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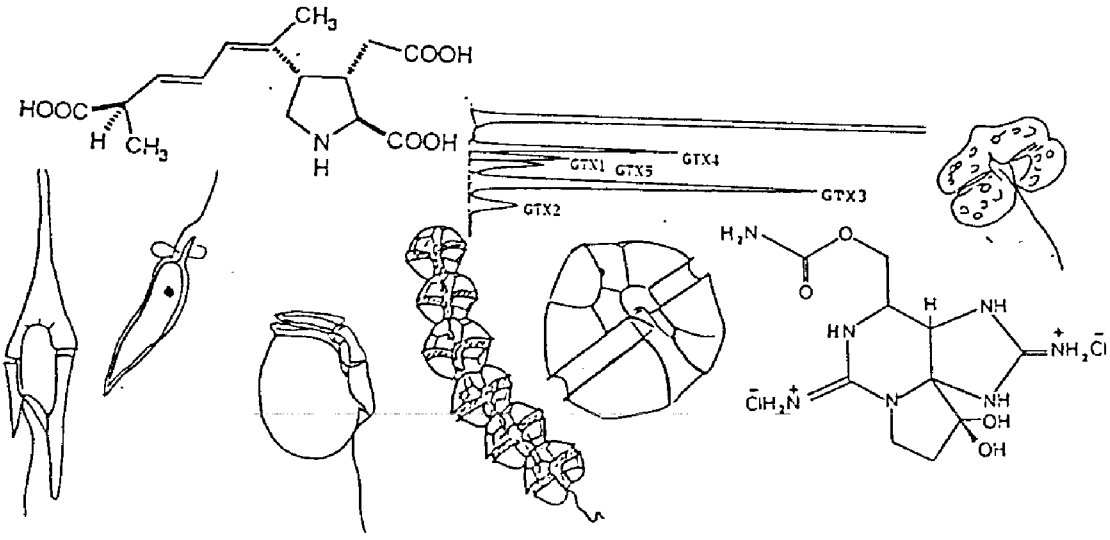
Volume 1

Amnesic Shellfish Poisoning (ASP)

HAB

Publication Series



Helle Ravn, 1995
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ABSTRACT

In this manual a review is provided of the chemical and toxicological aspects of Amnesic Shellfish Poisoning (ASP). The document contains information on chemical structure, chemical data, where to obtain standards and reference materials, the origin and occurrence, chemical analysis, mouse bioassay, epidemiology, mechanisms of action, symptoms and therapeutics. The practical use of this document has been highlighted in agreement with the Members of the Task Team on Aquatic Biotoxins.
This document is prepared by Dr. H. Ravn, IOC together with Prof. T. Yasumoto, Tohoku University, Japan, Chairman of the Task Team on Aquatic Biotoxins. Dr. J. Ramsdell, National Oceanic and Atmospheric Administration (NOAA), Charleston Laboratory, USA has supplied parts of the toxicological information, and the document has been kindly reviewed by Dr. M.A. Quilliam, National Research Council Canada, Institute for Marine Biosciences, Canada.

FOREWORD

This is the first volume in a new series called **HAB Publication Series**. Volume 1 in this series is a supplement to Chapter 7."Methods for Domoic Acid, the Amnesic Shellfish Poisons" in the IOC Manual on Harmful Marine Microalgae.

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1. INTRODUCTION TO INFORMATION ON AMNESIC SHELLFISH POISONING

The international IOC-FAO Harmful Algal Bloom (HAB) Programme was established in 1992 as part of the Ocean Science in Relation to Living Resources (OSLR) Programme. This was done in view of global interest in problems of phytoplankton blooms, red tides associated with mass mortality of marine organisms and through various recommendations of major IOC scientific and regional subsidiary bodies. The HAB Programme was endorsed by the Seventeenth Session of the IOC Assembly in February-March 1993.

The HAB Programme consists of three elements: a Scientific Element, an Operational Element and an Educational Element. A part of the Scientific Element is Toxicology and Toxin Chemistry.

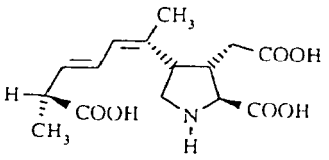
During several workshops organized as activities of the IOC-FAO HAB Programme, a strong need for training and capacity building within the field of Toxicology and Toxin Chemistry has been expressed. This was done by the scientists, as well as participants responsible for monitoring in both developed and developing countries.

During the Second Session of the IOC-FAO Intergovernmental Panel on Harmful Algal Blooms, a Task Team on Aquatic Biotoxins was established. The Task Team on Aquatic Biotoxins was formed in order to initiate, catalyze and activate interaction between relevant organizations and Member States with interest in the chemistry and toxicology of algal toxins. The members of the Task Team are representatives from IUPAC, FAO, WHO, EU, IST, NOAA-USA, GIPME/GESREM, GIPME/GEMSI, GIPME/GEEP, and OSLR/HAB.

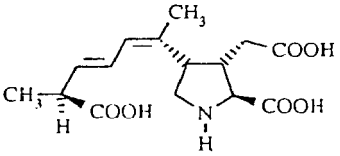
This document is a product of the collaboration between Organizations and IOC Member States participating in the Task Team on Aquatic Biotoxins.

2. CHEMICAL STRUCTURE OF AMNESIC SHELLFISH POISONS

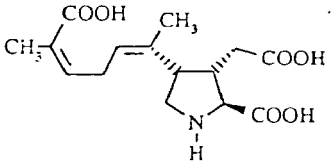
Domoic acid:



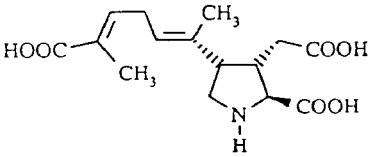
C5'-Diastereomer:



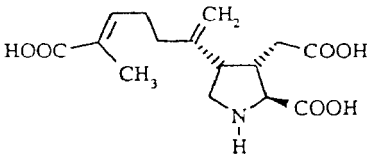
Isodomoic acid A:



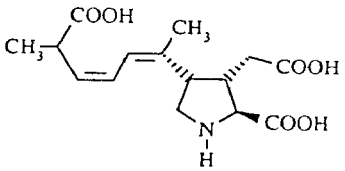
Isodomoic acid B



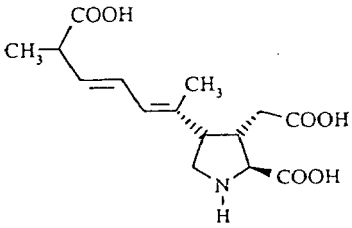
Isodomoic acid C:



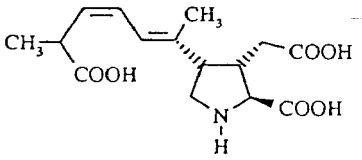
Isodomoic acid D:



Isodomoic acid E:



Isodomoic acid F:



3. Chemical data

Domoic acid (DA) (major component), isodomoic acid D, isodomoic acid E and isodomoic acid F are involved in Amnesic Shellfish Poisoning (ASP). These are all plankton products. The activity of some of these isomers is weaker than DA though isodomoic acid D to F are about the same (Hampson et al. 1992). The chemical group is kainates (Wright et al., 1990).

Domoic acid was named after the local Japanese name for *Chondria armata domoi* or hanayanagi (Takemoto & Diago, 1958; Daigo, 1959). Isodomoic acids A,B, and C were found in *Chondria armata*, but have never been isolated from *Pseudo-nitzschia* spp. (Maeda et al., 1986).

Domoic acid ([2S-[2 ,3ß,4ß (1Z,3E,5R)]]-2-Carboxy-4-(5-carboxy-1-methyl-1,3-hexadienyl)-3-pyrrolidineacetic acid), mol. wt: 311.34, $C_{15}H_{21}NO_6$ is white solid powder, mp 223-224°C. Store tightly sealed at -4°C. Soluble in water (8 mg/ml); slightly soluble in methanol (0.6 mg/ml). Avoid freezing and strongly acid solutions. UV $_{max}$ = 242 nm (log = 4.41, H_2O). DA is an excitatory secondary amino acid, containing the structure of glutamic acid and resembling kainic acid (Todd, 1990; Wright et al., 1989).

Domoic acid in acetonitrile/water (1:9, v/v) is stable for 1 year at -12°C (or lower) in dark conditions. Mussel reference material is stable at 4°C for 1 year.

Caution: Wear gloves and mask when handling this product. Avoid contact by all modes of exposure.

4. STANDARDS AND REFERENCE MATERIALS

Sigma, Chemical Company, P.O. Box 14508, Saint Louis, Missouri 63178, USA, Fax: USA/CANADA 1-800-325-6052, Outside USA/CANADA 314-771-5757, Tel.: USA/CANADA 1-800-325-3010, Outside USA/CANADA 314-771-5750, Telex: 910-761-0593 or 434475 ANSWERBACK "SIG OK COLLECT"

Name	Product no.	Purity o/o	Amount mg	Price US $ (1995)	Ref.
Domoic acid	D 6152	90	0.5	50	Briscoe, T.J. et al. (1976)
Domoic acid	D 6152	90	1	82.8	Briscoe, T.J. et al. (1976)
Domoic acid	D 6152	90	5	272.9	Briscoe, T.J. et al. (1976)

NRC-CNRC, Shellfish Toxins, Marine Analytical Chemistry Standards Program, Institute for Marine Biosciences, 1411 Oxford Street, Halifax, Nova Scotia, Canada B3H 3Z1, Fax.: (902) 426-9413, Tel.: (902) 426-8280

Name	Sales name	Purity o/o	Units	Price US $ (1994)	Ref.
Domoic acid	MUS-1	N/A (100 µg/g)	4 x 15 g	400	NRC-CNRC
Domoic acid	DACS-1B	99 (99.5 µg/ml)	4 x 0.5 ml	190	NRC-CNRC

Wako Pure Chemical Industries, Ltd., 1-2 Dashomachi 3-Chome, Chua-Ku, Osaka 541, Japan, Fax.: (81) 6 222 1203, Tel.: (81) 6 203 3741, Telex: 65188 wakoos

Wako Chemicals USA, Inc., 1600 Bellwood Road, Richmond, VA 23237 U.S.A., Fax.: (804) 271 7791, Tel.: (804) 271 7677, Telex: 283208 wako ur (RCA)

Wako Chemicals GmbH, Nissanstr. 2, W-4040 Neuss 1, Germany, Fax.: (02101) 3800 10, Tel.: (02101) 3800 0, Telex: 8517001 wako d

Name	Sales name	Purity o/o	Amount mg	Price US $ (1995/96)	Comments	Ref.
Domoic acid	Code no. 530-26173 (Domoic acid)	95.0%	1	-	Purity: HPLC	Grimmelt et al. (1990)

LC Laboratories, 165 New Boston Street, Woburn, MA 01801, USA, Fax.: (617) 938-5420, Tel.: (617) 938-1700

Name	Sales name	Purity o/o	Amount mg	Price US $ (1993)	Ref.
Domoic acid	D-1462 (Domoic acid)	-	0.500	28.70	Briscoe et al. (1976)
Domoic acid	D-1462 (Domoic acid)	-	1	46.25	Briscoe et al. (1976)
Domoic acid	D-1462 (Domoic acdi)	-	5	155.00	Briscoe et al. (1976)

Research Biochemicals International, Customer Service Dept., One Strathmore Road, Natick, MA 01760-2447, USA, Tel.: (1) 508 651 8151 (Tech. service), Tel.: (1) 508 651 8151, (1) 800 736 3690 (USA/Canada), Fax.: (1) 508 655 1359, (1) 800 736 2480 (USA/Canada).

Name	Sales name	Purity o/o	Amount mg	Price US $ (1995)	Ref.
Domoic acid	D-135 (Domoic acid)	-	0.500	45.00	Wright et al. (1989)
Domoic acid	D-135 (Domoic acid)	-	1	72.00	Wright et al. (1989)
Domoic acid	D-135 (Domoic acdi)	-	5 x 1 mg	240.00	Wright et al. (1989)

5. ORIGIN AND OCCURRENCE

Domoic acid was first isolated from the red alga *Chondria armata* (Takemoto & Diago, 1958). In 1975 it was identified as coming from the Mediterranean *Alsidium corallinum* (Impellizzeri et al, 1975).

Microalgae species* (Diatoms)	References
Pseudo-nitzschia pungens f. *multiseries (Nitzschia pungens* f. *multiseries)*	Subba Rao (1988); Bates et al. (1989); Dickey et al. (1992)
Pseudo-nitzschia pseudodelicatissima (Nitzschia pseudodelicatissima)	Martin et al. (1990)
Pseudo-nitzschia australis (Nitzschia pseudoseriata)	Buck et al. (1992); Garrison et al. (1992)
Nitzschia actydrophila	Worms et al. (1991)
Pseudo-nitzschia seriata	Lundholm et al. (1994)
Amphora coffaeiformis Cl. (Culture)	Shimizu et al. (1989); Maranda et al. (1990)

Macroalgae species* (Red algae)	References
Chondria armata	Takemoto & Daigo (1958); Daigo (1959)
Chondria baileyana	Takemoto & Daigo (1958); Todd (1990)
Alsidium corallinum	Impellizzeria et al. (1975)

* The table indicate the species in which domoid acid has been identified.

Domoic acid was not found in culture of *Pseudo-nitzschia pungens* f. *pungens* (Wang et al 1993). Most published results indicate that domoic acid production occurs at the onset of the stationary phase (Bates et al., 1991; Garrison et al., 1992; Lewis et al., 1993; Subba Rao et al., 1988; Worms et al., 1991).

6. CHEMICAL ANALYSIS

The most common analytical method for detection of domoic acid is High Performance Liquid Chromatography (HPLC) (Lawrence et al., 1989; Quilliam et al., 1989; Pocklington et al., 1990; Quilliam et al., 1995). The first method applicable to shellfish and phytoplankton was developed by Quillam and collaborators in 1989. Two methods with diode array detection (UV) were presented: a rapid isocratic reversed-phase (5 min) and a sensitive gradient HPLC method. The detection limit was 0.3 ng domoic acid for the most sensitive method (Lawrence et al., 1989; Quilliam et al., 1990).

During the last few years other HPLC methods with the same principle for determination of domoic acid have been used. In 1989 Pocklington and collaborators developed a reversed-phase gradient HPLC method with fluorometric detection of trace of domoic acid in seawater after derivatisation with fluorenylmethoxycarbonyl chloride. The detection limit was 15 pg/ml (Pocklington et al., 1990).

An improved solid phase extraction (SPE) clean-up procedure has been proposed (Hartfield et al. 1994; Quilliam et al., 1995).

6.1 Analytical methods used in different countries

High performance Liquid Chromatography (HPLC) Methods

Country	Comments	Monitoring by regulations since (private)	Shellfish or Plankton	Detection limit µg/kg	Ref.
Canada	Isocratic HPLC System, 11% Acetonitril in water (0.1% TFA modifier), RP-18 Column, (13 min/analysis)	1988	Shellfish	300 soft tissue	Internal document (1988)
Canada	Isocratic HPLC System, (Acetonitril, hydrochloric acid, phosphoric acid), C-18 column (150 x 4.6 mm) Recovery: 75% (18.9 µg/kg soft tissue) Validation (AOAC)	1988	Shellfish		Lawrence et al.(1991)
Canada	10% Acetonitril/0.1 % TFA DAD/UV detection 242 nm; Vydac 20ITP Column (250 x 4.6 mm)	1988 (1988)	Mussels	< 500	Quillam et al.(1989)
Canada	9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) derivatization and fluorescence detection (Ex. 265 nm, Em. 305); Vydac 20ITP Column (250 x 4.6 mm)	1988 (1988)	Plankton	< 5 ng/ml	Pocklington et al.(1990)
Spain	C-18 Column (150 x 4.6 mm); Reproducibility: 7.5-19.4%	-	Shellfish Plankton		Lawrence et al.(1991)

High performance Liquid Chromatography (HPLC) Methods

Country	Comments	Monitoring by regulations since (private)	Shellfish or Plankton	Detection limit µg/kg	Ref.
Australia	C-18 Column (150 x 4.6 mm); Validation: AOAC Recovery rate: 98% at 0-1 µg/kg, Repeatability: 2% Reproducibility: 10%	1993	Mussels Algae	1 µg/g extract solution	Lawrence et al.(1991)
Denmark	C-18 Column (150 x 4.6 mm); Rate of recovery: 75-85% (15 µg/kg mussels), Repeateability: 3-7% at 4-500 mg/kg sample	1990	Mussels	500 µg/kg seafood samples	Lawrence et al. (1989)
USA	Isocratic, 242 nm detection, C-18 Column (Vydac 20ITP, 250 x 4.6 mm)	1976	Shellfish	150	Quilliam et al. (1989)
New Zealand		1993	Shellfish		Lawrence & Menard (1991); Wright et al. (1989); Lawrence et al. (1991)

7. MOUSE BIOASSAY

Another method for detection of domoic acid is the Association of Official Analytical Chemists (AOAC) PSP mouse bioassay with a longer observation time (normal 15 min, here 4 hours)(AOAC, 1984; Todd, 1990). Shellfish containing > 40 µg domoic acid per g wet weight of mussel meat caused mouse symptoms (Iverson et al., 1989)

The AOAC bioassay method from 1984 on PSP toxins was used when domoic acid was identified in shellfish extracts from eastern Prince Edward Island in Canada in 1987 (Wright et al., 1989). A unique scratching syndrome of the shoulders by the hind leg followed by convulsions was a typical sign of the content of domoic acid in extracts. Three mice were used instead of two mice and the time of observation was extended from 15 min to 4 hours after which time the mice were periodically examined for up to 18-24 hours. Deaths associated with the contaminated mussels containing the domoic acid were never observed after 135 min (AOAC, 1984; Quillam et al., 1989; Todd, 1990).

7.1 Receptor Bioassay Method:

Country	Comments	Detection Limit µg/kg	Reference
USA	Microplate scintillation counter ([3H] Kainic acid) Lowest validated level 0.025 µg/kg mussel; Rate of recovery: 104% at 0.125 µg/kg in mussels; Repeateability: 0.131 $\pm$ 0.012 Assay validated in shellfish, crab hepatopancreas and serum	0.001 (mussels)	Van Dolah et al. (1994)

Forty-nine laboratories from twentythree countries kindly filled in the distributed Questionnaires from IOC, and nine laboratories perform ASP analysis:

Canada: **Stephen J. Stephen**, Fisheries and Oceans Canada, 200 Kent Street, STN 906, Ottawa, Ontario, Canada, KIA OE6, Tel.: (613) 990-1603; Fax.: (613) 990-4668;**Roger Pocklington**, Bedford Inst. Oceanography Chemical Oceanography Div., P.O. Box 1006, Dartmouth NS B2Y 4A2, Tel.: (902) 426-8880; Fax.: (902) 426-6695; Internet: pocklington@bionet.bio.dfo.ca;

Spain: **Maria Luisa Fernandez**, Ministerio de Sanidad y Consumo, European Communities Reference Lab. on Marine Biotoxins, Dirección Territorial en Galicia, Unidad Administrativa de Vigo, Estacion Maritima s/n, Apartado 90, 36200 Vigo, Spain, Tel.: (34) 86 43 41 31: Fax.: (34) 86 43 21 88; New Zealand: **Desmond Till**, ESR Communicable Disease Centre, P.O. Box 50348, Porirua Wellington, New Zealand, Tel.: (63) 42 37 01 49; Fax.: (63) 42 37 23 70;

Australia: **Christopher Soames**, Chemistry Centre of WA, 125, Hay Street, East Perth, 6004 Western Australia, Tel.: (61) 92 22 30 72, Fax.: (61) 93 25 77 67;

Denmark: **Benedicte Hald**, Dept. of Veterinary Microbiology, The Royal Danish Veterinary & Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark, Tel.: (45) 35 28 27 60, Fax.: (45) 35 28 27 57;

USA: **NOAA-NMFS**, Southeast Fisheries Science Center, Charleston Laboratory, P.O.Box 12607, Charleston, SC, 29422-2607, Tel.: (1) 803 762 8500, Fax.: (1) 803 762 8700

8. EPIDERMIOLOGY

8.1 Absorption, distribution, biotransformation and excretion:

Domoic acid (DA) present in seafood is absorbed through the gastrointestinal mucosa, but the rate of absorption is very low (Iverson et al., 1990). In its purified form, DA poses a risk via respiratory, corneal and dermal entry.In the blood stream DA exists as a charged hydrophillic molecule, available to all peripheral tissue (Suzuki & Hierlihy, 1993). The ionic form of DA and its inability to effectively conduct through glucose and amino acid transport mechanisms restricts its entry into the central nervous system (Preston & Hynie, 1991). Once the active level of domoic acid reachs the CNS, disruption of blood brain barrier by localized seizure may substantially promote its entry into the brain (Zucker et al., 1983).

DA cleared into urine exists predominately unmodified, suggesting that metabolism of DA *in vivo* is minimal (Suzuki & Hierlihy, 1993). DA administered orally is excreted almost entirely in the feces (Iverson et al., 1990). The excretion of DA by the kidneys is a critical event in the toxicity of DA (Suzuki & Hierlihy, 1993).

8.2 Mechanism of action:

DA acts as an agonist to glutamate receptor (Takemoto, 1978), and binds with high affinity to glutamate receptors of the quisqualate type (Stewart et al. 1990; Hampson et al., 1992). The glutamate receptor conducts Na^+ ion channels in the postsynaptic membrane inducing depolarization. This in turn increases the Ca^+ ion permeability which ultimately leads to cell death.

DA is an excitatory amino acid derivative, a neurotoxin and a neurotransmitter in the central nervous system (CNS), in a manner analogous to the structurally related compound, kainic acid (Hampson and Wenthold, 1988; Laycock et al., 1989). DA is 2-3 times more potent neuroexcitator than kainic acid, and up to 100 times more potent than glutamic acid (Biscoe et al., 1975,1976; Coyle, 1983; Debonnel et al., 1989; Olney, 1990). A synergistic effect between DA and other neurotoxic amino acids normally present in mussels is possible (Novelli et al., 1990; Tasker et al., 1991).

The toxic mechanism of DA are believed to be mediated at the level of the mitochondria, where uncoupling of oxidative phosphorylation decreases membrane permeability causing cell swelling and ultimately lysis.

Apart from neurotoxic and gastrotoxic activity, DA has shown no mutagenic action on pulmonary fibroplasts of hamster V79 (Rogers and Boyers, 1989).

8.3 Symptoms:

After consumption of DA contaminated seafood following gastro intestinal symptoms within 24 h have been observed: nausea, vomoting, headache, diarrhea, or abdominial cramps, or at least one of the following neurological symptoms or signs within 48 h: confusion, memory loss, disorientation, or other serious neurologic signs such as seizures or coma and death (Todd, 1993). Furthermore, symptoms as hypoactivity, sedation-akinesia, rigidity, sterotypy, loss of posteral control,tremors convulsion has been observed in mice (Tasker et al. (1991).

DA exposure causes a strong induction of c-fos in the brain, lesser effect in the heart and no effect in the kidney or liver. Within the brain the induction of c-fos has been located to two predominant areas, the brain stem and the limbic system (Peng et al., 1994). These effects in the brain result in vomoting and memory deficit. These regions are sensitive at doses as low as 0.25 mg/kg. Toxic effects of domoic acid resulting in structural damage to hippocampus have been correlated to behavioral effects of domoic acid predictive of anterograde memory deficit (Sutherland et al. 1990; Petrie et al., 1991). The anterograde memory deficit can last for more than five years in individuals.

Monkey studies with pure DA have been performed and symptoms were observed. The monkeys dosed orally with blended mussel digestive glands to give doses of 20 - 29 µg/(3.4 and 5.1 kg) domoic acid developed gastroenteritis such as vomiting, anorexia, diarrhea and some neurologic effects e.g. withdrawal and wet-dog shakes, disorientation, such as glassy-eye stares and prostration or weakness and trembling. The gastrointestinal

symptoms began between 2.75 and 6.0 hours and the neurologic ones between 0.25 and 6.0 hours. The last endured for up to 120 hours. Salvation, a feature noticed in severe human cases, occurred in 2 of the 5 animals. In other studies with pure domoic acid given to monkeys, the effects became stronger as the level was increased. No significant inner lesions were observed (Todd, 1990). Experimental studies in mice have indicated that neonates are at least 10 times more sensitive to lethal effects of DA (Xi, 1994). Thus neonates must be considered to be a high risk to DA with nursing or transplacenta as possible
routes of exposure. Nephromectimized animals are much more sensitive to DA (Preston and Hynie, 1991).

The mussels containing DA have the same effect as pure domoic acid (Iverson, et al., 1989, Todd, 1990). The action level for DA in shellfish is 40µg/g wet weight.

LD_{50}(IP) = 3.6 mg domoic acid in mussel extracts per kg mouse (Grimmelt et al., 1990). Mouse deaths occur at or above 100 µg in mussel extracts (5 mg/kg)(Iverson et al., 1989).

For shellfish containing more than 20 µg domoic acid/g the harvest is recommended to be stopped (Hallegraeff, 1993). The amount of domoic acid accumulated in Bay of Fundy mussels was 74 µg/g (Martin et al., 1990), whereas the level of those in the eastern Prince Edward Island in Canada in 1987 was 900 µg/g of wet tissue (Quillam et al., 1989).

Elderly individuals and individuals with compromised renal function should be considered to be a high risk group.

8.4 Therapeutics:

Therapy at present is limited to life support. Work with laboratory rats indicate that **diazepan** (5 mg/kg) reduces convulant behaveours but does no reduce impairment of spatial learning or damage to the hippocampus (Nakajima and Potvin, 1992).

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10. LIST OF ACRONYMS

AOAC	Association of Official Analytical Chemists
ASP	Amnesic Shellfish Poisoning
C	Carbon
CNS	Central Nerve System
DA	Domoic acid
DAD	Diode Array Detector
Em	Emission
Ex	Exitation
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FMOC-Cl	9-fluorenylmethoxycarbonyl chloride
GEEP	Group of Experts on Effects of Pollutants

GESREM	Group of Experts on Standards and Reference
GEMSI	Group of Experts on Methods, Standards and Intercalibration
GIPME	Global Investigation of Pollution in the Marine Environment
HAB	Harmful Algal Bloom
HPLC	High Performance Liquid Chromatography
IOC	Intergovernmental Oceanographic Commission
IST	International Society of Toxinology
IUPAC	International Union of Pure and Applied Chemistry
LD	Lethal Dose
NOAA	National Oceanic and Atmospheric Administration
OSLR	Ocean Science in Relation to Living Resources
PSP	Paralytic Shellfish Poisoning
RP	Reverse Phase
SM&T	Standards, Measurements & Testing Programme
SPE	Solid phase extraction
TFA	Trifluoroacetic acid
UV	Ultra violet radiation
WHO	World Health Organization

11. MEMBERS OF THE TASK TEAM

The members of the Task Team on Aquatic Biotoxins are as follows: Prof. T. Yasumoto (Chairman), Japan; Prof. D. Park (Co-Chairman) and Dr. J.M. Fremy, IUPAC; Dr. C.A. Lima Dos Santos, FAO; Dr. R. Plestina, WHO; Dr. A. Boenke, SM&T Programme, CEC, EU; Prof. G. Habermehl, IST; Dr. R. Kiefer, NOAA, USA; Dr. R. Boyd, GIPME/GESREM; Dr. R. Dawson, GIPME/GEMSI; Dr. M. Moore, GIPME/GEEP, and Dr. H. Ravn, IOC/HAB.

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