Pacific Region FDA office in San Francisco is a compilation of transcriptions of the semi-formal talks and discussions of the participants. Other than minor editing of typed transcripts, no formal peer review of the presentations was made.
ties of the time and the necessity to be conservative in the face of sketchy epidemiologic and toxicologic data. However, the value of this work, done up to 60 years ago in the case of PSP, is exemplified by quite literally "standing the test of time". Those levels, set in the 1930s, are still used today with only minor modification to the assay method itself.

Of course all regulatory risk managers would like to reduce risk to zero, but they know that this is not possible in the real world.

The management of marine toxins is a balance between a reasonable level of safety and complete closure/embargo of all seafood products. Considering the wide diversity of marine phytoplankton species, the number and variety of harmful marine natural products must also be significant. The development of reasonable safety guidelines is complicated by our limited knowledge of the known marine biotoxins, particularly how, when, where, and why harmful algae produce them.

LITERATURE CITED


