An Investigation of the Substitution of Scrapie Brain Pool Samples

A report for DEFRA

November 2001
Summary

The BSE/Scrapie Rendering and Subsequent Experiments

The Institute for Animal Health (IAH) carried out two series of experiments during the 1990’s to establish which sort of rendering process would be the best way of destroying Transmissible Spongiform Encephalopathy (TSE)-infected material. The first series, in 1990, involved the brains from cows with destroying Bovine Spongiform Encephalopathy (BSE). The second series, in 1992, involved brains from sheep with scrapie. In each set of experiments, brains from hundreds of animals from all over the country were collected, pooled and thoroughly minced and mixed. This produced a “brain pool” of several hundred kg of material – enough to divide into about 20 lots, each big enough to put through a small-scale rendering process.

In 1997 IAH started a second series of experiments using the scrapie brain pool material stored since 1992, to see whether scrapie-infected sheep material might have caused BSE. These experiments were inconclusive, so were extended in 2000. The new aim was specifically to test whether BSE was present in the scrapie brain pool (SBP) used in the rendering experiment.

Was it Contamination? - Previous Work

If a BSE-like signal was confirmed in the scrapie brain pool, one possible explanation for this was that it might have been contaminated with some BSE-infected cow brain. DEFRA commissioned two pieces of work, from us (Risk Solutions) early in 2001, and from the Laboratory of the Government Chemist (LGC) in September 2001, to explore this possibility.

We were asked to assess the risk of contamination occurring. Our conclusion, on the basis of the information available up to August 2001, was that low-level contamination of the scrapie brain pool with BSE was quite likely, but that much greater levels of contamination would be needed to explain the observed results, and that this was very unlikely.

LGC were commissioned to test a sample of the remaining scrapie brain pool material that had been stored since 1992 at IAH, to see whether it contained any cow DNA. The clear answer was that the sample received and tested at LGC was substantially cow material suggesting that substitution of the scrapie brain pool material had occurred at sometime prior to the test being carried out.

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1 Risk Solutions is a trading name of Risksol Consulting Limited, the wholly owned management consulting subsidiary of AEA Technology plc.
This Study

In October of this year DEFRA commissioned Risk Solutions to carry out an investigation with the specific objective of determining that if the sample analysed by LGC was cow:

- How this could have happened
- When this might have happened - what experiments would be affected
- What was the most likely source of substituted material

How and When Could Substitution Occur?

Substitution could in principle have occurred at any one of five points:
1. at the rendering plant where the scrapie brain pool was prepared,
2. at IAH before the rendering experiments were begun in 1992,
3. at IAH before beginning the experiments in 1997,
4. at IAH when samples where taken to send to LGC, or
5. at LGC.

The last (point 5) of these possibilities was outside the scope of this work. To evaluate the other possibilities, we examined documentary evidence, interviewed people involved in the experiments, and examined the facilities where various stages of the experiments were carried out.

We cannot be certain where and when the apparent mix-up occurred. Neither can we establish definitively where the cow material found in the LGC tests could have come from. There are several sources of uncertainty. Many of the relevant events happened a long time ago. Documentation has not always been retained. Some people have left the organisations involved and could not be traced, others’ memories are sketchy. The labelling and control of samples at key points in the process was of variable quality.

On the basis of the information provided we cannot rule out the possibility that substitution occurred at Point 2 above, and that the original rendering experiment was also carried out on the wrong material. However, we believe that the most plausible scenario is that incorrect material was selected in 1997 at the beginning of the second series of experiments (Point 3 above), and that this material was then submitted for the LGC tests. Our principal reasons are:

2 We think it most likely that it came from the parallel rendering experiments on a cow brain pool that started in 1990. Our reasons are:
- both came from the same source (i.e. the rendering plant) in similar containers in the 1990’s
- the same project manager was involved in both experiments, and the containers were thus labelled in a similar manner
- both samples were stored in the same freezer (i.e. that of the Project Manager at IAH)
- no sample has been found that can be clearly identified as the remaining cow brain pool material
- the material sent to LGC was consistent in appearance with previous descriptions of the cow brain pool.
• some key staff at IAH changed between the 1992 and 1997 experiments, and

• we know from the present day staff at IAH, who carried out the 1997 experiments, that they were unsure about the labelling on the samples of brain pool material they found in the freezer.

The staff at IAH were sufficiently unsure about the samples that they commissioned a DNA genotype test on the brain pool material in the freezer before starting the 1997 experiments. This showed that the sample definitely contained sheep material, but did not exclude the possibility of other material also being present.

We cannot resolve the uncertainties by examining currently available information. There are still samples available in frozen storage of several, though not all, of the materials used in the experiments. If a suitable test could be defined (bearing in mind the nature and condition of the samples) to determine which species these samples came from, then further analyses might help reduce the uncertainty about when the mix-up took place. Any such tests would need to be very carefully designed and executed in negotiation with DEFRA to ensure that they provide auditable and unambiguous results. They would not tell us any more about the source of any cow material substituted.

Whatever the results of any future tests and analyses it is clear that the scrapie brain pool material and the experiments carried out on this material from 1997 onwards cannot be relied upon to determine whether BSE was present in the British sheep flock in the early 1990s because:

• The standard of quality control applied to this experiment, in particular the standard of labelling and control of storage, was not sufficiently rigorous for this application.

• The provenance of samples cannot be determined with absolute certainty. The tests can only reduce uncertainties with respect to the origin of materials used in the experiments not eliminate them entirely.

• The results of the experiments will be difficult to interpret. There is no definitive test that can unambiguously differentiate BSE from scrapie where mixtures may be present, and in particular there is little experience of working with brain pools.

• We cannot rule out the possibility that the scrapie brain pool is contaminated with sufficient cow material to affect the experimental results.

Acknowledgements

Risk Solutions are a consultancy specialising in the management of risk and the review and improvement of management systems from a risk based perspective. While we are familiar with the techniques applied in the experiments we are not experts, nor are we experts in genotype tests. We are very grateful to the experts at IAH, VLA and DEFRA who have helped us with some of the technical aspect of this investigation.

We are especially grateful to the people involved in the experiments and the staff of IAH, Prosper de Mulder, DEFRA and VLA Weybridge for the information they have provided and the help they have given us throughout the audit.
An Investigation of the Substitution of Scrapie Brain Pool Samples

Customer: DEFRA

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1 Introduction

This is the final report of an investigation commissioned by DEFRA from Risk Solutions. It addresses when, how and with what the apparent substitution of bovine (cow) for ovine (sheep) brain material happened in experiments carried out at the Institute for Animal Health during the 1990’s to the present day. This introduction explains the background and scope of the investigation.

1.1 Background

In 1992 a complex series of experiments were initiated at the Institute for Animal Health (IAH) involving sheep brains collected and macerated to form a brain pool containing scrapie agent (the scrapie brain pool (SBP)). The experiments aimed to evaluate the efficacy of alternative rendering methods for destroying scrapie infective agent.

In 1997 a second series of experiments were begun designed to see if:

- the process of rendering selected strains of TSEs with BSE-like properties from the pool of natural scrapie infected sheep brains; or
- whether the rendering processes in some way induced changes in the infectious agent such that it took on BSE-like properties.

The aim of the experiments was to explore whether a scrapie agent could have caused BSE. The results of these experiments were inconclusive and the experiments were extended in 2000. At this stage the objective was restated as being ‘to test whether evidence can be obtained to support the suggestion that BSE was present in the sheep brain pool used in the rendering experiment.’

If BSE is confirmed in the SBP this could have occurred for a number of reasons. Risk Solutions were previously asked by MAFF (now DEFRA), early in 2001, to examine the possibility that the results could be explained by contamination with bovine material at some stage during the experiments. In this initial study, with the evidence presented to us at that stage, we concluded that contamination was a possible but unlikely cause of the result. The likelihood of getting sufficient contamination to produce the results obtained, as then described to us, was very low.

DEFRA also commissioned a DNA analysis from LGC to establish whether bovine material was present in the SBP (samples of which were still available held in cold storage at IAH), and at what level, to determine whether contamination could have been a possible cause.

These DNA tests have also recently been completed. They concluded that the material was substantially bovine; suggesting that substitution of the samples with bovine material occurred, either at some point during the experimental process, or just prior to the LGC DNA test.

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1.2 Terms of Reference

The audit has the objective of determining the following:

1. The point at which we can say we are certain beyond ‘reasonable doubt’ that the substitution of scrapie brain pool for bovine material had occurred.

2. The point, at some stage prior to this that it seems likely substitution could have occurred – if this is different

3. The extent of substitution - for example could rendered samples be involved as well as the scrapie brain pool, are other experiments impacted

4. As far as possible, the provenance of the materials used in each experiment.

We were asked particularly to address the following points raised by the CVO (Chief Veterinary Officer):

i. The origin of the samples sent to the LGC in which only bovine brain was identified to assess the provenance of these samples, how far back they can be traced, and whether the mice from which the BSE-like strain had been identified were inoculated with this material.

ii. To assess the audit trail within the IAH of the brains collected during the 1990/92 period from the Veterinary Investigation Centres (VICs) and submitted to the IAH - E. There may also be some need to check the audit trail from the VICs as well as through the IAH.

The CVO also asked for a report on the audit trail for archived material in general, which is held by the IAH and compare the situation at IAH to the standard that would be seen in any other research institute in the country. This issue has been addressed by UKAS in their audit of procedures and documentation at IAH, and therefore has not been addressed in detail in this audit.
1.3 Structure of this Report

The structure of the report is as follows:

Section 1 Background: Provide background information on experiments carried out, the terms of reference.

Section 2 Method: Describes the approach we have adopted and the challenges we are addressing in this work.

Section 3 Description of experiments: Details of our current understanding of the experiments, including purpose of the experiments and the process followed including handling, storage and labelling of the samples.

Section 4 Review of process, experiment and test results: General observations regarding standards of quality assurance applied, discussion of experimental and test results and our conclusions based on these investigations.

Section 5 Substitution scenarios: Identification and discussion of the various different substitution scenarios.

Section 6 Conclusions: Provides our conclusions regarding the likelihood of substitution scenario occurring and details tests that might reduce the outstanding uncertainties.

Section 7 Recommendations: Summarises our recommendations, which will further assist the investigation into when the substitution may have occurred.

Annex 1 Provides a summary of key abbreviations and terms.

Annex 2 Contains a description of the list of institutions and associated activities undertaken for this study.

Annex 3 Contains a detailed process map of the various experiments reviewed in this study.
2 Method

2.1 Our Approach

We have gathered information through:

1. Interviews - as far as possible with people directly involved in the experiments,
2. Examination of documentary evidence, including:
   a. Laboratory books, files and diaries recording day to day activities, observations and experimental results,
   b. Central filing systems providing records of experiments, and
   c. Observation of materials still in storage.

Our interviews and visits have all now been completed. A list of institutions visited and the interviews and activities undertaken is provided in Annex 2.

We have used the information collected to identify and evaluate the most likely substitution scenarios, using a systematic process as follows.

• Working forward from collection of brains at Veterinary Investigation Centres (VICs) and backwards from despatch of samples from IAH for DNA analysis at LGC, we have developed a detailed process map showing how the scrapie brain pool (SBP) samples (pure SBP and samples containing SBP prepared using different rendering protocols) were handled.

• As part of this process we tried to identify experiments involving bovine material that could have been candidates for substitution. We were told that a wide range of bovine experiments had been carried out. We could not include all these in the study in detail but one clear candidate emerged. This was a previous rendering experiment carried out by the same team on BSE brain pool (BBP) material. The process map was expanded to include explicitly movement of samples from this experiment. The possibility that an unknown experiment was the source of the substituted material was not ruled out.

• Using the process map, we developed a complete list of all possible points at which substitution could have occurred prior to that point (substitution scenarios).

• We examined the documentary and interview evidence, test results and other data to try to establish the point at which we can say we can be sure beyond ‘reasonable doubt’ that the substitution of scrapie brain pool sample for bovine brain pool occurred.

• We reviewed the evidence for and against each scenario and rated each in terms of an estimate of the likelihood of its occurring at that point.

• The detailed findings were supplied to IAH so that factual information could be checked and additional information supplied. Their comments were taken into account when preparing the final report.
• Finally we have recommended tests that can help establish the nature of the material used in each experiment.

2.2 Challenges to Investigation

Retrospective investigations and assessments carried out some significant time after events of significance occurred (as is the case here) can typically suffer from:

1. Sparse documentary evidence because of destruction and loss of files, coupled with less rigorous quality and documentation systems than are common today
2. Movement of samples, removal or refurbishment of facilities
3. Difficulty tracing people who were personally involved in the experiments
4. Unclear or distorted memories
5. A reluctance to share knowledge fully.

To counter these potential problems we aimed to find and interview multiple witnesses at each stage of the process and to review in detail files kept at the time the experiments were carried out. We have, as far as possible observed the experimental facilities, storage locations and samples from the experiments remaining in situ. Difficulties arise where the memories or expert input of just one or two people are central to understanding what has occurred.
3 Description of the Experiments

In this section we describe experiments carried out on the SBP materials and on BSE (bovine) brain pool (BBP) materials that we believe are relevant to this study. In Section 3.1 we provide an overview of the experiments. In Section 3.2 we describe the experimental process concentrating on movement, storage and labelling of materials.

It needs to be recognised that the events in question happened a long time ago, and that the original experiments were designed for a quite different purpose other than to determine whether there was BSE present in the brains of sheep with scrapie. Some people have left IAH and the other organisations involved and could not be traced, others’ memories are understandably sketchy. Not all of the relevant documentation has been retained, and much of the earlier documentation was not concerned (given the purpose of the original experiments) with the detailed provenance of the brain pool material used.

By reviewing documentation, interviewing key people and visiting stores we have developed what we believe is a reasonably accurate description of the movements of the SBP and BBP samples used in the IAH experiments from 1990 to the present day.

3.1 Overview of the Experiments

Three sets of experiments were carried out by the IAH on rendered bovine or ovine material.

The 272R series of experiments carried out between 1990 and 1996 was designed to explore the efficacy with which different rendering processes destroyed the scrapie infective agent. It was carried out on materials derived from a pool of macerated (or minced) sheep brains (the SBP) suspected to be infected with scrapie.

Three types of sample were prepared:

- Macerated Brain Pool Material - designated Protocol A - a sample of pure brain pool analysed to determine the starting titre of infectivity.
- Meat and Bone Meal (MBM) from different rendering processes - designated Protocols B to U - samples “spiked” with SBP material so that the efficacy of each of the protocols at destroying the infective agent could be judged.
- Tallow formed in rendering processes S and T - designated Protocol St and Tt.

Aliquots taken from each of these protocols were mixed with varying concentrations of antibiotic solutions to make an inoculum, which was injected into mice. The mice were then monitored for signs of disease and examined on death for evidence of BSE or scrapie.

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4 Based on the information supplied to us.

5 The leading number (2 or 3) in the experimental reference number indicates whether the material is expected to contain scrapie (2) or BSE (3) the next two or three digits refer to the experimental series.
depending on the type of brain material used (this type of experiment is called mouse bioassay).

The series of experiments begun in 1997 was carried out on what was believed to be a subset of the materials used for the 272R experiments. The experiment was initially designed to determine if:

- the process of rendering selected strains of TSEs with BSE-like properties from the pool of natural scrapie infected sheep brains; or

- whether the rendering processes in some way induced changes in the infectious agent such that it took on BSE-like properties.

The experiments were extended in 2000 to test whether evidence could be obtained to support the suggestion that BSE was present in the sheep brain pool used in the rendering experiment. This experiment involved a full strain typing analysis carried out by mouse bioassay. Samples from protocols A, F and H were used.

The series of experiments was a companion experiment to the 272R series and was designed to explore the efficacy with which different rendering processes destroyed the BSE infective agent. It was carried out on materials derived from a pool of macerated (or minced) cow brains (the BBP) known to be infected with BSE. Collection of brains started at the same time as the 272R experiments but sufficient bovine brains were obtained very quickly and the experiment ran about 2 years ahead of the scrapie experiment. The experimental processes followed for the 372R and 272R experiments were almost exactly the same. Samples from this experiment were considered the most likely source of any substituted bovine material.

### 3.2 Experimental Processes

#### 3.2.1 The Process Map

A detailed process map has been developed to show our understanding of:

1. Movements in and out of stores,
2. Interventions carried out on the samples,
3. Details of containers, labelling and sampling, and
4. Results of tests and experiments.

The detailed process map is included as Annex 3. It illustrates the complexity of the experiments and the relatively large numbers of movements of material involved.

Figures 3.1 and 3.2 summarise our understanding of the principal tests and movements between facilities of SBP materials (or materials believed to be SBP materials) and BBP materials. The letters in brackets indicate the protocols involved. Additional requests for materials were received from time to time from other institutions, these are not shown on the figures.

Figure 3.3 illustrates the timing of the experiments.
Figure 3.1 – Movement of SBP & SBP Rendering Samples

Figure 3.2 – Movement of BBP & BBP Rendering Samples
Figure 3.3 - Time Line of Experiments

Injection of Mice 372R Series

- Collection of Brains
- Trials
- Mice
- Incubation Period
- Injection Toxicity 1W
- Tallow 1st & 1st
- Toxicity 1A
- 1V

Injection of Mice 272R Series

- Collection of Brains
- Trials
- Incubation Period
- Injection Toxicity 1W
- Tallow 1st & 1st
- Toxicity 1A
- 1V

Protocol A- Genotype test

Injection of Mice 246A-1 Series

- Incubation Period
- Injection

Glyco-typing tests

LGC DNA Tests

Remaining 372 Samples in Freezer ET033 in IAH-E

272 samples in Freezer ET033
246 samples in Fridge/freezer 58

Incubation Period - 246 2nd Passage

Tallow sent to Lab from IAH-E?
3.2.2 Storage and Movement of Material

Table 3.1 describes the different storage locations used during the course of the experiments. In the sections below we describe how materials were moved between these stores using information derived from examination of documentation, interviews and examination of the storage facilities themselves where possible.

Table 3.1: Summary of Storage Locations

<table>
<thead>
<tr>
<th>Organisation</th>
<th>Storage</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosper de Mulder</td>
<td>Chest Freezer</td>
<td>Used to store raw materials for rendering experiments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No other materials stored in these freezers when preparation of scrapie protocols carried out</td>
</tr>
<tr>
<td></td>
<td>Upright freezer(s)</td>
<td>Used to store excess materials from both BSE and scrapie rendering products in a range of different sized pots, also some intermediate products during preparation of rendering protocols and final samples for transport to IAH - E (probably in ~100ml Medfor, clear, poly pots or ziplock bags).</td>
</tr>
<tr>
<td>IAH - E</td>
<td>Freezer ET 0333 (-20°C chest freezer)</td>
<td>Used to store protocols during BSE and scrapie rendering experiments. BSE materials seem to have been stored in a polystyrene box (later a yellow bag marked BSE rendering samples) in the bottom of the freezer, scrapie materials in a black plastic bag (later a yellow bag marked scrapie rendering samples) in a freezer basket. Containers were generally ~100ml Medfor clear polypots or ziplock bags. The McCartney tubes used in 246 (see below) were taken from this freezer, (from the freezer basket) prior to preparing the inoculum in March 1997. Other materials were stored in the freezer and in the freezer basket throughout the experiments.</td>
</tr>
<tr>
<td></td>
<td>Fridge/freezer 58 (up right freezer)</td>
<td>Used to store the brain pool samples (the 3 McCartney tubes) transferred from the freezer ET 0333 and supernatant prepared from the rendered protocols F and H (25 ml universals) used in the full strain typing study (246) and subsequently samples taken for the DNA tests.</td>
</tr>
<tr>
<td></td>
<td>Ultrafreezer ET 0821</td>
<td>Freezer used to store all relevant samples found at IAH - E (other than those stored in the animal facility), following release of the LGC test results in 2001.</td>
</tr>
<tr>
<td>Freezers in Animal Facility</td>
<td>Fridge/freezer</td>
<td>Used to store inoculum for the mouse bioassay experiments (272R, 372R and 246)</td>
</tr>
<tr>
<td>IAH - C</td>
<td>Freezers in Animal Facility</td>
<td>Used to store supernatant and brain pool material received from IAH - E and inocula for the mouse bioassay experiments (272R and 372R)</td>
</tr>
<tr>
<td>VLA Weybridge</td>
<td>Storage</td>
<td>Used to store excess material from Prosper de Mulder</td>
</tr>
</tbody>
</table>

Note that the naming convention adopted for the freezers in this report has been chosen to be consistent with the UKAS audit report.
• **Movement of material from VICs to PDM**

The brains of sheep suspected to have scrapie and of cows suspected to have BSE were collected at Veterinary Investigation Centres (VICs) in the early 1990s. The brains were stored at the VICs and in late 1990 cow brains were transported to a rendering plant run by Prosper de Mulder (PDM) for preparation of the samples for the 372R experiment. The brains were macerated and mixed in a pilot scale rendering plant in dedicated areas segregated from the full-scale rendering plant, and one or more samples of pure brain pool material were taken. Aliquots of the remaining brain pool material were then taken to prepare the rendering protocols.

The same process was followed for the scrapie experiment in September 1992. VICs collected approximately 3000 sheep brains that were transported to PDM for preparation of the macerate. While it is possible that some cow brain parts or other material may have been included with the brains delivered from the VIC centres, records and interviews confirm that these could only have formed a small proportion of the brain pool.

Prior to maceration, the sheep brains were stored in chest freezers which were completely empty prior to delivery of the sheep brains. At the time the scrapie samples were being prepared no whole bovine brains left over from the previous experiment remained in storage on site. However, there was excess brain pool macerate and rendered samples prepared for the bovine experiments were kept on site. They were kept in a storage location that was also used to temporarily store intermediate products produced in the scrapie experiment and final samples prior to dispatch to IAH. The samples should have been kept in separate freezers though we have been unable to verify this.

• **From PDM to IAH - E**

The BSE material was despatched in one batch to IAH - E in 1991. Detailed records kept at PDM of the preparation and transport of the samples have now been destroyed. From the memories of people involved in the preparation of the protocols and from observation of material remaining at IAH - E, it seems clear that BSE brain pool and rendering samples prepared at PDM were packaged for transport to IAH - E in ~100ml Medfor clear plastic pots. These pots were very simply labelled with the protocol number, and generally some details of the rendering process e.g. maximum or average temperatures. Neither the species or date were recorded. The BSE brain pool sample (protocol A) is noted on receipt as appearing “mushy” as if it had defrosted and been refrozen.

The scrapie material for the 272R experiments was sent in 2 or 3 batches in 1992 and 1993. How this was packaged is less clear. Fewer samples remain in storage at IAH - E and a number of these were additional samples forwarded from PDM in 1995 when the 272R experiments were well underway. The samples remaining in store are generally packaged in ziplock bags labelled with the protocol letter, date and a single letter G. We believe the G probably stands for greaves7.

The samples that arrived in 1995 are packaged in medfor pots and labelled with the protocol letter and the letter M (for meal?). There is no indication of species or date but correspondence on file suggests that these are scrapie samples.

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7 Greaves is a product produced towards the end of the rendering process. Meal is produced by grinding the greaves. It is not clear why greaves and not meal are in store at IAH - E. We understood that the experiments intended to use meal.
• At IAH - E

Materials were stored in a -20°C chest freezer (referred to throughout this report as freezer ET 0333). Throughout the experimental period this freezer was used to store a large number of experimental samples. Including bovine samples. The freezer was tidied from time to time and samples without labels discarded. No logs of the freezer were kept. Staff at IAH - E who had access to the freezer remember BSE rendering experiment samples being stored in a polystyrene box (later a yellow plastic bag) both marked BSE rendering samples, on the floor of the freezer. Scrapie rendering experiment samples were stored in a black bag (later a yellow plastic bag) both marked scrapie rendering samples in a basket of the freezer.

After receipt of the BSE rendering experiment samples at IAH - E the labelling convention was changed and the containers additionally labelled with the new scheme on autoclave tape consisting of two letters, the protocol letter and M (for meal?). It is not clear why the convention was changed but as the new scheme also employed letters, opportunities for confusion would have increased. There is no indication from the project files which scheme was adopted for the scrapie samples. As the final published papers clearly adopt the new scheme for both BSE and scrapie experiments it is likely that the new scheme once adopted was used from the beginning for the scrapie experiments.

The labelling schemes do not uniquely identify the species, but examination of containers remaining in store at IAH - E and the VLA (see below) allow patterns to be identified. For example BSE samples generally include the protocol letter and details of the rendering process. Samples received at IAH - E for the 372R experiments were given additional labels at IAH on autoclave tape using the new scheme. Scrapie samples are very often dated with the date they were prepared (which clearly distinguishes them from BSE samples) and often, but not always, with two letters - the protocol letter followed by an M (for meal?), G (for greaves?) or T (for tallow).

We observed a number of samples in store at IAH - E that do not fit clearly into either scheme. These include a number of duplicate samples of some rendered protocols found in the BSE bag. The duplicates are not labelled in either the conventional BSE or scrapie fashion. There is no evidence that these are not BSE samples, but we can have less confidence in the provenance than for those samples labelled with the BSE convention.

Duplicate samples of tallow (2xST and 2xIT) were found in the scrapie bag. All four samples are in 500ml bottles. One set is labelled directly on the bottle with the date establishing them as scrapie samples. The other is labelled on autoclave tape and additionally on coloured labels. They are not dated. No tallow was found in the BSE bag but there were two empty ziplock bags labelled ST and IT. It is possible that BSE tallow samples have been stored in the scrapie bag at some stage by error. The two undated containers are marked Scr (for scrapie) on the lid but these markings were added relatively recently on the basis that the samples were found in the scrapie bag. It was noted in the project files in 1996 that only a very small amount of tallow remained in the BSE bag, and that this had been dispatched for testing. This provides a possible explanation for why no tallow samples are to be found in the bag now, though of course this could be because the main sample bottles had been returned to the wrong bag previously.
• **Aliquots for 272R to IAH - C**

Once received at IAH - E, aliquots were taken at various times from the samples for various tests and experiments. In particular brain pool material, brain pool homogenate and supernatant prepared from the rendered protocols was dispatched in batches to IAH - C in 1991 for the 372R (BSE) and in 92 and 93 for the 272R (scrapie) mouse bioassay experiments. A sample of brain pool homogenate was also prepared and submitted to the animal facility at IAH - E. Previously samples for the BSE rendering study (372R) had been dispatched to IAH – C in 1991.

We have no documentary evidence of how the aliquots were packaged, but observation of samples remaining at IAH - C shows that homogenate and supernate material supplied by IAH - E was packaged in glass universals with gold metal tops. Labelling conventions (and handwriting) varies indicating that different people were probably involved in preparation of the aliquots at IAH – E. Glass universals were also used at IAH - C to store inoculate prepared from these materials.

• **From freezer ET 0333 (chest freezer) to fridge/ freezer 58 (upright freezer)**

The 246 series of experiments was begun in 1997 and memories of these are clearer. File notes indicate both the type of container and the labelling. The material believed to be the Protocol A SBP material was located by the principal technician in the freezer ET 0333 adjacent to, but not in, the scrapie bag. The material was packaged in 3 McCartney tubes, labelled with unusual orange labels in the hand of the project manager. The labels identified the material as ‘rendering sample A (brain pool)’ but didn’t identify the experiment reference, species or date. The technician was not involved in the 272R experiments and was sufficiently uncertain about the sample to query its origin with the group leader and to subject the material to a genotype test. The result of this test confirmed that ovine material was present in the sample and the 246 series of experiments was commenced on the basis that this must be the SBP material prepared for the 272R rendering experiments.

From the time the McCartney tubes were located to the time the samples were taken for DNA analysis they were kept in a separate freezer (called here the fridge/ freezer 58) under the control of the principal technician who has a reputation as a careful and reliable worker.

A Medfor pot labelled ‘A S...’ has, since the start of this audit, been found with scrapie rendering samples in the freezer ET 0333. The labelling is blurred but it seems very likely that this is the original brain pool sample; this doesn’t necessarily preclude the material in the McCartney tubes being sub aliquots of this.

Two samples of rendered materials (bags of greaves labelled F and H and clearly dated) were also removed from the freezer ET 0333 at this time. Aliquots were removed from which supernatant was prepared for the 246 experiments. The greaves were returned to the freezer ET 0333 and the supernatant stored in glass universals was labelled and stored in the fridge/ freezer 58.

• **Aliquots for 246 inocula, glyctype tests and DNA tests**

A sample was removed from one of the McCartney tubes (labelled 1 by the technician) and submitted for genotyping within IAH in 1997 as described above. The same tube was then supplied to the animal facility at IAH – E for the 246 mouse bioassay experiment. An aliquot of brain pool material was removed for preparation of inocula and the tube
returned. There are reasonably clear memories of the type of container and labelling supplied and the nature of the material that was noted to be in poor condition, very dark and liquid and unlike brain pool, but consistent with a sample that had been defrosted completely and refrozen. These tubes were only handled after this point to remove samples for glycotyping (from tubes 1 and a second tube labelled at this time 2) and the samples for the LGC DNA analysis (all three tubes). They were always handled by the principal technician who has a clear memory of all the interventions.

The glass universals containing the F and H supernatant were also supplied to the animal facility, aliquots were removed there for preparation of inoculum and the containers were returned to the 246 freezer.

- **From freezer ET 0333 and fridge/freezer 58 to ultrafreezer ET 0821**

On discovery of the possible substitution in September 2001, materials considered relevant were moved from the freezer ET 0333 and fridge/freezer 58 to an ultrafreezer ET 0821 that could be locked.

- **Excess materials at PDM and VLA**

Excess material was stored in a range of different types of container and kept at Prosper de Mulder before transport to VLA Weybridge in 1997, where it remains. The BSE material was labelled with the protocol number, and generally some details of the rendering process e.g. maximum or average temperatures as described above. The scrapie material was labelled with the protocol number and the date. Some samples are labelled very simply, with no dates. It is not possible to know what these samples derive from simply from the label.

From time to time additional samples were sent to IAH - E or to other research institutes direct from Prosper de Mulder. These include BSE samples sent to continental research institutes in 1991, and unspecified samples sent to IAH in 1995. IAH also received a request for materials from MAFF (now DEFRA) in 1995, it is not clear if these were sent.

The Prosper de Mulder scientist who understood the significance of the materials left the company in 1993. While we understand that BSE and scrapie materials were originally kept in separate freezers, certainly by 1996 they had been moved into one freezer. These two factors would considerably increase the risk of incorrect samples being supplied by PDM when requested.

Table 3.2 summarises the remaining samples that have been located.
### Table 3.1: Location of remaining samples (note a range of excess samples from PDM are now also stored at VLA)

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Samples still at IAH - C Freezers</th>
<th>Samples still at IAH - E</th>
<th>Animal facility</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>372R-1A various dilutions no undiluted brain pool 272R-1A brain pool and various dilutions</td>
<td>Freezer ET 0333 BSE Bag</td>
<td>Medfor labelled A S ...</td>
<td>372R-1A Brain pool 372R-1V</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Freezer ET 0333 Scrapie Bag</td>
<td>3x McCartney tubes labelled Rendering Sample A (brain pool)</td>
<td>272R-1V</td>
</tr>
<tr>
<td>B-U</td>
<td>372R-1B, C, D, E, I, J, K, L</td>
<td>Medfors labelled B, C, D, E, J, batch F Medfors with rendering details and following: On tape on label BM F CM J DM I FM G GM K HM L IM N JM M KM P LM Q MM B RM D SM C TM R UM Q QM E</td>
<td>Compton sample- 372R-1B dated Jan ’92. And labelled BSE Brain - Should not be labelled as BSE Brain as it is a rendered sample. Compton sample- 372R-1S dated Dec 92. Date not correct for BSE. Samples B, C, D, E, J in BSE bag not consistent with BSE labelling. A fax dated Aug 95 states more Protocol B, D, E, J samples will be sent (details supplied consistent with scrapie samples) these not found in scrapie bag with samples delivered in 9/95 and mentioned in the same fax. There is however no memory of receipt of additional samples at that time and a likely explanation is that they were not sent.</td>
<td></td>
</tr>
<tr>
<td>Protocol</td>
<td>Samples still at IAH - C Freezers</td>
<td>Samples still at IAH - E</td>
<td>Fridge/freezer 58</td>
<td>Animal facility</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------</td>
<td>--------------------------</td>
<td>------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Tallow St</td>
<td>Non located</td>
<td>Large empty bag with St on it no date. Large empty bag with It on it no date.</td>
<td>2 Large containers. 1 with ST only, the other with ST and dated Sept 92. 2 Large containers. 1 with IT only, the other with IT and dated '92.</td>
<td></td>
</tr>
</tbody>
</table>
4 Review of Processes, Experiment and Test Results

In this section we review the experimental processes, experiment and test results.

4.1 Quality Assurance of Experiments

Our review of the experiments has been limited to matters relevant to the substitution, that is the information on handling, storage and labelling of materials. We have not carried out a formal audit against modern standards (refer to the UKAS audit report for this) but our review of the files and visits to facilities revealed a number of instances of good practice and some significant areas for improvement.

4.1.1 Project Management Files

The rendering studies (372R and 272R) were project managed by IAH - E. The Project Manager’s files were available for inspection. The file for the Scrapie rendering study (272R) was found to be particularly sparse. More information was available on the BSE rendering study (373R) completed some two years earlier. Unfortunately files kept at PDM that described the preparation of samples were destroyed some time ago. It was not possible from the information kept on the files to trace all the movements of the samples.

The subsequent strain typing study (246) was also project managed at IAH - E by the same project manager. The working files of the principal technician, working under the supervision of the project manager, were provided to Risk Solutions. The project managers’ files were we understand also available, but were not reviewed in detail. The working files provided a reasonably complete picture of the movement and use of the 3 McCartney tubes located prior to the 246 series of experiments and believed to contain SBP material.

4.1.2 Storage and Labelling of the Samples Outside the Animal Facilities

Labelling of the samples was generally poor. Poor labelling started from the packaging of the samples before they left the rendering facility and were sent to IAH - E for testing. Dates, experimental references and species were all omitted making it impossible to distinguish between the BSE rendering experiment samples and scrapie rendering experiment samples.

Samples were not fully labelled on receipt at IAH - E and storage was informal. No logs of the relevant freezer (the -20°C chest freezer designated the freezer ET 0333 here) were kept, samples were stored loose in large bags and boxes in the freezer. Most of the samples still retained at IAH - E have a style of labelling consistent with their storage location (ie samples labelled with what appears to be the standard BSE labelling convention were found in the BSE bag). A small number of samples (specifically 4 or 5 samples observed in the BSE bag and two tallow samples observed in the scrapie bag) have less characteristic labelling and we can have less confidence that these have been stored correctly (see Section 3.2.2).

Labelling of samples and control of storage improves at the start of the 246 experiments but is still largely informal. For example supplementary labelling was added to the outer
packaging of the McCartney's indicating that material had been submitted for testing. Materials for the 246 experiments were stored in a separate location and access to them informally controlled.

Unfortunately prior to our arrival to carry out the audit at IAH - E, materials considered relevant were moved from the freezer ET 0333 and fridge/freezer 58 to an ultrafreezer ET 0821 that could be locked. We were therefore unable to observe materials in situ. We have no reason to believe that what we were told concerning the handling of the McCartney's was incorrect.

4.1.3 Record Keeping, Storage and Labelling of the Samples Within the Animal Facilities

Quality assurance and control within the animal facilities in IAH - E and IAH - C where the 372R and 272R mouse bioassays were carried out generally appears good. This appears to have been true throughout the time the experiments were carried out. Materials were logged into freezers and experimental records provide information on origin of samples, purpose and results of experiments. Samples prepared within the facility are fully and clearly labelled. Unfortunately, we were told that the 372R and 272R experiments were considered "sensitive" at the time, perhaps because of commercial confidentiality, and full information on the samples was not recorded in the experimental files. The reader was generally simply referred to the project manager for information.

There is evidence at IAH - C of incomplete logging of samples out of the facility; undiluted BBP Protocol A brain pool material is shown as being stored in the freezer in the log, but none has yet been located. It is not clear whether this sample was returned to IAH - E. Two samples had been labelled as a BSE experiment but dated with the date of the scrapie experiment.

4.1.4 Conclusions

No clear audit trail exists for the samples used in the rendering experiments (372R and 272R). Different labelling conventions were adopted for BSE and scrapie samples, but these do clearly identify the species and do not, in most cases, allow the species to be uniquely identified. A number of samples do not clearly follow either convention and we can be less confident of the provenance of these samples. There is evidence of much better (though informal) control of samples in the 246 experiments outside the animal facilities and for all experiments once samples passed into the animal facilities.
4.2 Results of Tests and Experiments

4.2.1 Results of experiments

Both the 372R and 272R experiments involved the same basic steps shown below.

![Diagram showing the steps of experiments: Collect Brains, Macerate Brains, Prepare Protocols, Storage and Sampling, Infectivity titrations, and 1V- TSE Strain Variation tests.]

Experiment 246 involved additional mouse bioassay of SBP and scrapie rendering experiment samples stored at IAH - E.

It is reasonable to ask “why did the experimenters not notice that they were working with cow brains not sheep brains?” and “can’t the results of the experiments tell us what material was used?” We have examined both these questions below.

“Why did the experimenters not notice that they were working with cow brains not sheep brains?”

The simple answer is because for the most part they were working with brain pool macerate (minced brain material) not brains. It is not credible that staff collecting brains at VICs would have uniformly supplied cow brains or cow brain parts in mistake for sheep. We have interviewed staff at VICs and we understand from the VLA that records do not support the possibility that significant numbers of cow brains were sent to PDM in place of sheep brains. It is also very unlikely that the people preparing the scrapie brain pool would not have noticed if they were for the most part handling cow brains or cow brain parts in place of sheep brains. We cannot rule out the possibility that some cow brain material entered the brain pool at this stage but it is not feasible that the majority of the material was bovine. The substitution, if substitution occurred, must have involved brain pool macerate or rendered products.

“Why can’t the results of the experiments tell us what material was used?”

The experiments had a number of features that make the results of the mouse bioassay difficult to interpret unambiguously and lead to the possibility that substitution of the samples would be difficult to detect by examining the results of the experiments:

1. The original experiments were not designed to determine whether BSE was present in sheep. Reasonable efforts were taken to ensure that the brain pool remained free from...
contamination during preparation but the level of control applied during the earlier experiments (272R and 372R) was not to the standard applied later.

2. Mouse bioassay as a method of diagnosing TSEs is not based on a full understanding of biochemical and physical processes. It is an empirical technique that has been widely applied, for example to show v-CJD is similar to BSE and different from scrapie. It is a complex process and the results need to be interpreted by experts. It can take several years to generate a firm result. The principal data collected in the experiments are lesion profiles (patterns of lesions in the mice brains) and incubation period (time from injection of mice to onset of clinical symptoms). The type of TSE is identified by comparing the results with those of known provenance. There is no good agreed test of “sameness of lesion profile”, so in marginal cases we are reduced to using subjective observations of the form “somewhat similar” and interpretation is difficult. The incubation times in principle give a more objective signal, but the effect of concentration has to be controlled.

The mouse bioassay data that we understand has been collected and analysed at each stage of the experiments is summarised in Table 4.1. Several features of these experiments are not commonly encountered in mouse bioassay of TSEs and this makes determining the origin of the original material from the experimental results extremely difficult. They include:

a. Mouse bioassay is generally carried out on individual brains; experience of working with brain pools is very limited.

b. The BBP exhibited a low titre of infectivity, which can confound interpretation of results.

c. The BBP comprised bovine brains with the hindbrains removed. By contrast most of the BSE strain typing has been carried out on the hindbrains, which may give a different pattern of results.

d. The 272R titrations used a different strain of mice than the 372R titrations, so direct comparison of the resulting lesion profiles cannot be made.

e. The 246 experiments used brain pool which was in an unsatisfactorily autolysed state.

f. The strain typing data collected (incubation time and lesion profiles) are very sparse.

Judging the sameness or difference of samples is a less challenging task for strain typing than identifying a strain and it may be possible to compare data from the 246 experiments with both the 272R and 372R experiments to determine whether the samples are similar or clearly different. However, the data are sparse and the result is unlikely to be clear cut. Much of this work is currently unpublished.
Table 4.1: Data collected from the mouse bioassay experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Data obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>372R-1A-U (all protocols)</td>
<td>Infectivity titrations</td>
</tr>
<tr>
<td>372R-1V (Protocol A – brain pool)</td>
<td>Incubation times in a limited number of two different mice strains</td>
</tr>
<tr>
<td>272R-1A-U (all protocols)</td>
<td>Infectivity titrations</td>
</tr>
<tr>
<td>272R-1A, F, H (Protocol A – brain pool, Protocols F and H )</td>
<td>Some of the A, F and H titration mice (C57BL) were lesion profiled.</td>
</tr>
<tr>
<td>272R-1V (Protocol A – brain pool)</td>
<td>Incubation times and a lesion profile were obtained from 4 C57BL mice.</td>
</tr>
</tbody>
</table>

4.2.2 Results of tests

A number of tests have been carried out on the material believed to be the SBP brain pool sample (Protocol A) in order to determine its speciation or genotype:

1. A genotype test carried out by IAH in 1997 just prior to the 246 series of experiments
2. A DNA test carried out at the request of IAH on brain pool material in store at VLA and a protein speciation test carried on the same material

(In addition a number of glycoform tests were carried out on the material. We have been told that these were inconclusive due to the poor condition of the material.)

The tests carried out at VLA (point 2 above) were carried out on spare brains collected at the VICs and forwarded directly to VLA. These therefore confirm that sheep brains were collected but doesn’t provide any information about the point of substitution.

The results of the two remaining tests can appear to be contradictory:

IAH, 1997 ⇒ the sample contained ovine material (pure bovine ruled out));
LGC, 2001 ⇒ the sample was substantially bovine.

This can be explained in one of four ways:

1. The IAH genotype test is somehow in error (samples mixed during execution of the test, results mixed up or misinterpreted) – we have no evidence that this happened,
2. Substitution of samples occurred after the IAH test and prior to the LGC test – what we know about control of the samples during this time make this unlikely; or
3. The LGC DNA test is somehow in error (samples mixed during execution of the test, results mixed up or misinterpreted) – we have been told that the quality assurance processes applied at LGC make this very unlikely.
4. The results of the two tests are not in fact contradictory – see below.
The 1997 test at IAH used the state-of-the-art method at that time for telling apart different versions of the PrP gene; the expert who carried out the test told us that the result obtained is not consistent with a sample that was all bovine because it detected a clear signal of an allele which is found in sheep and not in cows. It could, however, be consistent with a bovine sample contaminated with ovine. We do not know how sensitive the test is (i.e. how much ovine material would have to be present in a bovine sample to produce the result observed) or how likely contamination of the sample is. This does however provide one possible explanation of the result.

4.3 Conclusions

Opportunities for substitution

The process diagrams illustrate that there were numerous occasions when material could have been confused. The poor labelling of the samples and informal storage arrangements make a swap occurring a very real possibility. Poor labelling started from the packaging of the samples before they left the rendering facility and were sent to IAH opening up the potential for substitution to occur at that point or at any time later on.

What was substituted?

The BSE (bovine) brain pool material used for a previous rendering experiment (372R) appears to be the best candidate for substitution. Our reasoning is:

- Both came from the same source (i.e. the rendering plant) in similar containers in 1990’s
- The same project manager was involved for both experiments, and the containers were thus labelled in a similar manner. (The suspect samples are labelled Rendering Sample A (Brain Pool) in the project manager’s handwriting. We are not aware of any other rendering experiments being carried out at this time.)
- Both samples were stored in the same freezer (i.e. that of the Project Manager at IAH - E)
- No sample has been found that can be clearly identified as the remaining BBP Protocol A material
- The material sent to LGC was consistent in appearance with previous descriptions of the BBP

Evidence from results of tests

The results of the tests and experiments are not consistent with substitution occurring at any point prior to sampling for the LGC DNA test in September 2001.

However, consideration of the relative degree of care taken in handling the samples at this stage, both at IAH and, we understand, LGC makes both these scenarios seem unlikely.

The possibility remains that the test results have been misinterpreted.

Overall Conclusion

The documentary and interview evidence, experiment and test data cannot tell us unambiguously, where the substitution occurred or with what material.
5 Substitution Scenarios

5.1 Identification of Scenarios

We have demonstrated above that it is impossible to say, from the documentary evidence or from the results of tests and experiments, whether substitution of materials occurred. To try to better define the possible alternatives and identify ways of narrowing down the possibilities we have therefore identified from the process map the places where substitution could have occurred.

Figure 4.1 is a simplified process map indicating the outcomes if substitution had not occurred. Green (or dark) process streams represent experiments and movements of bovine material, yellow (or light) process streams represent ovine material. The diagram assumes that the source of the bovine material substituted is BBP material from the 372R experiments; this does not exclude other sources that may be equally valid. The LGC result is not consistent with this scenario and this is indicated by the bold outlined box.

Figure 4.1 - Simplified Process Map - No Substitution

It can be seen from the figure above that there are a number of key points where substitution may have occurred, these are:

1. at the rendering plant (PDM) where the scrapie brain pool was prepared,
2. at IAH prior to the rendering experiments begun in 1992,
3. at IAH prior to beginning the 246 experiments in 1997,
4. at IAH when samples were taken for dispatch to LGC for genotyping; or
5. at LGC, (or LGC DNA test misinterpreted).

We have developed these into a number of specific substitution scenarios as described below and subjected these to systematic scrutiny.

### Substitution of Brain Material - Protocols A

<table>
<thead>
<tr>
<th>Step in Process</th>
<th>Date</th>
<th>Substitution Scenario</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection of brains at VICs</td>
<td>Sept ‘92</td>
<td>1. Bovine not ovine brains sent to PDM and/ or processed at PDM All experiments affected</td>
</tr>
<tr>
<td>Preparation of brain pool at PDM</td>
<td></td>
<td>2. Excess bovine samples not ovine samples transported from PDM All experiments affected</td>
</tr>
<tr>
<td>Preparation and dispatch of samples from PDM</td>
<td>Sept ‘92</td>
<td>3. Wrong samples selected for aliquoting from freezer at IAH - E 272R experiment affected and possibly all subsequent tests and experiments</td>
</tr>
<tr>
<td>Preparation of aliquots at IAH - E for use in the rendering experiment (Experiment reference 272R)</td>
<td>Set/Nov '92</td>
<td></td>
</tr>
<tr>
<td>Preparation of aliquots at IAH - E for use in the full strain typing mouse bioassay (Experiment reference 246)</td>
<td>Mar 97 Oct 97</td>
<td>4. (a) Wrong samples selected from freezer at IAH - E before IAH genotype test. 246 experiment and IAH and LGC DNA tests affected (b) Wrong samples selected from freezer at IAH - E after IAH genotype test. 246 experiment and LGC DNA test affected</td>
</tr>
<tr>
<td>Preparation of aliquots at IAH - E for use in the DNA analysis at LGC (A only)</td>
<td>Sept 2001</td>
<td>5. Wrong samples selected from freezer at IAH - E before DNA testing LGC DNA test affected</td>
</tr>
<tr>
<td>DNA analysis at LGC</td>
<td>Sept 2001</td>
<td>6. Wrong samples processed at LGC of results misinterpreted (A only) LGC DNA test affected Examining this scenario was outside the scope of this report</td>
</tr>
</tbody>
</table>

For each of Scenarios 3 and 4(b), substitution could have occurred:
1. when samples were removed from storage at IAH - E for processing and submission to the Animal Facilities for mouse bioassay at IAH - E or IAH - C
2. within the Animal Facilities prior to injection.

We have not in general examined the second possibility; firstly because this scenario cannot explain the LGC DNA test results and secondly because the animal facilities are more closely quality controlled than other parts of the process.
## Substitution of Rendered Material - Protocols B-U

<table>
<thead>
<tr>
<th>Step in Process</th>
<th>Date</th>
<th>Substitution Scenario</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection of brains at VICs</td>
<td>Sept ‘92</td>
<td>7. Bovine not ovine brains sent to PDM and/or processed at PDM All experiments affected</td>
</tr>
<tr>
<td>Preparation of brain pool at PDM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparation and dispatch of samples from PDM in 2 or more batches</td>
<td>Sept ’92 - ‘93</td>
<td>8. Excess bovine samples not ovine samples transported from PDM All experiments involving affected batch</td>
</tr>
<tr>
<td>Preparation of aliquots at IAH - E for use in the rendering experiment (Experiment reference 272R)</td>
<td>Set/Nov ‘92</td>
<td>9. Wrong samples selected for aliquoting from freezer at IAH - E 272R experiment affected - one or more protocols</td>
</tr>
<tr>
<td>Preparation of aliquots at IAH - E for use in the full strain typing mouse bioassay (Experiment reference 246)</td>
<td>~Oct 97</td>
<td>10. Wrong samples selected from freezer at IAH - E 246 experiment affected - F and/or H protocol</td>
</tr>
</tbody>
</table>

## Substitution of Tallow Samples

<table>
<thead>
<tr>
<th>Step in Process</th>
<th>Date</th>
<th>Substitutions Scenario</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation of aliquots for experiments 272R-St and 272R-It</td>
<td>~Feb 94</td>
<td>11. Wrong tallow samples used 272 experiment - St and/or It</td>
</tr>
</tbody>
</table>

## 5.2 Ranking Scenarios

Evidence for and against each scenario occurring has been collated systematically and used to establish a likelihood of the scenario occurring. The results are presented in Section 6. The ranking scheme is described below.

<table>
<thead>
<tr>
<th>Likelihood</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negligible</td>
<td>Evidence indicates that this scenario couldn’t have occurred.</td>
</tr>
<tr>
<td>Very Low</td>
<td>Evidence suggests this is possible but highly unlikely.</td>
</tr>
<tr>
<td>Low</td>
<td>Evidence suggests that this is not a likely possibility.</td>
</tr>
<tr>
<td>Medium</td>
<td>Possible, limited evidence against.</td>
</tr>
<tr>
<td>High</td>
<td>Evidence suggests that this is a likely scenario.</td>
</tr>
</tbody>
</table>
6 Conclusions

6.1 Scrapie Brain Pool - Protocol A

We have set out below our best judgement of how, when and with what the apparent substitution occurred. We conclude:

- **HOW**: Poor labelling of the samples combined with poor control of storage provided opportunity for substitution to occur.

- **WHEN**: Based on the information we have been given we have developed what we believe\(^8\) is a reasonably accurate description of the movements of the SBP and BBP samples used in the IAH experiments from 1990 to the present day. By applying systematic analysis to this movement “map”, we have been able to determine what we believe\(^2\) is a complete set of possible substitution scenarios. Assessment of these scenarios against the evidence currently available does not allow us to select any one clear candidate. Our analysis concludes that, if a substitution has occurred, then Scenario 4a (wrong samples selected at IAH - E for genotype testing at IAH and full strain typing (Experiment 246A)) is the most likely. Our principal reasons are:

  - some key staff at IAH changed between the 1992 and 1997 experiments, and
  - we know from the present day staff at IAH, who carried out the 1997 experiments, that they were unsure about the labelling on the samples of brain pool material they found in the freezer.

We cannot rule out Scenario 3 (wrong samples selected at IAH - E for rendering experiment 272R)

- **WITH WHAT**: Our judgement at this stage is that the BSE (bovine) brain pool material used for a previous rendering experiment (372R) has been mistaken and substituted for the scrapie (ovine) brain pool in question at some time prior to being sent to LGC.

One of the reasons for the uncertainty is the apparently contradictory evidence from the audit trail (documentary records, interview evidence and observation of samples) and the results of the two genotype tests carried out by IAH in 1997 and LGC in September. The audit trail strongly suggests that the same sample was used for both these tests (McCartney tube 1). The results of the genotype experiment carried out by IAH - E was that the sample contained ovine material and that of the DNA test carried out by LGC that it was substantially bovine. It is difficult to find any substitution scenario that is consistent both with the test results and what we can conclude about the care taken in handling, storage and labelling of the materials at each point of the experiment.

However, it is possible that the IAH result can be explained by a relatively small amount of ovine contamination and in this case the two tests may not be inconsistent but we do not

\(^8\) Based on the information supplied to us.
know the sensitivity of the test, or the likelihood that the material tested was contaminated with ovine material.

If the results of the two tests cannot be reconciled then substitution must have occurred just prior to sampling for the LGC tests, or there was an error at the LGC. Other evidence suggests that both of these are very unlikely, though examining the latter was outside the scope of this audit. The conclusions from the two sources of evidence are illustrated in the table.

**Comparison of Evidence**

<table>
<thead>
<tr>
<th>Substitution Scenario</th>
<th>Likelihood from audit trail</th>
<th>Likelihood from tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bovine not ovine brains sent to PDM and/or processed at PDM</td>
<td>Negligible</td>
<td>-</td>
</tr>
<tr>
<td>2 Excess bovine samples not ovine samples transported from PDM</td>
<td>Very Low</td>
<td>-</td>
</tr>
<tr>
<td>3 Wrong samples selected from freezer at IAH - E for aliquoting for the 272R experiments</td>
<td>Medium</td>
<td>-</td>
</tr>
<tr>
<td>4 (a) Wrong samples selected from freezer at IAH - E before genotype tests at IAH - E</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>(b) Wrong samples selected from freezer at IAH - E after genotype test and before the 246 experiment</td>
<td>Low</td>
<td>High*</td>
</tr>
<tr>
<td>5 Wrong samples selected from freezer at IAH - E for aliquoting for LGC DNA test</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>6 Wrong samples processed at LGC of results misinterpreted (A only)</td>
<td>Outside scope</td>
<td></td>
</tr>
</tbody>
</table>

*note: test cannot distinguish between 4(b) and 5

We cannot resolve these contradictions by examining currently available information. The possibilities can be narrowed down by testing samples used in the experiments to determine their species, provided suitable tests can be devised. For the 272R experiments a reasonably complete set of samples exist in the well controlled environment of the animal facilities where the experiments were carried out. These samples are excess brain pool material and inoculum used in the actual experiments and therefore will provide the most direct evidence of the species of the material used in the tests.

Unfortunately, no brain pool material or excess inoculum submitted for the 246 experiment remains in the animal facilities at IAH – E. There is, however, good circumstantial evidence that the McCartney tube labelled 1 was submitted and used for the experiment and we recommended that this is tested. Spare material prepared for the LGC DNA test could also be retested. We have indicated appropriate materials to test in the table overleaf.

These tests will tell us little about the source of any substituted material. For the reasons described in Section 4.3 above we believe the best candidate is the BBP material used in
the bovine rendering experiments (372R). If tests suggest that material was substituted at some stage prior to the 246 experiments then analysis of mouse bioassay data obtained from the 372R, 272R and 246 experiments may indicate whether the material used in the 246 experiments is similar to, or very different from, material used in the other two experiments; however the data is sparse and the results are unlikely to be clear cut.

**Recommended Tests**

<table>
<thead>
<tr>
<th>Sample to Test</th>
<th>Location of Sample</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘A S…’</td>
<td>Ultrafreezer ET 0821 at IAH - E</td>
<td>Is this ovine and therefore most probably the scrapie brain pool Protocol A?</td>
</tr>
<tr>
<td>272R-1A brain pool and inoculum</td>
<td>Animal facility at IAH - C</td>
<td>What material was supplied/ used for the 272R-1A titration?</td>
</tr>
<tr>
<td>272R-1V 10⁻¹</td>
<td>Animal facility at IAH - E</td>
<td>What material was used for experiment 1-V?</td>
</tr>
<tr>
<td>3 x McCartneys</td>
<td>Ultrafreezer ET 0821 at IAH - E</td>
<td>What material do we believe was submitted for the IAH genotype test, 246 series of experiments and LGC DNA test?</td>
</tr>
</tbody>
</table>

In addition spare material prepared for the LGC DNA test and retained at IAH could also be retested to provide a full picture of the movement of samples. This will not however tell us anything more about the material used in the experiments.
6.2 Other Experiments Impacted

Rendered Samples

The rendering experiments involved a range of samples prepared using different rendering protocols. These samples were designated B to U depending on the rendering protocol applied. We have assessed the likelihood that these were substituted for BSE protocols at some stage in the process and find that we cannot rule out the possibility that one or more samples were substituted at the beginning of the experiments (Scenarios 8 and 9). This may not effect all protocols. The protocols were transported from PDM in batches and aliquoting of the samples for the 272R experiment took place over an extended time period and appears to have involved more than one person. It may be that only one batch or part of a batch is affected.

Substitution Scenarios

<table>
<thead>
<tr>
<th>Substitution Scenario</th>
<th>Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>7  Bovine not ovine brains sent to PDM and/or processed at PDM</td>
<td>Negligible for all protocols</td>
</tr>
<tr>
<td>8  Excess bovine samples not ovine samples transported from PDM</td>
<td>Low</td>
</tr>
<tr>
<td>9  Wrong samples selected for aliquoting from freezer at IAH - E prior to 272R</td>
<td>Low</td>
</tr>
<tr>
<td>10 Wrong samples selected from freezer at IAH - E prior to 246.</td>
<td>Very low</td>
</tr>
</tbody>
</table>
Recommended Tests

<table>
<thead>
<tr>
<th>Sample to Test</th>
<th>Location of Sample</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rendered protocol samples in “scrapie bag”</td>
<td>Ultrafreezer ET 0821 at IAH - E</td>
<td>Sample all, or selection: is ovine material present in MBM samples stored at IAH - E?</td>
</tr>
<tr>
<td>272R - 1D, F, G, H, Q, R, S, U</td>
<td>Behind the barrier at IAH - C</td>
<td>Sample all: was ovine material used in inocula in 272R experiment?</td>
</tr>
<tr>
<td>372R-1S IAH – C [date looks wrong on latter]</td>
<td>IAH - C</td>
<td>Is date wrong, or material wrong?</td>
</tr>
<tr>
<td>Test F and H supernate dated1997</td>
<td>Ultrafreezer ET 0821 at IAH - E</td>
<td>Was the supernatant for the 246 experiments on rendered samples F and H prepared from the correct samples?</td>
</tr>
<tr>
<td></td>
<td>Ultrafreezer ET 0821 at IAH - E</td>
<td>Have scrapie samples been placed in the BSE storage area?</td>
</tr>
</tbody>
</table>

**Bold tests provide direct or near direct evidence of materials used in experiments and should be given higher priority**

In addition:

- Duplicate B, C, D, E, F and J samples from the “BSE bag” could be tested. This test will not tell us whether BSE samples may have been substituted for scrapie samples. It only provides additional evidence relating to the control of samples in store at sometime between their receipt and the present day.

- Excess materials from PDM retained in storage can be tested to establish that ovine material was used in the preparation of the protocols in the first instance. However, from evidence reviewed during this and the previous contamination risk assessment we are confident that ovine material was collected and used to prepare the protocols. This test would not tell us what material was transported to IAH.

**Tallow Samples**

Two tallow samples were analysed from each of the BSE rendering experiment and the scrapie rendering experiment, we believe with the objective of determining whether tallow could carry TSE infectivity. Four tallow samples were observed at IAH - E, all stored with scrapie samples. Two were labelled with a date compatible with preparation of the scrapie samples. The other two are not labelled with the date and could be the BSE samples. There is no direct evidence that samples have been stored incorrectly, but we can have less confidence in the provenance of the undated samples. It is possible that BSE samples were used in place of scrapie samples but we rate the likelihood as low.
**Substitution Scenario**

<table>
<thead>
<tr>
<th>Substitution Scenario</th>
<th>Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrong samples selected for aliquoting from freezer at IAH - E prior to 272R</td>
<td>Low</td>
</tr>
</tbody>
</table>

**Recommended Tests**

We are not aware of any inoculum left behind the barrier at IAH - E. The tallow samples at IAH - E could be tested, if these experiments are considered important, but whatever the result this would not confirm unambiguously the material used in the original experiments.

**Cattle Challenge Experiments VLA Weybridge**

We understand that rendered protocol labelled with a date compatible with preparation of the scrapie rendering experiment samples were used in these experiments. The probability that this material was derived from the bovine brain pool will be very low. Contamination with some small amount of bovine material cannot be ruled out.
7 Recommendations

Our recommendations are:

1. That a testing scheme is devised with DEFRA based on the recommendations made in Section 6 to help establish if and where substitution occurred and the experiments affected.

2. That the tests are very carefully designed and executed in negotiation with DEFRA to ensure that they provide auditable and unambiguous results. Due regard should be given to the nature and condition of the samples when devising the scheme.

3. If testing indicates that substitution probably did not occur prior to the 246 experiments, that great care is taken in interpreting the results of these experiments for three reasons:

4. Whatever the results of any future tests and analyses it is clear that the scrapie brain pool material and the experiments carried out on this material from 1997 onwards cannot be relied upon to determine whether BSE was present in the British sheep flock in the early 1990s because:
   a. The standard of quality control applied to this experiment, in particular the standard of labelling and control of storage, was not sufficiently rigorous for this application.
   b. The provenance of samples cannot be determined with absolute certainty. The tests can only reduce uncertainties with respect to the origin of materials used in the 246 experiments not eliminate them entirely.
   c. The results of the experiments will be difficult to interpret. There is no definitive test that can unambiguously differentiate BSE from scrapie where mixtures may be present, and in particular there is little experience of working with brain pools.
   d. The possibility that sufficient contamination of the scrapie brain pool with cow material occurred during collection of brains to affect the experimental results cannot be completely ruled out.
## Annex 1: Key Abbreviations & Terms

<table>
<thead>
<tr>
<th>Abbreviation/ Term</th>
<th>Description/ Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>246</td>
<td>The strain typing experiments carried out on the (putative) SBP brain pool material (protocol A) and the protocol F and H MBM materials. The first passage began in October 1997, the second passage is still underway.</td>
</tr>
<tr>
<td>272R</td>
<td>The SBP titration experiments, started at IAH - C in November 1992. 272R-1A used the SBP brain pool material, while the experiments 272R-1B, ... , U used the various MBM samples. 272R-1V was a quick-look strain typing experiment carried out at IAH - E in June 1993. Some limited lesion profiling was carried out on the 272R-1A, 272R-1F and 272R-1H mice.</td>
</tr>
<tr>
<td>372R</td>
<td>The BBP titration experiments, started at IAH - C in September 1992. 372R-1A used the BBP brain pool material, while the experiments 372R-1B, ... , S used the various MBM samples. 372R-1V was a quick-look strain typing experiment carried out at IAH - E in June 1993. Some limited lesion profiling was carried out on some of the mice from some of the MBM experiments.</td>
</tr>
<tr>
<td>Aliquots</td>
<td>Equal amounts of some material for testing, in this case to be injected into panels of mice for titration or strain typing (mouse bioassay).</td>
</tr>
<tr>
<td>Animal Facility</td>
<td>Facility where mouse bioassay is carried out.</td>
</tr>
<tr>
<td>Barrier</td>
<td>Term relates to special security measures in place at entrance to animal facility to prevent infections being carried into the facility.</td>
</tr>
<tr>
<td>BBP</td>
<td>Bovine Brain Pool</td>
</tr>
<tr>
<td>Bovine</td>
<td>Pertaining to cattle</td>
</tr>
<tr>
<td>BSE</td>
<td>Bovine Spongiform Encephalopathy. A TSE in cattle; A progressive, fatal disease commonly known as ‘mad cow disease’.</td>
</tr>
<tr>
<td>CVO</td>
<td>Chief Veterinary Officer</td>
</tr>
<tr>
<td>DEFRA</td>
<td>The Department for Environment, Food and Rural Affairs, formed in June 2001 by the merger of the Environment Department and the Ministry of Agriculture, Fisheries and Food (MAFF).</td>
</tr>
<tr>
<td>DNA speciation tests</td>
<td>Testing of DNA to distinguish between different species eg cow and sheep.</td>
</tr>
<tr>
<td>Abbreviation/ Term</td>
<td>Description/ Explanation</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Experiment reference numbers used by IAH</td>
<td>For the 272R and the 372R series of experiments the last letter of the experiment code relates directly to the protocol used, e.g. 372R-1A refers to the experiment using Protocol A.</td>
</tr>
<tr>
<td>Genotyping</td>
<td>Testing of DNA to distinguish between different genotypes within a species.</td>
</tr>
<tr>
<td>Glycoform typing</td>
<td>This method of attempting to distinguish between TSE strains is based on the variability in the glycosylation of the prion protein. The result is given in terms of the relative apparent amounts corresponding to diglycosylated, monoglycosylated and unglycosylated forms of the proteins. Some analyses also assess the molecular weight of the aglycosyl fragment.</td>
</tr>
<tr>
<td>Greaves</td>
<td>Intermediate product in manufacture of meat and bone meal</td>
</tr>
<tr>
<td>Inoculum</td>
<td>The material, usually in dilute saline solution, which is injected into the test animals (in this case, mice).</td>
</tr>
<tr>
<td>IAH</td>
<td>The Institute for Animal Health (IAH) is the largest research institute in the United Kingdom dedicated to the health of farm animals.</td>
</tr>
<tr>
<td>IAH - C</td>
<td>The laboratory of the IAH at IAH - C. It carries out research on endemic diseases of cattle, pigs, poultry and sheep.</td>
</tr>
<tr>
<td>IAH - E</td>
<td>The IAH laboratory, also known as the Neuropathogenesis Unit (NPU). It is concerned exclusively with research on the TSEs of sheep (scrapie), cattle (BSE) and humans (CJD).</td>
</tr>
<tr>
<td>LGC</td>
<td>Formerly the Laboratory of the Government Chemist, LGC was privatised in 1996. The DNA testing of the supposed SBP material which showed it to be bovine was carried out there.</td>
</tr>
<tr>
<td>MAFF</td>
<td>Ministry of Agriculture, Fisheries and Food. In June 2001 it was merged with the Environment Department and the name was changed to the Department for Environment, Food and Rural Affairs (DEFRA).</td>
</tr>
<tr>
<td>MBM</td>
<td>Meat and bone meal</td>
</tr>
<tr>
<td>McCartney’s</td>
<td>Narrow necked glass container, now largely replaced by universals</td>
</tr>
<tr>
<td>Ovine</td>
<td>Pertaining to sheep</td>
</tr>
<tr>
<td>PDM</td>
<td>Prosper de Mulder – operators of rendering plant</td>
</tr>
<tr>
<td>Polypots</td>
<td>White plastic pots.</td>
</tr>
<tr>
<td>Abbreviation/ Term</td>
<td>Description/ Explanation</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Protocol A to U</td>
<td>In the Scrapie Brain Pool, protocols B to U were different specifications for rendering the porcine material spiked with SBP material to simulate the production of meat and bone meal. Protocol A was simply to take a sample of the unprocessed SBP material. The term “protocol X” is often used to refer to the material produced by protocol X. For example “protocol H” can refer to the material prepared using protocol H.</td>
</tr>
<tr>
<td>Q-labs</td>
<td>Microbiological testing labs.</td>
</tr>
<tr>
<td>Rendered Sample</td>
<td>One of the samples of simulated meat and bone meal or tallow, produced by the BBP or SBP rendering experiments.</td>
</tr>
<tr>
<td>SBP</td>
<td>Scrapie Brain Pool</td>
</tr>
<tr>
<td>Scrapie</td>
<td>A TSE which has infected sheep in the UK for centuries. It is not thought to infect human beings.</td>
</tr>
<tr>
<td>Strain</td>
<td>A strain is a line of inbred experimental mice selected for its genetic predisposition to react to material infected with TSE. Strains used in these experiments include RIII and C57BL. A strain of TSE is usually defined in terms of results of strain typing by mouse bioassay. BSE is thought to consist of a single strain, that have the same characteristics as v-CJD in humans, while scrapie is thought to consist of a number of distinct strains.</td>
</tr>
<tr>
<td>Strain Typing</td>
<td>A technique which seeks to tell apart different types (strains) of TSE. Originally this was based on two sorts of data derived from mouse bio-assay: the incubation times and the lesion profiles, as functions of the strain of mouse into which the material was injected. The underlying assumption is that these signals stabilise under repeated passage through mice and are indicative of information that is preserved through the passages. More recently PrP distribution mapping and glyco-typing have been put forward as relevant to TSE strain typing.</td>
</tr>
<tr>
<td>Supernate</td>
<td>Liquid that is separated from solids via centrifuge.</td>
</tr>
<tr>
<td>Tallow</td>
<td>Fat extracted as part of rendering process.</td>
</tr>
<tr>
<td>Titration</td>
<td>The technique of injecting into test animals progressively more dilute samples to determine at which dilution the infectivity drops to 50% (known as ID50). The infectivity of any sample expressed relative to ID50 is known as the titre of that sample.</td>
</tr>
<tr>
<td>TSE</td>
<td>Transmissible Spongiform Encephalopathy. A degenerative brain disease which can be contracted by ingesting or being injected with tissue from an infected animal. The infective agent is not known for certain; one suggested candidate is that the unusual form of the prion protein is infective by itself.</td>
</tr>
<tr>
<td>UKAS</td>
<td>United Kingdom Accreditation Service</td>
</tr>
<tr>
<td>Abbreviation/ Term</td>
<td>Description/ Explanation</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Universals</td>
<td>Wide Necked Glass Sample containers</td>
</tr>
<tr>
<td>v-CJD</td>
<td>A (supposedly) new variant of the human TSE, Creutzfeld-Jakob Disease, distinguished from the form of the disease known earlier (s-CJD) by the relative youth of its victims. It is thought to be caused by the ingestion of material contaminated by BSE.</td>
</tr>
<tr>
<td>VICs</td>
<td>Veterinary Investigation Centres – these were the labs at which the cow and sheep brains which went into the BBP and SBP respectively were collected. In 1995 they were merged to form the Veterinary Laboratories Agency (VLA).</td>
</tr>
<tr>
<td>VLA</td>
<td>The Veterinary Laboratories Agency is an Executive Agency of DEFRA. It is a regional network of laboratories in England, Scotland and Wales supported by a central research facility near Weybridge in Surrey.</td>
</tr>
</tbody>
</table>
Annex 2: Activities Carried Out

We visited the following organisations:

1. The Prosper de Mulder rendering plant where the brain pool macerate and rendering protocols were prepared, we interviewed staff.

2. IAH - E, where the protocols were stored and the experiments managed. We observed samples kept in freezers, reviewed freezer logs, experiment and project files and interviewed staff.

3. IAH - C, where the rendering experiment titrations were performed. We observed samples kept in freezers, reviewed freezer logs, experiment files and interviewed staff.

We interviewed the following staff members:

1. The rendering plant scientist and an assistant who helped prepare the SBP and BBP samples.

2. An officer from the State Veterinary Service who assisted in the preparation of rendering samples at the rendering plant and provided a number of containers and equipment.


4. The Group Leader at IAH - E who project managed the 272R, 372R and the initiation of the 246 experiments.

5. The animal facility manager at IAH - E responsible for overseeing the 246 series of experiments and various experiments from the 272R and 372R series.

6. The technician involved in preparation of samples for the 246 series of experiments and the genotype test and the DNA analysis.

7. IAH - E staff members responsible for managing the relevant freezers throughout the experimental period.

8. IAH - E scientists and technicians who carried out tests on the various materials.

We have followed up information through telephone interviews with VLA, DEFRA and Rendering plant staff.

As part of the earlier contamination risk assessment we visited and interviewed staff at Veterinary Investigation Centres and inspected QA systems in the animal facilities at IAH - E.
Annex 3: Process Map