Annex 1

WORKING DOCUMENT – INTERIM PROGRESS REPORT

AGENCY'S RESPONSE TO THE FINDINGS OF PROFESSOR MAKIN'S REPORT
Implementation of the agreed actions will be taken forward with the assistance of the UK-NRL.

	AUDIT FINDINGS AND RECOMMENDATIONS	AGENCY RESPONSE AND ACTION AGREED BETWEEN THE AGENCY, CEFAS, DARD AND FRS	STATUS
1	No evidence emerged from this audit to support the view that the atypical response is due to the presence of ether in the Tween extract (the report notes that this is being separately investigated by the FSA).	FSA notes that the audit did not find any evidence to suggest that the atypical response to the DSP MBA is due to the presence of ether remaining in the final extract. FSA commissioned separate solvent carry over investigations which provide further evidence to suggest that ether is not the cause of the atypical response. The Agency agrees that solvents should not be present at levels which could affect the test result. **Measures are to be introduced to minimise solvent levels before extract is tested in MBA; discussions will take place at the UK-NRL Network meeting on 8/9 October 2003.**	The updated DARD interim SOP was discussed at the October UK NRL Network meeting and converted it to a UK-NRL DSP SOP which all statutory monitoring labs agreed to introduce. Additional safeguards were agreed at the meeting to help ensure all laboratories operate it in the same way, minimise solvent carry over and achieve improved consistency in determination of test end points. These measures have been built into the UK NRL DSP SOP which was implemented in all labs on 17 November 2003.
2	If evaporation has been carried out correctly, ether and/or acetone should not be present in significant amounts and it should not be necessary to leave the extract over-night to allow further evaporation of ether.	FSA agrees that the evaporation stages must be carried out so as to minimise volumes of ether and acetone in sample extracts. It also agrees that it should not be necessary to leave the extract over-night to allow further evaporation of ether. **CEFAS, DARD, and FRS have been asked to ensure solvents are not carried over into the extract at levels that could affect the result.** A number of improvements are being made to tighten up operating procedures and help improve consistency in the way the extraction is carried out; **discussions will take place at the UK-NRL Network meeting on 8/9 October 2003.**	Prior to introduction of the UK NRL DSP SOP, tests carried out at each of the labs indicated that extra safeguards included in the SOP had been effective in minimising solvent carry over to levels which were acceptable to the Home Office (i.e. below levels causing clinical signs in the MBA). It is not technically possible to totally eliminate solvent carry over. The UK NRL DSP SOP post implementation trial, which runs until the end of December, will provide further data to demonstrate that this is still the case.

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3	While each laboratory operated a different protocol for the routine DSP assay, all were in accord with the basic methodology outlined by Yasumoto (1984). No evidence emerged from this audit to obviously support the argument that the cause of the atypical DSP response is a methodological or procedural artefact. However, if the atypical response is in fact due to a new toxin, what appear to be slight differences in methodology may well have a profound effect on what is present in the final extract and thus injected into the mouse. Under these circumstances, it would be sensible to ensure that all three laboratories operate identical protocols for the DSP assay.	FSA notes that the audit did not find any evidence to suggest that the cause of the atypical response to the DSP MBA is a methodological or procedural artefact. The MBA is the EU reference method for the detection of DSP toxins in shellfish.[1] There is currently no standardised procedure at EU level for carrying out the DSP MBA. The EU Community Reference Laboratory (CRL) is trying to address this matter, on behalf of the EU but progress is slow. FSA has funded an extensive programme of work at LGC to identify the agent responsible for the atypical response to the DSP MBA. We are also commissioning work to assess its implications for human health. **FSA is taking action to ensure all the statutory monitoring laboratories operate the DARD sample preparation procedure in the same way. Audits will be undertaken to check that the sample preparation procedures, including extraction, are being followed consistently.** **The sample preparation and extraction stages of the interim SOP applied by DARD will be used by all laboratories since the independent audit and solvent investigations have found it to consistently result in low levels of solvent carry over, and to be capable of detecting the atypical response. Target date for implementation is end of October 2003.**	At the October UK NRL Network meeting all laboratories agreed to adopt the DARD Interim SOP for sample extraction, once updated, to take account of comments made in the Makin report. At the same meeting this SOP was converted to a UK-NRL DSP SOP and additional safeguards were incorporated into the SOP to help ensure all laboratories operate it in the same way, minimise solvent carry over and achieve improved consistency in determination of test end points. Prior to its introduction on 17 November, all laboratories tested out the UK NRL DSP SOP and undertook a training exercise to ensure consistency in operating practices. Since then the Agency has commissioned an extensive trial of the UK NRL DSP SOP. The trial involves testing monitoring samples to check solvent levels (gastec and GC-MS), check for the presence of known toxins (LC-MS), data on mouse symptoms, end points and test results. Once the trial has been completed a full report will be produced which will include all the data generated from the labs. This will be made publicly available (probably during January).

[1] EU Directive 91/492/EEC and Commission Decision 2002/225/EC.

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4	The procedures used for routine DSP assays in all three laboratories differ to varying degrees from the method described in the SOP. All three laboratories need to address this and ensure that the SOPs in place accurately describe the procedures used in the laboratory, and ensure that SOPs in place are accurately followed. All laboratories must ensure that procedures are regularly audited to maintain compliance.	FSA believes it is imperative that procedures are applied consistently and effectively and that application is independently monitored through established auditing arrangements. **CEFAS, DARD and FRS have agreed to take action to address this point; discussions will take place at the UK-NRL Network meeting on 8/9 October 2003.**	All labs adopted the UK-NRL DSP SOP which covers the extraction stages of the test on 17 November 2003. As part of the UK NRL DSP SOP trial study, which runs until the end of December, labs have been sent some known positive and known negative shellfish samples to check that when they use the UK NRL DSP SOP they minimise solvent levels, interpret and report the clinical symptoms and test results in the same way and thereby provide confidence that the test is being applied correctly and in a consistent manner. Discussions on standardising the bioassay part of the test are underway between the Agency and the Home Office. Arrangements for auditing the application of the test are being discussed. The UK NRL will carry out this function.

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5	There are different approaches to the determination of positive/negative results [of DSP] by each laboratory. The end-point of the assay, irrespective of differences in analytical procedure prior to that point, has to be standardised. CEFAS require 2/3 or 1/2 mice (depending on amount of shellfish material analysed) to present symptoms within the 5 hour period for a sample to be declared positive. FRS need to observe only symptoms in 1/2 mice - they observe mice closely and kill any that suffer distress, often well before the 5 hour period of observation has ended. DARD observe for 24 hours with death as the end point. When the laboratory audit mussel homogenate was injected into the mice at CEFAS the symptoms observed were considered "mild" and as such the result was reported as NEGATIVE, but the same symptoms were observed at FRS and DARD where it was reported as POSITIVE. This is clearly not acceptable. It is strongly recommended that descriptions of symptoms of typical DSP and atypical responses to the DSP MBA are agreed between all three laboratories and clearly tabulated.	FSA agrees that the assay end point and interpretation of test results is important and has to be standardised. This will need to be discussed with the Home Office Results obtained from the MBA take precedence over those obtained by any other testing means because it detects the full range of shellfish toxins. Article 6 of Commission Decision 2002/225/EC states that where there is a discrepancy between test results the MBA shall be considered to give the definitive result. **The UK NRL is already undertaking work with the assistance of CEFAS, DARD and FRS to define common symptoms associated with typical and atypical DSP test responses and a suitable objective end point.** **Measures to standardise the approach to identification of symptoms and end point will be taken, following full consideration of legal, consumer protection and animal welfare aspects. Issues will be discussed with the Home Office, and the project and personal licence holders at the laboratories.** **Studies to achieve a more objective interpretation of mouse bioassay responses will continue.** **Work to generate robust statistical data to assess whether fewer mice can be used without jeopardising consumer health protection will be undertaken once a standardised test method is applied.**	At the October UK NRL Network meeting all laboratories agreed a common set of descriptions which would be used to interpret clinical symptoms in the MBA at 5 hours. The labs have applied them since 15 October 2003. The FSA discussed the need for standardised MBA test conditions with the Home Office at a meeting on 30 October. Discussions are continuing. In the absence of more detailed information, it has been agreed with the Home Office that for the time being animals will continue to be observed for 5 hours and any animals that show severe symptoms, as defined recently by the monitoring labs, are immediately culled to minimise animal suffering. To generate data on the most appropriate observation period for onset of symptoms, the observation period will be continued to 24 hours at DARD if no symptoms are seen in 5 hours. The number of mice used in each test is being discussed with the Home Office. The views of monitoring labs have also been sought. Discussions are continuing.

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6	There is a need to establish the cause of the atypical response and further research is recommended. A possible route would be a comparative LC-MS analysis of extracts that produced negative responses, typical DSP and atypical DSP responses to the MBA. This may indicate a possible cause, but until this research is complete and the cause established, changes in the methodology/procedures used for routine DSP assay should be avoided as the effect of such changes will be unknown, thus possibly exacerbating the problem.	FSA supports this recommendation and has funded work at LGC using LC-MS since May 2003 to detect and possibly identify the agent causing the atypical response to the DSP MBA. **Until such time as this work is complete CEFAS, DARD and FRS will use the DARD interim SOP for sample preparation and extraction procedures. The UK NRL will assure the interim SOP is applied in a consistent manner in the statutory monitoring laboratories.**	The LGC work has been completed and will be published on the Agency's website when the final report has been signed off (probably January 2004). The UK-NRL DSP SOP is to be applied in all laboratories until the cause of the atypical response has been characterised or shown to be of no known risk to public health. The UK NRL is responsible for auditing implementation of the UK NRL DSP SOP at the labs and is in the process of working up a protocol for undertaking this task, and planning its first audit. The Agency awaits notification of the UK NRL's proposals.
7	There appeared to be no satisfactory internal quality assurance (QA) for the shellfish monitoring protocols in place at any of the three laboratories visited. While the difficulties of setting up an effective procedure are recognised, it is felt that they can, at least partially, be overcome and some form of internal QA **MUST** be instituted in each laboratory.	The FSA supports this recommendation. **The UK NRL, FSA, FRS, DARD and CEFAS are already considering QA issues in general and how best to introduce effective measures suitable for a routine monitoring programme.** The introduction of internal QA will require careful consideration and take cost, sample throughput and ethical issues into account.	Issues relating to QA and control measures were discussed at the October UK NRL Network meeting and the meeting with the Home Office on 30 October 2003. An approach to addressing controls without the use of mice has been proposed. The Agency is considering the matter further, taking advice from the Home Office as appropriate.

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8	The staff of the UK-National Reference Laboratory (NRL) for biotoxins (UK-NRL) are not independent of FRS and, in effect because of their funding arrangements, serve two masters. It is recommended that if possible steps should be taken to establish more clearly the independence of UK-NRL and at the same time consider the role of this laboratory. I suggest that the remit of the UK-NRL should include *inter alia* responsibility for: • QA of statutory monitoring laboratories. • Liaison with the CRL. • Monitoring performance of all UK statutory monitoring laboratories. • Providing independent objective advice to the FSA and statutory monitoring laboratories, regarding methodology and procedures. • Undertaking independent research to improve methods with intention of providing alternative assay system to present MBA (e.g. Liquid Chromatography – Mass Spectrometry (LC-MS).	FSA agrees that the UK NRL should be seen to be independent. **FSA and the UK NRL are in the process of reviewing the NRL role, remit and functions.**	The role, remit and functions of the UK-NRL have been revised to take account of the recommendations in the Makin report. The UK-NRL has been asked to produce a paper by the end of the year outlining proposals on how it can be more independent from FRS.

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9	The UK-NRL should seek to set up at least a UK wide external QA scheme, which in co-operation with the Community Reference Laboratory (CRL) could be extended to the whole of the EU.	The FSA supports this recommendation. The UK NRL oversees implementation of external QA measures and ensures consistency in performance of statutory biotoxin testing within the UK and with other member States. **Consideration is being given to how best to set-up and carry out proficiency schemes for the shellfish biotoxin area.**	On-going.
10	Telephonic/oral transmission of results should be avoided as it may lead to errors. There should be a clearly described procedure in all laboratories for the approval of results by a named certifying scientist, which would require scrutiny of all the data, including quality control (QC) results, before they are released from the laboratory.	Biotoxin test results are transmitted by electronic means to FSA offices in London, Aberdeen and Belfast. **CEFAS, DARD and FRS will review, in conjunction with the FSA, the procedures used to report and check data before results are released from the laboratory and implement any measures which may be identified to improve current arrangements.**	On-going.
11	CEFAS laboratory has no prior notice of the numbers of samples that are sent for analysis and 20 samples could, with present staffing numbers, be close to overload. Large numbers of samples in a batch increases the possibility of mis-labelling and overload could cause errors in applying SOPs. If numbers of samples in batches exceed those which can be handled easily in one day, overnight storage is required.	FSA believes an early warning arrangement may help sample handling and testing efficiency. **FSA and CEFAS are considering ways in which sample management arrangements can be optimised in the interests of efficiency.**	On-going.