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Detoxification of *Pyrodinium*-Generated Paralytic Shellfish Poisoning Toxin in *Perna viridis* from Western Samar, Philippines[1]

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Introduction

Until recently, the occurrence of "red tides" in the Philippine marine waters is almost unknown. If ever, the areas affected involved only small coves or, at most, a few hundred metres of shoreline.

In July-August 1983, a red tide occurred in Western Samar and Leyte, Philippines, the extent of which covered coastlines and lagoons approximating 300 km. The causative dinoflagellate, which previously had never been the cause of red tides in the country, fitted the description for *Pyrodinium bahamense* var. *compressa* (Steidinger et al. 1980) and is the same organism that caused several large red-tide outbreaks in Papua New Guinea (Maclean 1973, 1975a, b, 1977; Worth et al. 1975), Sabah, Brunei, and Palau (Maclean 1979; Beales 1976).

In the aftermath of its accompanying effects, i.e., paralytic shellfish poisoning (PSP), the occurrence left scores of townsfolk in the provinces of Western Samar and Leyte dead and more than 300 persons hospitalized after eating toxic mussels and fish and was responsible for the near collapse of the mussel *Perna viridis* Linnaeus industry in Western Samar and other regions of the Philippines. For the 8 months that a ban on the gathering, sale, and consumption of the Western Samar mussel was in effect, the crop, worth ₱30 million (US$2.2 million), remained at sea, untapped for food.

A combination of PSP toxins had been found in a variety of shellfish that were able to absorb *P. bahamense* var. *compressa* in Palau (Harada et al. 1982). The shellfish, namely *Spondylus butleri, Tridacna corcea,* and *Septifer bilocularis,* contained 62-97% saxitoxin and lower amounts of a novel toxin coded PBT_1. Also found were neosaxitoxin (neoSTX), gonyautoxins III and V (GTX_3, GTX_5), and PBT_2, the latter being found only in the dinoflagellate cells. If true to form, Western Samar mussels would contain the same toxins, if not more, but in varying proportions.

There is clearly a need for the development of acceptable detoxification procedures to counteract the unusually prolonged retention time of neurotoxins in mussels. These procedures must assure modest gains by the farmer, but more importantly, the health and safety of the consuming

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public. It is with this in mind that research efforts were directed at probable solutions.

This paper will report on the results of detoxification producedures using ozone, chlorine, and PVP-iodine (polyvinylpyrrorlidone-iodide-iodine). Earlier, ozone was shown to be effective in detoxifying toxins from *Mya arenaria* exposed to *Gonyaulax tamarensis* (Dawson et al. 1976) and toxins from *Gymnodinium breve* (Blogoslawski et al. 1975; Thurberg 1975).

Materials and Methods

Packing and Transport of Mussels

Approximately 50 kg of 8-month old *Perna viridis* was collected from mussel farms in Maqueda Bay, Jiabong, Western Samar, Philippines, on 10 August 1983 — a few days after the height of a red-tide episode that took place from 23 July — 7 August 1983. The mussels were packed in plastic bags and placed in styrofoam boxes. The temperature inside the boxes was maintained at 22-25° C using crushed ice. The boxes were transported to Tacloban and then flown to Iloilo where studies were carried out in the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC).

Acclimation and Detoxification

The mussels were acclimated by floating the bags on 32 ppt seawater at a temperature of 26-28°C for 30 min. The samples were then taken out of the bags, distributed randomly on plastic trays, and placed in 4600-L fibreglass tanks for detoxification. The system consisted of a simple modification of the setup used by Blogoslawski et al. (1979).

Three chemicals were used: (1) chlorine, (2) ozone, and (3) PVP-iodide-idoine complex. To the first treatment, calcium hypochlorite was aded to the water to a concentration of 0.5 ppm. In the second treatment, ozone was generated from a Sandér ozonizer (Erwin Sander Elektro-apparatebau, Eltze/Peine Am Osterberg, West Germany) unit at the rate of 25 mg 0_3/hour. In the third treatment, polyvinylpyrrorlidone-iodide-iodine complex (Actomar[R] K-30, Ciba-Geigy Ltd., Basle, Switzerland)[2] was added to produce a final concentration of 2 ppm. The tanks were maintained for 24 hours, after which the water in each setup was replaced with fresh solutions having the same amount/discharge rate of each chemical as stipulated earlier.

For the control treatment, a lot of undeclustered shells were placed in a fourth tank and supplied daily with a mixture of *Dunaliella salina* and *Tetraselmis chuii.* As with the three chemical treatments, water replacement and feed renewal were carried out on a 24-hour basis.

Extraction of Toxin and Quantification

The standard mouse bioassay as advocated by APHA (1970) was modified for use in this study. Four or five pieces of mussel were declustered from each lot, washed with water, and opened by cutting the adductor muscles. The meat was then removed with a minium of disturbance, rinsed with water and collected in a beaker, transferred onto wire sieves, drained for 5 min, and homogenized in a blender.

A 100-g lot of homogenized flesh was mixed with 100 mL 0.1*N* HCl and boiled slowly for 5 min. The mixture was cooled and the pH adjusted to between 2 and 4 by adding 0.5*N* HCl or 0.1*N* NaOH. The volume was adjusted to exactly 200 mL by adding distilled water and the extract was mixed thoroughly. The extract was centrifuged for 5 min. The supernatant was taken for injection into experimental mice. Extractions were carried out after 3, 5, 6, 7, 8, and 15 days.

For quantifying the toxins, mice weighing 17-21 g were used. Orienting titrations were conducted with each extract by injecting a 1.0 mL aliquot into a mouse intraperitoneally. The time of death, estimated from the exact moment of injection to the last obvious yawning pant, was determined to the nearest 5 sec. Whenever possible, the time of death was adjusted to between 5 and 7 min by diluting the extract with distilled water. In all final injections, three mice were used.

The average time of death for each extract was computed and the corresponding mouse equivalent (ME) was drawn from Sommer's table (Halstead 1965). The value was multiplied by the degree of dilution; by 200, representing the total amount of extract; and the correction factor for the weight of the mouse. The resulting value represents the toxin content of the sample in mouse units (MU)/100 g mussel meat.

Results

The average toxicity of the mussels collected a full week after the height of the red tide and prior to experimental use was 1165 MU/100 g (range 945-1310 MU/100 g). On the basis of the equivalence of 1 MU to 0.183 μg of saxitoxin, the toxicity was 213 μg/100 g, almost triple the threshold set by the United States Food and Drug Administration for

[2]Reference to a true name does not necessarily mean endorsement of the product.

closure of shellfish beds (i.e., 80 $\mu g/100$ g).

Figure 1 shows the changes in toxicity of the mussels using four methods of detoxification. Ozone and PVP-iodide-iodine appear to effectively lower toxicity between the 6th and 7th days; in fact, to a value lower than the threshold level. The steep reduction in toxicity during this period was, to a certain extent, observed in those mussels continuously fed with the phytoplankton *Dunaliella salina* and *Tetraselmis chuii.* This trend did not continue, however, as indicated by a gradual insignificant decrease from the 8th day onward. The toxicity on the 8th day was calculated to be 67 $\mu g/100$ g and on the 15th day 44 $\mu g/100$ g, just barely lower than the threshold level. Chlorine was not as effective a detoxifier as the other treatments. By interpolation from the curve in Fig. 1, it would take 14 days to lower the toxicity to the safe level using chlorine.

At the concentrations the three chemicals were administered in the experiment, the mussels did not seem to have undergone any metabolic stress, as indicated by 100% opening up and unabated pumping activity. Similar studies on surf clams *Spisula solidissima* exposed to doses of ozone between 4.5 and 7.5 ppm for 2 weeks led to considerable stress (Blogoslawski et al. 1979), a condition that was improved by lowering the ozone levels to 0.7-1.2 ppm.

Discussion

The toxicity of the mussel *Perna viridis,* which accumulated toxins from a heavy bloom of *Pyrodinium bahamense* var. *compressa,* in Western Samar was considerably elevated. By standard mouse assay a full week after the red-tide bloom, the mussels had an average toxin content of 213 $\mu g/100$ g.

The values obtained were comparable to those of shellfish also made toxic by *P. bahamense* elsewhere in the Western Pacific and a variety of shellfish made toxic by *Protogonyaulax tamarensis* in Japan. For example, the butter clam *Spondylus butleri* collected from Palau in 1981 had a peak toxicity of 1100 MU/100 g (Harada et al. 1982).

When our extracts were injected into mice undiluted, killing times were, on average, between 2.5 and 3.5 min, similar with most results obtained by Maclean (1975b), using the shellfish *Spondylus,*

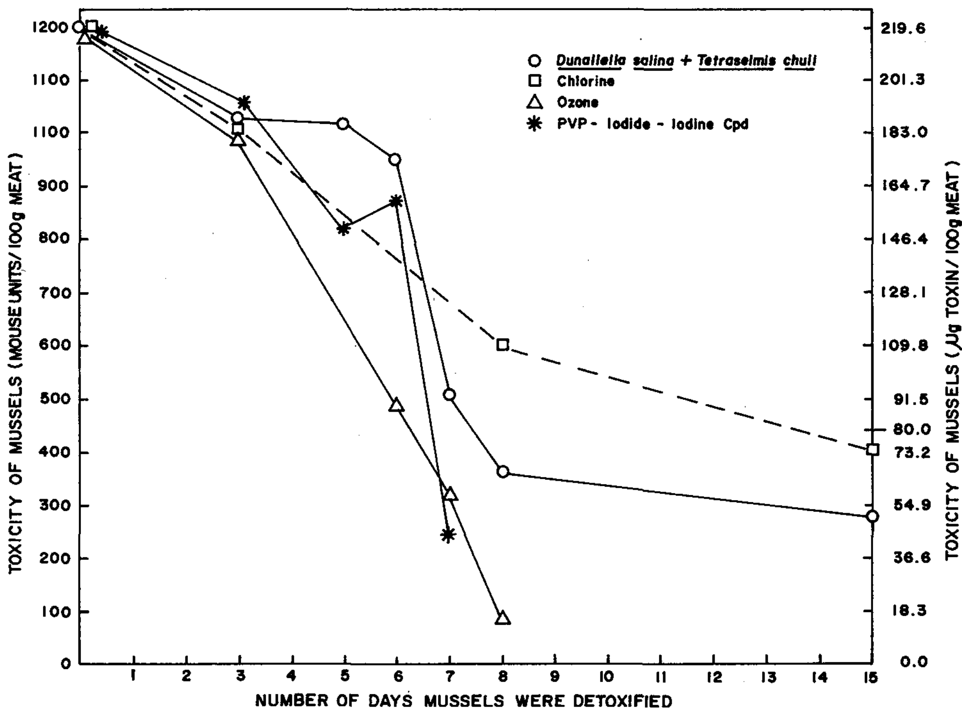

Fig. 1. Changes in the toxicity of mussel Perna viridis *L. from Maqueda Bay, Western Samar, after detoxification.*

Chama, *Ostrea trapezina*, and *Barbatia parvillosa* collected from Papua New Guinea. Based on his data, the shortest killing time was 1 min 30 sec. This should have given a toxicity of between 3130 and 3470 MU/100 g.

The potency of toxins from a variety of shellfish that assimilated from *P. tamarensis* during a red tide in Japan varied. The highest toxicity recorded in the scallop *Patinopecten yessoensis* from Ofunato Bay was 3500 MU/100 g in 1981, taking only digestive glands (Maruyama et al. 1983), and 1700 MU/100 g with pooled organs in 1979 (Oshima et al. 1982).

Although the characteristics of PSP due to *P. bahamense* have been fully documented (Maclean 1973, 1975b, 1977; Beales 1976; Worth et al. 1975) and its toxin components fractionated and characterized (Harada et al. 1982), no study has dealt with the detoxification or inactivation of these components.

Detoxification at the concentrations used was able to reduce the toxicity by one-half in 6 days and to a value lower than the threshold level between the 7th and 8th days. Although a shorter period should have been targeted, such as the 48-hour time constraint for shellfish depuration, the detoxification period might have been shorter had there been a flow-through system instead of the one at hand. As emphasized earlier, the water in all four tanks was static and was changed only once every 24 hours.

Although there have been a handful of similar studies on the effective inactivation by ozone of PSP toxins in mussels, oysters, and other shellfish that were absorbed from *Gonyaulax tamarensis*, *G. catenella*, and *Gymnodinium breve* blooms (Thurberg 1975; Dawson et al. 1976; Blogoslawski and Stewart 1978; Blogoslawski et al. 1979), none have dealt with *Pyrodinium* PSP.

Mya arenaria, which readily and rapidly assimilates PSP from *G. tamarensis* when subjected to flowing water with either high and low doses, lost a considerable amount of toxin within 24 hours (Blogoslawski et al. 1979). In both doses, the toxins after 48 hours were so low that the shells were considered safe for human consumption, whereas the controls did not come near the threshold level even after 72 hours. When the initial level was high (775 μg/100 g), however, reductions after 48 hours were 44.4 and 23.7%, respectively, and 10.6% in the controls. The above was also found true in our experiments.

A number of workers used isolated and semi-purified toxins by bubbling ozone. *G. breve* toxins reacted at 20-110 mL/min and were progressively inactivated (Blogoslawski et al. 1975, 1978), but not the untreated extracts, which brought total mortality to mice in 7-10 min and the killifish *Fundulus heroclitus* in 15-30 min (Blogoslawski et al. 1975). PSP toxins from *Mytilus edulis* and *Modiolus demissus* exposed for 5 min at 110 L/min were completely inactivated at pH 7.8 but not at pH 3.8, an indication that toxins are stable at low pH or even become much more potent. It is important to note that many of the people who died in Western Samar and Leyte ate mussels or noneviscerated fish boiled in vinegar or mussels simply broiled and dipped in vinegar.

The use of PVP-iodide-iodine is still not widespread, although there are many studies showing its ability to act against a wide variety of microorganisms (Casagrande 1978). At SEAFDEC, PVP-iodide-iodine has been used extensively to depurate heavily contaminated oysters, *Crassostrea iredalei*. At 2.0 mg/L, it has the same effect as chlorine at 0.5 ppm in purging the coliforms from oyster guts.

Chlorine is not suitable for detoxification work as suggested by our data. It would take 14 days to bring down the toxicity to slightly lower than the safe level. In studies with *G. catenella* toxins, sodium hypochlorite neutralized the toxin over long periods (Chin 1970). Likewise, it lowers flavour quality and decreases acceptability despite the fact that it is cost effective.

The setup fed with *Dunaliella salina* and *Tetraselmis chuii* was originally intended to indicate and approximate the reduction of toxicity in the field where there is no further absorption of toxin from the seawater and live cells or cysts from the mud. The data indicated that reduction was rather slow as on the 15th day the toxicity had not gone below acceptable levels. It should be noted that the ban on shellfish gathering in Maqueda Bay was finally lifted during the first week of March, 8 months after it was established. In the field, mussels are continually supplied live *Pyrodinium* cells, or in cases of slight turbulence generated by winds, with cysts that may be toxic as well (Dale et al. 1978).

There are not many data on the persistence of PSP toxins generated by *P. bahamense* var. *compressa*. Worth et al. (1975) observed that *Crassostrea echinata* was toxic for 15 weeks after the complete disappearance of the dinoflagellate from Papua New Guinea waters, a finding repeatedly demonstrated by northern-hemisphere researchers (Prakash et al. 1971; Quayle 1969). The decrease in toxicity proceeded at different rates in different shells.

PSP toxins produced by *Pyrodinium bahamense* var. *compressa* are as problematic as those produced by *Protogonyaulax catenella*, *P.*

acatenella, and *P. tamarensis* in Japan, United States, and Canada, in terms of the time needed for the toxins to be eliminated. In Japan, *Mytilus edulis* kept in a laboratory tank lost about half of the toxin within 1 week (Yasumoto et al. 1979).

With *Patinopecten yessoensis*, toxicity declined rapidly during 3 days from 1700 MU to 200 MU, went up to 520 MU for unknown reasons, and then declined gradually thereafter until it levelled off at 100 MU after 5 months (Oshima et al. 1982). *Mytilus edulis* PSP had a shorter period for toxin reduction: peak toxicity was observed on 15 June and no toxicity was demonstrable 6 weeks later when *P. tamarensis* had disappeared (Oshima et al. 1982).

Upward inflection from a lower level may mean the existence of bioconversion or toxin transformation to another form, as confirmed in Bay of Fundy scallops *Placopecten magellanicus* (Hsu et al. 1979; Shimizu and Yoshioka 1981). The methods we used did not fulfill the requirement for replications for the samples to be thoroughly declustered and randomized prior to the start of the detoxification run, so our results should be interpreted as preliminary.

Ozone gas and PVP-iodide-iodine may effectively inactivate PSP toxins from *P. viridis* without affecting the pumping ability. It is to be noted that recent collaborative work in Canada by White and Blogoslawski has shown no effect of ozone on depuration of toxins from clams (A. White, personal communication, 1984). Similar negative results have been found in Japan.

Due to public health and safety considerations, and everybody's desire for the mussel industry to flourish, work on ozone and PVP should be intensified with the purpose of finding the effective time and dosage for total inactivation of the toxins. In view of toxin interconversion, which accounts for increased toxicity scores, more work needs to be conducted. To obtain definite results, more replications must be set up.

The complexitiy of ozone's chemistry has led many to investigate its effects. When added to seawater, it reacts with free bromide ions to form hypobromite ions and hypobromous acid. Blogoslawski et al. (1979) believed that hypobromous acid, in addition to dissolved ozone itself, was responsible for inactivating PSP. There is, however, a paucity of information on PVP-iodide-iodine.

Ozone is now widely used to depurate coliform-loaded oysters the world over. The establishment of an ozone depuration/detoxification station in Maqueda Bay, which henceforth will be subject to *Pyrodinium* blooms, must be considered to routinely purify mussels and inactivate PSP should any toxic red-tide episode arise.

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