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

STATEMENT ON AMNESIC SHELLFISH POISONING

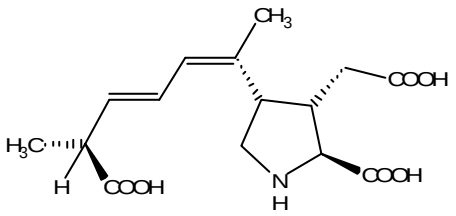
Introduction

1. We have been asked by the Food Standards Agency to review the issue of amnesic shellfish poisoning (ASP). The Committee was asked to consider whether the current EU action limit for bivalve shellfish (e.g. mussels, scallops, clams) of 20µg domoic acid/g tissue is adequate for the protection of public health. The Committee was also asked to comment on the public health implications of a proposed tired approach to scallop harvesting. This approach would allow harvesting of individual organs from whole scallops containing above 20µg domoic acid/g tissue. The individual organs could be marketed only if they contain levels less than or equal to 20µg domoic acid/g tissue.

Background

2. Amnesic shellfish poisoning was first recorded in Canada in 1987 following the consumption of contaminated mussels. Approximately 150 people became ill, and the outbreak resulted in the hospitalisation of 19 people and 4 deaths. The clinical effects were caused by domoic acid (figure 1), a water-soluble, amino acid which is produced by species of *Pseudonitzschia* phytoplankton, and accumulated by shellfish[1,2].

Figure 1: Chemical structure of domoic acid



3. Following the outbreak, the Canadian authorities imposed an action limit in mussels of 20 µg domoic acid/g tissue, above which harvesting of shellfish was suspended. The EU adopted this action limit for mussels and other bivalve shellfish including scallops and clams in 1997 (EC Directive 97/61/EC).

Toxicology

4. We reviewed the published toxicological data from animal studies and human case reports. There are limited data on the absorption, distribution, metabolism and excretion of domoic acid. However, these data indicate that domoic acid is not well absorbed in rodents and primates[3,4] and undergoes little metabolism prior to rapid excretion[5]. These data indicate species differences following oral exposure and suggest that primates have a relatively high sensitivity compared with rodents[4,6].

5. Domoic acid is a glutamate receptor agonist and binds with particularly high affinity to glutamate receptors in the central nervous system[7]. It is an excitotoxin and can produce a range of neuro-behavioural effects, which appear to be the most sensitive indicator of domoic acid toxicity.

6. Domoic acid is neurotoxic causing neuronal degeneration and apoptosis in specific regions of the hippocampus[8,9]. The lesions induced are consistent between rodent[11,12,13] and primate studies[6,14] and human[15,16] cases of ASP. Several mechanisms are thought to mediate the neurotoxicity. These involve perturbation of secondary messengers, including calcium and protein kinase C. However, it is thought that the critical toxic insult is the excessive accumulation of intracellular calcium[10].

7. The data indicate that rodent neonates are more susceptible to domoic acid toxicity than adults[17].

8. In rodent neonates, the spinal cord appears to be more sensitive than the brain to domoic acid toxicity[17]. However, a parallel comparison has not been carried out in adult rodents.

9. Domoic acid was not mutagenic *in vitro* in *V79* cells[18], but has not been tested in other systems. As there is potential for epoxide formation, it is suggested that further information on mutagenicity is required.

10. From review of the animal studies, we conclude that many used small group sizes and were inadequately reported. Additionally, many studies tested contaminated shellfish extract rather than purified domoic acid. This limited the utility of these studies, as the domoic acid content was not accurately quantified and the presence of other toxic components could not be excluded. Therefore, we consider the data were insufficient to identify a no-observed adverse effect level (NOAEL) or lowest-observed adverse effect level (LOAEL) which could support derivation of a Tolerable Daily Intake (TDI).

Human data

11. We reviewed the published case reports from two major outbreaks of ASP[1,2]. The most serious outbreak resulted in approximately 150 reported cases of ASP, the hospitalisation of 19 people and 4 deaths. The clinical

symptoms ranged from gastrointestinal (GI) effects, to neurotoxic effects such as hallucinations, memory loss and coma. GI disturbances appeared within 24 hours and neurological effects within 48 hours of consumption of contaminated shellfish[1,16]. We note that the quantity of shellfish consumed was based on the recollections of a small number of patients. Additionally, the concentration of domoic acid was estimated from analyses of mussels collected from the affected areas after the outbreak had occurred. Therefore, we were unable to correlate the range and severity of the adverse effects with the dose of domoic acid consumed.

12. It has been suggested that the elderly are particularly vulnerable to ASP as the reported deaths occurred in individuals over 70 years of age. However, we are unconvinced that these limited data support such a premise as co-morbidity present in this group may have contributed to the deaths[1,2]. We note there are no data on the susceptibility of infants or children to ASP.

13. Although there have been no recorded outbreaks of ASP in the UK, we recognise that food poisoning incidents are under-reported. We note that the symptomology and rapid elimination of domoic acid from the body make ASP difficult to verify clinically. However, we suggest that urinary domoic acid may serve as a potential biomarker of exposure to this toxin but only if analysed soon after ingestion.

14. Due to the limited data, we were unable to ascertain if the GI disturbances were direct effects of domoic acid or a manifestation of excitotoxicity in the central nervous system. The latter is a plausible mechanism although it does not preclude the possibility of direct effects occurring in tandem. We regard neurotoxicity as the most significant effect of ASP in terms of public health.

Action Limit

15. We note that the current action limit is based on consumption estimates from the 1987 Canadian ASP outbreak indicating that mussels contaminated with ≥200µg/g domoic acid resulted in human illness. However, this was a retrospective estimate from a small number of affected individuals. A ten-fold uncertainty factor was incorporated to give an action limit of 20 µg/g[19,20]. The EU subsequently applied this action limit to other bivalve shellfish.

16. We regard the action limit as a pragmatic guideline rather than a toxicologically based safety limit. As noted in paragraph 10, the available data are not adequate to identify a NOAEL or LOAEL. In view of the severe and potentially irreversible neurotoxicity of domoic acid, we consider that an uncertainty factor of 10 is inadequate to allow for inter-individual variability in addition to the uncertainties in the estimation of the domoic acid content and quantity of mussels consumed. However, we consider that at present the toxicological and shellfish consumption data are too limited to support derivation of an alternative action limit. We suggest that further long-term toxicological studies are conducted, using appropriate models. Additional

information on shellfish consumption is required to allow derivation of a TDI and a more robust action limit.

Tiered approach

17. Currently, EU directive 97/61/EC prescribes an action level for domoic acid of 20 μg domoic acid/g tissue for bivalve shellfish. If concentrations exceed this, harvesting of shellfish is stopped until levels drop below this. Detection of domoic acid in a range of shellfish, in particular King Scallops, has resulted in the frequent closure of harvesting grounds in Scotland.

18. The Food Standards Agency in Scotland is investigating a tiered approach to harvesting. This approach would allow harvesting of individual organs from whole scallops containing above 20 μg domoic acid/g tissue. The individual organs could be marketed only if they contain less than or equal to 20 μg domoic acid/g tissue.

19. The accumulation of domoic acid in shellfish is unpredictable, as very little is known about the environmental conditions that trigger phytoplankton blooms and the consequent production of domoic acid. There is also considerable inter-scallop and inter-organ variability in concentrations of domoic acid. Additionally, cross-contamination can occur during processing. We have paid particular attention to these factors in considering the public health implications of ASP.

20. In order to ensure adequate protection of public health we advise that shellfish and shellfish parts at point of sale should not exceed the current action limit. Therefore, we recommend that rigorous monitoring and enforcement at point of sale is incorporated into a tiered approach, if introduced. This is essential to account for the:

- unpredictable nature of domoic acid contamination of shellfish,
- considerable variability in the inter-scallop and inter-organ concentrations of domoic acid,
- possibility of cross-contamination during processing,
- pragmatic nature of the action limit,
- risk of irreversible neurotoxicity.

Conclusions

21. We *consider* there are important and severe public health implications of ASP due to the irreversible neurotoxicity of domoic acid.

22. We have reviewed the toxicological data on domoic acid but *consider* these insufficient to establish a NOAEL that is appropriate for regulatory purposes. This is a reflection of the paucity of these data rather than the

absence of harm. We *suggest* that if a TDI is to be established further toxicological studies using appropriate animal models are required.

23. In view of the small margin of safety between the current action limit of 20 μg domoic acid/g tissue and the concentration of domoic acid resulting in human illness we *consider* this limit as a pragmatic guideline and not a toxicologically based safety limit. We *advise* that shellfish at point of sale should not exceed the current action limit.

24. We *strongly recommend* that if a tiered approach is introduced it will require rigorous monitoring at point of sale and enforcement to ensure protection of public health.

25. We *consider* that more information on shellfish consumption is required.

26. We *note* that to date, there have been no reports of ASP in the UK and therefore, the current action limit may protect against major outbreaks. However, we *recognise* that in general, food poisoning incidents are under reported.

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References

1. Perl TM, Bedard L, Kosatsky T, Hockin JC, Todd EC, McNutt LA, Remis RS (1990). An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N Engl J Med* **322**: 1775-1780.

2. Todd EC. Domoic acid and Amnesic Shellfish Poisoning – review (1993). *J Food Protect* **56**: 69-83.

3. Iverson F, Truelove J, Nera E, Tryphonas L, Campbell J, Lok E (1989). Domoic acid poisoning and mussel intoxication: preliminary investigations into the response of mice and rats to toxic mussel extract. *Food Chem Toxicol* **27**: 377-384.

4. Truelove J, Mueller R, Pulido O, Martin L, Fernie S, Iverson F (1997). 30-day oral toxicity study of domoic acid in cynomolgus monkeys: lack of overt toxicity at doses approaching the acute toxic dose. *Nat Toxins* **5**: 111-114.

5. Suzuki CAM and Hierlihy SL (1993). Renal clearance of domoic acid in the rat. *Food Chem Toxicol* **31**: 701-706.

6. Tryphonas L, Truelove J, Iverson F. Acute parenteral neurotoxicity of domoic acid in cynomolgus monkeys (M fascicularis) (1990a). *Toxicol Pathol* **18**: 297-303.

7. Hampson DR, Manalo JL (1998). The activation of glutamate receptors of kainic acid and domoic acid. *Nat Toxins* **6**: 153-158.

8. Nijjar MS (1993). Effects of domoate, glutamate and glucose deprivation on calcium uptake by rat brain tissue in vitro. *Biochem Pharmacol* **46**: 131-138.

9. Novelli A, Kispert J, Fernandez-Sanchez MT, Torreblanca A, Zitko V (1992). Domoic acid containing toxic mussels produce neurotoxicity in neuronal cultures through synergism between excitatory amino acids. *Brain Res* **577**: 41-48.

10. Xi D, Ramsdell JS (1996). Glutamate receptors and calcium entry mechanisms for domoic acid in hippocampal neurons. *Neuroreport* **7**: 1115-1120.

11. Nakajima S, Potvin JL (1992). Neural and behavioural effects of domoic acid, an amnesic shellfish toxin, in the rat. *Can J Psychol* **46**: 569-581.

12. Sobotka TJ, Brown R, Candour DY, Jackson R, Smith M, Long SA, Barton CN, Rountree RL, Hall S, Eiders P, Johannessen JN, Scallet AC (1996). Domoic acid: neurobehavioural and neurohistological effects of low-dose exposure in adult rats. *Neurotoxicol Teratol* **18**: 659-670.

13. Tryphonas L, Truelove J, Nera E, Iverson F (1990b). Acute neurotoxicity of domoic acid in the rat. *Toxicol Pathol* **18**: 1-9.

14. Scallet AC, Binienda Z, Caputo FA, Hall S, Paule MG, Rountree RL, Schumed L, Sobotka T, Slikker W. Domoic acid treated cynomolgus monkeys (M fascicularis) (1993): effects of dose on hippocampal neuronal and terminal degeneration. *Brain Res* **627**: 307-313.

15. Cendes F, Andermann F, Carpenter S, Zatorre RJ, Cashman NR (1995). Temporal lobe epilepsy caused by domoic acid intoxication: evidence for glutamate receptor-mediated excitotoxicity in humans. *Ann Neurol* **37**: 123-126.

16. Teitelbaum JS, Zatorre RJ, Carpenter S, Gendron D, Evans AC, Gjedde A, Cashman NR (1990). Neurologica sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *N Engl J Med* **322**: 1781-1787.

17. Wang GJ, Schumed LC, Andrews AM, Scallet AC, Slikker W, Binienda Z (2000). Systemic administration of domoic acid induced spinal cord lesions in neonatal rats. *J Spinal Cord Med* **23**: 31-39.

18. Rogers CG, Boyes BG (1989). Evaluation of the genotoxicity of domoic acid in a hepatocyte mediated assay with V79 Chinese hamster lung cells. *Mutat Res* **226**: 191-195.

19. Viviani R (1992). Eutrophication, marine biotoxins and human health. *Sci Total Environ Supplement* 631-622.

20. Waldichuck M (1989). Editorial: Amnesic Shellfish Poison. *Mar Pollution Bull* **20**: 359-360.