Last updated June 2004

PROGRESS REPORT

AGENCY'S PROGRAMME OF WORK TO INVESTIGATE ISSUES ASSOCIATED WITH ATYPICAL RESPONSES IN THE MOUSE BIOASSAY USED TO MONITOR FOR DIARRHETIC SHELLFISH POISONING (DSP)

Since the Summer of 2001, atypical responses have been observed in the DSP Mouse bioassay (MBA) for some shellfish samples from England, Wales and Northern Ireland tested under the statutory biotoxin monitoring programme.

A programme of work is being undertaken to find out what causes the atypical results, and to assess the public health implications.

The results of all this work will be made public and will inform the Agency's policy in relation to the closure of shellfish production areas that give atypical responses in the current test.

All work carried out in 2002 is reported in the updating report which was considered by the Board in December 2002 and which can be downloaded from the Agency website (www.food.gov.uk). This report concerns work during and since 2003.

	Work	Purpose	Completion Date	Status
1	Agreement and implementation of harmonised Standard Operating Procedures (SOP) for the DSP test used by UK monitoring labs.	Introduction of a standardised DSP SOP.	All labs to implement an agreed UK-NRL DSP SOP (version 1) from 17 November 2003.	**Complete.** Solvent carry over studies found that the DARD[1] SOP gave consistently low solvent carry-over levels. It was agreed that all labs should follow the DARD interim SOP, which was converted to a UK-NRL DSP SOP at the October 2003 UK-NRL[2] Network meeting. Extra solvent carry over safeguards were also incorporated into the SOP. All labs agreed to move to the standardised UK-NRL DSP SOP for the sample preparation and extraction stages from end of October 2003. Solvent carry-over checks carried out prior to introduction of SOP, in all labs from 17 November 2003. A trial of the UK-NRL SOP (version 1) was undertaken during first 5 weeks of implementation to check solvent levels were minimal, observe clinical signs in mice and screen for known toxins using LC-MS. A report of this trial is to be written up and published at the earliest opportunity (on-going). The UK-NRL will audit the application of the UK-NRL SOP (version 1) at each of the labs, during June and July 2004. A report will be placed on the Agency's website in due course. The CRL[4] is being kept informed of developments.

[1] DARD – Department of Agriculture and Rural Development which undertakes statutory biotoxin monitoring for Northern Ireland on behalf of the FSA.
[2] UK NRL – UK National Reference Laboratory for biotoxins.
[4] CRL – EU Community Reference Laboratory for biotoxins.

	Work	Purpose	Completion Date	Status
2	Solvent carry-over investigations.	To determine whether solvent carry-over could be the cause of the atypical response in the DSP test.	This was a multi-phased project. All experimental work was completed by September 2003. A final report was published in October 2003.	**Complete.** Meeting held to discuss findings and agree action with the labs on 1 October 2003. Meeting held to share findings and actions with industry and enforcers on 2 October 2003. **Report published on Agency website on 2 October 2003:** http://www.food.gov.uk/science/research/researchinfo/foodborneillness/shellfishresearch/b16programme/shellfish_toxins Since publication new information has come to light which has meant that some data has had to be re-analysed and an addendum to the report has been produced. **Addendum to the report published on Agency website on 2 February 2004.** Meeting held with industry and enforcers on 13 November 2003 to discuss the report and the draft addendum in detail. Further data analysis was undertaken to take account of comments made at the meeting. This analysis has been included in the addendum report.

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3	Independent audit by Professor Makin.	To determine whether the test procedures being followed by each laboratory are equivalent and whether adequate QA measures are in place. Will identify any issues that may explain any discrepancies or deviations in experiences of using the test method and recommend a course of action to help standardise test arrangements.	Audit report to be signed off by end of September 2003 and made publicly available at the start of October 2003.	**Complete.** Report finalised, having taken account of comments from CEFAS[5], DARD and FRS. Meeting held to discuss findings and agree action with the labs on 1 October 2003. Meeting held to share findings and actions with industry and enforcers on 1 October 2003. **Report and action plan published on Agency website on 2 October 2003:** http://www.food.gov.uk/science/research/researchinfo/foodborneillness/shellfisheresearch/b16programme/shellfish_toxins Meeting held with industry and enforcers on 13 November 2003 to discuss the report in detail. Audit Actions table updated with progress at regular intervals and posted on the Agency's website.

[5] CEFAS – The Centre for Environment, Fisheries and Aquaculture Science which undertakes statutory biotoxin monitoring for England and Wales on behalf of the FSA.

	Work	Purpose	Completion Date	Status
4	CSL[6] project (B16002).	Optimisation of extraction and particulate removal stages of SOP for DSP test.	Draft report due April 2004.	**Work in progress**. Draft report has been received and is being appraised internal and externally. A summary of the final report will be placed on the Agency's website when finalised. The final report will be available from the Agency's library. Some additional validation work is being undertaken to address issues raised at the NRL Working Group meeting on lipophilic toxins in January 2004. The method is expected to by ring-tested by the CRL in 2004. Work will be fed into the CRL Working Group discussions to develop a European standard operating procedure for the DSP MBA.
5	LGC[7] project (B16001).	Study to detect and possibly identify the causative agent(s) responsible for the atypical response to the DSP MBA by Liquid Chromatography-Mass Spectrometry (LC-MS).	November 2003.	**Complete.** A summary of the final report was published on the Agency's website and the report placed in the Agency's library on 11 March 2004.

[6] CSL – Central Science Laboratory
[7] LGC – Laboratory of the Government Chemist

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6	Preliminary toxicological Study.	To provide preliminary information to allow an assessment of the implications for human health of the atypical responses seen in the DSP assay.	Completed April 2004.	**Complete.** Stored cockle extract (shown to contain acceptably low solvent levels) has been tested and found to be as potent in the repeat bioassay as it was in the original bioassays. The study investigated the behavioural responses after intraperitoneal and oral administration of the extracts, and vehicle control, with post-mortem of the animals and histopathological examination. The results, together with the input from the COT[8] and additional independent experts, provided the basis for further studies to yield information on the toxicity of the extracts (see 9-11 below). A report will be placed in the FSA library in the near future. The papers presented to the COT (TOX-2004-05 and associated Annexes) and the draft minutes of the meeting can be found on the Agency's website.
7	Fractionation study.	To arrange for bulk samples of cockles giving atypical responses to be sent to Japan for analysis by Professor Yasumoto (expert who developed the DSP test)	Samples to be sent to Japan as soon as sufficient atypical shellfish material can be collected.	**Planned new work**. Study protocol has been drawn up with input from the parties involved in the initiative. A copy of Professor Yasumoto's study protocol is awaited. Professor Yasumoto has recently confirmed that he is able to undertake this work, and has made provision in his research work schedule to fit it in. He has estimated that the work could take a year to complete. Bulk cockle material showing the atypical response is needed before this study can begin. The Agency is working with industry and enforcement authorities to collect approximately 30kg of cockle meat.

[8] COT – Committee of Toxicity

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8	Screen shellfish extracts for a range of biogenic amines.	To develop a method to analyse shellfish extracts for a range of biogenic amines and to measure the levels of these compounds in shellfish samples from the monitoring programme.	A study was commissioned during May 2004. The work is due to be completed by the end of July 2004.	**On going**. An analytical method for this work is currently being developed.
9	Cytotoxicity study	To examine the possible toxicity of cockle extracts to cells.	To be completed by the end of September 2004.	**Work in progress**. A study has been commissioned and started on the 24 May 2004.
10	Neurotoxicity study	To examine the possible binding of components in cockle extracts to a range of neurotransmitter receptors.	To be completed by the end of September 2004.	**Work in progress**. A study has been commissioned and started on the 1 June 2004.
11	Neurotoxicity study	To examine the possible electrophysiological responses induced by cockle extracts in the central nervous system.	To be completed by the end of October 2004.	**Work in progress**. A study has been commissioned and started on the 31 May 2004.
12	Screen cockle and mussel meat for a range of known hydrophilic and hydrophobic shellfish toxins.	To analyse atypical and negative shellfish samples for known shellfish toxins and close structural analogues.		**Under consideration**. No study commissioned at present.

	Work	Purpose	Completion Date	Status
13	Meetings with industry.	To update industry with progress and discuss further work.	On-going	**On-going.** Since the beginning of May 2004, the Agency has met with representatives from industry on 5 separate occasions. Further meetings are planned.
14	Meetings with the Commission.	To push forward issues concerning the DSP test to produce an EU standard method for DSP detection.	On-going	**On-going.** The Agency continues to press the Commission at appropriate opportunities. A meeting of NRLs is to be held in July to discuss standardisation of test methods.
15	Establishment of a Stakeholder Forum.	To improve co-operation and communication with industry and local enforcement authorities, and also with others with an interest such as consumer organisations and other Government Departments. This forum will also consider what further research might be needed to resolve the atypical DSP issue.	By the end of July 2004	**On-going.** The Agency is considering the composition and the terms of reference for the forum which will be agreed with stakeholders and published on the Agency web-site. It is anticipated that this process should be completed by the end of July 2004. The date for the first meeting of the forum will be dependent on the availability of the various representatives.