SOUTH PACIFIC COMMISSION

EXPERT COMMITTEE ON CIGUATERA

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REPORT

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I. INTRODUCTION

1. The SPC Expert Committee on Ciguatera Fish Poisoning met in Suva, Fiji on 26 February, 1981 immediately after the three-day meeting of the World Health Organization Working Group on Public Health Aspects of Marine Food Fish Poisoning. During this meeting many aspects of the subject were discussed at length which provided the members of the Expert Committee with a current up-to-date background upon which to base their recommendations.

2. For several years the South Pacific Commission has supported and promoted studies into fish poisoning. The incidence of the condition in the region has been estimated on an annual basis by country through the South Pacific Epidemiological and Health Information Service project. These data indicate that ciguatera fish poisoning is a widespread problem in the countries of the region and is of considerable nutritional and economic importance. The need for improved methods of diagnosis and control have increased along with the demand for increased fishing activities in the region.

3. In recent years the South Pacific Commission has formed a collaborative working group to examine the nature and causes of ciguatera fish poisoning. The group is formed of three independent groups of investigators, at the University of Hawaii, Institut Louis Malardé in Tahiti and Tohoku University in Sendai, Japan. The investigators heading each of these groups constitute the membership of the Expert Committee.

II. BACKGROUND

4. Ichthyosarcotoxism or marine food fish poisoning has become of great concern to populations in the South Pacific because of the illness and death it causes, the impact on the nutrition of the people and, also, on the development of the local shallow water fishing industry.

5. This problem has been under intensive investigation since 1974 when the South Pacific Commission organized the United States-French Polynesia-Japan tripartite cooperative programme and gave limited financial support to each group. In 1975, Professor Yasumoto, in co-operation with the research group at the Louis Malardé Institute of Medical Research in Tahiti, identified the cause of ciguatera poisoning (one of the most serious and widespread forms of ichthyosarcotoxism), as a unicellular dinoflagellate, Gambierdiscus toxicus, and demonstrated that the toxins contained in G. toxicus were ciguatoxin and maitotoxin.

6. In 1977, successful culturing of the organism led to the isolation and purification of ciguatoxin. As a result of ecological studies, it was found that there appeared to be a direct relationship between the distribution and density of G. toxicus and the incidence of ciguatera.

III. FREQUENCY OF CIGUATERA POISONING IN SOUTH PACIFIC COUNTRIES

7. Fish poisoning is included along with thirty-four communicable diseases reported from the countries of the South Pacific region. The frequency of reported cases by country in the years 1977-79 are summarised in Table 1.

8. The overall reported incidence in the above years was about 100 per 100,000 (93,120 and 82 per 100,000 in the years 1977, 1978 and 1979). Incidence rates of more than 100 per 100,000/year (in the years 1977-79) were reported from French Polynesia, New Caledonia, Trust Territory of the Pacific Islands, and Tuvalu, with the highest reported (more than 300 per 100,000) in French Polynesia and Tuvalu.

9. Four countries (Cook Islands, Niue, Papua New Guinea, and Wallis and Futuna) did not report fish poisoning in the period under concern, but in each the disease is known to occur.

10. The disease also occurs in tropical and sub-tropical waters elsewhere, such as in the Caribbean and Indian Oceans and in other areas of the Pacific, such as along the coast of Queensland and the Hawaiian Islands.

Table 1. Fish Poisoning in the South Pacific countries in the years 1977-79
(Rates per 100,000/year)

Country	1977		1978		1979	
	No.	Rate	No.	Rate	No.	Rate
American Samoa	0	0	0	0	70	226
Cook Islands	0	0	0	0	0	0
Fiji	69	12	201	33	131	21
French Polynesia	502	361	821	578	677	467
Guam	6	11	6	7	9	9
Kiribati	41	47	38	70	78	136
Nauru	0	0	0	0	1	14
New Caledonia	487	350	488	344	188	135
Niue	0	0	0	0	0	0
Papua New Guinea*	-	-	-	-	-	-
Solomon Islands	6	3	6	3	0	0
Tokelau	0	0	0	0	14	700
Tonga	43	47	13	14	8	8
T.T.P.I.	326	251	296	223	191	144
Tuvalu	44	550	71	888	21	429
Vanuatu	50	50	53	52	67	58
Wallis and Futuna	0	0	0	0	0	0
Western Samoa	81	53	179	115	62	40
GRAND TOTAL	1655	93	2172	120	1517	82

* Data not available

IV. SUMMARY OF RECENT PROGRESS

11. The proceedings of the meeting of the WHO Working Group on Public Health Aspects of Marine Food Fish Poisoning detail the recent work in the field.

12. The following is a brief summary of the recent developments and remaining problems. For a more detailed account of these, the complete report of the preceding meeting should be consulted.

University of Hawaii - Dr Banner

13. Research in ciguatera was initiated in 1955 in Hawaii. In 1975 research leading to the development of an immunological test for ciguatoxin was commenced by Dr Hokama, and in 1978 work on the culture of *Gambierdiscus toxicus* was initiated. Meanwhile, work on the chemical structure of ciguatoxin isolated from *Gymnothorax* spp. from Johnston Island continues.

14. We have continued to receive support from the South Pacific Commission, now entirely allocated towards finding a reliable bioassay that utilizes far less toxin (and, we hope, in less purified states) than the other bioassays and immunological tests presently available.

15. We have five nominally separate but mutually dependent projects at this time.

(a) Laboratory studies on *Gambierdiscus toxicus*
(P.I.: Dr Nancy W. Withers; fund sources: Sea Grant, NMFS)

Biological requirements for maximum growth and maximum toxin production are being explored in petri dish and 50 to 100 ml flask cultures; the toxin production is measured in 10 l. bottle cultures. We have begun to explore a variety of ecological parameters.

(b) Distributinn of *G. toxicus* in Hawaiian waters (Dr N.W.Withers, P.I., source of funds: Sea Grant and NMFS)

Using the technique of sampling developed by Dr Yasumoto, to date we have sporadically sampled the inshore reef areas about the major Hawaiian Islands, almost always with low counts (less than 500 cells per 100 g of algal host). We have recently initiated a regular two-week sampling program in Kaneohe Bay (where our laboratory is located). While Kaneohe Bay has the highest counts of *G. toxicus*, including the initial bloom discovered in 1978, there is no record of any fish ever caught from its waters causing ciguatera.

Because of extensive fishery investigations in the leeward chain, we have also started to sample *G. toxicus* along the chain. While the majority of the islands have marginally toxic to highly toxic fish, of the 5 islands we were able to sample during the trip of 5-21 November 1980 (trip limited by cruise plans and storms), only one, Laysan, had *G. toxicus* cell counts greater than 100 per 100 g. of algal substrate.

(c) The development of a more sensitive test for ciguatoxin (Dr Martin Rayner, P.I.; source of funds: SPC)

We have been forced to date, as have been our colleagues in Tahiti and Japan, to rely upon mouse bioassay for our measurement of toxin production. This requires 5-10 l. cultures with 10^6 - 10^8 cells and therefore an extremely long culture period that is fraught with many hazards. If toxin production could be measured with lesser amounts of toxin, all biological studies could be expedited.

Dr Rayner's work on the Na^+ ion effect of ciguatoxin explains the neurological effects of ciguatoxin. The disruption of the Na^+ ion balance causes a change in electrical potential of the excitable membrane. Dyes, newly developed, change in colour with this change in membrane potential.

Dr Rayner proposes to investigate the use of these dyes upon a crayfish giant axone immersed in a microbath and to measure the colour change with a spectrophotometer set at the region of maximal change. He believes that he could do this with a semipurified extract (as at the purity level of the mouse test, or lower) with perhaps 2 orders of magnitude less toxin than the mouse test. This could be from 10^4 - 10^6 cells.

(d) The molecular structure of ciguatoxin (Dr Paul Scheuer, P.I.; fund sources: NMFS and FDA)

In 1955-60, when we were setting forth our basic ground rules for the study of ciguatera, we decided to limit all of our work to the study of one ciguatoxigenic fish in one limited area, and then to apply that knowledge to other fishes and other areas. Our original toxic fish was Lutjanus bohar and our area was Palmyra; as Palmyra became logistically closed to us, we changed to Gymnothorax javanicus from Johnston Atoll.

Dr Scheuer continues to explore the molecular structure of ciguatoxin, but he has now only 0.9 mg. of toxin left. As it is not crystalline, many of the modern nondestructive techniques for analysis of molecular structure, such as x-ray spectroscopy, are denied to him.

As all studies, be they biological, pharmacological, immunological or medical, depend upon the ultimate precise definition of ciguatoxin through molecular structure, this work has, in all reality, the highest priority of all studies. To our knowledge, no one else is presently working in this field.

(e) Immunological Tests for Ciguatoxin (Dr Hokama, P.I.)

Utilising the RIA, Dr Hokama is continuing to test the most potentially toxic commercial fish in Hawaii. About 4,600 specimens have been tested, with a reject rate of 2-3 per cent. None of those passed have resulted in known human toxicity to this time.

Tohoku University - Dr T. Yasumoto

Assessment of ciguateric endemicity

Dinoflagellate population assay

16. We have established an assay method for measuring the population density of Gambierdiscus toxicus, the causative organism of ciguatera. In the survey conducted in French Polynesia it was found that the population density of the organism well reflected ciguateric endemicity estimated by the epidemiological information. The result strongly suggested that such population assay method would make an excellent tool to assess the toxicity level of the area. Seeking the firmer evidence to prove the correlation between the dinoflagellate population and fish toxicity we conducted similar survey in more expanded areas. The results of the surveys undertaken at Okinawa, Guam, Tahiti, Gambier Islands, and New Caledonia are given in Table 2.

Table 2. Correlation between the fish toxicity and G. toxicus population

Place	Population density*1	Fish toxicity*2
French Polynesia		
Gambier Islands	54000	125
Tahiti (Hitiaa)	250	12
Tahiti (Hitiaa)	1.5	1
Tahiti (Popoti)	41	16
Tuamotu (Apataki)	0.5	2*3
Guam	9.8	1 - 6*3
Okinawa	0.05	2.4*3
New Caledonia	0 - 780	5 - 6

*1 Number of G. toxicus cells attaching 1 g. of algae.
*2 MU/g of the pooled livers.
*3 Measured on carnivorous fish, others were measured on the surgeonfish Ctenochaetus striatus.

17. The dinoflagellate population well reflects the fish toxicity and the assay method has a practical value to monitor the level of fish toxicity. As construction on coral reef or environmental changes in coral reefs often trigger outbreaks of fish poisoning, monitoring on the dinoflagellate population by this method will make it possible to predict when the fish may become toxic.

Assessment by liver toxic score

18. While the flesh of fish shows considerable variation in toxicity, the liver shows far less fluctuation and bears toxin even when the flesh is non-toxic. Thus, by testing a few specimens of liver (preferably that of herbivorous fish), we can predict the toxic level of an area. Also, testing a few liver specimens will tell us the potential toxicity of a school of pelagic fish. This technique was applied to commercial fish in French Polynesia.

Distributional study on toxic dinoflagellates

19. Besides Gambierdiscus toxicus two other benthic species (Prorocentrum lima and Ostreopsis sp. nov.) are a possible source of secondary toxins. The distribution of these dinoflagellates has been examined in Tahiti, New Caledonia, Guam and Okinawa.

20. In Tahiti island the micro-regionality of their distribution between the shore and the reef edge was examined. The population of G. toxicus at both Tahiti and the Gambier Islands were remarkably lower than those found previously, which is in accord with a declining trend of poisoning due to the local fish. Both P. lima and Ostreopsis sp. nov. were distributed widely but not in high density. With their rather weak toxin productivity their contribution to the toxicity of fish will be limited.

21. In New Caledonia three species of dinoflagellates were examined on various species of substrative algae. The population of G. toxicus and other species were not very high but were more abundant than in Tahiti island. The result seems compatible with the fairly high incidence of ciguatera in this area (344 patients per 100,000 population in 1978). The dinoflagellates were shown to attach to various algae. Interestingly, the highest number of cells were attached to the blue-green algae, which is hypothesized to play an important role in the "new surface theory" proposed by Dr Randall.

22. In Guam one only sample collected gave a significant number of G. toxicus in keeping with the marginal toxicity of the area. In Okinawa G. toxicus was found in all but one collecting station, but the population density was low.

Environmental study

(a) Analysis of water

23. In a search for the nutritional factor(s) which allegedly stimulate the growth of the toxic dinoflagellate, chemical analyses were conducted on sea water samples collected at various places of different degrees of ciguateric endemicity.

24. Careful examination of the data points to a conclusion that there is no single nutrient which can be correlated to the population density of G. toxicus. The absence of the assumed growth stimulants in the water suggests that the "new surface theory" still holds true. A new surface created by the death of coral offers place for fine algae which in turn attracts the epiphytic toxic dinoflagellate.

(b) Evaluation of environmental factors by culture

Physical factors

25. Our experiments indicate that the low salinity and strong light intensity act as deterrent factors. The results explain why G. toxicus is less dense near the mouths of rivers or in shallow lagoons with a bright sandy bottom. Microscopic observation revealed that G. toxicus attached to algae is covered by mucus membrane. Thus, turbulence may help the organism exchange nutrients and eliminate the silt from the surface. Thus, the salinity, light entensity, and the turbulence of water may be the physical factors governing the micro-regionality of the organism.

Chemical factors

26. In the nutritional study it was found that increased concentration of phosphates, both organic and inorganic, resulted in the increased growth. Also, addition of soil extracts to the culture medium promoted the growth. These two factors offer a possible explanation for the effects of reef construction in fish poisoning, and if the land is rich in phosphate the effect will be more enhanced.

The possible source of secondary toxins

27. The presence of secondary toxins, besides maitotoxin, was often noticed in the viscera of herbivorous fish. We hypothesized that these secondary toxins originate from the benthic dinoflagellates we found co-habiting with G. toxicus. Subsequently we isolated 8 species of benthic dinoflagellates which were grown unialgally. The harvested cells were tested for toxin production. The following 5 species were found to produce toxins lethal to mouse: Amphidinium carteri, A. klebsii, Prorocentrum lima, Prorocentrum sp. nov. and Ostreopsis siamensis.

28. The toxin produced by O. siamensis resembled the acute toxin found in the parrot and surgeonfish, as was the water soluble toxin produced by P. lima. Two fat soluble toxins (PL toxins I and II) of P. lima were practically indistinguishable from scaritoxin and ciguatoxin, respectively, in chromatographic properties. The chemical properties of PL toxin I are also similar to those of scaritoxin and ciguatoxin. The pharmacological action of PL toxin II resembles that of ciguatoxin (Dr Miyahara). To our surprise, however, the subsequent chemical and spectral analysis of PL toxin II led to a conclusion that it is identical with okadaic acid, recently isolated by Dr Scheuer as the cytotoxic component of a sponge. Some similarity in chemical structure is observed between PL toxin I and II. Judging from the presence of such a variety of toxins, it seems likely that these benthic dinoflagellates are the source of secondary toxins found in herbivorous fish.

Institut Louis Malardé - Dr R. Bagnis

Comparative studies of production and evolution of Ciguatera in New Caledonia and French Polynesia - R. Bagnis, A. Inoue, Y. Fakuyo, T. Yasumoto, P. Laboute, E. Changue and J. Bennett.

29. Extensive studies of the ecology of G. toxicus have been conducted in conjunction with Dr Yasumoto and his colleagues in New Caledonia and French Polynesia.

30. The distribution of G. toxicus was determined using simple quantitative techniques, which are suitable for monitoring the likelihood of ciguatera in affected or potentially affected areas.

31. Various parameters in water were determined, such as phosphates, nitrites and nitrates, but no differences were found between areas with high populations of G. toxicus and low populations. Likewise, G. toxicus was absent in Papeete where concentrations of these nutrients were high due to effluent from the city.

32. Although only a limited number of substances were examined, the negative results suggest that growth promoting factors are more closely related to the benthic community in corals, benthic microalgae and macroalgae. The flora of the areas with many dinoflaggelates, such as in the Gambier Islands, is poor with only scanty calcareous algae and blue-green algae, whereas it was rich, both in variety and quantity, in areas where G. toxicus was sparse.

33. In previous studies we have shown that any environmental change leading to death of corals may be followed by increased outbreaks of ciguatera. The disturbances, to which corals are very susceptible, may be either natural (storms, cyclones, tsunamis, seismic shocks, heavy rains, red-tide phenomenon etc.), or artificial and related to human interventions made upon the highly dense, living coral biotopes (undersea works of all types, immersion of wastes or foreign bodies, groundings of ships, and chemical, thermic, or organic pollution of telluric or other origins). The different evolutionary modalities of ciguatera in New Caledonia, Tahiti and Gambier Islands seem to depend on the kind of intensity of these aggressions over time and space.

34. Thus, it appears that localized aggressions, which are relatively limited in time and exerted upon the protected, fringing reefs of high islands or along the atoll coastline, are at the source of ciguatera, which then progresses along a portion of the reef or lagoon coast. The ciguaterigenic zone increases in surface, starting from the points of aggression and moves gradually up to the higher levels of the food pyramid. Human aggression on the living coral environment, or episodic red tides, are most often the cause of this evolutionary pattern characteristic of Gambier Islands.

35. Natural aggressions on the other hand engender diffuse ecological disturbances. They strike the fringing or barrier reefs exposed to the dominant winds, the bays encircled by cliffs, and the coral banks in the open sea. Certain ones (the force of the sea, seasonal rains - for example) assure the perpetuity of ciguatera across the centuries, while others (cyclones, tsunami - for example) are responsible for episodic flare-ups in most of the islands where human aggressions are non-existent. They entail an evolution of the endo-paroxysmic type. During endemic periods the production of toxin is reduced overall and is insufficient to achieve a pathogenic threshold in fish of lower trophic levels. The toxic substances produced in the marine environment must be concentrated along the length of the food chain in order to be noxious. Only the larger, carnivorous fish, of sedentary or mobile habits, are usually involved: this is the case for New Caledonia. During flare-ups, the process is completely altered and the evolution follows the modalities described for human aggressions: this the case for Hitiaa fringing reefs where episodic flare-ups are reported.

36. Whatever the disturbance may be, the death of corals creates newly denuded surfaces suitable for certain species of algae which, in turn, attract the epiphytic organisms such as G. toxicus. The Gambier Islands represent the best example of this thesis. The flare-up of ciguatera in the area has been preceded by mass mortality of corals which, during our survey, were found still dead in most of the grounds, though in some places fresh coral growth is presently visible. Less spectacular in the other surveyed areas, the role of disturbances on reefs is nevertheless patent. We have not found G. toxicus in places covered completely by living corals, in mangrove areas, near the mouths of rivers or on a sandy bottom without current. On the other hand, in New Caledonia, the best location for this dinoflagellate seems to be near passes with much current and turbulence.

37. Since the micro-organism attaching to algal surface was observed to be covered with mucus membrane, its abundance near the pass may be accounted for by the fact that hydrodynamism helps the organism exchange nutrients and other substances through the membrane. It may be also advantageous for the dinoflagellate to live in turbulent places because the silt, sand and other sediments can be removed from its surface. The laboratory experiments on cultured specimens of G. toxicus have indicated that strong light and low salinity act as deterrent factors which may affect the distribution of this organism. Therefore, the absence of the organism near the mouth of a river or in a shallow lagoon with bright sandy bottom may be associated with the low tolerance of the organism to the above factors.

Radioimmunoassay for detecting ciguatoxin in fish tissues : Absence of specific immunoserum - F. Parc, S. Chanteau, R. Ducousso, M. Lafon, I. Lechat and R. Bagnis.

38. Some authors have reported a radioimmunological assay for cituatoxin in fish samples. However, we were unable to reproduce the results using identical experimental conditions. No significant differences were found between the respective amount of binding to presumed specific anti-ciguatoxin sheep immunoglobulins and control immunoglobulins from normal sheep serum. There is only a non-specific physiocochemical reaction between ciguatoxin and protein. This is often observed with lipidic antigens of small molecular weight.

Tentative detection of ciguatoxin in fish tissues by an immunoenzymatic method - S. Chanteau, I. Lechat, F. Parc and R. Bagnis.

39. The ELISA method has been used to detect possible anticiguatoxin antibodies in the tissues of toxic fishes. Thin lamellae of toxic fish muscles have been brought into contact with antibodies obtained after immunization of the rabbit and the mouse by a human serum albumin ciguatoxin conjugate and with human ciguatera convalescent serum. These various antibodies have been recognized by peroxidase labelled anti-rabbit, - mouse and - human antibodies. It has not been possible to demonstrate specific antibodies to the ciguatoxin in the various immunosera tested by this immuno-enzymatic method.

Preliminary study of fat-soluble toxins from Lethrinus mahsena (Forskal) - E. Chungue, I. Lechat and R. Bagnis.

40. One case of poisoning by ingestion of this variety of fish collected in New Caledonia was reported by Bagnis et al[1]. The patient showed a clinical pattern similar to parrotfish poisoning in Gambier Islands.

41. The crude toxin was fractionated by column chromatography using silicic acid, DEAE cellulose and Sephadex LH20. Evidence of the presence of two toxins was obtained. The Lethrinus toxins (Toxin 1 and Toxin 2) gave Rf values on Silica-gel G 60 F 254 nm plates (Camag). These values are rather similar to those of ciguatoxin and scaritoxin.[2]

42. The major toxin (Toxin 2) is similar to ciguatoxin and the minor one (Toxin 1) to scaritoxin as judged by chromatographic behaviour. The occurrence of Scaritoxin-like toxins in fishes other than Scarus and in uni-algal cultures of toxic dinoflagellates involved in ciguatera etiology (Gambierdiscus, Prorocentrum) is remarkable and may lead to understanding of the biosynthesis of ciguateric compounds.

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1. R. Bagnus, J.A. Bronstein, G. Jouffe, R. Forrestier, JL Meunier, J. Lejan, D. Brulefer, F. Parc and C. Tetaria (1977). Bull. Soc. Path. Exot., 70 (1) 89-93.

2. E. Chungue, R. Bagnis, N. Fusetani and T. Yasumoto (1977). Biochimie No. 59, 739-741.

Pharmacological studies of ciguateric toxins - A.M. Legrand and M. Galonnier.

43. The three principal toxins of ciguatera: ciguatoxin (CTX) scaritoxin (STX) and maitotoxin (MTX) were studied by both in vivo and in vitro pharmacologic tests. Their effects were compared on anesthetized cats, anaesthetized rats, on the isolated atrias of the rat and on the intestinal smooth muscle of the rabbit.

Research for a pharmacological protection against the ciguateric toxins : Study of a medicinal plant, Ximenia elliptica, belonging to the family of the Olacacens - M. Galonnier and A.M. Legrand.

44. The therapeutic properties of the medinical plant Ximenia elliptica were studied in experiments carried out in cats and in mice.

45. Given orally to mice, Ximenia elliptica presented toxic effects with doses higher than 10 mg/g. The L.D 50 is about 15 mg/g.

46. At doses such as L.D50/5 and L.D50/10, injected in one or several times, Ximenia elliptica had protective effects against a lethal dose of CTX extracted from the liver of a moray eel Pymnothorax javanicus and against a lethal dose of CTX extracted from muscle of Lethrinus mahsena. In contrast, Ximenia elliptica was inactive in case of experimental intoxication by MTX.

47. In cats intoxicated by ingestion of a parrotfish, Scarus gibbus, a daily oral administration (4g/Kg) of Ximenia elliptica seemed to diminish the toxic effects, but no statistically significant differences were observed between control group and treated group of animals.

V. DEVELOPMENT OF ASSAY PROCEDURES FOR CIGUATERA

48. The lack of a rapid, practical, reliable and simple test for ciguatera has greatly hindered work in many aspects of fish poisoning and the development of suitable control measures. While a radio-immunoassay has been developed, this is expensive and its reproducibility and reliability have been questioned.

49. Recent Australian experience in preparing and clinical tests of diagnostic tests for the venoms of poisonous snakes suggests that a similar approach might offer the possibilities for the rapid recognition of ciguatoxin.

50. The test procedure for the venoms involves an ELISA test in which the inner wall of a capillary tube is covalently coated with IgG antivenoms. Test material (many crude materials, e.g. blood, urine, etc. suffice) are then drawn into the tube(s). The antigen (venom) and antibody then combine. A second multivalent antivenom with an enzyme attached is then drawn into the tube which binds with the venom of the venom-antivenom complex. A substitute for the enzyme is then drawn into the tube after washing out the second antivenom. The substitute then reacts with the enzyme to produce a colour change, which indicates a positive test.

51. The key to success has been the ability to produce a very high titre antivenom (in rabbits). The ability to secure an adequate test for ciguatoxin will depend on the ability to produce antibodies to ciguatoxin either in rabbits or other animals or from man who has been repeatedly exposed to the toxin.

52. Dr S.K. Sutherland, Head of the Immunology Research Branch of the Commonwealth Serum Laboratories, Parkville, Victoria 3052, Australia, has been responsible for the development of the ELISA test for snake venoms, and has conducted some preliminary work towards the development of a similar test for ciguatera. If successful, the availability of such a test would greatly enhance the possibilities of successful practical ciguatera control programmes, as well as facilitate investigations in many aspects of the problem of fish poisoning.

VI. PROBLEMS ASSOCIATED WITH RESEARCH WORK IN CIGUATERA

53. A major problem in ciguatera research has been the difficulty in performing assays for the ciguatoxin. Very crude approaches to detect ciguatoxin in fish have included feeding fish to the mongoose or to cats. Neither approach is satisfactory as a reliable assay of toxicity in fish.

54. A bioassay of ciguatoxin using intraperitoneal injection in mice has been devised and has provided an assay to assess the potency of ciguatoxin containing extracts. One mouse unit represents approximately 0.0005 mg. of ciguatoxin.

55. The supply and purity of ciguatoxin have represented difficulties to investigators. The molecular weight of the toxin is about 1100 daltons, but the exact chemical composition is not established with certainty (C_{53} H_{77} $O_{24}N$ has been suggested) and tentative chemical configuration has been suggested, but work continues on this subject.

56. A major difficulty remains the lack of a quick, reliable and simple assay of the toxin. While radio immunoassay has been developed by one laboratory, high levels of non-specific activity are encountered, and the practicability of such procedure for practical aspects of the control of fish poisoning remain to be developed.

57. The discovery that the dinoflagellate, Gambierdiscus toxicus is implicated as the only presently recognized source of ciguatoxin has led to attempts to understand the ecology of this organism, of factors that lead to the increased occurrence of this organism on blue-green algae and to the possible factors which are associated with the organism and the recovery of toxic fish. It is now generally accepted that the herbivorous fish consume the algae and accumulate the toxin, which are in turn consumed by carnivorous fish, and hence the toxin moves up the food chain.

58. Factors such as reef destruction, both man-made and natural, have been implicated to some extent (but not invariably) in the occurrence of the ciguatoxin poisoning. Attempts to correlate events of this sort with the bloom of the G. toxicus and the subsequent occurrence of toxic fish continue. However, the predictability of fish poisoning from such events is presently uncertain.

59. The extent of the problem in terms of human cases of ciguatera poisoning and their geographic distribution are not well documented, in part as a result of the wide spectrum of clinical symptomatology, in part because of the lack of systematic data collection and reporting of cases, and in part because of a lack of a standardized definition of the disorder. Furthermore, the localized and scattered nature of the problem, both in terms of place and time, make the presently collected national data in the South Pacific region of only limited value.

VII. SOURCES AND SUPPLIES OF CIGUATOXIN

From Gymnothorax

60. Obtaining a supply and extraction of ciguatoxin have represented a significant problem for investigators. About 10 kg. of toxic moray eel (Gymnothorax spp.) liver is needed to prepare c.1 mg. of toxin.

61. About 1 ton of moray eel is needed to get 1 mg. of toxin from the liver and 1 mg. of toxin from the flesh. Both Dr Banner's group and Dr Bagnis' group have each processed between 1-2 tons of Gymnothorax spp. each year to obtain the supplies of toxin for the ongoing work by their groups and to supply others.

62. No other source of toxin is presently available.

From Gamierdiscus toxicus Culture

63. About 175,000 cells of G. toxicus grown in culture will yield about 1 MU (mouse unit) of toxin with a cell density of 1000 cells/ml (4-5 weeks of culture).

64. At the present time, however, this is not a practical method to produce toxin in the required amounts, but further development of the culture techniques should be continued.

65. A continued supply of ciguatoxin is a critical issue for the continued investigation of the chemistry, immunology, pharmacology and ultimately the practical control of ciguatera poisoning.

66. In view of this, the Committee drafted a proposal specifically relating to this matter for consideration by the Commission. The draft proposal is shown in the Annex.

VIII. FEASIBILITY OF POSSIBLE CONTROL MEASURES

67. Various possible strategies which might lead to the control of ciguatera poisoning were discussed. These included:

(a) increased surveillance of the disease in man on a national and regional basis;

(b) surveillance and monitoring of the environment for G. toxicus in areas known to produce toxic fish and in areas where developmental activities which lead to disturbance of the reef ecosystem;

(c) the practice of restricting the sale and harvesting of fish of particular species from particular locations which represent a public health hazard;

(d) the possibility of sampling fish for toxicity to monitor the catch and location of toxic fish to facilitate decisions to permit or prohibit the marketing and sale of fish. This could be done using the presently available mouse assay or possibly, as in Hawaii, using a radio-immunoassay procedure. However, the development of a simple, rapid assay for ciguatoxin would make such a scheme feasible and practical.

68. In the absence of further knowledge, it was the consensus that no general recommendations for the implementation of a control programme, except on an experimental basis, could be made at this time.

69. Until such time that an effective testing system is available, the practice of the local population of avoiding certain high risk toxic fish is to be encouraged. In general, large barracuda and moray eels are to be avoided.

IX. RECOMMENDATIONS

The Expert Committee expressed its gratitude to the Commission for ongoing support. The Committee endorsed the recommendations of the WHO Working Group on Public Health Aspects of Marine Food Fish Poisoning (see Annex). The following recommendations were made to the Commission as areas in which the Commission's contribution and support would be most suitable and helpful.

The Expert Committee recommended :

Recommendation n° 1

The availability of ciguatoxin is essential for continuing research in the chemistry, pharmacology and immunology of ciguatera, and for the development of suitable assays. The committee strongly urges the Commission to seek funds, including those from external sources, to facilitate a continued and increased production of the toxin for these purposes and arrange for the distribution to appropriate investigators.

Recommendation n° 2

The epidemiological surveillance of fish poisoning in the South Pacific Region should be continued and upgraded as there is evidence that the disease in underreported. The active cooperation of the Heads of Health Services should be solicited and appropriate information shall be distributed to health personnel and fisheries officers.

Recommendation n° 3

In order to institute a control programme, knowledge and recognition of the source of the toxin, and advice of the ecology of the dinoflagellate, Gambierdiscus toxicus, is needed. The development and publication of a technical information document on this organism for distribution to fisheries officers is recommended.

Recommendation n° 4

The Commission should support the development of a practical, simple and reliable assay for ciguatoxin. Such an assay could revolutionize the practical recognition and control of ciguatera by facilitating the identification of toxic fish and of human beings with the disease. The ELISA test and histochemical tests for the recognition of ciguatoxin represent promising developments in this area.

Recommendation n° 5

The Committee commended the SPC on the production of the Second Edition of the Fish Poisoning Handbook and recommended that it be made available free of charge to Heads of Fisheries Departments, Heads of Health Departments in the Region, and be advertised more widely than the previous edition. Making the handbook available to Fisheries and Health Departments would create a greater awareness of the problems and potential control methods for ciguatera poisoning.

Recommendation n° 6

Studies of the requirements for growth and toxin production by Gambierdiscus toxicus should be continued. This may lead to alternate ways of producing the toxin, understanding critical factors in the environment which relate to outbreaks of fish poisoning and possible control measures.

Recommendation n° 7

The Commission should lend its support to studies on ciguatoxin including its chemical structure, its pharmacological action and its immunological properties, as these may lead to the development of specific therapeutic measures to combat human diseases caused by ciguatera, as well as enhance the likelihood of developing suitable assay procedures and control measures.

Recommendation n° 8

Additional meetings of the Committee are desirable and may be coordinated with those of the WHO or other foundations or organisations on this subject.

ANNEX I

RECOMMENDATIONS OF WHO WORKING GROUP ON PUBLIC HEALTH ASPECTS OF MARINE FOOD FISH POISONING - SUVA, FIJI, 23-25 FEBRUARY 1981

The group recognizes ciguatera fish poisoning as a serious problem in tropical and subtropical regions. However, more data and more research information are required to formulate practical control measures and be able to arrive at developing an effective surveillance system. It is noted that since the FAO/WHO meeting in 1973 on the subject, the causative organism Gambierdiscus toxicus has been identified. The organism has now been isolated and has opened up entirely new research possibilities. In view of the present state of affairs, the following recommendations are made.

(1) Surveillance of fish poisoning in the South Pacific at present shows an incidence of about 100 per 100,000 population. There is sufficient evidence that the figure is underreported and, therefore, the system of surveillance needs to be strengthened. For obtaining the required epidemiological data, training courses for nationals of endemic areas should be carried out as soon as possible.

(2) The training course should include people in the following fields :

2.1. People who are doing epidemiological reporting and surveys

2.2. Medical officers and health educators

2.3. Fisheries people

(3) Routine surveillance and monitoring programme for population of G. toxicus should be carried out in the following areas :

3.1. Areas known to be producing toxic fish

3.2. Areas where developmental activities are taking place causing disturbances of reef ecosystem

3.3. Other environmental parameters should be measured when possible

3.4. Monitoring of potentially toxic commercial fish by current available tests to give general information to health and fisheries authorities

(4) The basic research on all aspects of the ciguatera problem has been carried on for sometime in the Central Pacific by investigative groups, mainly in French Polynesia, Hawaii and Japan; they have been supported by a variety of international, regional, national and private institutions. New programmes have been or are being started in the Carribean and Australia. Most aspects of the problem are becoming well-defined and, with the discovery of G. toxicus, much better results may be achieved in a shorter period of time.

The Group also feels the urgent need for closer cooperation and coordination of the different research groups. This can be achieved through the intermediary of the WHO Regional Office in Manila which could serve as the focal point for collation and rapid dissemination of new information to various workers engaged in research on ciguatera fish poisoning. In addition, periodic conferences on the subject may be desirable.

4.1. The availability of more toxin is fundamental for continuing research in chemistry, pharmacology, immunology, etc. It is essential to coordinate and maximize efforts with appropriate groups to ensure a continuing supply, national distribution, standardization and comparability of results.

4.2. Practical, simple and reliable assay method is urgently needed for laboratory studies and field monitoring.

4.3. Chemical structure of ciguatoxin should be determined as this knowledge may coincidentally facilitate advances in other areas of research and control. Chemical studies of related toxins should also be encouraged.

While current immunological studies may lead to the production of antibodies to ciguatoxin which in turn would be employed in simple diagnostic tests and possibly as specific treatment, it is necessary to have a better knowledge of the chemical structure of the toxin before more sophisticated immunological procedure can be explored.

4.4. More pharmacological and neurophysiological studies are required for understanding the basic action of ciguatoxin. This may lead to the finding of a more rational and specific therapy.

4.5. The study of environmental requirement for growth and toxin production of G. toxicus in the laboratory and in the field is necessary for the explanation, the possible prediction and even control of ciguatera outbreaks.

4.6. In addition to studies on ciguatera, which has the highest priority, investigations on clupliod, tropical paralytic shellfish and other marine intoxications should be encouraged whenever possible.

(5) Funding

5.1. For initiating the training courses planned, the Working Group requests the Regional Director to find seed money in the amount of US$50,000 for 1982/83.

5.2. The assistance of other multi-lateral, bilateral, regional, national and private agencies be solicited for the production of toxin, environmental and other research recommendations made by the group.

5.3. The valuable contributions of the various investigations in the past were made mostly under national, territorial, regional and private funding; therefore, the RD is urged to encourage these agencies to continue the funding at the present or higher levels.

ANNEX 2

PRODUCTION OF CIGUATOXIN

Introduction

Ciguatera is a neurological disease caused by eating certain tropical fishes that are tied to coral reefs through their food chains; the appearance of toxic fish is sporadic and unpredictable both in geographic distribution and in time. When the disease occurs, it presents severe economic problems both to commercial fisheries in larger centers and to subsistance fisheries in smaller islands; in all areas it is a grave threat to public health. While the disease is also found and is a serious problem in the Caribbean and parts of the Indian Ocean, because of its regional importance, the principal investigators of the disease have been in the Pacific, where intensive studies have been performed for about 25 years, the last 15 years under a tripartite international co-operative investigation sponsored by and funded in part by the South Pacific Commission.

This research has now reached a critical point and applied results in prediction and possible control of outbreaks, as well as clinical diagnosis and treatment, may be expected within the decade or possibly sooner, provided sufficient supplies of the toxin are available.

The causative toxin, ciguatoxin, has been isolated and studies are underway to determine its structure. The original elaborator of the toxin in the coral reef ecosystem, a benthic dinoflagellate, Gambierdiscus toxicus, has been identified; studies have been initiated in the laboratory and in the field to find those environmental parameters that cause the dinoflagellate to reproduce in such numbers as to cause epidemics of the disease from its toxigenic "blooms". The principal pharmacological action has been found. Initial work has been done on the mammalian immunological response, a response that may be use to produce a reliable assay for the toxin in the laboratory and for monitoring of fish in commercial markets; this immunology may also give rise in the future to a specific clinical therapy.

The international co-operative program of research depends upon the availability to the investigators of the participating nations of adequate supplies of the ciguatoxin (see paragraph below). It can be produced in quantities only by the Institut de Recherches Médicales "Louis Malardé" in Tahiti, which alone, among the participating institutions, lies in a chronic ciguatera area.

A Working Group of investigators and advisors gathered under WHO sponsorship in February 1981 at Suva, Fiji placed the procurement and distribution of the toxin as their highest priority for advancement of the knowledge and ultimate control of ciguatera poisoning. As this would be an international service not only to the research program but also to all of the smaller but developing island nations who do not yet have the expertise to participate in the research, it is felt that the production and distribution of ciguatoxin must be funded by an international agency.

Approximately only 1 mg of the toxin is available in the world at the present time. The purpose of the present proposal is to obtain within 2 years the 5 or 6 mgs of pure toxin necessary to have a complete knowledge of the chemical structure of ciguatoxin and to investigate its pharmacology and immunology, and to devise simple reliable assays for poisoned fish and human beings.

Source of Toxin

Toxic fishes will be collected in the most toxigenic areas from French Polynesia (mainly Gambier Island and, if possible, in Marquesas and in other islands where ciguatera rates are high). The species involved will be primarily moray eels, snappers, groupers, emperors, and jacks which are in these areas the fish which contain the largest amount of toxin. Approximately 2 tons of highly toxic fish of the above species will be required to obtain the toxin.

Additional help in the procurement of toxic fish from fisheries agencies of other countries would be desirable but not essential. The sampling should be limited to liver of the fish belonging to the above species or to barracuda, spanish mackerel or dog-tooth tuna that can concentrate also ciguatoxin. The liver should be kept frozen till an opportunity to be sent to Tahiti where the extraction of ciguatoxin will be processed.

The toxicity of the fish will be checked with routine methods used at the Malardé Institute by feeding on cats (for raw flesh) or on mice (for fat soluble extracts). The potency of the extracts produced will be determined by a standardized mouse assay.

The most toxic flesh will be retained for chemical extraction. The liver will be systematically processed as it always contains a suitable amount of ciguatoxin for extraction purposes.

Assay

Quantitative determination of toxin is carried out by injecting various doses of the toxin suspended in 1% Tween 60 solution into mice intraperitoneally. The minimum amount of toxin to kill a mouse weighing 20 g within 24 hours is defined 20 mouse unit (MU).

To evaluate the toxic level of fish, the tissue to be tested is extracted first with acetone and the procedure to prepare the methanol residue is followed. A series of dilutions of methanol residue in 1% Tween 60 are given to mice intraperitoneally and the minimum dilution of the toxin to kill mice in 24 hours is sought. The toxicity of the tissue is expressed in terms of MU/g tissue.

Extraction Procedure

Both the flesh and the liver will be used for the extraction employing the same procedures. The initial extraction is made with acetone (three times). After evaporation of the solvents the extracts are then partitioned between water and diethyl ether to remove the non-lipids contaminant in the aqueous phase. The extracts are partitioned between aqueous ether extracts and then between aqueous methanol and hexane to eliminate non-toxic neutral lipids in the hexane phase. The crude toxin obtained after evaporation of the aqueous methanol layer is called methanol residue and is the material which is to be distributed to the investigators in other areas of research.

The approximate amount of solvents required to process 1 kg of the flesh or liver are as follows: 8 l of acetone, 2.5 l of diethyl ether, 1.5 l of hexane, and 0.5 l of methanol.

Distribution of Ciguatoxin Extract

It is proposed that the initial distribution of the toxin will be for the following purposes and to the following institutions and investigators:

Structural chemistry of ciguatoxin	1. University of Hawaii Honolulu	Dr Paul J. Scheuer
	2. Tohoku University Sendai	Dr Takeshi Yasumoto
Pharmacology of ciguatoxin	1. University of Hawaii Honolulu	Dr Martin D. Rayner
	2. Institute Louis Malardé Papeete	Dr Anne Marie Legrand
Immunological response to ciguatoxin	1. Commonwealth Serum Laboratories Melbourne	Dr Struan K. Sutherland

Other groups may be added or deleted from the list. It is proposed the allocations be made by a committee of advisors appointed by the South Pacific Commission.

Budget Requirements *

Budget for one year	US Dollars
Animal colony expenses	2,000
Chemical extraction and initial purification expenses	8,000
Half salary for a technician	10,000
Total costs for harvesting and extraction	20,000
Administrative costs	2,500

Running time of the program - 2 years

Total cost over two-year period - US$45,000

* Funds to obtain the necessary supplies of toxic fish from Gambier and Marquesas Islands will be furnished by the Institut Malardé

Administrative Arrangements

The South Pacific Commission will serve as the executing agency for the project. The SPC will seek the collaboration and co-operation of the Institute Louis Malardé in the preparation and isolation of the materials by means of a request to the High Commissioner of French Polynesia and the Director of the Institute. The SPC will also consider requests for the material and make known its availability to interested parties and institutions throughout the region and elsewhere with consultation and advice of a Committee on Ciguatoxin Distribution.