Seafood Safety Monitoring Program for Ciguatera: Assessing Aquatic Product Safety

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ABSTRACT

Ciguatera toxins are odorless, tasteless, and generally undetectable by any simple test: therefore, bioassays have been used traditionally to monitor suspect fish. Programs designed to provide some degree of assurance that foods susceptible to natural toxicant contamination are wholesome and safe to eat require several facets including marketplace screening of suspect foods for identification of contaminated product, separation of adulterated product to less risk uses, and where feasible, development of systems or monitoring programs designed to predict potential hazardous food production/collection areas.

With respect to public health concerns, prevention of ciguatera would be the best strategy; however, because of its complexity, this approach has been very difficult. The next alternative would be a monitoring program for screening market place seafoods as well as harvesting areas. For a method to be of value in screening marketplace seafoods, it must meet the following criteria: (a) facile to use and interpretation; (b) rapid, *i.e.*, able to test a large number of samples in a short period of time; (c) accurately differentiate between toxic and non-toxic samples; (d) low cost; (e) available in sufficient quantity to meet private, industrial, and regulatory agency testing demands; and (f) where feasible, provide for a means of confirmation of identity.

The solid-phase immunobead assay (S-PIA, Ciguatect™), currently available from HawaiiChemtect International, has potential for application to screening market place fish for ciguatera toxicity. The kit can be used at several points, *i.e.*, harvesting, processing, distribution, retail, etc. Testing fish early is recommended, thus minimizing expenses incurred from handling the product. The self-contained assay is available as a single analysis kit designed for non-laboratory use by untrained personnel. Organizations conducting large numbers of analyses would be more inclined to use the laboratory kit which contains sufficient material for 50 tests.

In conclusion, the seafood safety monitoring program would entail a testing program which includes: (a) monitoring fish harvesting areas to determine ciguatera potential, (b) developing a sampling plan applicable to screening fish in the market place for ciguatera, (c) screening fish at various points in commercial channels for ciguatera, *i.e.*, on-board fishing vessels, at receiving docks, processing plants, distribution organizations, retail outlets, consumers, and regulatory agencies, (d) possible re-analyzing of fish testing positive using alternative extracting and analytical methods, and (e) diverting toxic fish to lower risk uses. The S-PIA method (Ciguatect™) can be used to monitor reef fishing areas for ciguatera potential and screen for toxic fish in the market place.

KEY WORDS: analytical methodology, Ciguatera, seafood safety.

INTRODUCTION

Seafood safety monitoring programs designed to provide some degree of assurance that foods susceptible to natural toxicant contamination are wholesale and safe to eat require several safety measure. These include screening of suspect foods for identification of contaminated products, separation of adulterated product to less risk uses, and development of systems or monitoring programs designed to predict potential hazardous food production/collection areas. The author, in cooperation with other scientists at the FDA and the NMFS, developed a critical path analysis (CPA) which was designed to establish a seafood safety monitoring program for ciguatera. Principal aspects of the CPA are presented in Figure 1.

When this CPA was prepared, major needs were identified and efforts were initiated to meet these needs. Initially, major efforts focused on obtaining sufficient quantities of toxins involved in ciguatera. Since attempts to isolate and purify toxins from contaminated fish involved in the ciguatera phenomena were labor intensive, expensive, and yielded minimal amounts of toxins, activities to culture toxin-producing organisms (dinoflagellates) were intensified. As a result of large-scale culturing efforts at FDA, NMFS, and selected laboratories in academia, sufficient quantities of okadaic acid (OA) were obtained to concentrate on method development studies.

Federal health research activities (Food and Drug Administration (FDA) and National Marine Fisheries Service (NMFS)) have focused on the protection of human health and the enhancement of commerce of subtropical reef fish. This goal can only be realizes, however, by having an effective control program that would remove ciguatoxic fish from the market place. Adequate standards and rapid screening methods for monitoring the presence or absence of these toxins in fish has not yet been attained. Rapid, reliable analytical methods are crucial to an effective seafood safety monitoring program. Analysis for ciguatoxin has been labor-intensive, time-consuming, and lacking specifity. The evolution of ciguatera-related analytical methodology is described below.

Several toxins may be responsible for ciguatera. The primary toxin (CTX), has been isolated from large carnivores and in smaller amounts in herbivores. CTX may accumulate in large carnivores due to its greater lipid solubility. Considerable circumstantial evidence has linked *Gambierdiscus toxicus* to this toxin; Murata *et al.* (1990) reported the structures of ciguatoxin from the Moray eel (*Gymnothorax javanicus*) and its likely precursor from the dinoflagellate *Gambierdiscus toxicus*. The cogener was shown to be less oxygenated analog of ciguatoxin. However, it has not yet been conclusively demonstrated that the toxin produced by the dinoflagellate is the precursor to ciguatoxin(s) accumulating in fish. Until suitable detection methods for these toxins are developed, it will be difficult to determine toxin properties. There are at least five toxins involved in ciguatera, which have been named ciguatoxin (CTX),

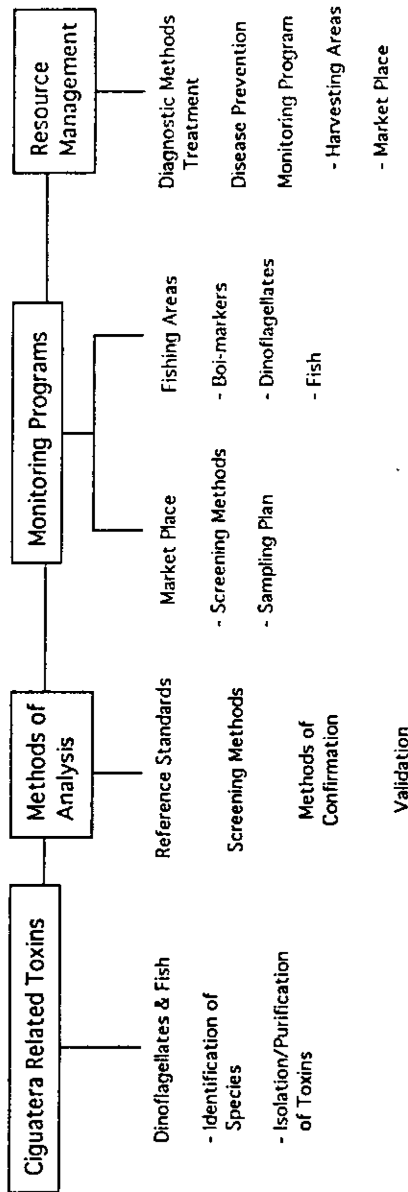

Figure 1. Key aspects of critical path analysis for Ciguatera research.

maitotoxin (MTX), scaritoxin STX), okadaic acid (OA, and a recently named toxin called Prorocentrolid (Bagnis *et al.*, 1974; Chungue *et al.*, 1977; Tindall *et al.*, 1984; Yasumoto *et al.*, 1971; Yasumoto and Murata, 1988a; Yasumoto and Murata, 1988b). The structures of ciguatoxin and akadaic acid are presented in Figure 2. Recent studies suggest than an excess of 20 toxins may be involved in the ciguatera phenomenon (Juranovic *et al.*, 1992; Park *et al.*, 1992d; LeGrande, 1991). Reef fish become toxic through consumption of toxigenic dinoflagellates and/or ciguatoxic fish which have been previously exposed to these toxins. Man is exposed to these toxins through the consumption of ciguatoxic herbivores and carnivores.

Most seafood avaiable to the U.S. consumer are wholesale and unlikely to cause illness. There are some areas of risk, however, such as the consumption of raw shellfish and naturally occurring toxins, *i.e.*, ciguatera and paralytic shellfish poisoning (National Academy of Science, 1991). Ciguatera is the most common non-bacterial food poisoning disease associated with the consumption of fish in the United States and its territories. Ciguatera poisoning outbreaks occur primarily in tropical regions of the world, *i.e.*, Caribbean area, Atlantic, Indian and Pacific ocean regions, Middle eastern and Australian areas (Bagnis *et al.*, 1979; Bagnis, 1970; Banner, 1965; Lewis, 1986; Maharaj *et al.*, 1986; Royal, 1982). Although, in the past, toxic outbreaks were limited to the endemic areas, interregional transport of fish can result in outbreaks in nontropical parts of the world. Ciguatera is considered a world health problem.

METHODS

Analytical Methodology

The format of analytical methods vary according to the application or purpose of the method, *i.e.* screening, confirmation of identity, reference, etc. Since ciguatera toxins are odorless, tasteless and generally undetectable by any simple chemical tests, bioassays have been traditionally used to monitor suspect fish. Most earlier methods were based on biological endpoints which had major limitations on levels of detection and specificity. Many native tests for toxicity in fish have been examined, including discoloration of silver coins, or copper wire, the repulsion of flies or ants, and rubbing the liver on the gums to ascertain if it causes a tingling feeling. But all of these, with the possible exception of rubbing the liver on the sensitive tissues of the mouth (Lewis, 1986), have proven invalid. As more reference material and standards became available, the emergence of chemical and immunochemical methods became apparent. Finally, before any method can be of value in a monitoring program, precision and accuracy parameters must be determined through an inter-laboratory validation study, similar to those sponsored by AOAC International (AOAC) and the International Union for Pure and Applied Chemistry (IUPAC).

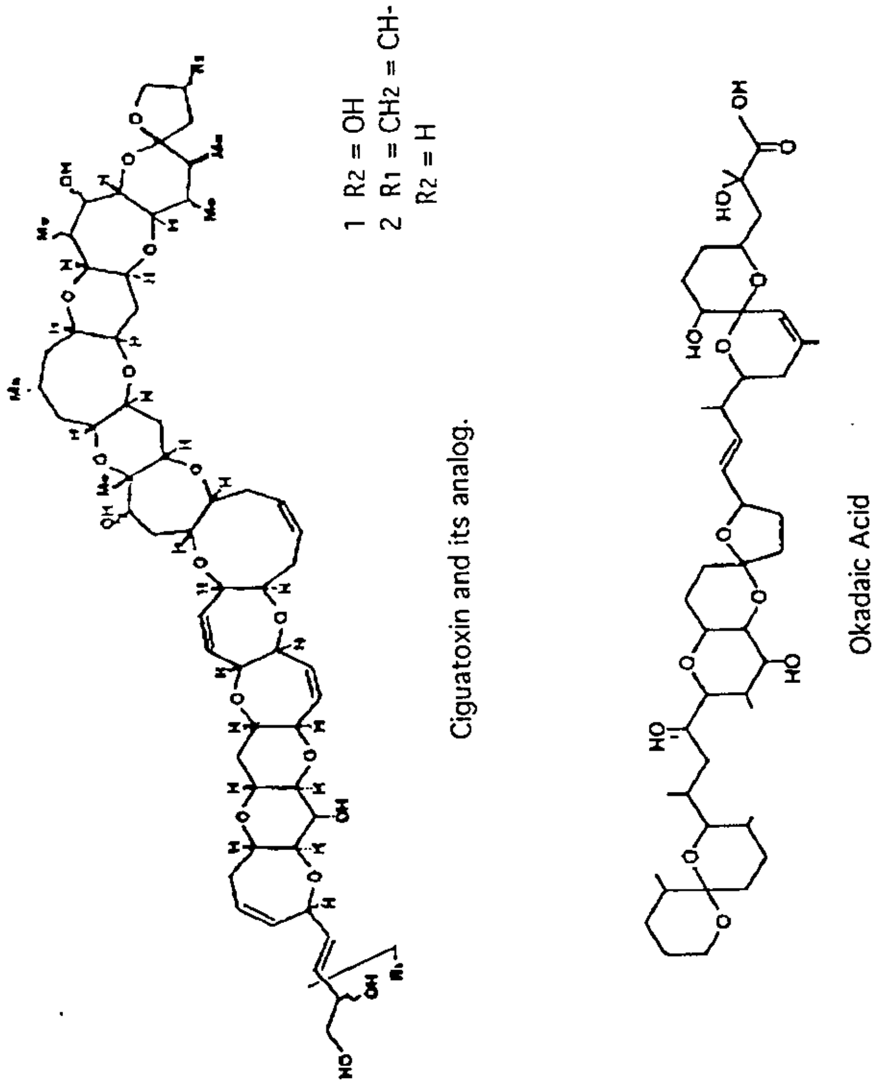

Figure 2. Ciguatoxin and Okadaic acid analog.

Biological Methods

Whole Animal Assays: The mouse assay has been used traditionally; however, it involves a time consuming process of obtaining the lipid-soluble extracts and it lacks specificity (Yasumoto *et al.*, 1971). Other major disadvantages include expenses associated with maintaining a mouse colony, subjective death time, and the nonlinear relationship of death time to dose. The method consists of injecting serially diluted semi-purified or crude toxic extracts into mice (usually intraperitoneal (IP)) and observing the symptons for 24 to 48 hours. the results are expressed in mouse units where one mouse unit is identifies as the amount of toxin that kills a 20-g mouse in 24 hours (World Health Organization, 1984). This assay is unsuitable as a market test. The mouse assay gave reproducible results following IP injections of toxic fish or dinoflagellate extracts (Gamboa and Park, 1985; Park *et al.*, 1992d; Sawyer *et al.*, 1984; Gamboa *et al.*, 1992). The measurement of rectal temperature of the animals immediately before the extracts and periodically for 16 or 48 hours showed a pronounced drop in the body temperature of the mice following exposure to the toxins (Park *et al.*, 1992; Gamboa *et al*, 1992; Gamboa and Park, 1985; Hoffman *et al.*, 1983; McMillian *et al.*, 1980). A symptomatological analysis has been prepared to facilitate the comparison between ciguatera research reports (Hoffman *et al.*, 1983). A dose-response curve was prepared for purified ciguatoxin obtained from toxic blackfin snapper collected from the Virgin Islands. The mouse bioassay has been used extensively in the Pacific and is described in detail by Yasumoto *et al.* (1984).

Cat and mongoose bioassays for ciguatoxin in fish have been developed by Bagnis and Fevai (19710 and Banner (1965). The test is based on feeding the animals rations containing 100 g of the fish to be tested per kg ration. These feeding tests are simple and useful for testing fish for toxicity, but they are cumbersome, not quantitative, and not suitable for screening market fish. The results of these assays with moral eels were compared to the mouse assay by Yasumoto and Scheuer (1969), showing that livers of all eels assayed were toxic to the mouse regardless of the cat or mongoose test results of the eel flesh. A mosquito bioassay was developed by researchers from the French Polynesia (Bagnis *et al.*, 1987; Chungue *et al.*, 1984; Pompon and Bagnis, 1984; Pompon *et al.*, 1984b). This procedure involves intrathoracic injection of serially diluted extracts from fish into mosquitos, and the toxicity of the fish expressed as mosquito LD50. It was recently used by Bagnis *et al.* (1987) to obtain a dose-response relationship between ingested ciguatoxin and clinical symptoms in man. A good correlation between this assay and the cat and mouse bioassay was observed. In addition, it is more rapid than animal assays, depends on a simple extraction, and requires only a small amount of fish for analysis. Still the assay is nonspecific and non-quantitative.

Banner *et al.* (1961) tested 37 species of animals and found only five were sensitive to oral administration of toxic fish flesh. The mouse was eliminated as a feeding-test animal because of its high tolerance. Turtle and crayfish test specimens were rejected because of the difficulty in quantifying symptoms for the former ans meals for the latter. The cat was rejected for the reason explained above and the mongoose remained the chosen species. The brine shrimp (*Artemia salina*) has been used to screen extracts from toxic fish and dinoflagellates (Juranovic *et al.*, 1992; Park *et al.*, 1992d).

In Vitro Assays: Many other bioassays have been developed using the guinea pig ileum, guinea pig atrium, isolated frog nerve fiber, crayfish nerve cord, and human and mouse blood cell hemolytic tests (Benoit *et al.*, 1986; Dickey *et al.*, 1982; Escalona De Motta *et al.*, 1986). Ether and butanol soluble fractions from *Amphidinium* showed hemolytic activity, although toxicity to the mouse was detected only for the butanol soluble fraction.

Miller *et al.* (1986) successfully used the crayfish nerve cord (CNC) as an assay for the extracts of the dinoflagellate *Prorocentrom concavum*. Three of the *P. concavum* extracts reduced the activity of the CNC. Purified CTX from moray eel induced spontaneous action potentials upon the node of Ranvier of frog isolated nerve fibers under current and voltage clamp condition. This spontaneous activity is reversible upon the removal of the toxin from the external solution (Benoit *et al.*, 1986).

Immunochemical Assays: Alternative assays based on immunochemistry have been developed which overcome the common disadvantage of traditional bioassays, *i.e.*, the lack of specificity for individual toxins. A radioimmunoassay (RIA) for ciguatoxin (isolated from toxic moray eel) injected into sheep and rabbit (Hokama *et al.*, 1977). The sheep antibody to ciguatoxin was then purified and coupled to 125I label to be used in the RIA. This assay was used successfully in the screening of amberjacks (*Soriola dumerili*) where 15% of the fish were rejected during a 2-year study on the Hawaiian market (Kimuar *et al.*, 1982). No poisonings were attributed to amberjacks during the 2-year study, although other untested species did cause illness. Despite this success, the assay was not suitable for routine use due to high cost, instrumentation requirements, and time involvement. The cost of the RIA limits its use to fish weighing more than 9 kg.

A follow-up competitive enzyme immunoassay (EIA) was developed and evaluated on Hawaiian reef fishes (Hokama *et al.*, 1983; Hokama *et al.*, 1984). Other researchers such Berger and Berger (1979) and Chanteu *et al.* (1986) have also developed immunoenzymatic methods for the detection of ciguatoxin in fish tissues. As with its predecessor, this characteristic was used effectively to demonstrate the close structural similarity of CTX, MTX, brevetoxin, and OA. Subsequently, an enzyme immunoassay, based on a horseradish peroxidase labeled sheep anti-ciguatoxin antibody applied to liquid-paper coated bamboo

sticks, was developed (Hokama, 1985). This assay was able to distinguish between toxic and nontoxic fish. Test results revealed a high number of false-positives, although no false-negatives were observed (Hokama *et al.*, 1987; Hokama and Miyahara, 1986).

The stick test has been further modified using monoclonal antibodies specific for OA and CTX that are more specific than the sheep antibody (Hokama *et al.*, 1986; Taizo, 1987). A preliminary collaborative evaluation study of the rapid enzyme immunoassay stick test has been concluded. Eight of the nine laboratories involved obtained results that were within acceptable limits for each of the three fish cake samples homogenized with ciguatoxin (Ragelis, 1988). Attempts to validate the method were unsuccessful due to the lack of reference material.

Emmerson *et al.* (1983) described a counter immunoelectrophoresis (CIEP) method designed to discriminate between nontoxic and toxic Caribbean fish involved in ciguatera outbreaks. This procedure, however, needs to be modified and tested further before it can be used as a screening test for ciguatoxic fish (Ragelis, 1984).

A rapid solid-phase immunobead assay (S-PIA, Ciguatect™) for the detection toxins associated with ciguatera (CTX) and diarrhetic shellfish poisoning (DSP) outbreaks has been developed (Park and Goldsmith, 1991; Park *et al.*, 1992c). The presence or absence of the toxins is determined by binding the toxins to a membrane attached to a plastic strip and exposing the toxin ladened membrane to monoclonal antibody-colored latex bead complex, which has a high specificity for the toxins of interest. The intensity of the color on the membrane denotes the presence of the toxins. CTX and DSP toxicity potential can be determined directly on edible tissue or following specific extraction procedures. The method has been used to evaluate CTX potential in fish obtained from Hawaii, Australia, and the Caribbean (Park *et al.*, 1992b) and DSP potential in mussels collected from Denmark and France (Park *et al.*, 1992c). This study confirmed the presence of okadaic acid and related DSP toxins in mussels implicated in a DSP poisoning outbreak (Denmark) and mussel depuration operations (France). The precision of the assay has been evaluated through the AOAC/IUPAC inter-laboratory validation mechanism (Park *et al.*, 1992a and 1992b). Analysis of toxic and non-toxic amberjack, surgeon, and parrot fish flesh and extracts showed acceptable repeatability and reproducibility parameters (Table 1).

Chemical Methods

Methods based on thin layer (TLC) and high performance liquid chromatography (HPLC) have been developed for selected individual toxins associated with ciguatera fish poisoning. These methods can be applied as a regulatory tool where sophisticated laboratory facilities are available. HPLC

Table 1. Statistical analysis of collaborative data for solid-phase immunobead assay determination of ciguatoxins and related polyether compounds.

Sample	Mean	S_r	S_2	RSD_r (%)	RSD_R (%)
Fish Fillets					
Parrot Fish	1.2	0.16	0.53	13.5	44.4
Surgeon Fish	1.7	0.15	0.50	9.00	29.7
Amberjack Fish	3.6	0.15	0.51	4.30	14.3
REM					
Parrot Fish	3.1	0.18	0.37	5.80	11.9
Surgeon Fish	3.8	0.18	0.37	4.80	9.90
Amberjack Fish	4.9	0.18	0.37	3.70	7.60

techniques have been applied to the analysis of okadaic acid in fish tissue (Yasumoto, 1985; Lee *et al.*, 1987; Dickey *et al.*, 1990). Since okadaic acid is the principle toxin associated with diarrhetic shellfish poisoning (DSP), this methodology has been applid to shellfish (Stabell *et al.*, 1991; Lee *et al.*, 1989). Park and co-workers (Personal Communications) have developed a TLC method for okadaic acid in fish tissue and dinoflagellate cultures. Specificity of this methodology is obtained following exhaustive purification of toxins extracted from fish tissue.

HPLC methodology has also been reported for ciguatoxin and several analogs (Murata *et al.*, 1990; Lewis *et al.*, 1991). These studies reported four major ciguatoxins. LeGrand and co-workers (LeGrand *et al.*, 1990; LeGrand, 1991) used HPLC methodology to isolate multiple ciguatera toxins from wild *Gambierdiscus toxicus* and toxic herbivorous and carnivorous fish.

Method Validation

Any method intended to be used in a seafood safety monitoring program must be validated through an inter-laboratory study to determine the precision and accuracy parameters of the method. Method validation programs are administered by AOAC and IUPAC. The precision of the solid-phase immunobead assay (Ciguatect™) to detect toxins associated with ciguatera poisoning has been evaluated through analysis of toxic and non-toxic fish fillets (amberjack, surgeon, and parrot fish) and REM™ extracts obtained from fishing areas around the Hawaiian Islands. The precision of the assay has been evaluated through the AOAC/IUPAC inter-laboratory validation mechanism (Park *et al.*, 1992a and 1992b). Analysis of toxic and non-toxic amberjack, surgeon, and parrot fish flesh and extracts showed acceptable repeatability and reproducibility parameters (Table 1). FDA and NMFS laboratories participated

in the study. The study confirmed excellent performance of the method and interpretation the results, and demonstrated acceptable precision parameters (Park *et al.*, 1992a; 1992b).

SEAFOOD SAFETY MONITORING PROGRAMS

An effective food safetymonitoring program comprises primarily three components; (1) monitoring fish harvesting areas for ciguatera potential, (2) establishment of regulatory limits, and (3) a screening program for testing fish in commercial channels designed to test a large number of samples in a short period of time so that toxic or high risk products can be separated from healthful foods. Violative or unacceptable product can then, if desired, be subjected to additional testing to confirm the presence and identity of the toxin(s).

Monitoring Fish Harvesting Areas: A key aspect of an effective seafood safety monitoring program, where feasible, is establishing a program of predicting and identifying high risk fishing areas. This is accomplished through the testing of marine specimens endemic to fish harvesting locations. Since seafoods commonly associated with ciguatera poisoning outbreaks come from highly mobile fish, the collection and testing of the fish alone could provide incorrect information of both false positive and false negative predictions. This possible problem can be corrected by identifying and testing a specimen or bio-marker of limited motility, *i.e.*, invertebrates, endemic to the fishing area.

The Ciguatect™ solid-phase immunobead assay has been used to screen 36 species of near shore invertebrates off the coast of the Island of Hawaii for ciguatoxin and related polyether compounds (Figure 3) (R.G. Kvitek and D.L. Park, Unpublished Data). Specimens included snails, sea urchins, sea cucumbers, crabs, brittle stars, bivalves, and zoanthids. Invertebrates were collected at six "toxic" locations having documented history of ciguatera fish poisoning along the Kona coast, and at three "non-toxic" sites along the Hamakua coast where there had been only one reported case of ciguatera since 1980.

A significant positive correlation between assay results and site-specific ciguatera history was found for the cowry (*Cypraea maculifera*). While assay results for most other species indicated ver low or no ciguatoxin present, cone snails (*Conu* spp.), ophiuroids (*Ophiocoma* spp.) and sea cucumbers (*Holothuria* spp.) tested positive frequently. There was no correlation, however, for these three genera between assay results and site history. These results suggest that invertebrates, particularly grazers and deposit feeders, and especially cowries, accumulate ciguatoxins and related polyether compounds at sites known for ciguatera fish poisoning outbreaks and have potential utility of being bio-indicators of reef toxicity. This marine specimen, or other invertebrates native to the area under study, could be an integral part of the ciguatera monitoring program.

This assay has been used to test for diarrhetic shellfish poisoning (DSP), toxicity potential in mussels implicated in a DSP poisoning outbreak in Denmark, and DSP depuration monitoring studies along the Atlantic coastline of France (Figure 4, Table 2) (Park *et al.*, 1992c). Study results demonstrated that the assay could identify DSP contaminated shellfish as well as serving as a useful too to monitor shellfish to determine at what point shellfish can enter the market following a *Dynophisis* sp. bloom.

Establishment of Regulatory Limits: An important aspect of any food safety monitoring proggram is the establishment of regulatory limits designed to assure wholesomeness of the food supply. Animal toxicological and human clinical data are crucial information needed for the determination of this value. Since multiple toxins are involved with ciguatera poisoning outbreaks, it is not practical to use a single compound for this regulatory limit. Historically, the establishment of a seafood safety monitoring program for ciguatera has been hampered by the lack of reference standards. At the present time, okadaic acid is the only toxin associated with ciguatera poisoning outbreaks in sufficient quantities to serve as a reference standard. Therefore, the term okadaic acid equivalents (OAE) can be used in the establishment of regulatory limits being aware of the relative potencies of other toxins involved with ciguatera phenomenon. Again, the term OAE is used because multiple toxins are involved in ciguatera poisoning outbreaks.

At the present time, okadaic acid is the only toxin associated with ciguatera poisoning outbreaks in sufficient quantities to serve as a reference standard. The Ciguatect™ test kit can determine the ciguatera potential directly on the fish fillet or after specific toxin extraction methods. Okadaic acid is used as the reference material for this kit. The limit of detection for direct analysis of fish for ciguateric potential is <1ng (nanogram) okadaic acid equivalents (OAE). The method can differentiate 1 ng increments of OAE. The University of Arizona and HawaiiChemtect International have developed a rapid extraction method (REM™) capable of extracting and partial purification of toxins associated with ciguatera poisoning in less than 30 minutes (Park *et al.*, 1992a) Toxins are extracted with a chloroform:water:methanol mixture and partitioned into selected phases by varying polarity. When the REM™ is used to extract and purify toxic components, the limit of detection for the Ciguatect™ test kit is <0.05 ng. Also, at this point chemical methods based on thin layer (TLC) and high performance liquid chromatography (HPLC) technology can be used to confirm the presence of individual toxins.

According to Yasumoto and co-workers (1984), ingestion of as little as 100 ng ciguatoxin is sufficient to be a health risk for an adult. Bagnis et al. (1987) reports a dose of 0.06 ng ciguatoxin/kg body weight is sufficient to observe evidence of pathological symptoms, an LP50 of 2 ng/kg, and LP100 of 8 ng/kg. This suggests a dose as low as 1.2 µg for a 60 kg man would result in symptoms

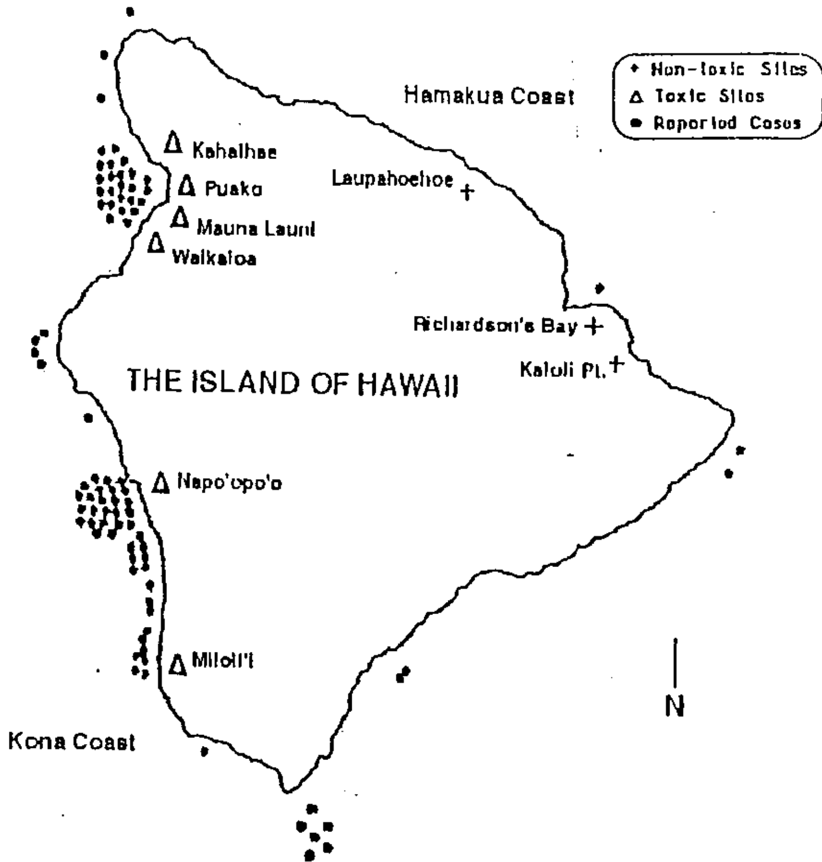

Figure 3. This Hawaii State Department of Health map of the Island of Hawaii shows reported cases of ciguatera outbreaks since 1980. The Hamakua coast, a non-toxic site, had very few ciguatera intoxications. Study sites are noted.

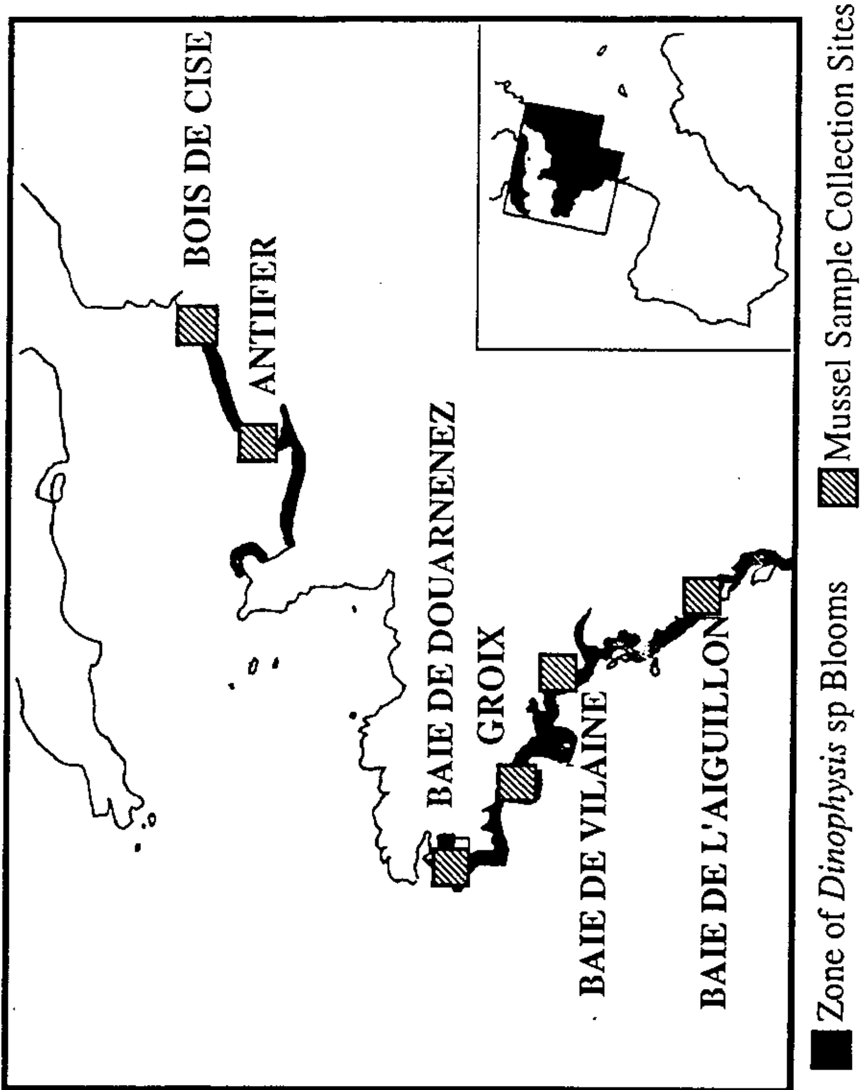

Figure 4. Zones of *Dinophysis* sp. blooms and mussel depuration study collection sites for diarreic shellfish poisoning contamination.

Table 2. Comparitive results between HPLC and Ciguatect™ for detection of okadaic acid in digestive gland tissue from mussels collected along the Atlantic Coastline of France.

Sampling Location	Date Year/Month/Day	ug OA/g DG HPLC	ng OAE Ciguatect
Groix	86/6	5.0	4.0
Bois Cise	87/5/21	<0.5	5.0
Douarnenez	88/3/14	<0.5	1.0-2.0
	88/6/12	11.5	>100.0
	88/8/23	<0.5	0.0-1.0
Baie Vilaine	88/3/21	<0.5	2.0
	88/6/06	3.7	100.0
	88/6/27	2.0	10.0
	88/8/17	<0.5	0.0-1.0

of ciguatera poisoning. Since these studies use bioassays to determine the levels of concern, it is not known which toxin(s) were responsible for the symptom observed. Lewis *et al.* (1991) estimated that >0.1 nmole ciguatoxin/kg fish would be sufficient to cause human intoxication. Therefore, fish fillets containing in excess of 1 ng OAE/g (ppb, parts per billion) should be considered a potential health risk.

Screen Fish in the Marketplace/Commercial Channels: With respect to public health concerns, prevention of ciguatera would be, of course, the best strategy; however, because of its complexity, this approach has been very difficult. For a method to be of value in screening marketplace seafoods, it must meet the following criteria: (a) be facile to use and interpret; (b) be rapid, *i.e.*, able to test a large number of samples in a short period of time; (c) should accurately differentiate between toxic and non-toxic samples; (d) be inexpensive; (e) be available in sufficient quantity to meet private, industrial, and regulatory agency testing demands; and (f) where feasible, should provide for a means of confirmation of identity.

The S-PIA method (Ciguatect™), currently available from HawaiiChemtect International, has the highest potential for application to screening market place fish for ciguatera toxicity. The kit can be used at several points along the marketing plan, *i.e.*, harvesting, processing, distribution, retail, etc. Testing fish early in the plan is recommended, since this will minimize the cost expended for the product and potential economic lost to the industry. The kit can be used on-board fishing vessels, at receiving docks, processing plants, distribution organizations, retail outlets, consumers, and regulatory agencies. The self-contained assay is available as a single analysis kit designed for

non-laboratory use by untrained personnel. Organizations conducting large numbers of analyses would be more inclined to use the laboratory kit which contains sufficient material for 50 tests.

The Seafood Safety Monitoring Program would involve large-scale testing according to an acceptable sampling plan. Fish or lots testing negative to the screening procedure would be allowed to proceed in commercial channels. Each point identified above would be a quality control point. Product testing positive for toxic potential would be diverted to lower risk uses or retested to confirm toxic potential. This can be done by using the REM procedure which concentrates the toxins and retesting or by using alternative test methods for specific toxins.

CLINICAL DIAGNOSIS OF CIGUATERA POISONING

Eventually, the ciguatera strategy would include clinical methods for diagnosing illness, confirmation of the presence of the toxins, and treatment of the symptoms and/or toxin removal. Park and co-workers (University of Arizona), through funding provided by HawaiiChemtect International, have developed immunochemical methods based on S-PIA, ELISA and affinity column (AC) formats for the detection of ciguatera-related toxins in human serum. The S-PIA format utilizes the REM™ extraction procedure to isolate and purify the toxins and the Ciguatect™ test kit for quantitation. Tha AC technique utilizes an affinity column which contains monoclonal antibodies specific to ciguatera-related compounds and an ELISA quantitation step. These methods have been used to confirm the presence of ciguatera toxins in victims of ciguatera fish poisoning from, Australia, Venezuela, Pompei as well as Hawaii, Puerto Rico, Florida, North Carolina, and California (D.L. Park, University of Arizona, Personal Communication). Studies evaluating the role these toxins play in other human illnesses are under way.

CONCLUSION

In conclusion, the seafood safety monitoring program would entail a testing program which includes: (a) monitoring fish harvesting areas to determine ciguatera potential, (b) developing a sampling plan applicable to screening fish in the market place for ciguatera potential, (c) screening fish at various points in commercial channels for ciguatera potential, *i.e.* on-board fishing vessels, at receiving docks processing plants, distribution organizations, retail outlets, consumers, and regulatory agencies, (d) possible re-analyzing of fish testing positive using alternative extracting and analytical methods, and (e) diverting toxic fish to lower risk uses.

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