COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

COT STATEMENT ON RISK ASSESSMENT OF MARINE BIOTOXINS OF THE OKADAIC ACID, PECTENOTOXIN, AZASPIRACID AND YESSOTOXIN GROUPS IN SUPPORT OF HUMAN HEALTH

Introduction

1. A number of marine phytoplankton produce biotoxins that can be bioconcentrated by shellfish. Consumption of shellfish sufficiently contaminated with these toxins can result in human illness. Marine biotoxins have previously been categorised on the basis of clinical signs, but are increasingly being categorised by chemical structure. The structural toxin groups that are generally considered to be of relevance to shellfish harvested in European waters are:

- Domoic acid group (DA)
- Saxitoxin group (STX)
- Okadaic acid group (OA)
- Pectenotoxin group (PTX)
- Azaspiracid group (AZA)
- Yessotoxin group (YTX)
- Cyclic imine group

2. Marine biotoxins can also be categorised according to their water solubility which determines the extraction protocol required for analysis. The DA and STX groups are hydrophilic, while the OA, PTX, AZA, YTX and cyclic imine groups are all lipophilic.

3. The DA group is associated with amnesic shellfish poisoning (ASP), the STX group with paralytic shellfish poisoning (PSP) and the OA group with diarrhetic shellfish poisoning (DSP).

4. The Committee was asked for its views on the risk assessment of biotoxins of the STX, OA, AZA, PTX and YTX groups in order to support protection of consumer health. A statement on STXs was published in September 2006[1]. The present statement addresses the OA, PTX, AZA and YTX groups.

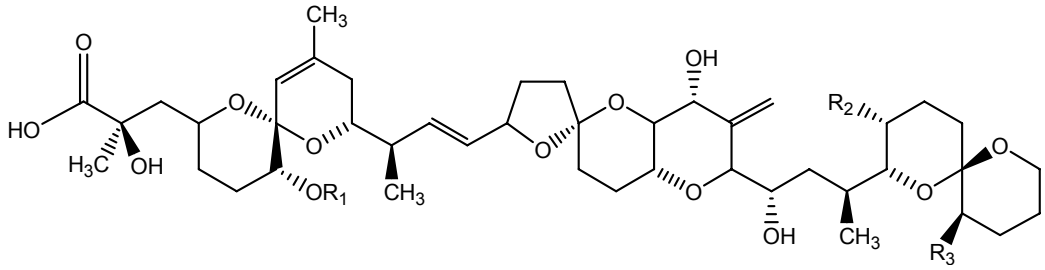
Okadaic Acid group

Background

5. Toxins from the okadaic acid (OA) group are known to cause diarrhetic shellfish poisoning (DSP), a gastrointestinal illness that was first identified in the late 1970s[2]. The effects of OA toxins are considered to be a result of the ability of these compounds to inhibit the activity of the protein phosphatases 1 and 2A [3,4].

6. The OA group comprises OA and its analogues dinophysistoxin (DTX)-1, DTX-2 and DTX-3. 'DTX-3' originally referred to a group of 7-O-acyl derivatives of DTX-1. More recently, however, it has been demonstrated that OA and DTX-2 can also be acylated to give 'DTX-3' compounds [3]. The structures of these compounds are shown in Figure 1. These esters are considered to be relatively unstable and are expected to be hydrolysed in the body to give the free toxins [5,6]. Alkaline hydrolysis of ester forms to the free toxins is required for detection by methods other than *in vivo* assays.

Figure 1. Chemical structures of OA group toxins[7]



Toxin	R1	R2	R3
OA	H	CH_3	H
DTX-1	H	CH_3	CH_3
DTX-2	H	H	CH_3
DTX-3	Acyl	CH_3	CH_3

7. In Japan, DSP outbreaks have mainly been associated with DTX-1, while OA has been more frequently associated with DSP incidents in Europe. DTX-2 has previously been reported to be the predominant diarrhetic shellfish toxin in Ireland [8]. A number of outbreaks associated with DTX-3 toxins have recently been reported in Chile, Norway and Portugal [6,5,9].

8. Current legislation sets out a maximum permitted level for OA toxins together with PTXs of 16 µg OA equivalents (eq)/100 g shellfish meat, although there have been recent proposals to regulate PTXs separately. Mouse bioassays (MBAs), involving intraperitoneal (i.p.) injection of shellfish extract, are prescribed in EU legislation as the reference methods for the detection of these toxins. The regulatory MBA provides a positive/negative result rather than

quantitation of toxin concentration, and a positive result is considered to indicate the presence of OA toxins and/or PTXs above the regulatory limit.

Previous COT evaluations

9. The COT previously considered DSP toxins in 1994, when it reviewed a Ministry of Agriculture, Fisheries and Food (MAFF) food surveillance paper on Naturally Occurring Toxicants in Food [10]. The COT recommended:

- That the surveillance programme for detecting shellfish contaminated with DSP toxins as described in the surveillance paper be continued
- That exposure to OA should be kept below those concentrations which cause toxicity as any tumour promoter activity would also then be minimised
- That the mouse bioassay be used for the detection of DSP in shellfish at present but that efforts be made to develop a more quantitative assay

10. Research into alternative methods is ongoing, but a method considered appropriate for use in the UK shellfish monitoring programme for lipophilic biotoxins (incorporating OA toxins, PTXs, YTXs and AZAs) is not yet available. Progress has been limited by a lack of analytical standards for many toxins and problems in achieving the required sensitivity and specificity. However, it is hoped that an appropriate method will become available within a couple of years.

Toxicology

Toxicokinetics

11. Research indicates that OA is widely distributed following oral administration. In Swiss mice, OA was detected in intestinal content (36.3% of given dose) > urine (11.6%) > skin (8.3%) > faeces (6.6%) > blood (4.3%) > muscle (3%) > intestinal tissue (2.6%) > liver and gallbladder > stomach > kidney > brain > lung > spleen > heart (all ≤ 1.0%) 24 hours after administration of a non-diarrhetic dose (50 µg/kg bw) [11]. The distribution was similar in animals given a dose that did cause diarrhoea (90 µg/kg bw), although the OA content was significantly decreased in the stomach and significantly increased in the intestinal tissue and contents compared with animals given 50 µg/kg. The authors suggested that OA largely underwent enterohepatic circulation, which they also observed following intramuscular injection[12].

12. In a later study, OA was detected in the lungs, liver, heart, kidney, stomach and small and large intestines of male ICR mice within 5 minutes of administration of 150 µg OA/kg bw by oral gavage[13]. The site of absorption was reported to be the jejunum. OA continued to be detected in the heart, lung, liver, kidney and blood vessels for 2 weeks following administration. Excretion in

urine and from the cecum and large intestine started 5 minutes after administration, and continued via the intestinal contents for 4 weeks.

13. OA has been shown to cross the placental barrier to the fetus following oral administration to pregnant Swiss-Webster mice on day 11 of gestation[14].

14. In humans, faecal samples collected from individuals who developed DSP symptoms following consumption of shellfish contaminated with DTX-3 were found to contain DTX-1[5]. No DTX-3 was detected in the faecal samples, indicating complete transformation into DTX-1 within the body. This transformation was hypothesised to have taken place in the stomach.

Acute toxicity

15. The lethal dose of OA, DTX-1 and DTX-3 following intraperitoneal (i.p.) injection in mice is reported to be 200, 160 and ca. 500 µg/kg bw, respectively[15]. An i.p. lethal dose of 250 µg/kg bw has also been reported for DTX-3[16]. A recent study reported an LD_{50} for DTX-2 of approximately 350 µg/kg bw[17].

16. Estimates of the oral lethality of OA in mice vary considerably. Ito *et al.* reported a lethal oral dose of 400 µg/kg bw[13], while in another study no animals died following oral administration at 1,000 µg/kg bw and 4/5 died after administration of 2,000 µg/kg bw[18]. In a further study inconsistent results were observed, with mortality occurring in all mice given oral doses of 770 µg/kg bw and higher and in animals given 575 µg/kg bw, but not in those administered 610 µg/kg bw or doses of 525 µg/kg bw or lower[19].

17. Following oral administration of DTX-1 to male ddY mice at doses of 100, 200, 300 and 400 µg/kg bw, 1/5, 0/5, 2/4 and 3/4 animals died, respectively[20]. At the higher doses, mice died within 6 hours of dosing, while survival time was 30 hours at 100 µg/kg bw.

18. Diarrhetic effects of OA, DTX-1 and DTX-3 have been reported in several studies with suckling mice [20,21,22]. Intestinal fluid induction has also been reported following administration of single oral doses of 75 µg OA/kg bw and above to 4-week old mice[13], and in adult mice following an oral dose of 90, but not 50 µg OA/kg bw[11]. OA, DTX-1 and DTX-3 have all been found to induce severe diarrhoea in ICR mice and Wistar rats following oral administration at 750 µg/kg bw[23]. Research presented at a recent conference reported a lowest observed adverse effect level (LOAEL) and no observed adverse effect level (NOAEL) for intestinal fluid accumulation by OA following oral administration of 75 and 50 µg/kg bw respectively[24]. In rats, a LOAEL of 400 or 200 µg/kg bw was reported, depending on the vehicle used (saline or triolein-oil, respectively).

19. Damage to the absorptive epithelium of small intestinal villi has been reported in several studies following oral treatment of mice and rats with OA, DTX-1 and DTX-3 [23,25]. All toxins were reported to induce intestinal injury at 750 µg/kg bw, while it was also noted that the minimum dose of DTX-3 that induced collapse of the villous architecture in mice was 150 µg/kg bw. OA and DTX-1

also caused intestinal injury following i.p. administration (≥200 and 375 µg/kg bw, respectively), but DTX-3 only induced significant injury by the oral route. Damage was almost completely repaired within 48 hours. DTX-1 has also been shown to induce mucosal injuries in the small intestine within 1 hour of i.p. administration at doses ranging from 50-500 µg/kg bw[26]. No discernible changes in organs and tissues other than the intestine were observed in this study.

20. Terao *et al.*[23] also examined the effects of i.p. (375 µg/kg bw) and oral (750 µg/kg bw) administration of OA, DTX-1 and DTX-3 to mice and rats on the liver. Adverse effects were observed following administration of DTX-3 by the oral and i.p. route, whereas OA and DTX-1 only induced damage when given intraperitoneally.

21. In a further study, injuries were reported in the lung, stomach, small and large intestines and cecum, but not the liver, of male ICR mice following oral administration of 150 µg OA/kg bw[13]. Degenerative lesions to the small intestine, forestomach and liver have been reported in CD-1 mice following oral administration of OA at doses of 1,000 and 2,000 µg/kg bw, while slight splenic atrophy was also observed at the higher dose[18]. In a recent study, apoptosis was detected in the liver, ileum and kidney of Swiss mice at various time points between 24 and 48 hours after oral administration of 115 and 230 µg OA/kg bw[19].

22. Atrophy and structural alteration of the thymus has been reported in mice 24 hours following consumption of shellfish tissue contaminated with OA or OA and YTX over a 24 hour period (estimated intakes of approximately 18 µg OA and 1.4 µg YTX/kg bw)[27]. Histopathological changes were also observed in the spleen immediately following consumption of contaminated shellfish, but these effects were less marked 24 hours following consumption.

23. A summary of acute toxicity studies is included in table 1.

Mechanism of action

24. OA is a potent inhibitor of the serine-threonine protein phosphatases (PP) 1 and 2A, and the adverse effects of OA group toxins are considered to be mediated by this activity. It has been proposed that OA may induce diarrhoea by stimulating the phosphorylation of proteins that control sodium secretion by intestinal cells, or by enhancing phosphorylation of cytoskeletal or junctional elements resulting in increased permeability to solutes, leading to passive loss of fluids[28].

Table 1. Acute toxicity studies with OA group toxins

Toxin	Species	Parameter	Route of administ-ration	Dose (µg/kg bw)	NOAEL (µg/kg bw)	Comments
OA	Mouse	Lethality	i.p.	200[15]	ND	
OA	Mouse	Lethality	Oral	400-2000[13,18,19]	ND	Varying estimates reported
OA	Mouse	Diarrhoea	Oral	75-750[11,13,23,24]	50[24]	
OA	Rat	Diarrhoea	Oral	200-400[24]	ND	LOAEL varied depending on vehicle of administration
OA	Mouse	Intestinal injury	i.p.	200[25]	ND	
OA	Rat	Intestinal injury	i.p.	375[23]	ND	
OA	Mouse	Intestinal injury	Oral	150-2000[13,23,25,18]	ND	
OA	Rat	Intestinal injury	Oral	750[23]	ND	
OA	Mouse + Rat	Liver injury	i.p.	375[23]		
OA	Mouse	Liver injury	Oral	115-2000[19,18]	750 (mouse + rat)[23]	Apoptosis: 115-1300 Degenerative lesions: 1000-2000
DTX-1	Mouse	Lethality	i.p.	160[15]	ND	
DTX-1	Mouse	Lethality	Oral	100-400[20]	ND	
DTX-1	Mouse	Diarrhoea	Oral	750[23]	ND	
DTX-1	Mouse	Intestinal injury	i.p.	50-500[26,23,25]	ND	Injury observed in mice and rats at 350 µg/kg bw
DTX-1	Mouse + Rat	Intestinal injury	Oral	750[23,25]	ND	
DTX-1	Mouse + Rat	Liver injury	i.p.	375[23]	ND	
DTX-1	Mouse + Rat	Liver injury	Oral		750[23]	
DTX-2	Mouse	Lethality (LD_{50})	i.p.	350[17]	ND	
DTX-3	Mouse	Lethality	i.p.	250-500[15,16]	ND	
DTX-3	Mouse	Diarrhoea	Oral	750[23]	ND	
DTX-3	Mouse + Rat	Intestinal injury	i.p.	375[23,25]	ND	Only minor injury observed with i.p. administration
DTX-3	Mouse	Intestinal injury	Oral	150[25]	ND	
DTX-3	Rat	Intestinal injury	Oral	750[23]	ND	
DTX-3	Mouse + Rat	Liver injury	i.p.	375[23]	ND	
DTX-3	Mouse + Rat	Liver injury	Oral	750[23]	ND	

ND: Not determine

Genotoxicity

25. OA did not induce mutations in the *Salmonella typhimurium* strains TA100 and TA98 in the presence or absence of metabolic activation, but was mutagenic in Chinese hamster lung cells without activation, using diphtheria toxin resistance as a marker[29].

26. OA has been reported to induce DNA adducts with no clear concentration-response relationship using the ^{32}P-postlabelling technique in BHK21 C13 fibroblasts and HESV keratinocytes[30]. OA was negative in the Chinese hamster ovary cell hprt mutation assay conducted to OECD guidelines (with and without metabolic activation) and an in vitro unscheduled DNA synthesis (UDS) assay in rat hepatocytes[31].

27. Using the cytokinesis-block micronucleus assay coupled to fluorescence in situ hybridization (FISH), OA has been shown to induce aneuploidy in CHO-K1 cells in the presence and absence of rat liver S9[32,33,34]. The authors suggested that this effect was due to inhibition of chromosome attachment to the mitotic spindle, possibly related to the effects of OA on protein phosphatases. Induction of micronuclei by OA has been reported in Caco-2 human colon cells at doses sufficient to induce apoptosis, but this was not confirmed in colon epithelial cells of mice given OA by oral gavage[19].

28. Overall, the data show some evidence for genotoxicity in vitro in non-standard assays, including evidence for DNA adduct formation in mammalian cell lines which are difficult to interpret, and thus it is noted some effects may be related to the toxicity of OA in the in vitro assays. There is evidence for aneugenicity in vitro in a mammalian cell line which is unlikely to be related to a direct effect of OA on DNA. Standard bacterial reversion, mammalian gene mutation assays and a UDS assay in rat hepatocytes were negative. The in vivo relevance of the positive in vitro findings is unclear and has not been investigated.

Tumour promoting activity

29. OA and DTX-1 have been shown to act as tumour promoters on mouse skin initiated with 7,12-dimethylbenz[a]anthracene (DMBA)[35,36]. In these studies 100 µg DMBA was applied once to mouse skin, followed by twice weekly application of OA (5 and 10 µg) or DTX-1 (5 µg) for 30 weeks. Administration of OA via drinking water has been found to promote tumour formation in the glandular stomach of rats initiated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water for the first 8 weeks of the study (OA administered at 10 µg/rat/day on weeks 9-55 followed by 20 µg/rat/day on weeks 56-72)[37]. In this study, the percentage of rats treated with MNNG and OA, MNNG alone or OA alone bearing neoplastic changes was 75, 46 and 0%, respectively.

30. The tumour promoting activity of OA and DTX-1 is considered to be mediated by inhibition of the protein phosphatases (PP)1 and PP2A, which results in increased protein phosphorylation leading to alterations in gene

expression. OA and DTX-1 have been found to induce ornithine decarboxylase activity in mouse skin, with OA also inducing this enzyme in the rat glandular stomach [35,36,37]. In addition, OA has been shown to induce tumour necrosis factor (TNF)-α gene expression in mouse skin[38] and synthesis and secretion of the murine cytokine CXCl1/KC, a promoter of tumour growth, in JB6 cells [39].

31. No data are available on reproductive toxicity or developmental toxicity.

Human data

32. DSP incidents have been reported in many countries around the world, including Japan[40], the Netherlands[41,42], Norway[43,6], Sweden[44], Belgium[45], Portugal[46,47], the UK[48], Canada[49], Chile[50,5] and New Zealand[51].

33. The predominant symptoms of DSP are diarrhoea, nausea, vomiting and abdominal pain. Symptoms are generally reported to occur between 30 minutes and a few hours following shellfish consumption, with patients recovering within 2-3 days.

Epidemiology data

34. Information provided in the majority of reports of DSP outbreaks is very limited. Many do not provide information on the amount of contaminated shellfish consumed by affected individuals, and where exposure assessments are reported, little information is given on how these estimates have been derived. There is also a potential for uncertainty due to disparities in toxin levels in tested shellfish compared with levels present in shellfish that were actually consumed. Limited information is available on the effects of cooking on levels of OA toxins in shellfish, however it is generally considered that cooking does not reduce the levels of these toxins in shellfish due to their chemical stability and lipophilicity[52]. Although many incidents of DSP have been reported globally, this paper focuses predominantly on reports where assessment of toxin intake has been conducted.

35. In several older human case reports, toxin levels in contaminated shellfish have been determined by mouse bioassay (MBA), involving i.p. injection in mice. MBA results are generally reported as either mouse units (MU) or OA equivalents (eq). For OA and DTXs, one MU was defined in these reports as either the minimum amount of toxin required to kill two of three 20 g mice within 24 hours following i.p. injection, or as the amount of toxin required to kill one mouse within 24 hours of i.p. injection. One MU is reported as corresponding to approximately 4 µg OA and 3.2 µg DTX-1, based upon the lethal dose of these toxins following i.p. injection in mice and assuming a 20 g mouse (200 and 160 µg/kg bw, respectively)[53,54]. For DTX-3, 1 MU has been reported as 5 µg, based upon the reported i.p. toxicity in mice of 250 µg/kg bw[55,16].

36. In June and July of 1976 and 1977, a total of 164 individuals in Japan were reported to have developed diarrhoea, nausea, vomiting and abdominal

pain following consumption of mussels or scallops[40]. Symptoms occurred between 30 minutes and a few hours following shellfish consumption, with time to onset rarely exceeding 12 hours. Mussels implicated in these incidents were found to contain a toxin, at that time unidentified, that killed mice following i.p. injection.

37. To assess the levels of toxin associated with human illness, three leftover mussel specimens from meals eaten by eight individuals who became ill in 1977 were tested by MBA. Toxin levels were quantified as MU/hepatopancreas, with a definition of 1 MU being the amount of toxin required to kill a mouse in 24 hours following i.p. administration. Details of shellfish consumption, severity of illness and toxin levels within the consumed shellfish are summarised in table 2.

Table 2. Comparison of human illness with toxin intake[40]

Individual (Age, Sex)	No. Mussels eaten	Toxicity (MU)		Symptom severity
		per g hepatopancreas	Total consumed*	
A (40, F)	3	5.0	12	Mild
B (15, M)	3	5.0	12	Mild
C (45, M)	5	5.0	20	Severe
D (10, M)	5	5.0	20	Severe
E (56, M)	10	5.0	40	Severe
F (52, F)	5	8.5	35	Severe
G (53, M)	10	8.5	70	Severe
H (68, M)	6	4.0	19	Severe

* Toxin intake estimated by study authors based upon an average weight of 0.8 g hepatopancreas per mussel

38. Mild symptoms were experienced by two of the affected individuals; nausea and mild diarrhoea was reported by a female aged 40 years while a male aged 15 years vomited but did not suffer from diarrhoea. The two individuals who experienced mild symptoms had both consumed 3 mussels containing 5 MU of toxin/g hepatopancreas. Assuming an average hepatopancreas weight of 0.8 g/mussel, the authors concluded that 12 MU of toxin was sufficient to induce mild illness in humans. This would correspond to an estimated 48 µg OA or 38 µg DTX-1, assuming that one MU is equivalent to 4 µg OA or 3.2 µg DTX-1. The other six patients, aged between 10 and 68 years, developed severe symptoms following an estimated intake ranging from 19-70 MU per person (corresponding to 76-280 µg OA or 60-224 µg DTX-1). The toxin involved in this incident was subsequently identified as DTX-1[56].

39. In October 1984, cases of gastrointestinal illness developing after consumption of mussels were reported simultaneously in Sweden and Norway[43]. In Norway, cases continued to be reported until April 1985. Mussels described as being ‘associated with cases of intoxication’ were collected and analysed by MBA. Further details on where these samples were obtained are

not reported. In addition, fortnightly sampling took place at three localities on the south-east coast of Norway and south-east coast of Sweden from November 1984 – April 1985.

40. Mussels associated with DSP cases in Norway were found to be 'slightly to highly toxic'[43]. It was also noted that some samples contained 1.5-2 MU of toxin per g of hepatopancreas but it was not specified whether these were samples associated with illness or samples collected from Norway or Sweden in the months following the incident. The amount of mussel meat consumed by affected individuals was reported to range from approximately 30-200 g, and it was suggested that an intake of between 10-15 MU (equivalent to 40-60 µg OA) caused gastrointestinal symptoms[43]. In a separate report, samples involved in the Swedish DSP cases were reported to contain DSP toxins at a concentration greater than 17 MU/100 g shellfish flesh[44], corresponding to 68 µg OA or 53 µg DTX-1/100 g.

41. At an opening ceremony of a new mussel farm in Norway, the 77 guests were served dishes containing blue mussels, and many subsequently developed DSP symptoms[2]. In total, 72 individuals were interviewed by the local Food Control Authority the following day, 39 of whom reported nausea, vomiting, stomach pain, diarrhoea and headache. Analysis of leftover mussels by high performance liquid chromatograpy (HPLC) without hydrolysis of DTX-3 (OA esters) indicated that they contained 55-65 µg OAeq/100 g mussel meat. No precise information on the amount of mussels consumed was available, but a crude estimate of 1-1.5 µg OA eq/kg bw was suggested, based on general information about consumption of blue mussels among Norwegians[57].

42. Several DSP incidents have been reported in the UK. In 1994, two patients developed DSP symptoms 1-2 hours after eating imported mussels[58]. Symptoms persisted for up to 36 hours. Uneaten mussels were tested by MBA and high performance liquid chromatograpy (HPLC), and HPLC confirmed the presence of OA at a concentration of 2030 µg/100 g shellfish flesh. Both individuals were reported to have each consumed 10 mussels weighing approximately 200 g in total including shells[48]. Estimates of meat yields vary, but information provided to the Food Safety Authority of Ireland by Bantry Bay Foods Ltd suggests that in a kg of mussels there are 80-105 mussels that yield between 180 g and 240 g of cooked meat[59], i.e. a meat yield of 18-24%. McCance and Widdowson's 'The Composition of Foods' reports a 30% tissue yield for boiled mussels[60], while an FAO review gives an average edible tissue yield for raw mussels of 24%[61]. Assuming a tissue yield of 25% in the absence of specific data, the individuals may be estimated to have eaten 50 g of mussels providing an OA intake of 1015 µg per person. It is notable that the toxin intake in this incident was considerably higher than that in the other DSP case reports.

43. In June 1997, 49 patients presented with acute onset (within 30 minutes) of DSP symptoms which persisted for more than 8 hours[62]. All individuals had eaten UK-harvested mussels at one of two London restaurants. No pathogenic bacteria or viruses were detected in stool samples taken from some of the patients. HPLC analysis of mussel samples indicated the presence of OA, at concentrations ranging between 25.3 and 36.7 µg/100 g shellfish flesh[48]. No

details on the amount of mussels consumed by the affected individuals were reported, although the authors noted that one patient who only developed diarrhoea that lasted for 8 hours had eaten mussel soup. They suggested that this meal may have contained less OA than other dishes.

44. Most recently, a DSP outbreak occurred in June 2006, involving approximately 159 individuals who ate mussels at a chain of restaurants in London. The majority of individuals became ill within 2-12 hours of eating mussels. A report from one restaurant indicates that 407, 242, 265, 239 and 297 mussel dishes were sold on the 17th, 18th, 19th, 20th and 21st of June, with 16 (4%), 25 (10%), 2 (1%), 4 (2%), and 25 (8%) people reporting DSP symptoms, respectively. Three samples obtained from the supplier that had been harvested on 14, 15 and 19 June and served in the restaurants were tested for the presence of norovirus and DSP toxins. Norovirus genogroups I and II were not detected in any sample.

45. The samples harvested on 15 and 19 June tested positive by MBA (indicating the presence of DSP toxins at a concentration > 16 µg/100 g shellfish flesh), while the sample collected on 14 June was negative. However, further analysis of the samples by liquid chromatography-mass spectrometry (LC-MS) indicated that all three samples contained OA and OA esters ('DTX-3'), while one sample also contained DTX-1 and DTX-1 esters. The total concentration of OA or OA and DTX-1, following hydrolysis of the samples to convert the esters to their parent compound, was 25.8, 26.5 and 30.2 µg/100 g shellfish flesh in the samples harvested on 14, 15 and 19 June, respectively, i.e. about 70% above the regulatory limit of 16 µg/100 g. These samples were also found to contain PTXs. Analytical results for these samples are summarised in table 3.

Table 3. OA group and PTX concentrations in mussels associated with June 2006 DSP outbreak.

Mussel sample	Date sample collected	MBA+ result	LC-MS results (µg/100 g shellfish meat)			
			OA/DTX	PTX-2	PTX-2 seco acid	7-epi PTX-2 seco acid
BTX/2006/936	19.06.06	+ve	30.2*	43.3	25.6	3.1
BTX/2006/937	15.06.06	+ve	26.5**	51.3	40.3	6.4
BTX/2006/938	14.06.06	-ve	25.8**	30.2	35.4	4.7

+ MBA analysis performed with a 5h observation period rather than the 24h required by EU legislation.

* Concentration of OA and DTX-1 following hydrolysis of shellfish extract (no evidence of DTX-2 or DTX-2 esters).

** Concentration of OA following hydrolysis of shellfish extract (no evidence of DTX-1, DTX-2 or their esters).

46. Information on the exact amount of mussels consumed is not available, although it is known that mussels were served at the restaurants in portion sizes

of 500 g and 1 kg including shells. Information from the suppliers of the mussels indicates that the meat yields of the affected batches were 28-30%. Assuming a meat yield of 29% and an OA/DTX concentration of 27.5 µg/100 g shellfish flesh (the average of the 3 values), it is possible that individuals who ate a 500 g portion of mussels may have consumed 145 g of mussels, providing a toxin intake of about 40 µg. Individuals consuming a 1 kg portion of mussels may have eaten 290 g of mussels, providing a toxin intake of about 80 µg. As noted above, these samples also contained PTXs and it is uncertain whether these toxins may have also contributed to illness.

47. In 1998, a DSP outbreak associated with OA esters (DTX-3) was reported in Portugal[46]. In total 18 individuals developed symptoms of DSP after eating *Donax* clams. The severity of symptoms was reported to be related to the amount of clams eaten, with those who ate little experiencing mild symptoms and those who ate 500 g presenting with the most serious symptoms. A sample of these clams was sent for analysis for bacteria and DSP toxins. *Salmonella spp.* were not detected in the clams, and HPLC analysis detected only low levels of OA (10 µg/100 g shellfish meat). However, following alkaline hydrolysis of the extract to release fatty acids from the esters, 130 µg OA/100 g shellfish meat was detected. Noting that the yield of edible tissue from *Donax* clams is approximately 18-20%, the authors calculated that individuals who consumed a 500 g portion would be expected to have eaten 90-100 g of tissue, suggesting a toxin intake of 117-130 µg OA eq per person.

48. A further DSP incident associated with OA esters occurred in Portugal in 2001[9]. Six individuals reported DSP symptoms after eating razor clams and clams that had been obtained locally. In total, 2 kg of razor clams and an unspecified amount of clams had been obtained by the affected individuals. Symptom severity appeared to be related to the amount of shellfish that had been eaten, and took 3 days to resolve in the most severe cases. LC-MS analysis of razor clams harvested the day after the affected individuals had become ill indicated the presence of OA at a concentration of 1 µg/100 g shellfish meat. Following hydrolysis of ester forms, 50 µg OA/100 g shellfish meat was detected.

49. Noting that the edible tissue yield of razor clams is 60%, giving a total edible mass of 1.2 kg from the 2 kg collected by the patients, the authors of the report hypothesised that individuals who reported eating ‘a lot’, ‘little’ or ‘very little’ may have eaten around 350 g, 150 g or 50 g, respectively. On this basis, it was estimated that the respective toxin intakes may have been 175, 75 or 25 µg OA eq per person. Low levels of domoic acid (DA; 490 µg/100 g), the biotoxin associated with amnesic shellfish poisoning (ASP), were also detected in razor clams harvested commercially at the same period. Although mild symptoms of ASP are similar to those of DSP, it was noted that the level was substantially lower than the regulatory limit of 2000 µg/100 g shellfish flesh and that DA was unlikely to have contributed to the poisoning.

50. In the same month, an individual living in the region developed DSP symptoms after eating green crabs harvested locally. Symptoms started 2-3 hours following ingestion and persisted over 3 days. Leftover crabs from the

meal were frozen and analysed by LC-MS 1.5 months later, and found to contain 32.2 µg OA equivalents following hydrolysis. It was estimated that around 30 crabs, consisting of approximately 140 g edible tissue, may have been consumed, which would correspond to an intake of around 45 µg OA eq. Very low levels of DA were detected in the cooked crab sample (40 µg/100 g), but were again not considered to have contributed to the poisoning. Noting the delay between sampling of the crab and analysis, and that OA esters are considered to be quite unstable, the authors suggested that the OA intake may have been underestimated in this case.

51. In 2002, several hundred people became ill after eating self-harvested brown crabs in southern Norway[6]. The symptoms were reported to be typical of DSP, although less severe and with a delayed onset. As no leftovers from any meals were available for analysis, fresh crabs from shallow waters in the same area as the original crabs had been harvested were collected and boiled prior to LC-MS analysis. Crabs move freely from area to area, and it is therefore uncertain how representative the crabs collected by the researchers are to those consumed by affected individuals. No DSP toxins were detected upon initial analysis, but analysis following alkaline hydrolysis indicated the presence of 29 µg OA/100 g and 2 µg DTX-2/100 g. Details of the amount of crabs eaten by affected individuals are not reported, but the report notes that a risk assessment was undertaken on behalf of the Norwegian Food Control Authority, resulting in the establishment of a temporary regulatory limit of 40 µg DTX-3 as OA eq/100 g brown meat in crabs. This limit was set on the basis of the analytical results and reports of illnesses available from the outbreak in 2002, indicating that 75-150 µg DTX-3 as OA eq per person would result in illness, and that an estimated average consumption of 2-3 crabs weighing 500 g at the level of 40 µg OA eq/100 g brown meat would give an intake of 28-42 µg DTX-3 as OA eq. Full details of this risk assessment have been submitted for publication, but are not currently available.

52. A summary of the estimated toxin intakes associated with DSP symptoms is provided in table 4.

53. In view of the tumour promoting effects of OA and DTX-1 in animal studies, researchers in France attempted to assess whether there may be a link between cancer risk and exposure to DSP toxins in humans[63]. Hypothesising that residual levels of OA may be present in shellfish harvested from beds recently re-opened following a contamination episode, the authors assessed mortality rates in coastal areas that had low, medium or high rates of harvesting bed closures for DSP toxin contamination and in areas where no closures had occurred. The authors considered their findings may suggest a possible association between living in areas with a high rate of closures and some digestive cancers, but acknowledged the large number of assumptions that had been made in the study. For example, consumption rates of locally harvested shellfish were not assessed, and it was not possible to confirm whether harvesting bed closure rates actually related to OA toxin exposure. Adjustment for confounding had also not been conducted, apart from for the presence of liver cirrhosis in men as a proxy for alcohol consumption.

Table 4 Summary of DSP epidemiology data

Cases	Reported concentration of OA group toxins in shellfish	Reported intake of OA group toxins	Derived dose calculated as µg OA eq/kg bw*	Assumptions
8 cases, 6 males and 2 females [40]	4-8.5 MU/g hepatopancreas	Mild symptoms: 12 MU/person Severe symptoms: 19-70 MU/person	Mild symptoms: 0.8 Severe symptoms: 1.3-4.7	Toxin intake calculated from reported number of mussels consumed and MBA result of toxin concentration in hepatopancreas of three leftover mussel samples. Average hepatopancreas weight of 0.8 g/mussel assumed. Estimated doses calculated assuming a bodyweight of 60 kg and that 1MU = 4 µg OA
Several hundred individuals[43]	Samples associated with cases of illness reported to be 'slightly to highly toxic' Samples of unspecified source contained 1.5-2 MU/g hepatopancreas	10-15 MU/person	0.7-1	Mussels associated with cases of illness analysed by MBA. Samples from an unspecified source contained 1.5-2 MU/g hepatopancreas. Amount of mussel meat consumed by affected individuals reported as 30-200 g. Value of 10-15 MU estimated by authors without precise basis for this reported. Estimated dose calculated assuming a bodyweight of 60 kg and that 1MU = 4 µg OA
2 cases, 1 male + 1 female[48]	2030 µg OA per 100 g shellfish		17	Both reported to have consumed 10 mussels weighing 200 g. Estimated dose calculated assuming a body weight of 60kg and assumption of 25% edible tissue yield from mussels. Toxin concentration derived by LC-MS.
49 patients[48]	25.3-36.7 µg/100 g shellfish flesh			No details available on amount of shellfish consumed by affected individuals.
18 cases[46]	130 µg OA eq/100 g shellfish flesh	Severe symptoms: 117-130 µg OA eq/person Mild symptoms: 'ate little shellfish'	Severe symptoms: 1.95-2.2 Mild symptoms: unknown	Individuals who ate 500 g clams reported to have most severe symptoms. Those with mild symptoms reported to have eaten 'little'. Authors estimated edible tissue proportion of *Donax* clams as being 18-20% of whole shellfish), suggesting consumption of 90-100 g edible shellfish in those who ate a 500 g portion. OA esters reported to be present in leftover shellfish tissue (HPLC data). Estimated dose calculated assuming a body weight of 60kg.

6 cases following consumption of razor clams; one case following consumption of crabs[9]	Razor clams: 50 µg OAeq/100 g shellfish flesh Crabs: 32.2 µg OAeq/100 g shellfish flesh	Razor clams: Symptom severity of +++++: 175 µg OA eq/person Symptom severity of +++: 75 µg OA eq/person Symptom severity of +: 25 µg OA eq/person Crabs: 45 µg OA eq/person	Razor clams: Symptom severity of +++++: 2.9 Symptom severity of +++: 1.3 Symptom severity of +: 0.4 Crabs: 0.75	Razor clams Toxin concentration measured by LC-MS in razor clams collected day after individuals became sick. Shellfish contained OA and OA esters. Authors estimated individuals eating 'a lot', 'little' or 'very little' clams consumed 350, 150 or 50 g respectively. Estimated doses calculated assuming a body weight of 60kg. Crabs: Toxins determined in leftover crabs that had been frozen for 1.5 months. OA esters present and authors suggest these toxins are unstable and some may have degraded during shellfish storage. Authors estimated individual may have eaten around 30 crabs containing around 140 g edible parts.
Several hundred individuals[6]		75-150 µg OA eq/person	1.25-2.5	Anecdotal mention in paper of risk assessment suggesting individuals became ill following an intake of 75-150 µg OA esters as OA eq/person. Risk assessment in press and currently unavailable. Estimated doses calculated assuming a body weight of 60kg.
39 cases from 77 individuals served mussels[2,57]	55-65 µg OAeq/100 g shellfish flesh		1.0-1.5	Information on amount of mussels consumed unavailable, but dose estimated on basis of general information about blue mussel consumption by Norwegians.
159 individuals	25.8-30.2 µg OA or OA+DTX-1/100 g shellfish flesh		0.7 (500 g portion size) 1.3 (1 kg portion size)	Toxin concentration of mussels supplied to the restaurant determined by LC-MS. Amount of mussels consumed unknown although it is known that restaurant served 500g and 1 kg portions. Yield of edible tissue reported as 28-30% of the affected batches, 29% used in estimation. Estimated dose calculated assuming a 60 kg bodyweight, and a toxin concentration of 27.5 µg/100 g shellfish meat, based on the average of the toxin concentration determined in 3 samples implicated in the incident.

*Derived okadaic acid group doses (µg OA eq/kg bw) estimated by COT based on reported intake data, where data on doses were not included in the original paper

Previous Risk Assessments

54. Three risk assessments of marine biotoxins, including those of the OA group, have been conducted by international bodies in recent years. Details of these assessments are summarised below.

55. A European Commission (EC) Working Group on Toxicology of DSP and AZA poisoning[52] based its risk assessment of OA group toxins on the data arising from the Japanese outbreak of 1976 and 1977 involving DTX-1 discussed in paragraphs 36-38 above[40]. The working group noted that 12 MU was the lowest observed adverse effect level (LOAEL) identified in this report, which would correspond to 48 µg OA eq, or 0.8 µg/kg bodyweight (bw) for a 60 kg individual. The working group applied a safety factor of 3 to this value to derive an allowance level of 0.27 µg/kg bw.

56. In 2004, a Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs was asked by the Codex Committee on Fish and Fishery Products (CCFFP) to perform risk assessments for a number of biotoxins that may be present in bivalve molluscs[64]. It was considered that the data from Japan indicated a LOAEL of 1.2-1.6 µg/kg bw, but no details were provided on what bodyweights were used to derive this value. The Expert Consultation also noted that in the outbreak in Norway described in paragraph 41[2] the affected individuals had an estimated toxin intake of 1.0-1.5 µg/kg bw.

57. FAO/IOC/WHO applied a safety factor of 3 to the LOAEL of 1.0 µg OA eq/kg bw to derive an acute reference dose (ARfD; i.e. the amount that can be ingested in a period of 24 hours or less without appreciable health risk) of 0.33 µg OA eq/kg bw. A safety factor of 3 was considered sufficient because of documentation of human cases including more than 40 persons and because DSP symptoms are readily reversible.

58. A further risk assessment was conducted by a Community Reference Laboratory on Marine Biotoxins Working Group on Toxicology in October 2005[65]. The Working Group's report is unreferenced, but notes that the LOAEL of OA in humans is 1 µg/kg bw. A safety factor of 3 was again applied to derive an ARfD of 0.33 µg OA eq/kg bw.

COT Evaluation

59. The Committee assessed the available epidemiology data on OA group toxins with a view to advising on an appropriate ARfD.

60. A large number of uncertainties were noted in the human data, including uncertainties in estimates of the amount of shellfish consumed by affected individuals, and potential disparities in toxin levels in tested shellfish compared with levels present in shellfish that were actually consumed. Reports generally provide only limited information on how exposure assessments have been calculated.

61. The COT considered that the totality of epidemiology data for OA toxins that was available prior to the recent UK DSP outbreak described in paragraphs 43-45 indicated a LOAEL of around 1 µg/kg bw. It was noted that the limited information from the 2006 UK incident may suggest a lower LOAEL of 0.7 µg/kg bw, based on a 60 kg bw. However, the data from this incident were difficult to interpret as the shellfish associated with the outbreak also contained biotoxins of the PTX group. It is uncertain whether the presence of the PTX toxins may have contributed to illness. In addition, two shellfish portion sizes had been sold at the restaurants involved in the incident, and it was not known which had been eaten by individuals who became ill. Had all individuals eaten the larger portion size, a LOAEL of 1.3 µg/kg bw would be indicated, in line with the earlier epidemiology data.

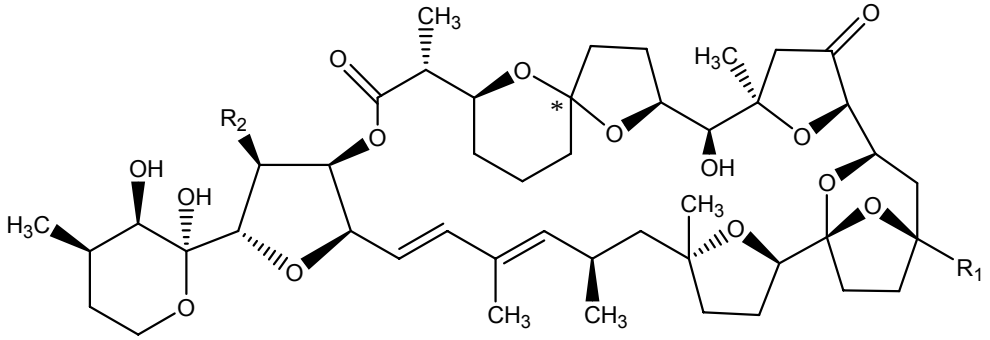
62. Overall, the Committee considered that 1 µg/kg bw should be viewed as the most appropriate LOAEL for deriving an ARfD for OA toxins. However, the 2006 UK DSP outbreak indicated a reported response rate of up to 10%, suggesting that more than the most susceptible minority were affected at this dose, and therefore that an uncertainty factor of 3 would not be sufficient for extrapolation from a LOAEL to a NOAEL due to potential human variability in susceptibility to the effects of these toxins. It was agreed that an uncertainty factor of 10 should be applied, resulting in an ARfD of 0.1 µg OA eq/kg bw.

Pectenotoxins

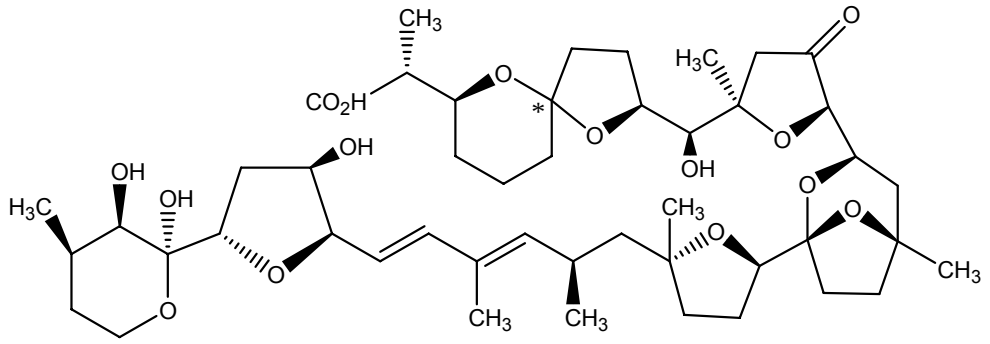
Background

63. The presence of pectenotoxins (PTXs) in shellfish was first discovered due to their high acute toxicity in mice following i.p. administration of lipophilic shellfish extracts[3]. More than 12 PTXs have been identified to date, and the structures of some of these toxins are shown in Figure 2.

Figure 2. Structures of PTXs[7,66]



Toxin	R1	R2	*C7
PTX-1	CH_2OH	H	*R*
PTX-2	CH_3	H	*R*
PTX-2b	CH_3	H	*S*
PTX-3	CHO	H	*R*
PTX-4	CH2OH	H	*S*
PTX-6	COOH	H	*R*
PTX-7	COOH	H	*S*
PTX-11	CH_3	OH	*R*
PTX-11b	CH_3	OH	*S*



Toxin	*C-7
PTX-2SA	*R*
7-epi-PTX2SA	*S*

64. PTXs exclusively arise from *Dinophysis spp.* which can also produce toxins from the OA group, and are therefore always accompanied by OA toxins[64]. This makes it difficult to assess the contribution of PTXs to human cases of DSP. It was previously suggested that PTX-2 seco acid (SA) and 7-epi-PTX-2 SA may have been responsible for outbreaks of human illness involving nausea, vomiting and diarrhoea in Australia in 1997 and 2000 following consumption of shellfish contaminated with these compounds[67]. However, the observed effects were later attributed to OA esters in the shellfish[64]. Mussels implicated in the recent DSP incident in the UK (paragraphs 43-45 above) also contained PTXs, and there is uncertainty as to whether these compounds may have contributed to the effects observed.

65. As noted in the OA group section, European legislation currently regulates for the presence of OA group toxins and PTXs together, but there have been recent proposals to regulate PTXs separately.

Toxicology

Toxicokinetics

66. The only data available on the toxicokinetics of PTXs are unpublished results from a PhD thesis summarised in the background document for the FAO/IOC/WHO evaluation of marine biotoxins[3]. Significant amounts of PTX-2 and PTX-2 SA were found in the gastrointestinal contents and faeces following oral administration, with only traces detected in tissue and urine. Following i.p. administration, PTX-2 and PTX-2 SA were detected in the blood and internal organs as well as in gastrointestinal contents and faeces. However, total recovery of toxins was reportedly low with both routes of administration.

Acute toxicity

67. Acute lethality data following i.p. administration of PTXs to mice are summarised in table 5. The minimum amount of PTX-2 reported to cause death in mice following p.o. administration is 25 µg/kg bw, with 1 of 4 mice dying[20]. However, the dose response in this study was unusual with no lethality observed in 4 mice given 100 µg/kg bw, while 1/5, 2/5, and 1/4 mice died following administration of 200, 300 and 400 µg/kg bw, respectively. In a subsequent study, no overt signs of toxicity were observed in mice following oral administration of PTX-2 or PTX-2 SA at doses up to 5000 µg/kg bw[68]. A recent study reported no signs of toxicity in mice given an oral dose of PTX-11 at 5000 µg/kg bw[66].

Table 5. Acute lethality of PTXs in mice following i.p. administration

Compound	Dose (µg/kg bw)
PTX-1	250[69]
PTX-2	230[69]
PTX-3	350[69]
PTX-4	770[69]
PTX-6	500[69]
PTX-7	>5000[70]
PTX-8	>5000[70]
PTX-9	>5000[70]
PTX-11	250[66]
PTX-2SA	No effects at 5000[71]
7-*epi*-PTX-2-SA	No effects at 5000[72]

68. Unlike OA group toxins, PTXs are not thought to inhibit protein phosphatases, and the potential for PTXs to induce diarrhoea has been a matter of some debate. In a study by Ishige *et al.* diarrhoea was observed in mice following oral administration of PTX-2 at doses of 1000 (1/5), 2000 (2/5) and 2500 (2/5) µg/kg bw, while at a lower dose of 250 µg/kg bw the small intestine was swollen and filled with fluid[52,73]. Vacuole formation was observed in the epithelial cells of the small intestine. In contrast, PTX-1 did not induce diarrhoea in suckling CD-1 mice following oral administration at doses up to 2 µg/mouse[21], or following i.p. administration to suckling BALB/c mice at doses from 150-1000 µg/kg bw, respectively[26]. In addition, no diarrhetic effects were observed in mice following oral administration of PTX-2 or PTX-2 SA at 5000 µg/kg bw[74], oral or i.p. dosing with PTX-11 at 5000 µg/kg bw or i.p. injection with PTX-2 SA[66] or 7-*epi*-PTX-2 SA at doses of 5000 µg/kg bw[72].

69. More recently however, a poster presented at the 12th International Conference on Harmful Algal Blooms reported that oral administration of PTX-2 resulted in intestinal fluid accumulation in mice at doses of 400 µg/kg bw and above[24]. Tissue damage was observed in the small intestine, characterised by vacuole formation in epithelial cells. No effects were observed at 300 µg/kg bw. Intestinal fluid accumulation was also observed in rats following administration of PTX-2 with a LOAEL of 300 or 400 µg/kg bw when administered in 2% lecithin water or in saline, respectively. Notably, no effect was observed when PTX-2 or OA were administered separately to mice at a concentration of 300 µg/kg bw or 50 µg/kg bw, respectively, whereas fluid accumulation was observed when the compounds were given together.

70. Reports of hepatotoxic effects of PTXs are also conflicting. Formation of non-fatty vacuoles, congestion and the appearance of granules were observed in mouse liver following i.p. administration of PTX-1 at doses of 150-1000 µg/kg bw[26]. Only slight injuries were seen in mice given 150 or 200 µg/kg bw in this study. In a later study, the same authors reported formation of non-fatty vacuoles in the liver and swelling of small intestinal villi in mice given PTX-1 or PTX-2 (375 µg/kg bw) by i.p. administration, but no adverse effects following oral administration of PTX-1 and PTX-2 at 750 µg/kg bw[23]. Ishige *et al.*,

however, reported that oral administration of PTX-2 to mice at doses of 250-2000 µg/kg bw resulted in hyaline droplet formation and granular degeneration in hepatocytes[73]. Increased granularity in the liver and increased serum activities of alanine aminotransferase, aspartate aminotransferase and sorbitol dehydrogenase were reported in mice given an i.p. injection of 200 µg PTX-2/kg bw[75].

71. In contrast to the above studies, several other studies have found no signs of toxicity in the liver or other organs following i.p. administration of PTX-2 SA or 7-*epi*-PTX-2 SA or oral administration of PTX-2, PTX-2 SA or PTX-11 at 5000 µg/kg bw[71,72,66].

Previous Risk Assessments

72. The EC Working Group on Toxicology of DSP and AZP identified an oral LOAEL of 250 µg/kg bw for PTXs, based on the report of fluid accumulation and injuries to the small intestine and liver at this dose by Ishige *et al.* (see paragraphs 68 and 70). A safety factor of 1000 (to account for use of a LOAEL and inter- and intra-species extrapolation) was applied to this value to derive an ARfD of 0.25 µg/kg bw[52].

73. FAO/IOC/WHO (2004) considered that the database was not sufficient to establish an ARfD for PTXs. However, it was noted that estimated human exposures to PTXs, assuming a 60 kg bw, for Canada (0.6 µg/kg bw) and Norway (1.6 µg/kg bw) are more than 8300 and 3100 lower, respectively, than the oral dose of PTX-2 or PTX-2 SA at which no adverse effects were observed in studies by Miles et al (5000 µg/kg bw; see paragraphs 67, 68 and 71)[64].

74. The information recently generated relating to induction of intestinal fluid accumulation and intestinal tissue damage in mice following oral administration of PTX-2 (see paragraph 69) was made available to the CRLMB Working Group on Toxicology for their discussions in 2005. The Working Group were also informed that pathological changes were observed in the stomach, lungs, liver, kidneys and intestines at 1500 µg/kg bw in this research, although these data were not presented at the 2006 Harmful Algae conference. It was noted that this work indicated a no observable adverse effect level (NOAEL) of 300 µg/kg bw. A safety factor of 100 was applied to this value to derive an ARfD of 3 µg/kg bw[65]. At the CRLMB meeting, it was noted that the discrepancies in findings relating to diarrhoea are to be investigated further.

COT Evaluation

75. While noting the conflicting results reported for PTXs, the COT considered that the studies reporting adverse effects following oral administration should not be discounted.

76. The Committee considered that it was appropriate to take the lowest identified LOAEL of 250 µg/kg bw, and apply an uncertainty factor of 1000 to

derive an ARfD of 0.25 µg/kg bw for PTXs. An uncertainty factor of 1000 was selected to account for extrapolation from a LOAEL to a NOAEL and to allow for differences between species and human variability.

77. In the *in vivo* study reported at the 2006 Conference on Harmful Algae, diarrhetic effects were reported following oral administration of PTX-2 and OA together at doses that did not produce adverse effects when administered separately. While the available data are limited, it was noted that it may be prudent to consider the potential for combined effects of these toxin groups given that they are known to co-occur in shellfish. However, a conservative approach incorporating a large uncertainty factor (1000) had been taken in advising on an ARfD for PTXs, while the ARfD for OA was based on human data which may be expected to include incidents of consumption of shellfish containing both OA toxins and PTXs. Indeed, shellfish associated with the 2006 DSP outbreak in the UK contained toxins from both groups. This provided some reassurance that the proposed ARfDs for PTXs and OA toxins would offer adequate protection for the consumer.

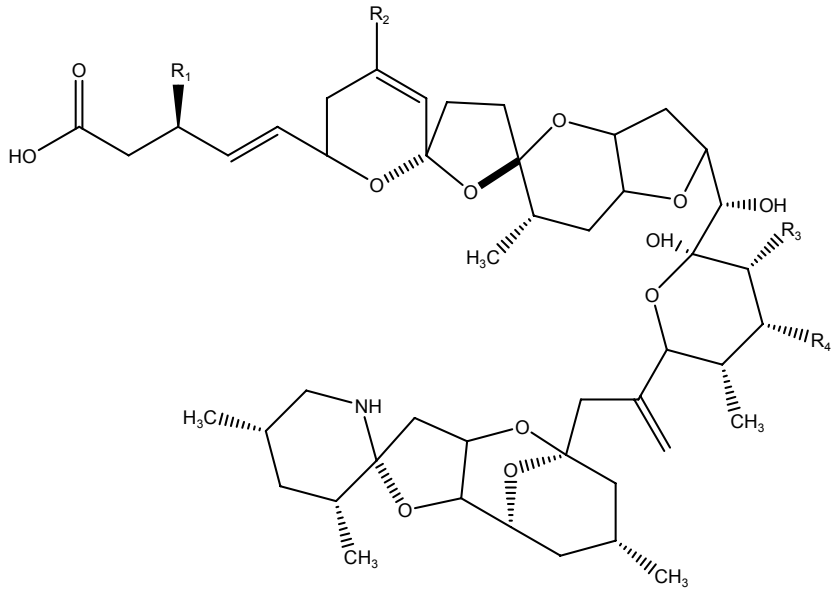
78. In view of the incomplete nature of the database for PTXs, the Committee recommended that the ARfD for these toxins should be reviewed when further data are available.

Azaspiracids

Background

79. Azaspiracids (AZAs) were first discovered in 1995 in Ireland, and have subsequently been reported in several other countries including the UK, Norway, France, Spain and Morocco. To date 11 different AZA congeners have been identified. The structures of AZAs-1 to -5 are shown in Figure 3.

Figure 3. Structures of AZAs-1 to -5[76]



Toxin	R_1	R_2	R_3	R_4
AZA-1	H	H	CH_3	H
AZA-2	H	CH_3	CH_3	H
AZA-3	H	H	H	H
AZA-4	OH	H	H	H
AZA-5	H	H	H	OH

80. Cases of human illness following consumption of shellfish contaminated with AZAs have been reported, and symptoms of AZA poisoning (AZP) are similar to those of DSP, including nausea, vomiting, severe diarrhoea and stomach cramps[77].

81. Current legislation prescribes a maximum permitted level for AZAs in shellfish of 16 µg/100 g shellfish flesh.

Toxicology

Toxicokinetics

82. No data have been reported on the toxicokinetics of AZAs.

Acute Toxicity

83. Lethal doses of AZA-1, -2, -3, -4 and 5 in mice following i.p. injection are summarised in table 6.

Table 6 Acute lethality of AZAs in mice following i.p. administration

Compound	Dose (µg/kg bw)
AZA-1	200[78]
AZA-2	110[79]
AZA-3	140[79]
AZA-4	470[76]
AZA-5	<1000[76]

84. The number of mice that died within 24 hours of a single oral dose of AZA-1 was 2/2 at 500 µg/kg bw (8 weeks old), 3/6 at 600 µg/kg (5 weeks old) and 1/2 at 700 µg/kg (5 weeks old)[80]. In a later study, the lowest lethal oral dose of AZA-1 was reported to be 250 µg/kg bw in 5-month old mice[81].

85. Following oral administration of AZA-1 at 300 µg/kg bw, adverse effects were seen in the small intestine of mice, including congestion, pooled watery substances in the lumen and necrosis of the lamina propria[80]. More severe effects were seen at doses of 600 and 700 µg/kg bw. Fatty changes were observed in the liver at doses of 300 µg/kg bw and higher, and necrotic lymphocytes were seen in the thymus, spleen and Peyer's patches at 500 µg/kg bw and above.

Repeat dose toxicity studies

86. Ito *et al.*[81] administered 2 oral doses of 300-450 µg AZA-1/kg bw to male ICR mice. Animals were treated on days 0 and 3, and surviving mice were sacrificed between days 7 and 90 to observe recovery of organ injuries. Slow recoveries from injuries were found, including erosion and shortened villi persisting in the stomach and small intestine for more than 3 months, oedema, bleeding, and infiltration of cells in the alveolar wall of the lung for 56 days. Fatty changes in the liver persisted for 20 days, and necrosis of lymphocytes in the thymus and spleen for 10 days.

87. In the same study, the cumulative effects of sublethal doses of AZA-1 were assessed. Mice received doses of 1, 5, 20 or 50 µg/kg bw AZA-1/kg bw p.o. twice weekly up to 40 times. At the higher doses, many mice became so weak they were sacrificed and subjected to autopsy before completion of 40 injections (9/10 at 50 µg/kg bw and 3/10 at 20 µg/kg bw). All these mice showed

interstitial pneumonia and shortened small intestinal villi in comparison with controls. Doses of 5 and 1 µg/kg bw did not cause death, even when administered 40 times. No signs of weakness or illness were observed in mice in these groups, but shortened intestinal villi were observed as with the animals given higher doses. Villi did not show full recovery 3 months after withdrawal of treatment. In the liver, focal necrosis, single cell necrosis, minor inflammation, mitosis and congestion were seen in a few mice, while 1/6 mice administered 1 µg/kg bw had a hyperplastic nodule 3 months following the chronic AZA treatment. Lung tumours were observed in four mice; one from the 50 µg/kg bw group after 32 injections and three from the 20 µg/kg bw group 2 and 3 months following completion of treatment. Tumours were not observed in mice treated with lower doses or in the control mice. Hyperplasia of epithelial cells was also observed in the stomach of 6/10 mice administered 20 µg/kg bw.

88. Brief details of a second, unpublished repeat-dose oral toxicity study are provided in a risk assessment of AZAs recently conducted by the Scientific Committee of the Food Safety Authority of Ireland (FSAI)[82] and in the background report for the FAO/IOC/WHO Expert Consultation[3]. In this study, groups of at least 10 mice were administered AZA-1 at doses of 5, 10 or 20 µg/kg bw once or twice weekly for 20 weeks. Surviving mice were then given doses ranging from 4-20 µg/kg bw for up to 1 year. The study also included 52 control mice. No tumours were observed among 66 mice sacrificed at 8 months, but 2 malignant lymphomas and 3 lung tumours were seen in the 20 remaining AZA-treated mice at 1 year. The dose at which all tumours occurred is not specified, although it is noted that three of the tumours again occurred at 20 µg/kg bw. The FSAI report notes that 9/126 (7%) mice treated in these two long-term studies developed tumours, compared with none of the control mice.

Reproductive toxicity

89. Microinjection of Japanese medaka finfish embryos with AZA-1 (≥ 40 pg AZA-1/egg) resulted in reduced somatic growth and yolk absorption within 4 days of exposure, as well as delayed onset of blood circulation and pigmentation[83]. Embryos had slower heart rates than controls for the 9 day *in ovo* period and reduced hatching success. These effects were dose-dependent, with failure to hatch occurring in approximately 50% of embryos exposed to ≥ 40 pg/egg, 90% of those exposed to 80-120 pg/egg, and all of the embryos injected with 120-160 pg/egg. Microinjection of a contaminated mussel extract containing AZA-1, -2 and –3, OA and DTX-2 resulted in similar responses.

Human data

90. In total, 5 AZP incidents have been reported in the Netherlands, Ireland, Italy, France and the UK since the identification of AZA in 1995, with all cases linked to the consumption of Irish shellfish prior to the introduction of a regulatory limit for AZAs in Europe in 2001[84]. Since the introduction of the regulatory limit, no cases of AZP have been reported despite evidence of two major incidents of AZA contamination of shellfish in this period.

Epidemiology data

91. No epidemiological details are available from the majority of these incidents, although limited information collected from an incident that occurred following consumption of mussels in Arranmore Island, Ireland in 1997[85] has been used in international risk assessments of AZAs.

92. Around 20-24 individuals are believed to have been affected in the Arranmore AZP incident, with 7-8 of these cases confirmed following consultation with a physician. Symptoms were reported as vomiting, diarrhoea and nausea, with all patients making a complete recovery after 2-5 days. There were no indications of any hepatotoxic effects and no individuals subsequently presented with illness that could be related to the initial poisoning. The lowest amount of mussels reported to have been consumed by an affected individual was 10-12.

Previous Risk Assessments

93. The Food Safety Authority of Ireland (FSAI) first performed a risk assessment of AZAs in shellfish in 2001[59]. This was largely based on the information obtained from the Arranmore incident, together with data on levels of AZAs present in the hepatopancreas of mussels collected from Arranmore in the months following the incident. Mussels were first collected 2 months after the incident and collections continued at regular intervals over the following 6 months. AZA levels were assessed by LC-MS.

94. Probabilistic modelling was applied to the data on AZA concentrations in mussels following the incident as well as data on variation in mussel tissue yields in order to estimate the likely AZA intake of the individual who became ill following consumption of 10-12 mussels. Evidence at this time suggested that AZA levels may be reduced by as much as 71% by cooking, and this information was also included in the assessment. Results of the modelling suggested that the AZA intake was likely to have been between 6.7 µg (5^{th} percentile) and 24.9 µg (95^{th} percentile).

95. The EC Working Group on Toxicology of DSP and AZP considered the FSAI risk assessment in the light of new data suggesting that AZA levels in shellfish are not reduced during cooking[52]. The range for the LOAEL was recalculated as being between 23 µg (5^{th} percentile) and 86 µg (95^{th} percentile). The Working Group applied a safety factor of 3 to these values to derive an ARfD within the range of 7.7 µg and 28.7 µg per person, or between 0.128 and 0.478 µg/kg bw assuming a 60 kg bw. The CRLMB Working Group on Toxicology also derived an ARfD of 0.128 µg/kg bw in its assessment of AZAs, based on the lower LOAEL of 23 µg/person[65].

96. FAO/IOC/WHO established a provisional ARfD for AZAs of 0.04 µg/kg bw, based on the lower LOAEL of 23 µg per person and a 60 kg bw, and applying a 10-fold safety factor to take into consideration the small number of people for whom data are available[64].

97. Most recently, the Scientific Committee of FSAI performed a re-evaluation of its 2001 risk assessment, in the light of relevant data published since 2001[82]. These data related to the tissue distribution of AZAs in mussels, the ratios of different AZAs in mussel tissue, and the influence of cooking on AZA concentrations within mussels.

98. Expert opinion on the relative proportions of AZAs in hepatopancreas versus whole flesh had been used in the 2001 FSAI risk assessment to calculate the likely concentration of AZA-1 in whole flesh, based on measurements in hepatopancreas. However, a recent publication reported a series of measurements of hepatopancreas:whole flesh ratios in 28 mussel samples collected in Ireland between 2001 and 2003[86]. These data were used to generate a cumulative distribution describing the variability of the measured ratios, which was then used to recalculate the range of estimates of AZA-1 levels in the whole flesh of mussels in the Arranmore incident. This indicated that AZA-1 levels within the mussels may have been higher than originally estimated, with an average estimate of 2 µg/g compared with the previous estimate of 1.3 µg/g.

99. The 2001 FSAI risk assessment used a single value for the proportion of AZA-2 and AZA-3 relative to AZA-1 likely to be present within contaminated mussels. Since this time, information from the 2005 Irish biotoxin monitoring programme has generated a range of 75 different proportions for AZA-2 and AZA-3 relative to AZA-1. These data were used in the 2006 risk assessment in order to provide what was considered to be a more accurate estimate of the total AZA concentration within the mussels associated with the Arranmore incident.

100. Recent data indicate that steaming of raw fresh mussels results in a 2-fold higher concentration of AZAs in the cooked flesh (whole flesh and hepatopancreas) compared with the uncooked flesh[86]. This was attributed to the loss of water/juice from the mussels. On this basis, it was considered appropriate to calculate mussel consumption by individuals during the Arranmore incident in terms of raw weight rather than having to account for the reduction in mussel meat weight during cooking (approximately 50%). However, it was acknowledged that a degree of uncertainty remains in this part of the exposure assessment due to a lack of knowledge on mussel meat weight in the Arranmore growing site in 1997.

101. The use of these new data in the probabilistic approach used by FSAI resulted in a substantially higher estimate of the AZA intake associated with AZP on Arranmore compared with previous assessments. The revised estimates of AZA intake associated with human illness were calculated to be between 50.1 µg (5^{th} percentile) and 253.3 (95^{th} percentile) per person.

102. The FSAI Scientific Committee applied a safety factor of 3 to the median AZA intake estimate associated with AZP (113.41 µg per person) to derive an ARfD of 0.63 µg/kg bw, assuming a 60 kg bw. This safety factor was applied to account for possible intra-species variation in the toxicodynamic effects of

AZAs. It was considered that a further safety factor was not required for intra-species variation in toxicokinetics, due to an absence of clear evidence for metabolism resulting in a more toxic compound. It was also suggested that metabolic activation is unlikely as the toxicity of AZA is targeted to the gastrointestinal tract.

103. The FSAI Scientific Committee noted that the derived ARfD of 0.63 µg/kg bw is comparable to the maximum intake value of 0.67 µg/kg bw for a 60 kg individual consuming 250 g of mussels at the current regulatory limit of 16 µg/100 g shellfish flesh. It was considered that the validity of the proposed ARfD is supported by the absence of reported incidents of AZP since the introduction of the 16 µg/100 g shellfish flesh regulatory limit for AZAs, despite evidence that approximately 216,000 portions of oysters have been legally placed on the market with AZA levels between 10 and 16 µg/100 g shellfish flesh. It was suggested that this information could be viewed as crude evidence of a much wider epidemiological data set than that provided by the Arranmore incident alone, indicating that a larger safety number was not required to account for the small number of people involved in the Arranmore incident for whom epidemiological data are available.

COT Evaluation

104. The COT considered the epidemiological data from the Arranmore AZP incident in the light of the new data presented in the 2006 FSAI risk assessment.

105. Concerns were raised that the absence of information on levels of AZAs in mussels associated with illness represented a significant source of uncertainty in the risk assessment.

106. In addition, the Committee expressed concerns over the appropriateness of the safety factor of 3 applied in the FSAI assessment. This was based on toxicodynamic variability with an assumption of no toxicokinetic variation due to a lack of clear evidence for metabolism of AZA resulting in a more toxic compound. The potential for variation in elimination of AZAs did not appear to have been considered.

107. In addition, it was noted that the limited animal data on acute oral toxicity of AZAs indicated a LOAEL of 250 µg/kg bw, the lowest reported lethal oral dose of AZA-1 in an acute study. Application of an uncertainty factor of 1000 to this value, to account for differences between species, human variability and extrapolation from a lethal dose, would indicate an ARfD of 0.25 µg/kg bw - lower than the ARfD of 0.63 µg/kg bw proposed by FSAI. While repeat-dose toxicity studies with AZA-1 had identified a potential for adverse effects following repeated oral administration at relatively low levels, it was noted that regular exposure to AZAs over a prolonged period was unlikely to occur in humans given general patterns of shellfish consumption in the UK.

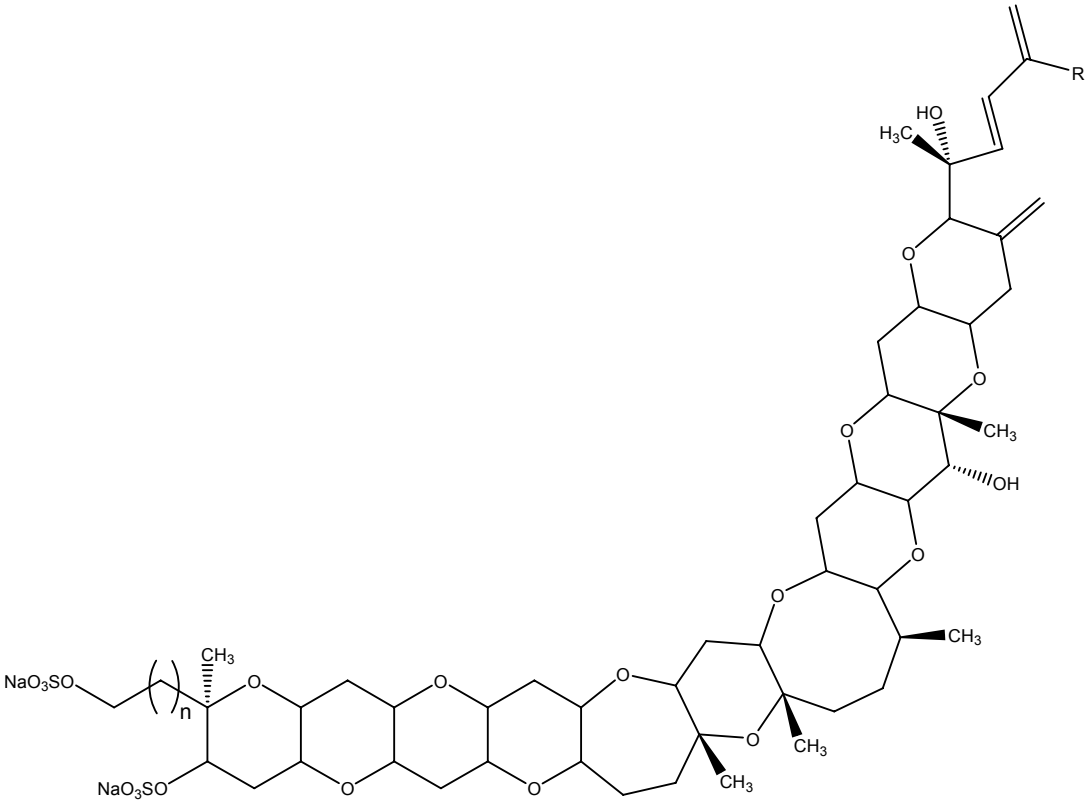
108. The absence of reported AZA poisonings since the introduction of the regulatory limit in 2001, despite evidence of two major incidents of AZA contamination of shellfish between 2001 and 2005, provided the COT with some reassurance that the ARfD proposed by FSAI was in practice sufficiently protective. However, it was noted that the absence of reported AZP incidents should not be taken as evidence that no such incidents have occurred in this time, and is not an adequate basis for risk assessment.

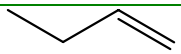
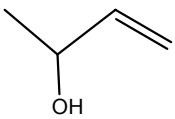
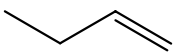
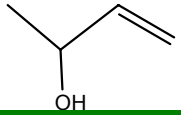
Yessotoxins

Background

109. Yessotoxins (YTXs) have been detected in microalgae and/or bivalve molluscs in Australia, Canada, Italy, Japan, New Zealand, Norway and the United Kingdom[64]. They have not been found to induce diarrhoea in animal studies, and there are no reports of human illness associated with these toxins. The structures of those YTXs for which most toxicological data are available are shown in Figure 4.

Figure 4. Structures of YTXs[7]



Toxin	R	n
YTX		1
45-hydroxyYTX		1
homoYTX		2
45-hydroxyhomoYTX		2

110. Current legislation prescribes a maximum permitted level for YTXs in shellfish of 100 µg/100 g shellfish flesh.

Toxicology

Toxicokinetics

111. No data have been published on the toxicokinetics of YTXs. However, an unpublished study reported in the background document for the FAO/IOC/WHO Expert Consultation found that the majority of an oral dose of YTX in mice was recoverable from the faeces, suggesting poor absorption from the gastrointestinal tract[3].

Acute toxicity

112. The acute lethality of YTX following i.p. injection in mice was originally reported as 100 µg/kg bw[69]. Subsequent studies have reported LD_{50}s of 286 µg/kg bw[87] and 512 µg/kg bw[18]. Ogino *et al.* reported that the minimum amount of YTX required to kill a mouse following i.p. injection was between 80-100 µg/kg bw[20], whereas in a later study death only occurred at doses ≥ 750 µg/kg bw[88]. The acute lethality of 45-hydroxyYTX has been reported as 100 or ca. 500 µg/kg bw[69,89].

113. The i.p. lethalities of homoYTX and 45-hydroxyhomoYTX were originally reported to be similar to those previously reported for YTX (100 µg/kg bw) and 45-hydroxyYTX (ca. 500 µg/kg bw)[90]. More recently, however, an LD_{50} of 444 µg/kg bw has been reported for homoYTX, while 750 µg/kg bw of 45-hydroxyhomoYTX did not cause death in this study[18].

114. No deaths have been observed following oral administration of YTX to mice at doses up to 10,000 µg/kg bw[88,18]. The FAO/IOC/WHO background document notes that unpublished research found no deaths at oral doses of 50,000 µg/kg bw. Oral administration of homoYTX and 45-hydroxyYTX at 1000 µg/kg bw also did not result in death[18].

115. Following i.p. administration in mice, a single dose of YTX at 300 µg/kg bw did not cause discernible changes in the liver, pancreas, lungs, adrenal glands, kidneys, spleen or thymus[87]. The intestines were also examined in this study, but the authors did not report whether or not adverse effects were observed. Electron microscopy (EM) showed severe cardiac damage including swelling and degeneration of the endothelium of capillaries, swelling of almost all cardiac muscle cells and rounding of mitochondria.

116. No changes were observed in the lung, thymus, liver, pancreas, kidney, adrenal gland, jejunum, colon and spleen of mice administered single i.p. doses of YTX up to 1000 µg/kg bw[88]. Slight intercellular oedema was detected by light microscopy in cardiac muscles of animals given 750 and 1000 µg/kg bw, while EM analysis of hearts of animals given 1000 µg/kg bw revealed swelling of

myocardial muscle cells and separation of organelles that was most pronounced near the capillaries.

117. Histological examination of major organs and tissues including the liver, heart, lungs, kidney, spleen, thymus and brain did not show morphological changes in mice given a single i.p. injection of YTX (265-750 µg/kg bw), homoYTX (375-750 µg/kg bw) or 45-hydroxy-YTX (750 µg/kg bw) [18]. TUNEL staining was performed on heart tissue but no apoptosis was detected.

118. Examination of the cerebellar cortex of Swiss CD-1 mice given a lethal i.p. dose of YTX (420 µg/kg bw) indicated damage to the Purkinje cells[91]. Immunocytochemical analysis indicated an increased positivity for S100 protein, and a decreased response to calbindin D-28K, beta-tubulin and neurofilaments. In a subsequent study using both lethal (420 µg/kg bw) and sublethal (10 µg/kg bw) doses of YTX, no effects were detected in the cerebellar cortex at the sublethal dose[92]. In the cerebral cortex, no morpho-functional alterations were observed at either dose. Morphological changes were detected in the thymus at both doses, including apoptosis, predominantly of thymocytes and increased mitosis. Alterations in cytokine levels were also observed.

119. The only adverse effects of YTXs reported following oral administration are ultrastructural alterations in cardiac muscle cells, similar to those observed following i.p. administration. Ultrastructural alterations in cardiomyocytes have been observed by EM in mice in a number of studies following single oral administration of YTX at concentrations of ranging from 1000-10,000 µg/kg bw, and at 1000 µg/kg bw of homo-YTX and 45-OH-homo YTX[88,18]. In the earlier of these two studies, no adverse effects were detected in cardiac muscle cells by light microscopy at a dose of 1000 µg/kg bw, although animals receiving this dose were not examined by EM. Light microscopy only detected adverse effects in myocytes at doses of 7500 and 10,000 µg/kg bw[88]. No changes were detected in the hearts of mice treated with oral doses of 500 µg YTX/kg bw[87].

120. In a short-term study, administration of YTX (2000 µg/kg bw), homoYTX (1000 µg/kg bw) and 45-OH-YTX (1000 µg/kg bw) to female CD-1 mice daily for 7 days resulted in ultrastructural changes in cardiac muscle cells, detected by electron microscopy (EM). No signs of toxicity were observed in the other organs or tissues that were examined, including the brain, thymus and spleen [93].

121. Information presented at the 2004 International Conference on Molluscan Shellfish Safety by Espenes *et al.* relating to a further study of repeated oral exposure to YTX is described in the background document for the FAO/IOC/WHO Expert Consultation. In this study, NMRI mice were exposed to YTX seven times in 21 days by oral intubation, at doses of 1000, 2500 and 5000 µg/kg bw. Mice were killed 3 days following last treatment, and major organs including the myocardium, brain, thymus and spleen were studied by light microscopy. The myocardium was also examined by EM. No clinical signs were observed in any of the groups exposed to YTX, and there were no differences in body weight gain between treated mice and controls. No pathologic effects were observed by light microscopy. By EM, some vacuoles

were observed in the myocardium of mice treated at the highest dose only. The authors suggested that the reason for the apparent conflict with previous studies showing ultrastructural changes in myocardium at lower doses may have been due to the 3-day delay between final dosing and sacrifice of the mice. They suggested that any damage occurring following treatment may have been repaired in this time[94].

122. In a recent study, atrophy and structural alteration of the thymus was observed in mice 24 hours following consumption of shellfish tissue contaminated with OA and YTX (estimated intakes of 18 and 1.4 µg/kg bw, respectively [27]. Histopathological changes were also observed in the spleen immediately following consumption of contaminated shellfish, but these effects were less marked 24 hours following consumption. However, similar effects were found with OA alone and it is therefore unclear whether YTX may have contributed to the effects observed.

Previous Risk Assessments

123. On the basis of the data available in 2001, the EC Working Group on Toxicology of DSP and AZP considered that findings of no adverse effects by light microscopy following a single oral administration of 1000 µg YTX/kg bw[88] represented a NOAEL[52]. The Working Group applied a safety factor of 100 to this to derive an ARfD of 10 µg/kg bw.

124. FAO/IOC/WHO (2004) concluded that the repeated administration study of Espenes *et al.* (see paragraph 121) indicated a NOAEL of 5000 µg/kg bw. A safety factor of 100 was applied to derive an ARfD of 50 µg YTX eq/kg bw.

125. The approach of FAO/IOC/WHO was also adopted by the CRLMB Working Group on Toxicology[65].

COT Evaluation

126. The Committee questioned the relevance of the observed alterations in cardiac myocytes, and of the apparent recovery from injury following treatment in the most recent study. Cardiac tissue does not readily regenerate following injury, and alterations were not observed by light microscopy at doses up to 5000 µg/kg bw. It was considered possible that the EM findings may have been artefactual, but the available evidence was insufficient to draw firm conclusions.

127. Despite the uncertainty over the significance of the reported alterations in cardiac muscle cells, it was considered that it would be conservative to use these data to establish an ARfD for YTXs. An uncertainty factor of 100 was applied to the NOAEL of 5000 µg/kg bw identified in the 21 day repeat-dose study, resulting in an ARfD of 50 µg/kg bw.

Conclusions

Okadaic Acid Group

128. We consider that human case reports should be used as a basis for risk assessment of OA group toxins, although we note the uncertainties relating to the amount of toxins consumed in many of these incidents.

129. We note that the totality of published epidemiology data for OA toxins indicates a LOAEL of around 1 µg/kg bw. While the limited information from the recent UK DSP incident may suggest a lower LOAEL of 0.7 µg/kg bw, the data from this incident are difficult to interpret as shellfish associated with the outbreak also contained PTXs, and there is also uncertainty with respect to which of two shellfish portion sizes sold at restaurants involved in the incident had been eaten by individuals who became ill. Had all individuals eaten the larger portion size, a LOAEL of 1.3 µg/kg bw would be indicated, in line with the previous epidemiology data.

130. The 2006 DSP outbreak indicated a reported response rate of up to 10%, suggesting that more than the most susceptible minority were affected at this dose. We therefore consider that an uncertainty factor of 3 would not be sufficient for extrapolation from a LOAEL to a NOAEL in this case. An uncertainty factor of 10 should be applied, resulting in an ARfD of 0.1 µg OA eq/kg bw.

131. We note that a portion size of 250 g is a reasonable estimate for high level consumption of shellfish in the UK[95]. We conclude that 2.4 µg OA eq/100 g shellfish meat would be the maximum concentration considered to be without appreciable health risk, assuming a 60 kg adult bodyweight.

132. We note that this concentration is lower than the current regulatory limit for OA group toxins together with PTXs of 16 µg/100 g shellfish meat. Furthermore, the MBA currently prescribed in EU legislation for detection of these toxins in shellfish monitoring programmes is not sufficiently sensitive to detect the presence of OA group toxins at this level.

Pectenotoxins

133. We consider that it is appropriate to use the lowest identified LOAEL in animal studies of 250 µg/kg bw as the basis for deriving an ARfD for PTXs. An uncertainty factor of 1000 should be applied to account for inter- and intra-species variation and extrapolation from a LOAEL to a NOAEL, resulting in an ARfD of 0.25 µg/kg bw.

134. However, we note the conflicting and incomplete nature of the database for PTXs, and recommend that the ARfD should be reviewed when further data become available.

135. On the basis of an ARfD of 0.25 µg/kg bw, a PTX concentration of 6 µg/100 g shellfish meat would be the maximum concentration considered to be

without appreciable health risk, assuming a 60 kg bodyweight. As with the OA group toxins, we note that this concentration is lower than the current regulatory limit, and that the MBA is not sufficiently sensitive to detect the presence of PTXs at this level.

Azaspiracids

136. We consider that the limited epidemiology data from the 1997 Arranmore AZP incident currently provide the best available evidence for risk assessment of AZAs, although we note that there are considerable uncertainties in the information derived from this incident, particularly with respect to a lack of accurate information on the levels of AZAs in mussels associated with illness.

137. The ARfD of 0.63 µg/kg bw proposed by FSAI is comparable to the maximum intake of 0.67 µg/kg bw for a 60 kg individual at the current regulatory limit for AZAs of 16 µg/100 g shellfish meat. We conclude that the absence of reported AZA poisonings since the introduction of the regulatory limit in 2001, despite evidence of two major incidents of AZA contamination of shellfish between 2001 and 2005, provides some reassurance that the ARfD proposed by FSAI would in practice be sufficient for the protection of the health of the consumer. However, we note that the absence of reported AZP incidents should not be taken as evidence that no such incidents have occurred in this time, and is not an adequate basis for risk assessment in isolation.

Yessotoxins

138. The relevance of the observed alterations in cardiac myocytes following oral administration of YTXs in animal studies is unclear. However, we consider that it would be conservative to use these data to establish an ARfD for YTXs.

139. A NOAEL of 5000 µg/kg bw can be identified on the basis of apparent absence or recovery from injury three days following final treatment in the repeat-dose study by Espenes *et al.* (paragraph 121). Application of an uncertainty factor of 100 to account for inter- and intra-species variation results in an ARfD of 50 µg/kg bw.

140. We conclude that a YTX concentration of 1200 µg/100 g shellfish meat would be the maximum concentration considered to be without appreciable health risk, assuming a 60 kg adult bodyweight.

Analytical methods

141. When the COT last considered DSP toxins in 1994, it recommended that efforts be made to develop a more quantitative assay to replace the MBA in the UK shellfish monitoring programme for lipophilic biotoxins. We reiterate this recommendation and, furthermore, we note that alternative methods may be required in order to support detection of OA group toxins and PTXs in shellfish at the concentrations identified as necessary for protection of public health. As alternative methods become available, it will be important to give careful

consideration to the most appropriate way to sum the concentrations of specific toxin analogues.

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December 2006

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