A Meta-analytic Approach To Evaluate The Reduction In Infectivity Of TSE Agents Exposed To Dry Heat.

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ABSTRACT

The objectives of this analysis were to summarize the scientific evidence of the reduction in infectivity of TSE agents exposed to dry heat and to develop a quantitative model to predict the relationship between dry heat treatment parameters and reduction in TSE infectivity. Published studies on the effect of dry heat on TSE infectivity on homogenized brain samples exposed to temperatures from 160 to 1000 ^{o}C were included. To estimate TSE infectivity reduction at these temperatures, a meta-analytical approach using a dose-response model was performed based on the scientific evidence available on inactivation of a rodent-adapted TSE agent. For each study, complementary log-log models were fit to obtain point estimates and standard errors for the initial and residual infectivity and the reduction in infectivity was then computed from these estimates. A meta-regression model was conducted to evaluate the effect of time and temperature on the infectivity reduction. Preliminary results suggested that on average a 11-$\log_{10}$ ID_{50}/gr reduction in TSE infectivity can be expected at 850ºC and the predictive interval at this temperature ranged from 8 to 14.

Keywords: BSE, TSE, dry heat, infectivity, inactivation, prion, meta-analysis

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs), such as bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in elk and deer, and Creutzfeldt-Jakob disease (CJD) in humans, are a group of neurodegenerative diseases. TSE agents are remarkably resistant to inactivation by physical or chemical procedures that successfully destroy conventional pathogens. Inactivation is less efficient under dry conditions because of the dehydration that occurs under these situations. When only dry heat is applied, an increase in inactivation of the TSE agent can be obtained by exposing the contaminated material to very high temperatures. However, residual infectivity can still be observed. *In vivo* procedures in laboratory animals are the most reliable methods for testing for reduction of TSE infectivity. However, these animal experiments are laborious, costly and time consuming due to extended TSE incubation periods. The experimental data on inactivation of the TSE agent by diverse temperature treatments are limited.

Many countries, including Canada, have adopted enhanced feed ban policies which require that all specified risk materials (SRM), the material known to contain infective prion, are destroyed and are not rendered in the animal food chain for possible recycling. These enhanced policies are extremely costly to the producers, industry, and governments and have stimulated research toward efficient means of destroying SRM. Incineration is being used in many countries as a means of SRM destruction, however there is a paucity of reports in the literature concerning the temperature required to achieve prion inactivation or destruction. This void makes the approval of methods for destruction challenging for regulatory control programs. Before regulatory approval can be granted, these methods must demonstrate prion inactivation in order to maintain confidence in the control program.

MATERIAL AND METHODS

Published studies used in this meta-analysis were obtained by a bibliographic search of the Pub Med (MEDLINE) and SCIRUS databases up to November 30, 2007 using the following keywords: “prion” or “scrapie” or “BSE” or “TSE” and “heat inactivation” or “dry heat” or “incineration”. The search was directed at publications appearing in English-language journals. Potentially relevant studies were also identified from the references listed in publications identified by the electronic database search. Studies were examined for duplications when they came from the same research group.

The criteria used to determine whether the results of a published study could be included in the analysis were: brain tissue sample had to be used in dry heat inactivation experiments, the TSE infectivity had to be evaluated by bioassay in animals, initial infectivity had to be documented, and the TSE agent had to be an

animal-related strain. Given that field incinerator conditions typically function at specified time intervals (in order to maximize burning efficiency), heat treatments longer than 240 minutes were not included in the modelling analysis.

To compute the $\log_{10}$-reduction in TSE infectivity (ID_{50}/g), first the initial infectivity was computed from each of the references using the following dose-response model:

$$\pi = 1 - e^{-p*dose}$$

where *"π"* is the probability for an animal to become infected by the "*dose*" of infectious material ingested and it is estimated from the number of animals that get the disease in the experiment; and "*p"* is the probability of infection. A generalized linear model with a binomial distribution and a complementary log-log (clog-log) link was used to estimate the *"p"* values and their standard errors. Under this model π is transformed according to:

$$\text{clog-log}(\pi) = \ln(-\ln(1-\pi)) = \ln(p) + \ln(dose)$$

Because the interest is in the estimation of the ID_{50}/g (e.g. the infectious dose that kill 50% of the injected animals), the following transformation was applied:

$$\ln(-\ln(0.5)) = \ln(p) + \ln(dose), \text{ then dose } (ID_{50}) = -\ln(0.5) / p = \ln(2)/p$$

Then, the same model as above was fit to the data to estimate the residual infectivity. Finally, the $\log_{10}$-reduction (ID_{50}/g) in TSE infectivity was estimated by computing the mean difference and standard error of the mean difference from estimates obtained from the clog-log models. The effect of TSE agent, temperature and time was estimated using a meta-regression approach.

RESULTS AND DISCUSSION

Seven published studies on dry heat inactivation of the TSE agent that met the inclusion criteria were initially selected (Brown et al.,1986; Brown, 1990;Taylor et al., 1996; Taylor et al., 1998; Brown, 2000;Brown et al., 2004;Fernie et al., 2007). The experimental data on inactivation of the TSE agent by high temperature treatment (incineration conditions) were limited to two studies (Brown, 2000;Brown et al., 2004). Experiments that focused on the effect of dry heat on TSE infectivity were performed primarily between 160 and 300 ^{o}C. One reference (Fernie et al., 2007) could not be used to estimate the residual infectivity because the authors did not provide the total number of animals used and the total number of positives at each dilution.

Initial infectivity values ranged from 5.29 to 10.95 $\log_{10}$/ gr. On the other hand residual infectivity values after different temperature and time combinations ranged from -2.28 to 5.37 $\log_{10}$ / gr. The unconditional effects of temperature, time and TSE agent on the infectivity reduction are summarized in the following table:

Table 1. Coefficients and P-values from the three meta-regression models on the infectivity reduction (log10/gr) of TSE after dry heat treatment exposure

Predictor	Log_{10} / gr reduction	P-value
TSE agent		0.01
22A	0.09	
263K	7.80	
301V	0.32	
ME7	3.78	
Temperature ºC		0.0001
150	1.77	
160	1.74	
200	3.47	
300	3.95	
600	8.60	
1000	10.0	
Time (minutes)		0.012
15	8.69	
20	-6.93	
40	-4.82	
60	-4.16	
240	-5.20	

The three predictors were unconditionally associated with the reduction of TSE infectivity after exposure to heat treatment. Some strains showed a lower reduction in infectivity values. A linear trend in infectivity reduction was observed for temperature and time. But it is likely that the effect of each one was likely to be confounded by the effect of the other predictors. For instance, samples from all the experiment using high temperatures (eg >600ºC) were exposed for 15 minutes. Due to the low number of observations in these models (n=16), a multivariable model was not fit.

However, the amount of the between-study variance that is explained by each of them is another way to identify important factors in meta-analysis. The total between-study variance estimated by the meta-regression model without any predictor (null model) was 12.95. TSE agent, time and temperature explained 53%, 57% and 92% of this between-study variance, respectively.

This suggested that temperature at which samples were exposed explained almost of the variability in infectivity reduction in the experiments selected for this analysis.

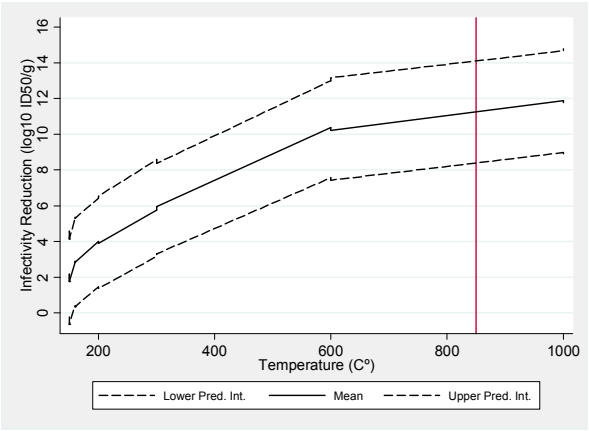
Based on the model including only temperature, predictive intervals at each temperature were computed and plotted against temperature to depict the relationship between them (Figure 1). The solid vertical line represents the current policy in Canada for SRM destruction (850ºC).

CONCLUSIONS

The limits of extrapolation from the laboratory scale to full scale (industrial) procedures have to be acknowledged, partially because it is not always possible at the industrial scale to achieve temperature conditions which were directly equivalent to those in laboratory studies. Thus, the final products of industrial (large) and laboratory (small) scale processes have to be carefully investigated for their equivalence.

Overall, the model reflected quite well the pattern depicted by the raw data (data not shown). Temperature was the most important factor explaining most of the variation in the infectivity reduction of TSE, with values below 400ºC having a low effect. According to this model, at 850ºC, the mean predicted $\log_{10}$ reduction was 11, with a predictive interval that ranged from 8 to 14.

Figure 1. Relatioship between infectivity reduction of TSE (log10/gr) and dry heat temperature exposure of brain homogenates.



The estimates from this model can be used to produce a quantitative estimate of inactivation of TSE infectivity at different temperatures and may be a very important component of future risk assessments.

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