REPORT OF MEETING

SECOND EXPERT COMMITTEE ON CIGUATERA
SUVA, FIJI
22 JANUARY 1983

South Pacific Commission
Noumea, New Caledonia
June 1983

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*Following the retirement of Dr Albert Banner it was decided by the Hawaii group that Dr Paul Scheuer should represent them at the second Expert Committee Meeting.

I. INTRODUCTION

1. Ciguatera fish poisoning is of considerable concern in the Pacific region because of the morbidity it causes in certain countries, and because of its adverse effect on subsistence and commercial reef fisheries. Although it is not a significant cause of mortality in the region, and not a major cause of morbidity either, a few cases of fish poisoning can have a drastic effect on the utilisation of the shallow water fish resource. Avoidance of reef fish can have an adverse economic impact on small-scale artisanal fisheries, and, in a subsistence economy, can create an undesirable dependence on imported canned substitutes. Thus the effects of ciguatera fish poisoning are much greater than the occasional death and recorded morbidity.

2. There are several varieties of food poisoning resulting from the ingestion of marine animals, fish and other organisms. The main types are as follows:

1. Bacterial food poisoning from contaminated fish and marine animals.

2. Allergic reactions to seafood (especially shellfish).

3. Scombroid fish poisoning. Improper preservation of scombroid fishes (such as mackerel and tuna) can lead to the conversion of histidine to a histamine-like substance by bacterial action. Ingestion of these fish will lead to allergic-type manifestations.

4. Puffer fish poisoning. Tetrodotoxin is present in the viscera, skin and gonads of the puffer fish and can cause fatalities.

5. Clupeoid poisoning may result from the ingestion of sardines, anchovies or herring in the tropical Pacific and Caribbean. The condition is very uncommon, but may be fatal.

6. Ciguatera fish poisoning. A result of ingestion of fish containing ciguatoxin which is derived ultimately from the dinoflagellate Gambierdiscus toxicus.

7. Paralytic shellfish poisoning results from ingestion of shellfish contaminated with a toxin-producing dinoflagellates (Protogonyaulax catenella and P. tamarensis)

8. Toxic crabs. The ultimate source of the toxin is from red algae (Jania species) upon which the crabs may feed.

9. Toxic snails. Toxin-producing algae are thought to be the origin of the poison.

10. Turtle poisoning (particularly the hawksbill turtle). Source of toxin unknown.

3. Ciguatera poisoning is characterised by an initial phase of gastroenteritis (nausea, vomiting, abdominal pain, diarrhoea) beginning at about 4-6 hours after ingestion. This is followed by neurological symptoms (beginning at about 12 hours after ingestion) which include parasthesae (numbness), pruritis (itchiness), and other abnormal skin sensations. Myalga (muscle aching), bradycardia (slow pulse) and hypotension (low blood pressure) also occur. The gastrointestinal symptoms usually last only 6-12 hours, but the neurological symptoms persist for days or weeks. In some cases recurrence of the neurological symptoms may be precipitated by eating non-toxic fish or drinking alcohol. The treatment of ciguatoxic fish poisoning is symptomatic, and there are no specific remedies.

4. Investigations into the epidemiology and ecology of ciguatoxic fish poisoning have revealed a correlation between outbreaks of the condition and natural or man-made disruption of reef structures and coral death. The dead coral becomes colonised with certain algae which attract the epiphytic dinoflagellate, Gambierdiscus toxicus. Ciguatoxin is elaborated by the dinoflagellate and passes through the food chain via herbivorous fishes ultimately to the big carnivores (especially red snapper, moray eel, barracuda, etc), in whose viscera the highest concentration of ciguatoxin is found. Research in French Polynesia has demonstrated that the density of G. toxicus is positively correlated with the geographic distribution of toxic fish and clinical cases of ciguatera.

5. Control measures used at present involve the avoidance or banning sale of certain categories of fish which are known to be subject to ciguatoxicity. This is unsatisfactory because the vast majority of these fish will not be toxic, and some species may never be toxic. Monitoring of the population density of G. toxicus may be of value in predicting outbreaks in certain areas, and surveillance of toxicity of fish and clinical cases of ciguatoxic fish poisoning is of value in detecting epidemics early so that preventive (avoidance) measures can be taken. Individuals should treat with extreme caution the viscera of large fish, and testing the flesh on a cat is recommended if the fish is suspect.

6. The greatest need at present is a simple test which can be rapidly applied in the field and which will differentiate between a toxic and non-toxic fish. The present bio-assay using the mouse has yielded satisfactory and reproducable results; however, it is cumbersome, time consuming, expensive, exacting and must be performed in a laboratory. Little progress has been made recently in perfecting a simple and reliable field test for ciguatoxicity.

II. BACKGROUND

7. The South Pacific Commission has promoted and supported studies into fish poisoning for a number of years. The incidence of reported cases by country is included in the returns of the South Pacific Epidemiological and Health Information Service. The Commission has published a handbook on ciguatera fish poisoning prepared by Dr R. Bagnis, and is involved in its republication in an updated and amended form.

8. The South Pacific Commission has encouraged collaborative arrangements in order to facilitate research into the nature and causes of ciguatera fish poisoning. The problem of ciguatera fish poisoning has been under intensive investigation since 1974 when the South Pacific Commission organised the United States-French Polynesia-Japan tripartite co-operative programme and gave limited financial support to each group. Investigators from each of these groups constitute the membership of the Expert Committee. In 1975, Professor Yasumoto, in co-operation with the research group at the Louis Malarde Insitute of Medical Research in Tahiti, identified the cause of ciguatera poisoning as a unicellular dinoflagellate, Gambierdiscus toxicus, and demonstrated that the toxins contained in G. toxicus were ciguatoxin and maitotoxin.

9. The first meeting of the South Pacific Commission (SPC) Expert Committee on Ciguatera was held in Suva, Fiji, on 26 February 1981. At this meeting recent data on the epidemiology and biology of ciguatera fish poisoning were reviewed, and recommendations were made for continuing research and training. It was also recommended that additional meetings of the Expert Committee be held, and co-ordinated with World Health Organization (WHO) activities.

10. A meeting of the SPC Expert Committee on Ciguatera was incorporated into the 1983 work programme of the South Pacific Commission, and the date and venue of this meeting were scheduled (in consultation with WHO) so that it immediately followed the WHO Training Course on fish poisoning (Ciguatera type) held in Suva, 11-21 January 1983.

III. REPORTED MORBIDITY FROM FISH POISONING IN THE PACIFIC REGION, 1973-1982

by

Richard Taylor, Epidemiologist, South Pacific Commission

The number of cases of fish poisoning reported to the South Pacific Epidemiological and Health Information Service is set out in the following table.

The coverage and accuracy of reporting of this condition in the various Island nations is not known. Although there is considerable under-reporting, the number of cases reported, and the rate per population, gives some indication of the magnitude of the problem in the region, and the variation between countries, and over time.

The number of cases reported from each country depends on many factors, including: the awareness of the condition amongst the population and medical staff, frequency of contact between those with fish poisoning and the medical care system, efficiency of morbidity recording and the extent of its coverage, proportion of the population living near the sea and their degree of dependence on reef fish, extent of and variation in ciguatoxicity amongst edible species, local knowledge and avoidance of toxic fish, and the existence and success of official programmes for the control of ciguatoxic fish poisoning. It has been estimated that approximately 10-20% of cases are reported.

French Polynesia has consistently reported high numbers and rates for fish poisoning over the last decade. This is probably a reflection of a high level of awareness amongst medical staff and an efficient reporting system, as well as a relatively high frequency of ciguatoxic fish.

The atoll countries of Micronesia and Polynesia also report relatively high rates per population, probably related, in part, to the extent of dependence of the population on reef resources for food.

The high rate reported from New Caledonia is probably influenced by the extent of awareness amongst the population and medical staff, the high doctor/population ratio, and an efficient morbidity recording system.

Ciguatera fish poisoning causes a well-recognised clinical syndrome of gastroenteritis and numbness or itchiness of the skin; and this may lead to neurotic after-effects. Mortality is rare (less than 1%), and the magnitude of reported morbidity does not indicate that it should be considered a high priority public health problem in most Pacific countries. However, ciguatera fish poisoning has an adverse effect on utilisation of the reef fish resource out of proportion to the reported morbidity. Only symptomatic treatment is available, and prevention is the only avenue to control of the problem.

In some parts of the Pacific the extent of fish poisoning may curtail the consumption of fresh fish, but protein deficiency is unlikely as canned protein foods are usually available and affordable. However, a dependence on imported canned substitutes usually has adverse economic and nutritional effects.

Table 1: Number of cases of fish poisoning reported to the South Pacific Epidemiological and Health Information Service, by country, by year

Country (estimated mid-1981 population)	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982+	1973 - 1982+		
											Total	mean	rate**
American Samoa (33,200)	4	-	-	-	-	-	70	30	31	29(58)	193	19.3	63.3
Cook Islands (17,700)	-	-	-	-	-	-	-	1	2	-	3	0.3	1.6
Fiji (637,000)	6	26	150	29	69	201	131	256	123	89(178)	1169	116.9	19.7
French Polynesia (149,800)	607	867	625	660	502	821	677	937	1145	181(362)	7203	720.3	525.8
Guam (Civilians) (106,400)	-	-	21	16	6	6	9	-	4	-	62	6.2	7.1
Kiribati ✱ (59,900)	101	175	187	77	41	38	78	-	286	102(204)	1187	118.7	221.9
Nauru (7,300)	-	-	-	-	-	-	1	5	-	-	6	0.6	8.2
New Caledonia (142,500)	-	200	518	647	487	488	188	147	107	51(102)	2884	288.4	212.1
Niue (3,200)	7	1	35	4	-	-	-	-	3	-	50	5.0	131.6
Papua New Guinea (3,066,000)	-	-	16	-	-	-	-	-	-	-	16	1.6	-
Pitcairn (100)	-	-	-	-	-	-	-	-	-	-	-	-	-
Solomon Islands (235,000)	1	7	-	7	6	6	-	4	4	-	35	3.5	1.7
Tokelau (1600)	-	-	-	8	-	-	14	-	3	7(14)	39	3.9	243.8

Table 1: (contd)

Number of cases of fish poisoning reported to the South Pacific Epidemiological and Health Information Service, by country, by year

Country (estimated mid-1981 population)	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982+	1973 - 1982+		
											Total	mean	rate**
Tonga (98,400)	11	58	12	17	43	13	8	7	2	13(26)	197	19.7	21.9
TTPI (129,000)	240	264	208	313	326	296	191	217	163	54(108)	2326	232.6	180.3
Tuvalu ✱ (7,600)	-	-	-	49	44	71	21	27	73	19(38)	323	32.3	430.7
Vanuatu (120,000)	-	-	35	28	50	53	67	-	32	10(20)	285	28.5	28.6
Wallis et Futuna (11,200)	-	-	3	7	-	-	-	-	-	-	10	1.0	10.3
Western Samoa (157,000)	65	89	15	17	81	179	62	115	127	71(142)	892	89.2	58.7

✱ Prior to 1976 the Gilbert and Ellice Island Colony was composed of what is now Kiribati and Tuvalu

TTPI: Trust Territory of the Pacific Islands.
Includes what is now the Northern Marianas, Palau, Federated States of Micronesia and Marshall Islands.

† January-June 1982

( ) = Estimated for year

\+ Calculated using 1982 estimates

** rate per 100,000 population (1977)

IV. REVIEW OF RECENT RESEARCH RELATED TO CIGUATERA AT THE LOUIS MALARDE INSTITUTE FOR MEDICAL RESEARCH, TAHITI, FRENCH POLYNESIA

by

Raymond Bagnis, Scientific Director of Programme

THE FOLLOWING PEOPLE ARE PARTICIPATING IN THE PROGRAMME

1. Full time

R. BAGNIS, Medecin du Service de Sante des Armees, docteur es-Sciences et en Biologie Humaine, Head of the Medical Oceanography Unit.

A. INOUE, Biologiste marin, Professeur a l'Universite de Kagoshima, Japon (March 1981 - March 1982).

E. CHUNGUE, Biochimiste, doctorat de specialite.

S. CHANTEAU, Biologiste, doctorat de specialite.

A.M. LEGRAND, Physiologiste, doctorat de specialite.

A. HUYARD, Etudiant en 3eme cycle en Biochimie (VSNA : December 1980 - April 1982).

J.Y. MICOUIN, Agrege biochimie (VSNA, since April 1982).

J.H. DROLLET, Licencie en Biologie Animale.

I. LECHAT, Technicienne superieure de laboratoire.

M. GALONNIER, Aide-laborantine (until July 1981).

A. DU BARRY, Aide-laborantine (SMCB, since March 1982).

J. BENNETT, Agent technique en biologie marine.

G. JACQUET, Agent technique animalier.

J. TEORE, Agent technique de laverie.

2. Part time

F. PARC, Medecin du Service de Sante des Armees, biologiste des Hopitaux, Head of the Medical Biology Unit (until July 1982).

S. RONGERAS, Technicienne superieure de laboratoire (toxin extraction).

C. LOTTE, Technicienne superieure de laboratoire.

V. GAY, Technicien superieur de laboratoire (Seawater bacteriology).

M. BARSINAS, Agent technique (preparation of biological material, diving).

M. CHEBRET, Agent technique (mosquito testing, diving).

E. FULLER, Agent technique (mosquito testing, laboratory assistant, site worker).

M. GERMAIN, Aide-laborantin terrain, plongee.

R. TETIARAHI, Agent technique plongee.

R. SPILLIAERT, Aide-laborantin, SMCB (until July 1981).

R. TEHINA, Manoeuvre d'animalerie.

E. AA, Manoeuvre d'animalerie.

VARIOUS AVENUES OF INVESTIGATION

1. Pharmacological studies on the action of ciguatoxin, scaritoxin and maitotoxin, the three main toxins involved in ciguatera.

1.1 In vivo studies on anaesthetised cats.
- (a) Cardiovascular and respiratory effects of each poison.
- (b) Tests to determine if modification of the specific effect of ciguatoxin results from (i) the administration of substances such as hexamethonium, atropine, propanolol, phentolamine, and clonidine; and (ii) bilateral adrenalectomy and bilateral vagotomy.

1.2 In vitro studies on isolated organs.
- (a) Rats' atria.
- (b) Segments of intestine of rabbits.
- (c) Segments of aorta of rabbits and rats.

1.3 Principal results.
- Ciguatoxin and scaritoxin behave alike, while maitotoxin acts in a slightly different manner.
- The effects of each toxin are roughly the same whether the extracts used are crude or purified.
- In small doses ciguatoxin stimulates cholinergic activity, while in stronger doses it acts as a stimulant of alpha-adrenergic activity.
- The overall findings reflect a series of complex effects which indicate that there are several target organs. But it is difficult at the present stage to locate the exact place or places where the effect occurs (for instance: central, ganglial, post-ganglial with release of acetylcholine, or in the post-synaptic muscarinic receptors).
- Other findings indicate that strong doses of ciguatoxin may have an effect on certain central cholinergic receptors.

2. Study in Vanuatu and New Caledonia* of a natural antidote for ciguatera poisoning: the medicinal plant Ximenia elliptica

2.1 Toxicological and pharmacological effects on live rats and mice.

2.2 Physiological effects on a rat's atrium in vitro.

2.3 The general findings reveal the following:
- the toxicity of plant filtrates and lyophilised filtrates, in strong doses;
- attenuating and sometimes protective effect, in cases of mild forms of ciguatera poisoning.

3. Research to establish whether ciguatoxin has any affinity for certain known receptors of the nervous system (preliminary in vivo and in vitro studies on binding)

- Cholinergic receptors of the muscarinic type.
- Alpha 1 and 2 adrenergic receptors.

This study has begun very recently, and only practical work for purposes of methodological standardisation has been carried out.

4. Algae culture in the laboratory

4.1 Isolation of several peridinians from ciguateric biotopes. In vitro acclimatisation, with varying degrees of speed and facility, of about twenty epibenthic species.

4.2 Semi-mass culture of Gymnodinium toxicus, Ostreopsis lenticularis, Ostreopsis ovata, P. lima, Gymnodinium sp. for testing production of toxin. Speed of reproduction varies from one species to another, the population doubling on average every 36 hours in the case of P. lima, every 2.7 days for G. toxicus, and every 5 days for O. lenticularis.

4.3 Study to determine the optimal light intensity, temperature and salinity for culture of the foregoing species on a medium of seawater enriched with 0.5% Provasoli ES 1 supplement.
In respect of G. toxicus these tests revealed the following: 2500 lux, 25°C, 34 to 35ppt. The most interesting finding is the relatively weak luminosity required by this peridinian in comparison with all the others, which require light intensity of between 7000 and 8000 lux.

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* In conjunction with ORSTOM-Noumea.

4.4 Axenic culture of G. toxicus, O. lenticularis and O. ovata.
This is achieved by repeated washings in sterile seawater and successive sub-cultures; the axenisation is monitored fortnightly by inoculation of one drop of the culture medium onto sterile peptone seawater enriched with yeast extract.

4.5 Nutrient requirements of the three above-mentioned species in artificial seawater medium.
Various Provasoli ASP media have been tested: ASP 7 so far appears to be the best, but the rate of multiplication is no better than with ES 1.

4.6 Toxin production of peridinians grown in semi-mass culture. Of the six species from a single clone stock that have been tested, only G. toxicus has proved positive to any significant extent. Production of maitotoxin was less than expected (1000 cells producing 1.5 mouse units). Although the production of ciguatoxin was small (1000 cells giving 0.6 mouse/unit), it was relatively high in comparison with maitotoxin production.

5. Biochemical aspects

5.1 Effects of ciguatoxin on the serum cholinesterase activity of experiperimental animals was studied. The toxin was administered to mice by intra-peritoneal injection and to cats orally. In both cases quantitaive analysis of pseudo-cholinesterase, using the Bohringer kit system (based on the Ellman reaction), showed no modification of any significance, or that could be interpreted.

5.2 Comparative study of serum proteins (by disc electrophoresis) of healthy fish and poisonous fish belonging to two microphagus and potentially ciguaterigenic species, i.e. the surgeonfish C. striatus and Acanthurus lineatus.
The results obtained after separating the protein fractions on a polyacrylamid gel without gradient, while not significant, do suggest the possibility of an increase in the amount of lipoproteins and very slow-globulins.

6. Research to establish a ciguatoxin tracer

Bearing in mind the results of earlier studies carried out at the "Institut de chimie des substances naturelles" (Institute for chemistry of natural substances) which showed the presence of 1,2 propanediol in certain ciguatoxic portions of muscle taken from toxic fish, we have tried to find evidence of this substance in ciguatoxic extracts from fish belonging to different links in the ciguaterigenic food chain.

Three approaches were used in the assays:

. chemical approach: identification by chromatography in the gaseous phase;
. biochemical approach: measurement of the induced anticholinesterase effects;

. pharmacological approach: examination of the toxicity found in the whole animal and in isolated organs (e.g. rat's atrium).

The results of all the abovementioned research were negative, which led us to suppose that the presence of propanediol originally found in ciguatoxic extracts was probably caused by contamination of laboratory equipment or reagents.

7. Study on the sensitivity of different kinds of mosquito to ciguatoxin

Liver homogenates and more or less purified lipid extracts from the muscle of fish with varying degree of toxicity have been administered intra-thoracically to batches of mosquitoes of various species, including Toxorhynchites amboinensis, Aedes polynesiensis, Aedes aegypti, Culex pipiens.

The difference between the toxic and the control substances was evident within one hour from their behaviour, inability to fly, or death of all the mosquitoes. However, it is not yet possible to establish a formula which relates the toxic effect on mice to that on mosquitoes. Furthermore, there are variations in the results according to the species of fish.

The most encouraging results, which might make it possible to have an additional, and more rapid means of testing for toxicity of fish, were obtained with Aedes aegypti. The mosquito test has the advantage of requiring less flesh or viscera, and less solvent for extraction.

8. Study on hypersensitivity to ciguatera

8.1 Development of an experimental model

. Familiarisation with techniques of injection in a mouse's foot pad and measurement of foot pad thickness using a micrometer.
. Preparation of toxin antigens of varying degrees of purity and various controls.
. Immunisation by intravenous primary injection, and subcutaneous boosters in the foot pad every 3 to 4 days.
. Monitoring of the volume of the foot pad for one month.
. Negative results that: (i) give no indication as to whether or not the hypersensitivity observed in man confers immunity, and (ii) a fortiori, are insufficient to determine whether the phenomenon is one of cellular mediation (as the human clinical picture suggests), or humoral mediation.

8.2 Amendment of protocol for study of delayed hypersensitivity

. Ciguatoxin (CTX) bound to sheep's red cells (SRC) is used as the experimental antigen, and a human serum albumen (HSA)-CTX conjugate is used as the control antigen.
. Preliminary selective inhibition of the activity of B. lymphocytes in mice by administration of cyclophosphamide.
. Research to establish the optimum immunising dose of SRC (10^8 cells):
  (i) Initial intravenous injection to 2 batches, comprising 21 and 30 mice, of 2 and 20 mouse units (MU) respectively, of CTX bound to 10^8 SRC.

(ii) Division of each batch into three groups, which, four days later, are given, respectively, SRC alone, SRC-CTX and HSA-CTX.
(iii) Measurement 24h. later.

. Results do not indicate, in the conditions of this experiment, any manifestation at all of hypersensitivity with conventional cellular mediation.

8.3 Cutaneous tests

. The Prick test technique was used, the antigen being a solution of purified ciguatoxin at 20 MU in 10 microlitres of physiological water (normal saline).
. The tests were carried out on two groups, each comprising 100 individuals, from a population which, during the last ten years, has been subject to a severe outbreak of ciguatera.
. The local signs observed are not interpreted as revealing evident or important symptoms of immediate hypersensitivity.
. On the other hand a few indications were noted of histamine release, but up to the time of our analysis it was not possible to establish any significant correlation between cases of alleged clinical hypersensitivity and the few cutaneous reactions.

9. Ecological and epidemiological aspects

9.1 Study of the experimental sites at Hitiaa, Tahiti

(a) Monthly fluctuations of the population of Gambierdiscus toxicus (the dinoflagellate which is the original elaborator of the toxin complex involved in ciguatera) on a fringing reef observed since June 1976: consistently low density.

(b) Comparative evolution of populations of three other species of dinoflagellate more or less frequently found in ciguaterigenic biotopes. Ostreopsis lenticularis, O. ovata, Prorocentrum lima (non-toxigenic or only partial producers of the ciguatera toxin complex). A much higher density of these species was found, especially of the first two, which are practically non-toxigenic (although there is less bloom).

(c) Some marine environmental factors associated with these populations: temperature, salinity, dissolved nutrients (nitrates, phosphates, silicates), light intensity, etc. No significant correlation between any of the measured parameters and the population density of any of the monitored dinoflagellates species has been noted.

(d) Evolution of ciguatoxicity of the fishes from the first links of the benthic foodweb: the amount of toxin in the digestive tract, liver and muscles of the surgeonfish (Ctenochaetus striatus) has been stationary during the last 18 months at a low level, with a good correlation with the G. toxicus population since 1977.

9.2 Yearly surveillance of above-mentioned (b) dinoflagellates around Tahiti island, for observation of the occurrence of bloom. The data obtained did not show any significant increase of the populations, which remain at a very low level.

9.3 Survey in Mataiva atoll (in Tuamotu archipelago)

(a) Geographical distribution of G. toxicus, with regard to the incidence of the drilling works - undertaken since 1978 for dredging the phosphates of the lagoon area. No massive colonisation of large places of the reef or the lagoon, but a few small "explosive epidemics" on patches in the lagoon, on the outside reef, and in the pass. There has been no new big outbreak of fish poisoning during the past two years; nevertheless, 15 cases involving three different species (surgeonfish, mullet and jack) have been reported during the period.

(b) A trial of evaluation of ciguateric risk, using the local geomorphology of the atoll and the increase of sedimentation process, has been done. It seems that Mataiva atoll is naturally exposed periodically to sudden changes in environmental factors and hydrodynamism. The effect of past events and more recent drilling works for phosphate exploitation is difficult to evaluate at the present time. Monitoring of G. toxicus distribution and density has been undertaken, and will be pursued in the future. The occurrence of new outbreaks may depend on the additive effects of natural seasonal disturbances and man-made disturbances.

9.4 Occasional surveys on the geographic distribution of G. toxicus have been carried out on Moorea, Bora Bora, Tetiaroa, Hao, Huahine. No observations of any bloom have been made.

9.5 Survey on the ciguatoxicity of fishes from Marquesas Islands. Cat-tests and mouse-tests have been carried out on 13 large sized specimens of the red snapper Lutjanus bohar, the jack Caranx ignobilis, the emperor fish Lethrinus miniatus coming from various areas. Only one specimen was toxic.

9.6 Monitoring of the G. toxicus population in the lagoons located adjacent to the town of Papeete and its suburbs (with a special attention to an eventual relationship with bacterial pollution). No correlation has been found between the population density of G. toxicus and the number of fecal coliforms, Escherichia coli and fecal streptococci. On the contrary, most of the high densities of the above-mentioned bacteria are found along the fringing reef and the few positive results concerning G. toxicus come from the barrier-reef area.
The ciguateric risk in the whole area is low, in spite (and perhaps because) of the permanent and complex disturbance caused by a number of various different aggressive physical and chemical agents.

10. Analysis of the epidemiological and clinical forms filled at the Institut Louis Malarde clinic in 1982: 183 patients attended

One hundred qnd eighty-three patients attended for consultation and treatment. The cases involved 39 different species from 13 ichthyological families belonging to the various trophic levels. The origin of the fish, bought mainly in Papeete and Pirae market places, is often unknown, but most of them come from the Tuamotu atolls.

V. REVIEW OF RECENT RESEARCH RELEVANT TO THE MARINE-FOOD TOXIC POISONING, AT TOHOKU UNIVERSITY, SENDAI, JAPAN

by

Takeshi Yasumoto

1. Toxicological Research

1.1 Ciguatoxin

Purified ciguatoxin was separated into two components by high performance liquid chromatography on Bondapack C-18 column. Unlike Dr Scheuer who uses only moray eel for the source of toxin, Dr Bagnis, who supplied our sample, used various species of fish for extraction. It is likely, therefore, that our result was caused by the multiple toxin sources. An alternative explanation is that the different chromatographic behaviour was caused by difference in steric conformation of a single component. Such phenomenon is occasionally observed in certain ionophoric compounds. However, the latter explanation seems less probable.

1.2 Scaritoxin

Scaritoxin was purified by similar chromatographic procedures as were used for purification of ciguatoxin, with slight modification in solvent systems. The sample size was too small to attempt spectral measurement. Chemical reactions designed to test functional groups confirmed the presence of only hydroxyl and olefinic moieties. Pharmacological investigation carried out by Dr Y. Ohizumi of the Mitsubishi-Kasei Institute of Life Sciences indicates that the mode of action of scaritoxin closely resembles that of ciguatoxin.

1.3 Maitotoxin

Maitotoxin obtained by mass culture of Gambierdiscus toxicus was purified to a toxicity beyond 0.15 ug/kg (i.p. mouse). Quantitative analyses of sugar and fatty acid moieties which compose a part of the molecule are being carried out. The most prominent feature of maitotoxin is its pharmacological action. In experiments with rat pheochromocytoma cell line, maitotoxin induced a release of norepinephrine (NE) at a low concentration (10^{-8} g/ml). The maitotoxin-induced NE release was dependent on the calcium concentration in the medium, but was independent on sodium concentration. The NE release was not blocked by tetrodotoxin, a specific sodium channel blocker, but was blocked by verapamil, tetracaine and Mn^{++}. The results suggested that maitotoxin specifically activates the calcium channel.

1.4 Toxins of *Prorocentrum lima*

Prorocentrum lima is a benthic dinoflagellate of widespread distribution in coral reef areas. It often outnumbers *G. toxicus*. Two lipoidal toxins tentatively named PL toxin I and II ($PLT_{1,2}$) attracted our attention because of their close resemblances in chromatographic properties to scaritoxin and ciguatoxin. Both toxins were purified in a similar manner as employed for purification of ciguatoxin. PLT_1 as found identical with okadaic acid (Figure 1), a cytotoxic component isolated from sponges. PLT_2 as a mixture of diol esters of okadaic acid. Okadaic acid was also identified as one of the causative toxins of diarrhetic shellfish poisoning which is prevalent in Japan. Oral intake of a small dose (30-40 ug) of okadaic acid causes gastrointestinal disorders. Judging from the abundance of *P. lima* in reef areas and the chemical resemblences between its toxins and ciguatoxin, it is conceivable that both okadaic acid and its esters are accumulated by herbivores and contribute to the development of gastrointestinal symptoms when the fish are ingested.

2. Research in Non-Ciguatera Marine Food

2.1 Paralytic shellfish poisoning

Paralytic shellfish poisoning has been a subject of extensive studies in many northern countries because of its health hazard and impact on shellfish industries. On the other hand, information concerning paralytic shellfish poisoning (PSP) in the South Pacific Region has been limited to that of Maclean who reported occurrence of 163 cases, including 10 deaths, in Papua New Guinea during 1971-1973. In symptomatology the poisoning was similar to PSP in the north. The shellfish toxicity was associated with the blooms of a dinoflagellate *Pyrodinium bahamense*, which was later amended to *P. bahamense* var. *compressa*. However, no chemical study was undertaken to identify the toxins in the shellfish and the dinoflagellate.

Recently we collected *P. bahamense* var. *compressa* and bivalves infested by this dinoflagellate at Koror, Palau Islands, and proved the presence of gonyautoxin-V and VI ($GTX_{5,6}$). neosaxitoxin (neoSTX), saxitoxin (STX) and decarbamoylsaxitoxin (decSTX). Their chemical structures are shown in Figure 2. The relative abundance of toxic components are shown in Table 1., together with those of toxic crabs and gastropods. Examination of water samples from various stations at Palau indicated its abundance in Arumizu Bay, Koror. Toxicity levels of the shellfish in the Bay were extraordinarily high. Ingestion of only one individual of *Spondylus butleri* may cause death in an adult. It seems urgent to advise the island population about the potential danger of the shellfish in the Bay to visitors (the local population are aware of the problem and will not eat shellfish from this area).

Besides Papua New Guinea and Palau, the occurrence of *Pyrodinium* was confirmed by other workers in Brunei and Sabah, and in Oahu and Kauai of Hawaii by our group. Shellfish poisoning resembling PSP has also occurred in Fiji and Solomon Islands (personal communication from Dr Raj). Apparently, the danger of PSP in the tropical region is as common as in northern waters, and an establishment of an effective surveillance system seems urgent.

2.2 Toxic crabs

There has been a widespread rumour in the Pacific islands regarding the occurrence of toxic crabs. The following three species of crab belonging to family Zanthidae have been reported by Hashimoto and his co-workers to be highly toxic: Zosimum aeneus, Atergatis floridus and Platypodia granulosa. Z. aeneus is the one most frequently implicated in human poisoning and the presence of STX in this species was confirmed by the above group. In view of recent advances in techniques of analyzing saxitoxin analogues, re-investigation of the toxin composition in the above species was undertaken by the authors. In the former two species neoSTX was the major toxin, followed by STX and trace amounts of GTX_5 and decSTX. P. granulosa contained almost exclusively STX but trace amount of decSTX was recognized.

Subsequent ethiological investigation led us to identification of a red calcareous alga Jania sp. as the primary source of toxins in the crabs. Moreover, crabs other than the above three species were found to bear toxins when collected from a Jania-rich spot. The following seven species were newly found to be toxic: Neozanthias impressus, Actaeodes tomentosus, Eriphia scabricula, Pilumnus vespertilio of family Zanthidae; Schizophrys aspera of family Majidae; Thalamita sp. of family Portunidae; and Percnon planissimum of family Grapsidae. Toxin compositions in the representative species of crab are given in Table 1. The result suggests that crabs not listed here may become toxic if the toxic alga is abundant in their habitats.

2.3 Toxic marine snails

Based on a legend in Okinawa that ingestion of the visceral parts of gastropods may cause occasional intoxication, representative species in the area were analysed for the alleged toxins.

Two turban shells, Turbo argyrostoma and T. marmorata, and two top shells, Trochus nilotica maxima and T. pyramis, were found to contain paralytic shellfish toxins in the viscera. STX, neoSTX, trace of GTS_5 and a new component of unknown structure were identified. The new toxin was named turban shell toxin (TST). The toxin profile in each species is shown in Table 1. The highest toxic score recorded was 20 MU/g, which was well above the quarantine level for paralytic shellfish toxins (4 MU/g). However, a marked regional variation existed among specimens tested. As the toxic alga Jania sp. was present in the stomach of the gastropods, the alga was presumed to be the source of the toxins.

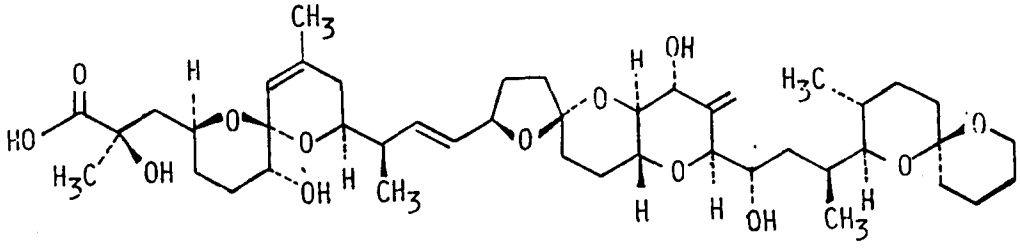
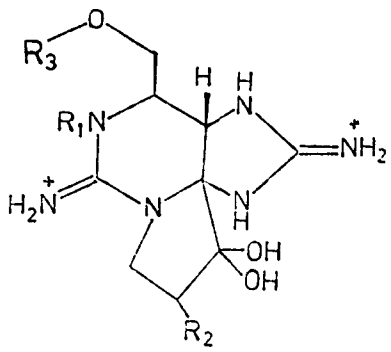


Fig. 1. Structure of okadaic acid.



		R_1	R_2	R_3	Lethality (MU/μmol)
1)	saxitoxin	-H	-H	$-\overset{O}{\overset{\|}{C}}-NH_2$	2,045*1
2)	neosaxitoxin	-OH	-H	$-\overset{O}{\overset{\|}{C}}-NH_2$	1,617*2
3)	gonyautoxin-I	-OH	$-\alpha OSO_3^-$	$-\overset{O}{\overset{\|}{C}}-NH_2$	1,638*1
4)	" -II	-H	$-\alpha OSO_3^-$	$-\overset{O}{\overset{\|}{C}}-NH_2$	793*1
5)	" -III	-H	$-\beta OSO_3^-$	$-\overset{O}{\overset{\|}{C}}-NH_2$	2,234*1
6)	" -IV	-OH	$-\beta OSO_3^-$	$-\overset{O}{\overset{\|}{C}}-NH_2$	673*1
7)	" -V	-H	-H	$-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-SO_3^-$	136*2
8)	" -VI	-OH	-H	$-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-SO_3^-$	108*2
9)	" -VIII	-H	$-\beta OSO_3^-$	$-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-SO_3^-$	277*3
10)	gonyautoxin-VIII epimer	-H	$-\alpha OSO_3^-$	$-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-SO_3^-$	20*3
11)	decarbamoyl-saxitoxin (PBT)	-H	-H	-H	1,378*4

*1 Genenah and Shimizu, 1981. *2 Harada et al., 1982a.
*3 Wichmann et al., 1981. *4 Harada et al., 1982b.

Fig. 2. Structures and specific activities of paralytic shellfish toxins.

TABLE 1. COMPOSITION OF PARALYTIC SHELLFISH TOXINS IN ORGANISMS OF TROPICAL WATERS

Organism	Locality	GTX_1	GTX_2	GTX_3	GTX_4	GTX_5	GTX_6	neo STX	STX	decSTX	TST
Rhodophyta											
Jania sp. 1	Okinawa	++++	+++	+	-	-	-	-	-	-	-
Decapods											
Zosimus aeneus	Okinawa	-	+	-	-	-	-	+++	+++	+	-
Platipodia granulosa	Okinawa	-	-	-	-	-	-	-	++++	+	-
Atergatis floridus	Okinawa	-	+	-	-	-	-	++++	+++	+	-
Eriphia scabricula	Okinawa	+	+	+	-	-	-	+++	+++	-	-
Pilumnus vespertilio	Okinawa	+	+	+	-	-	-	++	++++	-	-
Thalamita sp.	Okinawa	+	+	+	-	-	-	++	+++	-	-
Gastropods											
Turbo marmorata	Okinawa	-	+	-	-	-	-	+	+++	-	++++
Turbo argyrostoma	Okinawa	-	+	+	-	-	-	+	+++	-	+++
Tectus pyramis	Okinawa	-	+	-	-	-	-	+	+++	-	+++
Pelecypods											
Spondylus butleri	Palau	-	-	-	-	+	-	++	++++	+++	-
Tridacna crocea	Palau	-	-	-	-	+	-	+	++++	++	-
Septifer bilocularis	Palau	-	-	+	-	-	-	+	+++	+	-
Dinoflagellates											
Pyrodinium bahamense var. *compressa*	Palau	-	-	-	-	++	+	++++	+++	++	-
Protogonyaulax tamarensis	Iwate	++++	+++	+	++	-	-	++	-	-	-

VI. CIGUATERA RESEARCH AT THE UNIVERSITY OF HAWAII

by

Paul J. Scheuer, Professor of Chemistry

At the time for the previous meeting of ciguatera experts in February, 1981, three groups were working in Hawaii on ciguatera problems. One group directed by Professor A. H. Banner and Dr N. Withers, with funds from the National Marine Fisheries Service and from Sea Grant, was concerned with the ecology and the culturing of Gambierdiscus toxicus. A second group under Professor Y. Hokama, with funds from the U.S. Food and Drug Administration, concentrated on the establishment of a radioimmuno- or enzyme-linked bioassay for the rapid detection of ciguatoxin. A third group, under my direction and funded by the National Marine Fisheries Service, has had as its objective the elucidation of the molecular structure of ciguatoxin.

The first two research groups, while still in existence, are operating on a reduced scale because of diminished funding and Professor Banner's retirement at the end of 1982. The third (my) group has continued its activities at the previous level.

The most noteworthy, albeit serendipitous, event during the past two years has been the spontaneous crystallization of our ciguatoxin sample. This not only proved conclusively that our sample was homogeneous, but provided a solid foundation for the solution of the ciguatoxin structure by X-ray diffraction techniques. Our collaborating crystallographer, Professor J. Clardy of Cornell University, inspected the crystals and judged them too small ($<0.80 mm^3$) for direct study.

We have since attempted to grow larger crystals and to prepare a ciguatoxin derivative that might be crystallized. A functional derivative incorporating a heavy atom would be particularly useful as the size of the ciguatoxin molecule (1111.7 $\pm$ 0.3 daltons), encompassing as it does some eighty carbon and oxygen atoms, renders the structural solution by direct X-ray techniques extremely difficult and even doubtful. We have in fact succeeded in preparing a p-bromobenzoate derivative, which we are currently attempting to crystallize.

Our most serious handicap has been the lack of toxin. We had been able to isolate 1.3 mg of pure (LD_{50} 0.45 ug/kg, ip mice) ciguatoxin from approximately 60 kg of moray eel viscera. Slightly more than half of the sample was lost by spills and transfers while we attempted to obtain a ^{13}C NMR spectrum at a mainland U.S. laboratory. We were able to secure more toxin from a stored supply (25 kg) of eel viscera. We thus have now in hand a total of 1 mg of ciguatoxin. Although we are working on a microscale, each experiment (crystallization, derivatization), in order to have a minimum chance of success, needs at least 0.2 mg of material. Furthermore, we believe that it would be unwise to comit more than half of our supply at any given time. As a result of these constraints, our progress has been slow.

In order to conclude our structural studies of ciguatoxin, we urgently need a new supply of toxic eel viscera.

VII. COMMENTS BY OBSERVERS

Dr B. Dazo (WHO Regional Office) emphasised that there was a lack of good epidemiological data on ciguatera fish poisoning in the Pacific, with the notable exception of French Polynesia. He emphasised that it was too early to talk of prevention and control programmes to solve the problem, and that surveillance was the priority at present. The WHO training course had contributed to increased awareness of the problem in the region, and it was anticipated that more information would be available from returns of the clinical/epidemiological reporting form designed at the training course.

Dr Tony Lewis (Fiji Ministry of Agriculture and Fisheries) also pointed to the lack of epidemiological data on ciguatera fish poisoning. There was also a lack of biological information, and this resulted in blanket bans on certain genera (e.g. coral trout, barracuda) which were important economically (especially for export), when only certain species may be potentially ciguatoxic. The possibility of Fiji Fisheries supplying viscera of potentially ciguatoxic fish to researchers was discussed.

Dr Hazra Bibi Haq (Institute of Marine Resources, University of the South Pacific) explained her work in relation to ciguatera fish poisoning and the successful development of the mouse bio-assay technique by the USP group.

VIII. RECOMMENDATIONS

Preamble

The Committee felt that the highest priority for the eventual control of ciguatera fish poisoning was the development of a simple diagnostic test which could be used on fish in the field. Unfortunately, there have been no recent scientific breakthroughs which would suggest that such a test will be available in the near future.

Another priority, as expressed by doctors who deal with cases and the population at large, is the development of effective treatment. At present the only therapy available is symptomatic, and it is difficult to draw up definitive schedules because of the variability in manifestations of ciguatera fish poisoning. Work continues at Louis Malarde on a herbal remedy used in Vanuatu.

The specific recommendations of the Committee were:

Recommendation No.1:

The availability of ciguatoxin is essential for continuing research, and for the development of suitable assays. The Committee strongly urges the South Pacific Commission to assist with obtaining funds to facilitate continued and increased production of toxin.

Recommendation No.2:

The epidemiological surveillance of fish poisoning in the Pacific region should be continued and upgraded. Use of the new reporting form developed at the WHO Training Course on Fish Poisoning (Ciguatera type) in Suva, January 1983 should be encouraged (see Annex).

Recommendation No.3:

Endemic countries should be encouraged to institute surveillance for ciguatera and monitoring of the density of *G. toxicus*, including risk assessment programmes, so that rational prevention and control measures can evolve.

Recommendation No.4:

Recognising that considerable work has already been done on developing diagnostic tests for ciguatoxicity, the Committee recommends that the South Pacific Commission continue to support the development of more practical simple and reliable assays for ciguatoxin.

Recommendation No.5:

The Committee endorsed the recommendation of the WHO Training Course on Fish Poisoning (Ciguatera-type) which stated that:

"It would be extremely useful if endemic countries could be supplied with the SPC handbook on fish poisoning, and the group requests the SPC

to facilitate early re-publication and distribution of the document". The Committee suggested that a section on Gambierdiscus toxicus should be included in the revised edition.

Recommendation No.6:

The Committee recommended that studied be continued into the chemical structure, pharmacological action and immunological properties of ciguatoxins, and into the growth characteristics and toxin production of G. toxicus and other dinoflagellates.

Recommendation No.7:

It was recommended that the proposal for increased production of ciguatoxin required for further research, formulated by the last Expert Committee, be resubmitted by the South Pacific Commission to potential funding agencies.

Recommendation No.8:

In view of the increased awareness of ciguatera fish poisoning generated by the WHO training course it is anticipated that technical co-operation between ciguatera endemic countries in the region could develop. The Committee recommended that the South Pacific Commission assist with the development of project proposals for monitoring and research into ciguatera involving countries of the region on a joint collaborative basis.

Recommendation No.9:

Copies of the report of this Expert Committee Meeting should be sent to participants of the WHO training course, Directors of Health, and Fisheries Officers of the countries of the region, and other relevant people and organisations.

ANNEX

SEA FOOD POISONING

Please tick whichever is applicable

Consumer's Name _____ Age _____ Sex _____ Village _____

Country _____ Address _____ Clinic/Hosp. _____

Food: Fish ☐ Crab ☐ Shellfish ☐ Others (specify) _____

Local name _____ Date caught/bought: _____

English name _____ Place of catch _____

Method of preservation _____ No. of people eating same fish _____

Date of consumption _____ No. of people with symptom _____

Date of onset of symptoms _____ Method of preparation _____

No. of people hospitalised _____ Date _____ Duration _____

Died _____ Autopsy finding _____

Previous history of sea-food poisoning:

Date _____ Type _____ No. of people involved _____

Hospitalised _____ Died _____

SYMPTOMS

	YES	NO
1. Fever or chills	☐	☐
2. Vomitting	☐	☐
3. Diarrhoea	☐	☐
4. Headache.....	☐	☐
5. Muscle cramps.....	☐	☐
6. Joint aches	☐	☐
7. Tingling or numbness sensations	☐	☐
8. Pin pricking sensation upon touching water.....	☐	☐
9. Difficulty of walking	☐	☐
10. Difficulty of talking.....	☐	☐
11. Difficulty of breathing	☐	☐
12. Difficulty of urinating	☐	☐
13. Special taste in mouth	☐	☐
14. Skin itching or redness	☐	☐

How long after eating symptoms above developed? _____

_____

Complementary brief medical data.

Pulse ☐ BP ☐ ☐

Pupils ☐ Death ☐

_____

Additional information: _____

_____

Investigator _____ Date _____

Signature _____