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// Thermal inactivation of hazardous biological agents in animal carcasses //

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Introduction

Infectious waste as well as GMO-containing waste must be disposed safely. This includes animal carcasses derived from animal experiments with infectious and / or genetically modified organisms. The most suitable inactivation method is the incineration of those carcasses in a suitable plant. If this is not possible, the inactivation can also take place in an autoclave on site. In this case, the chosen autoclaving process must ensure an effective inactivation. Therefore the required parameters, i.e. the inactivation temperature of 121 °C with an inactivation time of 20 minutes (or 134 °C for 12 minutes alternatively) inside the carcasses have to be guaranteed. To determine the required conditions for the autoclaving process, experiments were carried out with carcasses of different animal species. It has been shown that the best results have been obtained by selecting a program with fractionated pre-vacuum for solid waste. As of yet, an inactivation process for batches with up to 100 mouse carcasses per sterilization unit could be validated. Currently equivalent tests with carcasses of larger experimental animals, such as ferrets are in progress. Our goal is to establish validated inactivation processes for carcasses of all animals commonly used in experiments with infectious and / or genetically modified organisms to ensure a safe disposal.

Materials and Methods

All carcasses were made available by the Paul-Ehrlich-Institut animal facility after killing the laboratory animals following animal experiments. A total of 7 experiments were required to find the appropriate parameters. Experiments 1 and 2 were carried out with the parameters preset in the autoclave in order to determine how far they deviate from the appropriate parameters. In experiments 3 to 7, these preset parameters were correspondingly adapted in a cycle development and subsequently validated. All experiments were performed in a Getinge GE 666 AR-2 autoclave with a chamber size of 298 l. Data loggers (EBI 125-A) from Ebro were used to determine the temperature profile inside the carcasses. In addition, the inactivation result was examined with the use of bioindicators (spores of Geobacillus stearothermophilus in 10^6 CFU), which was distributed in the mass of carcasses. Furthermore, a mathematical formula for calculating the preheating time should be determined. This formula can then be applied to all animal-carcass sizes and serves as a approach for the estimation of the entire process duration. The following assumptions were made:

. To calculate the carcass-surface, each animal has been equated with a cylinder.
. To calculate the inwards all other carcass-parts like muscles, bones, blood and other tissues have been equated with adipose tissue.

These assumptions simplify the calculation of the heat transfer to the center of the carcass. The calculation of the heating time is as follows:

Parameters for the calculation of Heat-Transfer inside a Body

Name	Animal weight	Specific Heatcapacity	Animal surface	Coefficient of heat conductivity	Heatingdistance	Temperature difference
	m	Cp	A	λ	s	ΔT
Unit	kg	J/kg*K	m^2	W/m*K	Meter (m)	K

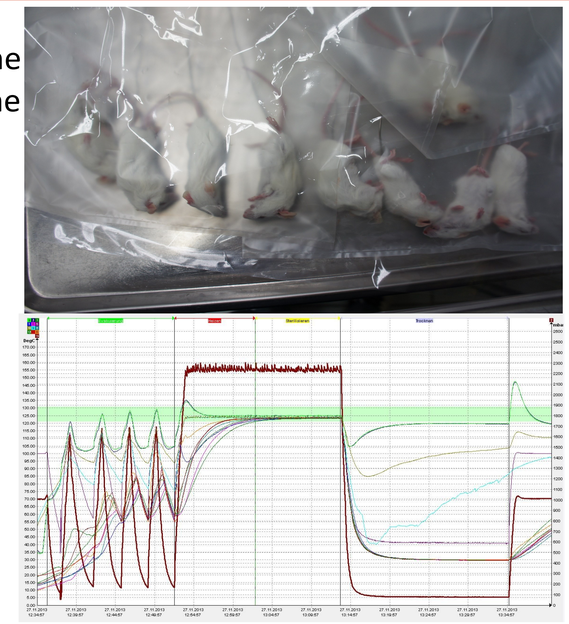
$$Heatingtime = \frac{Q}{\dot{q}} = \frac{m_{Mice} \cdot C_{p\,Fat} \cdot \Delta T}{\frac{\lambda}{s} \cdot A \cdot \Delta T}$$

Results

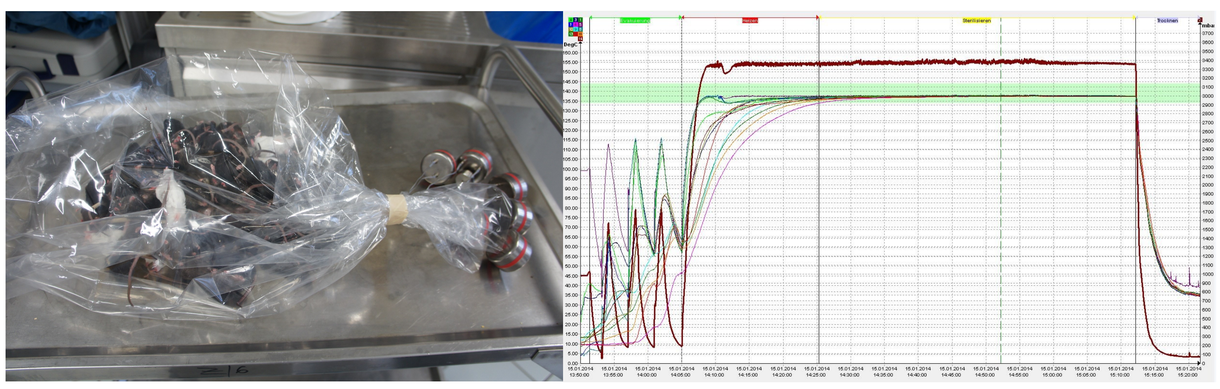
The results are presented in the table below. The blue table shows the results of the data logger and the successful inactivation. The red table compares the theoretical and measured heating time. The presented results consider the time that was needed for the first and the last mouse carcass to reach a temperature of 121°C.

Test No.	Material	Inactivationtemp.	Inactivationtime	Growth of Spore	Preheatingtime		Theoretical
					First	Last	Preheatingtime
1	10 Carcasses	121 min.	10 min.	Yes	4	10	4
2	20 Carcasses	121 min.	5 min.	Yes	6	15	*
3	40 Carcasses	134 min.	53 min.	No	5	7	*
4	80 Carcasses	134 min.	48 min.	No	4	12	*
5	100 Carcasses	134 min.	52 min.	No	4	8	13
6	100 Carcasses	134 min.	47 min.	No	6	14	13
7	100 Carcasses	134 min.	54 min.	No	4	6	13

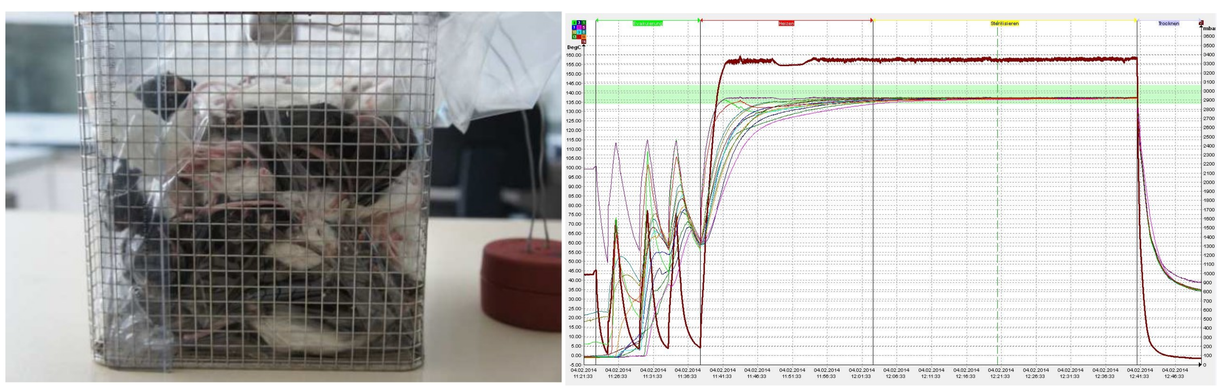
*no calculation possible, because of unknown heatingdistance.



Test No. 1: Seperately placed mouse carcasses, 121°C, 20 min



Test No.3: Mouse carcasses in a batch, 134°C, 60 min



Test No 5: Mouse carcasses in a „cubic“ shape, 134°C, 60 min

Conclusion

For the carcass inactivation experiments an autoclaving process for porous goods had been chosen, because it had been expected to remove potential air pockets between the carcasses and inside the fur better, since it guarantees fractionated vacuum during the heating period. The removal of air pockets had been expected to ensure a better heat transfer and thus a faster heating of the carcasses. An autoclaving process for liquids has also shown to be suitable, but the process takes much longer than the porous goods process (depending on the number of carcasses and the selected reference vessel 4-6h). Experiments 1 and 2 have shown that the required inactivation time of 20 minutes at 121°C is not reached inside the carcasses with the preset parameters. The porous goods processes are usually controlled by a temperature sensor in the chamber and not inside the waste (pourous goods). The chamber temperature usually reaches 121°C much faster than the inside of the carcasses. The inactivation temperature inside the carcasses thus could not be guaranteed for 20 minutes, before the cooling process was initiated. An extension of the entire process time therefore was necessary, to achieve a successful inactivation of all carcasses. The sufficient process time is dependent on the required heating time for the carcasses. The larger the number of carcasses and the greater the individual weight and volume of the carcass, the longer the required heating time will be. When the required heating time has been found for a certain number of carcasses, the required parameters for the entire process can be set. We were also able to show, that If saturated steam at a temperature of 134°C is used for the pourous goods process, a larger number of animal carcasses can be inactivated successfully. The calculation shown can be used as a reference for the expected preheating time. As shown in the table, the computed times correspond almost to the measured times in the process. In principle, a preheating time for individual carcasses can be calculated more accurate than for a higher number of carcasses, which must be autoclaved as a mass in bags.