The Director General

Maisons-Alfort, 19 February 2014

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the "microbiological safety and hygiene of pig carcasses refrigerated in chilling rooms and then transported in refrigerated trucks"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its Opinions are made public. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 19 February 2014 shall prevail.

On 3 January 2013, ANSES received a request from the Directorate General for Food (DGAL) for the following expert assessment: "Request for an Opinion on the microbiological safety and hygiene of pig carcasses refrigerated in chilling rooms and then transported in refrigerated trucks".

1. BACKGROUND AND PURPOSE OF THE REQUEST

EC regulation No. 853/2004 states that meat must reach (and maintain) a temperature below 7°C in all parts of the carcass before transport.

The Ministerial Order of 18 December 2009 (on health rules applicable to animal products and foodstuffs containing them), in accordance with EC Regulation 853/2004 and by way of derogation provided for therein, specifies that the transport and cutting of the carcasses of domestic ungulates be carried out during the chilling process subject to certain conditions: the temperature, in all parts of the carcass, must specifically be below 12°C upon departure from the slaughterhouse and transport time must be less than two hours.

In 2008, the French National Pork Trade Union, SNCP, based on a study report by the joint trade organisation, proposed easing these exemption criteria. First, it sought to raise the maximum permitted core temperature from 12°C to 15°C in all parts of pig carcasses upon leaving the slaughterhouse and second, it wished that the limitation on the transport time between the slaughterhouse and cutting plant be lifted. The DGAL then submitted a request to the French Food Safety Agency (AFSSA) on the amendment sought by the SNCP (Request No. 2008-SA-0283).

On 18 February 2009, AFSSA issued its Opinion on the transport of pig carcasses that have not reached the required temperature upon leaving the slaughterhouse. This Opinion indicated an

inability to comment on the suitability of using refrigerated transport as a means of chilling carcasses under the experimental conditions applied, and recommended conducting a new study.

In light of new data presented in the March 2011 French Pork and Pig Institute (IFIP) study, ANSES was asked the following questions:

1. Was the applicant's study relevant, both in terms of the protocol used and the microbiological criteria selected?
2. Are the study conclusions valid?
3. What would be the maximum acceptable temperature (i.e. not significantly increasing the risk to the consumer with respect to the pathogens studied) when carcasses leave the slaughterhouse, applying the two-hour transport time limit?
4. What would be the maximum acceptable values for the transport time and temperature of pig carcasses on the basis, if possible, of time and temperature data?

If ANSES confirms IFIP conclusions, France will approach the European Commission to request an amendment to EU regulations.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The expert assessment fell within the competence of the Expert Committee (CES) on the Assessment of the biological risks in food (BioRisk). ANSES entrusted the expert appraisal to external and internal *rapporteurs* at the CES and the Unit for Methodology & support in microbiology and animal health.

After consultation with the BioRisk CES, which met on 26 March 2013, ANSES requested additional information from the DGAL on 25 April 2013. On 29 October 2013, ANSES received all the information required to carry out the Expert Appraisal.

The work covering both methodological and scientific aspects was submitted to the BioRisk CES between 26 March 2013 and 26 November 2013. It was adopted by the aforementioned CES at its meeting on 14 January 2014.

First, the IFIP study of October 2013 was presented and its relevance discussed in response to the first question of the Request. ANSES then proposed a method for modelling the kinetics of chilling carcasses, together with a new assessment of scenarios. Lastly, some answers were provided for questions 2, 3 and 4 of the Request.

In addition to the technical records, sources for the data used were listed at the end of the report.

ANSES analyses the experts' declarations of interest prior to their appointment and throughout the course of the work, in order to avoid potential conflicts of interest with regard to the subjects covered in the context of the Expert Appraisal. The experts' declarations of interest are published on the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

3.1. Description of the IFIP study (October 2013)

3.1.1. IFIP study methodology

The objectives of the IFIP study were as follows:

- Estimate the difference in bacterial growth induced by refrigerated transport of pig carcasses loaded at a core temperature above 7°C, compared to these same carcasses had they remained in a chilling room;
- Assess the microbiological impact of refrigerated transport for pre-defined scenarios, in terms of carcass temperature at loading and of transport times.

IFIP used the following methodology:

- Acquire kinetic temperature data for pig carcasses in chilling rooms and refrigerated trucks. The core and surface temperatures of carcasses loaded at core temperatures above 7°C were measured for five different companies between 2006 and 2010. Forty-two kinetics of carcass temperature in chilling rooms and 141 in refrigerated trucks were obtained.
- Reconstruct, from temperature kinetics in chilling rooms and refrigerated trucks, the total temperature kinetics of the carcasses between their entry into the chilling room through to the end of refrigerated transport. Each temperature kinetic obtained for ham in chilling rooms was associated with the kinetics obtained for ham from the same company during transport. To remain compliant, these reconstructions had to meet certain criteria (for example, the temperature difference between leaving chilling rooms and entering the refrigerated truck had to be less than two degrees Celsius). After verifying that the criteria were satisfied for each association, 908 reconstructed temperature kinetics were retained.
- Simulate microbial behaviour on the surface of the carcasses (on both meat and rind sides) for the temperature kinetics obtained in chilling rooms or reconstructed (see above). This approach uses time/temperature integration. The theoretical growth potential of four microorganisms (*Salmonella*, *E. coli*, *Listeria monocytogenes* and *Pseudomonas*) were calculated from predictive microbiology models based on temperature kinetics measured on the surface of the carcasses. The growth simulations were carried out using a conservative approach: absence of lag-time, and constant values for the carcass pH and water activity (a_w). Different sources of variability were taken into account: the variability of chilling kinetics, the microbiological variability (especially the variability between strains for a single species), and the pH variability of different carcasses. The values of the parameters used for the probabilistic simulation were provided and supported by items available in the literature. Ten thousand simulations were performed for each of the four microorganisms studied by random selection of values used among the distributions presented.
- Compare microbial growth potentials on surface of carcasses loaded at a core temperature above 7°C (alternative scenarios) with (i) growth potential on carcasses kept in chilling rooms or (ii) on carcasses loaded at 12°C, and then transported for two hours (baseline scenarios).

Furthermore, in response to ANSES's request for additional information in April 2013, the SNIV-SNCP joint Trade Union of French Meat Companies described current practices for transporting pig carcasses. Temperature measurements were performed on carcass temperatures, in the core of the ham and on the belly surface, at loading (at 24 slaughterhouses, 9 carcasses per truck: 3 in the rear, 3 in the middle and 3 in front) and unloading. With regard to loading, 886 trucks were checked and the temperatures of approximately 6,000 carcasses were measured between 13 May

2013 and 19 June 2013. With regard to unloading, 407 trucks were checked and the temperatures of over 3,000 carcasses were measured.

3.1.2. Results and conclusions of the IFIP study

Two different strategies were used to compare microbial growth. Initially, bacterial growth on the surface of carcasses loaded at a core temperature above 7°C (all temperatures combined; between 9.8°C and 29.1°C) and then transported was compared with that of carcasses kept in chilling rooms (the time was equivalent for both categories of carcasses, transported or not). In a second phase, bacterial growth on the surfaces of carcasses leaving the slaughterhouse with core temperatures from 12°C, 15°C, up to 15°C, or 18°C for an illimited transport time (maximum 40h) was compared to that of carcasses leaving the slaughterhouse with a core temperature of 12°C, for a transport time of 2h (Ministerial Order of 18 December 2009 relating to warm cutting).

- Comparison of microbial growth for carcasses loaded at a core temperature above 7°C (alternative scenarios) compared with carcasses kept in chilling rooms (baseline scenario)

The characteristics relating to reconstructed temperature kinetics are available in Tables 7a, b and c of the IFIP study, referencing temperatures at loading, time in chilling room storage, in refrigerated trucks and total times (chilling room + refrigerated truck). For the 908 reconstructed temperature kinetics, the median core temperature at loading was 17.1°C [9.8°C; 28.9°C][1] and 10.5°C [7.4°C; 16.5°C] on the surface, median storage times were 5.04h [1.33h; 17.28h] in chilling rooms, 12.25h [4.25h; 26.42h] in refrigerated trucks, and 17.33h [9.17h; 32.58h] for total length of time.

The growth distributions obtained for the four microorganisms, for carcasses kept in chilling rooms, are shown in Figure 12 and Table 6 of the IFIP study. The distributions of the differences in growth obtained between carcasses kept in chilling rooms and those kept in chilling rooms and then in refrigerated trucks are shown in Figure 13 and Table 8b of the IFIP study. These differences in growth were estimated, meat-side, at values of 0.10 $\log_{10}$ [-0.08; 0.48] for *L. monocytogenes* and at 0.07 $\log_{10}$ [-0.06; 0.37] for *Salmonella*. Regarding the other two microbial indicators, these increases were estimated at 0.06 $\log_{10}$ [-0.08; 0.55] for *E. coli* and at 0.15 $\log_{10}$ [-0.14; 0.87] for *Pseudomonas*. A Multiple Analysis of Variance was suggested to identify the input parameters most influencing microbial growth.

The IFIP study concluded that compared to storage in chilling rooms without transport, the growth induced by transport was very limited, particularly in terms of the conservative assumptions of the modelling.

- Comparison of microbial growth for carcasses loaded at a core temperature above 7°C (alternative scenarios) with carcasses loaded at 12°C and then transported for 2h (baseline scenario)

The baseline scenario used by IFIP corresponds to carcasses leaving the slaughterhouse when the core temperature is 12°C, for a transport time of 2h (Ministerial Order of 18 December 2009 relating to warm cutting). From the available data sets, several alternative scenarios were defined:

- Carcasses leaving the slaughterhouse as soon as the core temperature reaches 12°C,
- Carcasses leaving the slaughterhouse as soon as the core temperature is lower than 15°C,
- Carcasses leaving the slaughterhouse as soon as the core temperature reaches 15°C,
- Carcasses leaving the slaughterhouse as soon as the core temperature reaches 18°C,

[1] Throughout the document, distributions are characterised as follows: median [2.5%; 97.5%].

No transport time limit was set for the assessment of these four alternative scenarios.

The objective was to compare microbial surface growth on carcasses loaded at a core temperature above 7°C (alternative scenarios) with carcasses loaded at a core temperature of 12°C and then transported for 2 hours (baseline scenario). To establish the link between surface and core temperatures of the carcasses, all the reconstructed temperature kinetics were sorted into four groups based on core temperature during loading (corresponding to four alternative scenarios: 12°C, lower than 15°C, 15°C and 18°C with a tolerance of +/- 1°C). Microbial growth was estimated from these four groups using surface rather than core temperature kinetics.

The characteristics for the four kinetic groups established are given in Tables 9a and b of the IFIP study, listing the kinetic number, temperatures at loading (core and surface) and duration of transport in refrigerated trucks. The distributions of the differences in microbial growth obtained for carcasses according to the baseline scenario (12°C, 2h) and those of the alternative scenarios (12°C, lower than 15°C, 15°C and 18°C) are shown in Tables 11a, b, c and d of the IFIP study. These differences in growth between the baseline scenario (12°C, 2h) and the alternative scenario (15°C–with no time limit) were estimated, for example, meat-side, to be 0.40 $\log_{10}$ [-0.50; 0.80] for *L. monocytogenes* and 0.10 $\log_{10}$ [-0.36; 0.41] for *Salmonella*, the latter's value being quite close to the differences estimated for *E. coli* (0.07 $\log_{10}$ [-0.50; 0.55]). For *Pseudomonas* the median value of these differences is greater (1.02 $\log_{10}$ [-1.00; 2.12]).

The IFIP study concluded that compared to the baseline scenario (12°C, 2h), there are few differences between the alternative scenarios assessed (12°C, lower than 15°C, 15°C and 18°C, with no time limit). The differences in growth between the baseline scenario (12°C, 2h) and loading at 12°C with no time limit are solely due to transportation exceeding two hours. The loading scenario at temperatures "lower than 15°C" corresponds to changing practices in the context of the exemption (L2009-n°0824). For loading at 18°C with no transport time limit, the median difference with the baseline scenario was a maximum value of 0.3 $\log_{10}$ for pathogens, greater than for spoilage flora (0.7 $\log_{10}$ for *Pseudomonas*).

- Current practices for transporting pig carcasses

The results from the compilation of measures of current practices (May 2013 – June 2013) for transporting pig carcasses are shown in Tables 3a and 3b of the IFIP study. Temperatures at the core of the ham were 11.80°C [4.10°C; 15.20°C] at loading and 5.80°C [2.20°C; 9.50°C] at unloading; while belly surface temperatures were 4.10°C [1.00°C; 11.10°C] at loading and 4.00°C [1.20°C; 6.70°C] at unloading.

Of the 886 trucks inspected at loading and the 407 inspected at unloading, 189 were checked at both loading and unloading, making it possible to characterise temperature trends during transport on the same carcasses and to estimate the variability of storage times in refrigerated trucks. The average temperature decrease at the core was 5.8°C in refrigerated trucks for a period of 10.8h [3.4h; 60.9h]. As mentioned in the IFIP study, transport time of over 55 hours corresponds to the time typically observed between loading on Friday and unloading on Monday morning.

3.2. Relevance of the applicant's study

3.2.1. Comments on the overall approach and microbial indicators used

With respect to the flora tested, the two selected pathogens (*Salmonella* and *Listeria monocytogenes*) are the most relevant for the pork industry. Both the microbial indicators used by the applicant—*E. coli* and *Pseudomonas*—are justified by technical and historic rationales. They do indeed adequately represent the evolution of *Enterobacteriaceae* populations and aerobic flora respectively, selected as process hygiene criteria in European Regulation (EC) No. 2073/2005.

The experimental protocol used is described in detail. The wish to demonstrate the comparability of situations is obvious. This concern with experimental accuracy is illustrated by the reconstruction of

temperature kinetics in case of transport (taking into account chilling in the chilling room, then chilling in refrigerated trucks) and by adjusting the duration of the compared temperature kinetics (reconstructed or not). The power of the study is enhanced by the increased number of temperature kinetics in the chilling rooms used (three temperature kinetics in 2008 compared to approximately 40 in the new study). Using data from about five companies and eight carcasses per company reveals the variability between and within slaughterhouses.

The simulation assumptions, identical to those of the 2008 study (simulated growth meat-side and rind-side, no latency (lag time), surface growth, etc.) remain relevant and conservative. The parameters used for the probabilistic simulation are properly identified in the report and the values used require no comment. All the core and surface kinetic temperature data during transport and in chilling rooms were made available.

The experimental protocol and general methodology (time-temperature integration) were quite appropriate for helping the risk manager define conditions for allowing an exemption from current regulations that would lead to an increased level of acceptable risk. Uncertainties from the first report were resolved: the applicant draws on more data and so the basis of comparison between carcasses transported before reaching a core temperature of 7°C and those remaining in chilling rooms is relevant.

3.2.2. Comments on differences in microbial growth between carcasses remaining at the slaughterhouse and those transported before reaching a core temperature of 7°C

Results presented for one of the bacterial flora (*L. monocytogenes*) were verified using kinetic data sent by the petitioner, equations and input parameters for the model used. The growth results obtained are consistent with those presented in the IFIP study.

Simulation results are not used by the petitioner to justify a new exemption but rather to compare growth characteristics for different scenarios. Moreover, the petitioner uses simulations for identifying, by means of an analysis of variance, the main factors influencing bacterial growth comparing the carcasses chilled solely in slaughterhouse chilling rooms and those chilled in chilling rooms then loaded onto refrigerated trucks at temperatures above 7°C (the core temperature at loading time ranged between 10°C and 30°C).

3.2.3. Comments on the proposal for alternative exemption scenarios and assessment of these scenarios

Use of the existing exemption (12°C, 2h) as a baseline for evaluating other scenarios is questionable. Carcasses transported under exemption conditions should be cut immediately upon arrival at the cutting plant.

An analysis of transport time data submitted by the applicant shows that 90% of truck journeys took between 3 and 18 hours. Consequently, the carcasses considered in the alternative scenarios arrived at the cutting plants at temperatures near or below 7°C. Thus the applicant's objective in requesting an exemption amounts to seeking time-savings in terms of slaughtering/cutting logistics, and not to providing cutting plants with "warm" carcasses. Therefore, it would have been more appropriate to compare alternative scenarios to the baseline situation, meaning carcasses that leave the slaughterhouse only if their core temperature is below 7°C.

Furthermore, assessment by scenarios prompted the applicant to choose specific kinetic temperature data from all the data available (see 3.1.2, point 2). The applicant chose the kinetics which, when the carcass leaves the slaughterhouse, have core temperatures matching the scenarios considered. Consequently, there are very few temperature kinetics available for assessing a given scenario, and the variability of the temperature profiles is very limited. The applicant thus loses much of the information collected.

3.2.4. Comments on carcass temperature data upon leaving the slaughterhouse and arriving at the cutting plants

The data sent by the SNIV-SNCP confirm that the chilling capacity of the trucks facilitates chilling to the core. However, the representativeness of these data and of the trucks' chilling capacity cannot be evaluated without knowing the sampling plan (method of choosing the trucks sampled). Figure 1 shows the core temperature of the carcasses during unloading of refrigerated trucks versus their core temperature when loaded into the trucks, and thus illustrates the lowering of carcass core temperature between loading and unloading.

As stated previously, according to the data provided by the applicant, the median length of transport time by a refrigerated truck is 10.8h [3.4h; 60.9h], 90% of the times lie between 3h and 18h, and the applicant indicates that a transport time of more than 55h corresponds to carcasses loaded on Friday and unloaded Monday morning. This therefore reflects two transport practices: transporting carcasses during the week (times ranging between 3h and 18h) and transporting carcasses on the weekend (times exceeding 55h).

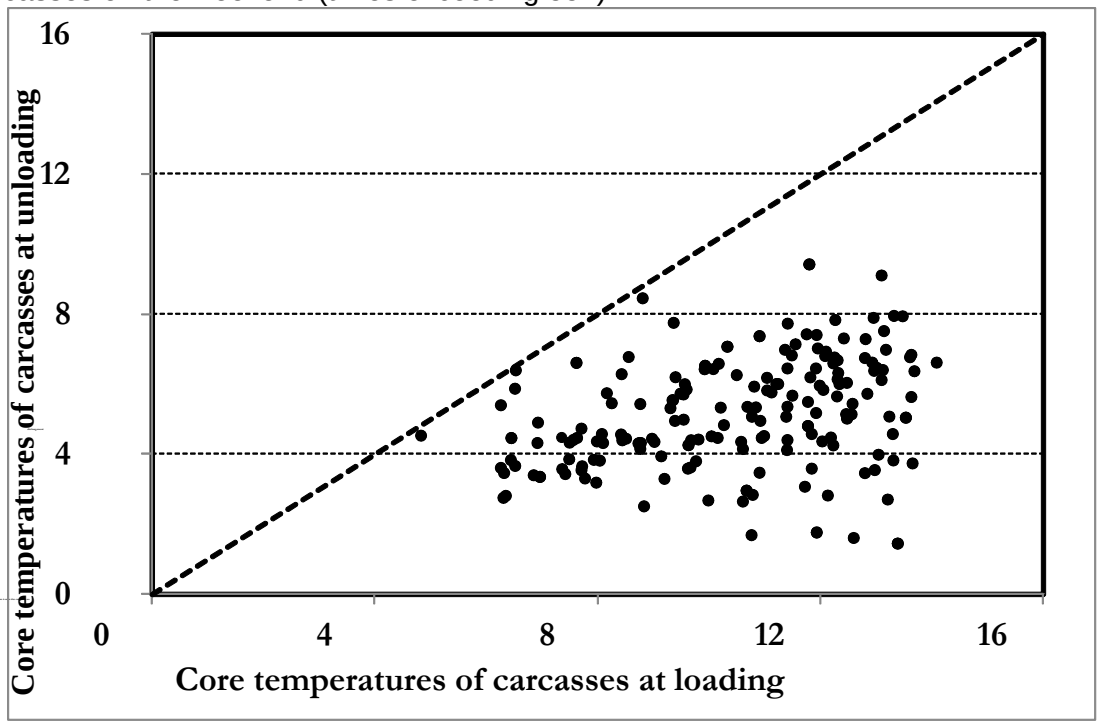

Figure 1. Mean temperatures (°C) measured at the core (ham) on carcasses arriving in cutting plants based on mean temperatures (n=9) taken upon leaving the slaughterhouse. Points under the bisecting line correspond to transport during which a decrease in temperature was observed.

3.3. Modelling carcass temperature kinetics

To assess bacterial growth during the chilling and transport of pig carcasses, the applicant uses the temperature kinetics directly. We suggest modelling instead. Modelling the temperature kinetics can be used to simulate growth for the time periods chosen at different stages (slaughterhouse chilling room, transport, storage) prior to cutting. In addition, modelling allows to keep all temperature kinetics, and thus ensures that the variability of situations encountered is correctly characterised.

3.3.1. Modelling core temperature kinetics

The core temperature of carcasses (denoted *Tc*) decreases exponentially in slaughterhouse chilling rooms. However, chilling kinetics vary depending on the slaughterhouse (due to the use of different refrigeration systems) and the carcasses themselves (particularly linked to their position in the chilling room, the loading? density of the chilling room, or even differences in carcass conformation).

Data provided by the applicant (5 slaughterhouses and 8 carcasses per slaughterhouse) were adjusted with an exponential decay model (See Annex 1). The variability of core temperature kinetics in chilling rooms is shown in Figure 2.

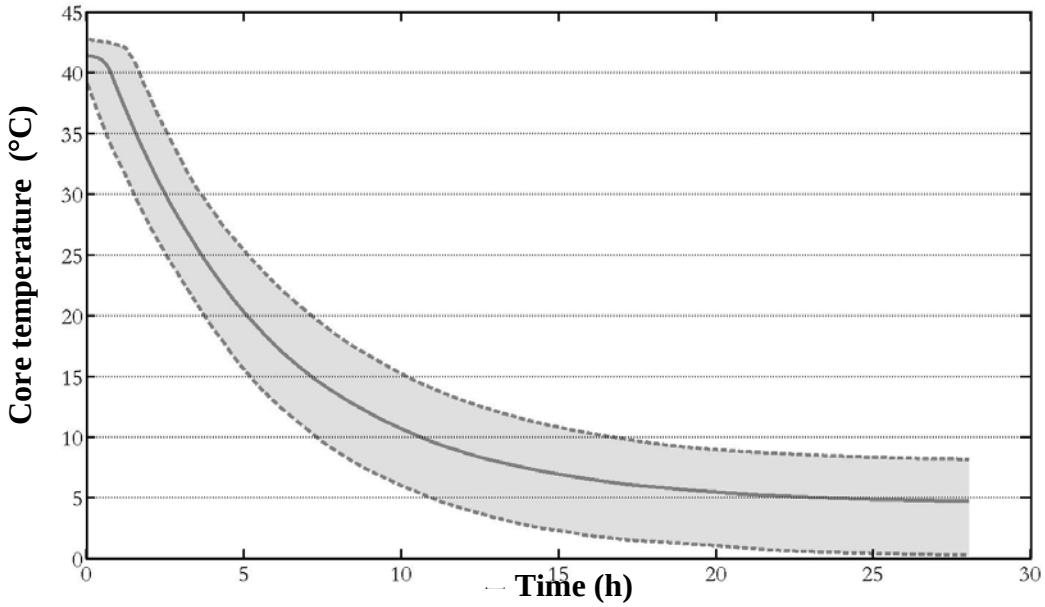

Figure 2. Chilling kinetics at the core of carcasses in a chilling room. Solid line, median chilling profile. Shaded area between the dotted lines, 95% variability range for all possible profiles.

These temperature kinetics of chilling room carcasses are characterised in particular by the exponential decay parameter, denoted k_1. The distribution of k_1 values obtained from the different carcasses is shown in Figure 3.

In refrigerated trucks, the core temperature profiles of the carcasses also follow an exponential decay model. The distribution of values taken for the exponential decay parameter (k_2) is also depicted in Figure 3. The values show that, on average, cooling in refrigerated trucks is less efficient than chilling room refrigeration systems.

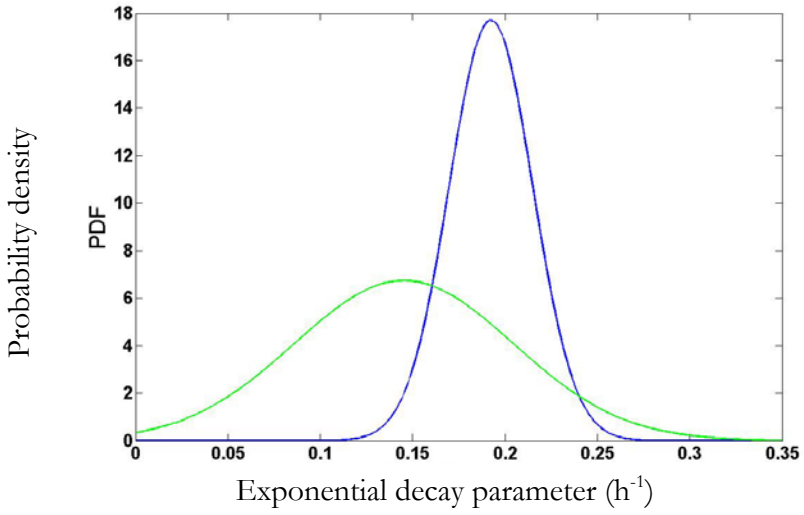

Figure 3. Distributions of estimated parameters k_1 (in chilling rooms; —) and k_2 (in refrigerated trucks; —), characterising exponential temperature decay at the core of the ham.

From the initial temperature of the carcasses and estimated values of the parameters that characterise chilling in slaughterhouses and refrigerated trucks (See Annex A.1), it is possible to simulate the core temperature of the carcasses for these two conditions.

3.3.2. Modelling the temperature difference at the core and on the surface of the carcasses

The temperature kinetics at the surface of pig carcasses do not exhibit a monotonic profile. At the end of the slaughter line (i.e. at the start of chilling), the temperature within a carcass is relatively homogeneous; there is a slight difference between surface temperature and core temperature. In the first hours, the surface temperature will decrease more rapidly than the core temperature. : During this initial phase, the more efficient the refrigeration system, the quicker the temperature will drop. After approximately 5 hours of chilling, the surface temperature increases slightly before declining thereafter.

The difference between the surface temperature and the core temperature can be described using a model (See Annex A.2). The result of this modelling is shown in Figure 4.

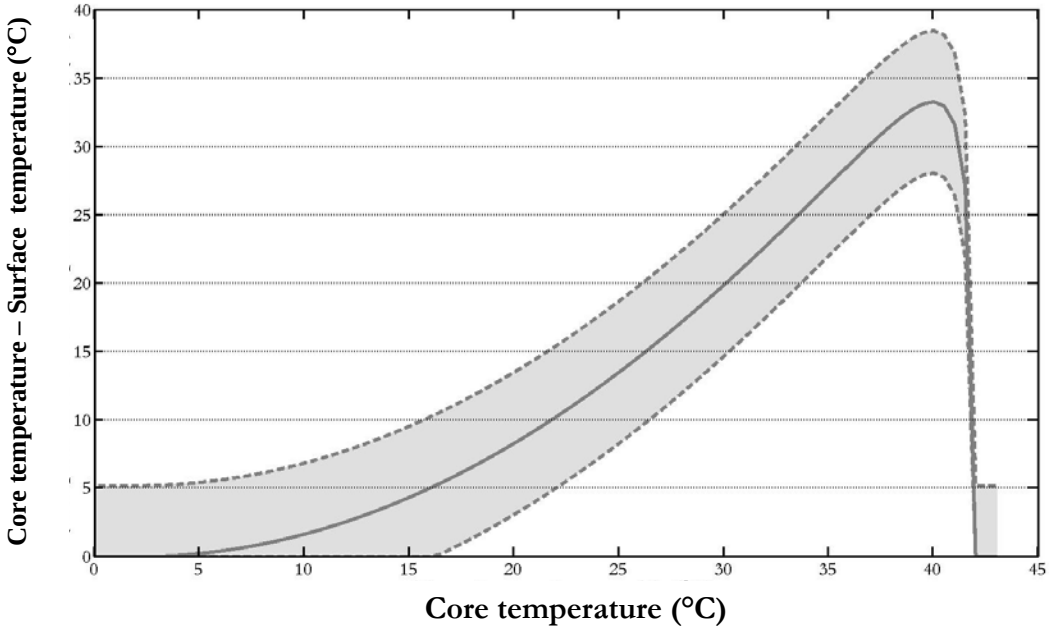

Figure 4. Temperature difference between the core and the surface of the hams during chilling as a function of core temperature.

3.3.3. Estimating changes in the surface temperature of the carcasses

The temperature on the surface, which is where bacterial growth occurs, can therefore be described using the previous two models. The temperature profiles simulated during chilling (see Figure 5) correspond to those observed by the applicant (see Annex A.3).

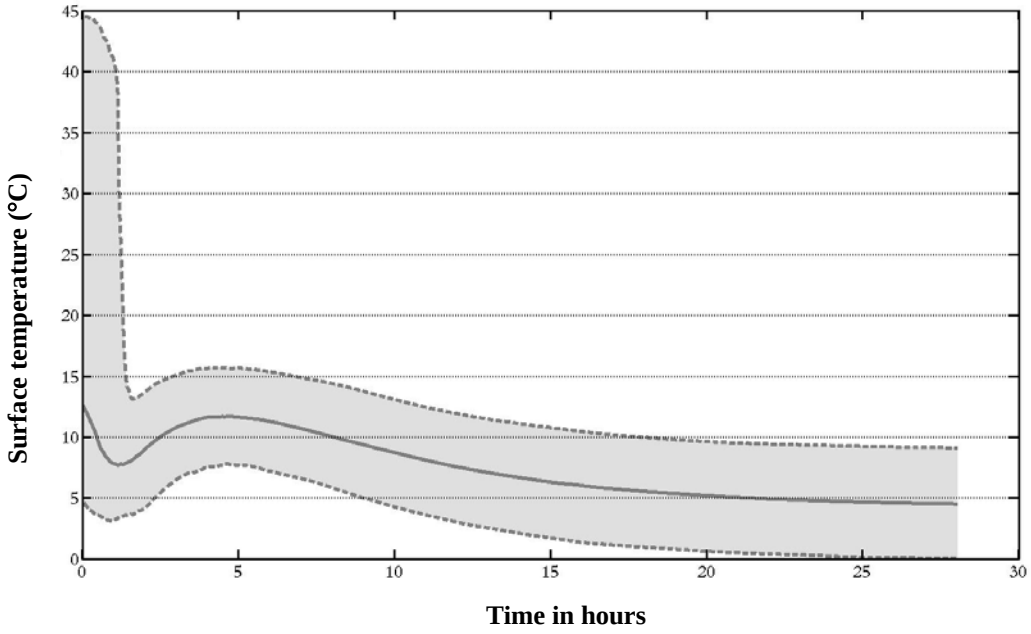

Figure 5. Chilling kinetics on the surface of carcasses in chilling rooms. Solid line, median chilling profile. Shaded area between the dotted lines, 95% variability range for all possible profiles.

3.3.4. Assessment of modelling the temperature kinetics

The models developed can replace the kinetics used by the applicant for estimating growth depending on temperatures at the core and surface of pig carcasses for different storage conditions in chilling rooms and different transport times. The distribution of values of the models' parameters reflect the variability in the chilling rooms of the five slaughterhouses included and all transport by truck (see 3.2.4).

3.4. New definition of baseline scenario and assessment of possible alternative scenarios

The subsequent analysis concentrates on two pathogens, *L. monocytogenes* and *Salmonella*, as only their growth assesment can address the risk issue. Only meat-side growth was considered because it corresponds to the most conservative situation. Moreover, the assessment of scenarios focuses solely on transport taking place during the week (lasting between 3 and 18h). Lengthy transport corresponding to loading carcasses on Friday for a Monday delivery were not included because the available data did not enable us to assess the chilling capacity of the refrigerated trucks when parked.

The assessment of alternative scenarios to those of the current regulation proposed by the applicant relies on core temperature at the time of loading. For example, it was suggested that the carcasses can leave the slaughterhouse chilling room when a core temperature of 15°C is reached. In practice, this decision-making rule for releasing the carcasses is not suitable because the temperature in a chilling room varies from one carcass to another based on its location and/or its particular characteristics. It is thus difficult to envisage releasing some carcasses after 9 hours of chilling, others after 10h and the last, after 12h for example. From a practical point of view, applied by the slaughterhouses, it seems more appropriate to define a chilling time in the slaughterhouse that enables them to ensure that a certain proportion (95% for example) of carcasses reach a temperature lower than or equal to 15°C. Modelling the core temperatures for different slaughterhouses and different carcasses demonstrates that over 95% of the carcasses have a core temperature below 18°C, 15°C and 12°C after 8 hours, 10 hours and 15 hours respectively (see Figure 2). These chilling room storage times were used in the following simulations to characterise the carcasses corresponding to core temperatures below 18°C, 15°C and 12°C.

To test whether alternative scenarios to the transport of pig carcasses chilled to a core temperature of 7°C are feasible, it is necessary to define baseline scenarios, i.e. to consider different situations observed in the industry and compliant with current regulations. Three baseline scenarios (case A, case B and case C) were therefore chosen and described below. The growth of *L. monocytogenes* and *Salmonella* corresponding to these baseline scenarios were evaluated and compared to alternative scenarios in which the carcasses are at a temperature other than 7°C when loaded into trucks.

3.4.1. Case A: baseline scenario (12°C, 2h), alternative scenarios (release of carcasses at 12°C, 15°C or 18°C prior to transport)

The baseline scenario in the first case was derived from the current exemption for warm cutting, used as a reference in the IFIP study. In this situation the carcasses leave the slaughterhouse when the core temperature stands at 12°C or below (which is after about 15 hours in the chilling room) and are then transported for up to a maximum of two hours. The growth of *L. monocytogenes* and *Salmonella* in this baseline scenario was compared to that obtained for carcasses leaving the slaughterhouse at 12°C, 15°C and 18°C. For these three alternative scenarios, it was considered that transport times were variable, ranging between 3 and 18 hours, an interval corresponding to 90% of transport times according to the data submitted by the applicant.

Figure 6 shows an example of the time-temperature profile for the baseline scenario (12°C, 2h; black curve) and the alternative scenario (15°C, no time limit; grey curve).

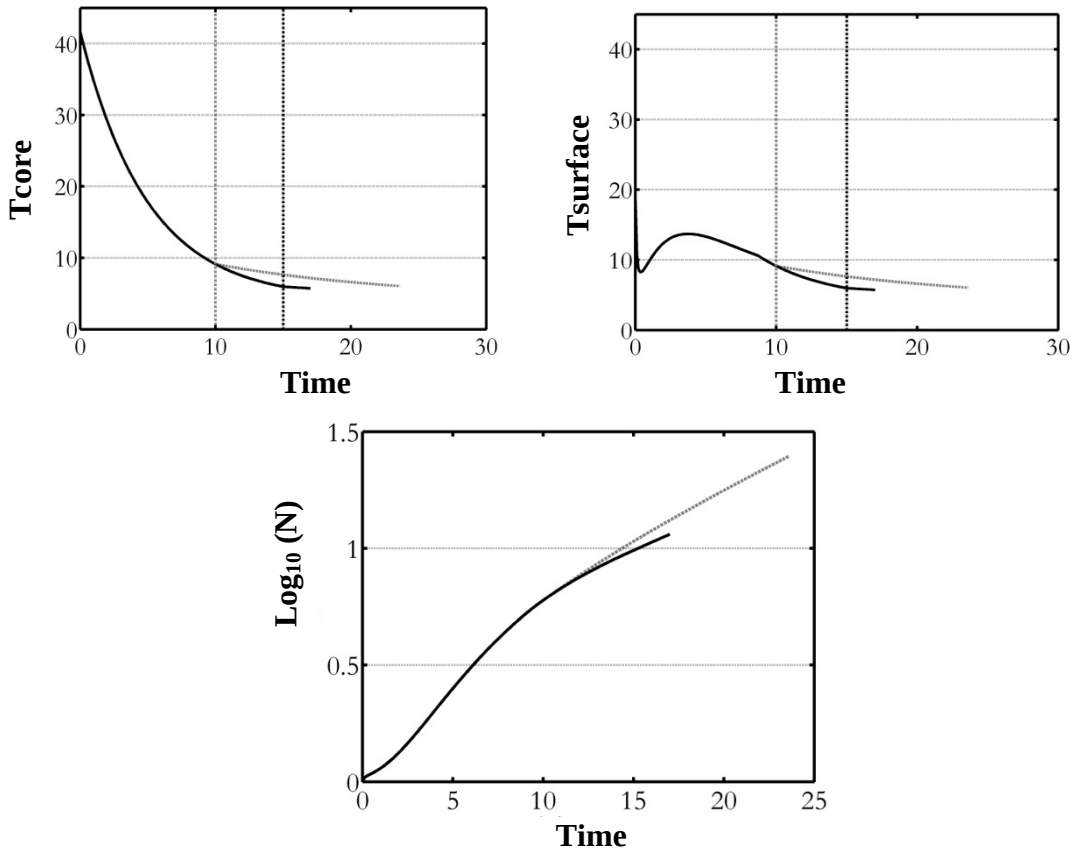

Figure 6. Example of chilling kinetics at the core (a) and surface (b) for the baseline scenario (black curve) and the 15°C alternative scenario (grey curve) out of the 10,000 simulations performed. The vertical dotted lines represent the instant of loading into trucks for both the baseline scenario (black dotted lines) and the alternative scenario (grey dotted lines). (c) An example of the growth of *L. monocytogenes* on the carcass surface (meat side) is shown for both situations.

A synopsis of differences in growth between the alternative scenarios and the baseline scenario used by the applicant (see Comment 3.2.4) is presented in Table 1. Estimated growth for the alternative scenarios is greater than for the baseline scenario. In the 12°C scenario (loading at a core temperature of 12°C equivalent to 15h in the chilling room), the longer transport time (2h for the baseline scenario versus a time ranging between 3 and 18h for the alternative scenario) alone explains this increased growth. For the 15°C and 18°C scenarios, the difference in growth is similar to that observed for the 12°C scenario.

Table 1. Differences in growth (log_{10} (CFU)) between alternative scenarios and the baseline scenario (case A)

Core temperature upon leaving the slaughterhouse	12°C scenario			15°C scenario			18°C scenario		
Percentiles	2.5%	50%	97.5%	2.5%	50%	97.5%	2.5%	50%	97.5%
Salmonella	0.00	0.03	0.19	-0.03	0.02	0.17	-0.07	0.01	0.22
L. monocytogenes	0.00	0.15	0.61	-0.12	0.07	0.46	-0.20	0.04	0.46

3.4.2. Case B: baseline scenario (release of carcasses at 7°C prior to transport), alternative scenarios (release of carcasses at 12°C, 15°C or 18°C prior to transport)

Another reference situation may be defined. This baseline scenario still complies with current regulations (EC No. 853/2004 and implementing French Ministerial Order) and current conditions for the transport of carcasses, apart from the context of warm cutting. The baseline scenario for case B is the following: the carcasses are transported when the core temperature reaches 7°C (the time required to reach a core temperature of 7°C in the chilling room is approximately 24 hours, according to the data submitted by the applicant). Then the carcasses are transported, their temperature remaining below 7°C throughout the transport period. As indicated above, transport times are variable, ranging between 3 and 18 hours in keeping with the data submitted by the petitioner (see Comment 3.2.5).

For the alternative scenarios, pathogen growth is estimated for carcasses with core temperatures below 18°C, 15°C and 12°C (i.e. chilled in chilling rooms for 8 hours, 10 hours and 15 hours respectively).

For this baseline scenario as for the alternative scenarios, growth is assessed between the end of the slaughter line and arrival at the cutting plant, assuming that the carcass is cut up immediately upon arrival at the cutting plant.

Figure 7 shows an example of a time-temperature profile for the baseline scenario and the 15°C scenario.

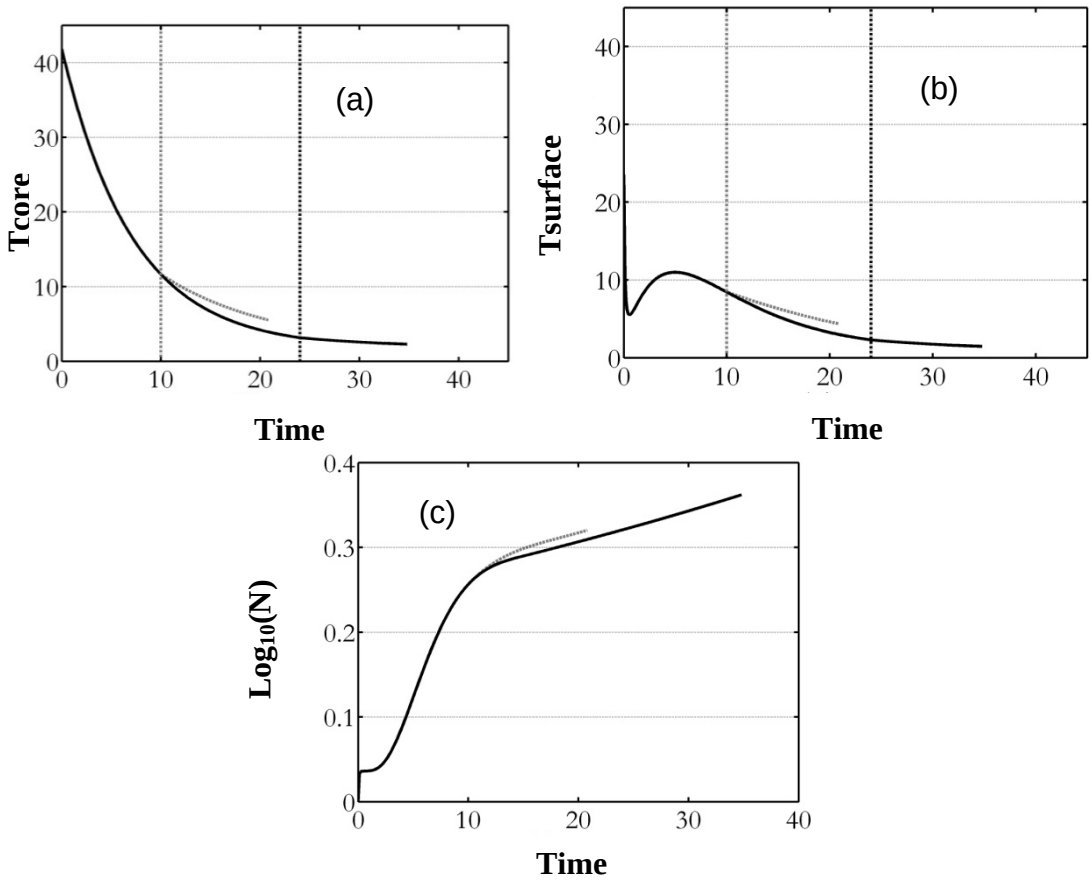

Figure 7. Example of chilling kinetics at the core (a) and surface (b) for the baseline scenario (black curve) and the 15°C alternative scenario (grey curve) out of the 10,000 simulations performed. The vertical dotted lines represent the instant of loading into trucks for both the baseline scenario (black dotted lines) and alternative

scenario (grey dotted lines). (c) An example of *Salmonella* growth on the carcass surface (meat side) is given for both situations.

A synopsis of the differences in growth between the alternative and baseline scenarios is provided in Table 2. There is less bacterial growth in the three alternative scenarios than in the baseline scenario. Carcass refrigeration is slower during transport than in chilling rooms, but in terms of pathogen growth potential, this reduced refrigeration capability is largely compensated by the time savings achieved in terms of logistics. In fact, for equal transport times, carcasses in the alternative scenarios arrive at the cutting plant 9, 14 and 16h respectively before the carcasses in the reference case. However, for these scenarios, the temperature of the carcasses upon arrival at the cutting plant is not consistently lower than or equal to 7°C.

Table 2. Differences in growth (log_{10} (CFU)) between alternative and baseline scenarios (case B)

Core temperature upon leaving the slaughterhouse	12°C scenario			15°C scenario			18°C scenario		
Percentiles	2.5%	50%	97.5%	2.5%	50%	97.5%	2.5%	50%	97.5%
Salmonella	-0.14	-0.05	0.00	-0.19	-0.05	0.02	-0.27	-0.05	0.04
L. monocytogenes	-0.47	-0.18	0.00	-0.71	-0.27	0.00	-0.83	-0.30	0.00

3.4.3. Case C: baseline scenario (carcasses released at 7°C, transport and storage – 72h between entry into slaughterhouse chilling room and entry into the cutting plant), alternative scenarios (release of carcasses at 12°C, 15°C or 18°C, transport and storage – 72h between entry into slaughter house chilling room and entry into the cutting plant)

For this third case, the situation is similar to case B except that case C takes into consideration storage upon arrival at the cutting plant, before the carcass is actually cut. For the baseline scenario as for the alternative scenarios, growth is assessed over three days (the time between slaughter and cutting).

Figure 8 shows an example of a time-temperature profile for the baseline scenario and the alternative scenario at 18°C.

Distributions of the difference in growth between the baseline scenario and the three alternative scenarios are given in Table 3.

In this case, the logistics time savings no longer play a role. The lesser refrigeration capability of trucks compared to chilling rooms explains the increased growth of pathogens in the three alternative scenarios, but the median deviations are at most 0.05 log_{10}.

Table 3. Differences in growth (log_{10} (CFU)) between the alternative and baseline scenarios (case C)

Core temperature upon leaving the slaughterhouse	12°C scenario			15°C scenario			18°C scenario		
Percentiles	2.5%	50%	97.5%	2.5%	50%	97.5%	2.5%	50%	97.5%
Salmonella	-0.01	0.00	0.05	-0.04	-0.01	0.21	-0.08	0.02	0.38
L. monocytogenes	-0.04	0.01	0.12	-0.10	0.03	0.36	-0.13	0.05	0.56

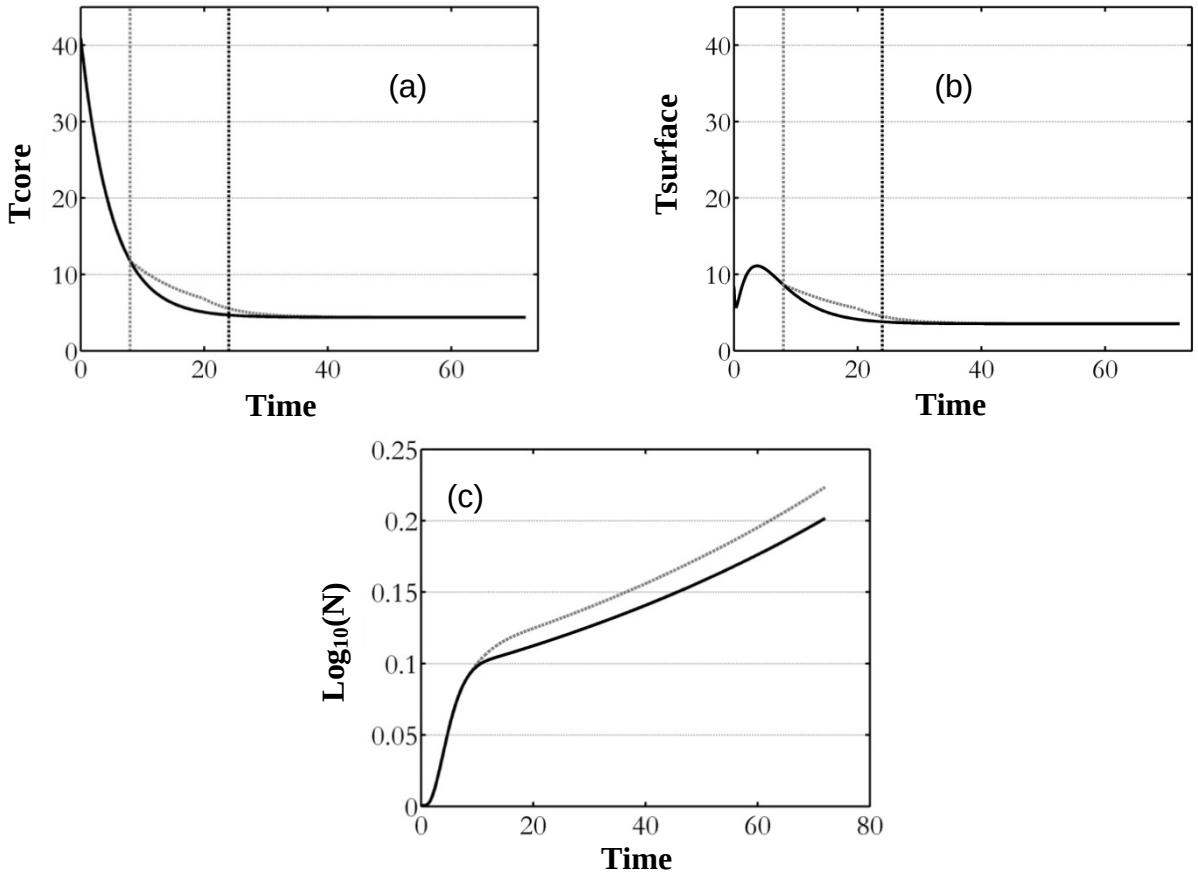

Figure 8. Example of chilling kinetics at the core (a) and surface (b) for the baseline scenario (black curve) and the 18°C alternative scenario (grey curve) out of the 10,000 simulations performed. The vertical dotted lines represent the instant of loading into trucks for both the baseline scenario (black dotted lines) and alternative scenario (grey dotted lines). (c) An example of growth of *L. monocytogenes* on the carcass surface (meat side) is given for both situations.

3.5. Response to terms of reference

ANSES was asked the following questions in the Request:

1. Was the petitioner's study relevant, as regards both the protocol and microbiological criteria used?

The experimental protocol and the general methodology (time-temperature integration) are appropriate for helping the risk manager define conditions for exemption from current regulations that would lead to an increase in the level of acceptable risk. Limitations from the first report have been resolved: the petitioner draws on more data and the basis of comparison between carcasses transported before reaching a core temperature of 7°C and those remaining in chilling rooms is relevant (see Section 3.2 "Relevance of the IFIP study").

However, the use of the current exemption (12°C, 2h) as the baseline scenario for assessing the alternative scenarios is questionable. Assessment by scenarios prompted the applicant to choose specific kinetic temperature data from all the data available. The applicant chose the data which, when the carcass leaves the slaughterhouse have core temperatures matching the scenarios considered. Consequently, there are very few temperature kinetic data available for assessing a given scenario, and the variability of the temperature profiles is very limited. Much of the information collected by the applicant is therefore lost.

To remedy these weaknesses, ANSES proposes in this Opinion to:

- Model the temperature kinetics of the carcasses (Section 3.3) to simulate growth for the time periods chosen at different stages (slaughterhouse chilling room, transport, storage) prior to cutting. Modelling ensures that no temperature data are excluded and ensures that the variability of situations encountered is taken into consideration.
- Define, by way of example, new potential reference and alternative scenarios in order to simulate bacterial growth differences between these various scenarios.

2. Are the study conclusions valid?

The petitioner's study produced a significant amount of satisfactory data that supported its conclusions cogently. It should be remembered that these data were obtained under conservative conditions that maximised the bacterial growth estimates. The additional information given by the petitioner help to (i) ensure a sound evaluation of the sources of variability (ii) compare alternative scenarios proposed by the applicant with a situation that seems more consistent with current regulations (case C, and to a lesser extent, case B).

As stated previously (see Sections 3.2.4 and 3.2.5), case A (baseline scenario: 12°C, 2h), may not be the most appropriate for establishing a new exemption. Regarding case B, microbial growth was lower under the alternative scenarios than under the baseline scenario due to time savings in the logistics. This situation corresponds to a theoretical case in which the carcasses are routinely cut up immediately upon arrival in the cutting plants. Case C more closely approximates field situations where the carcasses are stored in the cutting plants following transport.

This general modelling approach should help the risk manager. The tables providing differences in growth (see Tables 1, 2 and 3) between a situation similar to the current regulations and possible alternative scenarios for the main two pathogens in the pork industry may help the risk manager choose to grant an exemption. Given the data submitted (cases B and C), optimising storage time prior to operations at the cutting plant would appear to be an important additional parameter to consider when examining exemptions to regulations on transporting carcasses.

3. What would be the maximum acceptable temperature (i.e. not significantly increasing the risk to the consumer with respect to the pathogens studied) for carcasses leaving the slaughterhouse with a maximum two-hour transport limit?
4. Preferably on the basis of a time/temperature profile, what would be the maximum acceptable time and temperature values for the transportation of pig carcasses?

To fully answer these questions, it is important to define acceptable risk levels from which performance objectives can be deduced (maximum increase in flora during transport) and potentially to translate these performance objectives into process criteria (transport time and temperature, possibly supplemented by storage time upon arrival).

As a result of the evolving time/temperature kinetics of carcasses under dynamic conditions between the slaughterhouse and the cutting plant, it cannot be limited to a fixed time/temperature pair for transport. The models proposed may be used to optimise the chilling conditions in the slaughterhouse and during storage prior to cutting, by taking into account requirements and performance of the transport of carcasses between these two production stages.

Should the risk manager identify an "acceptable" scenario in terms of risk level, ANSES can help transcribe this scenario into the regulations using different process criteria: chilling time in the slaughterhouse, core temperature or even surface temperature at the time of loading.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions of the BioRisk CES.

Marc Mortureux

KEY WORDS

Carcasses; pig; chilling; exemption

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ANNEXES

A.1. Modelling chilling kinetics at the core of carcasses

A.1.1. Model for refrigeration in chilling rooms

The petitioner's data included recording the surface temperature (*Ts*) of carcasses in various slaughterhouses in France. The temperature kinetics recorded on the surface of pig carcasses in the five slaughterhouses were non-monotonic, i.e. their direction of variation changed according to the time interval measured. These temperatures do not continue to decrease, as increases in core temperature were occasionally observed, linked in particular to heat transfer.

For 37 carcasses, the surface temperature and core temperature (*Tc*) were available. Changes in core temperature (*T*c) over time (*t*) can be described by a model of exponential decay with time (See Equation 1):

$$Tc(t) = \begin{cases} T_0 & t \leq d \\ T_a + (T_0 - T_a) \cdot \exp(-k_1 \cdot t) & t > d \end{cases}$$

Equation 1

Where *Tc(t)* is the core temperature of hams at a given time (*t*), T_0 is the initial core temperature of the hams, T_a is the asymptotic core temperature of the hams, *d* is the lag time before exponential decay of the temperature, characterised by parameter k_1.

Figure A.1 shows an example of how to fit this model to one of the temperature kinetics the applicant recorded at the core of hams (of the 37 available temperature kinetics).

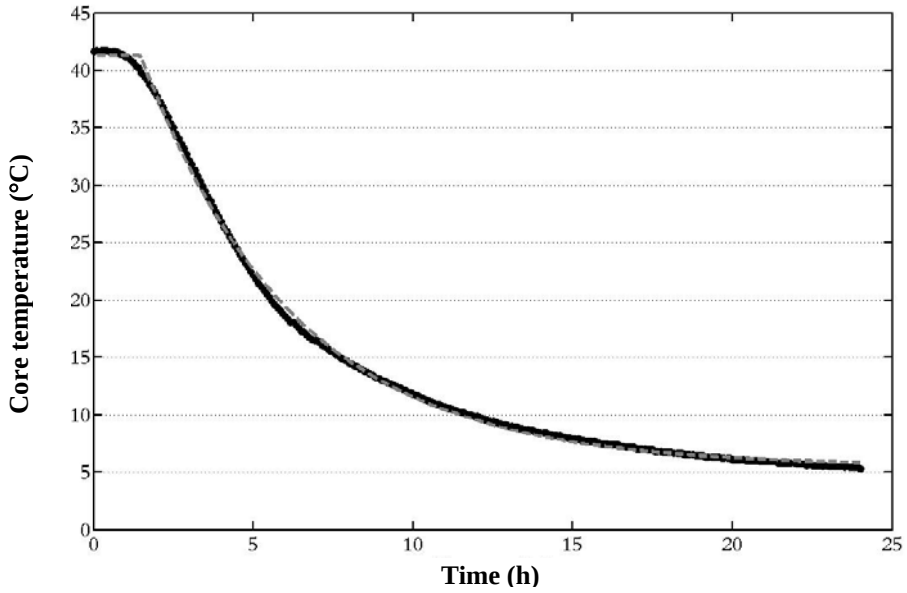

Figure A.1: Fit of one of the 37 kinetic temperature logs provided by the petitioner of core ham temperatures of carcasses chilled in chilling rooms.

A.1.2. Model for refrigeration in refrigerated trucks

The core temperature was available for 40 transported carcasses (loaded into over 35 different trucks from the five slaughterhouses). These kinetic data were fitted with the same model used for carcasses in chilling rooms but without any lag time before exponential decay:

$$Tc(t) = T_a + (T_0 - T_a) \cdot exp(-k_2 \cdot t)$$ Equation 2

The parameters are identical to those in Equation 1.

The values obtained for parameter k_2 indicate that the carcasses in trucks were chilled more slowly than in chilling rooms.

A.2. Modelling the difference between the core and surface temperatures of the carcasses

The difference between the surface temperature of the carcasses and the core temperature can be modelled using the following equation:

$$(T_C - T_S) = \frac{E_{\max}(T_C - 42)(T_C - T_{CE\min})^2}{(T_{CE\max} - T_{CE\min})[(T_{CE\max} - T_{CE\min})(T_C - T_{CE\max}) - (T_{CE\max} - 42)(T_{CE\max} + T_{CE\min} - 2T_C)]} + N(0,\sigma)$$

Equation 3

Where *Tc* is the core temperature, *Ts* is the surface temperature, Tc_{Emax} is the core temperature for which the difference (E_{max}) in temperature between the core and the surface is maximal, Tc_{Emin} is the core temperature for which the difference in temperature between the core and the surface is minimal and σ is the typical standard deviation of the variability between carcasses.

Figure A.2 shows the fitting of this model to the core and surface temperature data provided by the petitioner.

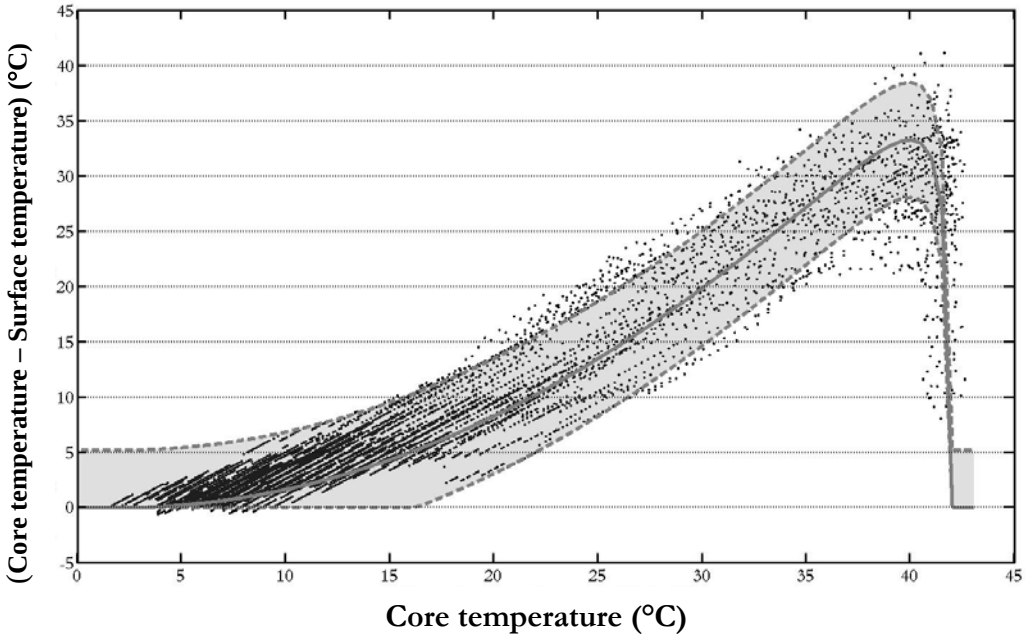

Figure A.2: Temperature difference between the core and surface of hams as a function of core temperature.

A.3: Estimating changes in the surface temperature of the carcasses

From equations (1) and (2), it is possible to predict the surface temperature of the carcasses from their core temperature (see Figure A.3).

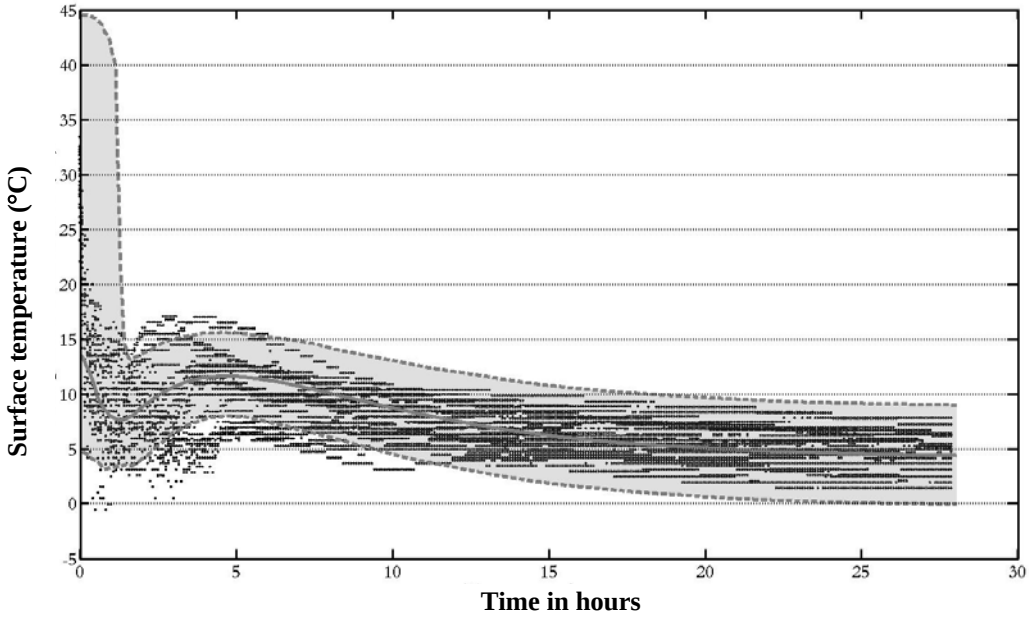

Figure A.3: Kinetics of the surface temperature of carcasses simulated from Equations 1 and 2 (and parameters from the tables below) and observed by the petitioner.

A.4. Calculating differences in microbial growth

A.4.1. Model for assessing the growth

The primary model used is the logistic model with time and delay described by Equation 4.

$$\ln N = \begin{cases} \ln N_0 & ,t \le lag \\ \ln N_{max} - \ln\left(1 + \left(\frac{N_{max}}{N_0} - 1\right)\exp\left(-\mu_{max}(t - lag)\right)\right) & ,t > lag \end{cases}$$

Equation 4

Where N is the bacterial density at time t (h), N_0 is the initial bacterial density, N_{max}, the maximum bacterial density, *lag*, the lag time (h) and μ_{max} the maximum growth rate (h^{-1}).

To describe the effect of temperature, pH and a_w on growth, the following model was used:

$$\mu_{max} = \mu_{opt} . CM_2(T) . CM_1(pH) . CM_2(a_w)$$

$$CM_n(X) = \begin{cases} 0, & X \le X_{min} \\ \dfrac{(X - X_{max})(X - X_{min})^n}{(X_{opt} - X_{min})^{(n-1)}\left[(X_{opt} - X_{min})(X - X_{opt}) - (X_{opt} - X_{max})\left((n-1)X_{opt} + X_{min} - 2X\right)\right]} & X_{min} < X < X_{max} \\ 0, & X \ge X_{max} \end{cases}$$

Equation (5).

Where μ_{max} is the maximum growth rate, μ_{opt} is the growth rate under optimal conditions of temperature, pH and a_w, X_{min}, X_{opt} and X_{max} are the minimum, optimum and maximum growth values respectively.

A.4.2. Calculations of growth potential

The calculations made in Section 3.4 take into account different sources of variability. These sources include the variability of the growth characteristics of the strains (see Table A.4.a), variability related to the specific characteristics of the carcasses (pH) meat-side and to the conditions under which they are chilled and transported (see Table A.4.b).

In order to calculate the growth for a given scenario:

- 10,000 core temperature profiles are generated (Equations 1 and 2),
- The surface temperature profiles are then calculated (Equation 3),
- Growth for both pathogenic flora is calculated on the basis of these temperature profiles (Equations 4 and 5).

Figure A.4 shows the general structure of the model:

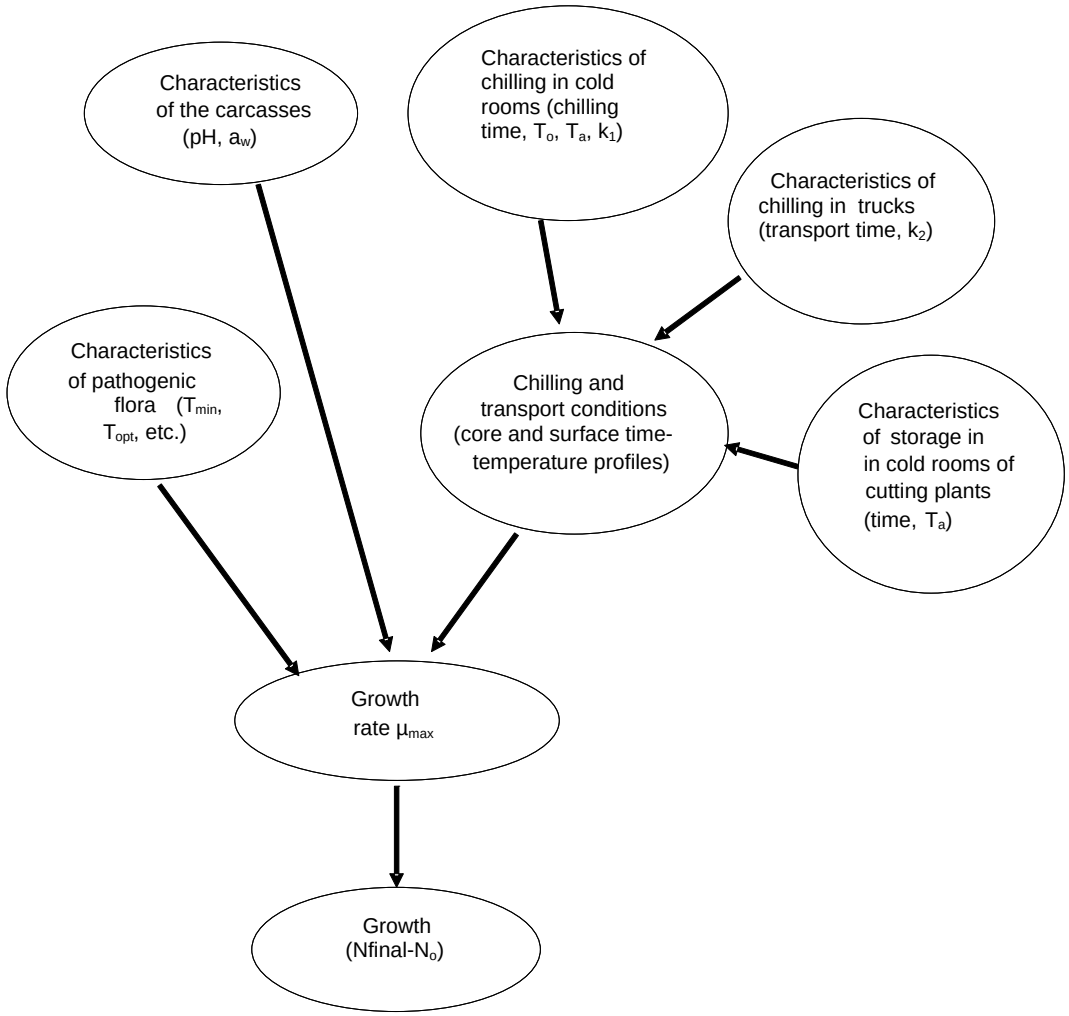

Figure A.4 Simplified representation of the modelling approach

Table A.4.a. List of microbiological parameters used to assess the growth of both pathogens (data taken from the applicant's report)

Microbiological parameters	Unit	Symbol	Type of parameter	*L. monocytogenes*	*Salmonella*
Primary model parameters (Equation 4)	CFU	N_0	F	1	1
	h	*lag*	F	0	0
	$\log_{10}$(CFU)	$\log_{10} N_{max}$	V	N(9.0; 0.1)	N(8.5; 0.13)
Secondary model parameters (Equation 5)	°C	T_{min}	V	N(-1.3; 1.1)	N(4.66; 0.63)
	°C	T_{opt}	V	N(38.2; 0.7)	N(39.56; 0.66)
	°C	T_{max}	V	N(43.3; 1.1)	N(45.88; 0.53)
		pH_{min}	V	N(4.2; 0.1)	N(3.17; 0.30)
		pH_{opt}	F	7	7
		$a_{w\ min}$	V	N(0.922; 0.009)	N(0.941; 0.006)
		$a_{w\ max}$	F	0.997	0.997
		$a_{w\ opt}$	F	1	1
	h^{-1}	μ_{opt}	V	N(1.24; 0.17)	N(1.74; 0.24)

F: Fixed parameter value; V: Variable parameter value; N: Normal distribution

Table A.4.b. List of parameters used to generate the time-temperature profiles and to characterise the carcasses (pH and a_w)

Technological parameters	Unit	*Symbol*	Type of parameter	Values
Carcass characteristics (meat side only)	-	pH	V	N(6.3; 0.5)
	-	a_w	F	0.995
Chilling to the core in chilling rooms (Equation 1)	h	d	V	U(0; 1.5)
	h^{-1}	k_1	V	N(0.192; 0.022)
	°C	T_a	V	N(4.5; 1.3)
	°C	T_0	V	N(41.5; 0.6)
Chilling to the core in trucks (Equation 2)	h^{-1}	k_2	V	N(0.145; 0.059)
	°C	T_a	V	N(4.5; 1.3)
Relationship between core and surface T° (Equation 3)	°C	E_{max}	F	33.2
	°C	Tc_{Emin}	F	2
	°C	Tc_{Emax}	F	40
	°C	σ	V	N(0; 2)
Length of stay in the chilling room	h	d_{cf}	V	8; 10; 15 or 24 according to alternative or baseline scenarios
Transport time in refrigerated trucks	h	$d_{transport}$	V	U(3; 18)
Storage time in cutting plants	h	$d_{cutting\ plant}$	V	0 (for cases A and B) $72-d_{cf}-d_{transport}$ (for case C)

F: Fixed parameter value; V: variable parameter value.
N: Normal distribution; U: Uniform distribution