A quantitative assessment of the TSE risk associated with wastewater from SRM handling facilities in Great Britain
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Summary

Wastewater derived from facilities processing livestock, that may harbour Transmissible Spongiform Encephalopathies (TSEs), is permitted under license for application to land where susceptible livestock may have access. Several previous risk assessments have investigated the risk of Bovine Spongiform Encephalopathy (BSE) associated with wastewater effluents, however the risk posed by classical scrapie and atypical scrapie has not been assessed. With the prevalence of certain TSEs (BSE in cattle and classical scrapie in sheep) steadily in decline in recent years, and with considerable changes in the structure of processing industries in GB, a reappraisal of the TSE risk posed by wastewater is required. This risk assessment estimates the annual number of new BSE infections in cattle and scrapie (classical and atypical) infections in sheep originating from the spread of wastewater arising from facilities which handle Specified Risk Materials (SRM).

Our results indicate that the predicted number of new TSE infections arising from production of wastewater over one year would be low, with a mean of one infection every 1,000 years for BSE in cattle (769, 555,556), and one infection every 30 years (16, 2,500) and 33 years (16, 3,333) for classical and atypical scrapie respectively. It is assumed that the values and assumptions used in this risk assessment remain constant.

For BSE in cattle the main contributors are abattoir and rendering effluent, contributing 35% and 22% of the total number of new BSE infections. For TSEs in sheep, effluent from small incinerators and rendering are the major contributors (on average 32% and 31% of the total number of new classical scrapie and atypical scrapie infections). This is a reflection of the volume of carcase material and Category 1 material flow through such facilities.

Such results need to be viewed as a snap shot of the industries concerned as they are constantly evolving.

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1 Introduction

Wastewater derived from premises handling Specified Risk Materials (SRM) can be applied to pasture land (land grazed by farm animals or land cropped for forage) in accordance with Annex II of Regulation (EC) No. 1774/2002 herein referred to as the ABP Regulation (EC, 2002). Facilities handling SRM include abattoirs where healthy animals are slaughtered, and those facilities handling fallen stock such as intermediate plants, collection centres, renderers and incinerators. Intermediate plants are sites where TSE testing may occur and where local materials are bulked up for onward transportation. In collection centres flesh from fallen stock is removed as food for dogs in kennels and zoo animals. However, the carcase is not tested and is certified as medication free.

The ultimate disposal of SRM as a Category 1 waste must be either rendering followed by incineration, or direct to incineration. Within the ABP regulations, there is a requirement for filtration of wastewater materials through a 6 mm trap prior to any treatment and direct spreading to land (image of trap shown in figure 8A in Appendix 3) These regulations aim to address the environmental impact of spreading wastewater but do not specifically consider the animal health impacts. Several previous risk assessments have investigated the risk of Bovine Spongiform Encephalopathy (BSE) associated with abattoir effluents in Europe (DNV, 1997a; Gale and Stanfield, 2001), Japan (Yamamoto et al. 2006), Australia (BRS, 2001) and for renderers and incinerators in the UK (DNV, 1997a, 1997b, 2001 and Comer and Spouge, 1998). However, the prevalence of certain TSEs (BSE in cattle and classical scrapie in sheep) has been steadily declining in recent years, and with considerable changes in the structure of SRM handling industries in GB, a reappraisal of the TSE risk posed by wastewater and controls is required.

This analysis was undertaken to assess the risk of livestock becoming infected with a TSE disease from wastewater that has been directly spread onto pasture land, both with and without treatment by filtration in accordance with the Regulations. The results from this assessment will be used to inform policy decisions in this area.

2 Methods

This risk assessment was undertaken using standard risk assessment methodology. It is a quantitative risk assessment and therefore quantitative estimates of risk are produced, along with the associated uncertainty and variability where possible. As outlined by the World Organisation for Animal Health (OIE), a risk assessment comprises a release assessment, an exposure assessment and a consequence assessment, culminating in a risk estimate. The output of the risk assessment is an estimate of the annual number of potential new BSE infections in cattle, and scrapie (classical and atypical) infections in sheep originating from the spread of wastewater derived from SRM handling facilities. The risk assessment is a stochastic model where uncertain and variable parameters are simulated using the software package @Risk (© Palisade) Version 5.0, an add-on package within Microsoft Excel (© Microsoft). The results presented follow the standard form of the arithmetic mean and the 5^{th} and 95^{th} percentile values. Accordingly the latter

represent the range of values for which we are 90% certain that the true value lies between.

2.1 Model overview

The scope of the risk assessment is wastewater derived from facilities which handle Category 1 SRM. Appendix 2 provides the full list of material types categorised as Category 1, 2 and 3. This risk assessment includes both those tissues designated as SRM in cattle and sheep; and where, at the time of disposal, specified risk material has not been removed, entire bodies of dead animals containing specified risk material, and the material removed from the drains from SRM handling facilities. SRM in cattle and sheep are those tissue types listed as high risk tissues which are required to be removed from animals and disposed of appropriately. Appendix 2 provides those tissues by age at removal listed as SRM in 2009.

The risk assessment has been divided into six specific components for ease of communication. The parameters requiring estimation within each of the modules are shown in Figure 1. Each of these parameters and any associated variability and uncertainty are described in each of the subsequent sections, with a summary table of input values provided in Table 1A in Appendix 1.

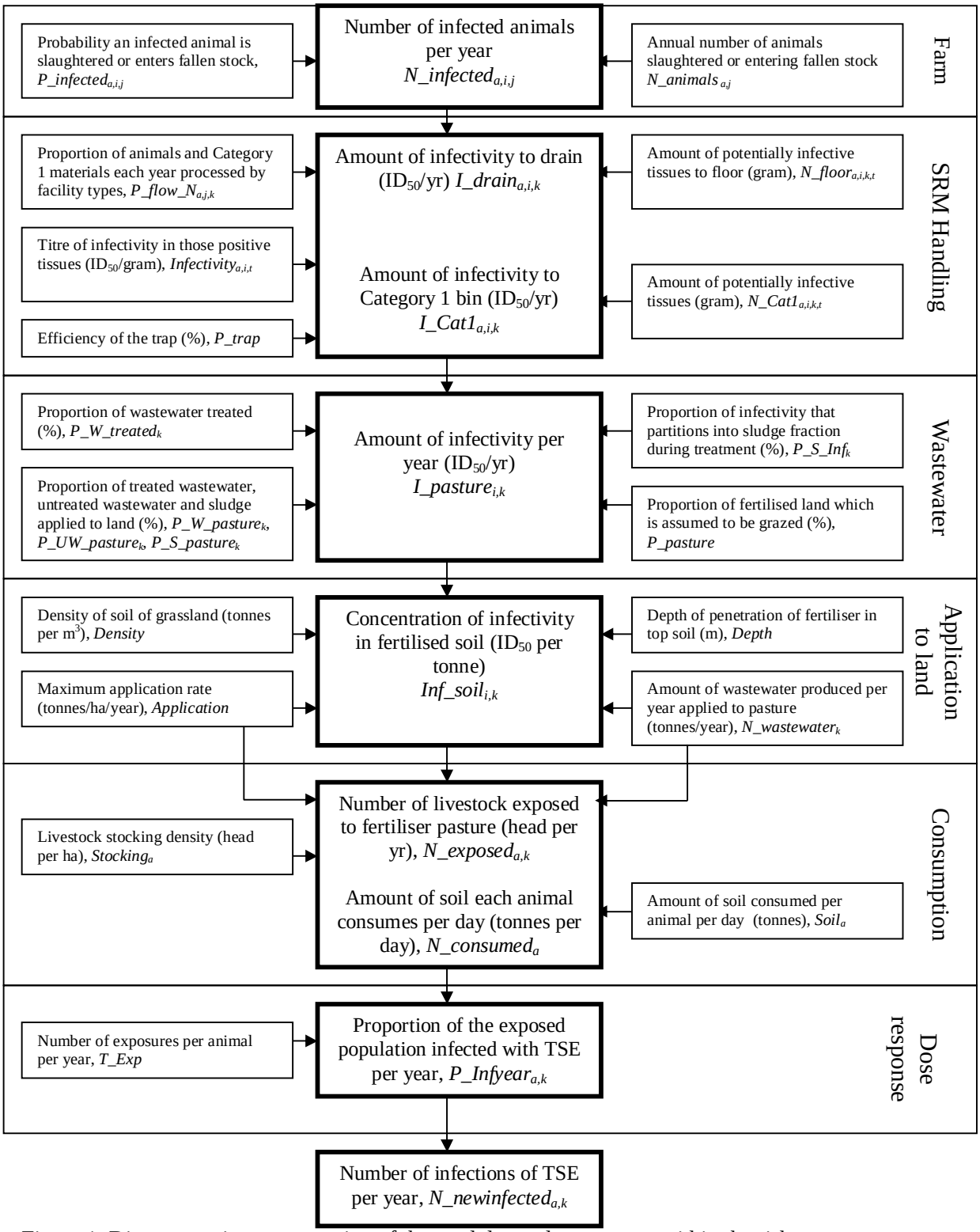

Figure 1: Diagrammatic representation of the modules and parameters within the risk assessment, where subscripts denote animal group $a \in \{C, S, l\}$, infectious tissue type $\in \{1$ to $14\}$, disease $i \in \{bse, sc, at\}$, exit stream $j \in \{HS, FS\}$, facility type $k \in \{AB, INT, CCN, REN, SIN, INC\}$.

2.2 Farm module

The number of infected animals that die or are slaughtered each year is dependent on the prevalence of infection and the annual number of animals dying via the various exit streams. The term 'exit stream' encompasses a general definition of streams of healthy slaughter (including emergency slaughter) at abattoir and fallen stock. Clinically suspect animals are not quantitatively considered as the risk of infectivity entering wastewater has been considered negligible due to stringent disposal procedures. The use of exit streams in this risk assessment is required as livestock in the different categories will have different TSE prevalences and will be diverted to different SRM handling facilities. The estimated numbers of infected animals ($N\_infected_{a,i,j}$), per animal group $a \in \{C, S, l\}$, by disease $i \in \{bse, sc, at\}$, by each exit stream $j \in \{HS, FS\}$, is given by the general equation:

$$N\_infected_{a,i,j} = P\_infected_{a,i,j} * N\_animals_{a,j} \quad (1)$$

where *HS* denotes animals slaughtered at abattoir, *FS* fallen stock; *bse* refers to BSE, *sc* denotes classical scrapie and *at* refers to atypical scrapie; *C* refers to cattle, *S* refers to sheep and *l* denotes lambs. For each possible animal group, disease and exit stream combination, $P\_infected_{a,i,j}$ denotes the probability that an individual animal is infected (i.e. prevalence of infection), and $N\_animals_{a,j}$ the number of animals.

In relation to prevalence, for BSE in cattle it is assumed that only cattle in the last 12 months of the incubation period will harbour significant levels of infectivity. The prevalence of infected cattle in the last 12 months of the incubation period has been previously investigated by Arnold and Wilesmith, (2003), and has been updated for this assessment with BSE surveillance data up to 2009 (Arnold, pers. comm. 2010). The uncertainty associated with the mean prevalence estimates is described in this assessment using a Betapert distribution for each exit stream (refer to Table 1A). The numbers of cattle slaughtered/dead in 2008 in the healthy slaughter (including emergency slaughter) and fallen stock streams were derived from data held by the British Cattle Movement Service (BCMS) (Woods, pers. comm. 2009) and an analysis of the VLA BSE testing data (Rajanayagam, pers. comm. 2009). In 2008, an estimated 2,301,868 cattle were slaughtered at abattoir, and 416,941 fallen stock were recorded.

The number of classical scrapie cases in the healthy slaughter stream is investigated each year by the Abattoir Survey with estimations made of the prevalence of infection. The abattoir survey for 2008 was used to estimate, by back-calculation, the mean prevalence of classical scrapie infection with the associated 5th and 95th percentiles (Ortiz-Pelaez and Arnold, VLA 2009). These values are represented in the risk assessment by a Betapert distribution. The Fallen Stock Survey for 2008 tested 10,128 samples from fallen sheep and detected 4 classical scrapie cases representing the prevalence of cases and described in the model using a Beta distribution. There are no estimates of the prevalence of infection for this stream, however, use of the prevalence of cases is likely to underestimate the true level of infection. The fallen stock stream is subject to more reporting and sampling anomalies than the abattoir survey, and the ratio between

prevalence of cases and infection is likely to vary. In theory infected animals in the fallen stock stream may be closer to clinical onset, such that the difference between prevalence and infection could be less than for abattoir animals. However, with the reduced reporting of clinical scrapie this may no longer be the case (Ortiz-Pelaez, pers. comm. 2009). In the absence of any further information, the prevalence of infection in the fallen stock stream is estimated by multiplying the prevalence of fallen stock cases by the estimated healthy slaughter prevalence of infection divided by healthy slaughter prevalence of cases.

In the absence of further data, the Abattoir Survey was used to estimate the prevalence of atypical scrapie. However, there are a number of key assumptions applied in using this data: (1) the incubation period of atypical scrapie is the same as that estimated for classical scrapie; (2) the survivability of sheep infected with atypical scrapie is the same as for classical scrapie; and (3) the sensitivity of the test for atypical scrapie is the same as for classical scrapie (Ortiz-Pelaez, pers. comm. 2009). The Fallen Stock Survey for 2008 detected 4 atypical scrapie cases out of the 10,128 samples tested. As with classical scrapie, a ratio between prevalence of infection and prevalence of cases in the healthy slaughter stream was calculated and used to modify the prevalence estimate for fallen stock animals.

The development of infectivity in the tissues of sheep infected with scrapie is dependent on the point of slaughter during the incubation period. In this risk assessment different assumptions concerning tissue infectivity are made for the two age classes where there are data available, lambs less than one year, and sheep greater than one year. The total number of sheep entering the healthy slaughter stream is recorded by Defra statistics. The number of lambs less than one year old slaughtered in 2009, $N\_animals_{l,HS,}$ was 13,357,036 with the number of sheep, $N\_animals_{S,HS,}$ 2,182,930 (Defra, 2010). There is no centralised recording of those sheep entering the fallen stock stream. Previously, estimates of the number of sheep dying on farm per year have been made by multiplying the percentage mortalities of sheep by the standing population (Bansback, 2006). The number of lambs dying on farm has been estimated by multiplying the number of adult sheep by the lambing rate and an estimate of the percentage mortality of lambs (Bansback, 2006). From discussions with stakeholders, not all fallen sheep and lambs are recorded as fallen stock and some burial of livestock still occurs. It is difficult to ascertain the exact proportion (Defra, pers. comm. 2009) and it was assumed that 50% to 75% of fallen sheep enter the fallen stock stream.

2.3 SRM handling facilities

The assessment includes the SRM facilities (k) of abattoirs (AB) where all healthy livestock are slaughtered and the SRM is removed and disposed of legally by either large incineration plants (INC) or rendering (REN) followed by incineration. For fallen stock, there are two types of licensed facilities, in addition to renderers and incinerators, where SRM may be handled: intermediate plants (INT) and collection centres (CCN). SRM from these facilities is disposed of legally by either small incineration plants (SIN) or by rendering. Thus $k \in \{AB, INT, CCN, REN, SIN, INC\}$.

The proportion of healthy slaughter and fallen stock each year that are processed by each of these facility types, $P\_flow\_N_{a,j,k,}$ is difficult to estimate as data on throughput are not collected centrally. However, various data sources have been used to estimate the proportion of the GB livestock handled by each facility type, $P\_flow\_N_{a,j,k}$: (1) the national database of ABP facility licences (AH, Database 2009), (2) number of livestock tests in total and by facility type (between 01/06/2008 to 31/05/2009), (3) expert opinion (Animal Health, UK Rendering Association (UKRA), European Fat Processors and Rendering Association (EFPRA), Licensed Animal Slaughterers' & Salvage Association (LASSA), Association of Independent Meat Suppliers (AIMS), and the British Meat Processors Association (BMPA)), and (4) a survey of renderers (Renderers Survey, 2010) and incinerators (Incinerators Survey, 2010). The most likely estimates (minimum and maximum) by animal population are shown in the flowcharts in Figures 1 and 2. The uncertainty associated with the estimated flow of materials is represented by a Uniform distribution where a minimum and maximum value are available, and Betapert distribution where additionally a most likely value is estimated.

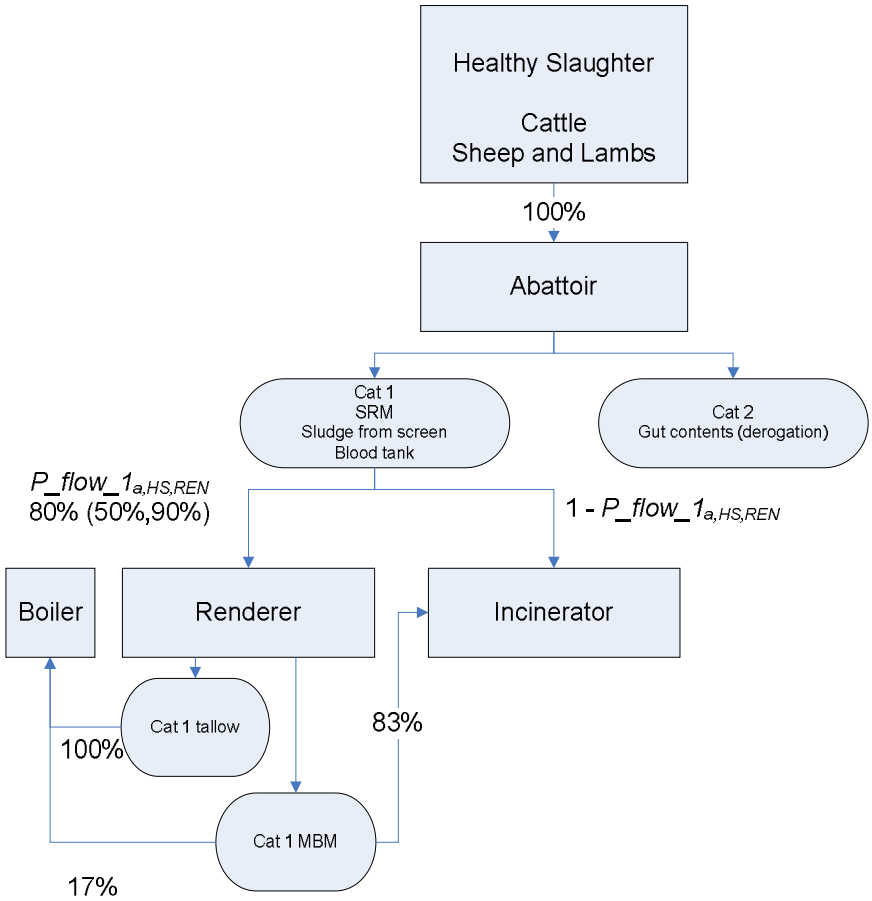

Figure 1: Flow diagram of the process of SRM flow for healthy slaughter and emergency slaughter cattle and sheep with estimated most likely rates of material flow (minimum and maximum).

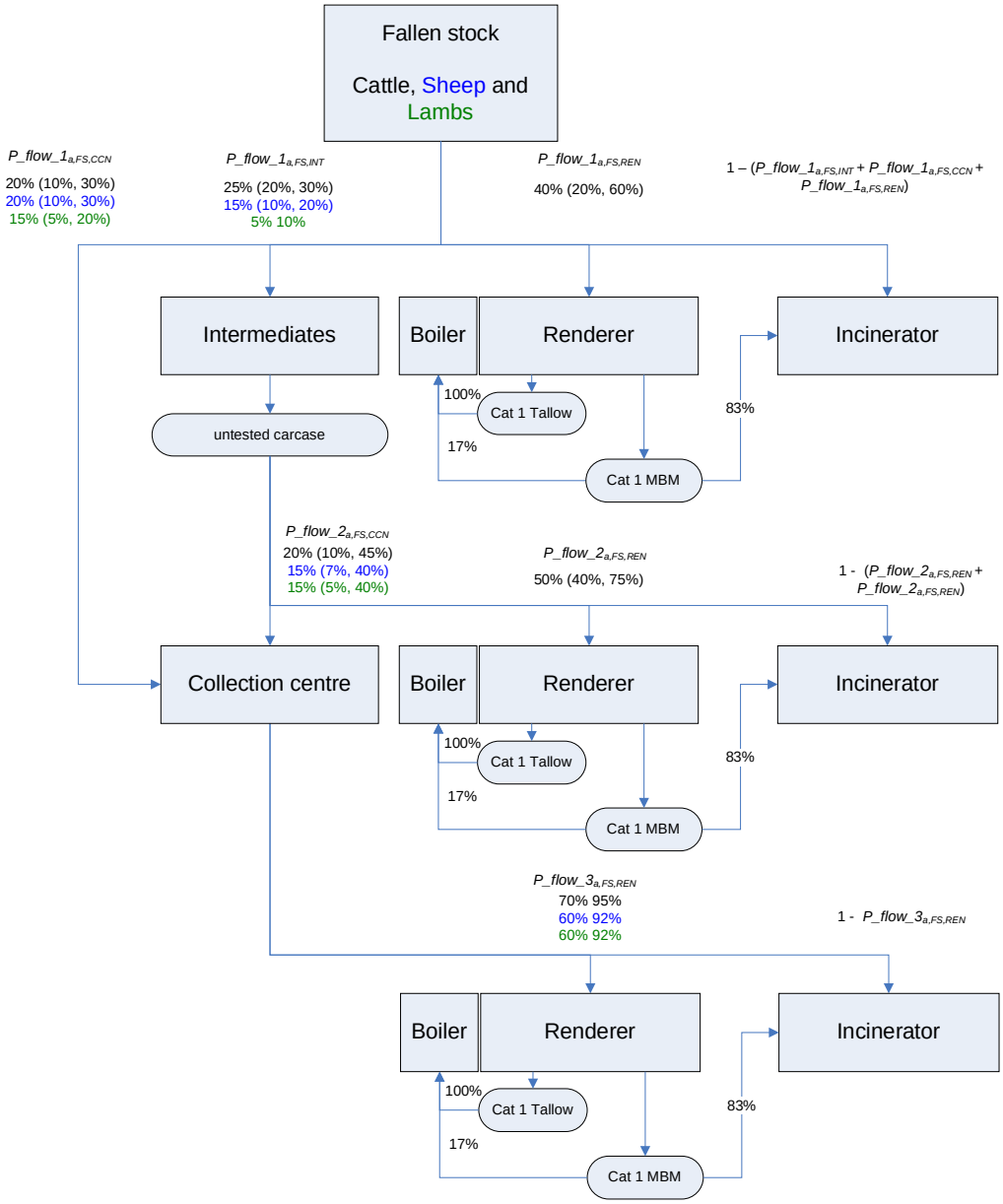

Figure 2: Flow diagram of the process of SRM handling for fallen stock with estimated mostly likely rates of material flow (minimum and maximum)

Black denotes cattle, blue denotes sheep and green denotes lambs.

For the amount of Category 1 MBM produced as a result of rendering, *P_MBM*, 83% is estimated to be sent to an incinerator or large technical facility for energy production, with the remainder used within the rendering plant for fuel (Renderers Survey, 2010). At the current time all Category 1 tallow from rendering is also used as a fuel source.

2.4 Amount of infectivity to floor per infected carcase component

The amount of infectivity that may ultimately enter the drains, $I\_drain_{a,i,k}$, is dependent on disease characteristics and the animal host, for example the tissues containing infectivity and the likely titre, and the type of activities conducted at each facility that could release quantities of infectivity to the floor. Finally the effect of the minimum 6 mm trap also needs to be considered. Due to these dependencies each facility type by animal population has been investigated.

The amount of infectivity passing through the trap (Oral ID_{50} per carcase) for abattoirs, intermediates, and collection centres is estimated by the following equation:

$$I\_drain_{a,i,k} = \left(\sum_{t} \left(N\_floor * Infectivity \right)_{a,i,k,t} \right) * \left(1 - P\_trap \right) \quad (2)$$

Where *k = AB*, *INT* and *CCN*, subscript *t* denotes infectious tissue type $\in$ *{1* to *14}*, $N\_floor_{a,k,t}$ is the amount in grams of infectious tissue that falls to the floor per carcase at each facility type, $Infectivity_{a,i,t}$, is the titre of infectivity (Oral ID_{50}/g) of each tissue type by disease and animal population, and the efficiency of the trap in retaining infectivity is denoted *P_trap*.

As the risk assessment follows the handling of materials identified as being infectious, all such materials generated from each infected healthy and fallen animal in one year in GB are included in the model. The amount of infectivity passing through each facility type (Oral ID_{50} per carcase) is estimated by calculating the total amount of infectivity of the tissues remaining (as a fraction of that fallen to the floor) and adding the material retained by the trap, as shown in the following equation:

$$I\_Cat1_{a,i,k} = \left(\sum_{t} \left(\left(N\_Cat1 - N\_floor + Trap \right) * Infectivity \right)_{a,i,k,t} \right) \quad (3)$$

$$Trap = N\_floor_{a,k,t} * P\_trap$$

Where *k = AB*, *INT* and *CCN*, $N\_Cat1_{a,k,t}$ is the amount of infectious tissue to Category 1 bins per carcase at each facility type , and *Trap* denotes the amount of infectivity that is retained in the trap and subsequently placed in the Category 1 bins for disposal.

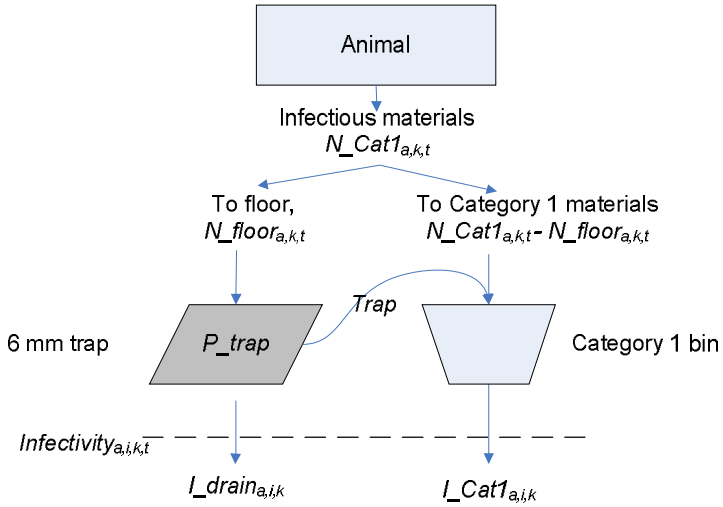

Figure 1: Flow of material at abattoir, intermediate and collection centre to trap and Category 1 bin

For rendering and incineration, estimates for the amount of material to floor for each unit operation was not available from the literature or estimates considered from visits made to facilities. However, estimates have been made in previous risk assessments (DNV, 1997a, 1997b and 2001) for the proportion of infectivity within a carcase that could enter the drainage system. The amount of infectivity to floor for rendering and large incinerators accepting whole fallen stock is estimated by the following equation:

$$I\_drain_{a,i,k} = \left(\sum_{t} \left(P\_floor * I\_carcase \right)_{a,i,k,t} \right) * \left(1 - P\_trap \right) \quad (4)$$

Where k = *REN* and *INC* nodes direct from the fallen stock node shown in Figure 2, $P\_floor_{a,k}$ denotes the percentage of total infectivity to floor (%), and $I\_carcase_{a.i,t}$ is the total infectivity in a carcase calculated by the sum of each infectious tissue weight, ($N\_floor_{a,k,t} + N\_Cat1_{a,k,t}$) by the titre of infectivity ($Infectivity_{a,i,t}$). For all other rendering and large and small incinerators nodes, the amount of infectivity to floor is estimated by the following simplified equation:

$$I\_drain_{a,i,k} = P\_floor_{a,k} * I\_Cat1_{a,i,k} \quad (5)$$

Where k if not denoted signifies *REN*, *INC*, and *SIN* nodes from all other flows and $I\_Cat1_{a,i,k}$ represents the calculated total amount of infectivity contained in the Category 1 bin transported from the node directly above (refer to Figures 1 and 2).

2.4.1 Cattle BSE infectious tissues and infectivity titres

A number of different tissues in cattle infected with BSE have been found to be infectious or positive for prion protein, however, at clinical onset the vast majority of infectivity is present in the brain and spinal cord of the animal. In this risk assessment,

only those tissues in the central nervous system (CNS) are included and as a worst case, infected animals are assumed to be at clinical onset - where the highest titres of infectivity in the CNS have been measured. The assumption is made that the brain and the spinal cord have equal titres of infectivity (Arnold et al., 2007), $Infectivity_{C,bse1,}$ (Bovine Oral ID50 per gram) estimated by the following equation with values shown in Table 1A:

$$Infectivity_{C,bse} = \frac{10^{MaxCNS}}{10^{BOunit}} \quad (6)$$

Uncertainty associated with the parameters is described in the assessment using a Normal distribution for the titre of infectivity in brain and spinal cord at clinical onset (Mouse intercerebral (ic) intraperitonial (ip) ID_{50} per gram), *MaxCNS*, and a Betapert distribution for the conversion factor from Mouse units to bovine oral units, *BOunit*.

2.4.2 Classical scrapie infectious tissues and infectivity titres

There are a number of tissues identified as carrying significant levels of classical scrapie infectivity in sheep as the organ and tissue distribution of infectivity is more widespread than in BSE in cattle. The titre of infectivity $Infectivity_{a,sc,t}$, (Ovine Oral ID_{50} per gram) is estimated by the following equation with values shown in Table 1A:

$$Infectivity_{a,sc,t} = P\_infectivity_{a,sc,t} * \frac{10^{Max_{sc,t}}}{10^{OOunit}} \quad (7)$$

Where $Max_{sc,t}$ denotes the maximum titre of infectivity (log 10 mouse ic ID_{50} per gram) for tissue type $t \in \{1 \text{ to } 14\}$. For tissues t = 1 to 9 estimates of infectivity are taken from Kimberlin & Wilesmith, (1994) which re-analyses data from Hadlow et al. (1980, 1982). However, these estimates have been obtained from experimental data and approximate the variability of the titres of infectivity. In order to obtain estimates of the uncertainty associated with the mean infectivity titre, the titres are firstly expressed as Normal distributions and then a parametric bootstrapping process is applied (Vose, 2000). Other tissues have also been found to harbour infection or had abnormal prion protein detected within them. Low levels of infectivity of the stomach, heart, and kidney (t = 10, 11 and 12) are described by point values (Gale, 2002). In the absence of information regarding the infectivity titre of the duodenum and jejunum (t = 13), it is assumed that the level of infectivity in these tissues is the same as in the ileum (t = 6). This is a worst case assumption since the ileum portion of the intestine is classified as SRM for sheep of all ages whereas the duodenum and jejunum are not. Evidence of classical scrapie infectivity has been detected in the blood of sheep (Terry et al. 2009). However, no data is available regarding the titre of infectivity. Therefore it is assumed that the value for blood lies between -1 and 0 log 10 mouse ic ID_{50} per gram. This uncertainty is described using a Uniform distribution. Low levels of infectivity have also been observed in the pituitary gland, cerebrospinal fluid and adrenal gland (Detwiler, 1996). These tissues are respectively paired with the brain, spinal cord and kidney for ease of assessing their weight. Other tissues containing low amounts of infectivity are the PNS (Groschup et al.,

1996, 1999), tongue (Casalone et al., 2005), bone marrow and supramammary gland (Detwiler, 1996). In the absence of any information regarding the titre of infectivity in these tissues, it is concluded that the infectivity levels are very low when compared to other infectious tissues which are being considered, and therefore are not quantitatively assessed.

P_infectivity $_{a,sc,t}$ refers to the proportion of maximum scrapie infectivity present when an animal is slaughtered or dies. The majority of clinical cases of classical scrapie appear in sheep between 2 and 5 years of age (OIE Terrestrial Manual, 2009). During the progression of the disease, infectivity accumulates in different tissues at different rates. It is also important to note that the vast majority of sheep are slaughtered before the age of 7 (Dawson, pers. comm., 2009) and therefore, in a small proportion of classical scrapie cases, the disease may not be fully developed in some of the sheep by the time of slaughter. An estimation of the percentage increase in infectivity at different ages is presented in DNV, (2002). In this risk assessment, two age groups are considered, lambs under one year old and sheep over 1 year old. Therefore the percentages are adjusted accordingly: for lambs under one year of age, the percentages are the same as those presented in DNV, (2002) for lambs over six months of age except for the lymph nodes and the intestine (duodenum and jejunum) where the percentage of infectivity has been lowered from 50% to 40%. For sheep over the age of one year, the percentage of infectivity in all tissues is estimated to lie between 70% and 100%. This range is described in the model by a uniform distribution. Infectivity of the ileum, pancreas and blood is not considered in DNV, (2002). Therefore the assumption has been made that the percentages of infectivity in the ileum are the same as those stated for other tissues of the intestine, namely the duodenum and jejunum. For the pancreas it is assumed that the percentages of infectivity are the same as those tissues having similar titres of infectivity such as the liver and thymus. For the blood it is assumed that infectivity is at 10% for lambs less than one year of age and between 70% and 100% (described by a Uniform distribution) for sheep more than one year of age.

A conversion factor, *OOunit*, is used to obtain approximations of these titres in units of ovine oral ID_{50}/g (Kimberlin & Wilesmith, 1994). Infection via the intracerebral route is associated with a titre of 7.03 ± 0.13 (arithmetic mean ± standard error of the mean, based on a sample size of 14) and infection via the intragastric route is associated with a titre of 2.03 ± 0.19 (based on a sample size of 7). The conversion factor is obtained by dividing the two routes resulting in a mean value of 10^5. However, it is important to note that these titres approximate the variability of infection via both of the routes. Therefore, in order to obtain an estimate of the uncertainty associated with the conversion factor *OOunit*, each of the samples was described by a Normal distribution and a parametric bootstrap process applied to obtain the distribution about the mean value (Vose, 2000). A simulation of 10,000 iterations was carried out with the most likely, minimum and maximum results described using Betapert distributions as shown in Table 3A.

2.4.3 Atypical scrapie infectious tissues and infectivity titres

The titre of infectivity (Ovine Oral ID_{50} per gram) for tissues from animals affected by atypical scrapie, $Infectivity_{a,at,t}$, is estimated by the following equation with values shown in Table 1A.

$$Infectivity_{a,at,t} = P\_infectivity_{a,at,t} * \frac{10^{Max_{at,t}}}{10^{OOunit}} \quad (8)$$

$Max_{at,t}$ denotes the maximum titre of infectivity (log 10 mouse ic ID_{50} per gram) by tissue type *t*. The number of potentially infectious tissues for atypical scrapie has been found to be much more restricted than for classical scrapie. Previous studies suggest that infection is limited to the central nervous system (Benestad et al., 2008). However, there is speculation that infection can also occur in the lymph nodes. The model considers infection of both the CNS (brain and spinal cord) and lymph nodes. The assumption is made that no other peripheral tissues contain infectivity. For those tissues which are infected, the titre of infectivity is not known. Therefore, for CNS tissues, it is assumed that the titre is the same as that measured for the CNS for classical scrapie. If infection is present in the peripheral tissues then the titre of infectivity is estimated to be 5 - 6 logs less than in CNS tissues (Simmons, per. comm., 2009). Therefore, in the model, the lymph nodes infectivity titre, $Max_{at,3}$, is obtained by subtracting a value in the range of 5 to 6 (described using a Uniform distribution) from the infectivity titre of the brain, $Max_{at,1}$. Based on only 8 clinical cases, the incubation period of atypical scrapie is at least 2 - 3 times longer than for classical scrapie (Simmons, per. comm., 2009). Hence the distribution of infectivity with age for CNS tissues and lymph nodes estimated for classical scrapie has been adjusted accordingly. For lambs under one year old the percentages for the brain, $P\_infectivity_{l,at,1}$, and spinal cord, $P\_infectivity_{l,at,2}$, are 0.1%. For the lymph nodes, the percentage of infectivity, $P\_infectivity_{l,at,3}$, is lowered to 0.1% as there is no evidence to suggest that the progression of the disease is faster in these tissues than in the CNS. For sheep over the age of one year, the percentage of infectivity in all of the tissues at the time of slaughter, $P\_infectivity_{S,at,t}$, is estimated to lie between 40% and 80%. This uncertainty is described in the model using a Uniform distribution.

2.4.4 Material to floor and Category 1 at Abattoir

There were 251 red meat abattoirs licensed in 2009 (MHS, 2009). Within these facilities there are two drain areas; one floor area includes the processing stages of stunning, head removal and bleeding and, if required TSE testing, and flows into the blood tank which is disposed of as Category 1. Wash down from the floor areas for processing the remainder of the carcase will flow via the trap into wastewater.

Cattle abattoir

A large number of studies have investigated the release of BSE infectivity or markers of infectivity during abattoir processing. The contributions of various tissues to infectivity entering the food chain and category 3 material have been previously summarised (Adkin et al., 2010; Cummins and Adkin, 2007). Various studies have been conducted to estimate infectivity contaminating the floor (Prendergast et al., 2003; 2004; Daly et al., 2002) with a key project undertaken by AFSSA. AFSSA conducted an experiment to

determine the amount of CNS going to the floor using glial fibrillary acidic protein (GFAP) detection, as it is specifically expressed in the CNS. An estimated total of 2.73 g of CNS material from each carcase flowed to the trap including that from the blood bath areas (AFSSA, 2003). In this risk assessment it is assumed that the content of the blood tank is disposed of correctly as Category 1 material and therefore, the only contribution to infectivity on the floor per carcase is equal to a mean of 1.03 g from the handling of head meat contaminated by brain, $N\_floor_{C,AB,1}$ (Cooper and Bird, 2002), and 0.27 g contamination from the spinal cord following splitting of the carcase, $N\_floor_{C,AB,2}$ (AFSSA, 2003). There is uncertainty in these estimates which is described in the assessment for brain contamination with 95th percentiles of 0.18 and 2.69. A Betapert distribution is fitted using the mean (1.03 g) and percentiles (0.18, 2.69). The amount of infectious CNS tissue for cattle is composed of tissues within the skull (brain 500 g, retina 1 g, trigeminal ganglia 20 g, Hart et al., 1997) and spinal cord (between 200 g and 482 g, Hart et al., 1997, SEAC, 1996). There is uncertainty associated with many of these values. However, only the uncertainty associated with spinal cord is described in the model using a uniform distribution. These weights for infectious cattle tissues, $N\_Cat1_{C,k,t}$, are used for the abattoir, intermediate plant and collection centre facilities and those renderers and incinerators accepting whole animals.

Sheep abattoir

It is assumed that blood lost at exsanguination is disposed of as Category 1 material through use of a blood tank. It is estimated that 40-60% (Warriss, 2000) of the total blood volume of a sheep (2,720 g) is collected here. The range of values for amount of blood lost at exsanguination for a sheep, $N\_Cat1_{S,AB,14}$ is described in the model using a Uniform distribution between 1088 g and 1632 g. The amount of blood loss due to further processing, which is not collected by the blood tank, $N\_floor_{S,AB,14}$, is assumed to go to the floor which is estimated to be 10-20% of the original blood content (272 - 544 g). This range is described in the model using a Uniform distribution. The tonsils and the skull including brain and eyes are classified as SRM for sheep over one year old. Therefore it is assumed that the head is removed in the blood bath zone and that all infectivity associated with the brain (160 g) and tonsils (3.2 g) is disposed of correctly as Category 1 material. It is assumed that all other tissues which are classified as SRM, namely the spinal cord (50 g - 64 g), ileum (200 g) and spleen (300 g), are removed as part of the evisceration stage and that a proportion of each of these tissues is lost to the floor as part of this process. The amount of spinal cord material going to the floor, $N\_floor_{S,AB,2}$, is assumed to be the same proportion by weight as the amount of spinal cord which is lost to the floor for cattle, estimated by the following equation where t = 2, spinal cord.

$$N\_floor_{S,AB,2} = \left(\frac{N\_floor_{C,AB,2}}{N\_Cat1_{C,k,2}} \right) * N\_Cat1_{S,AB,2} \quad (9)$$

For the spleen and ileum it is estimated that between 0.01% and 0.1% of these tissues goes to the floor (assessors assumption based on observation, 2009). Therefore the amount of material from the spleen and ileum going to the floor ($N\_floor_{S,AB,4}$ and $N\_floor_{S,AB,6}$) is given by the ranges 0.02 g - 0.2 g and 0.03 g - 0.3 g respectively. These

ranges are described in the model using a Uniform distribution. It is assumed that all other infectious tissues and blood remaining in the carcase either enter the food chain or are disposed of correctly as Category 3 materials.

Lamb abattoir

At exsanguination it is estimated that 40-60% of the total amount of blood in a lamb (1,700 g) is lost (Warriss, 2000). It is assumed that the blood which is lost is disposed of correctly as Category 1 material using a blood tank. Therefore the amount of blood from a lamb entering Category 1 waste from exsanguination, $N\_Cat1_{l,i,AB}$, is given by the range 680 g - 1,020 g. This range is described in the model using a Uniform distribution. The amount of blood which is lost through further processing and is assumed to go to the floor, $N\_floor_{l,i,AB,1}$, is estimated to be between 170 g and 340 g (10-20% of the original blood content). This range is described in the model using uniform distributions. Unlike sheep aged over one year the tonsils and skull including brain and eyes are not classified as SRM and therefore it is assumed that the head is removed as part of the evisceration process. The head can be disposed of as Category 2 material. However, most abattoirs dispose of the head as Category 1 material in order to reduce cost. Infectious tissues associated with the head are the brain (100 g) and tonsils (2 g). In the model it is estimated that the amount of brain material which is lost to the floor due to handling of the head, $N\_floor_{l,i,AB,2}$, is between 1 g and 2 g (i.e. 1% - 2% of all brain material). The tonsils and the remaining brain material are assumed to be disposed of correctly as Category 1 waste. The spleen (75 g) and the ileum (100 g) which are SRM for sheep of all ages are assumed to be removed at evisceration with the same proportions going to floor as for sheep. Hence the amount of ileum and spleen material going to the floor ($N\_floor_{l,i,AB,4}$ and $N\_floor_{l,i,AB,6}$) is 0.0075 g - 0.075 g and respectively 0.01 g - 0.1 g. These ranges are described in the model using a Uniform distribution. It is assumed that the spinal cord (which is not classified as SRM for lambs less than one year) and all other infectious tissues and blood remaining in the carcase either go to the food chain or are disposed of correctly as Category 3 materials.

2.4.5 Material to floor and Category 1 at intermediate plants

Intermediate plants carry out the collection of fallen stock from farms and, where a valid licence is held, carry out testing for TSEs. There were 61 intermediate plants licensed in 2009 (AH, 2009). Some intermediate plants do not test and only serve as points of bulking up whole fallen livestock carcases for further transportation to a larger facility where testing is conducted. Testing for TSEs involves the removal or partial removal of the head from the carcase. It is assumed in this assessment that there is no blood tank prior to collection of wastewater. From a visit conducted it is assumed that there are three routes in which CNS tissues may contaminate the floor and subsequently wastewater: (Route 1) brain fragments exiting the hole made by the captive bolt from those animals shot in the head when hoisted; (Route 2) brain fragments lost in blood from shot animals from the head/neck due to decomposition or emboli, and (Route 3) brain material released to floor during the TSE test. The estimated amounts of infectious material (in grams) of a carcase going to the floor from each of these 3 routes are given in Table 1A. It is assumed that all of the material which goes to the floor will go to the 6 mm trap. All material retained by the trap is disposed of as Category 1 waste.

For Route 1, the probability of adult livestock being shot dead on farm has been estimated and is represented in the model by a range of 60% to 85% of fallen stock entering intermediate facilities (INT, pers. comm. 2009). The majority of lambs are born dead or die shortly after birth. The probability of fallen lambs being put down is therefore low and estimated to be between 0.1% and 1%, with an estimated 10% of these shot (AH, pers. comm. 2009). These ranges have been represented in the model using a Uniform distribution. AFSSA investigated the amount of CNS material to floor during captive bolt stunning of cattle. 15% of animals' stunned lost brain fragments of an average weight of 0.34 g to floor (AFSSA, 2003). In the absence of data for the frequency of use of captive bolts, or in rare cases rifles, to put down animals on farm, it is assumed that, between 0.34 and 1 g of material may be lost from gun shot wounds during movement of the carcase in the yard area.

It has been estimated that for Route 2, approximately half of all animals at an intermediate plant had some bleed out, either due to being freshly slaughtered or particularly in the summer months, due to significant decomposition (INT, pers. comm. 2009). This has been represented in the model as a range between 40% and 60% using a uniform distribution. It is assumed for cattle that during these bleeds the amount of brain material lost is the same as that estimated for emboli caused by stunning, that is between 1 g and 10 g (Comer & Huntly, 2004). For sheep and lambs, it is assumed that the same proportion of total brain weight is lost during these bleeds.

The AFSSA study measured an average of 0.568 g of brain lost during testing for BSE in cattle (range of 0.01 g to 1 g), which is represented in the model using a Betapert distribution (AFSSA, 2003). A pessimistic assumption has been used that all cattle are tested for BSE, whereas at the current time only those cattle older than 48 months are tested. For sheep it is assumed that the same proportion of total brain weight is lost during testing, with the proportion of sheep subjected to an obex test estimated as 9300 (Jon Weston, VLA, pers. comm. 2010) divided by the total number of sheep in the fallen stock stream, $N\_animals_{S,FS}$. It is important to note that in contrast to sheep and cattle, lambs are not tested for TSEs. For sheep and lambs it is assumed that all other infectious tissues remain inside the carcase.

2.4.6 Material to floor and Category 1 at collection centres

Collection centres, also known as knackers yards, do not test for TSEs but are licensed to remove flesh (Category 2) for dogs (kennels) and zoo animals from animals that are certified not to contain any medication residues and have not been TSE tested. During processing, livestock have their heads removed and carcase eviscerated with the entire head and guts placed in the Category 1 bin (CCN visit, 2009). Meat from the legs is removed, and back meat and ribs removed by sawing and cutting. The vertebral column is not split and is placed in Category 1 waste with the remaining skeleton. It is assumed in this assessment that there is no blood tank prior to collection of wastewater. There were 190 collection centres licensed in 2009 (AH, 2010). From a visit conducted it is assumed that there are two ways in which CNS tissues may contaminate the floor and subsequently wastewater: (Route 1) brain fragments exiting the gun shot wounds when

the head is removed/handled from those animals shot in the head; and (Route 2) brain fragments lost in blood from shot animals from the head/neck due to decomposition or emboli when the carcase is handled for flesh removal.

It is assumed that the amount of infectious material to floor considered for Routes 1 and 2 following the handling of carcases at collection centres is the same as that estimated for intermediate plants. It is important to note that for sheep and lambs, the ileum may be removed and is disposed of as Category 1 waste. However, during this process it is assumed that a proportion of the ileum is lost to the floor. In the model it is assumed that the amount of the ileum material going to the floor, ($N\_floor_{S,CCN,6}$ and $N\_floor_{I,CCN,6}$), is the same as for sheep and lambs at abattoir (i.e. between 0.02 g and 0.2 g). It is assumed that all remaining infectivity enters Category 1 waste.

2.4.7 Material to floor and Category 1 during rendering

There are currently 8 rendering facilities accepting ruminant Category 1 and 2 material (Renderers Survey, 2010). The principle route of contamination into wastewater during rendering comes from TSE sampling and the crusher within the carcase receipt shed (Animal Health, 2009). Carcases enter the yard and are crushed and the minced material pumped to the raw material hoppers. TSE sampling may produce small amounts of material going to the floor and the crusher interior cleaning operation results in most of the solids being removed manually rather than washed down the drain. The proportion of infectivity falling to the floor, $P\_floor_{a,i,REN}$, is described in the model using a Betapert distribution with a most likely value of 0.003725, a minimum of 0.0001 and a maximum of 0.015 (DNV, 1997a, 1997b). Studies on the effectiveness of rendering processes on reducing BSE and classical scrapie infectivity have been previously summarised (Cummins and Adkin, 2007 citing Taylor et al. 1995; Taylor et al. 1997; and Schreuder et al. 1998). Based on these observations, the proportion of BSE infectivity remaining after rendering processes, REN_{bse}, is described in the model by a Betapert distribution with a most likely value of 0.01, minimum of 0.001 and maximum of 0.02. In the model, the proportion of classical scrapie infectivity remaining after rendering processes, REN_{sc}, is described by a Betapert distribution with a most likely value of 0.01, minimum of 0.00079 and maximum of 0.02. With no data available regarding the effects of rendering on atypical scrapie infectivity, the proportion of infectivity remaining, REN_{at}, is assumed to be the same as for classical scrapie. It is important to note that TSE agents are hydrophobic and will tend to be attached to solids (Gale et al., 1998). Thus any infectivity remaining after rendering will tend to be in the meat and bone meal (MBM) rather than in the tallow or liquid effluent (Comer and Spouge, 1998). It is therefore assumed that all remaining infectivity after rendering is assumed to go into the MBM fraction.

2.4.8 Material to floor and Category 1 during incineration

There are 280 incinerators licensed on the Animal Health database, the majority of which are relatively small in scale owned by groups incinerating a range of waste materials (Animal Health, 2009). These include crematoriums that also handle pets, intermediate plants and collection centres, and veterinary centre incinerators. The control of wastewater and processing at these facilities is significantly different from the large industrial incinerators and technical facilities. Therefore these two groups are considered

separately in this risk assessment. From a survey of lead VOs, only 10-12 operational high throughput incinerators were identified in GB that are licensed to process Category 1 materials and fallen stock, including those licensed under the Waste Incineration Directive, power stations, or technical facilities producing biodiesel.

The proportion of infectivity falling to the floor at small incinerators is assumed to be dependent on the type of material that is being processed. Therefore, for fallen stock arriving from farms the proportion of infectivity falling to the floor, $P\_floor_{a,SIN,1}$, is assumed to be the same as the proportion of infectivity from a single carcase going to trap at an intermediate plant. For materials transported from intermediate plants and collection centres, the proportion of infectivity per carcase falling to floor, $P\_floor_{a,SIN,2}$, is assumed to be the same as that estimated for a collection centre.

For high throughput incinerators and technical plants, it is assumed that Category 1 materials from abattoirs and rendering facilities such as SRM and MBM are processed. For these materials it is assumed that the proportion of infectivity to floor, $P\_floor_{a,INC}$, is the same as that estimated for a rendering facility.

2.4.9 Proportion of infectivity retained by 6 mm trap

It is a legal requirement for SRM handling facilities to have a 6 mm trap, with any sludge retained classified as Category 1 material (EC, 2002). The amount of infectious material that is retained by trap is not known. Research conducted by AFSSA attempted to measure the amount of CNS material that was retained at abattoir and the proportion that flowed through the trap (AFSSA, 2003). However, the experimental protocol used did not enable quantitative estimates. It is assumed that the baseline proportion of material which is retained by the trap, P_trap, is based on those estimates available in the literature between 0.8 and 0.9 (AFSSA, 2003; DNV 1997a). This associated uncertainty is described in the model by a uniform distribution. For abattoirs, intermediate plants and collection centres it is assumed that all material which is retained by the trap is disposed of as Category 1 waste. For rendering facilities, it is assumed that all material stopped by the trap is rendered. For incineration facilities, it is assumed that all material stopped by the trap is incinerated.

A key assumption in this risk assessment is that there is no illegal activity of lifting the drain and allowing the material retained to pass into wastewater. However, the efficiency of the trap is further explored by scenario analysis (refer to results section 3.5). In addition, in this risk assessment we have considered only a 6 mm trap. Many facilities use a 4 mm, 2 mm or even a 1 mm trap to retain material and therefore improve the quality of resulting wastewater. However, the numbers of plants that use more selective traps is not known and may vary between facility types.

2.5 Amount of infectivity to pasture per year

In this risk assessment, we are interested in how much infectivity from wastewater is spread on pasture land. Once through the trap, a range of downstream processes may take place and a number of other disposal options exist, other than land application:

1. Permit to discharge into sewage, with data held by water companies. Thresholds for contamination are attached to permit.
2. Consent to discharge to controlled waters (river/surface water).
3. Land Exemption if of proven benefit to land.
4. Use of storage tanks subsequently collected by an intermediary disposal company who then require a disposal permit/consent as listed above.

The disposal of wastewater produced from Category 1 and 2 facilities is not centrally regulated and data is not collected by any one government agency/department. The Environment Agency (EA) regulates facilities that process more than 50 tonnes of livestock per day (Environment Agency, pers. comm. 2009). Local Authorities regulate those facilities with throughputs below this threshold. Local Animal Health Veterinary Officers will check the paperwork for each ABP facility in their area to ensure that wastewater is being legally disposed of, however, centralised records are not kept (Animal Health, pers. comm. 2009). The Environment Agency holds the following data for ABP facilities (Environment Agency, pers. comm. 2009):

1. 21 sewer discharge consents - separate contracts issued by the sewage undertaker, usually a water company, for sewer discharge.

2. EA discharge consents: EA issues discharge consents to controlled waters such as rivers but not to sewer. There are 11 discharge consents for non-poultry slaughterhouses on their discharge consents database.

3. EA landspreading exemptions under paragraph 7 of the Environmental Permitting Regulations (EPR) include "abattoir, poultry or fish preparation plant wash waters": The law requires that the notifier informs the EA about their intention to spread and prove benefit to land. In total there were 16 notifications made by 6 notifiers onto 13 farms in 2009. Two of these 6 notifiers appear to be poultry producers, the remainder are assumed to be collection centres.

The Environment Agency also regulates EPR licences to operate abattoirs. There were 32 permits to operate slaughterhouses in 2009 and the remit is wider than discharges of wash water. Of these 32 permits, three list landspreading as a disposal route and three list discharge consents to controlled waters. These should be included in the landspreading exemptions listed above, but there is no mechanism to cross check as a contractor will remove the wash waters and spread on land under a notification registered in the contractors name (Environment Agency, pers. comm. 2010).

Due to the effect of dilution of material entering a water course, stream, sewer, lagoon, or soak-away, it is assumed that there is insufficient infectivity remaining for infection to occur following exposure of livestock to these sources, e.g. livestock drinking downstream or land application of sewage.

For each facility, the amount of infectivity spread on pasture (ID_{50} per year), $I\_pasture_{i,k,}$ is estimated from the addition of each amount of infectivity by wastewater type:

untreated wastewater, wastewater that has been treated and the sludge arising from such treatment, as shown by the following equations:

$$I\_pasture_{i,k} = \sum_{a}(I\_Uwastewater + I\_sludge + I\_wastewater)_{a,i,k} \quad (10)$$

Where for untreated wastewater:

$$I\_Uwastewater_{a,i,k} = \sum_{j} \begin{pmatrix} N\_infected * P\_flow\_N * I\_drain * (1 - P\_W\_treated) \\ * P\_UW\_pasture * P\_pasture \end{pmatrix}_{a,i,j,k}$$

For treated sludge:

$$I\_sludge_{a,i,k} = \sum_{j} \begin{pmatrix} N\_infected * P\_flow\_N * I\_drain * P\_W\_treated \\ * P\_S\_Inf * P\_S\_pasture * P\_pasture \end{pmatrix}_{a,i,j,k}$$

For treated wastewater:

$$I\_wastewater_{a,i,k} = \sum_{j} \begin{pmatrix} (N\_infected * P\_flow\_N * I\_drain * P\_W\_treated \\ * P\_W\_pasture * P\_pasture) - I\_sludge \end{pmatrix}_{a,i,j,k}$$

Where N = 1, 2, 3. The probability of wastewater being treated, $P\_W\_treated_k$ from abattoirs is assumed to be 7 of 14 abattoirs surveyed by Buncic, 2002, represented in the model using a beta distribution. There are no data on intermediate plants, collection centres and small incinerators, which are assumed to be the same as abattoirs. From a survey of the 8 renderers handling Category 1 and 2 material, it is estimated that 80% of wastewater is further processed with material sent to large incinerators. The probability of treated wastewater being applied to land, $P\_W\_pasture_k$, is assumed to be 2 out of 4 abattoirs and applies to other small facilities (Buncic, 2002) described using a Beta distribution. For renderers and large incinerators, it is assumed to be 25% (Renderers Survey, 2010). The proportion of untreated wastewater (1- $P\_W\_treated_k$), which is then applied to land, $P\_UW\_pasture_k$, is estimated as 2 out of 10 abattoirs, intermediate plants, collection centres and small incinerators (Buncic, 2002), and currently 3% for renderers and large incinerators (Renderers Survey, 2010).

$P\_S\_Inf_k$ denotes the proportion of infectivity that partitions into the sludge fraction when wastewater is further processed. The use of biological treatments, Dissolved-Air-Flotation (DAF) units and screening differ in their ability to remove suspended solids from the wastewater, and therefore any TSE agents that may associate with the sediment (BRS, 2001). However, the proportions of these treatments employed are not known for the different facility types, except for renderers, where a specific survey was performed. For small facility types, it is assumed that anywhere between 30% and 99% of infectivity will partition into the sludge phase when wastewater is further processed (assessors assumption based on BRS, 2001). This is described in the model using a Uniform distribution. For renderers where treatment was undertaken, both biological treatment and DAF was applied (Renderers Survey, 2010), and it is assumed that such treatments are

also employed by large incinerators. It is assumed that between 99% and 99.4% of infectivity partitions into the sludge phase during processing (Gale et al., 2000). The probability of sludge being applied to land, $P\_S\_pasture_k$, is assumed to be 6 out of 6 abattoirs and other small facilities (Buncic, 2002) described using a Beta distribution. For renderers and large incinerators, it is assumed to be 41% (Renderers Survey, 2010).

With no further information on the type of land to which the wastewater is applied, the proportion assumed to be grazed, *P_pasture*, is equal to the national proportion of grassland fertilised from the total area of grassland and crops fertilised, estimated to be 40% (Defra Agricultural Census, 2009, Defra, 2000).

2.6 Application of wastewater to grassland

The concentration of infectivity in soil where wastewater has been applied (ID_{50} per tonne soil), $Inf\_soil_{i,k}$, is dependent on the amount of wastewater produced, the application rate and density and depth of application and can be estimated by the following equation:

$$Inf\_soil_{i,k} = \left(\frac{I\_pasture_{i,k} * Application}{N\_wastewater_k * 10{,}000 * Depth * Density} \right) \quad (11)$$

Where $N\_wastewater_k$ denotes the amount of wastewater produced per year that is destined for pasture by each facility type. Estimates for wastewater production at abattoirs are based on the numbers of carcases processed, with 1000 Lt per carcase used by large facilities processing >150 carcases per day, and 2000 Lt per carcase used by abattoirs below this threshold (adapted from MHS throughput data, BAT guidance, AB visit, 2009). These rates produce a mean estimate of 3×10^7 tonnes of wastewater per year. Intermediate plants, collection centres and small incinerators are estimated to use less washing down water, approximately 10 to 100 Lt per carcase (LASSA, pers. comm. 2009). These rates produce respectively mean estimates of 1×10^4, 2×10^4, and 4×10^4 tonnes of water annually. Renderers were asked directly for water consumption data and it was estimated that the industry used 7×10^5 tonnes (Renderers Survey, 2010). It is assumed that on a per weight basis, large incinerators use similar amounts of water, estimated as a mean of 7×10^5 tonnes for the entire industry in one year. The annual amount of wastewater produced per facility type is dependent on the estimated number of carcases processed by each facility type within the model.

From a review of the literature, the application rate of wastewater to pasture, *Application*, is extremely variable and is dependent on the amount of nitrogen in the soil and wastewater as well as potential contaminants that may be present in the effluent. The maximum rate of application is applied as 250 tonnes/hectare/year (Mittal, 2006).

The depth of application, *Depth*, will vary, with some wastewater surface spread and sludge fractions injected to depths of 22-25 cm (Animal Health, pers. comm. 2010). Therefore the depth of application is estimated to be between 0 and 25 cm, described in the model using a Uniform distribution. The density of soil, *Density*, has been estimated

as between 0.88 and 0.92 tonnes per m3, with a most likely value of 0.9 tonnes per m3 (Engles, 1999).

2.7 Consumption by livestock

The number of livestock grazing on land is dependent on the quality of the pasture and seasonal weather conditions. In most parts of the country, cattle are housed over winter for several months and sheep may also be housed, particularly before and during lambing. During these housed periods, stock will not have direct access to fertilised pasture. However the worst-case assumption is made that during these periods animals are supplied with silage or hay that has been cropped from the fertilised fields. Consequently, animals are exposed year round.

In order to estimate the number of infected livestock per year, the number of livestock exposed to the fertilised pasture and amount of infectivity consumed per day can be estimated by the following equations:

$$N\_consumed_{a,i,k} = Soil_a * Inf\_soil_{i,k}$$

$$N\_exposed_{a,k} = \left(\frac{N\_wastewater_k}{Application} \right) \Big/ Stocking_a \quad (12)$$

Where $Soil_a$ is the amount of soil consumed per day. Grazing cattle involuntarily ingest each day between 1% and 18% of their dietary dry matter as soil. The average dry matter intake of cattle is 13.6 kg per day. Therefore, grazing cattle may ingest between 0.14 kg and 2.45 kg of soil per day (Thornton & Abrahams, 1983). Grazing sheep, due to cropping closer to the ground, ingest up to 30% of their dry matter as soil (Thornton & Abrahams, 1983). In a series of feeding experiments, Peterson and co-workers established that the dry matter intake of sheep ranged between 0.782 and 1.262 kg per day depending on the forage material (Peterson et al., 1974). Therefore, grazing sheep may ingest between 0.23 kg and 0.38 kg of soil per day. It is assumed that lambs consume the same amount of soil as adult sheep which is acknowledged as pessimistic. The average amount of soil consumed by sheep and cattle per day is associated with uncertainty that is represented in the assessment using a Uniform distribution between the minimum and maximum values previously provided. The assumption is made that any grazing cattle and sheep will have access to the full soil depth of fertiliser, that is, to a maximum of 25 cm. This is considered a worst-case precaution as it is acknowledged to be unrealistic but has been used inherently in previous risk assessments (for example, Cummins and Adkin, 2007).

Descriptions of stocking density for livestock, $Stocking_a$, vary in the literature. Ranges of 0.36 to 2.0 adult cattle per hectare were found (Scotland: Chadwick, 2003; England: MLC, 2001), and 14.1 sheep per hectare (UK: MLC, 2002). In a postal survey of sheep farmers in GB in November 2002, the stocking density of sheep > 1 year old per acre was found to be between 1.56 and 2.51 for the 2nd and 3rd quartiles of responses (McIntyre et

al., 2006). Given that 1 acre is equal to 0.404 hectares, this range equates to 3.9 to 6.2 sheep per hectare, with a mid point of 5.02 adult sheep per hectare. Another measurement used in the European Union agricultural statistics is the livestock density index, in livestock units (LSU) per hectare, where an adult cow is equal to 1 LSU and an adult sheep is equal to 0.15 LSU. There is an approximate upper constraint in the UK, using the livestock density index, of 2 LSU/ha to prevent overgrazing and a lower constraint of 0.5 LSU/ha to maintain grassland in good condition (Jones & Tranter, 2007). This would equate to 0.5 to 2 cattle per hectare, and 3.33 to 13.33 sheep per hectare. Finally, grazing management tools may use the measurement animal grazing days per hectare which is estimated from the stocking density and length of the grazing season (Dieguez Cameroni et al., 2006). With no further information available on the average stocking rate in GB of sheep and cattle, it is assumed that the stocking density for cattle per hectare of pasture per year has a minimum value of 0.36 and maximum value of 2.0. For sheep, the stocking density per hectare is a minimum value of 3.33, most likely of 5.02 and maximum of 14.1. It should be noted that these values apply to adult animals. The average yearly livestock stocking densities are associated with uncertainty. For cattle this uncertainty has been described in the model using a Uniform distribution between the minimum and maximum values. The uncertainty associated with the sheep distribution of stocking density is represented using a Betapert distribution. It is assumed that the stocking density for lambs is the same as the stocking density for sheep.

2.8 Dose response and Risk estimate

A dose response model is used to determine the probability of infection occurring as a result of exposure to a dose of a pathogen. The proportion of a population that becomes infected from a single dose, $P\_Infday_{a,k}$ can be estimated by the following exponential dose response equation (Teunis and Havelaar, 2000):

$$P\_Infday_{a,k} = 1 - e^{(-r * N\_consumed_{a,i,k})} \quad (13)$$

where r is the pathogen infectivity constant

This model assumes that each infectious particle's action is independent, that is, the probability of infection by each single agent is independent of the size of the dose. When $P\_Infday_{a,k} = 0.5$, that is, 50% of an exposed population becomes infected (ID_{50}), $-r = \mathrm{Ln}(0.5)$. Substituting into Eq 1:

$$P\_Infday_{a,k} = 1 - e^{(Ln(0.5) * N\_consumed_{a,i,k})} \quad (14)$$

For both cattle and sheep, in this risk assessment, the period of exposure is not limited to a single dose as successive doses are likely to be consumed over time as animals graze the land and may also consume derived hay and silage when housed. An annual risk of infection from T_exp exposures per year, assumed to be 365 days, to a pathogen dose can be estimated by the following equation:

$$P\_Infyear_{a,k} = 1\text{-}[1\text{-}\ P\_Infday_{a,k}]^{T\_exp} \quad (15)$$

Combining with Equation 14:

$$P\_Infyear_{a,k} = 1 - \left[1 - e^{(\ln(0.5) * P\_Infday_{a,k})}\right]^{T\_\exp} \quad (16)$$

where *T_exp* is the number of exposures per year, assumed to be 365 days as if livestock are housed for some of the year, grass may still be cropped for silage.

Finally, the number of livestock infected per year is estimated by the following equation:

$$N\_newinfected_{a,k} = N\_\exposed_{a,k} * P\_infyear_{a,k} \quad (17)$$

3 Results

Uncertainty and variability is considered in the model and represented by 5^{th} and 95^{th} percentiles (within parentheses), which indicate the range within which 90% of the results lie. The greater the range between the percentiles, the greater the total uncertainty. The model was run for 200,000 iterations using Latin Hypercube sampling. Convergence was achieved between 180,000 and 200,000 iterations. It should be emphasised that not all uncertainty and variability has been estimated in the calculations, as not all can be quantified. Therefore the 5^{th} and 95^{th} percentiles describe the amount of quantified uncertainty included in the model.

3.1 Percentage of infectivity going to floor by facility type

The first output of the risk assessment is the percentage of infectivity going to the floor due to the handling of infectious materials at each facility with results displayed in Table 1. At rendering facilities and large incinerators, the percentage of infectious material going to the floor is an assumed mean of 0.5% (0.12%, 0.98%).

Table 1: Estimated mean percentage of infectivity from a whole carcase to floor by facility type

Animal population and disease	Mean percentage of infectivity to floor (5^{th} , 95^{th} percentiles)		
	Abattoir (AB)	Intermediate/Small incinerators (INT/SIN)	Collection centre (CCN)
BSE in cattle	0.15 (0.06, 0.31)	0.31 (0.13, 0.52)	0.24 (0.07, 0.45)
Classical scrapie in sheep	0.03 (0.009, 0.05)	0.16 (0.04, 0.34)	0.16 (0.05, 0.34)
Classical scrapie in lambs	0.08 (0.03, 0.13)	7.9×10^{-7} (1.0×10^{-7}, 2.3×10^{-6})	0.005 (0.001, 0.009)
Atypical scrapie in sheep	0.02 (0.003, 0.05)	0.35 (0.11, 0.66)	0.35 (0.11, 0.66)
Atypical scrapie in lambs	1.5 (1.05, 1.95)	2.7×10^{-4} (4.9×10^{-5}, 6.6×10^{-4})	2.7×10^{-4} (4.9×10^{-5}, 6.6×10^{-4})

3.2 Total number of TSE infections per year due to wastewater disposal on pasture

The estimated mean number of TSE infections that arise per year due to the application of wastewater to pasture are displayed in Table 2. The mean values are low for each TSE disease considered, with 0.001 new infections of BSE in cattle, 0.033 new infections of classical scrapie in sheep per year and 0.030 new infections of atypical scrapie per year.

Table 2: Estimated mean number of TSE infections from grazing on pasture fertilised by wastewater per year

TSE disease	Mean number of TSE infections (5^{th} , 95^{th} percentiles)
BSE	0.0010 (1.8×10^{-6}, 0.0013)
Classical scrapie	0.033 (0.0004, 0.064)
Atypical scrapie	0.030 (0.0003, 0.061)

Such results can be represented as the number of years between one new infection if it is assumed that there is no change over time and equal intervals between infections. Using this assumption, the estimated mean number of years between infections for BSE in cattle is 1,000, and one infection every 30 years and 33 years for classical and atypical scrapie.

3.3 Percentage contribution to risk

The contribution to the total number of new TSE infections arising by facility type is given in Figure 2. For BSE in cattle, the largest contributions are from abattoirs (35%) and rendering facilities (22%). Intermediates, collection centres and small incinerators contribute 13%, 11% and 15% respectively with large incinerators contributing the least towards new BSE infections (4%). The largest contributions for classical scrapie are from rendering (31%), small incinerators (30%) and abattoirs (23%). Intermediates, collection centres and large incinerators contribute only small amounts to the total infectivity with between 3% and 8%. For atypical scrapie, the largest contributions are from small incinerators (34%), rendering (31%) and abattoirs (20%). The smallest contributions are from intermediates (3%), collection centres (4%) and large incinerators (9%).

BSE, classical scrapie and atypical scrapie are partitioned into each of the three wastewater types considered in this risk assessment (untreated/treated wastewater and wastewater sludge). The mean percentage contribution of each wastewater type to the total amount of infectivity is shown in
Figure 3. It can be seen that the sludge fraction arising during wastewater treatment contributes the most amount of infectivity for BSE and scrapie diseases, with between 47% and 62% contribution to the total risk respectively. Treated wastewater contributes the least on average, with an estimated 14% to 17% respective contribution to the total risk.

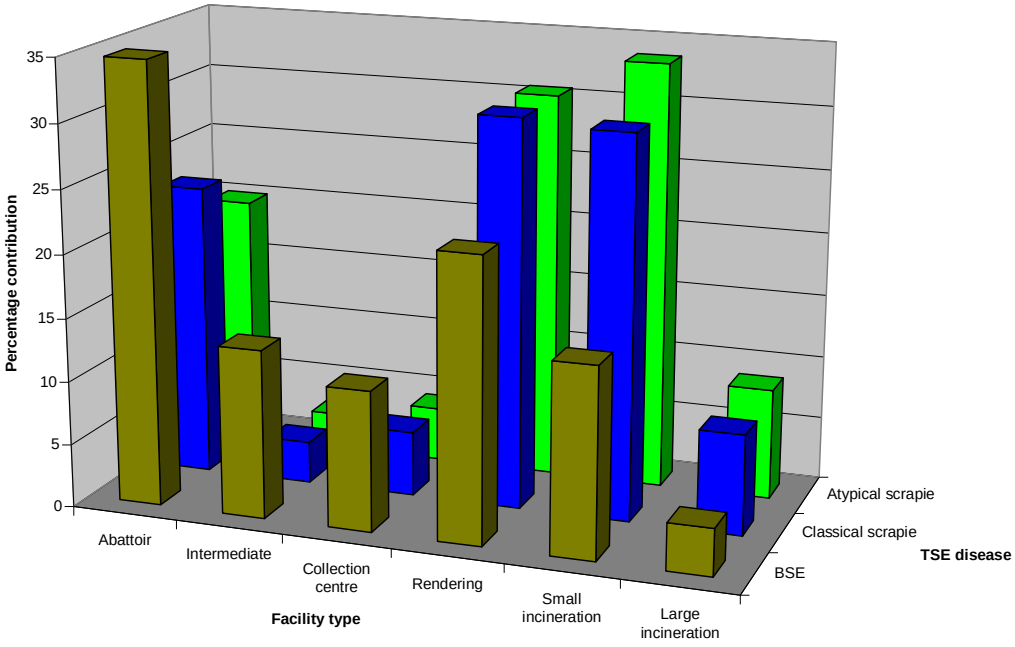

Figure 2: Mean percentage contribution to total infectivity by facility type

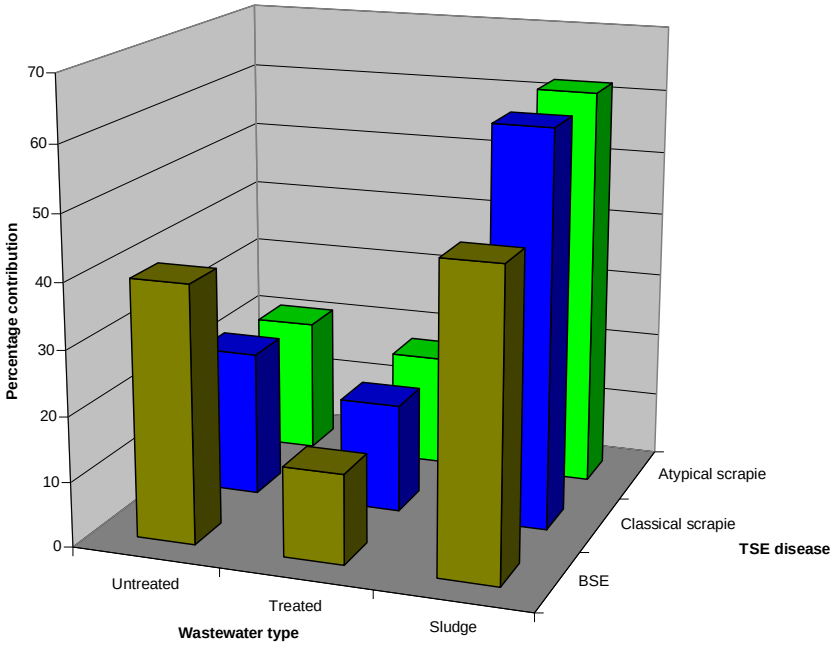

Figure 3: Mean percentage contribution to total infectivity by wastewater type

3.4 Sensitivity analysis

A multivariate stepwise regression analysis was used to calculate linear regression or sensitivity values for each input parameter in the model represented by a distribution. This method is preferred for large numbers of input parameters, as all values that provide an insignificant contribution are removed from the analysis. All parameters described by a range are included in the sensitivity analysis.

Estimated sensitivity values were estimated for the total number of TSE infections which occur due to the application of wastewater to pasture. For BSE in cattle, the results are strongly affected by four parameters: (1) uncertainty associated with the infectivity titre in the CNS at clinical onset, *MaxCNS*, (2) variability associated with the depth of application of wastewater, *Depth*, (3) uncertainty associated with the proportion of infectivity falling to the floor at rendering facilities, $P\_floor_{a,i,REN}$, (4) uncertainty associated with the conversion of mouse i.c. i.p. units to bovine oral units, *BOunit*.

For classical and atypical scrapie in sheep and lambs the results are strongly affected by three parameters: (1) variability associated with the depth of application of wastewater, *Depth*, (2) uncertainty associated with the titre conversion factor, *OOunit*, (3) variability associated with the amount of soil consumed by sheep per day, $Soil_S$.

It is interesting to note that although the risk assessment has a number of assessor assumptions based on facility visits, all significant parameters identified by the sensitivity analysis are based on data from the literature and surveys conducted.

3.5 Scenario testing

In order to assess the robustness of the model, selected scenarios have been completed to investigate the effect on the number of new TSE infections as predicted by the risk assessment: (1) the effectiveness of trap, (2) the amount of wastewater produced, and (3) the proportion of wastewater to pasture.

The effectiveness of trap in the baseline scenario has been implemented in the model by *P_trap*, as between 80% and 90% based on estimates in the literature (AFSSA, 2003; DNV 1997a). In order to investigate the impact of trap efficiency on the number of new TSE infections, values of 0%, 25%, 50% and 75% trap efficiency were simulated. The results are shown in Figure 4. It can be seen that the relationship between trap effectiveness and the mean number of new TSE infections is approximately linear. At 0% trap efficiency, the estimated number of new TSE infections rises to 0.0066 (1.3×10^{-5}, 0.0090) for BSE in cattle, 0.136 (0.0016, 0.273), for classical scrapie and 0.121 (0.0014, 0.239) for atypical scrapie, an increase of 6.6, 4.1 and 4.0 fold respectively.

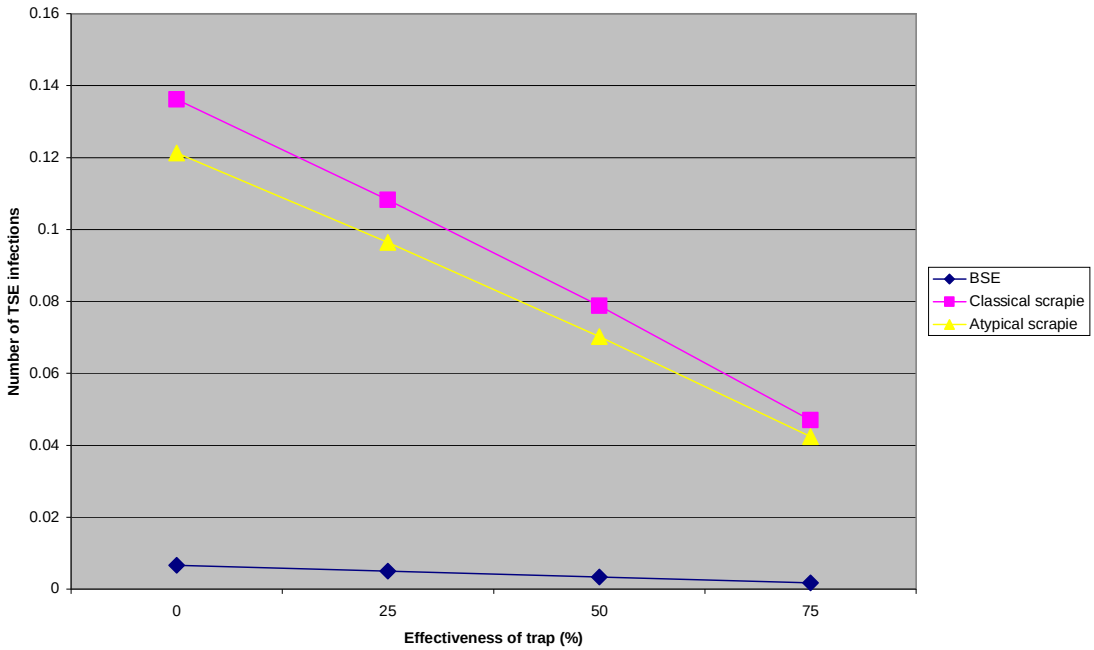

Figure 4: Contribution of trap effectiveness on the mean number of new TSE infections

The amount of wastewater produced at each facility was estimated using industry data and expert opinion based on the number of carcases processed, with resulting values associated with considerable uncertainty bounds. To investigate the impact of dramatic changes in wastewater production, the baseline figures were doubled and trebled. Neither scenario produced a significant change in the risk estimate in comparison with the baseline model.

In the baseline model, 40% of all wastewater applied to land is assumed to be spread on pasture. The calculation of this figure is based on the proportion of fertilised land in GB that is composed of grassland and is a point estimate in the model. To investigate the impact of unquantified uncertainty associated with this parameter, the baseline figure was increased to 70% followed by 100% (all fertilised land is grazed). When 70% of all wastewater applied to land is grazed (an increase by a factor of 1.75), the estimated mean number of new infections increases for BSE to 0.0017 (3.2×10^{-6}, 0.0023) cattle per year, 0.057 (0.0006, 0.112) new infections in sheep with classical scrapie, and 0.052 (0.0006, 0.106) new infections in sheep with atypical scrapie put in percentiles. These values describe an increase in the estimated number of infections in comparison to the baseline values by around a factor of 1.75. Hence the increase is linear with the additional amount of wastewater going to pasture. When 100% of all wastewater going to land is going to pasture (an increase by a factor of 2.5 over the baseline model), the mean number of infections are 0.0025 (4.6×10^{-6}, 0.0034) for BSE, 0.081 (0.0009, 0.160) for classical scrapie and 0.075 (0.0008, 0.152) for atypical scrapie. Again, the increase in size of these values over the baseline values is in line with the additional amount of wastewater going to pasture (i.e. an increase by around a factor of 2.5).

4 Discussion

In this report we have described the development and parameterisation of a quantitative risk assessment to assess the risk of cattle and sheep becoming infected with a TSE disease from wastewater produced in one year that has been directly spread onto pasture land. The key results indicate the number of new TSE infections arising would be low, with a mean of one infection every 1,000 years for BSE in cattle (769, 555,556), and one infection every 30 years (16, 2,500) and 33 years (16, 3,333) for classical and atypical scrapie in sheep, assuming that values and assumptions used in risk assessment remain constant. As shown by the range in brackets where 90% of the results are expected, the quantified uncertainty and variability associated with these estimates is large. From the sensitivity analysis the parameters with the greatest impact on the results are associated with variability of the depth of application of wastewater and with consumption of soil by livestock (*Depth, Soil* $_S$), and uncertainty associated with disease characteristics of tissue infectivity and ID_{50} conversion units (*MaxCNS, BOunit, OOunit*), which have been noted in previous TSE risk assessments (Adkin et al., 2010). The variability associated with parameters in an assessment cannot be reduced, but with further research uncertainty can be reduced. However, in view of the steady decline of BSE risk to public health, it would seem unlikely that any further large animal TSE disease research, giving rise to quantitative data appropriate for the risk assessment, will be funded. Finally, the parameter associated with the proportion of infectivity falling to the floor at rendering facilities, $P\_floor_{a,i,REN}$, was found to be influential. This parameter could be further enumerated by experimentation. However, the identified obstacles to such measurements identified in the AFSSA work (2003) would need to be considered when producing any experimental protocol.

The contribution to the number of new TSE infections by facility type differs by TSE disease and for sheep and lambs. This is due to each disease having a different predilection for different tissue types which varying in their probabilities of falling to floor by facility type, and the different processing that may be applied to lambs as opposed to larger adult sheep. For BSE in cattle the main contributors are abattoir and rendering effluent, contributing 35% and 22% of the total number of new BSE infections. For TSEs in sheep, effluent from small incinerators and rendering are the major contributors (on average 32% and 31% of the total number of new classical scrapie and atypical scrapie infections). Such contributions are due to the volume of material flow through such facilities. It needs to be remembered that an assumption is made in this risk assessment, that all facilities use a 6mm trap, whereas many facilities, particularly the rendering industry, are known to use traps of 4mm down to 1mm that may retain more material. Therefore, this assumption is worst case.

Another key assumption is that the risk assessment does not include illegal activities that may by-pass controls. Due to the permanent structures in place, it is less likely that large scale industries, such as rendering and large incinerators (which comprise a combined contribution to risk of 26% for BSE and 39% for sheep TSEs), can by-pass built in controls when compared to smaller more ad-hoc operations such as intermediate plants, collection centres, and small incinerators (which together contribute 39% for BSE and

40% for sheep TSEs to the risk estimate). Such facility types, together with smaller abattoir operations, are locations where illegal operations could occur and which contribute significantly to risk. For example, an abattoir operating illegally with no blood tank and no controls on wastewater could be illegally spreading such effluent direct to pasture. However, from discussions with Animal Health, such occurrences are likely to be rare although there are recorded incidents. Whilst current SRM controls are in place the amount of infectivity released by such rare events will still be small.

As stated in the introduction, a number of BSE in cattle risk assessments have been previously conducted. Where appropriate, data has been used from these publications in addition to the current estimates made by assessors from visits to GB facilities. Table 3 provides a comparison of values used in this risk assessment as compared to those used in previous risk assessments. There are two different units, firstly, the mean percentage of infectivity to floor (%) based on total infectivity per carcase, and secondly the equivalent weight of infectious tissue to floor (g).

Table 3: Estimated mean number of TSE infections from grazing on pasture fertilised by wastewater per year (min, max)

Facility type	Percentage of infectivity to floor (%)	Weight of infectivity to floor (g)	Estimates available in the literature
Abattoir (AB)	0.15% (0.04%, 0.6%)	1.3 g (0.4 g, 4.6 g)	2.7 g (AFSSA,2003) 1 to 20 g (BRS, 2001) 1% (Gale et al., 2001) 0.01% to 1% (Motes et al., 2008)
Collection centre (CCN)	0.24% (0.03%, 0.7%)	-	0.7% DNV, 1997a
Renderer (REN)	0.5% (0.01%, 1.5%)	-	0.5% DNV, 1997a
Large incinerator (INC)	0.5% (0.01%, 1.5%)	-	0.011% DNV, 1997a

From the table it can be seen that the values previously used in analyses are within the range used in this risk assessment with some exceptions. The Australian abattoir model used an upper limit of 20 g CNS tissue per carcase (BRS, 2001). This may be due to different abattoir controls in place in Australia when compared to Europe. In addition, the 1997 DNV estimate for abattoir is outside our maximum range. This is likely to reflect the change in abattoir controls brought about by the ABP regulations in 2002 (EC, 2002). For the AFSSA study the total 2.73 g of infectious material to the floor includes 0.82 g collected in the area of the blood bath that is collected separately at abattoir for Category 1 disposal under the ABP regulations. Of the remaining material 0.27 g is assumed to arise from carcase splitting and 1.64 g arising from “drain vats” (AFSSA, 2003). Contact with several of the AFSSA reports authors has not further elucidated the origin of this infectivity.

Three scenarios have been explored, the first to investigate the impact of retention of the 6 mm trap on the expected number of new TSE infections per year. By varying the efficiency of the trap from 0% to 75%, the relationship between the trap retaining material and new infections is approximately linear. When considering that the trap retains no material, the expected number of new infections increases approximately 6.5 fold for BSE and 4 fold for classical and atypical scrapie. When considering whether a 0% effective trap is the same as removing the trap, further investigation would be required. Additional supporting information would need to be collected to establish whether any operator behavioural changes may accompany the removal of the trap, for example, unconsciously would more material be allowed to fall to floor as there is no trap to lift and unblock when full?

The second scenario investigated the impact of the amount of wastewater produced per year. Increases in production did not affect the risk estimate. The occurrence of the parameter in Equation (11) and (12) indicate that as more wastewater is produced, any infectivity present becomes more diluted. However, that same total amount of infectivity is spread on more land, leading to more animals being exposed. Given that the dose response model does not have a threshold value, changes in the parameter have no effect on the number of new TSE infections. The third scenario investigates the amount of wastewater applied to pasture that is included in the baseline model as a point value of 40%. This parameter is extremely uncertain however, such uncertainty is difficult to qualify. Scenario testing established a linear relationship between pasture application and number of new TSE infections.

The risk assessment is based on wastewater production and application to land over one year. However, effluent is applied each year and in areas where repeated applications are made there may be an accumulation of infectivity on those fields. The rate of decay of TSEs in soil has been investigated with the longest study published in 1991 by Brown and Gadjusek. The authors demonstrated that hamster adapted scrapie infectivity reduced by approximately 99.7% to 98.3% after burial for three years from initial high levels of infectivity (170×10^6) (Brown and Gadjusek, 1991). However, long term environmental persistence has been recorded, for one farm in Iceland, epidemiological investigation into an outbreak of scrapie established with near certitude that the disease could not have been externally introduced and concluded that the agent may have persisted in an old sheep house for at least 16 years (Georgsson et al., 2006). Given the expected low numbers of BSE infected cattle and the random occurrence of disease amongst herds, it is unlikely at the current time for infected animals to be processed and any contaminated wastewater to be applied to the same location in successive years. However, for scrapie in sheep accumulation is more likely if certain areas have repeated application of wastewater in successive years. In order to investigate this phenomenon in more detail a survey of the contractors that carry out wastewater collection and spreading would need to be completed, with details collected of the locations where wastewater is applied and frequency of application.

The risk assessment focuses on an industry in GB that is not well characterised in the literature and is subject to constant changes due to market demands. Therefore, the results

are essentially a snap shot in time of a constantly evolving industry. During the facility visits undertaken and surveys conducted the changing nature of the industry was highlighted. There have been significant recent reductions in the usage of small incinerators and the moth balling of several large scale incinerators due to high fuel costs, and conversely a rise in the value of animal wastes to be used as a co-fuel to produce energy. Given the limitations of published accounts, several areas of the risk assessment are based on expert opinion and assessors assumptions based on viewing processes conducted. However, all significant parameters identified by the sensitivity analysis are based on data that was available from the literature and surveys conducted.

5 Conclusions

A quantitative risk assessment has been developed to assess the risk of cattle and sheep becoming infected with a TSE disease from wastewater that has been directly spread onto pasture land. The key results indicate the number of new TSE infections arising would be low. Our results indicate that the predicted number of new TSE infections arising from production of wastewater over one year would be low, with a mean of one infection every 1,000 years for BSE in cattle, one infection every 30 years for classical scrapie and 33 years for atypical scrapie, assuming no change in conditions.

For BSE in cattle the main contributors are abattoir and rendering effluent and for TSEs in sheep, effluent from small incinerators and rendering are the major contributors. This is a reflection of the volume of carcase material and Category 1 material flow through such facilities.

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Appendix 1: Table of input parameters

Table 3A: Parameter descriptions and values within wastewater risk assessment

Parameter	Symbol	Value	Unit	Reference
Farm				
Probability a cow is slaughtered at abattoir is infected with BSE and is in the last 12 months of the incubation period	$P\_infected_{C,bse,HS}$	Betapert (3.9×10^{-7}, 1.4×10^{-6}, 2.2×10^{-6})	%	Arnold, 2009, pers comm. based on method from Arnold and Wilesmith, 2003
Probability a cow enters fallen stock and is infected with BSE and is in the last 12 months of the incubation period	$P\_infected_{C,bse,FS}$	Betapert (3.3×10^{-6}, 4.4×10^{-6}, 1.8×10^{-5})	%	Arnold, 2009, pers comm. based on method from Arnold and Wilesmith, 2003
Probability a sheep slaughtered at abattoir is infected with classical scrapie	$P\_infected_{S,sc,HS}$	Betapert (0, 9.0×10^{-4}, 4.2×10^{-3})	%	Ortiz-Pelaez and Arnold, VLA 2009
Probability a sheep entering fallen stock is infected with classical scrapie	$P\_infected_{S,sc,FS}$	$Beta(5,10125) * \frac{P\_\text{infected}_{S,sc,Hs}}{Beta(3,10157)}$	%	Ortiz-Pelaez, pers. comm. 2009; Ortiz-Pelaez and Arnold, VLA 2009
Probability a sheep slaughtered at abattoir is infected with atypical scrapie	$P\_infected_{S,at,HS}$	Betapert (3.7×10^{-4}, 2.4×10^{-3}, 6.6×10^{-3})	%	Ortiz-Pelaez, pers. comm. 2009; Ortiz-Pelaez and Arnold, VLA 2009
Probability a sheep entering fallen stock is infected with atypical scrapie	$P\_infected_{S,at,FS}$	$Beta(5,10125) * \frac{P\_\text{infected}_{S,at,Hs}}{Beta(6,10154)}$	%	Ortiz-Pelaez, pers. comm. 2009; Ortiz-Pelaez and Arnold, VLA 2009
Number of cattle slaughtered	$N\_animals_{C,HS}$	2,301,868	Cattle	Adapted from British Cattle Movement Service (BCMS) (Woods, pers. comm. 2009) and VLA BSE testing data (Rajanayagam, pers. comm. 2009)
Number of cattle entering fallen stock	$N\_animals_{C,FS}$	416,941	Cattle	Adapted from British Cattle Movement Service (BCMS) (Woods, pers. comm. 2009) and VLA BSE testing data (Rajanayagam, pers. comm. 2009)
Number of sheep slaughtered	$N\_animals_{S,HS}$	2,182,930	Sheep	Defra Slaughter Statistics, 2010
Number of sheep entering fallen stock	$N\_animals_{S,FS}$	Betapert (149980, 264222, 446994)	Sheep	Adapting method of Bansback, 2006 using data from CiWF, 2008; SAC, 2009; Schoenian and Burfening, 1990; Defra, 2009

Parameter	Symbol	Value	Unit	Reference
Number of lambs slaughtered	$N\_animals_{L,HS}$	13,357,036	Lamb	Defra Slaughter Statistics, 2010
Number of lambs entering fallen stock	$N\_animals_{L,FS}$	Betapert (761381, 861399, 1718813)	Lamb	Adapting method of Bansback, 2006 using data from CiWF, 2008; SAC, 2009; Schoenian and Burfening, 1990; Defra, 2009
SRM handling facilities				
Proportion of healthy slaughter flow from abattoir to rendering	$P\_flow\_1_{a,HS,REN}$	Betapert (50%,80%,90%)	%	Assessors adaptation of data from BCMS (number of cattle tested in 2008), AH
Proportion of healthy slaughter flow from abattoir to incineration	$P\_flow\_1_{a,HS,INC}$	1- $P\_flow\_1_{a,HS,REN}$	%	(Location of TSE tests, number of ABPR facilities), expert opinion from AH,
Proportion of fallen stock flow from farm to collection centre	$P\_flow\_1_{a,FS,CNN}$	C= Betapert (10%,20%,30%) S= Betapert (10%,20%,30%) L= Betapert (5%,15%,20%)	%	LASSA and the rendering industry
Proportion of fallen stock flow from farm to intermediate	$P\_flow\_1_{a,FS,INT}$	C= Betapert (20%,25%,30%) S= Betapert (10%,15%,20%) L= Uniform(5%,10%)	%	
Proportion of fallen stock flow from farm to rendering	$P\_flow\_1_{a,FS,REN}$	Betapert (20%,40%,50%)	%	
Proportion of fallen stock flow from farm to incinerator	$P\_flow\_1_{a,FS,INC}$	1- ($P\_flow\_1_{a,FS,CNN}$ + $P\_flow\_1_{a,FS,INT}$ + $P\_flow\_1_{a,FS,INC}$)	%	
Proportion of fallen stock flow from intermediate to collection centre	$P\_flow\_2_{a,FS,CCN}$	C= Betapert (10%,20%,45%) S= Betapert (7%,15%,40%) L= Betapert (5%,15%, 40%)	%	
Proportion of fallen stock flow from intermediate to rendering	$P\_flow\_2_{a,FS,REN}$	Betapert (40%,50%,75%)	%	
Proportion of fallen stock flow from intermediate to incineration	$P\_flow\_2_{a,FS,INC}$	1- ($P\_flow\_2_{a,FS,CCN}$ + $P\_flow\_2_{a,FS,REN}$ + $P\_flow\_2_{a,FS,INC}$)	%	
Proportion of fallen stock flow from collection centre to renderer	$P\_flow\_3_{a,FS,REN}$	C=Uniform(70%,95%) S=Uniform(60%,92%) L=Uniform(60%,92%)	%	
Proportion of fallen stock flow from collection centre to incinerator	$P\_flow\_3_{a,FS,INC}$	1- $P\_flow\_3_{a,FS,REN}$	%	
Infectivity - BSE in cattle				
Conversion factor for Mouse ic ip ID_{50}/g to Bovine oral ID_{50}/g	*BOunit*	Betapert (1.81, 2.8, 3.79)	-	Wells et al., 2007
Infectivity of brain and spinal cord tissues in cattle at clinical onset of disease	*MaxCNS*	Normal (3.3, 0.3364), truncated between 2.4 and 5.2	Mouse ic ip ID_{50}/g	Arnold et al., 2009
Infectivity - Classical and Atypical scrapie in sheep/lambs				

Parameter	Symbol	Value	Unit	Reference
Maximum titre of infectivity of Classical scrapie in tissue *t* measured in units of Log_{10} Mouse ic ID_{50}/g	$Max_{sc,t}$	1, brain = 5.6 ± 0.2 (51) 2, spinal cord = 5.4 ± 0.3 (9) 3, lymph nodes = 4.2 ± 0.1 (45) 4, spleen = 4.5 ± 0.3 (9) 5, tonsils = 4.2 ± 0.4 (9) 6, ileum = 4.7 ± 0.1 (9) 7, liver = 2.0 ± 0.1 (9) 8, pancreas = 2.1 ± 0.1 (9) 9, thymus = 2.2 ± 0.2 (9) 10, stomach = 2* 11, heart = 1* 12, kidney= 1* 13, duodenum & jejunum = same as ileum ◊ 14, blood = Uniform(-1, 0) ◊	Log_{10} Mouse ic ID_{50}/g	Original data from Kimberlin and Wilesmith, 1994. Titres are expressed as mean ± SEM of (n) samples * Gale (2002) ◊ assessors assumption
Conversion factor from mouse ic log ID_{50}/g units to ovine oral ID_{50}/g	*OOunit*	Parametric bootstrap on Normal(7.03,0.2366) - Normal(2.03,0.2527)	-	Kimberlin & Wilesmith (1994) Vose (2000)
Percentage of maximum infectivity in tissue *t* at time of death for Classical scrapie in sheep	$P\_infectivity_{S,sc,t}$	Uniform (70%,100%)	%	Data adapted from Gale, 2002 and DNV, 2002
Percentage of maximum infectivity in tissue *t* at time od death for Classical scrapie in lambs	$P\_infectivity_{l,sc,t}$, *t = 1,2*	0.10%	%	Data adapted from Gale, 2002 and DNV, 2002
	$P\_infectivity_{l,sc,t}$, *t = 4,5,7,8,9,10, 11,12,14*	10%	%	Data adapted from Gale, 2002 and DNV, 2002
	$P\_infectivity_{l,sc,t}$, *t = 3,6,13*	40%	%	Data adapted from Gale, 2002 and DNV, 2002
Maximum titre of Atypical scrapie infectivity in tissue *t* measured in units of Log_{10} Mouse ic ID_{50}/g	$Max_{at,t}$	1, brain = Same as for classical scrapie 2, spinal cord = Same as for classical scrapie 3, lymph nodes = brain infectivity titre - Uniform(5, 6)	Log_{10} Mouse ic ID_{50}/g	Assessors assumption and expert opinion, VLA
Percentage of maximum infectivity in tissue *t* at death for Atypical scrapie in sheep	$P\_infectivity_{S,at,t}$, *t = 1,2,3*	Uniform(40%,80%)	%	Assessors assumption and expert opinion, VLA
Percentage of maximum infectivity in tissue *t* at death for Atypical scrapie in lambs	$P\_infectivity_{l,at,t}$, *t = 1,2,3*	0.1%	%	Assessors assumption and expert opinion, VLA
Tissue weights				
Total amount of CNS material in skull for cattle	$N\_Cat1_{C,k,1}$	500+20+1	g	Hart et al., 1997
Total amount of spinal cord for cattle	$N\_Cat1_{C,k,2}$	Uniform(200,482)	g	Hart et al., 1997; SEAC, 1996

Parameter	Symbol	Value	Unit	Reference
Tissue weights for lambs	$N\_Cat1_{l,k,t}$	1, brain = 100 2, spinal cord = 40 3, lymph nodes = 38 ◊ 4, spleen = 75* 5, tonsils = 2 ◊ 6, ileum = 100* 7, liver = 610 8, pancreas = 100 9, thymus = 50 10, stomach = 1,000 11, heart = 200 12, kidney = 100 13, duodenum & jejunum = 930 ◊ 14, blood = 1,700	g	Assessors assumption based on Hart et al., 1997 * DARD: VPHU Manual for Official Controls ◊ Assessors assumption based on Fryer et al., 2007
Tissue weights for sheep	$N\_Cat1_{S,k,t}$	1, brain = 160 2, spinal cord = Uniform(50*,64) 3, lymph nodes = 60.8 4, spleen = 300* 5, tonsils = 3.2 6, ileum = 200* 7, liver = 976 8, pancreas = 160 9, thymus = 80 10, stomach = 1,600 11, heart = 320 12, kidney = 160 13, duodenum & jejunum = 1,488 14, blood = 2,720	g	Application of 1.6 scale factor (Gale, 2002) to weight of lamb tissue * DARD: VPHU Manual for Official Controls
Abattoir				
Amount of brain to floor for BSE in cattle	$N\_floor_{C,AB,1}$	Betapert (0.135, 1.03, 4.659)	g	Cooper and Bird, 2002
Amount of spinal cord to floor for BSE in cattle	$N\_floor_{C,AB,2}$	0.27	g	AFSSA, 2003
Amount of blood lost to blood tank at exsanguination for sheep	$N\_Cat1_{a,AB,14}$	Sheep: Uniform(0.4,0.6) * 2720 Lambs: Uniform(0.4,0.6) * 1700	g	Warriss, 2000
Amount of blood to floor from further processing for sheep	$N\_floor_{a,AB,14}$	Sheep: Uniform(0.1,0.2) * 2720 Lambs: Uniform(0.1,0.2) * 1700	g	Assessors assumption based on observation
Amount of brain to trap for lambs	$N\_floor_{l,AB,1}$	Uniform(0.01,0.02) * 100	g	Assessors assumption
Amount of spinal cord to trap for sheep	$N\_floor_{S,AB,2}$	(0.27 / Uniform(200,482)) * 64	g	Assessors assumption based on proportion of spinal cord to trap for cattle

Parameter	Symbol	Value	Unit	Reference
Amount of spleen to trap for sheep	$N\_floor_{S,AB,4}$	Uniform(0.0001,0.001) * 300	g	Assessors assumption based on observation
Amount of spleen to trap for lambs	$N\_floor_{l,AB,4}$	Uniform(0.0001,0.001) * 75	g	Assessors assumption based on observation
Amount of ileum to trap for sheep	$N\_floor_{S,AB,6}$	Uniform(0.0001,0.001) * 200	g	Assessors assumption based on observation
Amount of ileum to trap for lambs	$N\_floor_{l,AB,6}$	Uniform(0.0001,0.001) * 100	g	Assessors assumption based on observation
Intermediate plant				
Amount of brain to trap for BSE in cattle	$N\_floor_{C,INT,1}$	Route 1: Uniform(0.6,0.85) * 0.15 * Uniform(0.34,1) Route 2: Uniform(0.6,0.85) * Uniform(0.4,0.6) * Uniform(1,10) Route 3: Betapert (0.01,0.568,1)	g	INT, pers. comm. 2009; AFSSA, 2003; Comer & Huntly, 2004
Amount of brain to trap for sheep	$N\_floor_{S,INT,1}$	Route 1: Uniform(0.6,0.85) * 0.15 * Uniform(0.34,1) Route 2: Uniform(0.6,0.85) * Uniform(0.4,0.6) * (Uniform(1,10) / 500) * 160 Route 3: (9300 / $N\_animals_{S,FS}$) * (Betapert (0.01,0.568,1) / 500) * 160	g	Assessors assumption based on expert opinion and values for amount of brain to trap for cattle; Jon Weston, VLA, pers. comm. 2010
Amount of brain to trap for lambs	$N\_floor_{l,INT,1}$	Route 1: 0.1 * Uniform(0.001,0.01) * 0.15 * Uniform(0.34,1) Route 2: 0.1 * Uniform(0.001,0.01) * Uniform(0.4,0.6) * (Uniform(1,10) / 500) * 100	g	Assessors assumption based on expert opinion and proportions/values for amount of brain to trap for cattle
Collection centre				
Amount of brain to trap for BSE in cattle	$N\_floor_{C,CCN,1}$	Route 1: Uniform(0.6,0.85) * 0.15 * Uniform(0.34,1) Route 2: Uniform(0.6,0.85) * Uniform(0.4,0.6) * Uniform(1,10)	g	CCN, pers. comm. 2009; AFSSA, 2003; Comer & Huntly, 2004
Amount of brain to trap for sheep	$N\_floor_{S,CCN,1}$	Route 1: Uniform(0.6,0.85) * 0.15 * Uniform(0.34,1)	g	Assessors assumption based on expert opinion and proportions/values for

Parameter	Symbol	Value	Unit	Reference
				amount of brain to trap for cattle
		Route 2: Uniform(0.6,0.85) * Uniform(0.4,0.6) * (Uniform(1,10) / 500) * 160		
Amount of brain to trap for lambs	$N\_floor_{l,CCN,1}$	Route 1: 0.1 * Uniform(0.001,0.01) * 0.15 * Uniform(0.34,1)	g	Assessors assumption based on expert opinion and proportions/values for amount of brain to trap for cattle
		Route 2: 0.1 * Uniform(0.001,0.01) * Uniform(0.4,0.6) * (Uniform(1,10) / 500) * 100		
Amount of ileum to trap for sheep	$N\_floor_{S,CCN,6}$	Uniform(0.0001,0.001) * 200	g	Assessors assumption
Amount of ileum to trap for lambs	$N\_floor_{l,CCN,6}$	Uniform(0.0001,0.001) * 100	g	Assessors assumption
Rendering				
Proportion of infectivity to trap	$P\_floor_{a,REN}$	Betapert (0.001,0.01,0.015)	%	Adapted from DNV 1997a, 1997b
Proportion of BSE infectivity remaining after rendering	REN_{bse}	Betapert (0.001,0.01,0.02)	%	Assessors adaptation of data from Taylor et al. 1995 and Schreuder et al. 1998
Proportion of scrapie infectivity remaining after rendering	REN_{sc} REN_{at}	Betapert (0.00079,0.01,0.02)	%	Assessors adaptation of data from Taylor et al. 1997 and Schreuder et al. 1998
Proportion of MBM from rendering to incineration	P_MBM	83%	%	Renderers Survey, 2010
Small incinerators				
Proportion of infectivity to trap from handling fallen stock sent from farms	$P\_floor_{a,SIN,1}$	Value is assumed to be the same as the proportion of infectivity for a single carcase going to trap at an intermediate	%	Assessors assumption
Proportion of infectivity to trap from handling materials sent from intermediates and collection centres	$P\_floor_{a,SIN,2}$	Value is assumed to be the same as the proportion of infectivity for a single carcase going to trap at a collection centre	%	Assessors assumption
Large incinerators/technical plants				
Proportion of infectivity to trap	$P\_floor_{a,i,INC}$	Betapert (0.001,0.01,0.015)	%	Assessors assumption
Effectiveness of 6 mm trap				
Proportion of material stopped by 6 mm trap	P_trap	Uniform(0.8,0.9)	%	Adapted from AFSSA, 2003 and DNV 1997a
Processing of wastewater after 6 mm trap				
Proportion of wastewater from abattoir, intermediate, collection centre and small incinerators that is treated	$P\_W\_treated_{AB}$ $P\_W\_treated_{INT}$ $P\_W\_treated_{CCN}$ $P\_W\_treated_{SIN}$	1 - Beta(Uniform(5,8)+1,14 - Uniform(5,8)+1)	%	Adapted from Buncic, 2002
Proportion of wastewater from rendering and large incinerator facilities that is treated	$P\_W\_treated_{REN}$ $P\_W\_treated_{INC}$	80%	%	Renderers Survey, 2010

Parameter	Symbol	Value	Unit	Reference
Percentage of infectivity removed from wastewater due to treatment at abattoir, intermediate, collection centre and small incinerator	$P\_S\_Inf_{AB}$ $P\_S\_Inf_{INT}$ $P\_S\_Inf_{CCN}$ $P\_S\_Inf_{SIN}$	Uniform(30%, 99%)	%	Assessors assumption based on Quinn and Fabiansson, 2001
Percentage of infectivity removed from wastewater due to treatment at rendering and large incinerator facilities	$P\_S\_Inf_{REN}$ $P\_S\_Inf_{INC}$	Uniform(99%, 99.4%)	%	Adapted from Gale et al., 2000
Wastewater				
Proportion of untreated wastewater to land from abattoirs, intermediates, collection centres and small incinerators	$P\_UW\_pasture_{AB}$ $P\_UW\_pasture_{INT}$ $P\_UW\_pasture_{CCN}$ $P\_UW\_pasture_{SIN}$	Beta(2+1,10-2+1)	%	Buncic, 2002
Proportion of untreated wastewater to land from rendering and large incinerators facilities	$P\_UW\_pasture_{REN}$ $P\_UW\_pasture_{INC}$	3%	%	Renderers Survey, 2010
Proportion of treated wastewater to land from abattoirs, intermediates, collection centres, and small incinerators	$P\_W\_pasture_{AB}$ $P\_W\_pasture_{INT}$ $P\_W\_pasture_{CCN}$ $P\_W\_pasture_{SIN}$	Beta(2+1,4-2+1)	%	Buncic, 2002
Proportion of treated wastewater to land from rendering and large facilities	$P\_W\_pasture_{REN}$ $P\_W\_pasture_{INC}$	25%	%	Renderers Survey, 2010
Proportion of wastewater sludge to land from abattoirs	$P\_S\_pasture_{AB}$ $P\_S\_pasture_{INT}$ $P\_S\_pasture_{CCN}$ $P\_S\_pasture_{SIN}$	Beta(6+1,6-6+1)	%	Buncic, 2002
Proportion of wastewater sludge to land from rendering facilities	$P\_S\_pasture_{REN}$ $P\_S\_pasture_{INC}$	41%	%	Renderers Survey, 2010
Proportion of land which is grazed	$P\_pasture$	40%	%	Defra Agricultural Census, 2009; Defra, 2007
Application to land				
Amount of wastewater produced per year applied to pasture	$N\_wastewater_{AB}$ $N\_wastewater_{INT}$ $N\_wastewater_{CCN}$ $N\_wastewater_{SIN}$ $N\_wastewater_{REN}$ $N\_wastewater_{INC}$	3×10^7 1×10^4 2×10^4 4×10^4 1×10^6 7×10^5 Mean values shown as production dependent on carcase number which varies by $N\_animal_{a,i}$	Tonnes per year	Adapted from MHS throughput data, BAT guidance, AB visit, 2009; LASSA, pers. comm. 2009; Renderers Survey, 2010

Parameter	Symbol	Value	Unit	Reference
Application rate	*Application*	250	Tonnes/ha/year	Mittal, 2006
Depth of application	*Depth*	Uniform(0,0.25)	m	Animal Health, pers. comm. 2010
Density of soil	*Density*	Beta Pert (0.88, 0.9, 0.92)	Tonnes/m^3	Engles, 1999
Consumption				
Amount of soil consumed per day per animal	$Soil_C$ $Soil_S$	Uniform(0.23,0.38)/1000 Uniform(0.14,2.45)/1000	tonnes	Thornton & Abrahams, 1983; Peterson et al., 1974
Stocking density of animals per hectare per year	$Stocking_C$ $Stocking_S$	Uniform(0.36,2) Beta Pert(3.33333,5.028595,14.1)	Head per ha	Scotland: Chadwick, 2003; England: MLC, 2001; UK: MLC, 2002; McIntyre, et al., 2006; Jones & Tranter, 2007
Dose response				
Number of exposures per year	*T_Exp*	365		Assessors assumption

Where
a = Animal population; *C* Cattle, *S* sheep, and *l* lambs
i = TSE disease; *bse* BSE, *sc* Classical Scrapie, and *at* Atypical Scrapie
j = Exit stream; *HS* Healthy slaughter and Emergency slaughter, *FS* fallen stock
k = SRM handling facility; *AB* abattoir, *INT* intermediate, *SIN* small incinerator, *CCN* collection centre, *REN* rendering, *INC* high throughput incinerator
t = Tissue type 1 to 14

Appendix 2: Legislation

Extract from:

REGULATION (EC) No 1774/2002 of THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption

Article 4
Category 1 material

1. Category 1 material shall comprise animal by-products of the following description, or any material containing such by-products:

(a) all body parts, including hides and skins, of the following animals:

- (i)animals suspected of being infected by a TSE in accordance with Regulation (EC) No 999/2001 or in which the presence of a TSE has been officially confirmed,
- (ii) animals killed in the context of TSE eradication measures,
- (iii) animals other than farmed animals and wild animals, including in particular pet animals, zoo animals and circus animals,
- (iv) experimental animals as defined by Article 2 of Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (2), and
- (v) wild animals, when suspected of being infected with diseases communicable to humans or animals;

(b) (i) specified risk material, and

- (ii) where, at the time of disposal, specified risk material has not been removed, entire bodies of dead animals containing specified risk material;

(c) products derived from animals to which substances prohibited under Directive 96/22/EC have been administered and products of animal origin containing residues of environmental contaminants and other
substances listed in Group B(3) of Annex I to Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC (1), if such residues exceed the permitted level laid down by Community legislation or, in the absence thereof, by national legislation;

(d) all animal material collected when treating waste water from Category 1 processing plants and other premises in which specified risk material is removed, including screenings, materials from desanding, grease and oil mixtures, sludge and materials removed from drains from those premises, unless such material
contains no specified risk material or parts of such material;

(e) catering waste from means of transport operating internationally;

And

(f) mixtures of Category 1 material with either Category 2 material or Category 3 material or both, including any material destined for processing in a Category 1 processing plant.

Article 5
Category 2 material

1. Category 2 material shall comprise animal by-products of the following description, or any material containing such by-products:

(a) manure and digestive tract content;

(b) all animal materials collected when treating waste water from slaughterhouses other than slaughterhouses covered by Article 4(1)(d) or from Category 2 processing plants, including screenings, materials from desanding, grease and oil mixtures, sludge and materials removed from drains from those premises;

(c) products of animal origin containing residues of veterinary drugs and contaminants listed in Group B(1) and (2) of Annex I to Directive 96/23/EC, if such residues exceed the permitted level laid down by Community legislation;

(d) products of animal origin, other than Category 1 material, that are imported from non-member countries and, in the course of the inspections provided for in Community legislation, fail to comply with the veterinary requirements for their importation into the Community, unless they are returned or their importation is accepted under restrictions laid down under Community legislation;

(e) animals and parts of animals, other than those referred to in Article 4, that die other than by being slaughtered for human consumption, including animals killed to eradicate an epizootic disease;

(f) mixtures of Category 2 material with Category 3 material, including any material destined for processing in a Category 2 processing plant; and

(g) animal by-products other than Category 1 material or Category 3 material.

Article 6
Category 3 material

1. Category 3 material shall comprise animal by-products of the following description, or any material containing such by-products:

(a) parts of slaughtered animals, which are fit for human consumption in accordance with Community legislation, but are not intended for human consumption for commercial reasons;

(b) parts of slaughtered animals, which are rejected as unfit for human consumption but are not affected by any signs of diseases communicable to humans or animals and derive from carcases that are fit for human consumption in accordance with Community legislation;

(c) hides and skins, hooves and horns, pig bristles and feathers originating from animals that are slaughtered in a slaughterhouse, after undergoing ante-mortem inspection, and were fit, as a result of such inspection, for slaughter for human consumption in accordance with Community legislation;

(d) blood obtained from animals other than ruminants that are slaughtered in a slaughterhouse, after undergoing ante-mortem inspection, and were fit, as a result of such inspection, for slaughter for human consumption in accordance with Community legislation;

(e) animal by-products derived from the production of products intended for human consumption, including degreased bones and greaves;

(f) former foodstuffs of animal origin, or former foodstuffs containing products of animal origin, other than catering waste, which are no longer intended for human consumption for commercial reasons or due to problems of manufacturing or packaging defects or other defects which do not present any risk to humans or animals;

(g) raw milk originating from animals that do not show clinical signs of any disease communicable through that product to humans or animals;

(h) fish or other sea animals, except sea mammals, caught in the open sea for the purposes of fishmeal production;

(i) fresh by-products from fish from plants manufacturing fish products for human consumption;

(j) shells, hatchery by-products and cracked egg by-products originating from animals which did not show clinical signs of any disease communicable through that product to humans or animals;

(k) blood, hides and skins, hooves, feathers, wool, horns, hair and fur originating from animals that did not show clinical signs of any disease communicable through that product to humans or animals; and

(l) catering waste other than as referred to in Article 4(1)(e).

Extract from:

THE MEAT HYGIENE SERVICE - MANUAL FOR OFFICIAL CONTROLS
Definition of SRM

MHS key issue It is imperative that all OVs and MHIs are aware of the parts of animal carcases that are classified as SRM by EU regulation 999/2001. MHS staff can use the following tables.

Cattle – All Member States	
All ages	• tonsils • intestines from the duodenum to the rectum • mesentery.
Over 12 months	**SKULL EXCLUDING THE MANDIBLE AND INCLUDING THE BRAIN AND EYES, AND SPINAL CORD.**

Over 30 months	Vertebral column including the dorsal root ganglia, but excluding: • vertebrae of the tail • spinous and transverse process of the cervical, thoracic and lumbar vertebrae • median sacral crest and wings of the sacrum.

Sheep and Goats - UK and all other Member States	
All ages	The spleen and the ileum. Note: To ensure that all of the ileum is removed, approximately 60 cm of the terminal small intestine should be removed and disposed of as SRM starting from the ileo-caecal junction, upwards and away from the caecum.
Over 12 months (or permanent incisor erupted)	Skull, including the brain and eyes, tonsils, spinal cord. Note: Skull does not include horns.

Exempt animals The SRM rules do not apply to animals whose origin is third countries with a negligible BSE risk as defined in Chapter C of Annex II of 999/2001 (as amended).

Appendix 3: Photos from facility visits

Abattoir

Figure 1A: Grids on abattoir floor to retain large fragments of carcase from drainage

Figure 2A: Area of abattoir discharging into Category 1 blood tanks

Figure 3A: Area of abattoir discharging into 6mm drainage trap

Figure 4A: Dividing line for wastewater in abattoir, to the right to blood tank and left to 3mm trap

Figure 5A: Screw and 3mm screen at abattoir

Intermediate and collection centres

Figure 6A: Dehiding and stripping of carcase for removal of Category 2 flesh

Figure 7A: Drainage in collection centre

Figure 8A: Trap with 6mm holes that is suspended below drain

Rendering facility

Figure 9A: Rendering facility unloading bay, material fed into crushers beyond barrier