Final

MINUTES OF THE MEETING TO DISCUSS THE AUDIT OF THE UK-NRL DSP SOP

Aviation House, London, 10 August 2004

PARTICIPANTS:

FSA:

KH	Kevin Hargin (FSA, Chair)
KP	Karen Powell (FSA)
TB	Tom Barlow (FSA)
HS	Heiko Stolte (FSA)

External:

CA	Clive Askew (SAGB)
GG	Gavin Grewar (UK-NRL)
CH	Cowan Higgins (DARD)
WH	Wendy Higman (CEFAS)
DK	David Kershew
FM	Fiona Mackintosh (UK-NRL)
DM	Douglas McKenzie (Integrin)
JP	Joyce Petrie (FRS)
AR	Andrew Rattely
BS	Ben Stubbs (CEFAS)

Welcome and Introductory Remarks by Chair (KH)

1. Apologies for absence:

   Susan Gallacher - UK-NRL

   Mike Scotter – CSL

2. The Chair welcomed the participants to the meeting which offered the opportunity to discuss the findings of the Audit report produced by the UK-NRL and any differences in the implementation of the UK-NRL DSP SOP by the three monitoring laboratories (CEFAS, DARD, FRS). It was agreed that the aim of the meeting would be to reach a consensus on the significance of the differences between the laboratories and decide which areas, if any, required further clarification. KH invited laboratory representatives to submit amendments and minor corrections on the report to GG for inclusion in the final document. Participants of the meeting praised the quality of the audit and its associated report.

Presentation by Gavin Grewar on the Audit report.

3. GG explained that the monitoring laboratories adopted the UK-NRL DSP SOP (version 1) in November 2003. The audit was carried out during June and early July 2004, the purpose being to ensure that all monitoring laboratories were implementing

the UK-NRL DSP SOP. The scope of the audit included sample login and storage conditions, sample preparation, the extraction procedure, use of Gastec kits and resuspension of the extracts into Tween. The mouse bioassay (MBA) was checked for consistency against each of the laboratories' own MBA SOP and the identification of mouse clinical signs was checked to ensure they followed those issued by the UK-NRL.

4. GG summarised the main findings and recommendations, as outlined in section 9 of the audit report. All laboratories were found to have adopted the UK-NRL DSP SOP and good consistency was found between and within laboratories. For the MBA procedure there was consistency in interpretation of mouse clinical signs between laboratories. GG noted that some areas of the SOP cannot be standardised and require judgement, which has to be left to the analyst to determine.

5. GG highlighted that there were some areas of the SOP that required further standardisation. These were:
    - Whether the use of automated rotary evaporation equipment was necessary.
    - The positioning of GASTEC probe.
    - Method used for resuspension of extract in Tween.

6. There were also a number of areas which required further clarification by the UK-NRL. These were:
    - Procedure for cleaning of Ultraturrax.
    - Filter paper manipulation during acetone extraction.
    - Continuation of timing of rotary evaporation procedure after end-point reached.
    - Minimum guidance on glass washing procedure.

Discussion on the findings of the report:

7. KH reassured participants that any names of staff would be removed from the MBA SOP before the report is placed in the public domain.

8. AR asked why the Home Office (HO) had not been invited to the meeting. KH replied that the HO had been invited and they had not indicated that they wished to attend.

9. DK queried the condition of samples on receipt by laboratories and whether this was covered in the audit. GG stated that the SOP does not include transportation of samples, and deals only with samples from the point of receipt at the laboratory. FM stated that SOP stipulates that samples should be preferably sent to the laboratory chilled in cold boxes. DK commented that transportation often takes a long time and samples are often not transported in cold boxes. From an industry point of view this was not acceptable and the issue had been taking too long to resolve. He did not feel that experimental data was required to make a decision to transport shellfish under temperature controlled conditions.

10. KH agreed and recognised the need to establish minimum standards on sample transport as well as the need to liaise with local authorities on the issue. However, work was being conducted on some aspects of toxin stability at different temperatures, and the results of this work would be fed into the Working Group on Transport and Storage. This was due to meet during November, but KH agreed this meeting should be brought forward.

    Action: NRL to trawl for earlier dates for the WG on sample transport and storage

11. All participants agreed that minimum standards for transport and storage of samples needed to be set. CEFAS have trialed lockable cold boxes with several of the local authorities which had worked well but would require significant additional resources. Several representatives noted that some species are more sensitive than others to changes in temperature and that setting a requirement of 4°C may not be appropriate for all species.

12. CH pointed out that samples from Northern Ireland (NI) are usually received directly from Local Councils with ice-packs within a matter of hours of being collected. WH noted that the labs have no control over the treatment of samples by local authorities prior to their arrival at the lab.

13. CA queried if the FSA will be looking to industry to provide samples in future. KH noted that these had been early discussions and that no policy decision had been made. It was likely that consolidation of hygiene legislation would require a number of changes.

14. It was noted that occasionally homogenates were stored overnight by FRS and DARD. DARD indicated that it was not always possible to conduct the entire procedure within one day due to their HO licence requirement to inject mice before noon. CH also asked for clarification on the best point at which to interrupt the procedure to store the sample overnight. WH advised that it was not best practice to store samples as homogenate, as the action of enzymes my result in degradation of toxins. CEFAS always stored whole shellfish. Clarification was also required on how long the homogenate may be in contact with acetone. BS replied that the SOP (7.7) states that homogenates should not be left in acetone for more than 2 hours, and that this was based on preliminary data looking at OA stability in acetone by the Central Science Laboratory.

15. Participants agreed to use Table 1 of the report as the basis for discussion on the significance of the differences. DM noted that the difference in rotary evaporator (RE) models between labs could potentially be of significance, as it could not be ruled out that different RE's were not the cause of the atypical response. The consensus of the group was that the interpretation of the evaporation end-point (removal of solvent) was far more important than the model of the RE, and that the end-point was checked through the use of Gastec equipment. DM indicated that a ring-trial using atypical material would provide a way of determining whether the differences in RE were significant in terms of the atypical response. If all labs did not produce the

atypical response then he would conclude that the differences would have to be further investigated. However WH and KH disagreed, noting that the SOP was for the detection of classic DSP and as such no conclusions could be drawn from a ring-trial with atypical material using a DSP SOP. Furthermore, KH said that since the audit had concluded that each laboratory had adopted the UK-NRL DSP SOP and that any differences in implementation related to interpretation, then it would be more appropriate to consider that there were differences in the samples, either in relation to homogeneity or stability of the atypical agent. CA pointed out that the water backwash volume remained minimal and possibly marginal and that if the atypical problem was caused by water soluble components this could still explain the apparent random nature of atypical results.

16. There was some discussion on the length of time of the evaporation stage at each of the laboratories. DM noted the difference in evaporation time (Table 1, section 7.25) between the labs and questioned if the SOP should state a minimum or maximum time for evaporation. The laboratory representatives thought that this might result in under or over evaporation and that the judgement as to when the end-point was reached should be left to the analysts to decide. This was considered appropriate because the end-point was verified using Gastec. CH stated that DARD always evaporate for a further five minutes after reaching the end-point and that their Gastec results were always negative. WH & BS pointed out that there will always be differences in the length of evaporation because of differences between laboratories, such as ambient temperature and air pressure. WH highlighted that the important point was that all labs used a consistent end point during RE and that the DSP SOP could not be dismissed just because there were atypical DSP results. KH agreed to note DM's concerns that differences in the length of evaporation between laboratories was significant, but KH indicated that the end-point was more critical. DM asked if the time taken to evaporate DEE for each species could be recorded by the laboratories so this information can be collated.

17. KH asked if the UK-NRL DSP SOP version 2 addressed the length of evaporation time required. FM replied that both SOP versions (1 & 2) only give guidance on the minimum number of minutes and that evaporation should be continued to dryness.

18. KH summarised that the differences of opinion had been noted, but that the endpoint as identified in the SOP was the critical step. The ring trial using classic DSP samples would show if any differences between the labs were significant. The NRL would clarify paragraph 7.26 of the SOP to include that the RE should be continued until a negative GASTEC (<10ppm) result had been achieved.

Action: NRL to clarify SOP (paragraph 7.26) to state that RE should be continued until a negative Gastec is achieved.

19. DM considered that the differences in the position of the Gastec tube during sampling was significant (Table 1, section 7.26). It was noted that the SOP did not specify the position of the Gastec during sampling and therefore this area would be clarified further by the NRL. CEFAS had been using the same method as DARD's current procedure, but changed after visiting DARD in November 2003 to remove the kit whilst the headspace gas was being extracted. However, for the last month since the audit CEFAS have reverted to their previous practice of leaving the Gastec tube in the flask while sampling, and WH noted that there had been no change in the incidence of positive Gastec results reported during this time despite the changes in operation of the kit. The majority of samples were still non-detect by Gastec after RE.

20. DM raised the issue of re-suspension of the dried extract (Table-1, section 7.27) and asked if the use of an ultrasonic bath (USB) makes any difference. He noted that CEFAS used an USB on every sample and DARD and FRS probably less so, although CH noted that he used it when necessary and that this was often on every sample. DM thought that the laboratories should move towards consistency in the use of USB and that some limits should be set on the dispersion procedure. BS was of the opinion that the use of an USB was necessary to properly re-suspend the

extract and therefore provides a better dispersion. FM stated the NRL would look at the USB procedure and make a recommendation in due course.

Action: NRL to consider the use of USB for resuspension and make recommendation.

21. There was some discussion on Table 1, section 7.3, whether the homogenate and acetone could be shaken by hand prior to the use of an ultraturrax. Both CEFAS and DARD shook mixtures prior to use of the ultraturrax because the mixture otherwise formed layers which was not conducive with the use of an ultraturrax. The NRL agreed to add a line to the SOP to include instruction on shaking of acetone and homogenate.

    Action: NRL to add line in SOP to advise shaking of homogenate/acetone prior to use of ultraturrax.

22. DM thought that the ultraturrax should be dismantled, washed and cleaned between samples, thus making an acetone rinse unnecessary (Table 1, section 7.4). FRS did not rinse the ultraturraxes in acetone as they were washed in a dishwasher and it was thought unnecessary. KH agreed that washing of the ultraturrax should take place between samples. The NRL agreed to change the SOP accordingly to include 'dismantle after every sample and wash' without referring to acetone rinse.

    Action: NRL to revise SOP accordingly.

23. CA noted that the shape of the separating funnels for the DEE extraction used by the laboratories was different. CEFAS used a conical shape while DARD and FRS used a column shape. The neck size was correct at all the laboratories. The laboratories considered that the shape of the funnel was not important in terms of the end-point. FM referred to an email from Mike Scotter where he indicated that the shape would be unlikely to make a difference, and that the glassware list in the SOP was to ensure that the laboratories were using the same volume flask, as this was more important. The laboratories were all using the same volume flask.

24. CA noted that the report stated that the ‘rocking motion’ used during the DEE extraction was broadly similar between laboratories. He considered that small changes in the ‘rocking motion’ may cause emulsions to form which could effect the test result, and that when he had been to CEFAS and observed the extraction procedure, he thought that the ‘rocking motion’ employed could easily have become too violent. WH stated that if the ‘rocking motion’ had been violent, an emulsion would be formed, and would have been removed following the instruction in the SOP.

25. CA considered that the difference in the swirling motion (section 8.5.4. of the report) was significant. CEFAS hold the separating funnel vertically while FRS and DARD hold it horizontally. CEFAS indicated that the funnel was held vertically as the shape allowed effective swirling. GG agreed that the surface area of the flask that was not in contact with DEE was minimal and that he did not think this was a significant issue.

26. KH suggested that there should be a consensus between labs on what shape of separating funnels to use. CEFAS agreed to purchase the same shape as DARD and FRS if resources were made available from FSA. The FSA would consider costings from CEFAS.

    Action: CEFAS to submit costings to FSA for the purchase of ‘column’ shaped separating funnels.

27. DM noted that the filter paper sometimes split while undertaking the acetone extraction (Table 1, section 7.26). CA asked whether vacuum filtration had been considered. KH noted that work was on-going to look at the differences between filtration and centrifugation in terms of toxin recovery and that once the draft report had been considered the NRL would make a recommendation as to which method to use for the UK-NRL DSP SOP. All parties agreed that centrifugation was preferred over filtration and was likely to be a much more accurate method.

28. DM noted that DARD currently record water bath temperature for each sample and queried why CEFAS and FRS only record once a day. CH explained that this was because DARD were going for UKAS accreditation and that as much information as possible was sought. JP suggested that FRS record temperature for every sample for a month and then less frequently if the temperature was found to be consistent. KH agreed with this procedure. The NRL indicated that if a calibrated water bath was used (as at CEFAS) recording of the temperature on every sample was unnecessary. The NRL has subsequently recommended that temperature be recorded for each sample.

    Action: All labs to record temperature of water bath for every sample for 1 month to determine consistency in water bath temperature during the day.

29. CA noted that Table 1, section 4.3.1, indicated that CEFAS used distilled water to make up the Tween extract while DARD and CEFAS used de-ionised. The UK-NRL DSP SOP states distilled water. CA asked whether the distilled water used for the preparation of Tween extract by CEFAS was made in-house. BS replied that the distilled water was not prepared in-house. After discussion it was agreed that all labs would move to using de-ionised water and the NRL would change the SOP accordingly.

    Action: All labs to use de-ionised water for preparing Tween and NRL to alter SOP accordingly.

30. DM inquired if different mice were used by different labs. BS replied that all labs used the same strain of mice (female CD-1), but that for logistical reasons different suppliers were used.

31. CA noted that the volume of water used for the backwash (2x5mL) was smaller than that used in other countries and suggested that this volume should be increased. KH noted that DM was producing a proposal for the Agency's consideration looking at the efficiency of the volume of water used for the backwash, compared to greater volumes.

32. KH clarified that some amendments to laboratory procedures had already been undertaken and other minor changes discussed today would be implemented immediately. Other amendments would be incorporated into Version 2 of the UK-NRL DSP SOP. There was general agreement that the laboratories were now consistently applying the SOP and used the same criteria to assess clinical signs to determine the bioassay end-point, and as such it was felt that a position had been reached whereby a ring-trial could now be undertaken.

Action: UK NRL to write to all labs with the agreed clarifications to the DSP SOP which are to be adopted immediately.

33. DM stated that if atypical samples where included in the ring-trial then it would demonstrate whether the atypical response can be reproduced between laboratories. Since all laboratories are using the same SOP, it should be possible to achieve reproducibility providing that the sub-samples are treated equally and homogeneity is not a problem. WH disagreed because nothing is known about the stability of agent(s) causing the atypical response. CH agreed with this point and as such one would have to be careful in drawing any conclusions from ring trials with atypical DSP samples. CH also pointed out that the current UK-NRL DSP SOP was designed for classic DSP toxins. WH thought that a ring trial with atypical DSP samples is not useful until something is known about its stability. KH agreed that there is confidence in the UK-NRL DSP SOP but that the ring trial results would have to be carefully interpreted as it was possible that, depending on outcome, a number of possible conclusions could be drawn.

Fish and Shellfish Branch
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