22. Management of Shellfish Resources

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Interactions between marine biotoxins and shellfish are complex, dynamic and vary between species and even within subpopulations. For detailed reviews the reader is directed to the following: Shumway (1990; 1994), Shumway *et al.* (1988, 1990), Shumway and Cembella (1993). In addition to commercial harvest areas, aquaculture facilities are adversely and often unpredictably affected by toxic blooms. Public health officials as well as harvesters, processors and dealers of shellfish must remain alert to outbreaks of toxic algal blooms to protect human health as well as preserving a high standard of quality assurance. Recent reviews of particular interest to managers include those by Maclean (1993), Cembella and Todd (1993) and van Egmond *et al.* (1992, 1993, 1994).

The following chapter provides a summary of available information on rates of loss of toxins by bivalve molluscs, regulations regarding paralytic (PSP), diarrhetic (DSP), amnesic (ASP) and neurotoxic (NSP) shellfish toxins worldwide and a detailed description of the United States Food and Drug Administration (USFDA) Interstate Shellfish Sanitation Commitee (ISSC) and National Shellfish Sanitation Program. In addition, the monitoring program of the State of Maine (USA) is described as an example of an efficient and highly reliable system. The appendix in Fig. 22.2 provides an example of a comprehensive Red Tide Response and Management Framework as developed by the ASEAN-Canada Cooperative Programme on Marine Science (Seagel, 1994).

INTOXIFICATION AND DETOXIFICATION OF SHELLFISH

Shellfish (bivalve molluscs, gastropods, crabs, lobsters and others) accumulate phycotoxins either by direct filtration of the plankton cells or by feeding directly on contaminated organisms (e.g. carnivores and scavengers). Rates of intoxification and detoxification of filter-feeding shellfish by toxic algae are species-specific and are, in most cases, directly related to the number of cells available to the animals (Sribhibhadh, 1963; Gilfillan *et al.*, 1976; Prakash *et al.*, 1971; Saunders *et al.*, 1982). Toxicity of individual shellfish in any given area is highly variable (see White *et al.*, 1993; Chebib *et al.*, 1993). The rate of loss varies with season (Prakash *et al.*, 1971) and low water temperatures apparently retard toxin loss; however, the degree to which temperature affects the uptake and release of toxins is not clearly understood (see Madenwald, 1985). Further, the rate of detoxification is highly dependent on the site of toxin storage within the animal i.e., toxins in the gastrointestinal tract (e.g. *Mytilus*) are eliminated much more readily than toxins bound in tissues (e.g. *Placopecten*, *Spisula*, *Saxidomus*), and on initial or peak level of toxicity. Few data are available for the retention times of toxins by crabs and carnivorous gastropods; however, the general trend appears to be towards long-term retention (Desbiens and Cembella, 1994; Shumway, 1994; Shumway, unpublished data).

The majority of information available concerns bivalve molluscs (see also Shumway, 1990), and as these are the species most commonly reared in aquaculture, they will be focussed upon here. Table 22.1 summarizes existing data on toxin retention for a number of bivalve species. Mussels (e.g. *Mytilus* spp., *Modiolus* spp., *Perna* spp.) are known to accumulate PSP toxins faster than most other species of shellfish and also to eliminate the poison quickly. While oysters do not accumulate the toxic species as readily as mussels, they take considerably longer to detoxify (Neal, 1967; Shumway *et al.*, 1990). In contrast, some species (e.g. *Saxidomus giganteus*, *Spisula solidissima*) may remain toxic for extended periods (e.g. in excess of 3

years) (Quayle, 1965; Blogoslawski and Stewart, 1978; Chambers and Magnusson, 1950; Cembella and Todd, 1993; Shumway *et al.*, 1994). In addition to the clams, scallop tissues (not adductor muscles) also store toxins for periods in excess of 2 years (Shumway and Cembella, 1993).

Some species of bivalves are known to avoid toxic dinoflagellates (see Shumway and Cucci, 1987). One species of particular interest is the northern quahog or hard clam, *Mercenaria mercenaria*. During an outbreak of a bloom of *Alexandrium tamarense (=Gonyaulax =Protogonyaulax tamarensis*) in 1972, the entire coastline of Massachusetts came under interdict. Monitoring of the coast indicated that some 2800 acres of shellfish harvesting areas were contaminated. Bioassays of shellfish samples showed toxin in the range of 3000-5000 μg per 100 g with the most heavily contaminated shellfish being the mussel (*Mytilus edulis*), soft-shelled clam (*Mya arenaria*) and bay scallops (*Argopecten irradians*). It was specifically noted that **no** quahogs (*M. mercenaria*) or oysters were affected. *Mercenaria mercenaria* was reported to be toxic in the Bay of Fundy and St. Lawrence regions by Bond and LaChance (1959). Studies in our laboratory have shown that in the presence of *A. tamarense* the quahog first retracts its siphons and then completely isolates itself from the external environment by means of shell valve closure. The animals did not re-open their shell valves until after the addition of clean sea water. Efforts to induce toxicity by feeding *A. tamarense* were unsuccessful. It is also possible that *Mercenaria* responds to the presence of other dinoflagellates in the same manner. Castagna (personal communication) has observed that quahogs exposed to red-tide blooms in Virginia (non-toxic) bury themselves very deep in the experimental trays. He further noted that 'wild' populations were found at depths of up to 35cm below the sediment surface as opposed to their usual 15cm during these blooms.

Subsequent studies by Bricelj *et al.* (1991) have shown, however, that the quahogs will ingest toxic dinoflagellates if fed mixtures containing both toxic dinoflagellates and other suitable food items, e.g. the diatom, *Thalassiosira weissflogii*. Perhaps more importantly, these workers have shown in both mussels (*M. edulis;* Bricelj *et al.*, 1990) and quahogs (*M. mercenaria*; Bricelj *et al.*, 1991) that ingestion rate of toxic cells, maximum body burden of toxin and initial rates of detoxification vary markedly in response to differences in cell toxicity and/or toxin composition of the dinoflagellate cells consumed.

Phycotoxins other than those associated with paralytic shellfish poisoning are also accumulated by filter-feeding molluscs and are also a threat to public health. Diarrhetic shellfish toxins (e.g. okadaic acid and its derivatives, dinophysistoxin-1 and dinophysistoxin-3; pectenotoxins and yessotoxins) associated with *Dinophysis* spp. and *Prorocentrum* spp. are readily accumulated by shellfish and little is known of the retention time of the toxins. Marcaillou-Le Baut *et al.* (1993) compared DSP depuration rates of mussels reared in an aquaculture pond and in the laboratory and found that the highly toxic (3 MU g^{-1}) mussels dropped to acceptable levels more quickly in the culture ponds than in the laboratory. They suggested that the quality of food available to the mussels during detoxification may affect the rate at which toxins are eliminated.

More recently, domoic acid has been identified as a potentially lethal phycotoxin associated with various species of the diatom, *Pseudo-nitzschia* (see Chapter 17).

Generalizations regarding the uptake and retention of phycotoxins by shellfish should be avoided. Differences in rates of toxin accumulation and retention are dependent upon the shellfish and algal species under consideration and these differences should be taken into account before choosing a species to be reared in areas prone to toxic algal blooms.

DETOXIFICATION - DEPURATION

Various attempts have been made at detoxifying shellfish contaminated with paralytic shellfish poisons in an effort to reduce the duration of 'off market' times. The most obvious method is to transfer shellfish to waters free of the toxic organisms and allow them to self-depurate. While

Table 22.1. Approximate times of toxin retention for various species of bivalve molluscs (represents time taken for toxin levels to fall below either quarantine or detection levels). Algal species are as given in original publications[1].

Species	Toxin source	Retention time	Reference
Ameghinomya antiqua	probably *Dinophysis acuta*	6 months	Lembeye *et al.* (1993)
Anadara maculosa	*Pyrodinium bahamense*	6 weeks	Worth *et al.* (1975)
Arctica islandica	*Protogonyaulax tamarensis*	2 months *in vivo*	Shumway, unpublished
Aulacomya ater	probably *Dinophysis acuta*	6 months	Lembeye *et al.* (1993)
Choromytilus meridionalis	*Gonyaulax catenella*	3 months	Popkiss *et al.* (1979)
Clinocardium nuttalli	*Gonyaulax acatenella*	9 weeks	Quayle (1965)
Crassostrea cucullata	not specifed, probably *Pyrodinium bahamense*	2 months	Karunasagar *et al.* (1984)
Crassostrea echinata	*Pyrodinium bahamense*	3 weeks in closed system; longer periods *in vivo*	Maclean (1975)
		4 months	Worth et al. (1975)
Crassostrea gigas	*Gonyaulax acatenella*	1-9 weeks	Quayle (1965; 1969); Sharpe (1981)
		1 month	Sribhibhadh (1963)
Crassostrea iridescens	*Gymnodinium catenatum*	>1 month	Mee *et al.* (1986)
Crassostrea virginica	*Gymnodinium breve*	2-6 weeks	Morton and Burklew (1969)
Meretrix casta	not specified, probably *Pyrodinium bahamense*	1 month	Karunasagar *et al.* (1984)
Modiolus auriculatus	*Pyrodinium bahamense*	6 weeks	Worth *et al.* (1975)
Modiolus modiolus	*Gonyaulax tamarensis*	up to 60 days[2]	Gilfillan *et al.* (1976)
Mya arenaria	*Gonyaulax acatenella*	5 weeks	Quayle (1965)
	Gonyaulax tamarensis	4-6 weeks	Prakash *et al.* (1971); Bicknell and Collins (1973)
		up to 45 days[2]	Gilfillan *et al.* (1976)
Mytilus californianus	*Gonyaulax catenella*	< 1 month	Sommer and Meyer (1937); Sharpe (1981)
Mytilus edulis	*Protogonyaulax tamarensis*	10 days- 7 weeks	Oshima *et al.* (1982);
		up to 50 days	Gilfillan *et al.* (1976); Prakash *et al.* (1971)
	Gonyaulax acatenella	11 weeks	Quayle (1965)
		4 weeks	Sharpe (1981)
	Gonyaulax excavata	2-3 weeks	Gaard and Poulsen (1988)
	Dinophysis spp.	1 week	Haamer *et al.* (1989)
	Dinophysis spp.	8 weeks	Marcaillou-le Baut *et al.* (1990)
	Dinophysis spp.	8->42 days[2]	Marcaillou-le Baut *et al.* (1993)
	Dinophysis spp. (?) *Prorocentrum* spp. (?)	=10 days	Quilliam *et al.* (1993)
	probably *Dinophysis acuta*	6 months	Lembeye *et al.* (1993)
Patinopecten yessoensis	*Protogonyaulax tamarensis*	6 weeks- 5 months	Oshima *et al.* (1982); Iioka *et al.* (1964)
*Perna canaliculus**	*Nitzschia pungens* f. *multiseries*	2 days	MacKenzie *et al.* (1993)
Placopecten magellanicus	*Protogonyaulax tamarensis*	6 month in closed system; can be toxic year round *in vivo*	Bourne (1965); Shumway *et al.* (1988)
Protothaca staminea	*Protogonyaulax acatenella*	5 weeks	Quayle (1965)
Saxidomus giganteus		2 years +	Quayle (1965); Anonymous (1974)
Saxidomus solidissima	*Gonyaulax catenella*	<1 month	Sommer and Meyer (1937)
Siliqua patula	*Pseudonitzschia* spp.?	>2 years	Wekell et al. (1993); Drum *et al.*(1993); Homer *et al.* (1993)
Spisula solidissima	*Protogonyaulax tamarensis*	up to one year	Medcof *et al.* (1947); Blogaslowski and Stewart (1978)
Spondylus sp.	*Pyrodinium bahamense*	still highly toxic after months	Worth *et al.* (1975)
Tresus capax	*Gonyaulax acatenella*	11 weeks	Quayle (1965)
Venerupis japonica	*Gonyaulax acatenella*	5 weeks	Quayle (1965)

[1] Note: *Gonyaulax* and *Protogonyaulax* = now*Alexandrium*; *Nitzschia* = now *Pseudo-nitzschia*

[2] Dependent on initial level of toxicity.; * laboratory study only; toxic organisms not identified in natural habitat

this may appear to be a satisfactory method for many species of shellfish, rates of detoxification vary considerably between species (See Table 22.1) and some species remain toxic for extended periods of time. Further, transferring large quantities of shellfish is labor-intensive and costly. Desbiens and Cembella (1993) investigated the potential use of vertical displacement of mussels in the water column as a means of minimizing PSP toxin accumulation. While they were able to demonstrate that transfer of mussels had an ameliorating effect on the toxin accumulation, they pointed out that the potential applications of vertical relaying for mussel culture are restricted by high levels of PSP toxicity observed in the region (eastern Canada). Detoxification of PSP-toxins using temperature or salinity stress has also been tried with marginal success (Gilfillan *et al.*, 1976; Blogoslawski and Neve, 1979). Instantaneous electrical shock treatments accelerated toxin excretion in scallops (Kodama *et al.*, 1989). Reduced pH has been tried as a means of detoxifying butter clams, but with no success (Anonymous, 1966; Neal, 1967). Chlorination has been used in France; however, this process alters the flavor of the shellfish and thus decreases marketability. Emergency relocation areas have been set aside in Tasmania (September 1994) to reduce the impact of upcoming paralytic shellfish poison blooms on shellfish stocks (Hallegraeff, personal communication).

Ozone has been touted as an effective means of reducing toxicity; however, its usefulness is questionable. Several early studies reported ozone to be effective in the inactivation of PSP toxins in shellfish exposed to *Alexandrium tamarense*, *A. catenella* and *Gymnodinium breve* blooms (Thurberg, 1975; Blogoslawski *et al.*, 1975, 1979; Dawson *et al.*, 1976; Blogoslawski and Stewart, 1978). Blogoslawski *et al.* (1973) also suggested that ozone could be used to inactivate *Gymnodinium breve* toxins. More recently, preliminary studies by Gacutan *et al.* (1984, 1985) demonstrated that both ozone gas and PVP-iodide-iodine may effectively inactivate PSP toxins from *Perna viridis* contaminated by *Pyrodinium bahamense*. However, a subsequent study by White *et al.* (1985) gave results totally contradictory to previous studies in that no detoxification occurred in *Mya arenaria* exposed to ozone treatments.

In a review (Blogoslawski, 1988), it was again suggested that ozonised seawater can be of value in detoxification of shellfish contaminated recently by the vegetative cell phase of toxic (PSP) dinoflagellates. In a study during a red tide outbreak, it was shown that ozone treatment of the seawater does prevent shellfish (*Mytilus edulis, Mya arenaria* and *Guekensia demissus*) from accumulating paralytic shellfish poison. Blogoslawski concluded that inactivation could be achieved in bivalves exposed to and contaminated by motile dinoflagellate cells bearing PSP without measurably altering the physical state of the treated bivalves and that this inactivation could be achieved in a marketable species such as *Mya* within an economically feasible time frame (Blogoslawski *et al.*, 1979). Ozone is useless in detoxifying cysts or in bivalves that have ingested cysts or have the toxins bound in their tissue over long periods of time. Further, detoxification of algal toxins, especially paralytic shellfish poisons, over long periods of time is not economically feasible. We do not recommend ozone as a practical or safe means of eliminating algal toxins from shellfish. At present the economic feasibility of efficiently detoxifying shellfish on a large scale in artificial systems is not promising. In areas prone to regular outbreaks of toxic algal species, culturists and commercial fishermen alike must still depend on reliable monitoring systems to warn of toxic shellfish and plan their activities accordingly. Through the combined efforts of an intensive monitoring program and culture of 'rapid release' species (e.g. *Mytilus edulis*), species known to avoid toxic dinoflagellates (e.g. *Mercenaria*, most oysters) or scallops (adductor muscles rarely if ever toxic), economic losses can be kept to a minimum (see also Shumway *et al.*, 1988).

Cooking has also been touted as a possible means of detoxifying shellfish contaminated with paralytic shellfish poisons. **Cooking does not eliminate the danger of intoxication**; however, it may reduce levels of toxins. If initial levels of toxicity are low, cooking may effectively reduce toxicity to safe levels. Pan frying seems to be more effective than other methods of cooking (Medcof *et al.*, 1947; MacDonald, 1970). When clams or mussels are steamed or boiled, toxins lost from the tissues are contained in the cooking liquid rendering the fluids extremely toxic.

Commercial canning has been shown to reduce toxicity (paralytic shellfish poisons) of soft shell clams, *Mya arenaria*, by as much as 90%. A toxicity level of 160 µg STX equiv.100

g^{-1} for soft-shell clams and mussels (*Mytilus edulis*) to be canned was established in the 1950's in Atlantic Canada and remains in effect today (Cembella and Todd, 1993). (Note: canning of soft shell clams contaminated by paralytic shellfish poisons in Canada is confined to those clams that were harvested prior to a closure. This is a method which allows a clam dealer to utilize these clams. No harvesting is permitted in any closed area.) Noguchi *et al.* (1980) (see also Nagashima *et al.*, 1991) showed that toxicity levels of PSP-infested scallops could be reduced during canning processes. They demonstrated that during retorting (110°C, 80min, or 122°C, 22min) most of the PSP-toxins could be eliminated (maximum initial level 102MU g^{-1} digestive gland) whereas heating (70°C, 20min) followed by washing was less effective in reducing the toxicity below the quarantine limit of 4MU g^{-1}. Recent efforts in Spain (Berenguer *et al.*, 1993) have demonstrated that toxicity levels of Mediterranean cockles (*Acanthocardia tuberculatum*) may be significantly reduced via the canning process. Total toxicity of cockles (initial levels of approximately 800 µg STXequiv.$100g^{-1}$) was reduced to <35 µg STXequiv $100g^{-1}$ after cooking. These authors believe that the decreases in PSP toxicity obtained by commercial processing is sufficient to warrant canning as a practical means of obtaining a legal and acceptable product. Similar attempts have been made to detoxify surfclams (*Spisula solidissima*) via the canning process, but results are thus far inconclusive. The effectiveness of canning as a means of reducing PSP-toxicity levels below quarantine levels is dependent upon the initial level of toxicity and should be approached with great caution.

With the exception of the study by Berenguer *et al.* (1993), there have been no useful methods devised for effectively reducing phycotoxins in contaminated shellfish. All methods tested to date have been either unsafe, too slow, economically unfeasible or yielded products unacceptable in appearance and taste. Given the apparent global increase in harmful algal blooms and the continually growing interest in culture of bivalve molluscs, further efforts are needed to develop effective means of detoxifying shellfish contaminated with phycotoxins. Failing the development of any such methods, increased efforts will need to be expended in monitoring shellfish for the presence of phycotoxins.

REGULATION AND MONITORING PROGRAMMES

There appears to be general, worldwide agreement on the need for measurements to control shellfish toxins in seafood and many countries have taken legal action to ensure that phycotoxin-contaminated shellfish do not reach the consumer.

Factors that may influence the regulation of shellfish toxins

Various factors may play a role in establishing regulatory criteria and limits for phycotoxins. These include:

- the availability of survey data
- the availability of toxicological data
- the distribution of phycotoxins throughout sampled lots and the stability of the toxins in the samples
- the availability of methods for analysis of toxins
- regulation in force in other countries.

Data on the occurrence of toxic algal species may indicate which toxins may be expected during periods of algal blooms and which seafood products should be considered for analytical monitoring. A problem is that certain algal species, which have never occurred in a certain area, may suddenly appear and then rapidly cause problems, e.g. the intoxication episode with

domoic acid in Canada (Wright, 1989) and recent, unprecedented outbreaks of neurotoxic shellfish poisoning in New Zealand (Jasperse, 1993).

Without toxicological information there can be no hazard assessment, one of the basic ingredients of risk assessment. Although there are many reported cases of human intoxications due to shellfish toxins, it is difficult to obtain reliable human toxicity data. For example, variations in PSP toxicity to humans may be due not only to variable sensitivity between people, but also to the composition of individual toxins in the samples. Toxin profiles can vary according to the species of shellfish consumed and the area of harvest (Krogh, 1988). In addition, toxic doses are often estimated from left-over toxic seafood, which may not be representative of the ingested food. Data from animal experiments are rather restricted.

The distribution and composition of the multiple toxins that make up many of the shellfish toxins throughout the sampled lots and within the individual shellfish, as well as the (in)stability of the toxins in the samples may pose certain difficult problems in establishing criteria. A few mussels may not be representative for the whole catch. For example, the composition of DSP toxins varies throughout the world, and sometimes in some areas from year to year. Mussels collected in Europe contain okadaic acid as the major toxin (Edebo *et al.*, 1988; Kumagi *et al.*, 1986); however, in Norway in 1985, mussels collected in one area, contained okadaic acid as the major toxin constituent while mussels collected in another area of Norway in 1986 contained dinophysistoxin-1 as the major toxin and yessotoxin as well as pectenotoxin-like compounds as minor toxins (Lee *et al.*, 1987). Scallops from Japan show the most complicated toxin profile; pectenotoxins have been detected and confirmed only in shellfish harvested there (Yasumoto and Murata, 1990). In addition, a high level of variability (±48%) in toxin concentration has been demonstrated in some species of shellfish collected from the same sample in the Gulf of Maine (White *et al.*, 1993). The risk to both consumer and producer must be considered when establishing sampling criteria to protect not only the public health, but also the fishery resources and the coastal economy.

Accurate methods of analysis have to be available, because legislation calls for methods of control. A big problem with many of the phycotoxins is that reliable, validated chemical methods of analysis are not yet available, or cannot be easily operated because of the lack of standards and reference materials (Van Egmond *et al.*, 1993). Most countries still rely on animal bioassays to detect PSP and DSP (see Tables 22.2 and 22.3). A main disadvantage of bioassays is the ethical aspects of these tests, which have led to growing resistance from animal welfare groups. It should also be borne in mind that it is not realistic to establish a tolerance level lower than the actual limit of detection, although this might be desirable from the toxicity point of view. Should the analytical methodology improve as desired, then it may be necessary to reconsider the tolerances.

Regulations presently in force in other nations, especially those of trading partners, should be considered when new domestic regulations are being put into place. Differences between nations in tolerances set for shellfish toxins can result in chaos and inconsistencies in the protection of public health. Differences can also raise unnecessary barriers to international trade.

Weighing the various factors that play a role in the decision-making process of establishing shellfish toxin tolerances may not be easy. Despite the dilemmas, there are a number of countries that have established limits and regulations for shellfish toxins.

Current limits and regulations for phycotoxins

Within the framework of a project of the International Union of Pure and Applied Chemistry (IUPAC) a project was initiated in 1990 to obtain a global overview of current legislation on phycotoxins and plant toxins. A substantial part of the information concerned shellfish toxins. The Agricultural Attachés or Counsellors of the Dutch Embassies were approached with the request to collect up-to-date information on the state-of-affairs of national regulations for these toxins in as many countries of the world as possible. For that purpose, enquiry forms together

Table 22.2. Phycotoxin - producing marine algae for which monitoring programs exist worldwide.

Country	Algal species monitored	Closure of fishery product harvesting area
Australia	*Alexandrium catenella* *Gymnodinium catenatum*	$>5x10^4$ cells l^{-1} based on level of toxin
Canada	*Alexandrium* spp. *Pseudonitzschia pungens* spp. *Dinophysis* spp. *Prorocentrum* spp.	when toxin levels in shellfish exceed tolerable limits
Denmark	*Amphidinium* spp. *Dinophysis* spp. *Alexandrium* spp. *Gyrodinium aureolum* *Gymnodinium* spp. *Noctiluca scintillans* *Prorocentrum* spp. *Protogonyaulax catenella* *Dictyocha speculum* *Chrysochromulina polylepis* *Prymnesium* spp. *Heterosigma* cf. *akashiwo* *Pseudonitzschia pungens*	at approx. $5x10^5$ cells l^{-1} depending on species
France	*Dinophysis* spp. *Alexandrium* spp. *Gambierdiscus toxicus* *Ostreopsis lenticularis* *Prorocentrum lima*	based on toxicity level in shellfish not applicable not applicable not applicable
Ireland	*Dinophysis* spp. *Alexandrium* spp.	>200 cells l^{-1} $>5x10^4$ cells l^{-1}
Italy	*Dinophysis* spp. PSP and DSP producing species	1000 cells l^{-1} and presence of DSP in mussels simultaneous presence of algae in water and toxin in mussels
The Netherlands	*Dinophysis acuminata* and other *Dinophysis* spp.	when DSP toxins detected in shellfish
Norway [1)]	*Prymnesium parvum* *Chrysochromulina polylepsis* *Dinophysis* spp. *Alexandrium* spp.	when detected during routine check around shellfish farm
Singapore	*Cochlodinium catenatum* *Chattonella* *Heterosigma* *Mesodinium*	not applicable
South Korea	*Chaetoceros* spp. *Skeletonema* spp. *Thalassiosira* spp. *Cochlodinium* spp. *Heterosigma* spp. *Prorocentrum* spp. *Protogonyaulax* spp.	$>10^6$ cells l^{-1} $>10^6$ cells l^{-1} $>10^6$ cells l^{-1} $>10^4$ cells l^{-1} $>10^5$ cellsl^{-1} $>10^5$ cells l^{-1} $>10^5$ cells l^{-1}
USA (Florida)	*Gymnodinium breve*	$>5x10^3$ cells l^{-1}

1) only in special situations (unusually large algal blooms)

Table 22.3. Regulations for paralytic shellfish poisons in various countries.

COUNTRY	PRODUCT	TOXIN(S)	TOLERABLE[1,2] LEVEL	RESPONSIBLE AUTHORITY	METHOD OF ANALYSIS	REMARKS	REFERENCE
Australia	shellfish	saxitoxin	80 µg 100g^{-1}	State authorities under supervision of the Australian Quarantaine and Inspection Service	mouse bioassy		
Austria	shellfish	saxitoxin	40 µg 100g^{-1}	Ministry of Public Health and provincial authorities	Spectrophotometric method; mouse bioassay for confirmation		Satzler (1990)
Canada	molluscs	PSP	<80 µg 100g^{-1}	Dept. of Fisheries & Oceans; Dept. of Health & Welfare	mouse bioassy	Products having levels between 80-160µg 100g^{-1} may be canned	Fish Inspection Regulations (1978)
European Union[3] (EU)	bivalve molluscs	PSP	80 µg 100g^{-1}	Various	(Mouse) bioassay in association if necessary with a chemical method for detection	If the results are challenged, the reference method is the biological method	Council of the European Communities (1991)
Guatemala	molluscs	saxitoxin	400MU 100g^{-1}	Ministry of Public Health; Fisheries	mouse bioassy		Rosales-Loessener *et al.* (1989)
Hong Kong	shellfish	PSP	400 MU 100g^{-1}	Dept. of Health; Agriculture and Fisheries Dept.	mouse bioassy		Wong and Wu (1987)
Japan	bivalves	PSP	400 MU 100g^{-1}	Ministry of Health & Welfare; Bureau of Environmental Health	mouse bioassy		Yasumoto (1991)
Korea	bivalves	gonyautoxins	400 MU 100g^{-1}	Ministry of Health & Social Affairs	mouse bioassy HPLC method		Il-Yong Ha (1990)
New Zealand	shellfish	PSP, NSP, DSP ASP		Department of Health; Ministry of Agriculture and and Fisheries; Fishing Industry Inspection and Certification Council		regulations being developed	Jasperse (1993)
Norway	all types of mussel	PSP	40-80 µg 100g^{-1}	Food Control Authority	mouse bioassy	40-80 µg 100g^{-1}: single case assessment; >80 µg 100g^{-1}: banned	Yndestad (1989)
Panama	bivalves	PSP	400 MU 100g^{-1}	Ministry of Public Health	mouse bioassy	proposal	De Solis (1990)
Singapore	bivalves	saxitoxin	80 µg 100g^{-1}	Ministry of National Development (Primary Production Department) Ministry of the Environment	mouse bioassy		Lim Lian Chuan (1989)
Sweden	molluscs	PSP	80 µg 100g^{-1}	Ministry of Public Health; Ministry of Agriculture	mouse bioassy	tolerance expressed as saxitoxin equivalents	Hagelom (1989)
United States	bivalves	PSP	80 µg 100g^{-1}	Interstate Shellfish Sanitation Conference (ISSC); Food and Drug Administration	mouse bioassy	ISSC coordinates the Shellfish Programs, administered by the individual states	NSSP (1990)

1) For reasons of uniformity in presentation, all tolerances are expressed as MU 100g^{-1} or µg 100g^{-1}; 2) MU = mouse unit;
3) European Union countries include:Belgium, Denmark, France, Germany, Greece, Ireland, Italy, Luxembourg, The Netherlands, Portugal, Spain, United Kingdom

with some background information about the phycotoxin problem were sent out in four languages (English, Spanish, French, and Dutch). The questions concerned:

- measures taken to restrain the presence of phycotoxins;
- surveillance programs to check for occurrence of toxic algal species in areas where shellfish are grown;
- types and concentrations of algal species leading to closure of harvesting areas;
- types of phycotoxins and fishery products for which legislation is in force, together with the maximum permissible levels;
- the rationales for the regulations;
- the authorities responsible for the control of phycotoxins;
- the use of official and published methods of sampling and analysis;
- the disposal of consignments containing inadmissible amounts of phycotoxins.

About half (47) of the countries that were approached, responded to the inquiry. For a few countries information (also) came from other sources such as publications and personal communications. Altogether at least 24 countries, including the 12 member States of the European Union (EU), seemed to have regulations or detailed proposals for regulations on toxin-producing marine algae or marine phycotoxins. Figure 22.1 illustrates where the countries with marine phycotoxin legislation are geographically situated (black areas), and where such regulation does not exist (white areas). The regulations are summarized in Tables 22.3-22.5 and they are further discussed in the following sections. Other responding countries (Bolivia, Brazil, Burkina Fasso, Cameroun, Chile, Colombia, Ecuador, Egypt, Ethiopia, Guinée Bissau, Honduras, Hungary, India, Jordan, Kenya, Malawi, Mexico, Peru, Romania, San Salvador, Sudan, Switzerland, Syria, Yemen) indicated they had not (yet) specific regulations for marine phycotoxins. The fact that the law is silent on the phycotoxin topic does not necessarily mean that the problem is non-existent or ignored. Several countries rely on general food legislation in this respect, and may take specific action as each case arises in respect of phycotoxin problems. Regulations are continually changing, e.g. the EU Commission recently decided that Morocco may be included in the list of countries which satisfy the "equivalency" conditions of Third Countries able to export live mollusc bivalves to the Community (Directive 387, June 1993 of the EU Commission). The Directive 397 also specifies the particular sanitary conditions requested for the import of these products.

Several of the countries surveyed indicated they have monitoring programmes to check for occurrence of (toxic) phytoplankton species in areas where shellfish are grown. Table 22.2 gives an overview of these programs. In 25 countries tolerances have been established or proposed for one or more phycotoxins in shellfish. Regulations exist for: PSP and specifically for saxitoxin and gonyautoxins; DSP and specifically for okadaic acid; domoic acid (one of the amnesic shellfish poisons [ASP]); brevetoxin (one of the neurotoxic shellfish poisons [NSP]); ciguatera toxins are currently limited to finfish and will not be discussed here. In Tables 22.3-22.5 tolerances for these toxins, responsible authorities, methods of analyses used, and relevant references are summarized. The data presented in Tables 22.3-22.5 may not be complete or fully correct in a number of cases, due to problems experienced with language, terminology and the interpretation of the authors of the responses in the inquiry forms. Moreover, new or modified regulations are continuously being established.

Monitoring programs for toxic algal species

Some countries monitor only for one or two algal species, others have a long list of species monitored for, Denmark being the most active in this respect (see Table 22.2). In some countries the shellfish harvesting areas are closed when the number of cells of certain algal species exceeds certain concentrations (Australia, Denmark, Ireland, South Korea, USA, State of Florida) the latter depending on the species. Other countries (Canada, The Netherlands) close their harvesting areas only when the toxic phytoplankton species have been detected in the

Table 22.4.Regulations for diarrhetic shellfish poisons in various countries.

COUNTRY	PRODUCT	TOXIN(S)	TOLERABLE LEVEL[1,2]	RESPONSIBLE AUTHORITY	METHOD OF ANALYSIS	REMARKS	REFERENCE
Canada	shellfish	DSP	0.2µg g^{-1}	see Table 2	mouse bioassay	not official Todd (1993)	Cembella and
European Union[3] (EU)	bivalve molluscs	DSP	not detectable	various	customary biological testing methods		Council of the European Communities (1991)
Japan	bivalves	DSP	5 MU $100g^{-1}$	See Table 2	mouse bioassay		Yasumoto (1991)
Korea	shellfish	DSP	5 MU $100g^{-1}$	See Table 2	mouse bioassay		IL-Yong Ha (1990)
Norway	all types of shellfish	DSP	5-7 MU $100g^{-1}$	See Table 2	mouse bioassay		Yndestad (1989)
Sweden	molluscs	DSP	40-60 µg $100g^{-1}$	See Table 2	HPLC; mouse bioassay for confir-mation	tolerance expressed as okadaic acid equivalents; 40 µg $100g^{-1}$: tolerance. 60 µg $100g^{-1}$: domestic tolerance	Hageltorn (1989)

1) For reasons of uniformity in presentation, all tolerances are expressed as MU $100g^{-1}$ or µg $100g^{-1}$.
2) MU- mouse unit. A mouse survival time equal to 5 hours corresponds to about 0.2MU g^{-1} digestive gland.
3) European Union countries include: Belgium, Denmark, France, Germany, Greece, Ireland, Italy, Luxembourg, The Netherlands, Portugal, Spain, United Kingdom

Table 22.5. Regulations for phycotoxins other than PSP and DSP.

COUNTRY	PRODUCT	TOXIN(S) LEVEL	TOLERABLE	RESPONSIBLE AUTHORITY	METHOD OF ANALYSIS	REMARKS	REFERENCE
Canada	molluscs	domoic acid	2mg $100g^{-1}$	See Table 22.2	HPLC		Bureau of Chemical Safety (1988)
Portugal	shellfish	domoic acid	2 mg $100g^{-1}$	See Table 22.2		proposal	
Italy	shellfish	NSP	n.d.	See Table 22.2			Ministerio della Sanità (1990)
United States	bivalves	domoic acid	2 mg $100g^{-1}$	See Table 22.2	HPLC	not official	
	bivalves	NSP	n.d.		mouse bioassay	only in certain states (Florida)	NSSP (1990)
viscera	cooked crab	domoic acid (mg kg^{-1})	30ppm	See Table 22.2	HPLC	(1992)	HHE NO. 2937

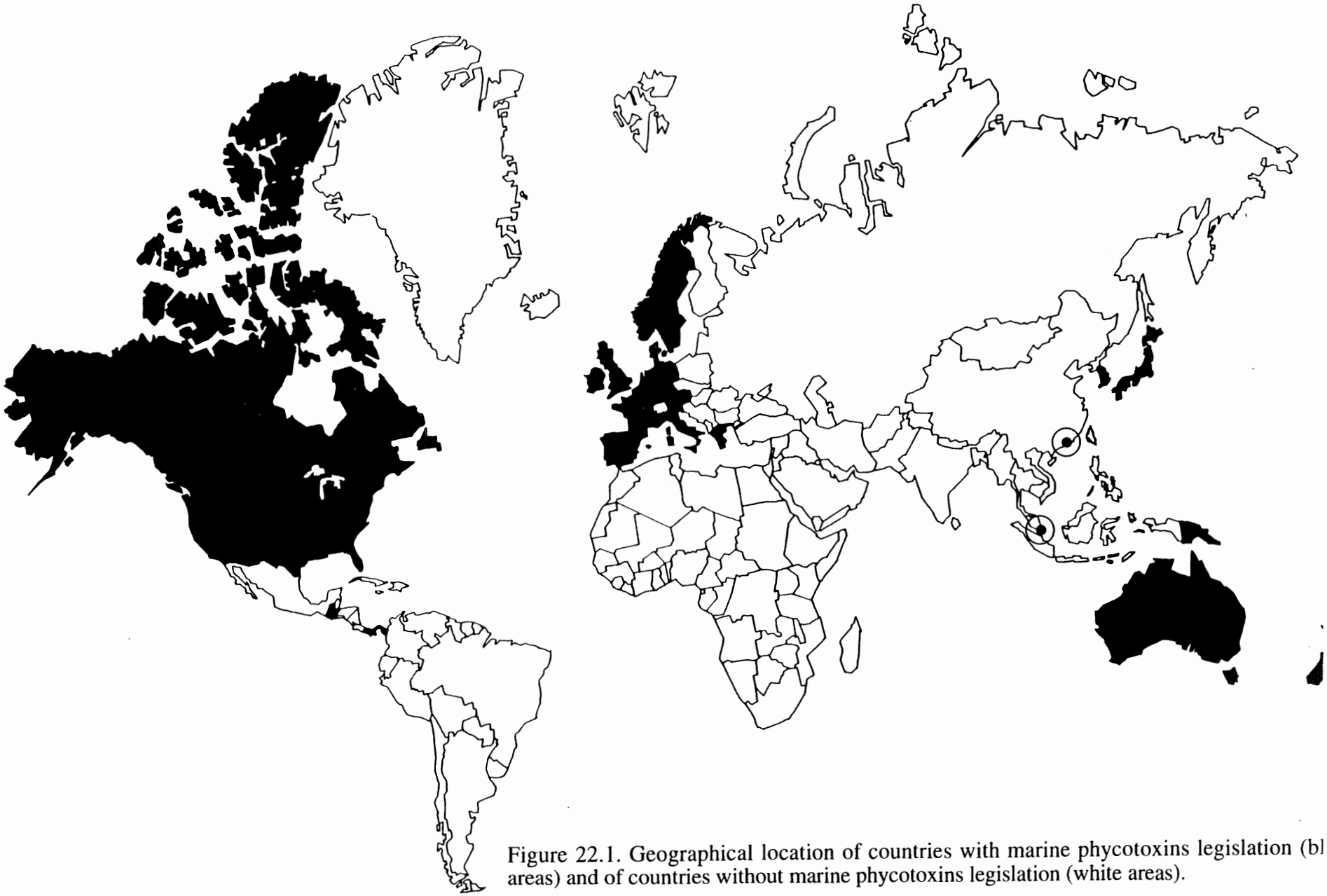

Figure 22.1. Geographical location of countries with marine phycotoxins legislation (bl
areas) and of countries without marine phycotoxins legislation (white areas).

shellfish. In Italy closure of harvesting areas occurs when simultaneous presence of toxic algae in water and toxin in mussels is noticed.

Regulations for paralytic shellfish poisons

The number of countries known to have in-force or proposed regulations for PSP was 25 at the time of writing (Table 22.3). Most regulations are set for paralytic shellfish poisons as a group. Some countries indicated specific regulations for one of the PSP toxins, mostly saxitoxin. In most cases the regulations concerned shellfish, but some countries mentioned more generally molluscs, or more specifically bivalves, as the types of products for which maximum permissible levels of PSP toxins were set.

Many countries indicated use of the standard mouse bioassay of the Association of Official Analytical Chemists International (AOAC) (Hollingworth and Wekell, 1990) as the method of analysis. A (non-selective) spectrophotometric method is applied in Austria (Hellwig and Petuely, 1980) and in Germany (Bundesgesundheitsamt, 1989). A few other countries apply an HPLC-method (Sullivan *et al.*, 1985), sometimes in addition to the mouse bioassay. In the EU a new directive came into force 1 January 1993 (Council of the European Communities, 1991), stating that the total PSP content in molluscs has to be determined according to the "biological testing method in association, if necessary, with a chemical method for detection of saxitoxin. If the results are challenged, the reference method shall be the biological method."

Different concentration units are used to express the tolerance level: mouse unit g^{-1} (MU g^{-1}) and $\mu g\ g^{-1}$ (incidentally $\mu g\ ml^{-1}$). The latter unit currently seems to be less appropriate in the countries that use the mouse bioassay, because they actually test for toxicity in the mouse. Expression of a tolerance level for PSP in $\mu g\ g^{-1}$ would be valuable, if the various PSP toxins exhibit the same toxicity, which is not the case (Krogh, 1988). If the unit $\mu g\ g^{-1}$ still would be preferred above MU g^{-1}, one might consider application of a toxic equivalence factor and expression of concentration of the various PSP (if these can be selectively measured) in concentration units of saxitoxin. The development of analytical-chemical methodology (HPLC) shows promise in selective toxin measurements.

The application of HPLC for regulatory purposes has been hampered by the lack of validated analytical methodology, pure analytical standards of the various PSP toxins, and reference samples for analytical purposes (Van Egmond *et al.*, 1993). Recent advances by the National Research Council of Canada (Marine Analytical Chemistry Standards Program, NRC, 1411 Oxford Street, Halifax, Nova Scotia, Canada B3H 3Z1) have made available certified solutions for paralytic shellfish poisoning poisons (standards for domoic acid and diarrhetic shellfish toxins are also available). To date, the AOAC mouse bioassay remains the only method of detection accepted by the United States Food and Drug Administration and members of the NSSP/ISSC agreement.

All countries that have a limit for toxicity apply a level of 400 MU 100 g^{-1} (which corresponds to approximately 80 μg STXequiv 100 g^{-1}). For those countries that apply tolerances expressed in physico-chemical concentration units, the situation is less uniform. As demonstrated in Table 22.3, the following variants occur: 80 μg PSP 100 g^{-1}, 40 μg PSP 100 g^{-1}, 80 μg saxitoxin 100 g^{-1}, 40 μg saxitoxin 100 g^{-1}. This situation cannot be beneficial to public health, nor to international trade, and it would be desirable to harmonize these limits on a commonly accepted basis.

Information was requested in the international inquiry as to the rationales that national authorities had applied in establishing their tolerance levels. Some type of response to the rationale question for PSP was received from 12 countries, but none of these indicated the risk considered acceptable. Australia indicated that tolerance levels are generally based on toxicity, taking account of analytical possibilities. The regulations of trading partners (e.g. the USA) also played a role in the establishment of the Australian limit for PSP. Canada mentioned that the rationale used to establish their PSP-tolerance was currently being re-addressed. France referred to the PSP bioassay of the AOAC (Hollingworth and Wekell, 1990), in which the threshold is

clearly indicated at 80 μg 100 g^{-1} and to the recommendations of a WHO Expert Consultation on PSP (Halstead and Schantz, 1984). Guatemala and Hong Kong referred to risk assessment studies based on WHO reviews of Halstead and Schantz (1984) and Wood (1976) respectively. Ireland referred to EU-documents and working group reports of the International Council for Exploration of the Sea (ICES) on harmful effects of phytoplankton, without further details. Japan set the PSP regulation level in accordance to that used in the USA and Canada, as this level was considered to be successful in both countries. The Netherlands indicated that a low tolerance for PSP of 40 μg kg^{-1} was desirable in view of the fact that a toxicological evaluation of the whole PSP-mixture was not available, and that most toxicological data relate solely to saxitoxin. The establishment of the desired Dutch tolerance was also influenced by the low Italian tolerance. Norway indicated that PSP limits were based on international risk studies and recommendations from a Norwegian expert group, without providing further details. In South-Korea, the risk had been determined in accordance with international specifications, supposedly those of the U.S.A. and Japan. Portugal also referred to international norms and standards, without giving specifics. Singapore referred to the AOAC standard mouse-bioassay, thereby obviously following the U.S. rationale in this respect. The U.S.A. indicated that no formal risk assessment had been completed and that the limit was primarily established on the basis of epidemiogical data and the capability of analytical methodology. However, should the methodology improve and the limit of determination decrease, this would not change the regulatory level in the U.S., because regulations in the U.S. were said to based on human health consequences and not on the idea that possible harmful substances must be avoided at any level.

In the European Union, where originally divergent tolerances for PSP-toxins were administrated, the limits were recently harmonized at 80 μg PSP/100 g mollusc flesh (Council of the European Communities, 1991), following the advice of the Scientific Veterinary Committee. This Committee agreed that levels up to 80 μg total PSP per 100 g mollusc flesh have not been shown toxic for consumers and that a tolerance of 80 μg total PSP per 100 g shellfish would ensure that no single component, such as saxitoxin, exceed 30 μg/100 g shellfish. Despite the preference of some EU-countries to go as low as 40 μg/100 g with their limit (Germany, Italy, The Netherlands) they follow the common EU-directive.

It might be appropriate for international authoritative bodies such as the World Health Organization (WHO) and the International Life Sciences Institute (ILSI) to re-evaluate current knowledge about toxicity of PSP and give guidance as to safe intake levels for PSP (Van Egmond *et al.*, 1993).

Regulations for diarrhetic shellfish poisons

There were 18 countries that had regulations for DSP at the time of writing, Canada and New Zealand being the most recent additions (Table 22. 4). Products for which limits were set were indicated with different degrees of specificity, e.g.: molluscs; shellfish; bivalves; mussels. The toxins covered by the regulations were mostly identified as DSP toxins and sometimes, more specifically okadaic acid. The tolerance levels for DSP were generally set at the limit of detection of the analytical method used, most often a mouse bioassay, originally developed in Japan (Yasumoto *et al.*, 1978). A few countries relied on a rat bioassay (Kat, 1983). HPLC was used in addition to the rat bioassay in Ireland and as the main method of detection in Sweden (Lee *et al.*, 1987). New regulations in the EC (Council of the European Communities, 1991) state that the normal biological methods of analysis may not lead to positive results for DSP in consumable parts of molluscs (whole animal or every separate consumable part). As with PSP toxins, analytical determination of DSP toxins has been limited. Efforts are underway in several countries to improve the situation. Neither the bioassays, nor the HPLC-procedure have been validated in international collaborative studies. Pure analytical standard and reference materials have not been readily available, and there is a great difference in performance of the mouse bioassay (toxicity criterion: animal death) and the rat bioassay (toxicity criterion: soft stool, diarrhoea and feed refusal), resulting in differences in specificity and detectability.

Presumably the mouse bioassay detects all DSP components, and probably also other toxins, whereas the rat bioassay detects only okadaic acid and the dinophysis toxins, because only these acidic components are known to cause diarrhoea in animals (Krogh, 1989). A new and promising analytical development is a solid phase immuno-bead assay, initially designed for ciguatera toxins. This assay cross reacts with other polyether compounds such as okadaic acid (Park, 1991). The recent development of certified calibration solutions of okadaic acid (OACS-1) and mussel tissue reference material for diarrhetic shellfish poisoning toxins (MUS-2) by the National Research Council of Canada (ibid.) should provide a viable means of standardizing methods of detection for DSP toxins worldwide.

The questions of rationale for established tolerances were only partially and superficially answered. Ireland and the Netherlands indicated that the limit should be established by the limit of the analytical method selected, as to avoid possible harmful substances in food at any level. In Italy the DSP-limit was said to be based on the experience of other countries, in particular France. Japan based its limit for DSP partly on a poisoning case study (Yasumoto *et al.*, 1978) and partly on the pracatical limit of detection of the mouse bioassay. In Sweden, risk assessment based on domestic experiences on the National Food Administration was the basis for the limit. South-Korea, Norway, and Portugal gave the same answers as for PSP.

As for PSP, the non-uniformity of tolerance levels for DSP requires harmonization for the benefit of public health and international trade. It might be desirable if international organizations would evaluate the hazards caused by DSP, and provide a basis for a common rationale for the establishment of DSP limits. Such an evaluation could include other adverse effects, such as the tumor-producing effect of okadaic acid, in addition to the acute effects of some DSP toxins on the gastro-intestinal tract.

Regulations for other marine phycotoxins

At the time of writing, only a few countries had established regulations for shellfish toxins other than PSP and DSP (Table 22.5). The countries that have (proposed) regulations for domoic acid (ASP) have a uniform tolerance level of 2 mg 100 g^{-1} product, based on a Canadian risk assessment (Bureau of Chemical Safety, 1988). In contrast to PSP and DSP toxins, the analytical determination of domoic acid is fairly straightforward. An HPLC method exists, which has been evaluated in a collaborative study (Bureau of Chemical Safety, 1989), and a certified instrument calibration solution and a certified mussel tissue reference material have been developed (Hardstaff *et al.*, 1990). The National Research Council of Canada has also developed a certified calibration solution for domoic acid (DACS-1B).

Regulations exist for neurotoxic shellfish poisoning (NSP), specifically for brevetoxin, and for ciguatera toxins in finfish. The acceptable level of brevetoxins (NSP) in shellfish is administrative zero, i.e. undetectable by bioassay. The United States and New Zealand are currently the only countries directly affected by NSP; however, as with PSP, ASP and DSP, all countries conforming to the NSSP/ISSC regulations adhere to the same limit.

Actions taken, when products contain unacceptable levels of toxins

Most countries surveyed indicated they did not allow entry of consignments of shellfish products containing an inadmissible amount of toxin. A few countries (Austria, United Kingdom) mentioned that condemned imported goods would be destroyed.

In the case of domestic produce, several countries (Canada, Guatemala, Ireland, Norway, Korea, Sweden, United Kingdom and USA) stop harvest of fishery products if levels of toxins exceed the limits and a waiting period is established until the concentrations of toxins are below acceptable limits. Harvested products containing too much toxin are usually destroyed. Canada mentioned that products containing PSP levels ≥ 80 µg 100 g^{-1} and ≤ 160 µg 100 g^{-1} may be canned and retorted.

CONCLUSIONS

Currently, limited information exists about limits and regulations on marine phycotoxins in 25 countries, located in North and Central-America, Western Europe, and Australasia. These regulations concern environmental surveillance for toxic algae and analytical monitoring of shellfish products for one or more of the toxins associated with PSP, DSP, ASP, and NSP.

The acceptable levels for the major toxins of concern (PSP, DSP) may differ between countries. It would be desirable if international organizations would (re-)evaluate the hazards caused by marine phycotoxins, to provide a common basis for risk assessment.

Moreover, further development and validation of analytical methodology (and reference materials) for marine phycotoxins is highly desirable, because the enforcement of phycotoxin legislation is ultimately based on the ability of analysts to identify and quantify accurately these toxins in seafood products.

INTERSTATE SHELLFISH SANITATION COMMITTEE AND NATIONAL SHELLFISH SANITATION PROGRAM (ISSC/NSSP, USA)

In the United States, the Federal Food and Drug Administration (USFDA) is charged with the responsibility of assuring that all food items shipped in interstate commerce are safely prepared, packed, and always held under sanitary conditions. Items must be correctly labeled and the conditions in which the food is prepared and held must also be safe and sanitary. In 1925, the F.D.A. was authorized to receive aid from State and local authorities in order to enforce laws to prevent and suppress communicable disease transmission. This led to the creation of the National Shellfish Sanitation Program (NSSP). The NSSP includes members of the F.D.A., State control agencies, and shellfish industry which set the standards to insure that sanitary conditions exist in the production and interstate shipment of shellfish, on a voluntary basis. The Interstate Shellfish Sanitation Conference (ISSC) was formed in 1982 and consists of members of State and Federal control agencies, the shellfish industry and the academic community. It is a voluntary organization and is open to all persons interested in assuring that shellfish reach the consumer under safe, sanitary conditions. The ISSC provides up-to-date sanitation guidelines for the regulation of harvesting, processing and shipping of shellfish. It also provides a forum for all interested persons to air their concerns regarding shellfish sanitation, and disseminates information of recent developments via publications, meetings and working with academic institutions as well as trade associations. Any country shipping shellfish to the United States must comply with these regulations (Hurst, personal communiation).

Within the United States, the State of Maine has one of the most comprehensive monitoring programs for paralytic shellfish poisons and will be described here as an example of a successful and effective monitoring system (see NSSP Manual - Part 1 Section C, Appendix A January 1990). This program was necessitated by yearly occurrences of toxic shellfish and has been used as a template for establishing monitoring programs in many other regions worldwide. The purpose of this program is to assure that only safe shellfish are harvested. Years of practical experience have afforded the opportunity to continually modify the sampling program, to better reflect increasing knowledge of potential toxic areas as well as the changing utilization of shellfish. Instead of "primary-key stations", areas of similar toxic patterns are used. At the beginning of the PSP testing year, shellfish samples are collected from each of these areas to determine the background level of toxicity. Sampling stations from these areas are sampled each week from April-October regardless of toxin patterns. When shellfish show any toxicity sampling is expanded until stations of no toxicity are found. This sampling program allows for closures to be made in a safe manner. Maine's law and regulation require the immediate closure of toxic shellfish harvest areas, embargo or confiscation of all suspect

shellfish. When necessary, licenses and certificates may be suspended. Administrative actions are accomplished on a same day basis.

THE SAMPLING PLAN

The Maine Department of Marine Resources is the state agency responsible for marine biotoxin monitoring. Personnel of the department located coastwide report any unusual occurrences such as bird or fish kills, water discoloration, and abnormal behavior of shellfish to the Marine Science Laboratory at Boothbay Harbor.

Channels of communication concerning fluctuations in shellfish toxicity have been actively established to provide updated information on shellfish toxicity with Canada, New Hampshire, Massachusetts and the FDA.

LAWS AND REGULATIONS

Paralytic Shellfish Poison Monitoring Program Section 6076

1. Purpose: A comprehensive Paralytic Shellfish Poison Monitoring Program is established to protect the public health while providing for the harvest of susceptible species of marine mollusks in areas not shown to be affected by contamination.
2. Responsibilities: The department shall be the state agency responsible for implementing the program. The department may adopt rules under section 6172 as may be warranted to provide for adequate protection of the public health.

Contaminated or Polluted Flats Section 6172:

The Commissioner may examine the coastal waters and intertidal zone and adopt regulations to close coastal waters or intertidal zone areas if he determines that any marine mollusks are or may become contaminated or polluted. The commissioner may adopt or amend regulations as he deems necessary, setting forth standards for contaminated or polluted areas, giving consideration to established state water quality standards, the most recently adopted federal sanitation standards, the most recent generally accepted research data and known sources of pollution in any area, in a manner so as to protect the public health and safety while allowing reasonable use of the state's shellfish. The commissioner may adopt or amend regulations under the emergency procedures, if immediate action is necessary to prevent the taking of polluted or contaminated marine mollusks.
SECTION 6192: *Notwithstanding any provisions of the Maine Administrative Procedure Act, an emergency regulation authorized by Section 6172, subsection 2 or 3 shall be effective immediately upon signature by the commissioner or his authorized designee. Upon promulgation of such an emergency regulation, the commissioner shall give oral notice of such an emergency closure to local governmental authorities and shall publish notice of closure as soon as possible in a newspaper of general circulation in the area of the State affected. Marine Patrol Officers shall take action to prevent taking of shellfish from that area, including the embargo of contaminated shellfish under section 6856, subsection 6 and the arrest of any person violating the emergency regulation.* SECTION 6856: *The commissioner or his agent, shall indefinitely embargo, condemn or order to be destroyed any shellfish product in any establishment whenever it is determined that the product is of unsound quality, contains any filthy, decomposed or putrid substance, or may be poisonous or deleterious to health, or otherwise unsafe. The commissioner and his agent shall cooperate with those state and federal*

agencies, having similar responsibilities, in the protection of public health and in enforcing the order to embargo or destroy.

Closure of Contaminated Areas Chapter 23.30

A. An area shall be closed to the harvest of shellfish immediately if the meats of shellfish harvested from that area contain 80 micrograms of Paralytic Shellfish Poison toxins per 100 grams of shellfish meats or contain concentrations of other toxins or contaminates known to be harmful to consumer health. The commissioner may also close surrounding areas of lower toxicity levels to provide a margin of safety in the event of rapidly changing toxicity levels.
B. The commissioner may close areas or fisheries if sufficient current information is not available to assure above conditions do not exist or current information does not permit prediction that the above conditions are unlikely to occur.

Chapter 23.40 Repeal of Polluted or Contaminated Area Closures

The Commissioner shall repeal polluted or contaminated area closure regulations when sanitary surveys reveal that pollution or contamination conditions no longer exist and that shellfish may be harvested from the area without threat to the public health.

PARALYTIC SHELLFISH POISON MONITORING PROGRAM

Maine's PSP monitoring program is continually modified to reflect increasing knowledge as to where toxic shellfish are likely to occur as well as changing utilization of shellfish. Shellfish sampling locations may be changed throughout the sampling year to reflect unusual conditions.

Maine's PSP monitoring program is conducted on a yearly basis from April until October. Based upon areas of similar toxin patterns the coast of Maine has been divided into 18 areas from southwest to northeast with Area 10 the most southerly and area 27 in Cobscook Bay the most northerly. At the beginning of the PSP testing year shellfish samples (mussels, *Mytilus edulis* and clams, *Mya arenaria*) are collected weekly from "primary" sampling stations from these areas. Early season shellfish samples determine the background level of toxicity in an area (hopefully below the limit of detection sensitivity of the test). Whenever there is any rise of toxin in an area the sampling of shellfish is increased to adjacent sampling stations so that when a closure is necessary adequate data is available to make the proper public health oriented closure. Closures are made with safety zones in place and frequently are made on the basis of previous PSP closures for that area. Shellfish collectors are trained by experienced staff on sample collection methods as well as how to locate the sampling stations. To aid in sampling all stations have been listed by area as well as which species of shellfish are present. Shellfish are returned to the lab under refrigeration and assayed as soon as possible under the standard mouse bioassay. Whenever toxin levels are found approaching the quarantine level of 80 micrograms STX equiv per 100 g of shellfish, the area is closed to shellfish harvesting. Closures are made on a species basis if adequate information is available to demonstrate that not all species are toxic. Species closures require increased sampling of the "toxin-free" shellfish. In areas where it can be justified, increased sampling will permit a partial opening of an area where part of the area is proven not toxic. Shellfish such as the ocean quahog, *Arctica islandica*, are sampled from contract fishing boats by department personnel. Areas where quahogs and similar species cannot be sampled are closed because they cannot be regarded as safe without sampling.Reopening of areas closed is dependent upon continued toxin levels less than 80 micrograms. Openings are made after evaluations of current and historical records. Areas in which historical data indicates that several rises of toxin may be expected remain closed throughout the PSP season, as making multiple openings and closures in these areas reduces the credibility of the PSP program. Areas in which there is a high value shellfish resource and

where there is reason to believe that there will not be another rise of toxin may be reopened after at least two weekly samplings below 80 micrograms. Reopenings are at best a judgment call and must be made upon the ability to assure that the area is safe.

TOXINS OTHER THAN PSP COVERED UNDER NSSP MANUAL REQUIREMENTS JANUARY 1990

The Department of Marine Resources (DMR) acknowledges that there may be toxins other than PSP in shellfish. DMR conducts a limited sampling program for Amnesic Shellfish Poison (ASP), domoic acid, in conjunction with it's PSP sampling program. Information from adjacent Canada concerning domoic acid is available on a up to date basis. Closures will be made whenever domoic acid levels reach 20ppm. Diarrhetic Shellfish poisoning (DSP) has not been reported from North America. Due to the presence of *Dinophysis* spp DMR recognizes that cases are likely to occur. DMR cannot assay for DSP toxins at this time.

Any area suspected of containing ASP, DSP or any other toxin defined or undefined will be closed under Chapter 23.30 until such time that area is deemed toxin free.

In order to sell shellfish to countries to of the EU shellfish must be accompanied by a Health Certificate. The USFDA has indicated that they will issue these certificates if the dealer involved is in compliance with applicable laws and regulations. This will involve giving consent for FDA to access quality control, production, and other relevant records. If the dealer is in compliance with NSSP regulations they will meet the EU requirement other than for DSP.

DSP may or not be a problem if the dealer has any records at all concerning the presence of toxic algae in the harvest waters.

There is at present no test for DSP in the US. The requirement for DSP in EU countries is a negative biological test. The FDA is assuming the responsibility until December 31, 1994 for issuing Health Certificates for molluscan shellfish to be exported to EU nations, they cannot certify them to be DSP free. It is reasonable to expect and suspect that DSP may at times be present in Maine mussels. The credibility of any certification of compliance with a biological standard without documentation of tests being run must be deemed questionable. Until the FDA can give us a standard testing method for DSP we should insist that *they* not the State laboratory certify the safety of these shellfish with respect to DSP.

There is a possible positive solution for determining the presence of DSP in mussels. A quick reading of the EU requirements indicates that knowledge of plankton in the harvest area is a important consideration. It is reasonable to assume that documented plankton absence is a good indication of the absence of DSP. We must recommend that anyone contemplating shipping shellfish to EU countries to collect data in their harvest area's on the presence or absence of potentially toxic plankton.

It is reasonable to expect the FDA to make the States assume the responsibility of determining any safety standards which is realistically theirs. If and when a Maine dealer(s) asks for certification to the EU market it must be pointed out that there is no way of determining a negative DSP assay.

Contingency Plan(s) for Toxic Shellfish Emergencies

Regardless of the overall success of a shellfish toxin monitoring plan designed to avoid toxin shellfish emergencies, they will occur, thus contingency plans should be developed to resolve these emergencies.

Contingency plans for toxin shellfish emergencies that affect the public health and safety should be developed so there will be no misunderstanding of what actions to take. It is very important that adequate legal authority to act immediately be in place and understood by all.

Updated lists of the person(s) responsible for decisions should be known to all persons involved so that proper actions can be taken in a timely manner. Emergencies by their very nature can be expected to occur at inopportune times, thus the chain of command must be defined in advance.

The extent of the toxin emergency should be defined if at all possible. Small local emergencies can be concluded quickly by the embargo and destruction of product and a closure of the harvest area(s). Attempts to salvage portions of confiscated shellfish should be discouraged, as the sampling method will always be in question.

Listed below are some of the information needed to evaluate a shellfish emergency:
Location of the shellfish in question:

Positive identification of harvest area(s)
Species of shellfish under question
The amounts of shellfish involved
Is the toxin identified?

In emergencies that occur due to unexpected rises of known toxins, quick positive action to close harvest areas and embargo and destroy shellfish in the market should be expected to solve the problem.Rises of unknown toxins require quick action to prevent real serious problems. Shellfish must be removed from the market and harvesting prohibited until the toxin is identified. It must be assumed that if the safety of shellfish is in doubt, they are not safe.

Public health and safety requires the as complete as possible removal of any toxic shellfish from the market and closure of any suspect harvest area. Economic value of the shellfish resource is secondary to public health and safety.

Unknown toxins present special problems as testing procedures have to be developed to determine when the shellfish will be safe. Modifications of shellfish monitoring plans must be made to include these new toxin(s).Cooperative agreements between laboratories to address the appearance of new toxins are desirable. These agreements should be general in nature as what expertise and needed equipment cannot be determined in advance.Contingency funding for shellfish emergencies while desirable cannot be anticipated in advance thus some idea of where funds can be obtained should be given some thought.

Shellfish Certification

Persons or Cooperations interested in selling shellfish in the International market should contact their respective National Health agency and their National Natural Resource agency for their current regulations concerning shellfish. In order to market safe shellfish, regulations must be in place and enforced concerning the sanitary suitability of the harvest area(s) and the safe processing of the shellfish. The exporting nation is responsible for determining the safety of shellfish being exported.If the potential shellfish market is the United States, there must be in place a Memorandum of Understanding (MOU) between the National Agency responsible for Shellfish Safety and the USFDA. Nations that have current MOU's with USFDA are: Australia, Canada, Chile, Japan, The Republic of Korea, Iceland, Mexico, England and New Zealand. The MOU's may restrict harvest areas and selected species of shellfish.Shipments of shellfish to nations that are members of the European Union (EU) - France, Germany, Italy, United Kingdom, Spain, Portugal, Greece, Netherlands, Belgium, Denmark, Ireland and Luxembourg must meet the EU requirements of sanitation under Council Directive (91/492/EEC) (Commission of the EU, 1992) which requires a Certificate of Health by a competent authority from the nation of origin. This certificate must address measures that assure that the shellfish meet the EU standards for shellfish sanitation. Nations not listed above can also be expected to require a MOU from the nation of origin concerning safety of shellfish being imported.The

USA regulations are carried out under the National Shellfish Program which is similar to other National Programs.

Toxin Management

The overall management of toxins in shellfish is in the developmental stage, and should be considered to be limited at best. Management of toxic shellfish requires extensive monitoring of the harvest areas and as demonstrated by the example of the State of Maine (USA) it can successfully combine public health assurances and commercial harvest realities. It must be understood that defining safe harvest areas is the real goal of management of shellfish resources as related to toxic shellfish. Monitoring of the toxic harvest areas allows for the reopening of these areas but does not protect the public health and safety.

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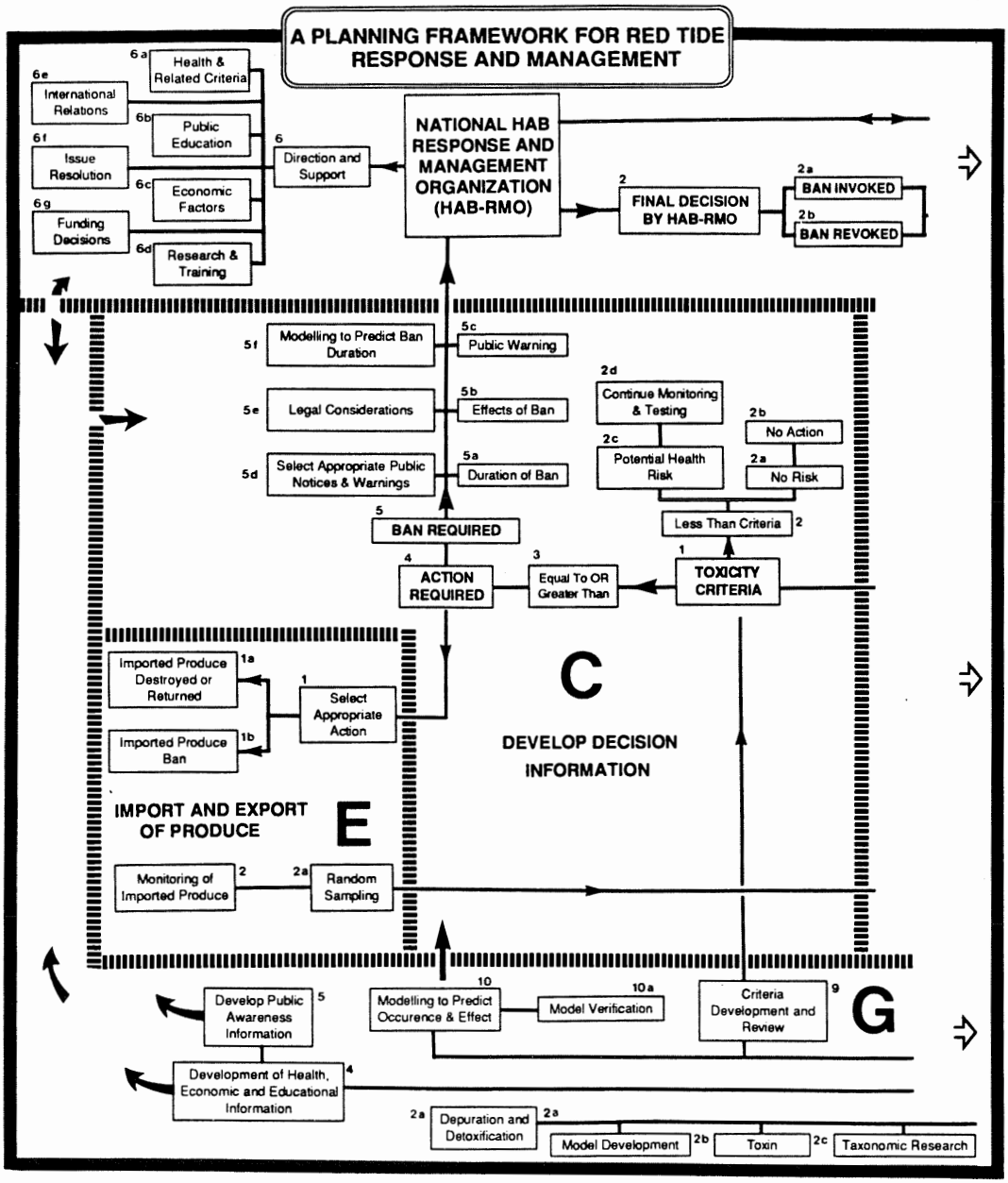
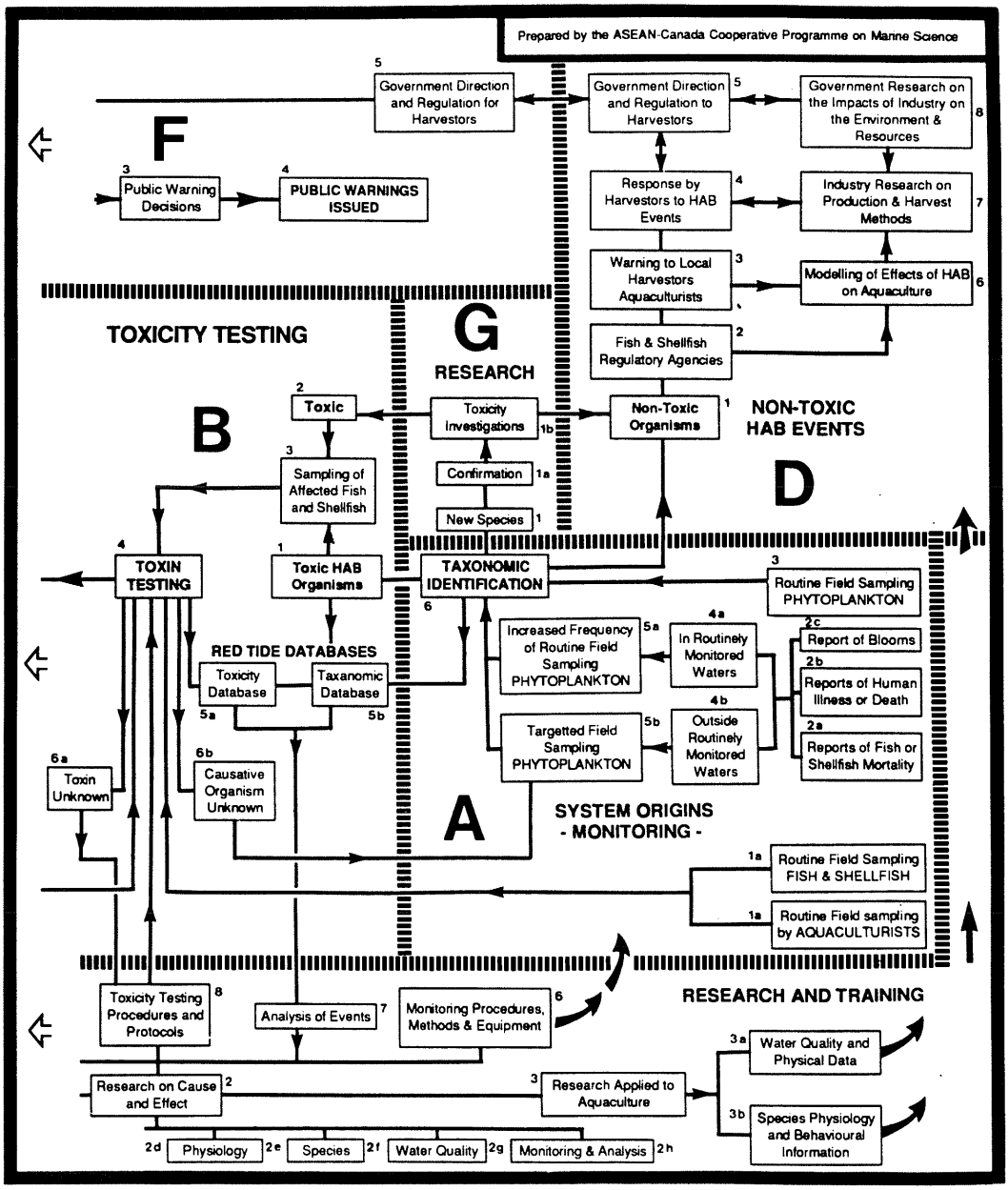


Figure 22.2. Red Tide Response and Management Framework, prepared by the Red Tide Technical Working Group under the ASEAN-Canada Cooperative Programme on Marine Science, as a tool for assessing and developing red tide response and management systems in ASEAN and its member states.


Prepared by the ASEAN-Canada Cooperative Programme on Marine Science
Government Direction and Regulation for Harvestors
Government Direction and Regulation to Harvestors
Government Research on the Impacts of Industry on the Environment & Resources
F
Public Warning Decisions
PUBLIC WARNINGS ISSUED
Response by Harvestors to HAB Events
Industry Research on Production & Harvest Methods
Warning to Local Harvestors Aquaculturists
Modelling of Effects of HAB on Aquaculture
TOXICITY TESTING
G
RESEARCH
Fish & Shellfish Regulatory Agencies
B
Toxic
Toxicity Investigations
Non-Toxic Organisms
NON-TOXIC HAB EVENTS
Sampling of Affected Fish and Shellfish
Confirmation
New Species
D
TOXIN TESTING
Toxic HAB Organisms
TAXONOMIC IDENTIFICATION
Routine Field Sampling PHYTOPLANKTON
RED TIDE DATABASES
Toxicity Database
Taxanomic Database
Increased Frequency of Routine Field Sampling PHYTOPLANKTON
In Routinely Monitored Waters
Report of Blooms
Reports of Human Illness or Death
Targetted Field Sampling PHYTOPLANKTON
Outside Routinely Monitored Waters
Reports of Fish or Shellfish Mortality
Toxin Unknown
Causative Organism Unknown
A
SYSTEM ORIGINS
- MONITORING -
Routine Field Sampling FISH & SHELLFISH
Routine Field sampling by AQUACULTURISTS
RESEARCH AND TRAINING
Toxicity Testing Procedures and Protocols
Analysis of Events
Monitoring Procedures, Methods & Equipment
Water Quality and Physical Data
Research on Cause and Effect
Research Applied to Aquaculture
Species Physiology and Behavioural Information
Physiology
Species
Water Quality
Monitoring & Analysis