A Quantitative Assessment of the Prion Risk Associated with Wastewater from Carcase-Handling Facilities

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Wastewater from facilities processing livestock that may harbor transmissible spongiform encephalopathies (TSEs) infectivity 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 of exposure to 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, and with considerable changes in the structure of carcase-processing industries in Great Britain, a reappraisal of the TSE risk posed by wastewater is required. Our results indicate that the predicted number of new TSE infections arising from the spreading of wastewater on pasture 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 plants 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.

KEY WORDS: Abattoir; bovine spongiform encephalopathy; QRA; scrapie; TSE

1. INTRODUCTION

In Great Britain, the occurrence of some prion diseases in cattle and sheep (bovine spongiform encephalopathy [BSE] and classical scrapie in sheep)

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has been greatly reduced. For BSE this has been achieved by stringent, long-term control measures and surveillance; indeed, the disease has almost disappeared. A voluntary program of genetic selection of breeding stock has reduced the prevalence of classical scrapie. However, until complete eradication, safeguards are still required to ensure that prion diseases are not recycled with animal by-product or waste disposal processing. Wastewater derived from premises handling and removing those parts of cattle and sheep carcases defined as specified risk material (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 Animal By-Products (ABP) Regulation.[1]

Facilities that handle SRM include 251 licensed abattoirs (*AB*) where healthy livestock are slaughtered.[2] There are 61 licensed intermediate plants (*INT*) that collect fallen stock from farms and, where a valid license is held, carry out testing for TSE. Collection centers (*CCN*), numbering 190 licensed facilities, do not test for TSEs, but are licensed to remove flesh to feed dogs (kennels) and zoo animals from animals that are certified not to contain any medication residues. The SRM from each of these facilities may be sent to rendering for disposal. Currently, there are eight rendering (*REN*) facilities accepting ruminant Category 1 and fallen stock in Great Britain (GB). The rendering process employed at these facilities produces Category 1 meat and bone meal (MBM) and tallow. The tallow is currently used as a fuel source, together with a significant proportion of the MBM, with the remainder sent to large incinerators (*INC*) or for energy production. There were 280 licensed incinerators in GB in 2010, the majority of which are relatively small in scale (*SIN*), owned by groups incinerating a range of waste materials. These include crematoria that handle pets and waste from *INT* plants and *CCN* and veterinary center incinerators. From a survey conducted, only 10–12 large incinerators were licensed to process Category 1 materials and fallen stock.

Within the ABP Regulations, there is a requirement for filtration of all wastewater from SRM handling facilities through a 6-mm trap prior to any treatment and direct spreading to land. 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 BSE associated with abattoir effluents in Europe,[3,4] Japan,[5] and Australia[6] and for renderer and incinerator effluent in the United Kingdom.[7–9] 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 is required.

This article, informed by the outputs of an accompanying paper, presents an analysis that 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

ABP Regulations. The results from this assessment will be used to inform policy decisions in this area.

2. METHODS

2.1. Model Overview

The output of the quantitative 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 model includes uncertain and variable parameters, which were simulated using the software package @Risk (©Palisade) Version 5.0, an add-on package within Microsoft Excel (©Microsoft). Convergence of all distributions was monitored, with the model being run for 200,000 iterations; this value ensured full convergence. The results presented follow the standard form of the arithmetic mean and the 5th and 95th percentile values. A multivariate stepwise regression analysis was used to calculate linear regression or sensitivity values for each input parameter in the model, which was represented by a probability distribution. This method is preferred for large numbers of input parameters, as all values that provide an insignificant contribution are removed from the analysis. In addition to this sensitivity analysis, the impact of uncertainty associated with specific key parameters was investigated. Selected scenarios were run to investigate the effect on the number of new TSE infections as predicted by the risk assessment. In these scenarios, parameters relating to (1) the effectiveness of trap, (2) the amount of wastewater produced, and (3) the proportion of wastewater spread on pasture were modified.

The scope of the risk assessment is wastewater derived from facilities that handle high-risk materials denoted as Category 1 SRM. To estimate the risk, we considered tissues designated as SRM, that have been removed from slaughtered cattle and sheep, entire bodies of dead animals containing SRM at the time of disposal, 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.[10] Although it was assumed that there are some unintentional losses of SRM to the floor and wastewater at facilities, intentional illegal behavior was not included. Figure 1 gives an overview of the model and parameters, estimates for which are provided in Table I
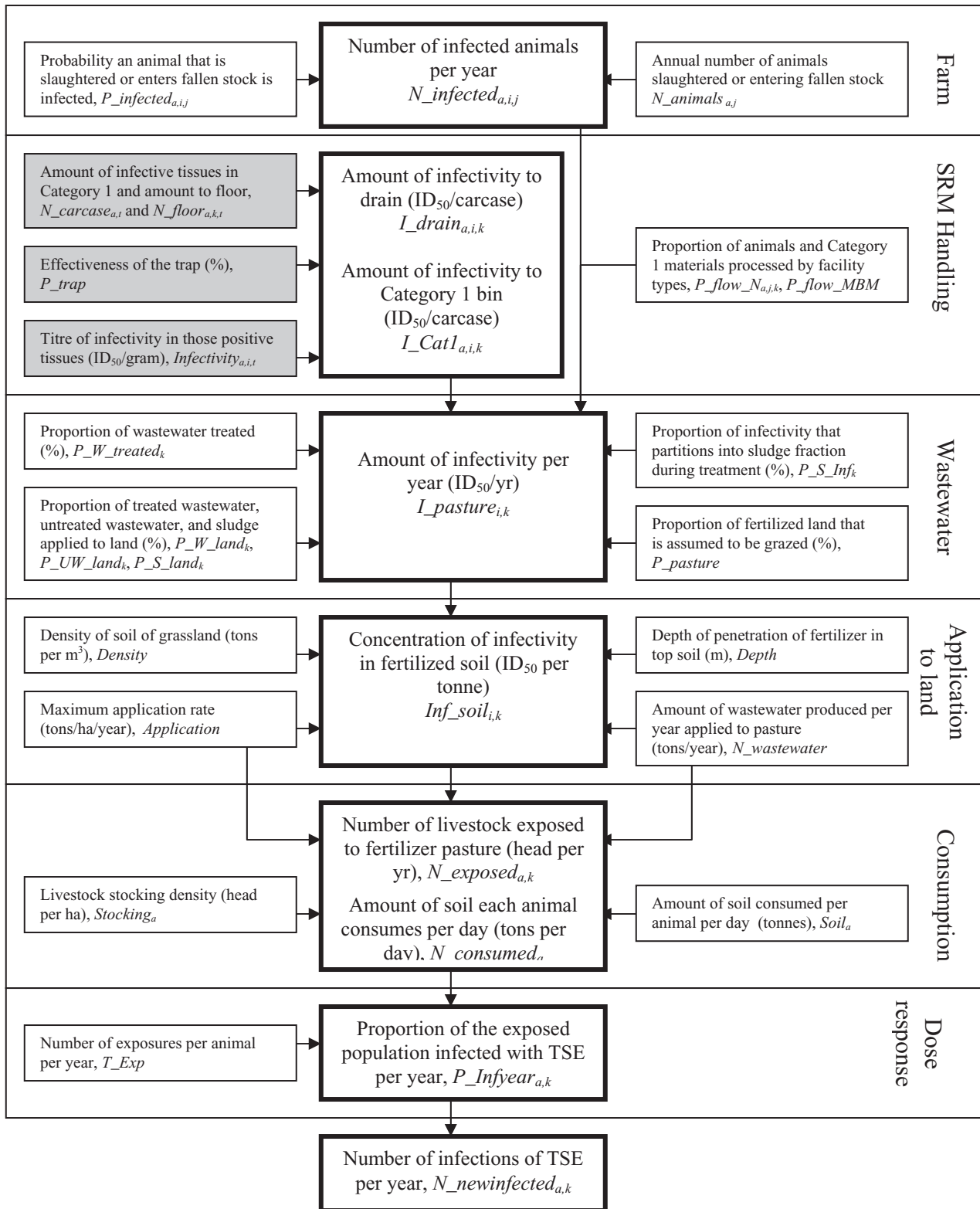
Fig. 1. Diagrammatic representation of the modules and parameters within the risk assessment, where subscripts denote animal group $a \in \{C, S, l\}$, infectious tissue type $t \in \{1 \text{ to } 14\}$, disease $i \in \{bse, sc, at\}$, exit stream $j \in \{HS, FS\}$, facility type $k \in \{AB, INT, CCN, REN, SIN, INC\}$. Shaded parameters indicate those described in the accompanying paper.

Table I. Parameter Descriptions and Values Within the Risk Assessment

Parameter	Symbol	Value	Unit	Reference
Farm				
Probability a cow 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})	%	11; Updated by Arnold, personal communication 2010
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})	%	11; Updated by Arnold, personal communication 2010
Probability a sheep/lamb slaughtered at abattoir is infected with classical scrapie	$P\_infected_{a,sc,HS}$, for $a = S,l$	Betapert (0, 9.0×10^{-4}, 4.2×10^{-3})	%	13
Probability a sheep/lamb entering fallen stock is infected with classical scrapie	$P\_infected_{a,sc,FS}$, for $a = S,l$	$Beta(5, 10125) * \frac{P\_infected_{S,sc,HS}}{Beta(6,10157)}$	%	13
Probability a sheep/lamb slaughtered at abattoir is infected with atypical scrapie	$P\_infected_{a,at,HS}$, for $a = S,l$	Betapert (3.7×10^{-4}, 2.4×10^{-3}, 6.6×10^{-3})	%	13
Probability a sheep/lamb entering fallen stock is infected with atypical scrapie	$P\_infected_{a,at,FS}$, for $a = S,l$	$Beta(5, 10125) * \frac{P\_infected_{S,sc,HS}}{Beta(6,10154)}$	%	13
Number of cattle slaughtered	$N\_animals_{C,HS}$	2,301,868	Cattle	12
Number of cattle entering fallen stock	$N\_animals_{C,FS}$	416,941	Cattle	12
Number of sheep slaughtered	$N\_animals_{S,HS}$	2,182,930	Sheep	15
Number of sheep entering fallen stock	$N\_animals_{S,FS}$	Uniform (149287, 447866)	Sheep	15, 16
Number of lambs slaughtered	$N\_animals_{L,HS}$	13,357,036	Lamb	15
Number of lambs entering fallen stock	$N\_animals_{L,FS}$	Uniform (750046, 1734921)	Lamb	15, 16
SRM handling				
Proportion of healthy slaughter flow from abattoir to rendering	$P\_flow\_1_{a,HS,REN}$	Betapert (50%, 80%, 90%)	%	12, 17, 18, 41
Proportion of healthy slaughter flow from abattoir to incineration	$P\_flow\_1_{a,HS,INC}$	1- $P\_flow\_1_{a,HS,REN}$	%	
Proportion of fallen stock flow from farm to collection center	$P\_flow\_1_{a,FS,CNN}$	$a = C$: Betapert (10%, 20%, 30%) $a = S$: Betapert (10%, 20%, 30%) $a = l$: Betapert (5%, 15%, 20%)	%	
Proportion of fallen stock flow from farm to intermediate	$P\_flow\_1_{a,FS,INT}$	$a = C$: Betapert (20%, 25%, 30%) $a = S$: Betapert (10%, 15%, 20%) $a = l$: 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- ($P\_flow\_1_{a,FS,CNN}$ + $P\_flow\_1_{a,FS,INT}$ + $P\_flow\_1_{a,FS,REN}$)	%	
Proportion of fallen stock flow from intermediate to collection center	$P\_flow\_2_{a,FS,CCN}$	$a = C$: Betapert (10%, 20%, 45%) $a = S$: Betapert (7%,15%,40%) $a = l$: Betapert (5%, 15%, 40%)	%	

(*Continued*)

Table I (*Continued*)

Parameter	Symbol	Value	Unit	Reference
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- ($P\_flow\_2_{a,FS,CCN}$ + $P\_flow\_2_{a,FS,REN}$)	%	
Proportion of fallen stock flow from collection center to renderer	$P\_flow\_3_{a,FS,REN}$	$a = C$: Uniform (70%, 95%) $a = S$: Uniform (60%, 92%) $a = l$: Uniform (60%, 92%)	%	
Proportion of fallen stock flow from collection center to incinerator	$P\_flow\_3_{a,FS,INC}$	1- $P\_flow\_3_{a,FS,REN}$	%	
Proportion of MBM from rendering to incineration	P_flow_MBM	83%	%	17, 18
Wastewater				
Proportion of wastewater from abattoirs, intermediate plants, collection centers, and small incinerators that is treated	$P\_W\_treated_k$, for $k = AB, INT, CCN, SIN$	Beta (7 +1,14−7 + 1)	%	42
Proportion of wastewater from rendering and large incineration facilities that is treated	$P\_W\_treated_k$, for $k = REN, INC$	80%	%	17, 18
Percentage of infectivity removed from wastewater due to treatment at abattoirs, intermediate plants, collection centers, and small incinerators	$P\_S\_Inf_k$, for $k = AB, INT, CCN, SIN$	Uniform (30%, 99%)	%	6, 19, 20
Percentage of infectivity removed from wastewater due to treatment at rendering and large incineration facilities	$P\_S\_Inf_k$, for $k = REN, INC$	Uniform (99%, 99.4%)	%	20, 21
Proportion of untreated wastewater to land from abattoirs, intermediate plants, collection, and small incinerators	$P\_UW\_land_k$, for $k = AB, INT, CCN, SIN$	Beta (2 + 1,10−2 + 1)	%	42
Proportion of untreated wastewater to land from rendering and large incineration facilities	$P\_UW\_land_k$, for $k = REN, INC$	3%	%	17, 18
Proportion of treated wastewater to land from abattoirs, intermediate plants, collection, and small incinerators	$P\_W\_land_k$, for $k = AB, INT, CCN, SIN$	Beta (2 + 1,4−2 + 1)	%	42
Proportion of treated wastewater to land from rendering and large incineration facilities	$P\_W\_land_k$, for $k = REN, INC$	25%	%	17, 18
Proportion of wastewater sludge to land from abattoirs, intermediate plants, collection centers, and small incinerators	$P\_S\_land_k$, for $k = AB, INT, CCN, SIN$	Beta (6 + 1,6−6 + 1)	%	42
Proportion of wastewater sludge to land from rendering and large incineration facilities	$P\_S\_land_k$, for $k = REN, INC$	41%	%	17, 18
Proportion of land that is grazed	$P\_pasture$	40%	%	22

(*Continued*)

Table I (*Continued*)

Parameter	Symbol	Value	Unit	Reference
Application to land				
Amount of wastewater produced per year	$Wastewater_{AB}$	3×10^7	Tons per year	15, 17, 18, 41
	$Wastewater_{INT}$	1×10^4		
	$Wastewater_{CCN}$	2×10^4		
	$Wastewater_{SIN}$	4×10^4		
	$Wastewater_{REN}$	7×10^5		
	$Wastewater_{INC}$	2×10^5		
		Mean values shown as production dependent on carcase number, which varies by $N\_animal_{a,j}$		
Application rate	$Application$	0.025	Tons/m^2/year	23
Depth of application	$Depth$	Uniform (0,0.25)	m	Animal Health, personal communication 2010
Density of soil	$Density$	Betapert (0.88, 0.9, 0.92)	Tons/m^3	24
Consumption				
Amount of soil consumed per day per animal	$Soil_C$	Uniform (0.23,0.38)/1000	Tons	25, 26
	$Soil_S$	Uniform (0.14,2.45)/1000		
Stocking density of animals per hectare per year	$Stocking_C$	Uniform (0.36,2)	Head per ha	Scotland: 28; England: 29; UK: 30, 31, 32
	$Stocking_S$	Betapert (3.33333,5.028595,14.1)		
Dose response				
Number of exposures per year	T_Exp	365		

of the supplementary materials. The model is split into six modules that track the TSE agent from the farm through to the wastewater being applied to land and the subsequent ingestion and infection of animals. Elements of the SRM-handling module, shown in gray in Fig. 1, are described in a separate paper.

2.1.1. 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 being processed via various exit streams. The term "exit stream" encompasses a general definition of streams of healthy slaughter animals (including emergency slaughter) at abattoir and fallen stock. Clinically suspect animals were not considered because the risk of infectivity entering wastewater has been considered negligible due to stringent disposal procedures. The use of exit streams is required as livestock in the different categories will have different TSE prevalences and will be diverted to different SRM-handling facilities. The estimated annual numbers of infected animals ($N\_infected_{a,i,j}$) per animal group $a \in \{C, S, l\}$, disease $i \in \{bse, sc, at\}$, and exit stream $j \in \{HS, FS\}$ is given by Equation (1):

$$N\_infected_{a,i,j} = P\_infected^*_{a,i,j} N\_animals_{a,j},$$

(1)

where *HS* denotes animals slaughtered at abattoir and *FS* denotes fallen stock; *bse* refers to BSE, *sc* to classical scrapie, and *at* to atypical scrapie; *C* refers to cattle, *S* refers to sheep over one year of age, and *l* denotes lambs (sheep less than one year of age). Sheep were divided into these two age groups because SRM controls are different for the two groups. For each 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 annual number of animals.

In relation to prevalence, for BSE in cattle it was assumed that only cattle in the last 12 months of the incubation period will harbor significant levels of infectivity. This is a very pessimistic assumption as infectivity increases significantly over this period from very low to high in the central nervous system. The prevalence of infected cattle in the last 12 months of the incubation period has been previously investigated[11] and was updated using BSE surveillance data up to 2009 (Arnold, personal communication 2010). The uncertainty associated with the mean prevalence estimates was described using a Betapert distribution for each exit stream (see Table I). 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).[12]

The mean infection prevalence and 5th and 95th percentiles for classical scrapie in the healthy slaughter stream has been estimated previously,[13] with values represented in the risk assessment by a Betapert distribution. In the absence of infection prevalence estimates for the fallen stock stream, case data were used (with a Beta distribution to describe the prevalence of cases) multiplied by the ratio of estimated healthy slaughter infection prevalence divided by healthy slaughter prevalence of cases (refer to Table I).

There are few data for atypical scrapie cases. In the absence of further information, the Abattoir Survey was used to estimate the prevalence of atypical scrapie. A number of key assumptions were applied when using these data: (1) the incubation period of atypical scrapie is the same as that estimated for classical scrapie (atypical scrapie is known to have a longer incubation period); (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. As with classical scrapie, a ratio between prevalence of infection and prevalence of cases was calculated and used to modify the prevalence estimate for fallen stock animals. It has been suggested that classical and atypical scrapie can coexist within the same host.[14] Therefore, it was assumed that all lambs and sheep entering the healthy slaughter and fallen stock streams could potentially be infected with both types of scrapie.

The total number of lambs and sheep entering the healthy slaughter stream is recorded by Defra statistics (standing population). There is no centralized 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.[16] 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.[15] From discussions with stakeholders, not all fallen sheep and lambs are recorded as fallen stock and some burial of livestock still occurs. It was difficult to ascertain the exact proportion and it was assumed that 50–75% of fallen sheep enter the fallen stock stream. Using these data, a uniform distribution was used between 149,289 and 447,866 adult sheep to describe the uncertainty associated with the number of dead sheep entering fallen stock, and between 750,046 and 1,734,921 for the number of lambs entering fallen stock.

2.1.2. SRM-Handling Module

SRM facilities, denoted by k, are defined as abattoirs (AB), intermediate plants (INT), collection centers (CCN), rendering (REN) facilities, small incineration plants (SIN), and large incineration plants (INC). Thus, $k \in \{AB, INT, CCN, REN, SIN, INC\}$. The proportion of healthy slaughter and fallen stock each year that are processed by each facility type was estimated and denoted by $P\_flow\_N_{a,j,k}$, where N is the tier of the flow as shown in Figs. 2 and 3. For healthy slaughter $N = 1$ since there is only a single tier. However, for fallen stock, $N = 1, 2, 3$ as there are three tiers. These proportions are difficult to estimate directly as data are not collected centrally. Various data sources were used to estimate the proportion of the GB livestock handled by each facility type: (1) the national database of ABP facility licenses, (2) the number of livestock tests in total and by facility type (between 01/06/2008 and 31/05/2009), (3) expert opinion (animal health [AH], 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) surveys of all eight renderers[17] and incinerators.[18] The estimated most likely, minimum, and maximum values for each
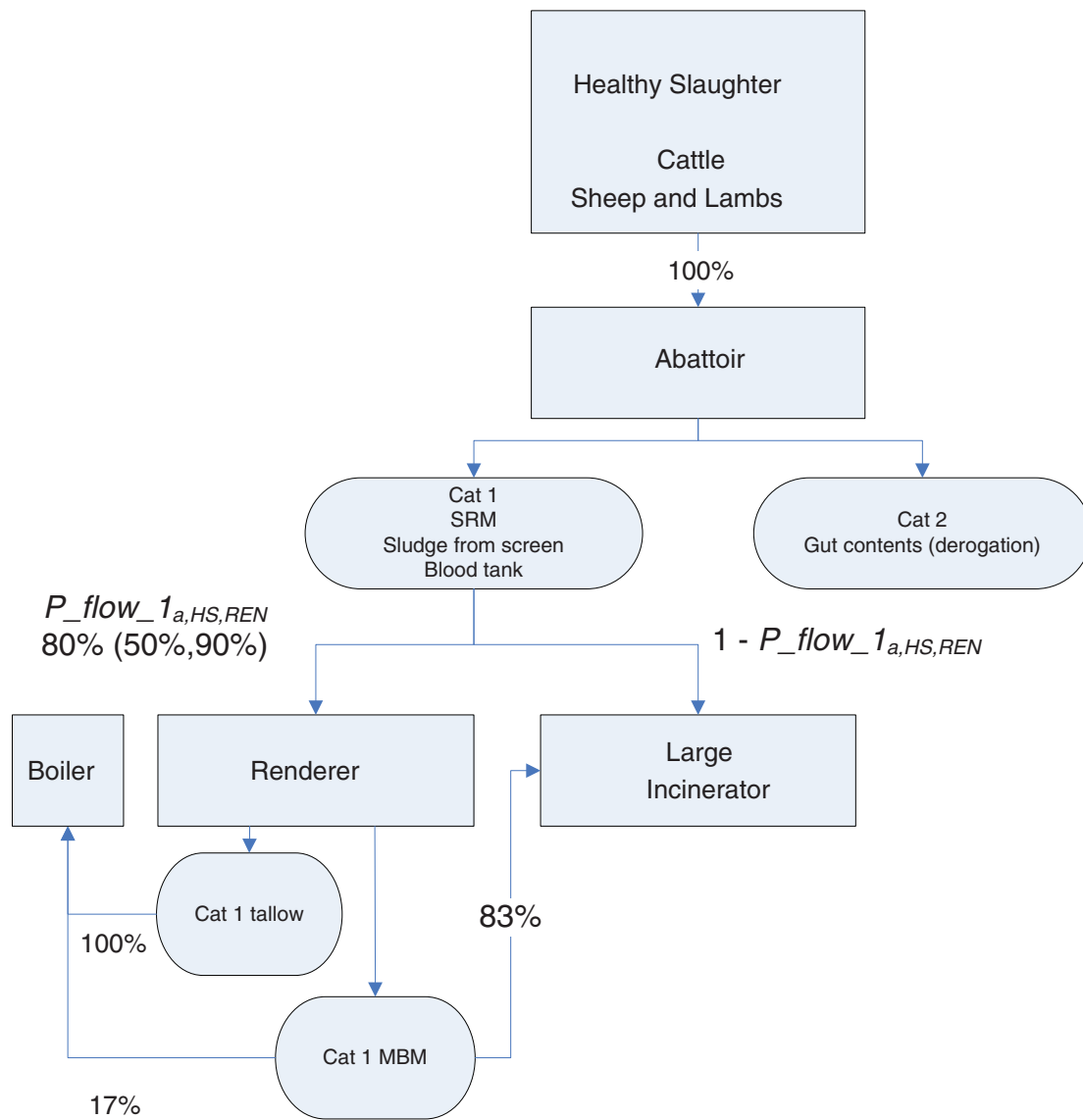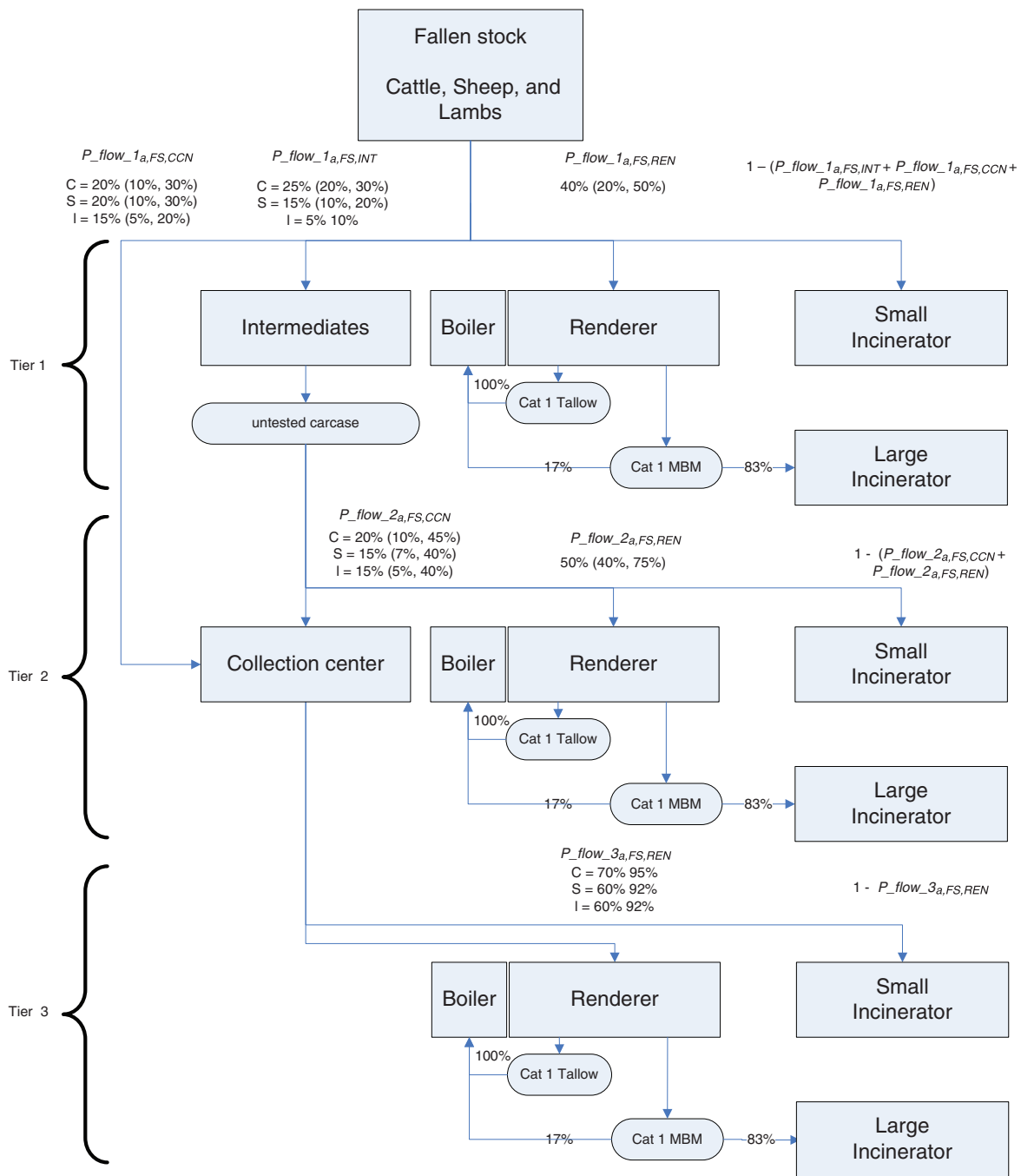
Fig. 2. Flow diagram of the process of SRM flow for healthy slaughter cattle, sheep, and lambs with estimated most likely rates of material flow (minimum and maximum).

animal population are shown in the flowcharts in Figs. 2 and 3 and in Table I. The uncertainty associated with the estimated flow of materials was represented by a uniform distribution in cases for which minimum and maximum values were available and a Betapert distribution in cases for which an estimate for the most likely value was also available.

Given the estimated proportion of livestock and associated Category 1 materials that entered each facility type per year, the SRM-handling module investigates the sequence of events for this infectivity to ultimately enter the drains, $I\_drain_{a,i,k}$, or be collected as Category 1 material for disposal, $I\_Cat1_{a,i,k}$, as shown in Fig. 1. The amount of infectivity released from carcases to the floor of facilities is dependent on the TSE disease predilection for certain tissues within livestock, the particular animal host, and the type of activities conducted at each facility that release infectivity to the floor. Finally, the effect of the minimum 6-mm traps needs to be considered. Several of the parameters used in this module have a number of dependencies (facility type by animal population, TSE agent, and tissue type) and therefore

Fig. 3. Flow diagram of the process of SRM handling for fallen stock with estimated most likely rates of material flow (minimum and maximum), where $a \in \{C, S, l\}$.

these parameters have been estimated and presented separately in the accompanying paper, which presents results on a per infected carcase basis. Fig. 1 indicates those parameters that are described in the accompanying paper in shaded boxes.

2.1.3. Wastewater Module

The amount of infectivity to pasture from each facility type depends on the estimated number of infected animals/carcasses processed through

that facility type ($N\_infected^*_{a,i,j} P\_flow_{a,j,k}$), the amount of infectivity that enters the drain per animal/carcase ($I\_drain_{a,i,k}$), and facility-dependent probabilities of wastewater treatment and disposal routes. Wastewater may be treated, with infectivity separated into the treated wastewater fraction ($P\_W\_treated^*_k(1 - P\_S\_Inf_k)$) or into the sludge fraction ($P\_W\_treated^*_k(1 - P\_S\_Inf_k)$), or untreated ($1 - P\_W\_treated$). The disposal route of interest is the proportion to land (P_land_k) that is pasture ($P\_pasture$). For each facility, the amount of infectivity spread on pasture (ID_{50} per year), $I\_pasture_{i,k}$, was estimated by adding together the amount of infectivity from each wastewater type (untreated wastewater, wastewater that has been treated, and the sludge arising from such treatment) as shown in Equation (2):

$$I\_pasture_{i,k} = \sum_a (I\_Uwastewater + I\_wastewater + I\_sludge)_{a,i,k}, \quad (2)$$

where for untreated wastewater,

$$I\_Uwastewater_{a,i,k}$$
$$= \sum_j {}^{N\_infected_{a,i,j} * P\_flow_{a,j,k} * I\_drain_{a,i,k} * (1 - P\_W\_treated_k)}_{* P\_UW\_land_k * P\_pasture}$$

and for treated wastewater,

$$I\_wastewater_{a,i,k}$$
$$= \sum_j {}^{N\_infected_{a,i,j} * P\_flow_{a,j,k} * I\_drain_{a,i,k} * P\_W\_treated_k}_{* (1 - P\_S\_Inf_k) * P\_W\_land_k * P\_pasture}$$

and for sludge arising from treated wastewater,

$$I\_sludge_{a,i,k}$$
$$= \sum_j {}^{N\_infected_{a,i,j} * P\_flow_{a,j,k} * I\_drain_{a,i,k} * 1 - P\_W\_treated_k}_{* P\_S\_Inf_k * P\_S\_land_k * P\_pasture}.$$

A number of different data sources were used to estimate the facility-dependent probabilities of wastewater treatment and disposal routes, provided in Table I, including published literature and two surveys.[17,18] However, data were absent for *INT*, *CCN*, and small incinerators. These facilities were assumed to treat and disposal of wastewater in a similar manner to *AB*.

$P\_S\_Inf_k$ 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 wastewater, and therefore any TSE agents that may associate with the sediment.[6] 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 was assumed that between 30% and 99% of infectivity will partition into the sludge phase when wastewater is further processed due to the range of treatments that are currently in use (assumption based on Refs. 6, 19, and 20) described using a uniform distribution. For renderers where treatment was undertaken, both biological treatment and DAF was applied, and it was assumed that large incinerators also employ such treatments. It was estimated that between 99% and 99.4% of infectivity partitions into the activated sludge solids during processing.[20,21] It was assumed that there is no loss of infectivity during any consequent treatments of the sludge, for example, anaerobic digestion.[4,20]

With no further information on the type of land to which the wastewater is applied, the proportion assumed to be grazed, $P\_pasture$, was set equal to the national proportion of grassland fertilized from the total area of grassland and crops fertilized, estimated to be 40%.[22]

2.1.4. Application to Land Module

The concentration of infectivity in soil where wastewater has been applied (ID_{50} per ton soil), $Inf\_soil_{i,k}$ is dependent on the estimated concentration of infectivity in wastewater ($I\_pasture_{i,k} / N\_wastewater_k$), the application rate (tons/m^2), density (tons/m^3), and depth of application (m) as shown by Equation (3):

$$Inf\_soil_{i,k} = \left(\frac{I\_pasture_{i,k} * Application}{N\_wastewater_k * 10,000 * Depth * Density} \right), \quad (3)$$

where $N\_wastewater_k$ denotes the total amount of wastewater produced per year that is destined for pasture, as shown in Equation (4):

$$N\_wastewater_k = (Wastewater_k * P\_pasture) * \left(\begin{matrix} (P\_W\_treated_k * P\_W\_pasture) + \\ ((1 - P\_W\_treated_k) * P\_W\_pasture) \end{matrix} \right). \quad (4)$$

Estimates for total wastewater production are shown in Table I. Estimates for *AB*, *Wastewater$_{AB}$*, are based on the numbers of carcases processed, with 1,000 L per carcase used by large facilities processing >150 carcases per day, and 2,000 L per carcase used by *AB* below this threshold (adapted from abattoir throughput data and discussions with *AB*). *INT*, *CCN,* and small incinerators are estimated to use less washing down water, with approximately 10–100 L per carcase (LASSA, personal communication 2009). Renderers were asked directly for water consumption data and it was assumed that large incinerators use similar amounts of water based on input weights as renderers.[17,18] 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 was estimated to be 0.025 tons/m^3/year.[23] The depth of application, *Depth*, will vary, with some wastewater surface spread and sludge fractions injected to depths of 22–25 cm. Therefore, the depth of application was estimated to be between 0 m and 0.25 m, described in the model using a uniform distribution. The density of soil, *Density*, was estimated as between 0.88 tons/m^3 and 0.92 tons/m^3, with a most likely value of 0.9 tons/m^3.[24]

2.1.5. Consumption by Livestock Module

To estimate the number of infected livestock per year, the number of livestock exposed to the fertilized pasture and amount of infectivity consumed per day was estimated using Equation (5):

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

$$N\_exposed_{a,k} = \frac{N\_wastewater_k}{Application^*Stocking_a}, \quad (5)$$

where *Soil$_a$* is the amount of soil consumed per day by livestock species and has been estimated based on the percentage of consumed dry matter as soil and average dry matter intake.[25,26] It was assumed that lambs consume the same amount of soil as adult sheep, which is acknowledged as overestimating risk. The assumption was made that any grazing cattle and sheep will have access to pasture all year round and that the full soil depth of fertilizer, that is, to a maximum of 25 cm. This was considered a worst-case presumption as it is acknowledged to be unrealistic but has been used inherently in previous risk assessments.[27]

Descriptions of stocking density for livestock, *Stocking$_a$*, vary in the literature. Ranges of 0.36–2.0 adult cattle per hectare (Scotland: Ref. 28; England: Ref. 29), and a maximum of 14.1 sheep per hectare were found (UK: Ref. 30). In a postal survey of sheep farmers in GB in November 2002 the midpoint stocking density of sheep >1 year old was found to be 5.02 adult sheep per hectare.[31] The lower European livestock density index for sheep to keep grassland in good condition equates to 3.3 sheep per hectare.[32] These data are used in the risk assessment as shown in Table I. It was assumed that the stocking density for lambs is the same as the stocking density for sheep.

2.2. Dose Response and Risk Estimate

A dose-response model was used to determine the probability of infection occurring as a result of exposure to a given dose of a pathogen. Research conducted with scrapie-adapted strains seems to indicate that, as yet, there is no clear consensus as to whether TSE doses act independently, cumulatively, or interact in succession.[33–35] Further, in regards to hamster and mouse experiments,[33,35] it is uncertain whether these results would apply to all TSE agents given the differences in species and agents. It is assumed that the proportion of a population that becomes infected from a single dose, *P_Infday$_{a,k}$*, can be estimated using the following exponential dose-response equation:[36]

$$P\_Infday_{a,k} = 1 - e^{(-r^*N\_consumed_{a,i,k})}, \quad (6)$$

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$_{50}$), and *N_consumed$_{a,i,k}$* = 1, then −*r* = Ln(0.5). Substituting into Equation (6):

$$P\_Infday_{a,k} = 1 - e^{(Ln(0.5)^*N\_consumed_{a,i,k})}. \quad (7)$$

For both cattle and sheep 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. An annual risk of infection from *T_exp* exposures per year, assumed to be 365 days, to a pathogen dose was estimated by

Equation (8):

$$P\_Infyear_{a,k} = 1 - [1 - e^{(In(0.5)^* P\_infday_{a,k})}]^{T\_exp}. \quad (8)$$

Combining with Equation (7):

$$P\_Infyear_{a,k} = 1 - [1 - e^{(In(0.5)^* P\_infday_{a,k})}]^{T\_exp}, \quad (9)$$

where $T\dot{\,}exp$ is the number of exposures per year, assumed to be 365 days. Finally, the mean number of livestock infected per year is estimated using Equation (10):

$$N\_newinfected_{a,k} = N\_exposed_{a,k}{}^* P\_infyear_{a,k.} \quad (10)$$

3. RESULTS

The results indicate the number of new TSE infections arising per year due to the application of wastewater to pasture would be low. The mean values are low for each TSE disease considered, with 0.001 (1.8×10^{-6}, 0.0013) new infections of BSE in cattle per year, 0.033 (0.0004, 0.064) new infections of classical scrapie in sheep per year, and 0.030 (0.0003, 0.061) new infections of atypical scrapie per year. 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, there is an estimated 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 the risk assessment remain constant.

The contribution to the total number of new TSE infections arising by facility type is given in Fig. 4. For BSE in cattle, the largest contributions are from *AB* (35%) and *REN* facilities (22%). Intermediates, *CCN,* and small incinerators contribute 13%, 11%, and 15%, respectively, with large incinerators contributing the least toward new BSE infections (4%). The largest contributions for classical scrapie are from rendering (31%), small incinerators (30%), and *AB* (23%). Intermediates, *CCN,* 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 *AB* (20%). The smallest contributions are from intermediates (3%), *CCN* (4%), and large incinerators (9%).

The estimated numbers of BSE, classical scrapie, and atypical scrapie infections were also parti-

tioned into each of the three wastewater types (untreated/treated wastewater and wastewater sludge). The mean percentage contribution of each wastewater type to the total amount of infectivity is shown in Fig. 5. 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 14–17% respective contribution to the total risk.

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 presented in the accompanying paper (*MaxCNS, BOunit, OOunit*), which have been noted in previous TSE risk assessments.[37] Finally, the parameter associated with the proportion of infectivity falling to the floor at *REN* facilities, *P_floor$_{a,i,REN}$*, described in the accompanying paper, was found to be influential. This parameter could be further enumerated by experimentation. However, the identified obstacles to such measurements identified in previous work[38] would need to be considered when producing any experimental protocol.

3.1. Scenario Testing

Selected scenarios were run to investigate the effect on the estimated number of new TSE infections arising.

(1) *The effectiveness of drain trap:* The effectiveness of trap in the baseline scenario was represented in the model by *P_trap* as between 80% and 90% based on estimates from the literature (refer to accompanying paper for further details). To investigate the impact of trap effectiveness on the number of new TSE infections, values of 0%, 25%, 50%, and 75% retention of infectious material were simulated. The relationship between trap effectiveness and the mean number of new TSE infections is approximately linear. At 0% trap effectiveness, the estimated number of new TSE infections is shown in Table II.
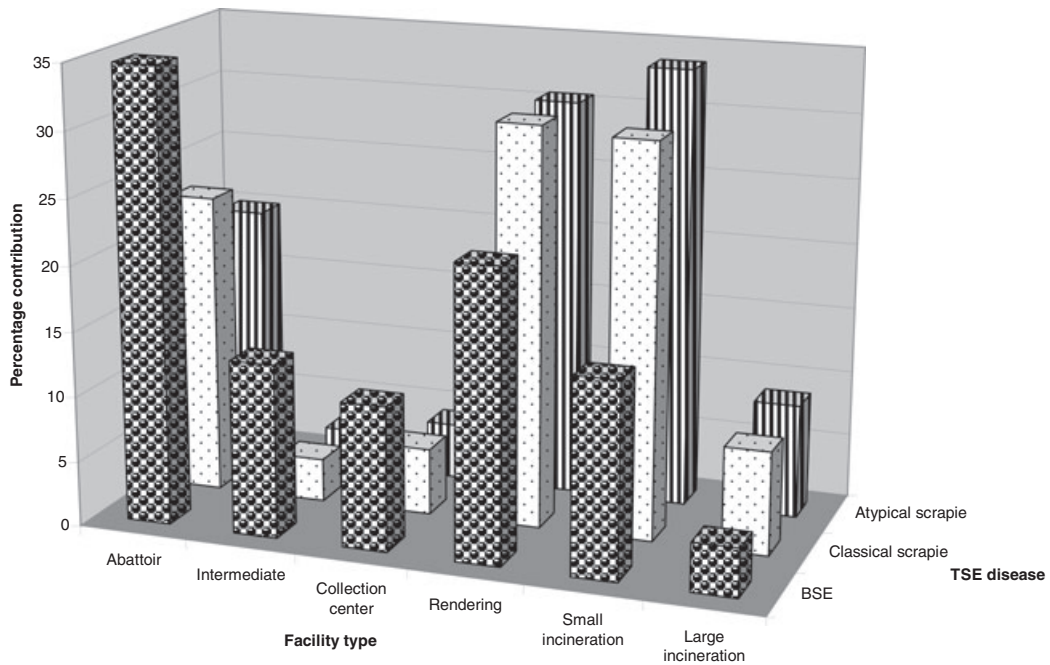
Fig. 4. Mean percentage contribution to total infectivity by facility type.
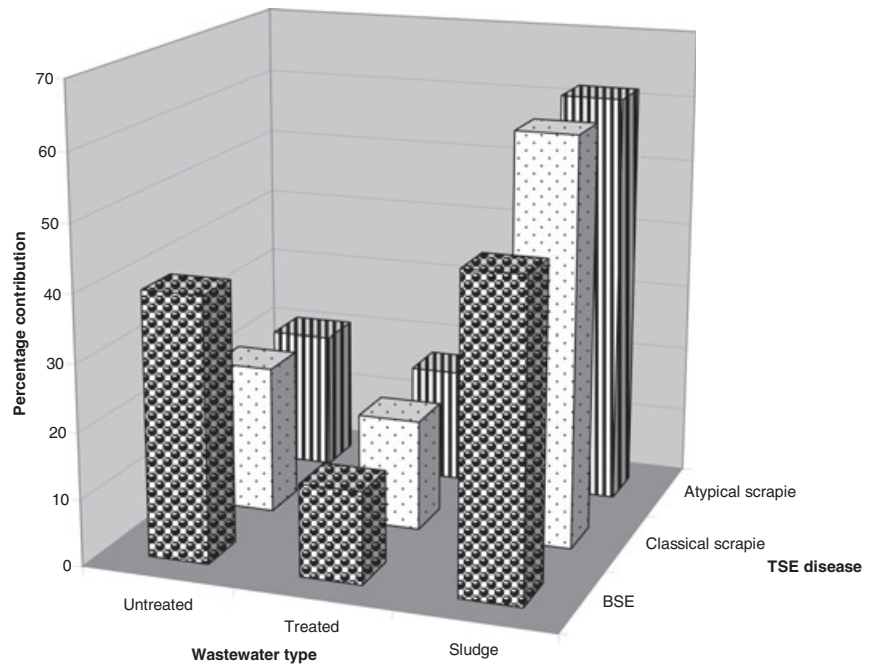

Fig. 5. Mean percentage contribution to total infectivity by wastewater type.

(2) *The amount of wastewater produced:* 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.

Scenario	Scenario Values	Risk Estimate
1. Effectiveness of trap	0	BSE 0.007 (1×10^{-5}, 0.01) Classical scrapie 0.136 (0.002, 0.27) Atypical scrapie 0.121 (0.001, 0.24)
2. Amount of wastewater produced per year	Baseline × 2 Baseline × 3	No significant change in mean results
3. Proportion of wastewater to pasture	Baseline ×1.75 Baseline × 2.5	Mean results × 1.75 Mean results × 2.5

(3) *The proportion of wastewater to pasture:* In the baseline model, 40% of all wastewater applied to land is assumed to be spread on pasture. The calculation of this figure was based on the proportion of fertilized 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 fertilized land is grazed). The relationship between the proportion of wastewater to pasture and the mean number of new TSE infections is approximately linear.

4. DISCUSSION

In this article, we have described the development and parameterization of a quantitative risk assessment 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 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 vary in their probabilities of falling to floor in each 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 also needs to be remembered that an assumption was made that all facilities use a 6-mm trap before collection of wastewater, whereas many facilities, particularly the rendering industry, are known to use traps of 4 mm down to 1 mm that may retain more material. Therefore, this assumption is worst case.

Other pessimistic assumptions need to be highlighted. It is assumed that any grazing livestock will have access to pasture all year round and have access to the full soil depth of fertilizer, that is, to a maximum of 25 cm. These assumptions are unrealistic as the majority of grazing livestock are likely to be housed with feed for several months of the year, and when grazing, a depth of 25 cm is unlikely to be realized. It is assumed that intentional illegal activities that may bypass controls do not occur. 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 bypass built-in wastewater and drainage controls when compared to smaller more *ad hoc* operations such as *INT*, *CCN*, 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 may occur and that 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 licensing authorities, such occurrences are likely to be rare, although there are recorded incidents. Although current SRM controls are in place the amount of infectivity released by such rare events will still be small.

By varying the effectiveness of the trap from 0% to 75%, the relationship between the trap retaining material and new infections was found to be 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 behavioral 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 form of Equations (4) and (5) indicate that as more wastewater is produced, any infectivity present becomes more diluted. However, that same amount of infectivity is spread on more land, leading to more animals being exposed. Given that the dose-response model does not have a lower bound value (that is infinitely divisible), changes in the amount of wastewater produced and applied to pasture have no effect on the 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. TSE agents are associated with long-term environmental persistence. Seidel *et al.* found by western blotting a strong decrease in the amount of extractable scrapie prion protein after 29 months in sandy loam soil. However, it is unclear whether this is due to molecular decay of the protein or due to tight binding to soil particles.[39] 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.[40] Despite this, given the expected low numbers of BSE infected cattle and the random occurrence of disease among herds, it is unlikely, at the current time, that infected animals will be processed and any contaminated wastewater 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. 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 characterized in the literature and is subject to constant changes due to market demands. Therefore, the results are essentially a snapshot 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 mothballing 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 cofuel 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 were available from the literature and surveys conducted.

5. CONCLUSIONS

A quantitative risk assessment was 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 from this practice 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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