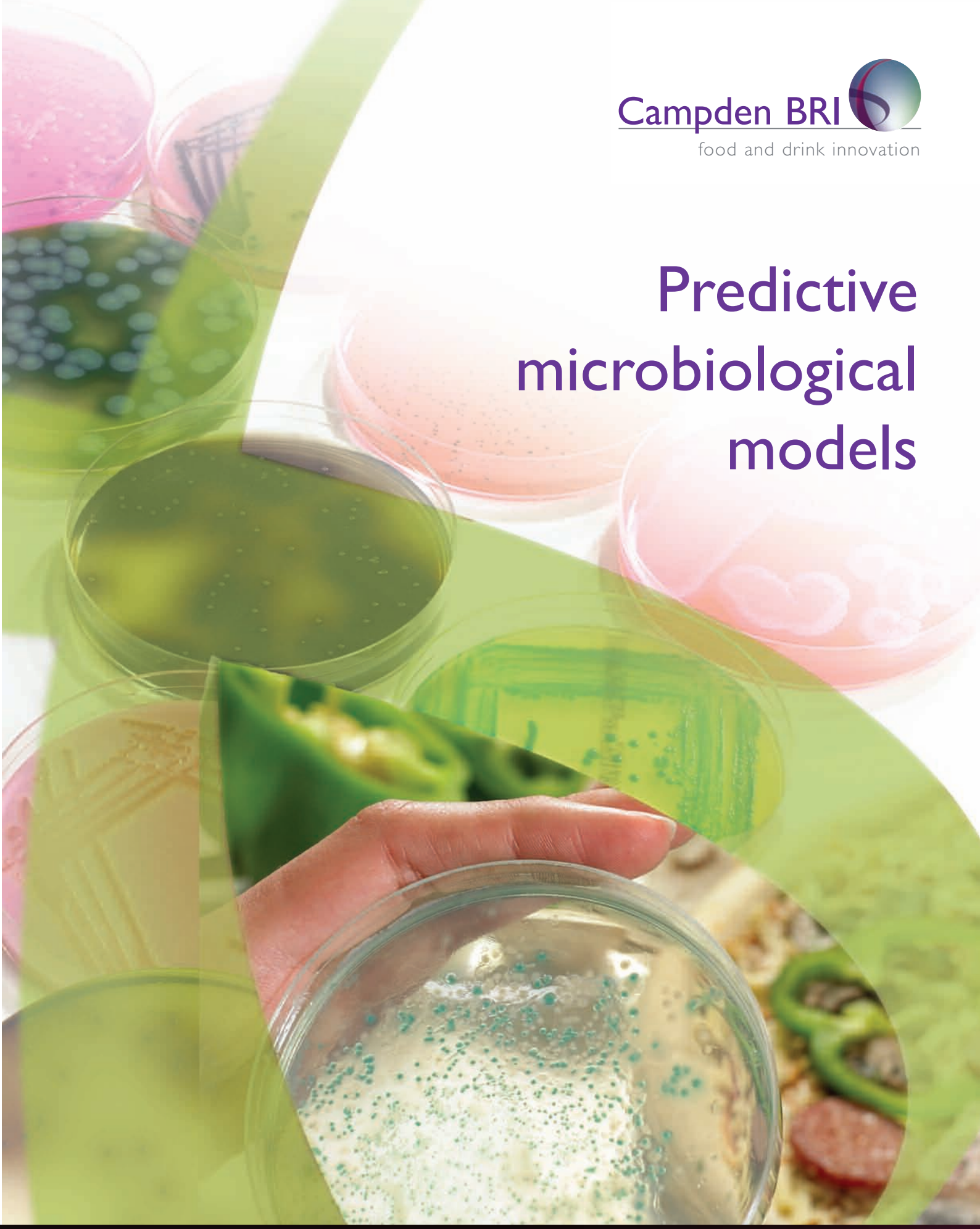
Campden BRI
food and drink innovation

Predictive microbiological models



What are they and how can they be used in the food industry?

PREDICTIVE MICROBIOLOGICAL MODELS:
WHAT ARE THEY AND HOW CAN THEY BE USED IN THE FOOD INDUSTRY?

WHY USE MODELS?

Predictive microbiological models are tools that can be used to assess product shelf-life and safety. Models can also be used:

- In product development
- To identify areas where challenge testing should be undertaken
- As a tool with HACCP and risk assessment plan development

THE SCIENCE

Predictive microbiological models are computer based software packages which allow the user to estimate the rate of microbial growth or get an indication of whether growth of a particular microorganism will occur under a specified set of conditions.

The models are based on laboratory generated data. Microbiological growth media (broths) are produced with different instrinsic parameters such as pH and salt level and are then inoculated with the relevant organism or cocktail of organisms. These broths are then stored at a range of temperatures and the microbial level present is assessed over time. These are known as **kinetic growth models**, as they allow assessment of the amount of growth that can occur.

A different approach is to note time to turbidity rather than assess microbial levels. These are **growth/no growth** or **time to growth models**, as they cannot estimate the level of growth, but simply if growth occurs.

Once the data is generated, statistical equations are fitted and these are then combined with a user-friendly interface.

Predictive models have been developed for both spoilage and pathogenic organisms and there are both growth and survival models available for use. The models will usually include the following variables:

- Temperature of storage, including fluctuating temperatures.
- pH.
- Salt or equivalent water activity.

Some models also take into account levels of preservatives such as nitrite, CO_2 and lactic and acetic acid.

Once parameters have been entered into the system, a prediction is produced. The prediction will usually be in the form of a growth curve (Figure 1), but parameters such as lag time, time to reach a specified microbial level and level at a specified time can also be predicted.

THE APPLICATION

Predictive models are a very quick, efficient and cost effective way of assessing the potential for growth of microorganisms under specific conditions without needing practical studies.

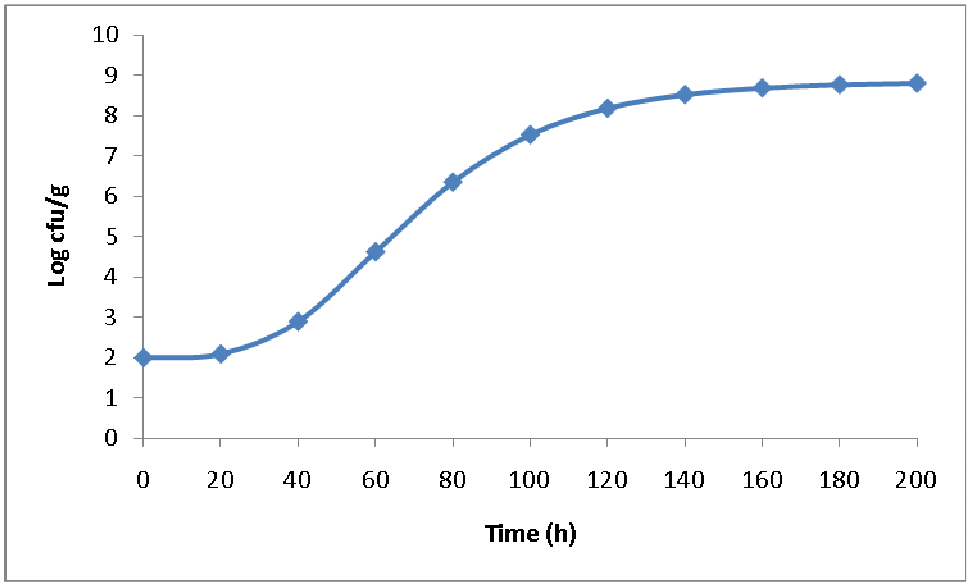
During product development, a product formulation may be changed several times before being finalised. Often there can be a pressure on time and money and therefore it is not possible to practically evaluate the shelf life of all possible formulations. It is important that the potential for growth and/or survival of all relevant organisms is assessed and practical trials are carried out on the final formulation. However, limiting the amount of practical work required will save time and money.

It is not only during product development that the potential for microbial growth is important; other examples include a product being subjected to a slightly elevated temperature or if there has been a problem with formulation of a batch of product.

With regards to spoilage organisms it is usually the time to reach a specified target level that is the most important parameter. However, for pathogenic organisms such as *Salmonella* or *C. botulinum*, it is the time for growth to begin (lag time) that is the most important parameter as any growth at all has to be avoided. For some organisms like *S. aureus* and *B. cereus*, which produce toxins and cause food poisoning when a specified level is reached, predictive systems can be used to predict the time taken to reach these levels. Predictive systems can also be used to predict if *Listeria monocytogenes* could reach a level of 100 cfu/g during a product's shelf life (the limit set in EU hygiene legislation).

Growth of spoilage organisms can be modelled using a Campden BRI developed system known as FORECAST. Also included in the FORECAST system are models covering specific food commodities. These models are based on the specific spoilage organisms for the relevant food commodity. There are currently models for fish, meat, fresh produce and yeasts in fruits and drinks, plus a range of models relevant to acidified foods. The acidified foods models and yeast in fruit and drinks models give predictions of time to growth rather than producing growth curves.

Figure 1
Microbial growth curve



Predictions are carried out by Campden BRI and a report including growth curves and time to reach specified levels is produced and interpreted for the Client.

In order for a prediction to be produced, the client must provide the following information: pH, water activity, % salt (all can be measured at Campden BRI) and temperature (either one static temperature or a fluctuating profile). Campden BRI can then advise as to which organisms or commodity models would be most relevant. For some models such as the acidified food models, level of preservative is also required. The models currently available in the FORECAST system are detailed below in Tables 1 to 4.

Acid tolerant organisms
The models developed for acidified foods are based on 3 groups of organisms. These models have been termed: cold fill spoilage, which includes acid adapted yeasts, moulds and lactic acid bacteria; cold fill pathogens, which includes *E. coli, S. aureus* and *Salmonella*; and hot fill spoilage, which includes sporeformers such as *B. coagulans* and *C. pasteurianum*.

Predictions generated from these models are based on 5 categories: category 1 growth in 1-14 days, category 2 growth in 15-30 days, category 3 growth in 31-60 days, category 4 growth 61-182 days, category 5 no growth in 6 months.

Data was produced and models were generated based on pH, Aw and preservative level as detailed in Table 2. Further cold fill spoilage and hot fill spoilage models were also produced based on amount of salt, sugar, acid and preservative plus acid type (Table 3) and a further cold fill spoilage model was produced based on pH, salt, sugar and preservative amount (Table 4).

Safety and stability of acetic acid based mayonnaises and sauces can also be assessed using the equations given in the CIMSCEE code. There are safety and stability formulae, both of which are based on pH, and amounts of acetic acid, salt and sugar in the water phase. A numerical value is calculated which has to be greater than 63 for the product to be considered safe and/or stable.

Campden BRI is able to perform these calculations for clients. If the required values are not available, Campden BRI can analyse products for salt, sugar, water, pH and acetic acid level.

Food pathogens

Pathogen predictions can be carried out using the Combase predictor system. This system is internet based and predictions are generated online. This system is freely available via *http://www.combase.cc/predictor.html*

Campden BRI is able to generate pathogen predictions using Combase predictor and interpret them for clients who do not have a microbiological background. The Combase system contains growth models but also thermal death models. These thermal death models predict log reductions of an organism at a specified temperature. There are also survival models for *Salmonella* and *Listeria* which predict the log reduction of the organism at various pH values, temperatures and salt levels. This can be useful to assess if pathogens can survive in, for example, acidified foods. Another useful model is one that allows the potential for *C. perfringens* growth to be predicted during cooling. The details of models and input parameters for models contained in the Combase predictor system are detailed in Tables 5 and 6.

A further set of pathogen models in the USA, known as the pathogen modelling program (PMP), is freely available via the internet. Details of the models included are given in Table 7. These can be generated online at *http://www.arserrc.gov/pmponline.htm* or a software package can be downloaded for stand-alone use. The stand alone system has more models available for use than the online system. Details of these additional models are given in Table 8.

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Table 1
Spoilage models available in FORECAST

Model	Temperature (°C)	NaCl (% aq)	Equivalent Aw	pH	Other Conditions
Pseudomonas	0 - 15	0.0 - 4.0	1.00 - 0.977	5.5 - 7.0	Fluctuating temperature, pH, salt
Bacillus spp.	5 - 25	0.5 - 10	0.997 - 0.935	4.0 - 7.0	Fluctuating temperature, pH, salt
Enterobacteriaceae	0 - 27	0.5 - 10	0.997 - 0.935	4.0 - 7.0	Fluctuating temperature, pH, salt
Yeasts (chilled foods)	0 - 22	0.5 - 10	0.997 - 0.935	2.6 - 6.0	Fluctuating temperature, pH, salt
Yeasts (fruit/drinks) (time to growth)	0 - 22	-		2.0 - 7.0	0 - 60% Sucrose (w/v) 0 - 20% Ethanol (v/v) Potassium sorbate 0 - 1000(ppm)
Lactic acid bacteria	2 - 30	0.5 - 10	0.997 - 0.935	3.0 - 6.0	Fluctuating temperature
Meat spoilage	2 - 22	0 - 6	1.00 - 0.964	4.6 - 7.0	0 - 240 KNO_2 (ppm) Fluctuating temperature, pH, salt
Fish spoilage	2 - 22	0 - 6	1.00 - 0.964	4.5 - 8.0	Fluctuating temperature, pH, salt
Fresh produce TVC	2 - 25	-	-	-	
Fresh produce Enterobacteriaceae	2 - 25	-	-	-	
Fresh produce lactic acid bacteria	2 - 25	-	-	-	
Fresh produce *Pseudomonas*	2 - 25	-	-	-	
Enterobacteriaceae death model	52 to 64	0 - 8	1.00 - 0.95	4.0 - 7.0	Predicts D value
Bacillus (time to growth)	8 - 45	0.5 - 10	0.997 - 0.935	4.0 - 7.0	

Table 2
Models for acidified foods in FORECAST

Organisms	Prediction categories/time to growth (G)	pH	Aw	Salt % w/v	Preservative ppm
Cold fill spoilage (yeasts, moulds, lactics)	1 = G in 14d 2 = G in 15 - 30d 3 = G in 31 - 60d 4 = G in 61 - 182d 5 = NG in 182d	2.8 - 5.0	0.85 - 1.00	0.5 - 18	Benzoate Sorbate 0 - 2000 (in total)
Cold fill pathogens (*E. coli, S. aureus, Salmonella*)	1 = G in 120d 2 = NG in 120d	3.9 - 5.0	0.87 - 1.00	0.5 - 16	Benzoate Sorbate 0 - 2000 (in total)
Hot fill spoilage (sporeformers)	1 = G in 14d 2 = G in 15 - 30d 3 = G in 31 - 60d 4 = G in 61 - 182d 5 = NG in 182d	3.7 - 5.2	0.86 - 1.00	0.5 - 18	Benzoate Sorbate 0 - 2000 (in total)

Key: G = Growth
NG = No Growth

Table 3
Models for acidified foods in FORECAST

Organisms	Prediction categories	Salt* % w/v	Sugar* % w/v	Acid %	Acid type	Preservative ppm
Cold fill spoilage (yeasts, moulds, lactics)	1 = G in 14d 2 = G in 15 - 30d 3 = G in 31 - 60d 4 = G in 61 - 182d 5 = NG in 182d	0 - 4	0 - 45	0.5 - 4.0	Acetic Lactic Citric	Benzoate Sorbate 0 - 2000 (in total)
Hot fill spoilage (sporeformers)	1 = G in 14d 2 = G in 15 - 30d 3 = G in 31 - 60d 4 = G in 61 - 182d 5 = NG in 182d	0 - 4	0 - 45	0.5 - 4.0	Acetic Lactic Citric	Benzoate Sorbate 0 - 2000 (in total)

Salt and sugar cannot exceed a total of 45%.

Key: G = Growth
NG = No Growth

Table 4
Models for acidified foods in FORECAST

Organisms	Prediction categories	Salt* % w/v	Sugar* % w/v	pH	Preservative ppm
Cold fill spoilage (yeasts, moulds, lactics)	1 = G in 14d 2 = G in 15 - 30d 3 = G in 31 - 60d 4 = G in 61 - 182d 5 = NG in 182d	0 - 4	0- - 45	2.9 - 6.1	Benzoate Sorbate 0 - 2000 (in total)

Key: G = Growth
NG = No Growth

Table 5
Combase Predictor growth models

Model	Temperature (°C) (fluctuating temperature profiles can be used)	NaCl (% aq)	Aw	pH	Other conditions (only one extra condition permitted per prediction)
Aeromonas hydrophila	2 - 37	0.0 - 4.5	1.00 - 0.974	4.6 - 7.5	-
Bacillus cereus	5.0 - 34.0	0.0 - 9.4	1.00 - 0.94	4.9 - 7.4	CO_2 0 - 60%
Bacillus licheniformis	13.0 - 34.0	0.0 - 13.5	1.00 - 0.907	4.0 - 7.6	-
Bacillus subtilis	10.0 - 34.0	0.0 - 10.3	1.00 - 0.933	4.3 - 7.8	-
Brochothrix thermosphacta	0.0 - 30.0	0.0 - 8.0	1.00 - 0.95	5.5 - 7.0	-
Clostridium botulinum (non-proteolytic)	4.0 - 30.0	0.0 - 4.5	1.00 - 0.974	5.1 - 7.5	-
Clostridium botulinum (proteolytic)	14.0 - 30.0	0.0 - 7.5	1.00 - 0.954	4.7 - 7.2	-
Clostridium perfringens	15.0 - 52.0	0.0 - 5.0	1.00 - 0.971	5.0 - 8.0	-
E. coli	10.0 - 42	0.0 - 6.5	1.00 - 0.961	4.5 - 7.5	0 - 100% CO_2
Listeria monocytogenes/ innocua	1.0 - 40	0.0 - 10.2	1.00 - 0.934	4.4 - 7.5	0 - 100% CO_2
Listeria monocytogenes/ innocua	1.0 - 40	0.0 - 11.4	1.00 - 0.924	4.4 - 7.5	0 - 20,000ppm lactic acid 0 - 10,000ppm acetic acid 0 - 200ppm $NaNO_2$
Salmonella	7.0 - 40	0.0 - 4.6	1.00 - 0.973	3.9 - 7.4	0 - 100% CO_2 0 - 200ppm nitrite
Staphylococcus aureus	7.5 - 30.0	0.0 - 13.5	1.00 - 0.907	4.3 - 7.1	-

Model	Temperature (°C) (fluctuating temperature profiles can be used)	NaCl (% aq)	Aw	pH	Other conditions (only one extra condition permitted per prediction)
Yersinia enterocolitica	-1 - 37.0	0.0 - 7.0	1.00 - 0.957	4.4 - 7.2	0 - 10,000ppm lactic acid 0 - 80% CO_2
Pseudomonas	0 - 20	0 - 6.5	0.961	5 - 7.4	
Shigella flexneri	15 - 37	0 - 5	0.971	5.5 - 7.5	0 - 1000 NO_2
Perfringens predictor - cooling model					
Cured/uncured	pH 5.2 - 8.0		0 - 4% NaCl		

Non thermal death	Temp (°C)	pH	NaCl (%)	Aw
L. monocytogenes/ innocua	0 - 20	3.5 - 7.0	0 - 25	0.793 - 1.0
Salmonella	0 - 40	4.3 - 7.5	0 - 26	0.781

Table 6
Combase Predictor thermal death models

Model	Temperature (°C)	NaCl (% aq)	Aw	pH
Bacillus cereus	90 - 100	2.5 - 7.5	0.986 - 0.954	4.5 - 7.0
Clostridium botulinum (non-proteolytic)	80 - 95	0 - 5.0	1.00 - 0.971	4.1 - 7.3
E. coli	54.5 - 64.5	0 - 8.4	1.00 - 0.947	4.2 - 8.0
Listeria monocytogenes/innocua	60 - 68	0 - 9.0	1.00 - 0.943	4.2 - 7.0
Salmonella	54.5 - 65	0 - 0.6	1.00 - 0.997	4.0 - 7.1
Yersinia enterocolitica	52 - 60	0.65	1.00 - 0.961	4.2 - 7.0
Brochothrix	40 - 55	0 - 2.0	1.00 - 0.989	5 - 7.0

Table 7
Models included in the Pathogen Modelling program on-line version

PMP Growth Models - Broth Culture						
Model	**Temp (°C)**	**NaCl (% aq)**	**Aw**	**pH**	**Initial level (log cfu/g)**	**Other conditions**
Aeromonas hydrophila aerobic	5 - 42	0.5 - 4.5	0.997 - 0.974	5.3 - 7.3	3 - 5.9	0 - 150ppm $NaNO_2$
Aeromonas hydrophila anaerobic	5 - 30	0.5 - 3.5	0.997 - 0.980	5.3 - 7.3	3 - 5.9	0 - 150ppm $NaNO_2$
Bacillus cereus aerobic	5 - 42	0.5 - 5.0	0.997 - 0.970	4.7 - 7.5	3 - 5.9	0 - 150ppm $NaNO_2$
Bacillus cereus anaerobic	10 - 42	0.5 - 5.0	0.997 - 0.970	5.0 - 9.0	3 - 5.9	0 - 150ppm $NaNO_2$
Escherichia coli O157:H7 aerobic	5 - 42	0.5 - 5.0	0.997 - 0.970	4.5 - 8.5	3 - 5.9	0 - 150ppm $NaNO_2$
Escherichia coli O157:H7 anaerobic	5 - 42	0.5 - 5.0	0.997 - 0.970	4.5 - 8.5	3 - 5.9	0 - 150ppm $NaNO_2$

Model	Temp (°C)	NaCl (% aq)	Aw	pH	Initial level (log cfu/g)	Other conditions
Listeria monocytogenes (NaCl) aerobic	4 - 37	0.5 - 10.5	0.997 - 0.928	4.5 - 7.5	3 - 5.9	0 - 150ppm $NaNO_2$
Listeria monocytogenes (NaCl) anaerobic	4 - 37	0.5 - 5.0	0.997 - 0.970	4.5 - 8.0	3 - 5.9	0 - 150ppm $NaNO_2$
Staphylococcus aureus aerobic	10 - 42	0.5 - 12.5	0.997 - 0.911	4.5 - 9.0	3 - 5.9	0 - 150ppm $NaNO_2$
Staphylococcus aureus anaerobic	12 - 42	0.5 - 16.5	0.997	5.3 - 9.0	3 - 5.9	0 - 150ppm $NaNO_2$
Shigella flexneri aerobic	10 - 37	0.5 - 5.0	0.997 - 0.970	5.0 - 7.5	3 - 5.9	0 - 150ppm $NaNO_2$
Shigella flexneri anaerobic	12 - 37	0.5 - 4.0	0.997 - 0.977	5.5 - 7.5	3 - 5.9	0 - 150ppm $NaNO_2$

Thermal Death Models

Model	Temp (°C)	NaCl (% aq)	Aw	pH	Other conditions
Listeria monocytogenes (ground beef)	55 - 65	0 - 6.0	1.00 - 0.963	4 - 7	Sodium pyrophosphate 0 - 0.3%

Other Models

Model	Input factors	Capabilities
L. monocytogenes transfer	Inoculation level (log cfu/g per blade) Temperature (°C) Attachment time (mins) A list of potential combinations is given	Calculates *L. monocytogenes* level on slices of ground salmon from slice 1 - 40
Growth of *L. monocytogenes* in a seafood salad	Aerobic/anaerobic Temp 4.0 - 12°C Mayonnaise pH -3.7 - 5.1 Time duration 0 - 28 days Time interval 0.1 - 2 days Initial level on seafood 0 - 7.0 log cfu/g Level of concern 1.0 - 7.0 cfu/g Lag or no lag	Calculates increase in level of *L. monocytogenes* after time and indicates time to level of concern
Growth of *L. monocytogenes* in ground ham	Anaerobic only Temp (°C) 6 - 36 Sodium lactate 1 - 4.2% Sodium diacetate 0.05 - 0.20% Initial level 1 - 3.0 log cfu/g	Calculates increase in level of *L. monocytogenes* over 250h
Salmonella Typhimurium in ground chicken with competitive microflora	Temp (°C) 10 - 40 Initial level set at 0.6 log cfu/g	Calculates growth of *Salmonella* over 154h

Table 8
Additional models included in pathogen modelling program - stand-alone version 7.0 not available via online

Model	Temp (°C)	Nacl (% aq)	Aw	pH	Initial level log cfu/g	Other conditions
Salmonella	10 - 30	0.5 - 4.5	0.997 - 0.974	5.6 - 6.8	3.0 - 5.9	N/A
Listeria monocytogenes Aw (aerobic)	4 - 37	-	0.928 - 0.997	4.5 - 7.5	3.0 - 5.9	Sodium nitrite 0 - 150ppm
Listeria monocytogenes Aw (anaerobic)	4 - 37	0.5 - 5.0	0.997 - 0.97	4.5 - 8.0	3.0 - 5.9	Sodium nitrite 0 - 150ppm
Yersinia enterocolitica aerobic	5 - 42	0.5 - 5.0	0.997 - 0.970	4.5 - 8.5	3.0 - 5.9	Sodium nitrite 0 - 150ppm

Thermal inactivation

	Temp (°C)	Nacl (%)	Aw	pH	Other conditions
Non proteolytic *C. botulinum* in turkey slurry	70 - 90	0 - 3	1.00 - 0.983	5 - 7	Sodium pyrophosphate 0 - 0.3%
E. coli O157:H7 in simulated beef gravy	55 - 62.5	0 - 6	1.00 - 0.963	4 - 8	Sodium pyrophosphate 0 - 0.3%
Listeria monocytogenes in ground beef	55 - 65	0 - 6	1.00 - 0.963	4 - 8	Sodium pyrophosphate 0 - 0.3%

Survival models

	Temp (°C)	Nacl	Aw	pH	Other conditions
E. coli O157:H7	4 - 37	0.5 - 15	0.997 - 0.887	3.5 - 7.0	Lactic acid 0 - 2%, $NaNO_2$ 0 - 75ppm
L. monocytogenes (NaCl)	4 - 42	0.5 - 19	0.997 - 0.845	3.2 - 7.3	Lactic acid 0 - 2%, $NaNO_2$ 0 - 150ppm
S. aureus	4 - 37	0.5 - 20	0.997 - 0.834	3.0 - 7.0	Lactic acid 0 - 1%, $NaNO_2$ 0-200ppm
Salmonella	5 - 42	0.5 - 16	0.997 - 0.887	3.5 - 7.2	$NaNO_2$ 0 - 200ppm

Other models

Model	Model Capability
C. botulinum (proteolytic) cooling profile model	Calculates increase in numbers during cooling of broth
C. perfringens cooling profile model	Calculates increase in numbers during cooling of beef broth, chicken or cured beef
Salmonella Typhimurium irradiation	Predicts decline in numbers following 0 - 3.6kGy treatment of chicken
E. coli O157 irradiation	Predicts decline in numbers following 0 - 2.0 kGy treatment in beef tartar
Spoilage flora (chicken leg) irradiation	Predicts decline in numbers following a 0 - 3.6kGy treatment in chicken meat
Time to turbidity proteolytic *C. botulinum*	Predicts probability of growth at 15 - 34°C, pH 5 - 7.2, Nacl (%) 0 - 4

Model	Model Capability
Non proteolytic *C. botulinum*	Predicts probability of growth at 5 - 28°C, pH 5 - 7.0, 0 - 4% Nacl
Time to toxin production for *C. botulinum* in fish	Lag time predicted at 4 - 30°C, initial spore level -2 - 4.0 log cfu/g and initial aerobic count -2 - 3.0 log cfu/g