Intergovernmental Oceanographic Commission
Training Course Reports

29

IOC-UNEP-WHO-FAO Training Course on Qualitative and Quantitative Determination of Algal Toxins

Friedrich-Schiller University of Jena
Jena, Germany
18-28 October 1994

UNESCO

IOC Training Course Reports

No.	Title	Language versions
1.	IOC Indian Ocean Region Training Course in Petroleum Monitoring Perth, 18 February-1 March 1980	English
2.	IOC Regional Training Course for Marine Science, Technicians Cape Ferguson, Queensland, 1-28 June 1980	English
3.	ROPME-IOC-UNEP Training Workshop on Oceanographic Sampling Analysis, Data handling and Care of Equipment, Doha, Qatar, 3-15 December 1983	English
4.	Stage COI d'initiation à la gestion et au traitement de l'information scientifique et technique pour l'océanologie, Brest, France, 28 novembre - 9 décembre 1983	French
5.	Curso mixto COI-OMM de formación sobre el Sistema Global Integrado de Servicios Oceánicos (SGISO), Buenos Aires, Argentina, 15-26 de octubre de 1984	Spanish
6.	UNESCO-IOC-NBO Training Course on Tidal Observations and Data Processing Tianjin, China, 27 August - 22 September 1984	English
7.	Stage COI sur la connaissance et la gestion de la zone côtière et du proche plateau continental Talence, France, 18 septembre - 4 octobre 1984	French
8.	IOC Regional Training Course on Marine Living Resources in the Western Indian Ocean Mombasa, Kenya, 27 August - 22 September 1984	English
9.	IOC-UNESCO Summer School on Oceanographic Data, Collection and Management Erdemli, Icel, Turkey, 21 September - 3 October 1987	English
10.	IOC-UNESCO Regional Training Workshop on Ocean Engineering and its Interface with Ocean Sciences in the Indian Ocean Region, Madras, India, 17 March - 5 April 1986	English
11.	IOC-UNESCO Training Course on the Use of Microcomputers for Oceanographic Data Management Bangkok, Thailand, 165 January - 3 February 1989	English
12.	IOC Advanced Training Course on Continental Shelf Structures Sediments and Mineral Resources Quezon City, Philippines, 2-13 October 1989	English
13.	IOC/IODE Training Course on GF3 Data Formatting System Obninsk, USSR, 14-24 May 1990	English
14.	IOC Training Course on Microcomputers and Management of Marine Data in Oceanographic Data Centres of Spanish-speaking Countries, Bogotá, Colombia, 21-30 October 1991	English Spanish
15.	IOC Advanced Training Course on Nearshore Sedimentation and the Evolution of Coastal Environments, Kuala Lumpur, Malaysia, 17-29 February 1992	English
16.	First IOC Training Course on the Applications of Satellite Remote Sensing to Marine Studies Caracas, Venezuela, 24-28 September 1990	English
17.	IOC-KMFRI-RECOSCIX (WIO) Regional Training Course on Microcomputer-based Marine Library Information Management, Mombasa, Kenya, 10-21 August 1992	English
18.	ROPME-IOC Regional Training Course on Management of Marine Data and Information on Microcomputers for the ROPME Region, Kuwait, 18-28 October 1992	English
19.	IOC-SOA Training Workshop on Environmental Effects on Benthic Communities Xiamen, China, 19-23 October 1992	English
20.	IOC Training Course for the Global Sea Level Observing System (GLOSS) directed to the African and South American Portuguese and Spanish-Speaking Countries São Paulo, Brazil, 1-19 February 1993	English
21.	IOC-SSTC-SOA Training Course on Marine Information Management and ASFA Tianjin, China, 19-30 October 1992	English
22.	First IOC/IOCARIBE-UNEP Training Course on Monitoring and Control of Shoreline Changes in the Caribbean Region, Port-of-Spain, Trinidad and Tobago, 21-30 July 1993	English Spanish
23.	IOC/WESTPAC Training Course on Numerical Modelling of the Coastal Ocean Circulation Matsuyama, Japan, 27 September - 1 October 1993	English
24.	IOC-JODC Training Course on Oceanographic Data Management Tokyo, Japan, 28 September - 9 October 1992	English
25.	IOC-JODC Training Course on Oceanographic Data Management Tokyo, Japan, 27 September - 8 October 1993	English
26.	IOC Training Course on Ocean Flux Monitoring in the Indian Ocean. Organized with the support of the Government of Germany, Mombasa, Kenya, 15-27 November 1993	English
27.	IOC-UNEP-SPREP Training Course on Coral Reef Monitoring and Assessment, Rarotonga, Cook Islands, 23 February - 13 March 1994	English
28.	IOC-JODC Training Course on Oceanographic Data Management Tokyo, Japan, 28 September - 9 October 1992	English
29.	IOC-UNEP-WHO-FAO Training Course on Qualitative and Quantitative Determination of Algal Toxins Jena, Germany, 18-28 October 1994	English

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ABSTRACT

The first IOC-UNEP-WHO-FAO Training Course on Qualitative and Quantitative Determination of Algal Toxins was held at the Friedrich-Schiller-University Jena, Germany, from 18-28 October 1994. The Course was jointly sponsored by IOC and UNEP through the Mediterranean Action Plan (MAP), and organized in the framework of the HAB and MEDPOL programmes. Furthermore, Germany (the University of Jena), Parapharm Laboratories Co., Ltd., Japan and Hewlett Packard, Germany supported the course.

The Course was organised and hosted by Prof. Dr. Bernd Luckas, Friedrich-Schiller-University, Jena, Germany, in co-operation with Dr. Helle Ravn, IOC, Paris, France, Dr. Malte Elbrächter, Biologische Anstalt Helgoland, Germany, and Prof. Dr. Takeshi Yasumoto, Tohoku University Sendai, Japan. Dr. Achim Boenke, CEC, Brussels and Dr. Marisa Fernandez, Ministerio de Sanidad y Consumo, Vigo, Spain, were also invited to give lectures. The 12 participants originated from 11 countries.

The goals of the course were to provide the participants with an overview of the state of the art of different analytical methods for qualitative as well as quantitative determination of phycotoxins. Lectures and demonstrations on all relevant toxins were performed, and the participants were encouraged to establish scientific collaboration.

Qualitative and quantitative determination of algal toxins using different analytical methods (Bioassay, ELISA, HPLC) were made on samples of algae and mussels provided mainly by the participants. The laboratory experiments focussed on the analysis of Diarrhetic Shellfish Poisoning (DSP), Paralytic Shellfish Poisoning (PSP), and Amnesic Shellfish Poisoning (ASP) by application of HPLC methods with chemical derivatization.

During various roundtable discussions the need for reference material and standards for international use was stressed. Currently standards are available only for a few toxins. The necessity to improve the methods for non-ambiguous determination of the different algal toxins in order to evaluate possible hazards originating from seafood and drinking waters contaminated by phycotoxins, was stressed.

1. BACKGROUND, GOALS AND CONTENT OF THE COURSE

BACKGROUND

At the Second Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, Paris, 14-16 October, 1993, a strong need for educational and scientific elements of the IOC-FAO Harmful Algal Bloom Programme was expressed. The present training course on Qualitative and Quantitative Determination of Algal Toxins was included in the Training Programme.

At the International Workshop on Marine Environmental Protection and Coastal Living Resources, Bremerhaven, Germany, 29 September - 3 October, 1992, the need for training courses in this field was stressed.

The IOC-FAO Intergovernmental Panel on Harmful Algal Blooms have given high priority to the initiation of training activities on harmful algae acknowledging that:

(i) toxin chemistry and toxicology skills need to be considerably improved in many parts of the world;
(ii) reliable qualitative and quantitative determination of toxins from marine organisms is crucial to the response to, and management of harmful algal events.

In a phytoplankton monitoring program, and especially with respect to harmful phytoplankton, the reliable qualitative and quantitative determination of toxins from marine organisms is an important step towards taking decisions to mitigate undesirable effects associated with harmful algal events.

The high priority given to the training of young scientists within the field of harmful algae is reflected in the structure of the IOC-FAO Harmful Algal Bloom Programme, where one of the three main elements is "Education: Training and Network Building". In general the training activities of IOC are focused and coordinated in the Programme for Training, Education and Mutual Assistance (TEMA).

GOALS OF THE COURSE

The goals of the training course were to give the participants an overview of the state of the art of the different methods used for toxin analysis as well as practical experience in qualitative- and quantitative determination of algal toxins. Furthermore, it was intended to encourage the individual participants from the various countries to establish scientific collaboration with other participants of the course.

This training course is also important as a pilot course which will serve as a basis for developing an envisaged toxin chemistry/toxicology training module. The toxin chemistry/toxicology training module can be held recurrently in developed countries where adequate facilities are available. Later on, the course can be held in different regions of the world where harmful algae are a major economic and health problem, if adequate equipment is available.

The toxin chemistry/toxicology module will be an element of a more comprehensive training program on harmful algae including various aspects of harmful algal events and related problems.

Priority in this and future training courses will be given to topics where the knowledge gained is of direct significance to research and monitoring activities in the participants' home countries.

The courses will also serve as an important mechanism to establish research links between the participants' and the hosts' Institutes, as well as between the participants themselves. As a long-term

perspective the training program should thus support the build-up of regional scientific networks of experts on harmful algae.

CONTENT OF THE COURSE

The course was organized on the basis of needs identified by participant with respect to toxins present in their countries, in order to enable them to use the knowledge gained in the course to develop appropriate studies in their own countries.

Lectures and experiments were organized. The practical aspect was highlighted in order to enable the participants to perform analytical experiments.

The theoretical part gave the participants an overview of the toxins: origin of toxins (microorganisms as well as contaminated seafood); physiological aspects (biosynthesis, physiological mechanisms related to growth under different conditions, accumulation, conversion, and depuration processes in contaminated seafood, together with processes of toxin degradation); chemical aspects (different toxicological groups, and structure of toxins); toxicological aspects (mechanisms of toxicity, bioassays).

2. OPENING AND INTRODUCTION

OPENING AND INTRODUCTION

The 12 participants from 11 different countries and the three external organizers, Dr. Elbrächter, Biologische Anstalt Helgoland, Germany, Prof. Yasumoto, Tohoku University Sendai, Japan, and Dr. Ravn, IOC, as well as Dr. Fernandez and Dr. Franco, Vigo, Spain, guest scientists at the Friedrich-Schiller University Jena who joined the training course, were welcomed by Prof. Bernd Luckas from the Institute of Nutrition and Environment, and Prof. Eberhard Müller, Dean of the Biological and Pharmaceutical Faculty of the University. Prof. Müller presented the history and traditions of the Friedrich-Schiller-University Jena.

Dr. Helle Ravn welcomed the participants on behalf of the IOC, the United Nations Environment Programme (UNEP), the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the Mediterranean Action Plan (MAP), and explained the importance of this first course in the field of toxin chemistry and toxicology. The first objective of the course was to learn new methods to detect toxins and to meet colleagues in the toxin chemistry and toxicological field, and thus to strengthen international research collaboration in phycotoxin research between the participants of the course. Most of the researchers are working alone or in very small groups. Therefore, the second objective of the course was to foster and develop further interaction within the field.

The IOC-FAO Harmful Algal Blooms Programme was presented. The objective of this course was to improve the participants' chemical and toxicological skills in order to enable them to produce reliable qualitative and quantitative determination of toxins from planktonic algae and contaminated food.

The experience obtained in the course will be used to design a comprehensive training program on harmful algae and in particular to prepare a training module on qualitative and quantitative determination of toxins from harmful marine phytoplankton. Training activities will focus on improving related human resources as well as technology and knowledge transfer in order to develop national capabilities for the management of harmful algal events.

3. NATIONAL REGULATIONS AND FOOD CONTROL

Prof. Luckas presented the facts needed to obtain and perform an effective food control. The stability of toxins is very important in the toxicity of seafood. It is of great importance to be able to use various methods of analysis in order to detect the different algal toxins. It is also necessary to know which algal species and which kind of toxins can be expected in different periods of the year in order to perform food control and in order to protect consumers. Prof. Luckas presented an overview of the different countries and their food control programs.

In Germany a chemical detection method with low detection level is used first and is followed by a bioassay, the mouse test. Most countries perform the mouse test first, followed by chemical detection methods.

The CEC has not yet regulated the nature of the detection methods and their order. Different toxicological and chemical tests were discussed with respect to false positive and negative results related to the various methods.

In addition, a study was presented on the current situation concerning worldwide regulations for marine phycotoxins. This study is based on an enquiry undertaken in 1990 within the framework of a project of the International Union of Pure and Applied Chemistry (IUPAC) in order to obtain up-to-date information about worldwide marine phycotoxin legislation.

Forty-seven countries responded to the enquiry. The response, supplemented with published information, showed that 21 countries have legislation for one or more phycotoxins in fishery products. Details about algal monitoring programs, tolerances, legal bases, responsible authorities and methods of analysis are given for paralytic shellfish poisons (PSP), diarrhetic shellfish poisons (DSP), amnesic shellfish poisons (ASP), neurotoxic shellfish poisons (NSP), and ciguatera toxins (H.P. van Egmond et al.: J. Nat. Toxins 1, 67-85, 1992). (Copies of this publication are available from the Harmful Algal Bloom Programme Office, IOC).

There were some differences noted between nations with regard to tolerance levels established for PSP and for DSP toxins. These differences should be eliminated in order to ensure consistency of public health protection and greater harmonization of international trade.

It was recommended that international organizations should re-evaluate the hazards caused by marine phycotoxins in order to provide a common basis for risk assessment. Moreover, further development and validation of analytical methods (and reference materials) for marine phycotoxins is highly desirable, because the enforcement of phycotoxin legislation is ultimately based on the ability to analyze these toxins in order to identify and quantify them accurately in seafood products.

4. NATIONAL REPORTS

The participants presented national reports from their respective countries with regard to toxin chemistry and toxicology. These statements are included in this training course report (see Annex III).

5. PREPARATION OF REFERENCE MATERIAL AND INTERCOMPARISON BETWEEN LABORATORIES WITHIN THE COMMISSION OF THE EUROPEAN COMMUNITIES (CEC)

Dr. Boenke, invited lecturer from the Measurement and Testing Programme of the CEC, Brussels, Belgium, presented the different programmes, projects and related activities on algal toxins. The DG XII of the CEC is responsible within the Measurement and Testing Program for the supply of certified reference materials with defined concentrations of phycotoxins.

The goals and procedure of two intercomparison studies on DSP and PSP toxins were described, and the importance of precise determination methods for algal toxins were highlighted. Furthermore, the CEC rules for certification of reference materials and standards were introduced, and the ongoing project on purification of specific PSP toxins, performed at the Centro Nacional de Alimentacion, Madrid, Spain, was mentioned.

Dr. Fernandez, Vigo, Spain, presented goals and the work of the European Community Reference Laboratory in Europe and their interaction with other laboratories. The possibilities for joint activities with respect to IOC-CEC training courses of this kind for developing countries were identified and discussed.

Regarding the identification of reference materials and standards from a global view point, Dr. Ravn, IOC, introduced on-going activities of the IOC-FAO Harmful Algal Bloom Programme, and guided a round-table discussion on problems and needs related to the global supply of reference materials and standards. Related activities in the Global Investigation of Pollution in the Marine Environment (GIPME) programme were also presented with information on the Group of Experts on Standards and Reference Materials (GESREM).

Prof. Yasumoto explained how important it was to have pure standards. In order to be able to determine algal toxins in different materials with high accuracy, pure standards are needed. Problems related to commercially available standards and the purity of these standards were discussed.

The matrix influence and differences in chemical reaction during a chemical and bioassay determination of the phycotoxins were presented. The participants identified the needs in their countries for reference materials and standards.

Dr. Boenke encouraged the participants to prepare proposals for the Measurement and Testing Program of the CEC in order to help attain adequate standards and reference methods. This invitation is brought to the attention of GESREM.

6. PHYCOTOXINS IN MARINE ORGANISMS

Dr. Malte Elbrächter presented an overview of the apparent worldwide increase and spreading of Harmful Algal Bloom events. He reviewed the different algal groups (dinoflagellates, diatoms, prymnesiophytes, blue greens) that produce the different toxins relevant to the course (PSP,DSP,ASP). He also mentioned other algal genera involved in different HAB problems, such as ciguatera ichthyotoxins, anoxia. He pointed out the importance of physical processes in the formation of high algal cell densities (e.g. accumulation at fronts).

Dr. Elbrächter reviewed the biological processes leading to the mass occurrence of harmful algae (e.g. vegetative and sexual reproduction, vertical migration, hatching from cysts etc.). Problems of long distance transport of toxic algal species with ballast water in ships or with sea food and shrimp larvae were also mentioned. Finally, he stressed that the toxin biosynthesis pathway in algal cells is, so far, poorly understood. In relation to this, he also mentioned the hypothesis that bacteria might be involved in the process of toxin production.

A lecture on chemical, physiological, and toxicological aspects of DSP toxins was given by Prof. Yasumoto who presented a review of algal toxins and their toxicology. Bioassay methods and toxicological mechanisms as well as ELISA and HPLC methods were presented. Prof. Yasumoto kindly offered reference materials on DSP toxins to all the participants to be used in their home laboratories.

Prof. Luckas presented the chemical, physiological, and toxicological aspects of PSP and ASP toxins. An introduction was given concerning the most often applied principles and techniques of the chromatographic separation and selective detection of PSP and ASP toxins.

7. QUALITATIVE AND QUANTITATIVE DETERMINATION OF ALGAL TOXINS

7.1. DETERMINATION OF DIARRHETIC SHELLFISH POISONING (DSP)

DSP determination by ELISA

A Japanese DSP Quick Test Kit using ELISA developed by Prof. Yasumoto was demonstrated by Dr. Tubaro, Italy, after a detailed introduction. The DSP-Test Kits were a gift from Prof. Yasumoto, Japan, to the course.

DSP determination by HPLC

Dr. Hummert introduced the preparation method of DSP samples (mussels). The problems related to the derivatization of the DSP toxins in order to detect the compounds in small amounts were clearly described. Three different derivatization reagents and methods were described. Techniques for analyzing raw extracts of samples were presented.

The participants were divided into four groups and practical experiments were performed in the laboratory. Two of the groups analysed DSP in mussels by HPLC with an automatic column-switching system after

derivatization with ADAM (Dr. Hummert), and the other two groups determined DSP toxins in algae by HPLC after derivatization with BrMmc (Dr. Kirschbaum).

The methods for HPLC determination of DSP toxins after derivatization with ADAM and BrMmc performed in the course are decribed in Annex IVA to this report.

7.2. DETERMINATION OF PARALYTIC SHELLFISH POISONING (PSP)

PSP determination using the Mouse Bioassay of the AOAC

Dr. Schubert organized a video presentation on the activities of the Institute. He introduced the AOAC Mouse Bioassay for PSP determination. The test was performed on standards of saxitoxin and PSP contaminated mussel extracts from Mexico and Spain. The symptoms were studied, and the results were calculated by the participants with the help of Dr. Sierra-Beltrán, Mexico.

PSP determination by HPLC

Dr. Kirschbaum introduced the different HPLC methods for PSP determination and the participants prepared their own samples to be analyzed by two different HPLC methods. All participants performed PSP analysis by application of both the Lawrence method with pre-column derivatization (Dr. Kirschbaum) and the Thielert method with post-column derivatization (Dr. Hummert).

In addition, the PSP toxin determination by combined ion-exchange HPLC and electrochemical oxidation was demonstrated. Dr. Hummert explained the advantages of using an electrochemical detector instead of a post-column derivatization unit for oxidation of the PSP toxins. The methods for HPLC determination of PSP toxins performed in the course are described in Annex IVB. The results were discussed and evaluated by all the participants, and the methods were discussed.

7.3. DETERMINATION OF AMNESIC SHELLFISH POISONING (ASP)

Prof. Luckas presented an overview of the methods for analytical determination of domoic acid. The determination of this ASP toxin is possible by HPLC separation and selective detection of domoic acid in a diode-array detector (DAD).

Dr. Hummert demonstrated an HPLC method for domoic acid determination, which was recently published by M. Quilliam *et al* (see Annex IVC).

8. FUTURE ACTIVITIES

It was generally agreed during the course, that other courses of this type should be organized, possibly as joint activities of IOC, CEC, and Member States of IOC. The Representative from the European Commission Reference Laboratory, Dr. Fernandez, Vigo, Spain, discussed the organization of training courses to cover, not only the European Union, but also international training courses for developing countries in collaboration with IOC.

Dr. Fernandez and Dr. Ravn, will co-operate in the preparation of such a joint activity in the future. Italy, represented by Dr. Tubaro, Trieste, expressed a wish to prepare a course for the Eastern Mediterranean and developing countries. Discussions concerning this training course were organized during the course.

9. CONCLUSIONS

In view of legislation calling for control methods, accurate methods of analysis must be available.

Due to a lack of standards and reference materials, strictly validated chemical methods of analysis are not yet available, or cannot be easily applied.

Many countries still rely on bioassays e.g., mouse bioassay to detect algal toxins. The main disadvantage of this method is the ethical aspect of these tests, which have led to growing resistance from animal welfare groups.

In view of this, many efforts have been made to determine algal toxins by chemical methods. In DSP determination ELISA (e.g. DSP Check Kit) and HPLC methods were developed. The HPLC methods are characterized by pre-column derivatization of the most important DSP toxins, followed by chromatographic separation and fluorescence detection.

PSP determination by chemical methods is mainly based on HPLC in connection with pre- or post-column derivatization to form detectable fluorescent oxidation products. However, there are some problems referring to the quantification of the individual PSP toxins after chromatographic separation.

No problems arise in the determination of domoic acid (ASP). Determination-efficient HPLC methods have been developed for ASP.

It can be stated that the application of chromatographic methods, as performed in the course, makes possible the rapid, sensitive and non-ambiguous determination of individual species of the most important groups of algal toxins.

These methods are therefore appropriate to solving problems regarding the contamination of seafood with algal toxins caused by marine dinoflagellates, as well as for control of drinking water containing higher concentrations of phycotoxins originating from blooms of blue greens.

ANNEX I

TIMETABLE

Tuesday, 18 Oct. 1994		Lecturer
10.00-12.00	Welcome, Technical and Practical Information	Ravn
14.30-16.00	General Introduction of HAB's	Elbrächter
18.00-20.00	National Reports of Participants, Part 1 (Problems in their Countries)	Ravn
Wednesday, 19 Oct. 1994		
10.00-12.00	Phycotoxins in Marine Organisms - An Overview	Yasumoto
13.30-15.00	Visit of the botanical garden	Dietrich
16.00-18.00	Phycotoxins in Seafood (National regulations and food control)	Luckas
Thursday, 20 Oct. 1994		
10.00-12.00	DSP-Toxins (Chemical, physiological, and toxicological aspects)	Yasumoto
14.00-15.00	Analytical Methods for DSP determination (Bioassays, ELISA, and HPLC methods)	Yasumoto
15.00-19.00	Determination of DSP toxins using ELISA (Demonstration)	Yasumoto/ Tubaro
19.00-24.00	Visit to Cospeda Battlefield (Napoleon, 1806), Supper in the restaurant "Napoleon"	
Friday, 21 Oct. 1994		
10.00-12.00	HPLC determination of DSP toxins (Introduction of the HPLC methods for DSP)	Hummert
14.00-20.00	**Experiment I** - DSP toxins in mussels (Determination by HPLC with column-switching system) Group: A,B	Hummert
	Experiment II - DSP toxins in algae (Determination by HPLC as BrMmc derivatives) Group: C,D	Kirschbaum
Saturday, 22 Oct. 1994		
10.00-16.00	**Experiment I** - DSP toxins in mussels (Determination by HPLC with column-switching system) Group: C,D	Hummert
	Experiment II - DSP toxins in algae (Determination by HPLC as BrMmc derivatives) Group: A,B	Kirschbaum

16.30-18.00	Preparation of reference material for algal toxin determination	Boenke
18.00-20.00	Problems of standardization of toxin analysis (Round Table)	Ravn
Sunday, 23 Oct. 1994		
9.00-18.00	Excursion to Weimar	
Monday, 24 Oct. 1994		
10.00-12.00	PSP toxins (Chemical, physiological, and toxicological aspects)	Luckas
14.00-16.00	Analytical methods for PSP determination (Bioassays, ELISA, and HPLC methods)	Luckas
16.00-19.00	PSP toxin determination using mouse bioassay (Demonstration)	Schubert
20.00-22.00	National Reports of Participants, Part 2 (Problems in their Countries)	Ravn
Tuesday, 25 Oct. 1994		
10.00-12.00	HPLC determination of PSP toxins (Introduction of the HPLC methods for PSP)	Kirschbaum
14.00-20.00	**Experiment III** - PSP toxins in algae (Determination by Thielert-chromatography) Group: A,B	Kirschbaum
	Experiment IV - PSP toxins in mussels (Determination by Lawrence-chromatography) Group: C,D	Hummert
20.00-22.00	National Reports of Participants, Part 3 (Problems in their Countries)	Ravn
Wednesday, 26 Oct. 1994		
10.00-12.00	PSP toxin determination by combined ion-exchange HPLC and electrochemical oxidation (Demonstration)	Kirschbaum
14.00-20.00	**Experiment III** - PSP toxins in algae (Determination by Thielert-chromatography) Group: C,D	Kirschbaum
	Experiment IV - PSP toxins in mussels (Determination by Lawrence-chromatography) Group: A,B	Hummert

Thursday, 27 Oct. 1994

10.00-12.00	HPLC determination of ASP in mussels (Demonstration)	Hummert
14.00-16.00	Discussion of the problems with regard to Phycotoxin determination (Round Table)	Luckas
16.00-18.00	Discussion of the tasks in future - Research activities - Monitoring activities (Round Table)	 Ravn Elbrächter Luckas

Friday, 28 Oct. 1994

Departure

ANNEX II

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ANNEX III

NATIONAL REPORTS

ARGENTINA

Determination of algal toxins in Argentina

The first toxic outbreak of Paralytic Shellfish Poisoning (PSP) was detected in the Argentina sea during the spring of 1980, as a consequence of the death of two fishermen due to intoxication by mussel ingestion. Since that initial bloom of *Alexandrium tamarense*, the toxic area has been expanded, in successive stages, to cover nearly all the Argentina coastal ecosystem.

During 1991/1992 an extremely high toxicity outbreak was detected produced by *Alexandrium catenella* in the Beagle Channel in southern Argentina. Mussel toxicity reached a maximum of 127 200μgSTXeq/100g that resulted in a high number of human intoxications.

A public health program was established in 1981. The shellfish areas are patrolled and closed for harvesting, when toxic levels reach 80μgSTX/100g of shellfish meat. In all the cases the analysis method used to detect PSP is the mouse bioassay (AOAC 1980).

The intoxication of a member of the Korean community after ingesting whole snails showed that some gastropodous accumulate PSP. The sea snails (*Zidona angulata, Adelomedon brasiliana and Pachicymbioal brasiliana*) can accumulate high toxicity levels in the viscera. These organisms show toxicity all year round, but with levels near to the limit of acceptance (80μgSTXeq/100g). There is a high variability of toxicity among different groups in the same fishing region. This produces on-going disputes between the control organizations and fishermen. The snails are destined for export with a considerable financial return.

During the spring of 1993, a high mortality of mackerel was detected for the first time in the Buenos Aires shelf waters. The viscera of these mackerel contained a high concentration of PSP. The highest level was in the stomach content (until 2800μgSTX/100g). Nevertheless, no toxins were detected in the mackerel muscle. At that moment the mortality of sea birds was also detected.

There have been no intoxications due to commercially harvested shellfish up to the present. Several human deaths and serious illnesses have been recorded, as well as an undetermined number of mild intoxications experienced by tourists and fishermen, who often disregard warnings and are the most common sufferers from PSP.

MONITORING PROGRAM

Toxicity testing:
The program include shellfish and snail PSP toxins determination.

Agencies involved:

National government:
INIDEP (National Institute of Fisheries Research and Development)
SENASA (National Service of Animal Health)

Provincial governments:
The provincial governments conduct their own programmes, they have the power to close the coastal waters under their jurisdiction:
Santa Cruz
Río Negro

Chubut
Tierra del Fuego e Islas del Atlántico Sur

Planned Expansion

In 1981, a red tides program was established in the INIDEP, when the chromatographic method (HPLC) was used in order to determine PSP toxins, and to begin studies on ASP and DSP toxins. For this purpose, HPLC equipment will be acquired in the near future.

CHILE

Harmful algal blooms in Chile

Red tides have been present in southern Chile since 1972. The toxic episodes became endemic and represented a serious threat to the shellfish industry and the public health. The dinoflagellates *Alexandrium catenella* and *Dinophysis acuta* are responsible for these outbreaks. *Pseudonitzschia australis*, a domoic acid producer, has also been found in the Chilean sea. The mussels *Mytilus chilensis* (chorito), *Choromytilus chorus* (choro) and *Aulacomya magellanica* (cholga), the clam *Chlamys patagonicus* (ostion) and the carnivorous gastropod *Concholepas concholepas* (loco) are the main risk species.

Paralytic Shellfish Poisons (PSP)

The first episode of human poisoning associated with the consumption of shellfish containing PSP was detected in October 1972 as a consequence of the death of three fishermen. This toxic phenomenon occurred in the central portion of the Strait of Magellan (Estrecho de Magellanes). A second bloom occurred in 1981 in the entire Strait of Magellan when two people died. The third outbreak was reported in 1988, with a low level of toxins and did not produce victims.

Since 1991, blooms of *Alexandrium catenella* in South Chile have been more extensive. Numerous poisonings have occurred (about 230) and twelve people have died in this period. The affected area extends approximately from 47° to 55° latitude south (Beagle Channel).

Diarrhetic Shellfish Poisons (DSP)

The first record of toxic *Dinophysis* in Chile occurred in April 1970. More than 100 people were intoxicated due to the ingestion of bivalves obtained from the Estuario of Reloncavi (41° 40' latitude south). Several blooms were reported with similar consequences. These episodes occurred in 1979, 1991 and 1994, and were higher in toxicity, extension and duration. At present, the results indicate the presence of *Dinophysis acuta* in the whole studied area. They are present in sounds and fjords between 44° and 51° latitude south.

In 1991, DSP produced 126 cases of human intoxication in the Aysen Region after those people ate fresh *Mytilus chilensis*. At the same time canned shellfish reached the Region Metropolitana (Santiago) where they were consumed, and 400 persons suffered DSP symptoms.

In Chile, the Ministry of Health, through its Health Services, and the Institute of Public Health (ISP) are responsible for food control.

The Institute of Public Health is the official laboratory of the Health Service Laboratories in the country. The laboratory of the ISP in Santiago was evaluated in 1990 by the Food and Drug Administration (FDA) for the analysis of paralytic shellfish poison for the export of molluscan shellfish to the USA. The ISP approved this evaluation.

Besides the ISP, each Regional Health Service (X, XI, XII Regions) has a laboratory that analyzes PSP and DSP. When the level of toxin increases, they forbid the consumption and close the areas for shellfish harvesting.

Samples

1707 samples collected from January 1989 to April 1994 were analyzed. 1183 for PSP and 524 for DSP. These samples came from Health Services of Magallanes, Aysén, Llanchipal, and the Chilean Shellfish Sanitation Program (CSSP). This program corresponds to an agreement of understanding between Chile and the Food and Drug Administration of USA.

Methods

The laboratory uses the following methods:

PSP: AOAC Biological method - mouse test;
DSP: Biological method - Yasumoto's method, according to the modification used in Spain.

Recently ASP by HPLC - acid extraction (AOAC 1991) has been used.

Mice

CF-1 from our stock colony were used for routine assays (19-21g). Mice weighing more than 22g are not used.

Standard

PSP: FDA std soln: 100 g/mL.
working std soln: 1μg/mL

DSP: std.soln. not available

ASP: FDA std soln: 100 μg/mL
working std soln: 1 μg/mL and 10 μg/mL

Results

Of the 1183 samples analyzed for PSP we found that 7,5% of them presented toxins. However, PSP toxins were not detected in any samples send by CSSP (Chiloé Island in X Region and Horcón in V Region). The highest PSP level was found in samples of *Aulacomya magellanica* from XII Region (9.884μg/100g) in April 1991. PSP toxins were also detected in the Region of Aysén in 1992, but in low levels (30-105μg/100g). 524 samples have been analyzed from the XI Region for DSP, since February 1991. 28,2% of them were DSP contaminated. Samples from X Region did not show the presence of DSP toxins.

CROATIA

Even though algal blooms (red tide blooms, "mucilage") have occurred in the Adriatic Sea for more than two decades, the first toxic blooms were recorded at the end of the eighties. Monitoring and research programmes were therefore mostly organized with the object of investigating the mechanisms of initiation and termination of excess blooms, and to mitigate the consequences of these events.

Studies of suspect toxic phytoplankton species began during the mid-eighties. At the beginning of the nineties, the monitoring of toxic phytoplankton species was included in the long-term eutrophication monitoring program of the mid-Adriatic. The war in Croatia hindered the organization of extensive research programmes throughout the Adriatic; thus, this is the only organized program of observation of toxic species in the Croatian part

of the Adriatic Sea. The delay in legislation regarding phycotoxins was due to the same, aforementioned reasons, but legislation was eventually implemented in 1994.

These legislative measures are still incomplete as they regulate only the maximum permissible limit of toxins in shellfish. Meanwhile, the monitoring and determination of toxic species in shellfish breeding areas are not mentioned at all. The Institute of Oceanography and Fisheries is intensively attempting to introduce this aspect of protection into the law. This is particularly important for the areas rich in wild shellfish populations, where large quantities caught remain uncontrolled.

The first event of shellfish toxicity occurred in the northern Adriatic in late summer of 1989, when the DSP phenomenon associated with the bloom of several species of *Dinophysis* genre was recorded. The first toxicity analyses in Croatia were carried out at the Institute of Oceanography and Fisheries in 1993.

In co-operation with IFREMER experts, the Institute organized a meeting of Mediterranean countries in Dubrovnik in the summer of 1994, to organize the monitoring of shellfish breeding areas. A training course on the analysis of toxicity by mouse bioassay was held as a part of this meeting.

GERMANY

HISTORY

PSP

In September 1880 and December 1883, the first reports of poisonings of mussel consumers were reported from Wilhelmshaven, North Sea. The symptoms reported can clearly be related to PSP. In October 1885, 19 people were poisoned after mussel consumption when four fatalities occurred. Again the reported symptoms were those of PSP. Extracts of the mussel were injected into guinea-pigs and rabbits, which died. These experiments showed that, in particular, the hepatopancreas of the mussels was toxic. In the following years, poisonings were reported.

Since the beginning of the century, no poisonings related to PSP were reported after the consumption of mussels collected in German waters. However, in 1976, several persons showed PSP symptoms after consumption of mussels originating from Vigo, Spain. Since then, mussels are regularly monitored for PSP toxins, and so far there are no reports of PSP poisoning in Germany.

DSP

DSP poisoning shows symptoms very similar to those of some bacterial or viral infections due to mussel consumption. Thus, there are no early reports which can unequivocally be attributed to DSP.
The organisms causing the DSP poisoning in North-European waters are dinoflagellates of the genus *Dinophysis*, which may reach very high cell numbers (up to 80 000 l^{-1}). Thus it is likely that DSP poisonings had occured but were not related to algal born toxins.

Since 1976, when DSP was reported from the Dutch coastal area, mussels have regularly been monitored for DSP toxins, and almost each year some mussels containing DSP have been found.

ASP

So far there is no report on ASP poisonings in Germany, either from mussels collected in German waters or from imported seafood.

OCCURRENCE OF CAUSATIVE ORGANISMS

PSP

In German waters, species of the genus *Alexandrium halim* like *A. tamarensis*, *A. minutum* and *A. ostenfeldii* occur, but only in limited numbers. Since March 1992, viable cysts of a species very similar or identical to *Gymnodinium catenatum* have been found in German coastal waters of the North and Baltic Seas. So far, the toxicity of this strain has not been tested. Several species of blue greens, which are known to produce PSP toxins, have been reported from German waters; they have not yet been tested for toxins. But it has been reported that dogs have died after drinking slightly brackish water in which there was a blue-green algae bloom.

In freshwater, PSP producing blue greens have been isolated. Other species known to produce PSP toxins have not yet been reported in German waters.

DSP

Several species of the genus *Dinophysis*, which have been shown to produce DSP toxins, regularly occur in German coastal waters and may reach high cell densities. Several species of the genus *Prorocentrum* such as *P. micans*, *P. minimum*, and *P. redfieldii* have been reported with high cell densities but are apparently not involved in DSP phenomena. So far, *Prorocentrum lima* and other species known to produce DSP toxins have not been found in German waters.

ASP

Species of the diatom-genus *Pseudonitzschia* as *P. pungens* forma *multiseries*, *P. pseudodelicatissima*, and *P. seriata* are known to produce domoic acid. Together with other species of the genus, they are regularly abundant in German coastal waters. So far, there is no positive proof of ASP toxin in seafood either collected in German coastal waters or imported.

Longterm Observations and Monitoring of Harmful Algal Blooms:

(i) Longterm observations

For more than 25 years the Biologische Anstalt Helgoland has performed longterm observations of phytoplankton, including harmful algae in coastal waters of the North Sea, Germany, at Helgoland and Sylt;

a) Helgoland: phytoplankton, salinity, temperature, pH, nitrite, nitrate, ammonium, phosphate, silicate (each working day)

b) List/Sylt: phytoplankton, salinity, temperature, pH, nitrite, nitrate, ammonium, phosphate, silicate (once a week)

(ii) Monitoring of Harmful Algae

a) State of Niedersachsen (Lower Saxony)

North Sea: coastal waters from the Dutch border to the river Elbe: 10 stations, every second week from March to October: harmful algal species, abundant phytoplankton species, salinty, temperature, pH, oxygen, nitrite, nitrate, ammonium, phosphate, silicate

b) State of Schleswig-Holstein

North Sea: coastal waters from the River Elbe to the Danish border : 15 stations every second week from April to October: harmful algal species, abundant phytoplankton species, salinity, temperature, pH, nitrite, nitrate, ammonium, phosphate, silicate

Baltic Sea: coastal waters from the Danish border to the island Fehmarn: 14 stations once a week: harmful algal species

c) State of Mecklenburg-Vorpommern

Baltic Sea : from the Fehmarn Island to the Polish border : 50 stations once per month : phytoplankton, salinity, temperature, pH, oxygen, nitrite, nitrate, ammonium, phosphate, chlorophyll a.

ISRAEL

Is it necessary to have a national Harmful Algal Bloom (HAB) programme in Israel?

The Israel Oceanographic and Limnological Research (IOLR) is a governmental research institute. It is responsible for exploration and investigation of the aquatic and marine environments in Israel for the development of marine biotechnologies of potential commercial application. Research is carried out at three different locations:

Haifa - National Institute of Oceanography and the headquarters
Lake Kinneret - Kinneret Limnological Laboratory
Eilat - National Center for Mariculture

Why there were no reports on harmful algal blooms from Israel?

(i) the coastal waters in Israel are very oligotrophic. Consequently, the fauna is limited in number of species and in biomass.

(ii) there are no significant populations of edible oysters and mussels, and a related industry has not yet been developed.

(iii) the consumption of oysters and mussles by the local population is very limited due to religious restrictions.

Do we need a national program for monitoring of harmful algal bloom in Israel?

(i) the spreading of HAB-related toxic events around the world should alert us too.

(ii) we expect the development of an oyster production industry in the next several years. This development is part of a general scheme which integrates the production of recently domesticated marine fish in inland intensive ponds with the production of oysters and algae.

The rationale is that oyster and algae production can benefit from the effluents of fish production ponds and at the same time will improve the effluent quality before it is discharged into the coastal marine environment, causing eutrophication.

Conclusion

Adequate preparation at the scientific and technical levels will allow us to be ready and to implement a monitoring program for harmful algal bloom and related toxic substances when needed.

ITALY

The problem of shellfish contamination by algal toxins in Italy

In Italy the shellfish market concerns chiefly mussels *(Mytilus galloprovincialis),* about 130,000 tons of mussels are consumed every year. More than 65% of the production comes from the Adriatic Sea and about 20% are imported from foreign countries (chiefly Spain).

In the Adriatic Sea dinoflagellate red tides are a recurring phenomenon and, despite the presence of potentially toxic species, such as *Alexandrium* ssp. (Protogonyaulax) and *Dinophysis* ssp., no shellfish poisoning was described until 1989.

In June 1989 several cases of Diarrhoeic Shellfish Poisoning (DSP) were recorded in people after consumption of mussels grown in the Adriatic Sea, along the Emilia Romagna and Marche coasts (Boni et al., 1992). Since 1989, the collection of shellfish along the Northern and the Middle Adriatic Sea coasts has been banned for long periods with considerable economical loss. Thus, DSP represents the main problem for aquaculture in Italy. Mussels, both from natural banks and from farms, appear to be the most contaminated shellfish. Other molluscs, such as scallops, oysters and clams generally appear to be free of contamination.

Until now, contaminated shellfish seem to come only from the Adriatic Sea, but shellfish grown in lagoons appear to be less contaminated. Contamination has not been detected in molluscs coming from other areas.

Various *Dynophysis* species have been found in the Adriatic Sea. In the Gulf of Trieste a direct correlation between the DSP contamination of mussels and the presence of *D.fortii* in seawater was found (Della Loggia et al., 1993). No toxins different from okadaic acid were identified with certainty, but the presence of YTX is suspected in some areas (Zaho et al., 1993).

Although the main problem in Italy is represented by DSP, recently some shellfish samples were found to be contaminated by PSP along the Emilia Romagna coasts. The phenomenon seems to be related to the presence of *Alexandrium cf. minutum* in seawater (Poletti and Honsell, personal communications). No PSP intoxication cases in humans were reported.

Italian law prescribes that shellfish growing areas must be monitored by Regional Health Authorities for algal toxins in shellfish and phytoplankton in seawater every two weeks. Seawater samples for qualitative and quantitative analysis have to be collected at three different depths (0,5m from surface, middle, and bottom). If more than 1.000 *Dinophysis* cells/l are present, or other potentially toxic algae are found, or toxins are detected in shellfish, the phytoplankton and shellfish control has to be carried out more frequently.

Shellfish harvesting and marketing is prohibited if the shellfish contain:

(i) a concentration of DSP toxins that gives positive results to the mouse bioassay (survival time: 5 hrs);
(ii) more than 40μg of PSP toxins/100g of soft tissue, determined by the AOAC method;
(iii) a concentration of NSP toxins detectable by the McFarren method.

Research work on algal toxins is very active, in particular in the search for new methods to determine DSP risk and to identify DSP toxins involved. Taxonomical studies on potentially toxic algae are carried out by different research groups in order to identify the causative agents for DSP and PSP contamination. The presence of cysts of potentially toxic algae represents another field of research.

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MEXICO

Qualitative and quantitative determination on algal toxins in Mexico

The presence of harmful algal blooms in México has been known since Precolombian times. According to the reports of Alvar Nuñez Cabeza de Vaca (Naufragios/ Shipwrecks), natives were aware of the risks of eating molluscs collected at the beginning of the year when blooms appeared and they banned the comsumption of shellfish during that period. The oldest scientific report (1955) describes a massive bloom of ***Gymnodinium brevis*** along the coast of Texas in the Gulf of México. No human poisonings were reported, other than several cases of eye and respiratory tract irritation in the local population. The bloom had a great impact on the marine environment as more than 1000 dead fish/km were observed along the coastline of Tamaulipas, México and Texas, USA.

A branch of the National Autonomous University, the Institute of Sea Science and Limnology (ICML/UNAM) at Mazatlán Bay, Sinaloa, is currently monitoring this coastal area in México for the presence and follow-up of these phenomena. They have shown that, at least in this area, there is a direct correlation between oceanic upwellings and occurrences of these events. During this study, eight species were reported as the main cause of formation of red tides, three of them being notoriously toxic (mainly ***Gymnodinium catenatum***); however, there are more than 21 other species that are potentially toxic (Cortés-Altamirano y Nuñez-Pasten, 1992). Blooms of organisms not reported to be toxic such as ***Noctiluca scintillans*** and ***Mesodinium rubrum*** are common along the coasts of the Gulf of California. Nevertheless, blooms of these organisms can be dangerous afterwards.

In México, the management of toxic red tide blooms is the responsibility of the Federal Health Secretary (Secretaria de Salud), through the Direction of Applied Epidemiology, which runs two officially certified laboratories, one in the city of Acapulco, Guerrero, and the other in the National Health Laboratories in México City. Once a red tide is reported, or when human poisoning occurs, a total ban on the commercialization and distribution of shellfish is put in place until the levels of PSP, determined by mouse bioassay, drop below the limit of 30 μg/100 g of shellfish.

Another branch of the Health Secretariat runs the Programa Nacional de Sanidad de Moluscos Bivalvos (National Program for the Health of Bivalve Molluscs). This organization develops the rules that govern how health authorities of each state must supervise the production, commerce, and distribution of all shellfish products, paying attention to the type and amount of toxins permitted in food destined for human consumption.

As mentioned above, the only institution that constantly monitors bloom activity is the ICML/UNAM at Mazatlán, Sinaloa. Their efforts are primarily oriented to oceanographic and physico-chemical aspects, and to the identification of organisms responsible for these blooms. At the Biological Research Center, Northwest (CIBNOR), La Paz, Baja California Sur, México, research is mainly devoted to the quantification of PSP activity in shellfish collected from the areas affected by blooms. Currently, the CIBNOR is doing two small follow-up studies on areas where blooms have recently occured. During these studies, high levels of phycotoxicity (460 μg/100g shellfish) were observed in bloomless areas having yearly occurences of toxicity in late winter and early spring.

Record of noxious red tides in México

YEAR	PLACE	ORGANISM	EFFECTS	TOXIN DETERMINED
1955	Veracruz	*G. brevis*	1000 dead fish/km	
1956	Tamaulipas	*G. brevis*		
1979	Mazatlan, Sinaloa	*Gymnodinium catenatum* $1.1*10^6$ cells/ml		
1985	Guerrero		7 people intoxicated/2 deaths	51 μg Sax/ g lioph. cells 1720 μg Sax/100 g shellfish
1986	Tamaulipas/Texas			
1988	Sinaloa (10 times)	*Gymnodinium catenatum* $0.94*10^6$ cells/ml	10 people intoxicated	
1989	Salina Cruz, Oaxaca	*Pyrodinium bahamense var. compresum* *G. catenatum*	99people intoxicated/ 3 deaths	380-570 μg Sax/100 g shellfish
1989	Guerrero, Chiapas	*Pyrodinium bahamense var. compressum.* *G. catenatum*		
1991	Sinaloa (7veces)			
1991	Bahía Concepción, Baja California Sur		Several tons of shellfish lost	
1992	Pto. madero, Chiapas		2 people intoxicated/ 1 death	45 mg Sax/100 g shellfish
1992	Ojo de Liebre, Baja California Sur (2 veces)		Several tons of shellfish lost	
1992	Bahía Magdalena, Baja California Sur		22 dead dolphins, also several sea lions, turtles, fish and sea-birds.	
1993	La Paz, Baja California Sur	*Trichodesmium spp.*		Mouse bioassays showed the presence of hepatotoxin-like effects.
1994	Mazatlan, Sinaloa	*Mesodinium rubrum* $0.45\text{-}2.0*10^6$ cells/l (feb) *Protoperidinium spp* $0.6\text{-}2.0*10^6$ cells/l (mar) *Gymnodinium catenatum* $16\text{-}52*10^6$ cells/l (mar) *Prorocentrum dentatum* $31*10^6$ cells/l (apr) *G. catenatum* $1*10^6$ cells/l (apr)		
1994	Acapulco, Guerrero	*Gymnodinium sp.* *Gonyaulax sp.*		57-93 μg/100 g shellfish
1994	Pta. Abreojos, Baja California Sur		Several sea-birds and many fish dead along the beach	No PSP present in samples of sea water.?!

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NEW ZEALAND

The monitoring and qualitative and quantitative determination of algal toxins in New Zealand

MONITORING

Toxins in shellfish: monitored by the Marine Biotoxin Surveillance Programme, which is controlled by a Marine Biotoxin Management Board comprising of members of the Ministry of Agriculture and Fisheries, the Ministry of Health, the Public Health Commission and the New Zealand Fishing Industry Board. Approximately 120 shellfish samples from around the coast of New Zealand are monitored per week. All are analysed for PSP, NSP and DSP, and 50 are analysed for ASP, including all scallop samples.Closures to harvesting areas are effected if assay results are higher than regulatory limits after just one test (see below). Areas are reopened after two successive negative tests.

Phytoplankton: following the 1992/93 toxic algal bloom event, the Ministry of Agriculture and Fisheries organised the Phytoplankton Research/Monitoring Programme. Samples from 4 different water depths at 17 sites in the bloom area were collected weekly and sent to the Cawthron Institute where they were examined under microscope for species composition and cell density. The information gained was passed on to the Marine Biotoxin Surveillance Programme to be used in conjunction with the shellfish assay information. Unfortunately this programme continued for just 9 months. Currently the Cawthron Institute is receiving samples from 30 sites per week (with water depths integrated into one sample per site) from the Marlborough Sounds and the Coromandel area, costs for this monitoring being incurred by the marine farmers. The information from this monitoring goes back to the farmers and is used by them to help decide where and when to harvest.

DETERMINATION OF TOXINS

PSP

Almost all known PSP toxins have been detected in NZ shellfish and indigenous microalgae. Analysis for PSP is carried out by mouse bioassay at Environmental Science and Research Ltd, Lower Hutt. Extraction is by the standard APHA method (Delaney, 1985), using 0.1N HCl. The sensitivity of this method for saxitoxin is 40μg/100g shellfish tissue, and any value above the regulatory limit of 80μg/ 100g shellfish tissue results in automatic closure of the sample area.

The Cawthron Institute uses the HPLC method of Oshima et al. (1992) to detect PSP toxins in shellfish and phytoplankton for internal research purposes. Extraction is by the standard APHA method (see above). The sensitivity of this method for saxitoxin is 1μg/100g shellfish tissue. The R-Biopharm Ridascreen saxitoxin ELISA test kit (sensitivity for saxitoxin 50pg/ 100g shellfish tissue) has been used at Cawthron to test the toxicity of dinoflagellate cultures.

NSP/DSP

Samples to be tested for NSP/DSP are extracted initially using acetone by the NZ method (Hannah et al. 1994). The extract is tested by mouse bioassay at Environmental Science Research Ltd. If there are no deaths after 6 hours of observation then NSP is said to undetectable to 10MU/ 100g. If the mice die then the toxin could be either NSP or DSP. The sample is then tested for DSP using the Sceti 'DSP-check' ELISA kit (sensitivity for okadaic acid / DTX1 10μg/ 100g shellfish tissue, regulatory limit 20μg/ 100g shellfish tissue). Further analysis for NSP involves extracting 100g of the original shellfish with ether and testing with the

standard APHA mouse bioassay (Delaney, 1985). Sensitivity of this method, and the regulatory limit for NSP toxicity is 20MU/ 100g shellfish tissue.

The Cawthron Institute has a post-doctoral researcher developing new methods for HPLC determination of DSP.

Neale Towers' group at AgResearch, Ruakura, Hamilton, uses a cytotoxicity assay based on that of Manger et al. (1993) to detect NSP and PSP for research purposes. The sensitivity for brevetoxin, and saxitoxin, is 20ng/ 100g shellfish tissue. This group plans to raise antibodies against several species of toxic dinoflagellates, and against derivatives of saxitoxin and brevetoxin for use in immunoassays.

ASP

Domoic acid is tested at Environmental Science and Research Ltd. for the surveillance programme, and at the Cawthron Institute for research purposes. It is extracted by methanol/water (Lawrence and Menard, 1991), and is analysed by HPLC (Wright et al. 1989). The detection limit for domoic acid is 4μg/ 100g shellfish tissue and the regulatory limit is 2mg/ 100g shellfish.

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PORTUGAL

Qualitative and quantitative determination on algal toxins in Portugal

In Portugal bivalve toxication is monitored at the Portuguese Institute for Marine Research, Lisbon (IPIMAR). Sampling of bivalve species is made monthly, and then weekly when toxic algae are found in monitored water samples.

PSP DETERMINATION

Determination

PSP toxins are monitored by mouse bioassay using the AOAC method (AOAC, 1984).

Management decisions

Bivalve species with PSP values greater than 80μg STXeq./ 100g are closed to harvest.

Occurrence

Since 1986 and until 1990 there were occurrences of PSP outbreaks at the Portuguese coast north from Roca cape. In 1991 the problem did not appear. In 1992 it appeared again but occurred also off the south coast of Lisbon and at the Algarve coast. In 1993 PSP was found almost all around year round, covering the entire coast. In 1994 the PSP outbreaks have occurred off the south coast of Portugal and off the Algarve coast.

Causative species

The main causative organism is *Gymnodinium catenatum*. *Alexandrium lusitanicum* was also found to be responsible for PSP outbreaks at the Obidos Lagoon.

Future trends

Shortly PSP toxins will be monitored by HPLC. HPLC studies of toxic dinoflagellate cultures are already carried out at the National Health Institute (INSA).

DSP determination

DSP toxins are monitored by mouse bioassay following a modified Yasumoto et al. (1978) method. Since the beginning of 1993 all samples screened for this kind of toxicity have also been studied by a HPLC technique (Lee et al., 1987).

Management decisions

The harvest of contaminated species is closed.

Occurrence

The first confirmed occurence happened in 1987. Until 1992 the problem increased in space: it occurred along the west coast (north of Sines) and south coast of Portugal. In 1992 DSP outbreaks occurred only in reduced areas: Minho estuary, Mondego estuary and Aveiro Ria. In 1993 there were no areas closed due to DSP toxication. In 1994 the same areas have been closed to harvest as during the 1992 outbreak, as well as the coast south of Lisbon.

Causative species

The causative species are *Dinophysis acuminata* and *Dinophysis acuta*.

Preliminary HPLC results

The most abundant DSP toxins found in Portuguese bivalves are OA and DTX-2 (in mussels for example) or a modified (acilated?) form of these two (in surf clam - *Spisula solida*, for example). Other

unknown peaks await further knowledge for identification. OA and DTX-2 have also been found in water samples from Aveiro Ria and Obidos Lagoon (the only water samples screened so far for this toxins).

ASP determination

ASP outbreaks have not yet been found off the Portuguese coast. Screening for this kind of toxicity has been carried out utilizing the mouse assay for PSP (mice were under observation for 24 hours).

ROMANIA

The increase of phosphorus, nitrogen, and organic matter induced by the enhancement of eutrophication during 1970-1990 have influenced microalgal bloom dynamics in Romanian coastal waters. During 1981-1990, we registered 46 monospecific blooms produced by 15 algal species, twelve of them achieved the highest densities known for the Romanian littoral up to the present time. Apart from these bloom species causing changes in water colour, numerous other algae realized remarkable developments. The numerial densities of the main phytoplankton species have increased during the last two decades.

Table 1: **Mean phytoplankton quantities offshore the Romanian littoral of the Black Sea (Constanta-Portita zone up to 30 miles seaward).**

Period	Cells L^{-1}	$Mg.m^{-3}$
1971-1975	259,600	719
1976-1980	789,489	2244
1983-1990	2,235,77	4105

Table 2: **Predominating algal species associated with red water blooms in the Romanian Black Sea littoral from 1981-1990**

BLOOM SPECIES	PERIOD AND MAXIMUM DENSITY (MILLION CELLS L^{-1})									
	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990
Prorocentrum cordatum	VI 175.0	VII 462.7	VI 43.5	VII 35.6		VI 276.6	VII 807.6	VII 22.7	VI 87.9	V 9.9
Prorocentrum scutellum									VII 7.2	
Scrippsiella trochoidea									VIII 25.8	
Heterocapsa triquetra	V 12.0	VI 5.3				V 12.8		V 4.7	V 3.6	
Eutreptia lanowii			V;VIII 20.0	VI;IX 51.5		V 5.9	VII 16.7		VII 108.0	VI 5.0
Emiliania huxlevi								VIII 291.2	VII 213.3	
Chromulina sp.	VII 1000.0									
Sceletonema costatum	V 13.2	V 36.8	VII 90.0	IV 141.4	IV 46.0	V 59.3	VI 14.3			V 17.9
Sceletonema subsalsum						VI 10.0		V 18.7		
Cyclotella caspia	VII 300.0					VI 23.0				
Chaetoceros similis f. solitarius								21.5		
Cerataulina pelagica				V 5.2	X 5.1					VI 6.0
Nitzschia closterium									VII 13.1	
Nitzschia tenuirostris									VIII 74.8	
Nitzschia delicatissima						V 17.2				
Total Blooms	5	3	4	5	2	7	3	5	8	4

SPAIN

In 1976 a major PSP outbreak occurred off the coast of Galicia (NW). The causative organism was *Gymnodiniumm catenatum*. Following this event, several monitoring and control programmes were established. In the following years other species such as *Alexandrium minutum* and *Prorocentrum lima* were identified in the same region. In addition to PSP, the presence of DSP toxicity, originating in species of the genus *Dinophysis*, was also detected. More recently, the Mediterranean coast has been affected by several toxic as well as non-toxic phytoplankton blooms. In the region of Cataluna (NE) both *Alexandrium minutum* and *Dinophysis accuminata* were detected. In Andalucia (S) shellfish toxicity provoked by *Alexandrium minutum, Prorocentrum lima* and several *Dinophysis* was recorded. In the region of Valencia (W), several non-toxic phytoplankton blooms have been observed over the last two years. Concomitantly relatively high numbers of potentially toxic species belonging to genera such as *Gymnodinium*, *Gonyaulax*, *Dinophysis*, *Prorocentrum* and *Gyrodinium* were also identified in the same waters. Concern about red tides is therefore very high in Spain.

MONITORING

Several monitoring programmes have been implemented in Galicia and Cataluna. The local governments in Galicia, Cataluna, Andalucia and Valencia also perform large public health and seafood safety control programs for PSP and DSP. The official method employed for toxin detection is the mouse bioassay. The use of HPLC methods is increasing. In addition to this, the EC Reference Laboratory for Marine Biotoxins has been established in Vigo, Galicia. This laboratory also serves as the National Reference Laboratory. Another institution, the National Food Laboratory is currently involved in the preparation of reference material (contaminated and uncontaminated liophilized mussel) for PSP analysis as part of a BCR project.

RESEARCH

On the scientific level, several lines of research are currently followed in Spain at the Spanish Oceanographic Institute, the National Research Council and several universities. Studies concern the ecology, taxonomy, genetics, toxicology and toxin chemistry of involved species. Emphasis is put on general oceanographic research, the improvement of monitoring and control methods, the physiology of toxin production and the interaction between mussels and dinoflagellates. The developement of molecular techniques for the accurate identification of toxic species as well as the determination of optimal procedures for the preparation of toxic samples and correct bioassay methods are a further priority. In parallel to this, several training courses in HPLC analysis, monitoring, and taxonomy as well as some intercalibration exercises have been organized. Spain also participates in international information networks. At the national level no harmful algal bloom (HAB) programme has been established yet, but communication among scientists and among institutions is very fluent.

TUNISIA

Monitoring of the presence of algal toxins in shellfish from Tunisia

Tunisia is situated in the north-eastern part of Africa, and has a population of about 8 millions. In the last decade tourism, finfish harvesting, and aquaculture have grown considerably in the coastal region of Tunisia, (about 1.300 km). In 1992, Tunisia produced 89,000 tons of shellfish and fish. 75% of shellfish production (12.000 tons) were exported mainly to France, Italy and Spain. It has been observed that marine dinoflagellates and diatoms occur seasonally (mainly in the summer time) within certain coastal waters. Luckily, the harmless species predominate, but as a precaution, the need to monitor water, coastal algal blooms and detection of toxins in shellfish is essential. Monitoring is also necessary to preserve natural resources.

The Tunisian government is interested in developing routine detection of toxins in shellfish. Several such programmes started last year. From November 1993 to June 1994, monitoring of mussel safety was performed weekly at five coastal sites for mussels, which were exported to European countries. Assessement of toxicity of the samples was carried out by the mouse bioassay method. Aqueous PSP containing solutions were obtained by extraction of shellfish samples. DSP extraction was carried out using acetone. These extracts were intraperitonial administered to mice. Mice injected with PSP were observed for at least 30 min. and those injected with DSP were observed for 24 hours.

During the past year about 200 biological tests were performed and it was found that from November 93 to June 94 mussels were not toxic. Also no PSP or okadaic acid were detected.

ANNEX IV

HPLC-METHODS FOR PHYCOTOXIN DETERMINATION

A. HPLC METHODS FOR DETERMINATION OF DSP

IMPROVEMENT OF HPLC FLUORESCENCE AND HPLC MS DETERMINATION OF DSP TOXINS

The method applied most often for DSP determination and described by Lee *et al* is based on the selective extraction of OA and DTX-1 with 80% methanol, followed by derivatization with ADAM, clean-up on silica gel and isocratic HPLC separation of 9-AM-OA and/or 9-AM-DTX-1 on a RP-column and fluorescence detection.

It has been proven that an acceptable recovery of the DSP toxins extracted from contaminated material (algae, mussels) is impossible when applying a clean-up procedure with silica gel after derivatization with ADAM. Silica gel purification of solutions containing 9-AM-esters, which have been formed by derivatization with ADAM, led to unreproducible results.

To overcome the shortcomings of the method developed by Lee *et al* (1) (i.e. to avoid losses of 9-AM-OA and 9-AM-DTX-1 within the clean-up procedure), Shen *et al* (2) proposed to modify the HPLC equipment. The use of an HPLC with column-switching system is necessary for the DSP determination by HPLC immediately after the derivatization of the extracts with ADAM. This HPLC device permits the injection of DSP containing extracts immediately after derivatization with ADAM.

This modified HPLC method allows a rapid and sensitive determination near to 100%. Yet, the handling of the HPLC-column-switching system demands skill and is connected with many operations (cuts).

Thus, the reaction of DSP toxins with BrMmc for derivatization was examined. The BrMmc derivatives of the DSP toxins are more stable than the ADAM derivatives. An additional clean-up step after the derivatization with BrMmc is not required, because the HPLC chromatograms revealed no interfering peaks originating from the BrMmc reagent.

Extraction of the homogenized mussel sample is carried out with methanol/water (80/20; v/v). A clean-up of the raw extract with n-hexane can be omitted. However, the application of an HPLC method based on the chromatographic separation of the BrMmc derivatives of the DSP toxins needs an additional clean-up step after extraction.

Further purification is advantageously carried out by SPE with a silica gel cartridge before the derivatization reaction. The purification procedure leads to thoroughly clean extracts containing DSP (3).

The purified solutions are suitable for both derivatization methods, the esterification with ADA connected with direct injection into an HPLC including a column-switching system, as well as the reaction with BrMmc followed by isocratic HPLC.

Fluorescence detection is applied for the determination of the ADAM derivatives and for the BrMmc derivatives of the DSP toxins after their chromatographic separation on RP-columns.

In view of the utilization of the HPLC-MS coupling in DSP analysis the HPLC method based on the BrMmc derivatization followed by isocratic HPLC separation of the coumarin esters of OA and DTX-1 is suggested (4).

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B. HPLC METHODS FOR DETERMINATION OF PSP

IMPROVEMENT OF THE HPLC-FLUORESCENCE AND HPLC MS DETERMINATION OF PSP TOXINS

Introduction

The nature and number of PSP compounds in shellfish depend on the toxin patterns produced by the algae, the conditions of storage, and on the metabolism of the PSP toxins in shellfish. In addition to the difference in their chemical structure the individual PSP toxins show various toxicities. It is possible that PSP toxins are converted by enzymatic processes, e.g. the hydrolysis of N-sulfocarbamoyl toxins which lead to the more toxic carbamate- and decarbamoyl toxins.

HPLC techniques allow the separation and sensitive detection of individual PSP toxins irrespective of their number and group. Therefore, HPLC methods have opened up a new dimension in phycotoxin analysis. However, the results obtained have to be comparable to those of the mouse bioassay (1). This requirement is partly fulfilled by the application of identical procedures for sample preparation.

Additionally, accurate HPLC determination of the various PSP components in the samples is necessary.The concentrations of individual PSP toxins were calculated on the basis of the PSP peaks in the HPLC traces, converted into their STX equivalent, and summed for comparison with bioassay values. Individual contributions to samples toxicity were calculated for each toxin using the following equation (2):

$$G = CTD/ 100$$

where C = toxin concentration (μm/100g), T = toxicity facto (μg STX/ μM toxin) and
D = dilution of sample (ml/100g of shellfish meat).

Sample preparation

The hydrophilic nature of PSP toxins complicated their isolation from biological material by organic solvents.

Therefore, often aqueous-acidic extracts were purified by gel-chromatography and/or slightly acidic cation exchangers. These techniques are very expensive. Furthermore, the toxins C1-C4 were not attached

to cation exchangers and they could only be isolated by repeated gel chromatography. To avoid these inconvenient steps of sample preparation, a simple extraction technique was developed.

The extraction technique was based upon the standard AOAC method for PSP determination by mouse bioassay, but the quantity of mussel material for extraction was reduced from 100g to 1 to 2g, because of the availability of small amounts of mussel material only.

After weighing 1 to 2g of mussel material into a centrifugation tube, the extraction solution was added, and the total mass was noted. Afterwards, the sample was heated for 10 min. in a steam bath and stirred several times.

After cooling, the initital mass was refilled with extraction solution and the sample was centrifuged at 1000g for 30min. in order to separate insolubles from the matrix. Prior to the injection into the HPLC the solution in the tube was filtered through a membrane filter (0.45μm).

Although this technique led to reliable results, this extraction procedure was time-consuming because of a low sample output. The application of sealed reacti-vials in a heating-module solves these problems. At the end of the extraction time the reacti-vials could be directly centrifuged, and the extract suitable for direct injection into the HPLC system with post-column derivatization unit as well as for pre-column PSP derivatization.

HPLC separation

(i) HPLC with pre-column derivatization

The detection of the PSP toxins is based on the fluorimetric assay described by Bates and co-workers (3). As PSP toxins show neither UV absorption nor fluorescence, STW was oxidized in alkaline solution in order to obtain derivatives detectable by common HPLC detectors. The derivatization reaction is based on the oxidation of STX to 8-amino-6-hydroxylmethyl-2-aminopurine-3-propionic acid, which reacts in acidic solution and gives a fluorescent pyrimidopurine.

For PSP detection some workers have used this reaction and measured the fluorescence of the oxidation products directly (4); others first separated the oxidation products by chromatography before subsequent fluorescence detection (5). Recently, this so called Lawrence method was improved (6).

Yet, it could be noted that the application of the oxidation reaction for pre-column derivatization has several drawbacks.

The PSP toxins were oxidized at room temperature under mildly basic conditions with hydrogen peroxide or periodic acid. The products were then analyzed by HPLC. The N-1-hydroxylated toxins (neosaxitoxin, B-2, GTX-1, and C-3) formed fluorescent products after periodate oxidation at ca pH 8.7, but did not form fluorescent derivatives with peroxide oxidation. The non-N-1 hydroxylated toxins (saxitoxins, B-1, GTX-2, GTX-3, C-1, and C-2) formed highly fluorescent derivatives with both peroxide and periodate oxidations. Individual toxins produced mainly single fluorescent peaks by reverse-phase HPLC. However, all GTX toxins eluted with the same retention time. Also, C-1 and C-2 eluted together, as did neosaxitoxin and B-2.

Therefore, the oxidation of the PSP toxins is usually carried out as a post-column reaction (2).

(ii) HPLC with post-column derivatization

Many chromatographic techniques have been developed for separating PSP toxins in their

underivatized form. At first these separations were carried out using ion-exchange and/or gel permeation techniques, and later silica-based HPLC-columns were applied. However, the breakthrough in HPLC for PSP separation was the introduction of ion-pair chromatography (7).

Sullivan *et al* (8) have developed an HPLC method involving ion-pair chromatography on a PRP-1 phase. Separation of PSP toxins is provided by a gradient elution using two phosphate buffers containing hexane-/heptanesulfonic acid as ion-pair reagents.

This HPLC method requires careful management of operating parameters in order to produce reliable results. The handling of the low buffer concentrations is relatively difficult because changes in pH and salt concentrations of injection solutions may cause variations in separation. Problems arise in case of contamination of seafood with decarbamoyl toxins (e.g. STX and cd-STX coeluate). Differences in findlings and poor correlation between HPLC and mouse bioassay may be explained by the presence of decarbamoyltoxins in the samples.

Oshima *et al* (9) proposed the application of three chromatographic runs for PSP determination. Three groups of PSP toxins (A: C1-C4; B: GTX1-GTX4, B1, B2, dc-GTX4; C: Neo, dc-STX, STX) are separated in three HPLC systems under isocratic conditions.

This method was modified by Franco *et al* (10) . However, an expensive HPLC equipment and time-consuming pre-chromatographic steps are serious drawbacks of these methods.

Luckas *et al* (11) proposed ion-pair chromatography on an RP-C18 column (Nucleosil 7-C18; Macherey-Nagel, Düren, Germany) with octanesulphonic elution to overcome the problem of dc-STX-STX separation. As the application of this method led to interferences in the chromatograms at the retention times of gonyautoxins, the chromatographic conditions were changed.

Thielert *et al* (12) proposed the application of an RP-C18 column and two phosphate buffers containing octanosulfonic acid and acetonitrile-THF as eluents. A two steps elution allows the separation of carbamate and decarbamoyltoxins, and good resolution of the more strongly retained toxins (NEO, dc-STX, STX) is achieved.

A disadvantage of both HPLC methods, the Sullivan-HPLC and the Thielert-HPLC, is the impracticability of the HPLC-MS coupling. The ion-pair reagents let to difficulties in mass spectrometry. Additionally, solutions containing phosphate are not suitable for HPLC-MS coupling. A new HPLC method was therefore developed.

This method is based on the HPLC separation of the underivatized PSP toxins on an exchange resin, post-column oxidation with an electrochemical detector, and fluorescence detection.

The chromatographic separation of the carbamate toxins (STX, dc-STX, NEO, GTXs) is achieved by a cation exchange column. The eluent contains ammonium acetate and the electrochemical oxidation potential is set up to +1050mV. The application of different potentials such as +1050mV for the PSP toxin detection in their oxidized form via a fluorescence detector and mass spectrometry and 0mV for the analysis of the underivatized PSP components by MS opens new doors in the PSP analysis by HPLC-MS (13).

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C. HPLC METHODS FOR DETERMINATION OF ASP

INTRODUCTION

From November 11 to December 12, 1987, 153 people suffered from acute poisoning after eating cultured blue mussels (*Mytilus edulis L.*) harvested from a localized area in eastern Prince Edward Island (P.E.I.). Symptoms included nausea and diarrhea which in some cases were followed by confusion, disorientation, loss of memory and even coma. Three elderly people died. In the other most severely affected cases neurological symptoms still persist. The term Amnesic Shellfish Poison (ASP) has been proposed for this new shellfish toxin. The toxic agent was first detected in mussel digestive glands using the Association of Official Analytical Chemists (AOAC) mouse bioassay for paralytic shellfish poison (PSP). Extracts of contaminated mussels injected intraperitoneally into laboratory mice produced an unusual scratching syndrome and eventual death. An intense investigation using bioassay-directed analysis led to identification of the toxin as domoic acid, a relatively rare naturally-occurring secondary amino acid that was first isolated in 1958 from the red alga Chondria armata Okamura (Rhodomelaceae family) (1).

HPLC played a key role in all stages of investigation which led to the identification of domoic acid as the mussel toxin. In the initial stages, HPLC with UV diode array detection (DAD) was used for „fingerprinting" extracts of toxic and control shellfish, and preparative HPLC was used for purification of

the toxin for structural elucidation by spectroscopic techniques. Later, HPLC was used for accurate determination of domoic acid in mussel tissue in order to account for the observed toxicity and also for survey work on a wide variety of samples including other shellfish and phytoplankton. Recently, it was shown that the pennate diatom *Nitzschia pungens* Grun. f. *mulitseries* Hasle produces domoic acid and was likely the primary source of the toxin in the P.E.I. incident (2).

The addition of domoic acid to shellfish monitoring programs will require a rapid, accurate, reproducible, and sensitive analytical method. The method must be applicable to a wide range of sample types and preferably provide at least two independent sources of evidence for identification purposes. Such a method based on high speed HPLC-ADA, was published by Quilliam *et al* and was presented at the training course (3).

SAMPLE EXTRACTION AND CLEAN-UP PROCEDURE

In a typical extraction, homogenized mussel tissue (100g) was heated and boiled for 5min. (stirring) with distilled water (100mL). The cooked mixture was centrifuged at 3500rpm (5min.), the supernatant decanted and the residual pellet washed with water (50mL) and recentrifuged. The combined supernatants were made up to 250.0mL.

Phytoplankton and seaweed samples were extracted by sonication in a minimum volume of cold water, followed by filtration to remove debris.

After the pH of the crude extract had been adjusted to pH 6 to 7 (usually not necessary with aqueous extracts), an accurate volume of up to 2ml was placed on a pre-rinsed (6mL acetonitrile and 6mL water) octadecylsilica LC-18 solid phase extraction cartridge (Supelco, Bellefonte, PA). The sample was eluted with 3mL of 10% aqueous acetonitrile, with the eluate being collected in a 5mL volumetric flask. The contents of the flask were made to volume with water. An aliquot was then passed through a dry 0.22μm filter and used for HPLC analysis.

HPLC SEPARATION AND DAD DETECTION

Analyses were performed on a Hewlett-Packard model 1090M HPLC equipped with a DR5 solvent delivery system, variable volume (1 to 25μL) injector and autosampler, built-in HP 1040 DAD and HP 79994 data system. Columns (25cmx4.6mm I.D. or 2.1mm I.D.) packed with 5μm Vydac 201 TP (Separations Group, Hesperia, CA) were used at 40°C. The mobile phase was aqueous acetonitrile with 0.1% v/v trifluoroacetic acid. High resolution separations were performed with the 4.6mm I.D. column using a 20μL injection and linear gradient elution from 5% to 25% acetonitrile over 25min. at 1mL/min. High speed analyses used the 2.1mm column with a 5μL injection volume and isocratic elution with 10% acetonitrile at 0.5mL/min. Detection was effected by monitoring absorption at 242nm with a 10nm bandwidth. UV spectral acquisition was either triggered by peaks or continuous at 640msec. intervals. Quantification was accomplished by comparing the areas of peaks from unknowns with those from standard solutions prepared from pure domoic acid.

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ANNEX V

LIST OF ACRONYMS

ADAM	9-Anthryldiazomethane
AOAC	Association of Official Analytical Chemists
ASP	Amnesic Shellfish Poisoning
BrMmc	4-Bromomethyl-7-methoxycoumarin
CEC	Commission of the European Communities
DA	Domoic Acid
DAD	Diode Array Detector
dc-STX	Decarbamoylsaxitoxin
DSP	Diarrhetic Shellfish Poisoning
DTX-1	Dinophysistoxin-1
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
HPLC	High Pressure Liquid Chromatography
GTX 1-4	Gonyautoxin 1-4
IOC	Intergovernmental Oceanographic Commission
IUPAC	International Union of Pure and Applied Chemists
MAP	Mediterranean Action Plan
NEO	Neo-Saxitoxin
OA	Okadaic Acid
PSP	Paralytic Shellfish Poisoning
STX	Saxitoxin
TEMA	IOC Committee for Training, Education and Mutual Assistance in the Marine Sciences
UNEP	United Nations Environment Programme
WHO	World Health Organization