Use of risk assessment and predictive microbiology in Regulatory Science related to the Scientific Opinions of the EFSA BIOHAZ Panel

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Abstract

EFSA's Panel on Biological Hazards (BIOHAZ Panel) deals with questions on biological hazards relating to food safety and food-borne diseases. This covers food-borne zoonoses, transmissible spongiform encephalopathies, antimicrobial resistance, food microbiology, food hygiene, animal-by products, and associated waste management issues. The scientific assessments are diverse and frequently the development of new methodological approaches is required to deal with a mandate. Among the many risk factors, product characteristics (pH, water activity etc.), time and temperature of processing and storage along the food supply chain are highly relevant for assessing the biological risks. Therefore, predictive microbiology becomes an essential element of the assessments. Uncertainty analysis is incorporated in all BIOHAZ scientific assessments, to meet the general requirement for transparency. Assessments should clearly and unambiguously state what sources of uncertainty have been identified and their impact on the conclusions of the assessment. Four recent BIOHAZ Scientific Opinions are presented to illustrate the use of predictive modelling and quantitative microbial risk assessment principles in regulatory science. The Scientific Opinion on the guidance on date marking and related food information, gives a general overview on the use of predictive microbiology for shelf-life assessment. The Scientific Opinion on the efficacy and safety of high-pressure processing of food provides an example of inactivation modelling and compliance with performance criteria. The Scientific Opinion on the use of the so-called 'superchilling' technique for the transport of fresh fishery products illustrates the combination of heat transfer and microbial growth modelling. Finally, the Scientific Opinion on the delayed post-mortem inspection in ungulates, shows how variability and uncertainty, were quantitatively embedded in assessing the probability of *Salmonella* detection on carcasses, via stochastic modelling and expert knowledge elicitation.

Keywords:

quantitative microbial risk assessment (QMRA), mathematical modelling, variability, uncertainty, expert knowledge elicitation

1. Introduction

In the European Union (EU) food legislation has to be based on "risk analysis" following Regulation (EC) No 178/2002 (General Food Law), which establishes the general principles governing food and feed safety. The risk analysis framework, as initially defined by FAO, WHO and the Codex Alimentarius Commission (CAC, 1999), consists of three components: risk assessment, risk management and risk communication. An updated guidance document on Microbiological Risk Assessment has been published in 2021. It incorporates new developments in the principles and methods for risk assessment of microbiological hazards (FAO and WHO, 2021).

At EU level, the European Food Safety Authority (EFSA), also established by the General Food Law and operating since 2002, is the body responsible for risk assessment and, more in general, for providing independent scientific advice to the EU risk managers in relation to legislation and policies in all fields which have a direct or indirect impact on food and feed safety in the EU. EFSA's founding regulation, the General Food Law Regulation (EC, 2002), introduced the functional separation of risk assessment and risk management in the risk analysis framework, with each being responsible for the communication of aspects that fall within their respective remits. Article 29 of the General Food Law Regulation states that EFSA shall issue Scientific Opinions in response to questions posed by the risk managers, including the European Commission (EC), European Parliament, and EU Member States

(MSs), as well as on its own initiative. The intention of the legislator was that scientific advice should be independent from undue influence by policy makers. Policy makers include the legislative and the executive branches of government, i.e., the European Commission, the European Parliament, and the executive and legislative branches of the EU Member States.

Food safety issues do not respect national borders. That's why scientific cooperation is central to EFSA's scientific work. EFSA works closely with partners and stakeholders across Europe and worldwide, sharing scientific expertise, data and knowledge. EFSA works in close collaboration with other EU agencies, such as the European Centre for Disease Prevention and Control (ECDC), the European Medicines Agency (EMA), the European Chemicals Agency (ECHA) and the European Environment Agency (EEA), sharing information used in risk assessments and producing joint Scientific Opinions and Reports with some of them in certain fields, such as on food-borne outbreak investigations and on antimicrobial resistance. EU Member State scientific support is also critical for the normal functioning of the EU food safety system. Over 300 universities, institutes, governmental, public and other scientific bodies currently form a network of Member State organisations active in fields within EFSA's mission. These so called 'competent organisations' in Member States carry out various tasks in support of EFSA's work. EFSA has also regular contacts with risk managers and decision-makers in the EU food safety system, and engagement with stakeholders is key to EFSA's work and reflects its commitment to openness, transparency, and dialogue. EFSA's stakeholders are representative organisations that have an interest in the Authority's work or in the wider food and feed sector. In addition, EFSA has developed close working contacts with international organisations and food agencies in different parts of the world, such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (WOAH).

The remit of the EFSA Scientific Panel on Biological Hazards (BIOHAZ Panel) is to provide independent scientific advice on biological hazards in relation to food safety and food-borne diseases, covering food-borne zoonoses (animal diseases transmissible to humans through food), transmissible spongiform encephalopathies, antimicrobial resistance, food microbiology, food hygiene, animal by-products and associated waste management issues[1] (Hugas et al., 2007; Latronico et al. 2017; Messens et al., 2018; Romero-Barrios et al., 2013). Risk assessments (RAs) carried out by the BIOHAZ Panel are usually provided to the risk manager in the form of Scientific Opinions and can use either quantitative or qualitative approaches, depending on the scope and extent of data, as well as resources and time available. The topics of the scientific assessments are diverse and frequently new approaches must be established to deal with a mandate. Romero-Barrios et al. (2013) summarised the first two full farm-to-fork quantitative microbiological RA (QMRA) for the whole EU that the BIOHAZ Panel used in their assessments, which concerned *Salmonella* in slaughter and breeder pigs (EFSA BIOHAZ Panel, 2010), and *Campylobacter* in broiler meat (EFSA BIOHAZ Panel, 2011). Latronico et al. (2017) reviewed the RAs delivered by the BIOHAZ Panel on food safety during 2012-2016 and identified future challenges and prospects, while the review by Messens et al. (2017) gave an overview of the EFSA BIOHAZ Panel activities published in the 2014-2016 period that used predictive microbiology. It highlighted the importance of predictive microbiology in risk assessment and in risk-based food safety management. Predictive microbiology models for exposure assessment, together with cross-contamination (transfer), mixing, partitioning and removal, are discussed in Costa et al. (2020), while more in general quantitative methods for microbial risk assessment in foods,

[1] www.efsa.europa.eu/en/panels/biohaz

including data resources and the modelling processes are summarised in Messens et al. (2000).

Uncertainty analysis is the process of identifying limitations in scientific knowledge and evaluating their implications for scientific conclusions, expressing the anticipated validity of the assessment. It is important in EFSA's scientific assessments, as it ensures that the assessment conclusions of the assessments provide complete and reliable information for decision making. To support its application, EFSA published a guidance document and supporting opinion on methods for uncertainty analysis, which explains how to identify appropriate options for uncertainty analysis in different assessments and how to apply them (EFSA Scientific Committee, 2018a, 2018b). EFSA also provided a guidance document on how to communicate on uncertainty, that is structured according to EFSA's three broadly defined categories of target audience: 'entry', 'informed' and 'technical' levels. Assessors should use the guidance for the technical level (EFSA, 2019). Methods described in these guidance documents are now applied in Scientific Opinions of the BIOHAZ Panel and allow to express the overall uncertainty of the conclusions based on modelling and expert judgements.

This paper aims to illustrate how predictive modelling, QMRA principles and uncertainty analysis are applied within the BIOHAZ Scientific Opinions. Four recent examples are used for that purpose. The Scientific Opinion on the guidance on date marking and related food information, part 1 (date marking) (EFSA BIOHAZ Panel, 2020a) gives a general overview of growth modelling for shelf-life purposes in the context of safety. The Scientific Opinion on the efficacy and safety of high-pressure processing of food (EFSA BIOHAZ Panel, 2022) provides an example of inactivation modelling, global fitting, and compliance with

149 performance criteria. The Scientific Opinion on the use of the so-called 'superchilling'
150 technique for the transport of fresh fishery products (EFSA BIOHAZ Panel, 2021) is used to
151 illustrate the use of heat transfer modelling. The Scientific Opinion on the public and animal
152 health risks, in case of a delayed post-mortem inspection (DPMI) in ungulates (EFSA
153 BIOHAZ Panel, 2020b) shows how uncertainty was quantitatively embedded in the
154 assessment. This advice informs European laws, rules and policymaking – and so helps
155 protect consumers from risks in the food chain. The last section (conclusions) contains the
156 follow-up actions that the risk manager (European Commission) has taken after the
157 publication of the Scientific Opinions.
158
159 **2. Guidance on date marking and related food information, part 1 (date marking)**
160
161 Food waste prevention is a priority set out in the EU Action Plan for the Circular Economy
162 adopted by the EC in December 2015. As part of that Action Plan, the Commission has been
163 called upon to examine ways to improve the use of date marking by actors in the food chain
164 and its understanding by consumers. 'Date marking' is used as an umbrella term to refer both
165 to the 'best before' and 'use by' dates. The development of EU guidance based on the
166 existing EU requirements to ensure more consistent date marking and related food
167 information practices was considered an immediate priority. The EC asked EFSA to deliver a
168 Scientific Opinion describing the development of a risk-based approach that would have to be
169 followed by food business operators when deciding on the type of date marking (i.e., 'use by'
170 date versus 'best before' date), the setting of shelf-life and the related food information that
171 should be provided on the labelling to ensure food safety. This work gives a general overview
172 of growth modelling for shelf-life purposes in the context of safety.
173

The Scientific Opinion provides scientific advice on the factors that make certain foods highly perishable, so they may constitute an immediate danger to human health after a short period, and on how those factors should be considered by food business operators when deciding whether a 'use by' date is required and when setting the shelf-life and required storage conditions.

The raw materials, the processing environment and the manufacturing steps determine the type and the levels of microorganisms in the food product when released to the market. The intrinsic (especially pH and water activity (a_w)), extrinsic (especially temperature and atmosphere) and implicit factors (such as interactions with competing background microorganisms) of the food product determine which microorganisms can grow, and their growth potential during subsequent storage until consumption.

The decision on the type of date marking (i.e., whether a 'use by' date is required or a 'best before' date is appropriate) needs to be taken on a product-by-product basis, considering the product characteristics (abovementioned factors), and the processing and storage conditions. A decision tree consisting of a sequential list of 10 questions was developed (Fig. 1), and supported with examples, to assist food business operators in deciding the type of date marking for a certain food product. In the case of food products processed in a way that eliminates pathogenic microorganisms and avoids recontamination, or which does not support their growth, the risk to consumer health would not increase during shelf-life, and a 'best before' date is appropriate. In contrast, if there is no pathogen elimination step, or there is the possibility of recontamination after such a treatment, and the food product supports the growth of the contaminating pathogen, the risk to the consumer is expected to increase during shelf-life and a 'use by' date is required. Overall, it is considered that the decision tree will

result in appropriate and consistent outcomes on the type of date marking within the interpretation of regulations and the assumptions made in its development, e.g., using growth or no growth as basis for decisions.

The Scientific Opinion also provides advice on the factors that make certain foods become unfit for human consumption without constituting an immediate danger to human health, and food business operators´ considerations related to setting of shelf-life and storage conditions.

Several factors determine the shelf-life of a food product. Those factors related to food quality include organoleptic changes due to physical (e.g., water loss or gain), chemical, biochemical/enzymatic and microbiological phenomena, while those factors related to food safety include growth of pathogens and/or toxin production. Referring to these as 'sensory shelf-life' (here only changes due to microbial growth of spoilage causing microorganisms) and 'safe shelf-life' (based upon potential growth or toxin production of pathogens) (NACMCF, 2005), respectively, the attributed shelf-life time (to be indicated in the labelling) should never be longer than the shortest of these. If the safe shelf-life is longer than the sensory shelf-life, then the sensory shelf-life should determine the length of the shelf-life for a 'use by' product, and vice versa (see Fig. 2). Which of these situations is relevant, depends on several factors, such as the types and initial levels of spoilage and pathogenic microorganisms, and may vary, depending on the intrinsic and extrinsic factors of the food. For instance, some factors may have inhibitory effects on the growth of spoilage microorganisms but be less effective in inhibiting the growth of the relevant pathogenic microorganisms.

223 Important in the setting of shelf-life is the interpretation of the term “reasonably foreseeable
224 conditions of distribution, storage and use in the post-processing stages of the food product”,
225 used in Regulation (EC) No 2073/2005 (EC, 2005). Potential factors to consider in the
226 determination of ‘reasonably foreseeable conditions’ for the determination of shelf-life
227 include consumer behaviour (intended use of food), storage temperatures at distribution,
228 storage, and retail level as well as storage temperature at consumer level.
229
230 A case-by-case procedure to determine and validate the shelf-life of a food product should be
231 applied as depicted in Fig. 3. Key steps are: (i) to identify the relevant pathogenic/spoilage
232 microorganisms and estimate their initial levels, (ii) to characterise the intrinsic, extrinsic and
233 implicit factors of the food product affecting the growth behaviour of the pathogenic/spoilage
234 microorganism and, (iii) to assess the growth behaviour of the pathogenic/spoilage
235 microorganism in the food product (based on literature, predictive models, challenge tests or
236 durability studies) during storage, from retail to consumption, to determine the time at which
237 the pathogenic/spoilage microorganism will reach maximum acceptable levels under the
238 appropriate reasonably foreseeable conditions.
239
240 Many EU Members States have written regional and national guidance documents on food
241 donations because the nature of and the way in which donated foods are collected, stored, and
242 distributed by food business operators and charity organisations may be different. Most of
243 these documents include tables with a list of food categories or specific food products that are
244 eligible to be donated. Marketing of food past the ‘best before’ date is allowed in several
245 countries under the responsibility of the seller provided that the food is fit for human
246 consumption. In such regional or national guidance documents, indicative time limits are
247 either not provided, or are indicated without providing their scientific basis. Due to the

variability, among MS, between types of food products, and consumer habits, in the EFSA Scientific Opinion it was not considered appropriate to present indicative time limits for food donated or marketed past the best before date. Further details can be found in EFSA BIOHAZ Panel (2020a).

3. The efficacy and safety of high-pressure processing of food

When raw milk, colostrum, dairy or colostrum-based products undergo heat treatment, such treatment must comply with the requirements listed in Regulation (EC) No 853/2004 (EC, 2004). These products need to be pasteurised achieving certain requirements (i.e., at least at 72°C for 15 s or at least at 63°C for 30 min or any other combination of temperature-time (T/t) conditions to obtain an equivalent effect) or be subjected to ultra-high temperature (UHT) treatment.

Triggered by an increasing demand to allow HPP as an alternative treatment because it is expected to keep the properties closer to those of raw milk and colostrum, the European Commission requested to assess the efficacy of HPP when applied to raw milk (and raw colostrum from ruminants and specifically to recommend minimum requirements as regards time and pressure of the HPP, and other factors if relevant, for the control of *Mycobacterium*, *Brucella*, *L. monocytogenes*, *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC), to achieve an equivalent efficacy to that of thermal pasteurisation. The end point in the assessment was the raw milk/colostrum for direct human consumption. This work provides an example of inactivation modelling, global fitting, and compliance with performance criteria.

Based on a previous Scientific Opinion on the public health risks related to the consumption of raw drinking milk (EFSA BIOHAZ Panel, 2015) and using food-borne outbreak (FBO) data, it was concluded that the relevant hazards to be reduced by thermal pasteurisation of raw milk/colostrum from ruminants are *Mycobacterium bovis*, *Brucella melitensis*, *L. monocytogenes*, *Salmonella* spp., STEC, *Campylobacter* spp., tick-borne encephalitis virus (TBEV) and *Staphylococcus aureus*.

First, the $\log_{10}$ reduction of those relevant hazards following thermal pasteurisation of raw milk and raw colostrum from ruminants was assessed. Thermal pasteurisation of milk according to the legal requirements specified above is expected to result in more than 10 $\log_{10}$ reductions of most of the pathogens (i.e., STEC, *L. monocytogenes*, *Salmonella* spp., *S. aureus* and *Campylobacter* spp.), while lower reductions are expected for *Brucella* spp. and *M. bovis* (using *Mycobacterium avium* subsp. Paratuberculosis (MAP) as surrogate) and even lower for TBEV for which there is a significant lack of data.

Next, the minimum HPP requirements were derived when treating raw milk/colostrum from ruminants to achieve an equivalent efficacy (in terms of $\log_{10}$ reduction) to that of thermal pasteurisation to control the relevant pathogens. Based on literature review, $\log_{10}$ reduction data of pathogens were retrieved (ideally the decimal reduction time at a given target pressure, D_p-value) upon treatment of raw milk or previously heat-treated milk using HPP. A total of 35 relevant studies were available for the evaluation of the HPP impact on the selected pathogens, in different human or ruminant milk types, including raw, pasteurised, or UHT milk, whole or reduced fat milk and colostrum. No records were found for the HPP impact on *B. melitensis* and TBEV and thus, no relevant conclusions could be drawn for these hazards.

298

299 A global modelling approach, with a log-linear (single inactivation phase) or a biphasic
300 primary inactivation model (Cerf, 1977), both encompassing a Bigelow secondary model
301 term for the impact of pressure on microbial inactivation, was successful in describing the
302 combined effect of pressure (MPa) and holding time (min) on the inactivation of six hazards
303 in various types of milk and colostrum, excluding UHT milk.

304

305 An example of the combined effect of pressure (MPa) and holding time (min) on the
306 inactivation (expressed as $\log_{10}$ reduction) of *S. aureus* in raw milk and colostrum is shown in
307 Fig. 4. Note that, for modelling purposes, the $\log_{10}$ reductions referring only to the holding
308 time were chosen for describing the reduction of pathogens as a function of pressure and
309 time, as the reductions in the compression stage could not (reliably) be attributed to a specific
310 pressure level.

311

312 The dependence of D_p-values on pressure as estimated from the global fitting of log-linear
313 (single-phase inactivation) or biphasic primary models is illustrated in Fig. 5. *S. aureus* was
314 the most resistant pathogen, followed closely by *M. bovis* (using MAP as surrogate), STEC
315 (using the entire body of evidence for any *E. coli* strain) and *L. monocytogenes*, at pressures
316 commonly applied by the industry (> 450 MPa). *Salmonella* was more sensitive to these
317 pressures, while *C. jejuni* was the most pressure sensitive pathogen.

318

319 HPP cannot achieve equivalent $\log_{10}$ reductions to those achieved by thermal pasteurisation
320 of milk according to these legal requirements, but equivalent conditions (at processing
321 temperatures < 45°C) can be identified for lower $\log_{10}$ reductions (performance criteria; PC)

322 as recommended for thermal pasteurisation by international agencies (i.e., 5-8 $\log_{10}$
323 reductions as summarised in Table 1).
324
325 Contour plots illustrate the equivalent HPP conditions (P/t combinations) that the global
326 model predicted (Fig. 6). However, there is uncertainty associated with these model
327 calculations due to the representativeness and sufficiency of the data (i.e., number of records,
328 impact of strain variability and physiological state of cells) used to fit the model and the
329 structure of the model used to describe the data. Therefore, assuming an arbitrary 1 log
330 margin of error, a conservative approach was applied in which the model was used to
331 estimate the P/t combinations needed to achieve 1 $\log_{10}$ reduction higher than each target PC.
332
333 Informed by the modelling results and all additionally collected scientific evidence, an expert
334 judgement was performed to conclude on the certainty that different PCs were achieved. For
335 STEC, *L. monocytogenes*, *Salmonella* spp., *S. aureus* and *Campylobacter* spp., it is judged
336 99–100% certain (almost certain) that the PC of 8 $\log_{10}$ reduction is achieved using thermal
337 pasteurisation of raw milk and by HPP treatment of raw milk/colostrum by using defined P/t
338 combinations. For example, by using 600 MPa – 8 min, 550 MPa – 10 min and 500 MPa – 15
339 min for *S. aureus*, the most HPP resistant of these biological hazards (see Fig. 6). For *M.*
340 *bovis*, it is judged 95–99% certain (extremely likely) that the PC of 5 $\log_{10}$ reduction is met
341 using thermal pasteurisation of milk. This 5 $\log_{10}$ reduction can be achieved with 99–100%
342 certainty (almost certain) by HPP treatment of raw milk/colostrum using, e.g., 600 MPa – 2.5
343 min, 550 MPa – 4.5 min and 500 MPa – 7.5 min.
344
345 The most stringent HPP condition currently used industrially (600 MPa for 6 min), based on
346 the information collected, would achieve the PC (i.e., 5 logs for *M. bovis* and 8 logs for *S.*

aureus, STEC, *L. monocytogenes*, *Salmonella* spp. and *Campylobacter* spp.), except for *S. aureus,* where this HPP condition would achieve 6 $\log_{10}$ reductions.

4. The use of the so-called 'superchilling' technique for the transport of fresh fishery products

This assessment focused on domestic trade and import into the EU/EEA regarding the transport and storage of unpackaged, wrapped, prepared fresh fishery products (FFP) from the first on-land establishment onwards, using the authorised practice 'in boxes with ice' (reference condition referred to as conventional FFP or 'CFFP'), in comparison with FFP that, after being superchilled in the first on-land establishment, are transported in 'boxes without ice' (alternative condition referred to as 'SFFP'). There is no commonly agreed definition of superchilling in the literature (Bantle et al., 2016). The definition of superchilling considered in the assessment was the process *'entailing lowering the fish temperatures between the initial freezing point of the fish (always below the temperature of melting ice, i.e., < 0°C) to about 1–2°C lower'*. For the Scientific Opinion, both CFFP and SFFP were considered until they are marketed or processed. The boxes used to store/transport CFFP and SFFP are generally made of expanded polystyrene (EPS), with other types of containers being outside the scope of the mandate. Fish were assumed to be kept in the boxes for a maximum duration of 5 days.

The requestor clarified that the purpose is to know if the SFFP stored/transported in boxes without ice is at least as safe (from a microbiological food safety perspective) as CFFP stored/transported in boxes with ice. In this context, it was agreed to follow a stepwise approach. Firstly, focusing the assessment on the reasonably foreseeable fish temperature

372 during the storage/transport of SFFP in boxes without ice compared to CFFP in boxes with
373 ice. This was translated into a first question: *"Which SFFP configurations (i.e., initial degree*
374 *of superchilling) ensure that the fish temperature, at any time of the storage/transport, is*
375 *lower or equal to CFFP when exposed to the same conditions of on-land storage and/or*
376 *transport?"*. Secondly, and only if higher temperatures can occur in SFFP compared to
377 CFFP, to assess the impact on the growth of biological hazards. The second question reads as
378 *"If the SFFP conditions allow fish temperature to be higher than in CFFP during the on-land*
379 *storage and/or transport, what is the potential increase of relevant biological hazards for on-*
380 *land SFFP compared to CFFP upon exposure to the same conditions of storage and/or*
381 *transport?"*
382
383 This work illustrates the use of heat transfer modelling in an EFSA assessment. A heat
384 transfer model was developed to identify under which initial configurations the fish
385 temperature of SFFP, at any time, is lower or equal to CFPP. This approach is feasible since
386 the boxes (EPS boxes) and the storage/ transport conditions were the same for SFFP and
387 CFFP. The capacity of CFFP and SFFP to maintain the temperature depends on their capacity
388 to absorb heat, as determined by their initial configurations: (a) for CFFP: the initial fish and
389 ice temperature and the ice:fish proportion in the box; and (b) for SFFP: the degree of
390 superchilling, i.e., the ice fraction in the fish matrix, which depends on the fish temperature
391 after superchilling and the initial freezing point of the fish. Fig. 7 represents the temperature
392 dynamics of the fresh fishery products when stored/transported as CFFP in comparison with
393 SFFP. Two cases of lean and fat sea fish species were used (cod and salmon) as well as a
394 species from temperate freshwater (Nile perch).
395

396 The questions that were assessed using heat transfer balance models included (i) the initial
397 configurations for both CFFP and SFFP that are equally capable of absorbing the same
398 amount of heat before all ice melts, i.e. the ice fraction in SFFP and the ice:fish proportion in
399 the box of CFFP and (ii) the ratio between the quantity of heat that predefined initial
400 conditions for SFFP and CFFP can absorb, before all ice melts. The first was used as an
401 illustrative example.
402
403 The model allows to derive the proportion of ice in CFFP to equal the absorbing heat capacity
404 of SFFP before all ice melts. The predictions for three different types of fish covering a high
405 fat content fish (salmon), a lean fish (cod) and a temperate freshwater fish (Nile perch) are
406 shown in Fig. 8. For example, salmon that is superchilled to –1.04°C (and contains an ice
407 fraction of 0.1704) can absorb the same amount of heat as conventionally chilled salmon
408 placed in a box with 0.18 kg ice per kg of fish. When the salmon is superchilled to lower
409 temperatures, it will contain a higher ice fraction and be equivalent to a conventionally
410 chilled salmon placed in a box with more ice per kg of fish. For cod and Nile perch, the
411 results are practically the same as for salmon: when these are placed in a box with 0.18 kg ice
412 per kg of fish, it would correspond to an ice fraction of 0.1733 and 0.1715, respectively.
413 Their ice fraction would however correspond to a higher initial temperature of the
414 superchilled cod (i.e. –0.85°C) and in particular for Nile perch (i.e. –0.34°C), compared to
415 superchilled salmon, due to the higher initial freezing point of these two lean species.
416
417 Based on the developed model, an MS Excel spreadsheet tool was built named the HTM-
418 SFFP Tool (heat transfer model tool for the heat absorption capacity of superchilled fresh
419 fishery products). It was made available through the Knowledge Junction under
420 https://doi.org/10.5281/zenodo.4304283 and can be used as part of the 'safety-by-design'

421 approach. It allows the food business operator to identify the initial configurations under
422 which SFFP have an equivalent or higher capacity to absorb heat than CFFP. In case the food
423 business operator wants to use an initial configuration with a lower degree of superchilling
424 than that provided by the tool, there would be a need to document the safety e.g. by
425 performing an experimental study for the specific supply chain and/or proving that the
426 temperature of SFFP at arrival to the EU establishment is ≤ 0°C. Provided that the initial
427 configuration of SFFP is properly adjusted to the envisaged outside conditions, the
428 temperature conditions of SFFP are equal or usually less favourable for microbial growth
429 compared to CFFP and thus the increase of relevant biological hazards (due to growth of
430 vegetative pathogens and/or histamine accumulation) will be equal or usually lower in SFFP
431 compared to CFFP.
432
433 **5. The public and animal health risks, in case of a delayed post-mortem inspection in**
434 **ungulates**
435
436 EFSA was asked to deliver a Scientific Opinion on the evaluation of the public and animal
437 health risks in case of a delayed post-mortem inspection (DPMI) of ungulates in any
438 slaughterhouse or game-handling establishment. The assessment focused on the reduction in
439 sensitivity of detecting diseases or health conditions after 24-h and 72-h delay of post-
440 mortem inspection (PMI) as compared to PMI immediately after slaughter. This review only
441 covers the assessment of the impact of DPMI on the sensitivity of *Salmonella* detection as
442 process hygiene criterion (PHC) for domestic ungulates. It is a case study, demonstrating how
443 uncertainty in the inputs of a stochastic model, that are elicited from literature data, predictive
444 models, or expert knowledge elicitation (EKE), affects the output of the assessment and how
445 this can be (quantitatively) expressed.

446

447 The sampling for testing compliance with the PHC is done after dressing of carcasses, but
448 before chilling (pre-chilling). For this assessment, the moment of sampling could be changed
449 to the moment of DPMI. The potential change in *Salmonella* concentration, and its impact on
450 the probability of detection, need to be assessed, assuming that carcasses are stored under
451 chill conditions immediately after slaughtering, with 7°C as the maximum permissible
452 temperature of carcass surface. The effect of DPMI on the probability of laboratory detection
453 of *Salmonella* (=sensitivity of detection hereinafter) was assessed per sample, without
454 assessing the performance of the regulated sampling plan.
455

456 The sensitivity in *Salmonella* detection (i.e., the probability of finding *Salmonella* when it is
457 present on a swabbed area of a carcass) is subject to limitations related to the number and
458 physiology of cells, the efficacy of sampling method (herein '*swabbing*') and the
459 performance of enrichment step of the detection method. Even though theoretically, a single
460 cell of *Salmonella* is expected to result in a positive swab sample, this may not always
461 happen, leading to false negative results. As such, it is conceivable that more than 1 cell
462 needs to be present in the sampled carcass area to ensure that the sample will be found
463 positive. In the current assessment, the minimum *Salmonella* levels required on a swabbed
464 carcass area for a positive sample is termed $Salm_{Min}$. Based on the above, the post-chilling
465 probability of detecting *Salmonella* on carcasses is defined as the probability of having at
466 least $Salm_{Min}$ cells in the sampled area. This probability depends on (Table 2):
467 (i) the levels of the organism on carcasses at the time of sampling (*Log No*);
468 (ii) the viability and culturability of *Salmonella* (*Phys*) as affected by the cold shock
469 caused by chilling and possible reduction of carcasses surface water activity (a_w);

(iii) the ability of the sampling method (swabbing; *SpEff*) to detach *Salmonella* from carcasses surfaces, depending on the attachment strength of *Salmonella* on the carcass and the distribution of *Salmonella* over the carcass surface. The momentary habitats of *Salmonella* on carcass during chilled storage depend on the surface characteristics of carcass, such as crevices and niches that are either naturally present due to the innate surface roughness of meat, or newly formed by the removal of surface moisture, possibly causing translocation of *Salmonella* cells;

(iv) the ability of low and possibly injured *Salmonella* cells, detached by swab and placed in the enrichment medium, to outcompete the background meat microbiota and achieve detectable levels by subsequent molecular or cultural methods (termed as *Compt* in the model).

To assess the impact of DPMI on the sensitivity of *Salmonella* detection, a stochastic model was applied to estimate the reduction in probability of *Salmonella* detection after a delay of 24- and 72-h (Fig. 9). The type and parameters of probability distributions describing the variability and uncertainty of each model variable are illustrated in Table 2. The log reduction (*SR*) of culturable *Salmonella* by the combined effect of dehydration and chilling was predicted by the non-thermal inactivation model of Pin et al. (2011), for constant values of a_w sampled from the relevant uncertainty distributions (Table 2) and a constant temperature of 7°C. The predicted log reductions were used as inputs to the overall probabilistic model, that collectively considers the uncertainty in the contribution of each of the above factors on the levels and recovery of *Salmonella* that may give a positive swab result, after 24- or 72-h of chilled storage. The model was run with Monte Carlo simulation for 10,000 iterations with @Risk Software.

Based on the structure of the model (Fig. 9) and the uncertainty of model inputs, the additive effect of the aforementioned factors on pre-chill population of *Salmonella* (*Log No*) is either negligible or causes a reduction of *Salmonella* partly below the $Salm_{Min}$ (*Log Nt*), depending on the combination of sampled values for each variable from its uncertainty distribution, and the variability of *Log No*. As a result, for each time-point of the assessment (24- and 72-h of chilled storage), two distributions of *Salmonella* population are compared (Fig. 10): (i) the initial distribution of *Salmonella* load on carcasses and (ii) the post-chill distribution of *Salmonella* on the same carcasses. The part of post-chill distribution that is not covered by the pre-chill distribution in their overlay (Fig. 10), is compared to the uncertainty distribution of $Salm_{Min}$ (Fig. 9; Table 2) for estimating the percentage of samples carrying *Salmonella* that will be found positive P_{after} or not (1-P_{after}; false negative). The same comparison is made for the pre-chill distribution, because the assumed variability in *Log No* may also lead to a percentage of contaminated pre-chilling samples assessed false negative (1-P_{before}).

The difference between the two probabilities P_{before} and P_{after} divided by P_{before} represents the percentage of reduction in the sensitivity of *Salmonella* detection for each DPMI time point. Because of the stochastic form of the uncertainty of model inputs, the final output is also expressed as a probability distribution describing the uncertainty about the % reduction in sensitivity of detection. The median estimate after 24 h of chilling is 66.5% and after 72 h increases to 94%, suggesting that prolonged chill storage intensifies the stress conditions that reduce the recovery of sufficient number of *Salmonella* cells required for a positive sample. The (cumulative) probability distributions of Table 3 represent the uncertainty about the reduction in sensitivity. The probabilities for each pre-defined reduction percentage can be interpreted as the probability of observing reduction from 0 up to this percentage.

520 The sensitivity analysis showed that the variance of the uncertainty distribution of the model
521 output is mainly associated with the uncertainty about the average initial *Salmonella*
522 contamination (explaining 59.3% of the variance) and the uncertainty in $Salm_{Min}$ (explaining
523 12.5% of the variance). Based on that, the % reduction in sensitivity of detection was
524 simulated assuming two hypothetical (non-variable) mean levels of *Salmonella* pre-chilling,
525 one high (1 log/100 cm^2) and one 10-fold lower (Fig. 11). The lower the pre-chill
526 contamination level of *Salmonella* on carcass, the higher the estimated reduction in the
527 sensitivity of detection. Decreasing the pre-chilling levels of *Salmonella* causes a shift of the
528 median of distributions representing the post-chilling levels of surviving, non-irreversibly
529 injured, detachable, and competent cells of *Salmonella* entering the enrichment, to values
530 lower than the $Salm_{Min}$, thereby reducing the probability of post-chilling detection.
531
532 **6. Conclusions**
533 The abovementioned Scientific Opinions have been taken into consideration by European
534 risk managers (EC), in consultation with their national counterparts in EU Member States,
535 while deciding on possible related risk mitigation measures, as outlined here below.
536
537 Based, amongst other, on the Scientific Opinion on the guidance on date marking and related
538 food information, the EC made a proposal to revise EU rules on the information provided to
539 consumers as part of the EU's 'farm-to-fork' strategy. It aims to ensure better labelling
540 information to help consumers make healthier and more sustainable food choices and tackle
541 food waste, by proposing to: (i) introduce standardised mandatory front-of-pack nutrition
542 labelling; (ii) extend mandatory origin or provenance information for certain products; and
543 (iii) revise the rules on date marking ('use by' and 'best before' dates). This proposal was

open for public consultation from 13 December 2021 until 7 March 2022 (EP, 2023) and the EC is expected to announce in near future possible related legislative actions.

As the HPP Scientific Opinion clearly indicates that there was no significant food safety concern by HPP treatment, there was no need for a direct regulatory action. As regards efficacy, the opinion concluded that HPP cannot be considered as an alternative to thermal pasteurisation of milk/colostrum and therefore was not introduced as an alternative treatment option in legislation (no regulatory action). The opinion provides input to the food industry on how food safety can be further approved by HPP compared to non-treated food and used as an additional food safety tool within its procedures based on the Hazard analysis and critical control points (HACCP) principles.

Considering the Scientific Opinion on the use of the so-called 'superchilling' technique, the EC revised the EU legislation (EC, 2022) to allow the use of superchilling for transporting fresh fishery products. The transport in boxes without ice shall be permitted under the condition that those boxes clearly indicate that they contain superchilled fishery products. During transport, superchilled fishery products must respect temperature requirements included in a range between – 0.5 and – 2 °C temperature in the core of the product. The transport and storage of superchilled fishery products must not exceed 5 days.

Currently, *Salmonella* control is largely based on sampling by food business operators immediately after slaughter. The Scientific Opinion on the public and animal health risks related to a DPMI in ungulates illustrated that a 72h-delay reduces the sensitivity in the detection of certain major animal diseases (results not discussed in this review), therefore no

legislative changes are expected to allow a 72h-delay of post-mortem inspection after slaughter or arrival in a game-handling establishment.

Highlights

- The use of predictive microbiology and QMRA in regulatory science is presented
- A decision-tree made available for deciding the type of date marking for food products
- HPP treatment of milk/colostrum is an additional tool to improve food safety
- A 'safety-by-design' approach allows setting the superchilling conditions for transport and storage of fresh fishery products
- Detection sensitivity of certain major animal diseases reduced through delayed post-mortem meat inspection for ungulates

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Disclaimer

The authors Winy Messens, Michaela Hempen and Pietro Stella are employed with the European Food Safety Authority (EFSA). More specifically, they are employed in the Unit on Biological Hazards & Animal Health and Welfare (BIOHAW Unit) and provide administrative and scientific support to the Panel on Biological Hazards. However, the present article is published under the sole responsibility of the authors and may not be considered as an EFSA scientific output. The positions and opinions presented in this article are those of the authors alone and do not necessarily represent the views/any official position or scientific works of EFSA. To know about the views or scientific outputs of EFSA, please consult its website www.efsa.europa.eu.

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765 **Tables**

766

767 **Table 1.** Overview of the performance criteria (PC) proposed by international agencies as
768 reference values for thermal pasteurisation of milk.

Agency	Reference	PC for thermal pasteurisation	Log_{10} reduction considered in the assessment
Codex Alimentarius Commission	CAC (2004)	As *Coxiella burnettii* is the most heat-resistant non-sporulating pathogen likely to be present in milk, pasteurisation is designed to achieve at least a 5 log_{10} reduction of *Coxiella burnettii* in whole milk (4% milk fat)	5, 6, 7, 8
Food Safety Authority of Ireland	FSAI (2020)	Minimum 6 log_{10} reduction in the number of vegetative cells of *Listeria monocytogenes* because it is currently regarded as the most heat-resistant foodborne pathogen that does not form spores	
New Zealand Government	MPI (2021)	5 log_{10} reduction of *Campylobacter* spp., *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*; and 6 log_{10} reduction for *Mycobacterium avium* subsp. paratuberculosis (used as surrogate for *Mycobacterium bovis*) (domestic market) > 7 log_{10} reduction for these hazards to achieve an equivalent outcome to thermal pasteurisation (export product should meet at least this standard)	
European Food Safety Authority	EFSA BIOHAZ	Usually 6 log_{10} reduction (according to a reported range from 4 to 8 log_{10} reduction[(a)]) of relevant vegetative	

	Panel (2020a)	pathogen depending on the type of commodity/raw materials used

769 (a): not necessarily related to milk.

770

771 **Table 2.** Probability distributions of the stochastic model input variables for assessing the
772 reduction in sensitivity of *Salmonella* detection[(a)].

Model variable	Element addressed	Probability distribution[(b)]	Justification or source of input values
Average (μ_0) pre-chilling contamination level of *Salmonella* (*Log No*), immediately after slaughtering	Uncertainty of the mean value of the variability distribution (*Log No*) according to the distribution form elicited by EKE	BetaGeneral (1.74,7.97, 0, 2.845) elicited by EKE	μ_0 elicited by EKE
Standard deviation of *Log No* (σ), independent of μ_0	Uncertainty of the standard deviation of the variability distribution of *Log No,* according to the distribution form elicited by EKE	BetaPert (0.05, 0.15, 0.3)	Input values discussed and agreed in the WG[(c)]
Log No	Variability	Normal (μ_0, σ)	Input values discussed and agreed in the WG
$Salm_{Min}$: minimum number of *Salmonella* cells required on carcass for a positive sample	Uncertainty: defined at the beginning and assumed not to be affected by chill storage	Normal (4.67, 1.59) elicited by EKE	Input values elicited by EKE
Water activity (a_w) of carcass surface after 24 or 72 h of chilled (7°C) storage: a_w is used as input variable in the non-thermal inactivation model of	Uncertainty	(1) 24 h post-chilling BetaPert (0.94, 0.95, 0.97) (2) 72 h post-chilling	Input values discussed and agreed in the WG

Salmonella for estimating the reduction of viable and culturable population on carcass		BetaPert (0.93, 0.95, 0.97)	
Phys: Proportion (0-100%) of surviving cells (*Log No-SR*) that are irreversibly injured	Uncertainty	(1) 24 h post-chilling Beta (1.30, 0.80) (2) 72 h post-chilling Beta (2.105, 1.319)	Input values elicited by EKE
SpEff: Proportion (0-100%) of surviving, non-irreversibly injured cells [(*No-10^SR*)×(1-*phys*)], that are detached from carcass by swabbing	Uncertainty	(1) 24 h post-chilling BetaPert (60, 90, 100)[(d)] (2) 72 h post-chilling BetaPert (50, 80, 95)	Input values discussed and agreed in the WG
Compt: Proportion (0-100%) of *SpEff* that is outcompeted by indigenous meat microbiota	Uncertainty	(1) 24 h post-chilling BetaPert (1, 3, 10) (2) 72 h post-chilling BetaPert (1, 6, 15)	Input values discussed and agreed in the WG

773 EKE: expert knowledge elicitation; WG: Working Group.

774 (a): Annotations are as in the Fig. 9. BetaPert values in parentheses represent minimum, most likely
775 and maximum values of the distribution.

776 (b): Values expressed as 0-100 % are converted to 0-1 for use in the model.

777 (c): Consensus by the WG members, without applying an EKE procedure.

778 (d): Corresponding to reduction in sponge efficacy by 40, 10 and 0% due to post-chilling storage.

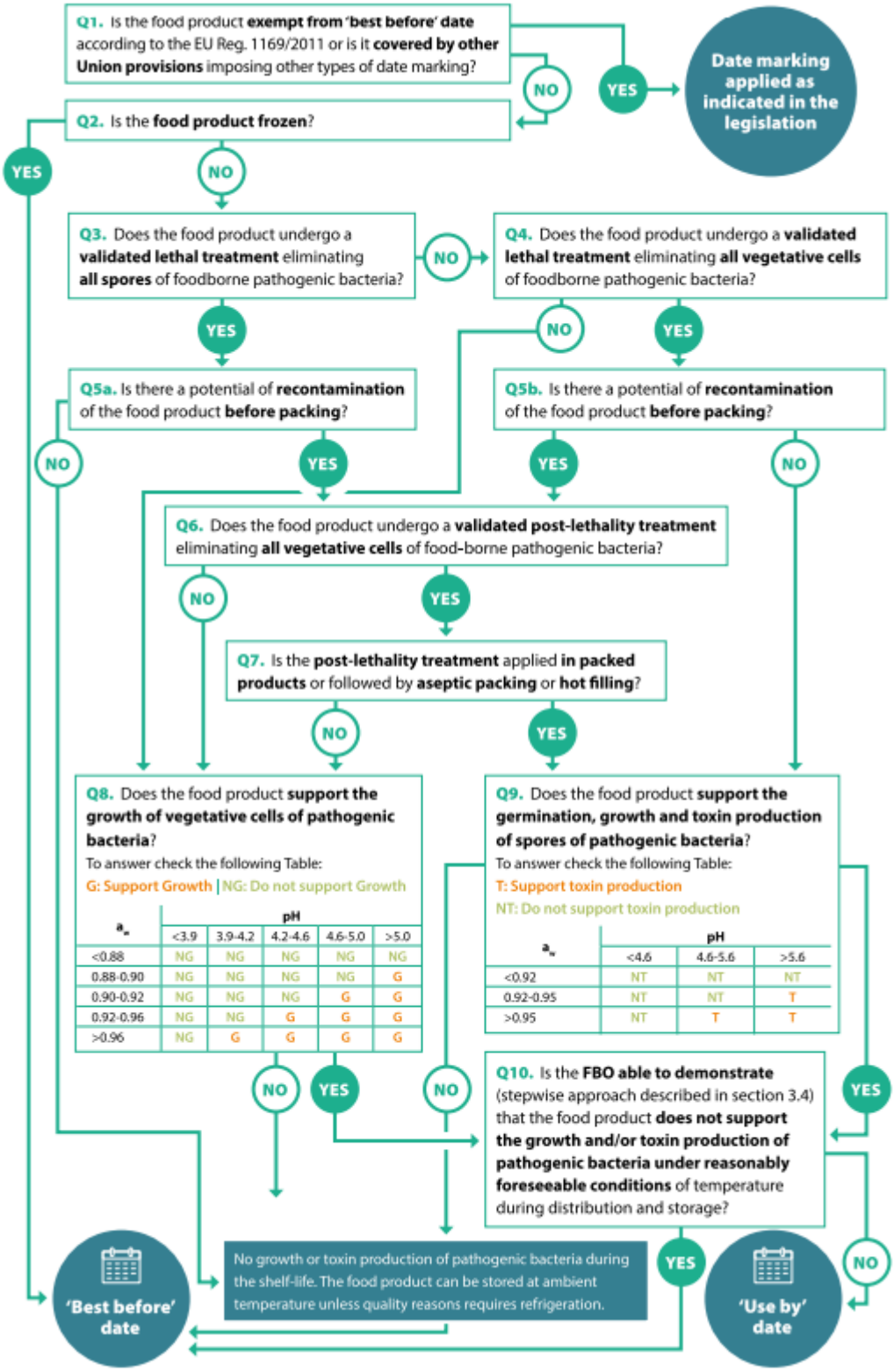
779

780 **Table 3.** Cumulative probabilities of reduction in sensitivity of *Salmonella* detection after 24-
781 and 72-h of chilled storage.

Percentage of reduction (%)	After 24 h		After 72 h	
	Cumulative probability	Probability of greater reduction	Cumulative probability	Probability of greater reduction
10	0.15	0.85	0.09	0.91
20	0.2	0.8	0.12	0.88
30	0.25	0.75	0.14	0.86
40	0.31	0.69	0.17	0.83
50	0.37	0.63	0.20	0.8
60	0.44	0.56	0.23	0.77
70	0.53	0.47	0.27	0.73
80	0.63	0.37	0.33	0.67
90	0.75	0.25	0.43	0.57

782

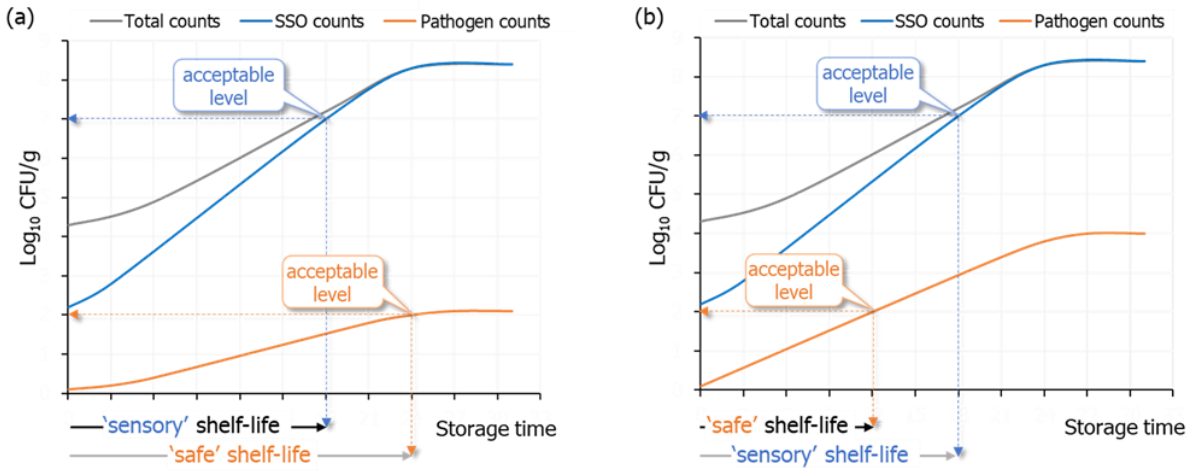
Figures



a_w	pH <3.9	3.9-4.2	4.2-4.6	4.6-5.0	>5.0
<0.88	NG	NG	NG	NG	NG
0.88-0.90	NG	NG	NG	NG	G
0.90-0.92	NG	NG	NG	G	G
0.92-0.96	NG	NG	G	G	G
>0.96	NG	G	G	G	G

a_w	pH <4.6	4.6-5.6	>5.6
<0.92	NT	NT	NT
0.92-0.95	NT	NT	T
>0.95	NT	T	T

Fig. 1. Decision tree on the appropriate date marking for temperature controlled prepacked foods © European Food Safety Authority.



787

788 **Fig. 2.** Food characteristics and storage conditions support the growth of both pathogenic
789 (hazard) and specific spoilage organisms (SSO) during storage. The use by date is determined
790 by the shortest shelf life considering the 'sensory' shelf life based on microbial growth of
791 SSO (a), or the 'safe' shelf life based on the potential growth of pathogens (b). © European
792 Food Safety Authority.

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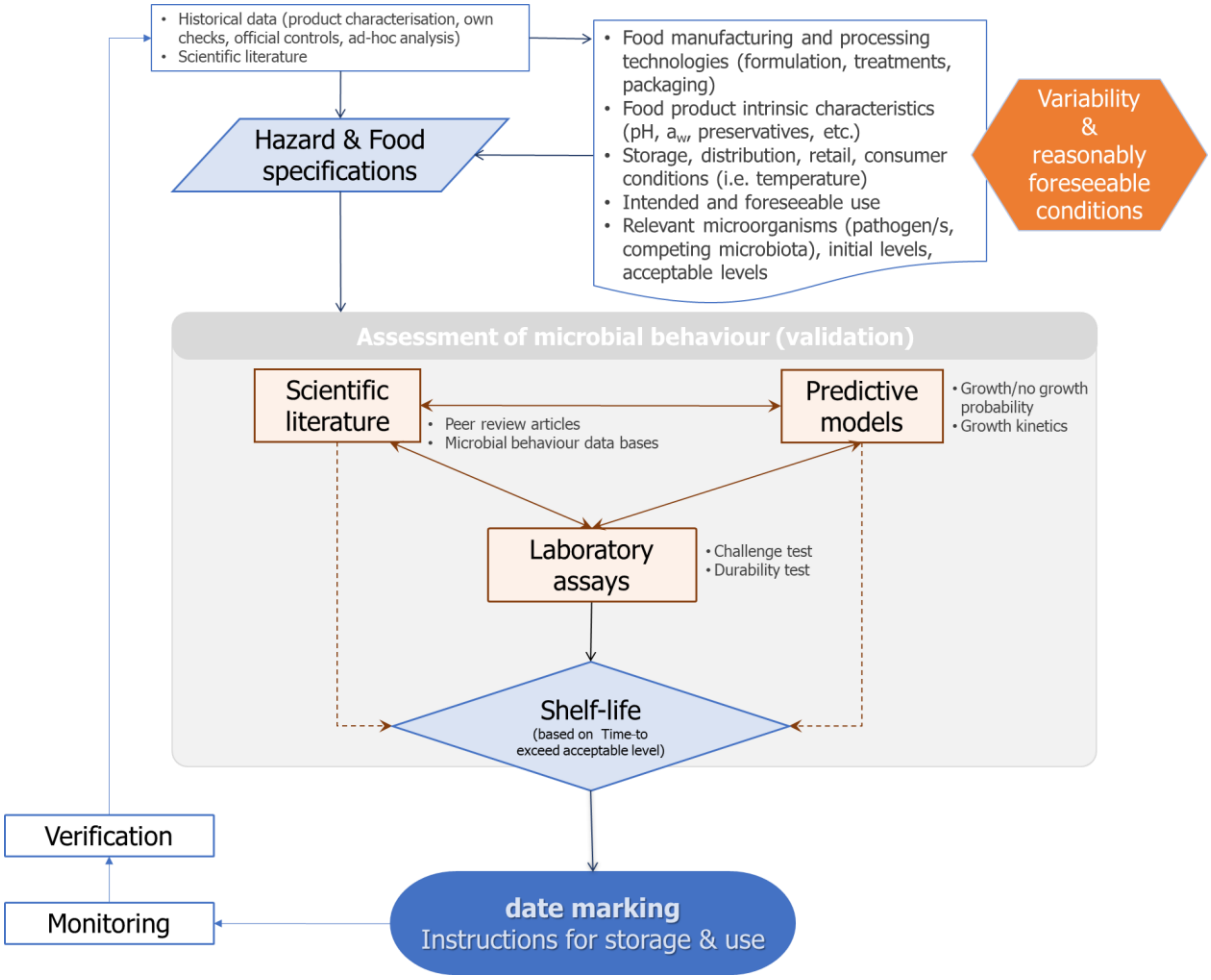

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795 **Fig. 3.** Flowchart summarising the stepwise approach to set shelf-life date © European Food

796 Safety Authority.

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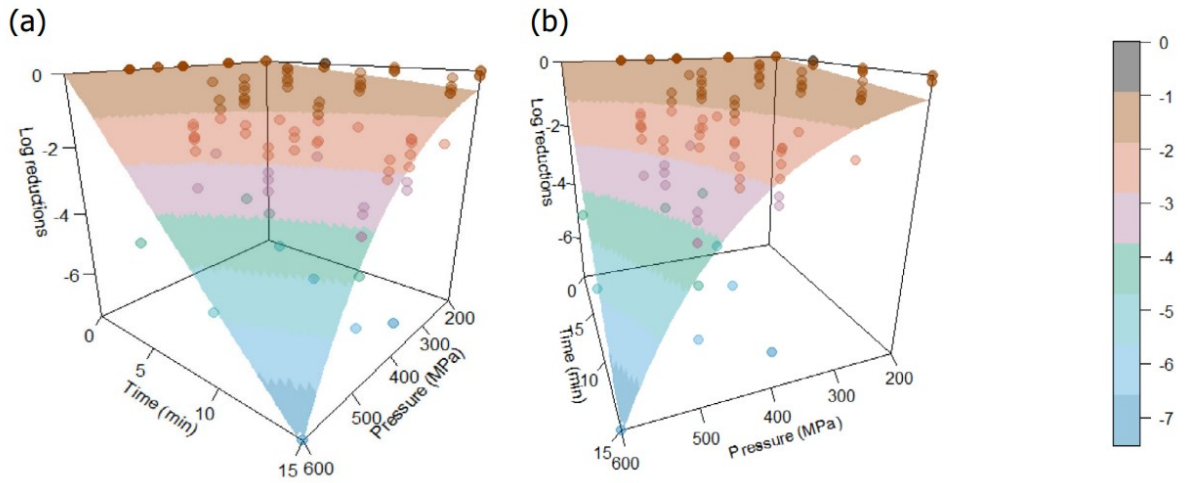
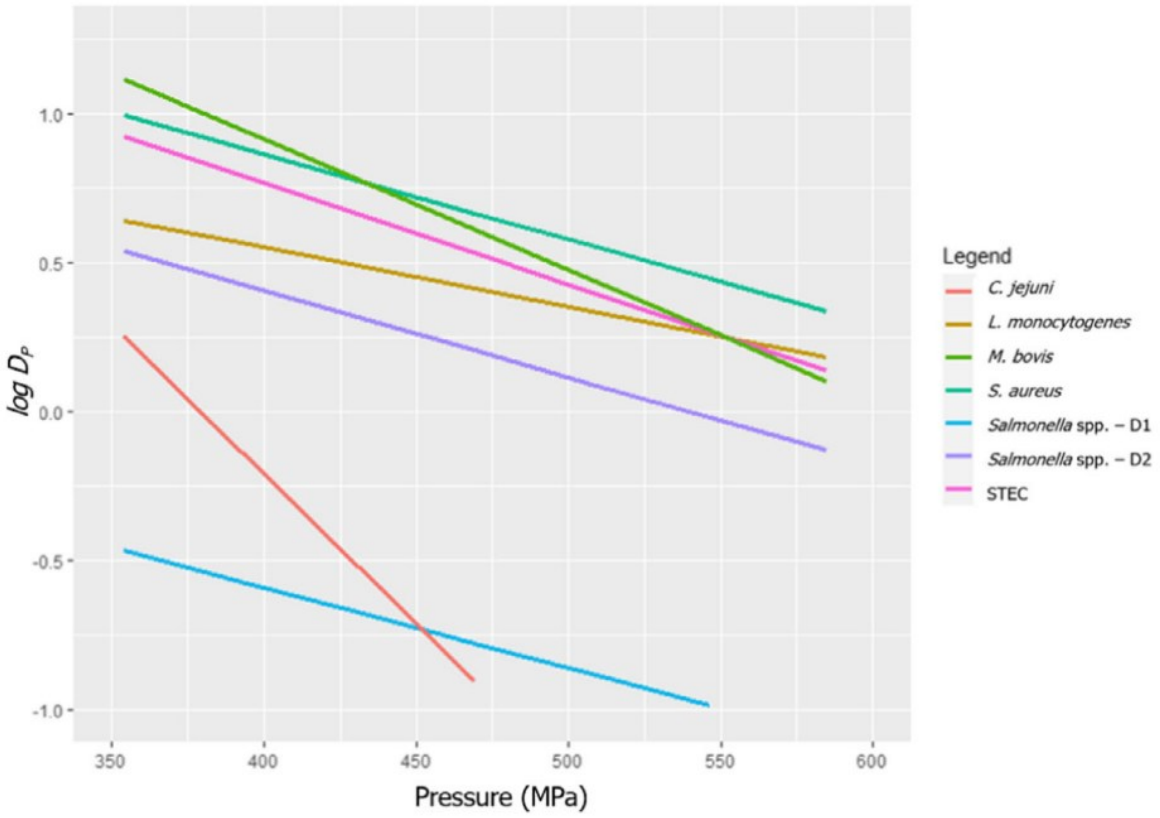


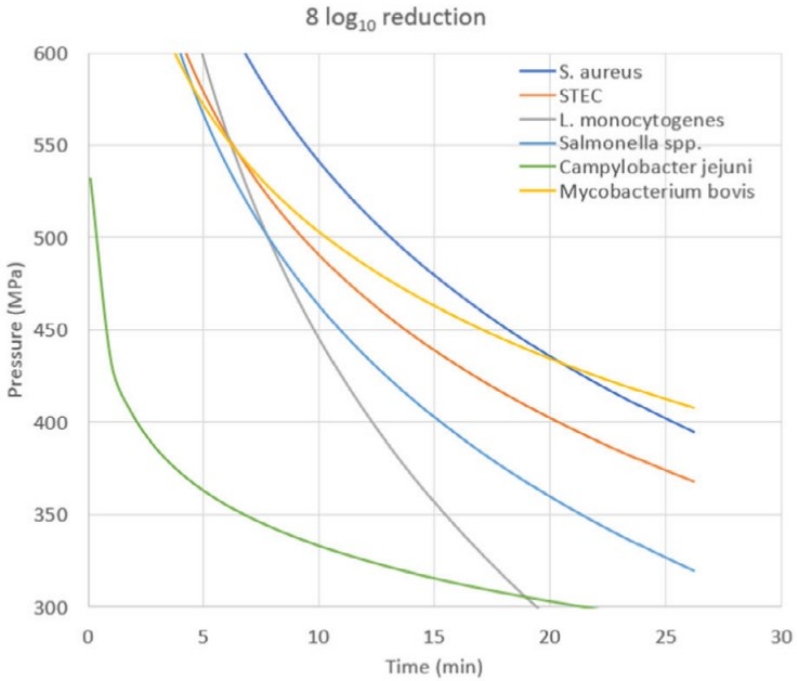
Fig. 4. Observed (points) and predicted (response surface) $\log_{10}$ reductions of *Staphylococcus aureus* in response to pressure (P, MPa) and holding time (min), in various milk types (excluding UHT milk). The two figures (a and b) represent two different angles of the same 3D graph. © European Food Safety Authority.



804

805 **Fig. 5.** Dependence of D_p-values on pressure, based on D_{ref} and z_p-values estimated by global
806 fitting of log-linear (single-phase inactivation) or biphasic primary models. Low log D_p
807 values indicate a higher sensitivity. For *Salmonella* spp., D1 considers the first rapid
808 inactivation phase while D2 considers the second slower death phase. © European Food
809 Safety Authority.

810



811

812 **Fig. 6.** Example of isoreduction curves of HPP conditions (pressure/holding time

813 combinations) needed to achieve a performance criterion of 8 $\log_{10}$ reductions, according to

814 the global model parameters for all six relevant pathogens in milk © European Food Safety

815 Authority.

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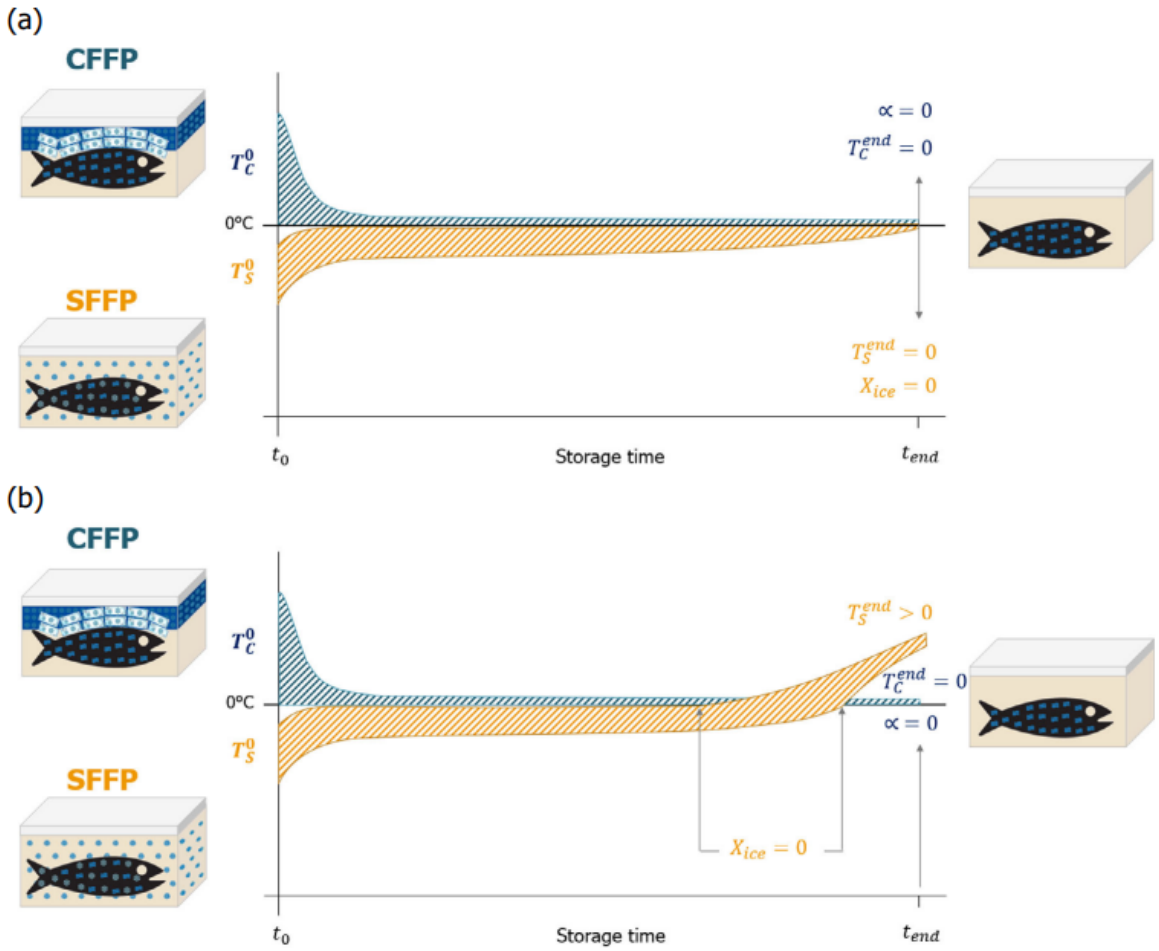
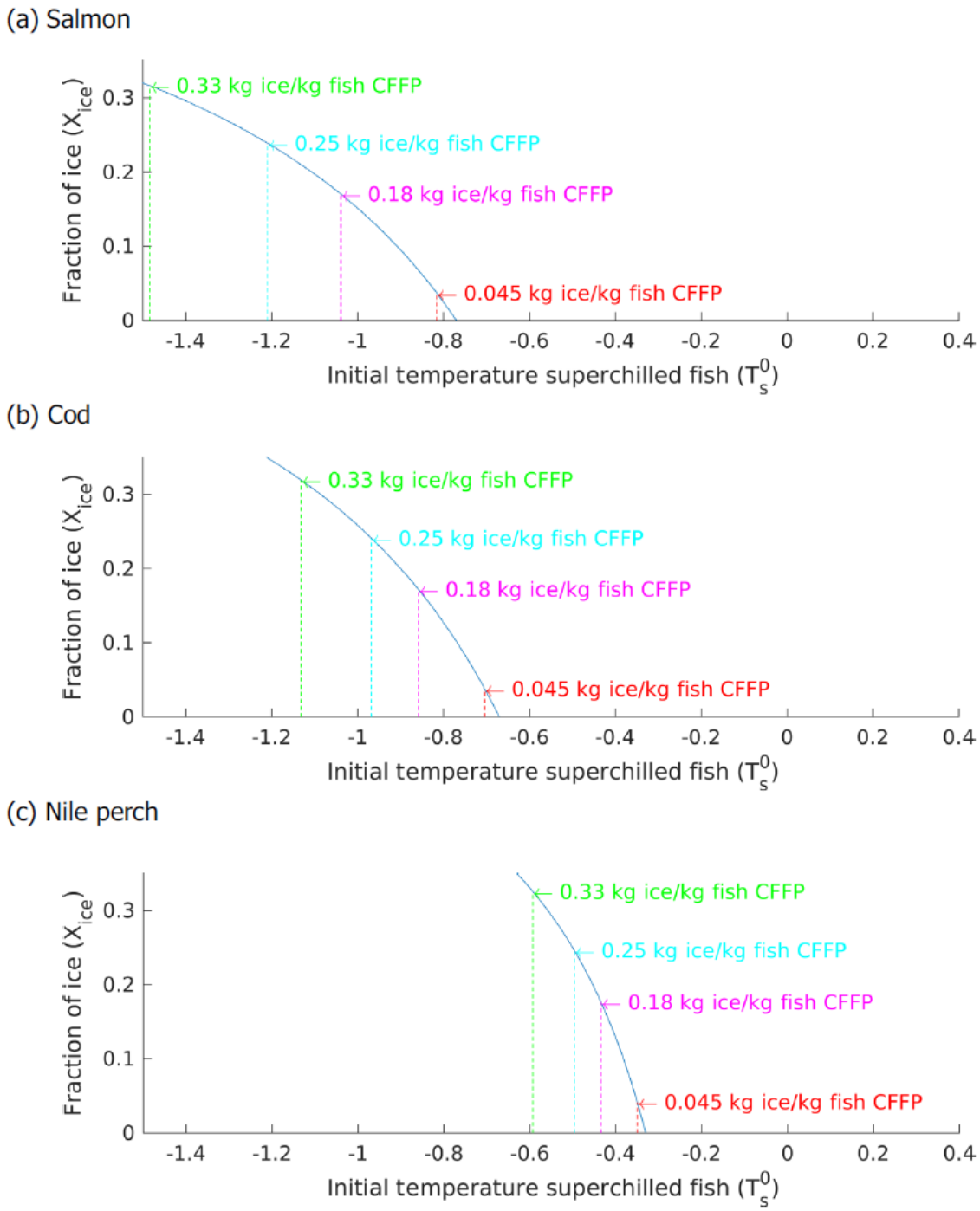


817

818 The initial fish temperature in the fish matrix is denoted by T_0 while the temperatures after
819 storage and transport are denoted by T_{end}. CFFP and SFFP are referred to by addition of C
820 and S as superscripts. The ice fraction in the SFFP is X_{ice}.

821 **Fig. 7.** Conceptual representation of the temperature dynamics of the fresh fishery products
822 when stored/transported as conventional fresh fishery products (CFFP) in comparison with
823 superchilled fresh fishery products (SFFP) to the point in which all ice has melted. It presents
824 the practice ensuring that there is ice in the box of CFFP during the whole period of storage
825 and transport while in (a) the SFFP maintains an ice fraction and in (b) the absorbed heat
826 during storage/transport completely melts the ice inside the SFFP and raises fish temperature
827 above 0°C. © European Food Safety Authority.

828



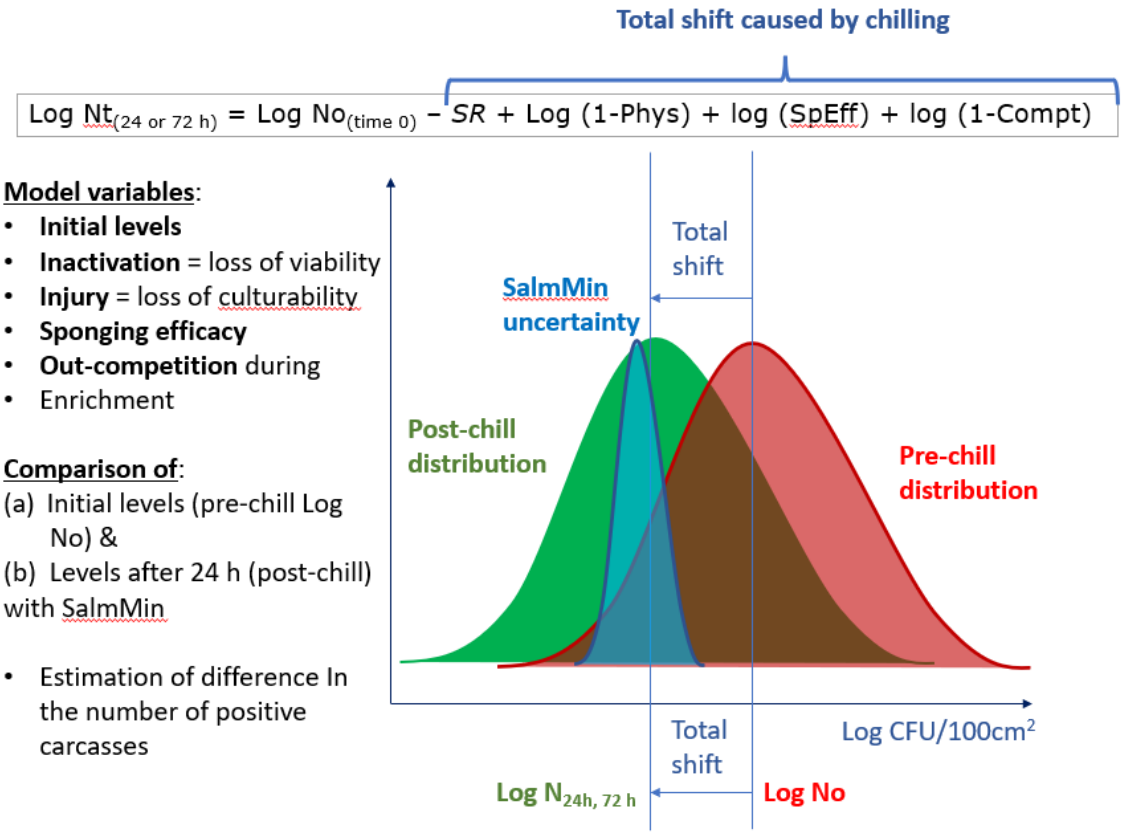
829

830 **Fig. 8.** Proportion of ice in CFFP (α, kg ice/kg fish) needed to equal the absorbing heat

831 capacity of SFFP as a function of the degree of superchilling (i.e., ice fraction or the

832 associated initial temperature) of the SFFP © European Food Safety Authority.

833



834

835 **Fig. 9.** The conceptual stochastic model (assumptions, variables, and mathematical

836 expressions) for assessing the impact of DPMI on the reduction in sensitivity of *Salmonella*

837 detection on carcass. Abbreviations of model variables are consistent with Table 2. ©

838 European Food Safety Authority.

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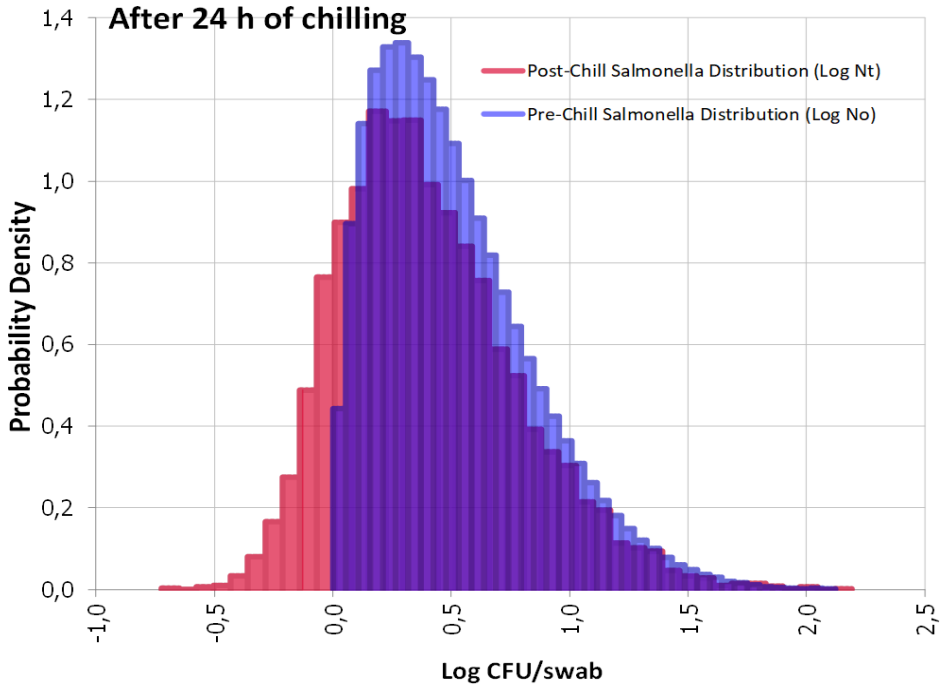

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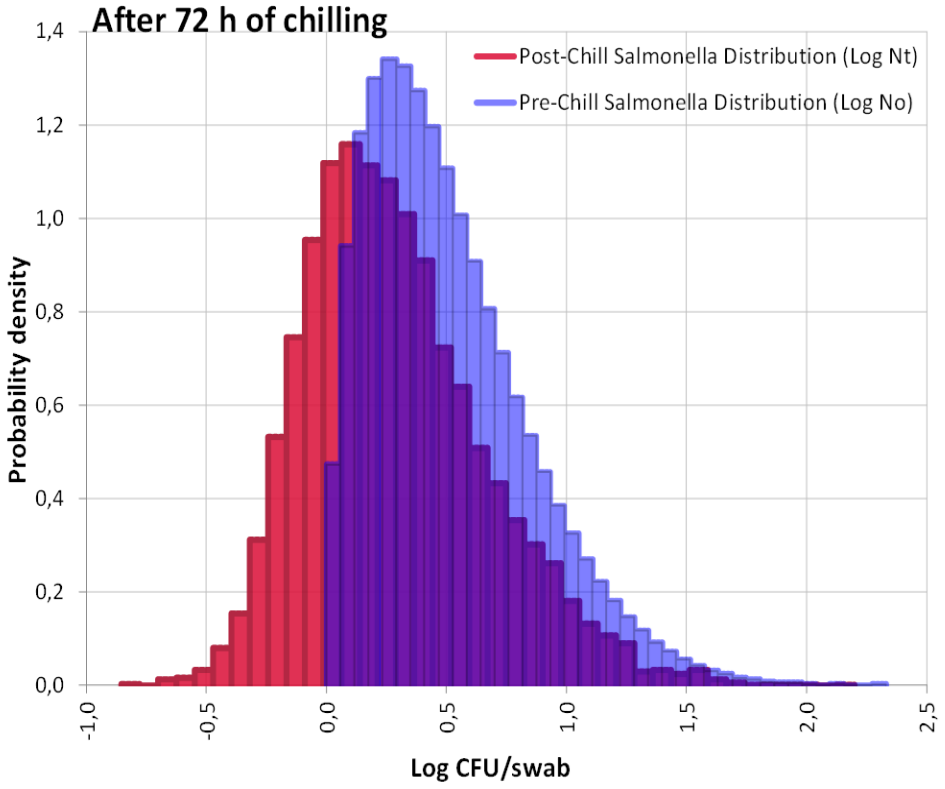
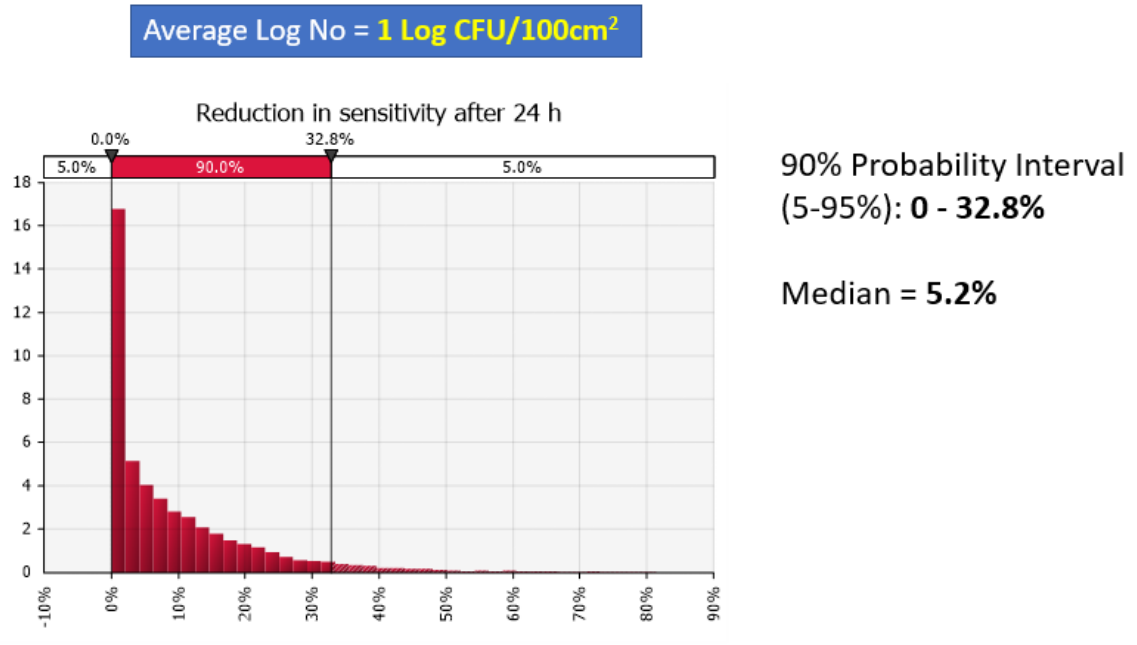


841

842 **Fig. 10.** Overlayed distributions of *Salmonella* variability (given the uncertainty about mean
843 and standard deviation) in log CFU/swab before (blue) and after (red) 24 h and 72 h of
844 chilling. © European Food Safety Authority.

845



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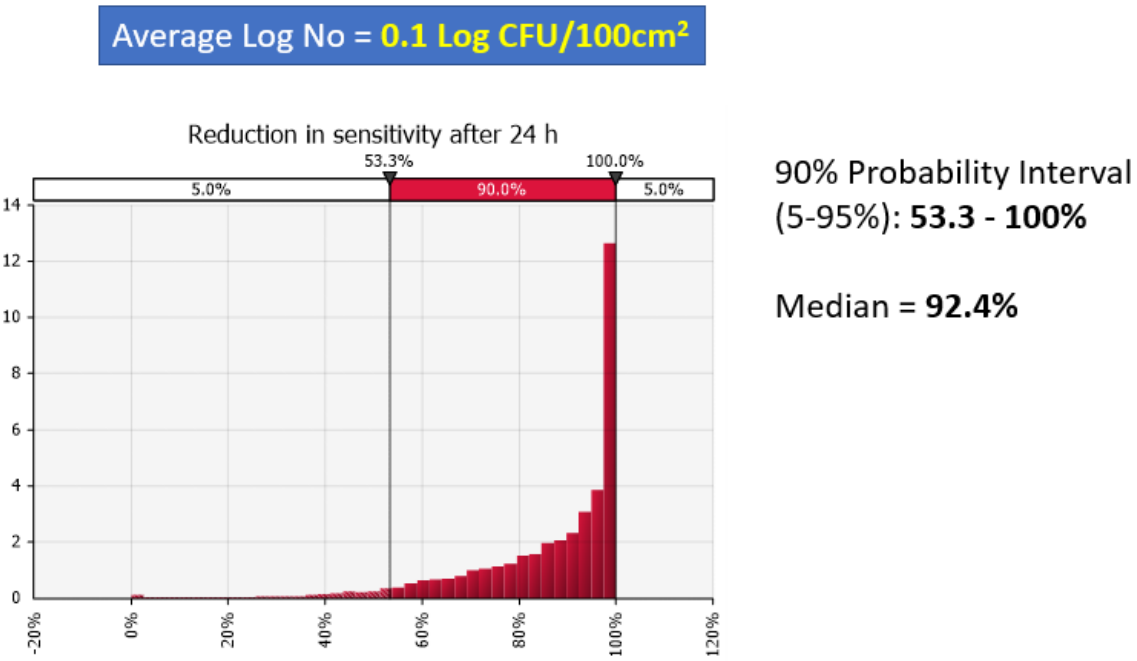

848

849 **Fig. 11.** Stochastic model outputs assuming two specific (non-variable) mean pre-chill levels

850 (Log No) of *Salmonella* on carcass: 1 log CFU/100 cm^2 and a 10-fold lower (0.1 log

851 CFU/100 cm^2). © European Food Safety Authority.