Detail of Consultation

Background

The Food Standards Agency is the Competent Authority in the UK responsible for the statutory monitoring programme for marine biotoxins in shellfish. EC regulations lay down both the specific method of analysis for the detection of marine biotoxins and the maximum toxin levels permitted in shellfish flesh. Regulation (EC) No. 2074/2005 (as amended) stipulates the detection methods for the various biotoxins and prescribes the MBA for the detection of PSP toxins in the edible parts of molluscs. This is also the reference method.

The same Regulation also allows the use of alternative methods that are internationally recognised. Over the past few years, Agency commissioned research has been undertaken to evaluate and refine the AOAC HPLC method 2005.06 (also known as the Lawrence method) to take account of UK conditions. The method has previously undergone international validation for a number of toxins in the PSP toxin group and a few shellfish species. The research took into account the toxins and shellfish species most prevalent in UK waters and the expected sample numbers typically tested in the monitoring programme.

Initial findings from this research allowed the implementation of HPLC as a screening method in the UK statutory monitoring programme at the end of 2006. Since then, only samples found to contain PSP toxins have been further tested by MBA for quantification of the toxin levels present. This change made a significant impact on the reliance on animal testing by reducing the number of animals used in the monitoring programme for PSP toxins by over 80% across the UK.

Evaluation and validation of the AOAC HPLC as a **quantitative method** followed the recommendations of various international and national scientific bodies, including the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) and the International Union of Pure and Applied Chemistry (IUPAC). The final report of the work, containing a description of the performance characteristics of the method, including the limits of detection and quantification, selectivity, precision, recovery and the measurement uncertainty, has been published on the Agency's website at:

http://www.food.gov.uk/foodindustry/farmingfood/shellfish/aoachplc

Planned implementation of HPLC

Following review of the available evidence and scientific data, the FSA considers that the refined AOAC HPLC method now provides a sound basis to replace the MBA in selected shellfish species in the UK. The Agency is proposing to introduce the method for the analysis of mussels in the first instance, with a target start date of the 5th of May 2008. Work to evaluate the method for use with other species is continuing, with the intention to introduce its use in the statutory monitoring programme as soon as practicable, before the end of 2008. Until then, the present arrangements for testing other species will remain in place. A further stakeholder consultation will be undertaken about this.

From the 5th of May it is proposed that all mussels submitted to the UK monitoring programme, and showing positive in the HPLC screen, would be tested for levels of PSP toxins by HPLC. This is a quantitative method and results will be expressed in terms of micrograms STX eq/kg shellfish tissue, similar to the MBA. The current statutory maximum permitted level will not be amended and samples found to exceed the maximum permitted level of 800 micrograms STX eq/kg shellfish will result in the closure of the shellfish production area. Please note that as with current practice two results below this level, each taken one week apart, will be required to re-open the area for harvesting.

The introduction of the new method in the monitoring programme will have implications in terms of results reporting as the MBA and the HPLC are quite different methods and operate and determine toxicity on a different basis.

The HPLC method takes more time to deliver results than the MBA. The analysis time (from sample receipt to reporting of results) will depend largely on the number of samples positive in the HPLC screen. Though negative results can generally be reported within 36 hours, any screened samples demonstrating the presence of toxins will need to be analysed further for full quantification. For these samples a minimum turnaround time of 52 hours is expected.

Calculation of toxicity of a sample

One of the biggest differences between the MBA and the HPLC methodology is that while the first assesses the toxicity of compounds, HPLC measures the individual concentration of each of the various toxins present, without giving any indication of their toxicity. Thus for the HPLC results to be meaningful, toxicity equivalence factors (TEF) need to be applied to the levels of each toxin compound present before summing the results to give the total toxicity of a sample in STX equivalent.

In the absence of TEF in EU legislation it is proposed that those TEF determined by Oshima (1995), listed in Table 1, are used to calculate a sample's toxicity since these appear to be the most widely used internationally and were recommended by an expert consultation of the WHO/IOC/WHO.

Table 1 Relative toxicities of PSP toxins in the mouse bioassay reported by Oshima, 1995

Toxin	Relative toxicity
STX	1
neoSTX	0.92
GTX1	0.99
GTX2	0.36
GTX3	0.64
GTX4	0.73
dcSTX	0.51
dcGTX2	0.15
dcGTX3	0.38
B1 (GTX5)	0.064
C1	0.006
C2	.096
C3	0.013
C4	0.058

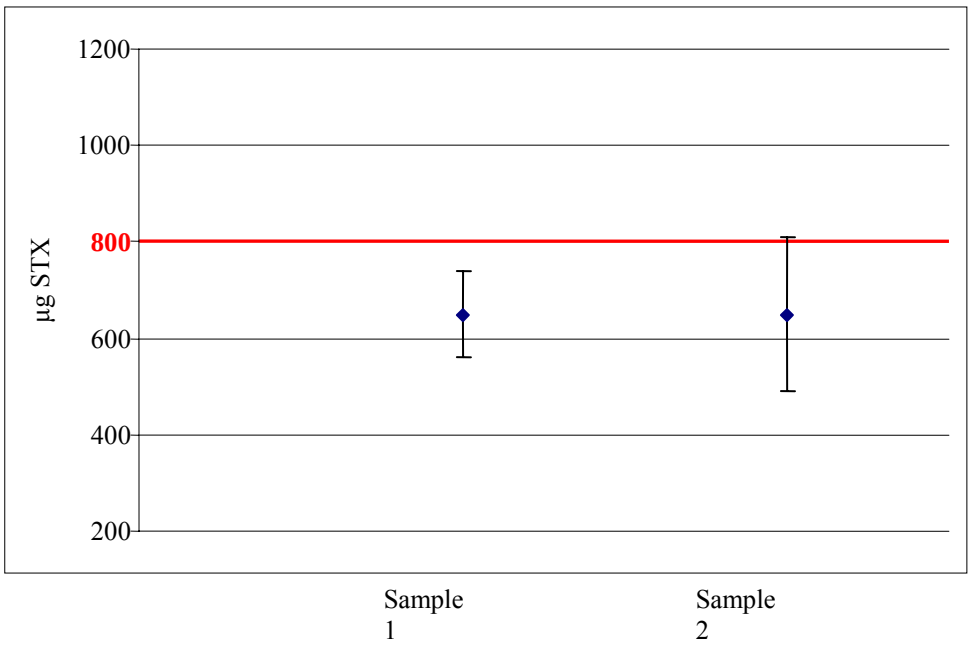
Additionally, a few of the PSP toxins ($GTX_{1,4}$, GT $X_{2,3}$, $C_{1,2}$ and $dcGTX_{2,3}$) consist of an isomeric pair of toxins which are detected together during the HPLC analysis. As a result, there is no way of knowing the proportion of each toxin in the pair present in a sample. Given this current constraint of the methodology, and that some of the analogues in the pairings have high toxicity values, the Agency proposes that samples containing any of these toxins will be reported using the highest relative toxicity for each pair. We consider this approach to be most protective of public health, although it may result in the over-estimation of toxicity in some circumstances.

Furthermore, the HPLC method, as do most analytical methods, has a degree of measurement uncertainty associated with it. Uncertainty defines the range of values that can reasonably be attributed to an analytical result and, essentially, can be regarded as the sum of all the experimental variations that can occur at each step throughout a procedure.

Measurement uncertainty is not taken into account with the current MBA methodology. This is due to the current international guidelines for application of the method and also the lack of knowledge of measurement uncertainty associated with the technique.

The evaluation work on the HPLC has demonstrated the range of uncertainty associated with the analytical method. The uncertainty associated with a sample is complicated and highly dependent on the toxin profile (i.e., the suite of toxin analogues present) of that sample. Since the toxin profile of samples can vary significantly, the *same* HPLC result can have *different* ranges of measurement uncertainty. A possible outcome of this is illustrated in Figure 1. Sample 1 and 2 have the same HPLC value (650μg STX eq/kg) but with significantly different toxin profiles. Thus despite having the same HPLC value, the range of measurement uncertainty in sample 2 takes it above the legal limit while the range in sample 1 remains below.

Figure 1
Graph showing how theoretical samples 1 & 2 relate to the legal limit of 800µg STX eq/kg when measurement uncertainty is taken into account



Given the seriousness of PSP toxins in terms of public health and the recent recommendations of the COT[1], the Agency is proposing to apply measurement uncertainty in a way that best protects public health. This means measurement uncertainty would be applied at 95% confidence level to all values obtained by HPLC. Decisions to close or open a bed will be based on the upper limit of the determined measurement uncertainty range for any given sample.

Results will continue to be reported weekly and as a single value, which will equate to the upper limit of the uncertainty range.

The FSA has a policy to issue public consultations on all changes in policy that may affect stakeholders, including those related to Official Controls. We will keep you informed of other developments in this area.

[1] http://cot.food.gov.uk/statements/cotstatements2006/cotstatementpsp200608