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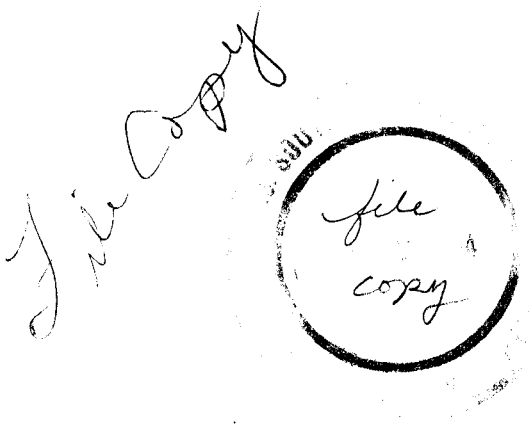

SUMMARY REPORT OF THE PACIFIC CIGUATERA WORKSHOP HONOLULU, HAWAII, 18-20 MARCH 1981

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Cosponsored by the Honolulu Laboratory and the
University of Hawaii Sea Grant

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The Hawaii Ciguatera Workshop, cosponsored by the Honolulu Laboratory and the University of Hawaii Sea Grant (UHSG), was held at the Honolulu Laboratory, Southwest Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Honolulu, Hawaii from March 18 to 20, 1981. Mr. Richard S. Shomura, Honolulu Laboratory Director and Chairman of the workshop, opened the workshop by welcoming all the participants and observers and made a few introductory remarks including the rationale for holding the workshop. The objective of the workshop was to bring together some of the principal, active ciguatera researchers in the Pacific to help develop working hypotheses to guide future research on the ciguatera problem. It is recognized that the ultimate goal of ciguatera research is to fully understand the mechanism underlying the origin and transfer of the toxin(s) in the ecosystem and to develop corrective measures to provide nontoxic fish for human consumption.

The workshop agenda (Appendix 1) included the consideration of four broad areas or aspects of ciguatera research: toxins, the detection of toxins, the medical/pharmacological aspects of ciguatera, and the ecological aspects. Seven background papers were assembled for the workshop (Appendix 2) and nine working papers which described ongoing ciguatera research in the Pacific were presented (Appendix 3). In addition to the oral presentations on ciguatera research in the Pacific, presentations were made on the results of the recent World Health Organization meeting on ciguatera held in Noumea, New Caledonia, and on the status of ongoing ciguatera research in the southeastern United States. The workshop participants and observers are listed in Appendix 4.

TOXINS (WP/2, WP/6)

The discussion on toxins covered the topics of the chemical and molecular structure of ciguatoxin and other toxins and the origin of the toxins. The problem of solving the molecular structure of ciguatoxin has been hampered by the lack of an adequate supply of pure toxin for analysis. The toxin is diffusely distributed in minute amounts in the flesh and viscera of fishes so large amounts of toxic fish are needed to extract the toxin in a complex purification procedure. Dr. Paul Scheuer of the University of Hawaii (UH) now has 0.9 mg of ciguatoxin and Dr. Takeshi Yasumoto of Tohoku University, Sendai, Japan, has 0.4 mg. Although the analytic methods used to determine the molecular structure of the toxin is basically non-destructive, minute amounts of the toxin are lost in the analyses. Dr. Scheuer's 0.9 mg of ciguatoxin is presently at the California Institute of Technology for analysis. The basic, empirical formula of ciguatoxin, $(C_{35}\ H_{65}\ NO_8)_n$, has been known for some time. Although the molecular structure of ciguatoxin is not completely known, it is believed that the problem will be solved when more pure toxin becomes available. In this regard, it was announced that the UH will soon be acquiring a 300 MHz Nuclear Magnetic Resonance Spectrometer for the analysis of the molecular structure of complex compounds. This will greatly increase the instrument time available for the analysis of the molecular structure of ciguatoxin.

A breakthrough in ciguatera research occurred within the past 5 years when the source of ciguatoxin in the coral reef ecosystem was determined to be a benthic dinoflagellate, Gambierdiscus toxicus, a microscopic unicellular alga that grows primarily as an epiphyte on certain seaweeds on reef flats. This same species has been found near the Hawaii Institute of Marine Biology (HIMB)

in Kaneohe Bay and at other scattered locations around the island of Oahu. The results of experiments conducted at HIMB to determine the factors controlling toxin production, growth rate, and yields of laboratory cultures of the HIMB strain of G. toxicus were discussed. A problem in the research on culturing G. toxicus is the tremendous variability in the levels and kinds of toxins present in the cultures.

Elsewhere in the Pacific, the possible sources of secondary toxins have also been investigated. In addition to maitotoxin, the presence of secondary toxins have often been detected in the viscera of herbivorous fishes and it can be hypothesized that these secondary toxins, like ciguatoxin, originate from other benthic dinoflagellates. It was found that several species of dinoflagellates found growing with G. toxicus also produced toxins that were lethal to mice. Of interest are two fat soluble toxins of Prorocentrum lima, PL toxins I and II, which are practically indistinguishable in chromatographic properties from scaritoxin and ciguatoxin, respectively. A chemical and spectral analysis of PL toxin II showed that it is identical with okadaic acid, the cytotoxic component of a sponge.

DETECTION (WP/4, BP/2, BP/3, BP/6)

The early tests for ciguatoxin have been bioassays in animals such as mouse, cat, and mongoose. Among the shortcomings of these bioassays are that they are subjective and, in the case of the mongoose, the test animals are obtained from the wild and, therefore, are not homogeneous. A breakthrough in testing for ciguatoxin occurred with the development of a radioimmunoassay (RIA) for the direct examination of fish tissues for toxin. The advantages of the RIA are that it is simple and practical, relatively specific, and sensitive. One of the objectives of current research is to evaluate the RIA procedure for ciguatoxin based on two approaches: 1) identifying the reactive factor in the sheep anti-ciguatoxin serum used in the RIA, and 2) assessing the RIA procedure on fishes from clinically defined and documented cases of ciguatera intoxication and comparing results with those from nontoxic fish of the same species. The RIA results are also being evaluated by supportive bioassays, the mouse bioassay, and the guinea pig atrium assay.

There was much discussion on the technical aspects of the immunological reactions of the RIA procedure including questions on dose responses, extracts, and homogenates versus solids. It was pointed out that the whole basis of the RIA procedure is the assumption that ciguatoxin, being a lipid, is bound on the membrane surface or cells so that it can be detected by an antibody. Some of the difficulties associated with the RIA procedure is 1) sampling, i.e., there is uncertainty in the distribution of ciguatoxin in the fish flesh, 2) the possibility of false positives occurring in the RIA test and, 3) reproducibility owing to the fact that tissues are used and even within close proximity "hot spots" of ciguatoxin may vary.

Beginning in April 1979, at the request of the Hawaiian fishing industry and in cooperation with the Honolulu Laboratory, the RIA test was used to screen commercially landed kahala, Seriola dumerili, for ciguatoxin. The testing program made possible the marketing of large numbers of kahala larger than 20 pounds which, before the testing started, were ordinarily not marketed in the belief that large fish were more likely to be toxic. The most

significant result of the testing program has been the absence of any reported ciguatera cases from marketed RIA-negative kahala.

Mouse bioassays and guinea pig atrium assays have been used to evaluate the RIA procedure. Although the number of test samples is small, the data suggest direct correlations among the three assays. It was noted that ciguatoxin causes an initial massive release of neuroepinephrine in guinea pig atrium. However, one of the clinical symptoms of ciguatera intoxication in humans is reduced cardiac activity. In light of the effect of ciguatoxin on guinea pig atria, the question was posed regarding the effect of ciguatoxin on humans in the early stage of ciguatera intoxication.

Research is also underway to develop an enzyme-linked immunosorbent assay (ELISA) for ciguatoxin. ELISA is simpler and more rapid and unlike the RIA procedure, is not dependent on the use of radioisotopes. Current research on ELISA includes the establishment of toxicity ranges based on absorbence values of samples from clinically documented tissues from ciguatera intoxications and nontoxic tissues. A comparison of RIA and ELISA tests on kahala indicated that ELISA gave a lower rejection rate, which may be more realistic in light of the incidence of reported ciguatera intoxications by kahala in the past.

MEDICAL/PHARMACOLOGY (WP/7, WP/8, WP/9)

This section of the workshop dealt primarily with the public health aspect of ciguatera intoxication. The results of a study to determine the effect of ciguatera on the overall health of Pacific islands populations and how it has affected the utilization of marine resources were discussed. The study was an extensive comparative investigation of the perceived distribution and occurrence of ciguatera in the late 1970s, folk beliefs as to the etiology of the disease, and other aspects of the health and resource problems stemming from ciguatera. Statistics on ciguatera morbidity rates from the South Pacific Commission Health and Epidemiological Information Service (SEPHIS) area were presented. In 1978 the mean repoted ciguatera morbidity rate for the area (excluding Papua New Guinea) was 12.01/10,000. The actual morbidity rate is undoubtedly considerably higher.

A summary of ciguatera intoxication in Hawaii covering a period from 1900-80 was presented. It was noted that cases of ciguatera intoxication were higher in the spring and autumn and that more patients rather than physicians are apt to report ciguatera poisonings. It was further noted that a 25% reporting rate is considered good.

A detailed account of the fatality caused by Marquesan sardine, Sardinella marquesensis, on Kauai in 1978 was also presented. Clinical data from this case were remarkably similar to what would be expected from a severe form of ciguatera intoxication, and it was suggested that clupeoid poisoning should not be excluded from the concern of those doing research on ciguatera poisoning. However, other workshop participants noted that clupeoid poisoning may be more closely related to paralytic shellfish poisoning (PSP) in ecological terms.

ECOLOGY (WP/1, WP/3, WP/5, WP/6, BP/1, BP/4, BP/5, BP/7)

Based on the correlation of the population density of G. toxicus with epidemiological information, the endemicity of ciguatera in various locations

in the Pacific was discussed. The results of surveys showed that the density of the G. toxicus population was correlated with fish toxicity. These results suggest that toxicity in any area can be predicted by the density of G. toxicus. Another possibility of a monitoring system for ciguatera is the assessment of the toxicity of fish livers. It has been shown that fish livers show far less variability in toxicity than the flesh of fishes.

The results of a study on the distribution of G. toxicus in French Polynesia (Tahiti and Gambier Island), New Caledonia, Guam, and Okinawa were also presented, including the effect of certain environmental factors on the density of G. toxicus. Temperature is not an important factor in the density of G. toxicus. However, salinity and light intensity appear to be important. Iron was not important in the growth of G. toxicus but phosphates significantly increased the growth.

Development of the RIA procedure made possible large-scale testing of fishes for ciguatoxin. As part of the cooperative survey of the marine resources in the Northwestern Hawaiian Islands (NWHI), the Honolulu Laboratory and the Hawaii Division of Fish and Game (HDFG) have been sampling inshore and nearshore fishes to determine the distribution of ciguatoxic fishes.

The Honolulu Laboratory's results of tests for ciguatoxin on fish and shellfish collected in the NWHI, Midway Islands, American Samoa and Western Samoa, and Guam and the Northern Mariana Islands were presented. The overall rejection (positive and borderline toxicity levels) rate was 15% for NWHI, 39% for Midway, 19% for American Samoa and Western Samoa, and 4% for Guam and the Northern Mariana Islands.

Flesh samples from fishes are obtained from the dorsal anterior region (A), ventral abdominal region (B), and the anal region (E). It was pointed out that there was no clear trend as to where (sites A, B, or E) the toxin is concentrated in the fish. The results were not only variable among species, but also between sexes. The results to date showed also that toxicity was not related to size or sex.

The results obtained by the HDFG indicate that fishes in the NWHI were relatively more toxic (in terms of numbers rejected in the RIA test) in the summers of 1977 and 1978 than in 1979 and 1980. In 1980 the nearshore fishes were more toxic in the fall than in the summer. Based on these results, it was suggested that the occurrence of ciguatoxic fishes appear to be cyclical. The feeding habits, distribution, and abundance of the fishes sampled were also described.

Also, some anomalous results in the HDFG data and the Honolulu Laboratory data were evident. In sampling around Midway in 1980, the Honolulu Laboratory samples indicated an overall high rejection rate for fishes caught from September 30 to October 4, whereas the HDFG samples collected around the same period showed a relatively low rejection rate.

The results of an analysis of data from the kahala sampling program between April 1979 and December 1980 were also discussed. The mean quarterly RIA scores showed a general trend of a decline in RIA scores from the second quarter of 1979 to a low at the end of 1979/beginning of 1980 and a subsequent increase in RIA scores through 1980. The trend was consistent for sampling

sites A, B, and E, for geographical origin of the fish (NWHI, Oahu, Penguin Bank, and Hawaii), and for both sexes. The correlation of RIA between sampling sites was high: (AB) = 0.74, (AE) = 0.69, and (BE) = 0.70. Also, the variation in RIA scores from two replicates from each sampling site (A, B, and E) was no greater than the variation among sampling sites A, B, and E. Other results indicated that: there was no relationship between kahala weight and RIA scores, and that sampling site B or E would be the best single site to sample for maximum RIA values.

Color slides of the various fish species implicated in ciguatera intoxication were shown and the distribution, food habits, and habitat of these fishes were described. The species that have caused ciguatera are shore fishes associated with reefs, are bottom-dwelling, but they may also be semipelagic open-water forms that range into the reef habitat to feed. They may be carnivorous or they may be benthic algal or detrital feeders. The carnivores that prey heavily on reef fishes are the most prone to be toxic whereas those that feed primarily on benthic crustaceans tend to be least toxic. The moray eel, Lycodontis (= Gymnothorax) javanicus, is highly toxic.

Other ecological aspects of ciguatera were also discussed. Young and old Hawaiian monk seals, Monachus schauinslandi, in the NWHI suffered high mortality in 1978. It was speculated that ciguatera intoxication could have been the cause of the mortality. In connection with this, the results of experiments where elephant seals, Mirounga angustirostris, were fed moray eels caught in the NWHI were described. Elephant seals were found to be highly susceptible to ciguatera intoxication.

RECOMMENDATIONS

The workshop identified three general objectives or goals for future ciguatera research. The first goal is to obtain an understanding of the factors or mechanisms triggering the chain of events leading ultimately to potential outbreaks of ciguatera intoxication so that these events could be predicted. The second goal is to elucidate the pharmacological aspects of ciguatera and to develop a viable treatment for ciguatera intoxication. The third goal is to develop and disseminate a quick and reliable field test to screen marine organisms for ciguatoxin and related toxins.

Several general areas of research were recommended to achieve the first objective:

1. Determine baseline conditions in (a) areas with no previous history of ciguatera outbreaks and (b) areas that could potentially produce ciguatera outbreaks. Related to this is the determination and verification of factors causing blooms of Gambierdiscus toxicus and the comparison of environmental conditions in areas containing and devoid of dinoflagellate blooms.

2. Verify the source of ciguatoxin in the reef ecosystem; determine the rate of toxin production from culture experiments and determine factors that influence the growth of toxin-producing dinoflagellates. Determine the source (cysts?) of dinoflagellate blooms.

3. Conduct field and laboratory studies to determine the transfer and metabolism of ciguatoxin in the food chain.

4. Determine the effects of ciguatoxin on fish including its toxicity to fish and its influence on growth rates; conduct experiments to determine if ciguatoxin is accumulated in fish or excreted and whether it would be possible to predict time/area fish toxicity based on dates/areas of dinoflagellate blooms; determine why some species are more toxic than others including a quantitative analysis of toxicity levels by species, size, and area, and compare findings with the food chain understanding; and determine whether crustaceans and molluscs in areas of dinoflagellate blooms are toxic.

5. Evaluate the ELISA technique for field work.

Research activities recommended to achieve the second goal were:

1. Determine source of toxin by locating toxic areas, e.g., by public health follow-ups, search for alternate source of ciguatoxin by bio-testing for possible new sources. A related activity is to determine factors that make dinoflagellates toxic.

2. Collect toxic fishes including moray eels, RIA-rejected kahala, and other species and develop cultures of Gambierdiscus toxicus to extract the crude toxins.

3. Assemble hardware and manpower to extract and purify toxins for experiments to a) compare toxin from moray eel with toxins from other species, b) determine the molecular structure of ciguatoxin and other toxins, and c) determine the metabolic pathways and target tissues/organs in tests on mice and other animals and muscle tissues, c) investigate the pharmacology of ciguatoxin to determine metabolic pathways and target tissues/organs, in tests on mice and other animals, and d) determine the efficacy of off-the-shelf drugs for the treatment of ciguatera in test animals and in clinical trials.

Research activities recommended for the third goal were:

Develop usable ELISA test for ciguatoxin and 1) improve specificity of ELISA test to sort out other toxins (scaritoxin, maitotoxin, okadaic acid) and 2) adapt ELISA procedure for a "dip stick" test.

APPENDIX 1

AGENDA

1. Introduction (R. Shomura)

    1.1 Participants
    1.2 Objectives of workshop

2. Report of SPC ciguatera meeting (A. Banner)

3. Toxins

    3.1. Chemical composition (P. Scheuer)
    3.2. Dinoflagellates (T. Yasumoto, N. Withers)

4. Detection

    4.1. RIA (Y. Hokama, L. Kimura)
    4.2 ELISA (Y. Hokama, L. Kimura)
    4.3 Muscle tests (J. Miyahara)

5. Medical/Pharmacology

    5.1. Case histories--Hawaii (M. Sugi)
    5.2 Case histories--Other Pacific areas (N. Lewis)
    5.3 Pharmacological (J. Miyahara)

6. Ecology

    6.1. Elaborators (T. Yasumoto, N. Withers)
    6.2. Fishes

        6.2.1. NWHI fishes (Honolulu Laboratory, Hawaii Division of Fish and Game)
        6.2.2. Kahala (Honolulu Laboratory)
        6.2.3. Other areas--American Samoa, western Pacific (Honolulu Laboratory)

    6.3. Other animals

        6.3.1. Hawaiian monk seal (Honolulu Laboratory)
        6.3.2. Others--birds, turtles (open)

APPENDIX 2

LIST OF BACKGROUND PAPERS

BP/1 Adachi, Rokuro, and Yasuwo Fukuyo.
1979. The thecal structure of a marine toxic dinoflagellate, Gambierdiscus toxicus gen. et sp. nov. collected in a ciguatera-endemic area. Bull. Jpn. Soc. Sci. Fish. 45:67-71.

BP/2 Hokama, Y., A. H. Banner, and D. B. Boyland.
1977. A radioimmunoassay for the detection of ciguatoxin. Toxicon 15:317-325.

BP/3 Hokama, Y., L. H. Kimura, and J. T. Miyahara.
In press. Immunological approaches to understanding marine toxins. Proceedings of the Sixth Food and Drug Administration Science Symposium on "Aquaculture - Public Health, Regulatory and Management Aspects," February 12-14, 1980, New Orleans, Louisiana (Office of Health Affairs, FDA).

BP/4 Humphreys, Robert L., Jr.
1980. Feeding habits of the kahala, Seriola dumerili, in the Hawaiian Archipelago. In Richard W. Grigg and Rose T. Pfund (editors), Proceedings of the Symposium on Status of Resource Investigations in the Northwestern Hawaiian Islands, April 24-25, 1980, University of Hawaii, Honolulu, Hawaii. Sea Grant Misc. Rep. UNIHI-SEAGRANT-MR-80-04, p. 233-240.

BP/5 Ito, Bernard M., and Richard N. Uchida.
1980. Results of ciguatera analysis of fishes in the Northwestern Hawaiian Islands. In Richard W. Grigg and Rose T. Pfund (editors), Proceedings of the Symposium on Status of Resource Investigations in the Northwestern Hawaiian Islands, April 24-25, 1980, University of Hawaii, Honolulu, Hawaii. Sea Grant Misc. Rep. UNIHI-SEAGRANT-MR-80-04, p. 81-89.

BP/6 Miyahara, J. T., C. K. Akau, and T. Yasumoto.
1979. Effects of ciguatoxin and maitotoxin on the isolated guinea pig atria. Res. Commun. Chem. Pathol. Pharmacol. 25:177-180.

BP/7 Randall, John E.
1980. A survey of ciguatera at Enewetak and Bikini, Marshall Islands, with notes on the systematics and food habits of ciguatoxic fishes. Fish. Bull., U.S. 78:201-249

APPENDIX 3

LIST OF WORKING PAPERS

WP/1 Polovina, Jeffrey J., and Bernard M. Ito.
An analysis of data from the kahala ciguatera sampling program, April 1979-December 1980.

WP/2 Withers, Nancy.
Pacific ciguatera.

WP/3 Uchida, Richard N., Bernard M. Ito, Paul M. Shiota, Darryl T. Tagami, Karen P. Wendel, Victor A. Honda, and Michael P. Seki.
Status of the Honolulu Laboratory ciguatera research on fishes of the Northwestern Hawaiian Islands, American Samoa, Western Samoa, Guam, and the Northern Mariana Islands.

WP/4 Hokama, Y., L. Kimura, and J. Miyahara.
Status report on detection methods for ciguatoxin.

WP/5 Okamoto, Henry.
Status report of ciguatera research in the Northwestern Hawaiian Islands by DLNR, Division of Fish and Game.

WP/6 Yasumoto, Takeshi, Yasukatsu Oshima, Akio Inoue, Yasuwo Fukuyo, and Takako Harada.
Studies on ciguatera.

WP/7 Kubota, Wilbert.
Ciguatera fish poisoning cases: A summary from 1900 - December 1980.

WP/8 Lewis, Nancy Davis.
Ciguatera morbidity in the island Pacific.

WP/9 Melton, Robert J.
Summary of data on fatal case of fish poisoning associated with Marquesan sardine, Kauai, 1978.

APPENDIX 4

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