Exploitation of challenge testing in exposure assessment of foodborne pathogens

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Objectives

- You will learn:
  - The types of microbial exposure assessment and the requirements to conduct one
  - The key aspects for consideration when building an exposure assessment model
  - How to exploit challenge test data when building an exposure assessment model
  - How to make use of predictive microbiology models or kinetic parameters when building an exposure assessment model
  - How to conduct a step-by-step analysis to incorporate growth and inactivation models in an exposure assessment model programmed in R

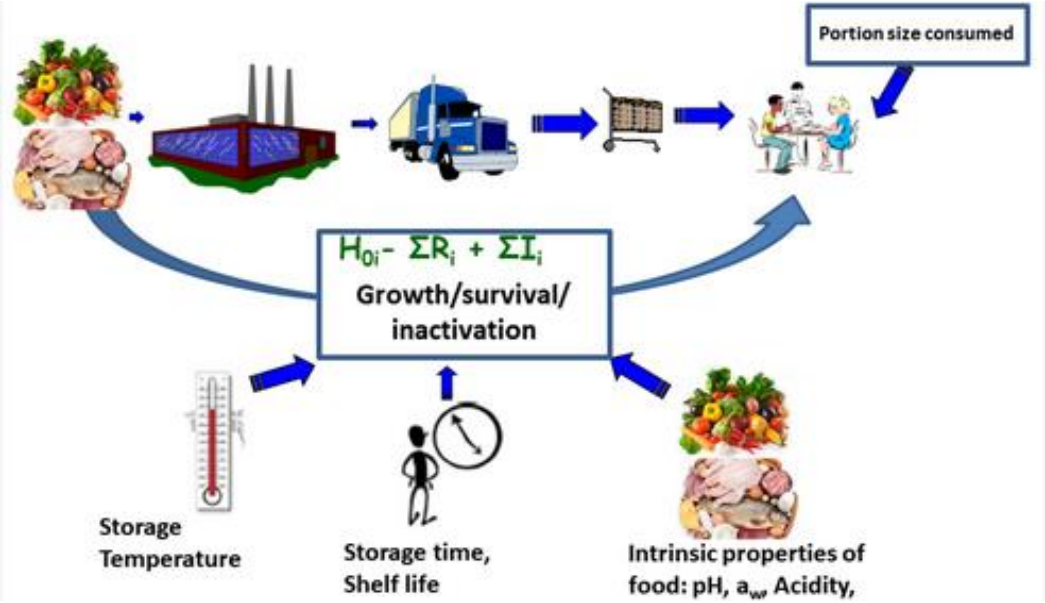
- Materials needed:
  - This presentation
  - Three R scripts: StirFry_MildHeat.R, StirFry_Storage.R, StirFry_Baseline.R
  - The accompanying video

Content

- Microbial exposure assessment
  - Definition, requirements, types
- Model development
  - Key aspects, conceptual model
- Growth and inactivation models in exposure assessment
  - Critical features, use of challenge test data, use of kinetic models/parameters
- An example of use of growth/inactivation models in exposure assessment
  - Conceptual model
  - Baseline scenario in R

Microbial exposure assessment

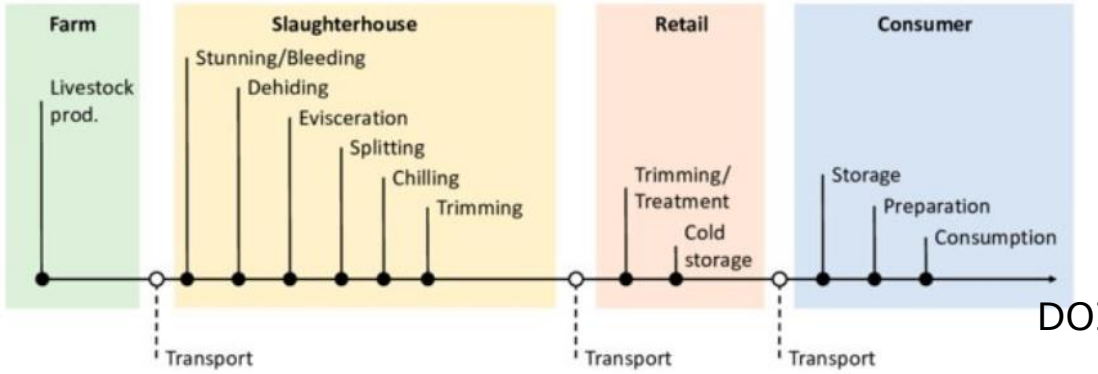
- Definition
- Requirements
- Types



Microbial exposure assessment

- Microbial exposure assessment is the **qualitative** and/or **quantitative** evaluation of the likely intake of a microbial hazard via specific foods

- The exposure assessment should describe the pathways of exposure that are relevant to the risk assessment:
  - If the purpose was to compare strategies from production to consumption, then the various stages from **farm to fork** should be assessed
  - If the purpose was to assess the effects of consumer handling, then only the pathway from **retail to consumption** may be relevant



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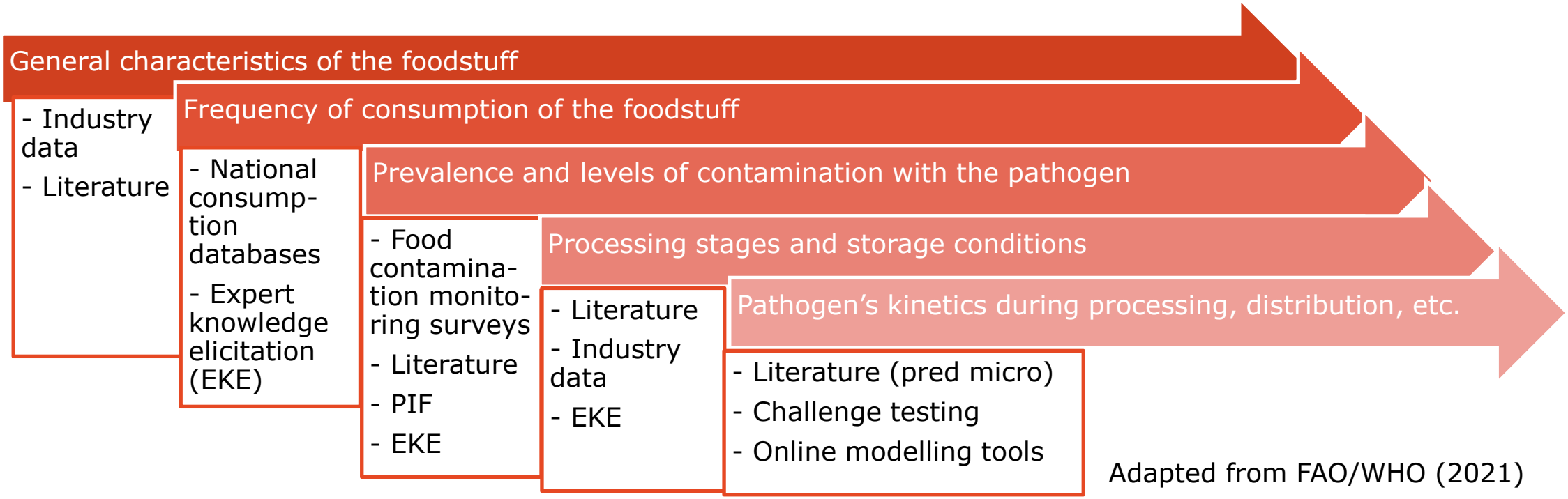
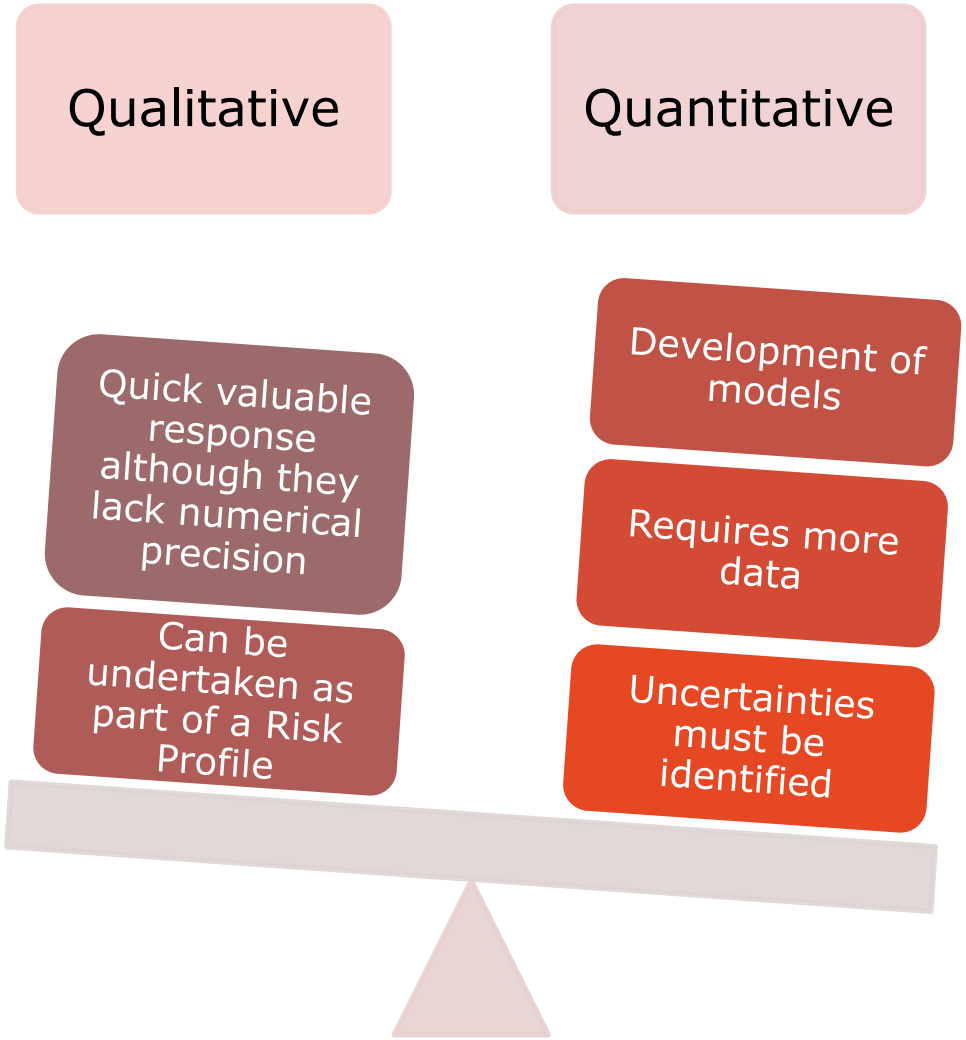
Requirements for microbial exposure assessment

- One must consider the factors that have a direct effect on the consumer's exposure to the hazard

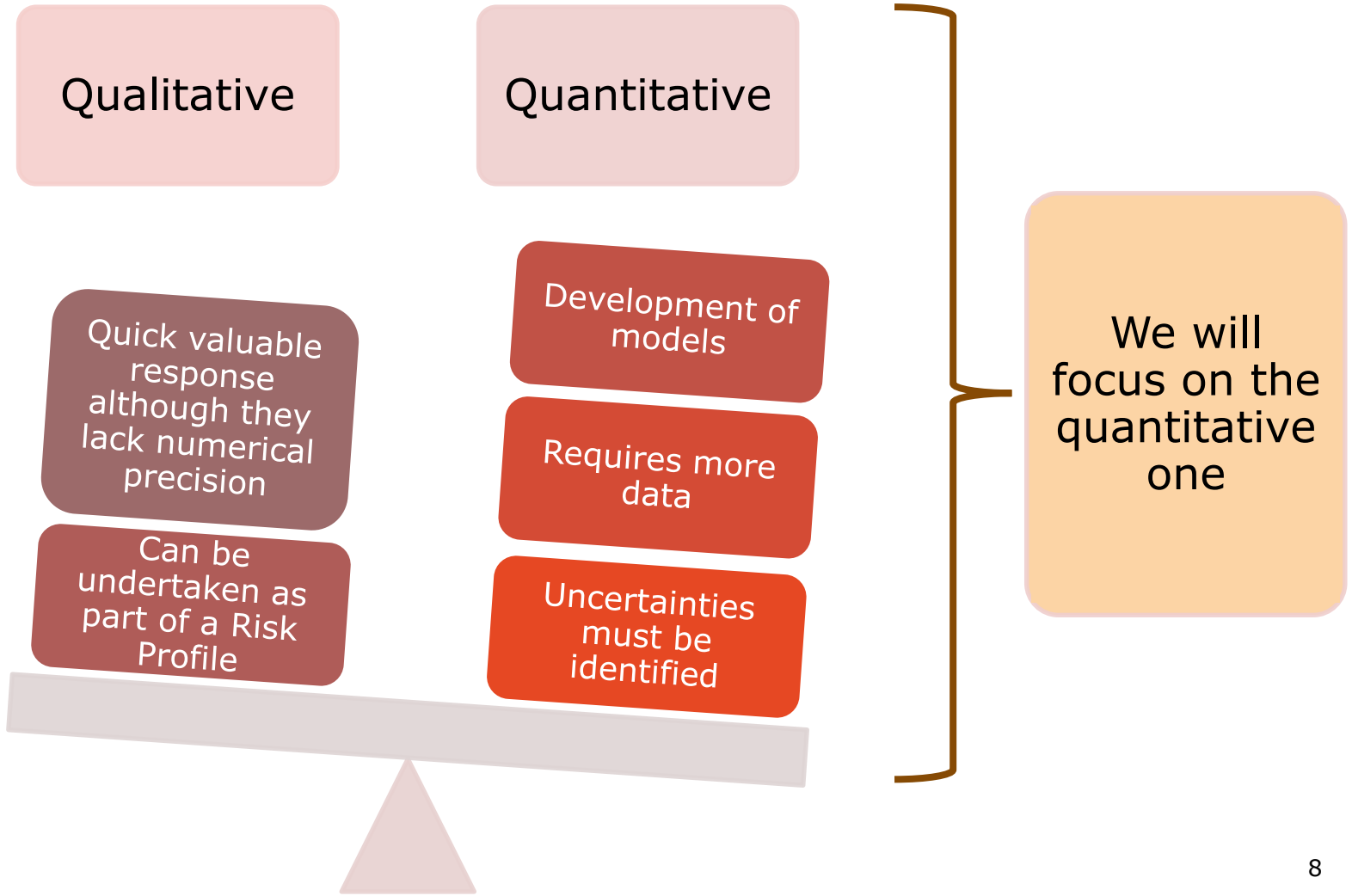


Adapted from FAO/WHO (2021)

Types of microbial exposure assessment



Types of microbial exposure assessment

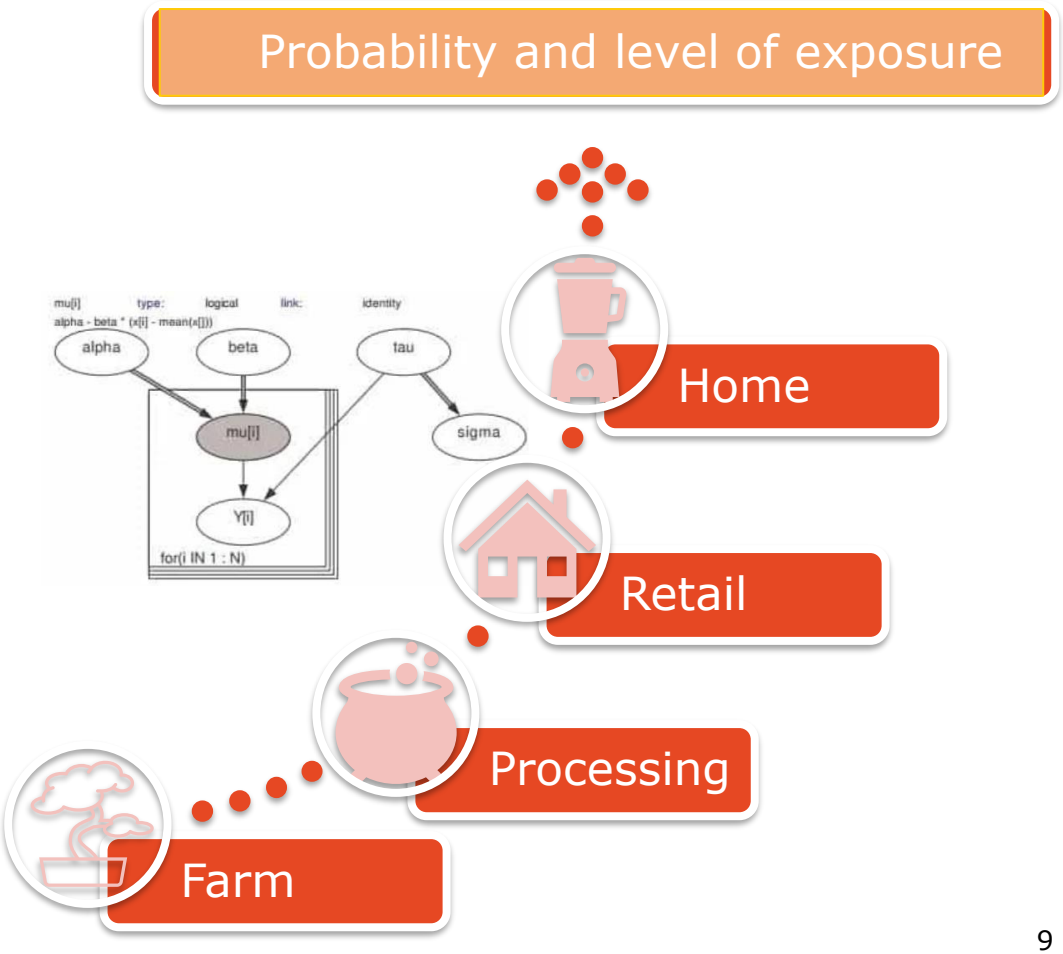


Quantitative microbial exposure assessment

- The goal from the quantitative exposure assessment is to deduce, **from the available information**, the probability and magnitude of the exposure to the hazard

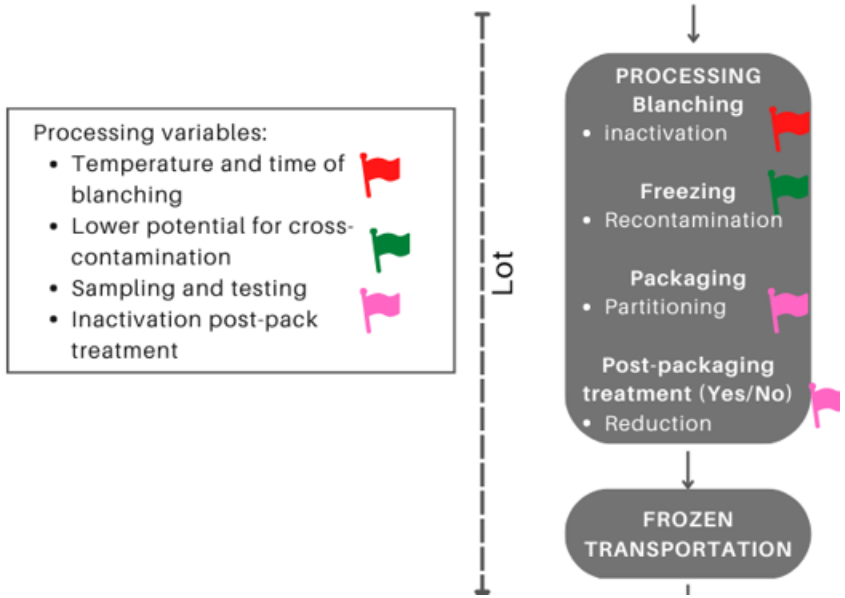
- Exposure data characterising the occurrence of the microbial hazard in food at the point of consumption is usually not available.

- Thus, an exposure assessment model will estimate prevalence and concentration in a food serving

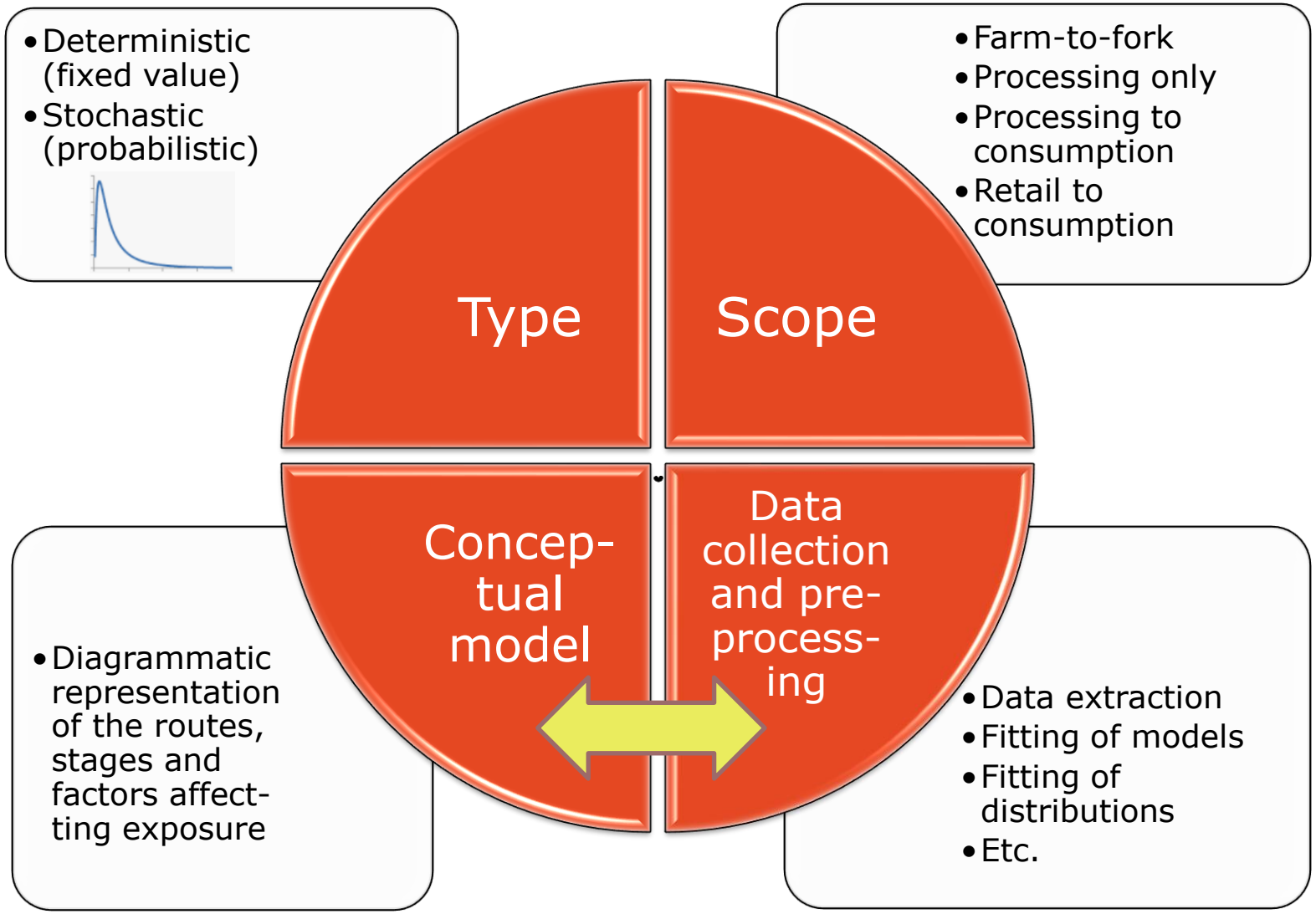


Model development

- Key aspects
- The conceptual model

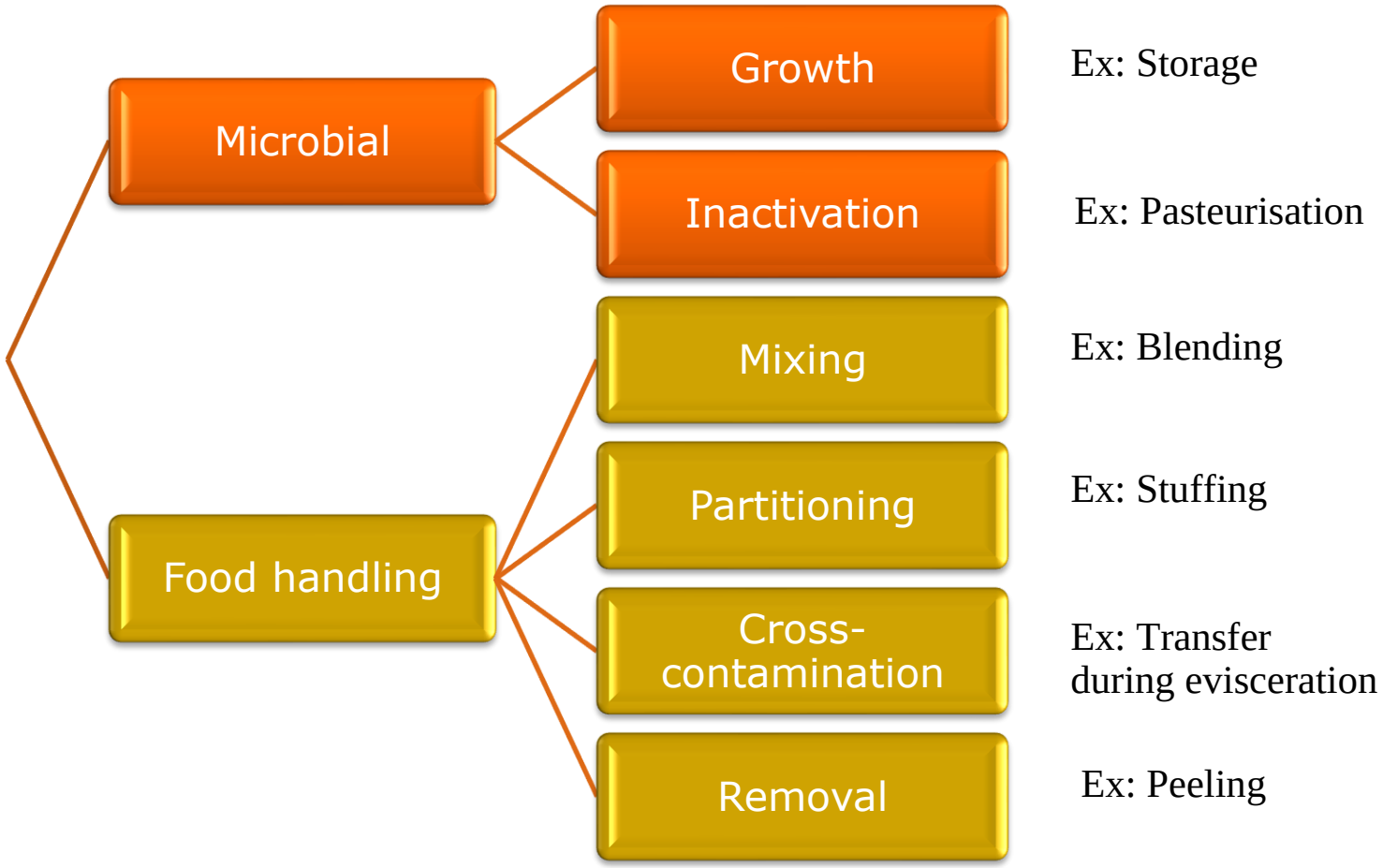


Key aspects of model development



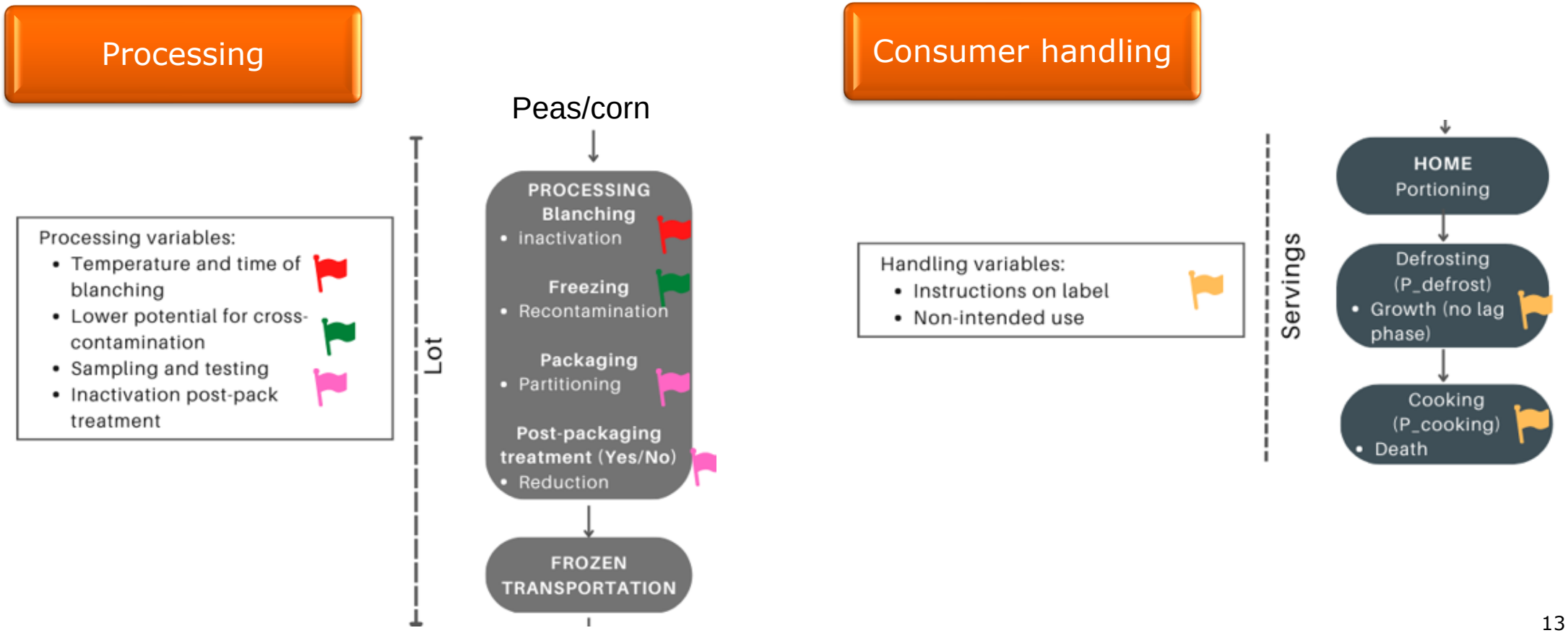
Conceptual model

- Often based on the Modular Process Risk Model (MPRM, Nauta et al., 2002)
  - There are two types of processes: **microbial** processes and **food handling** processes
  - At least one of them should be assigned to the modelled stages



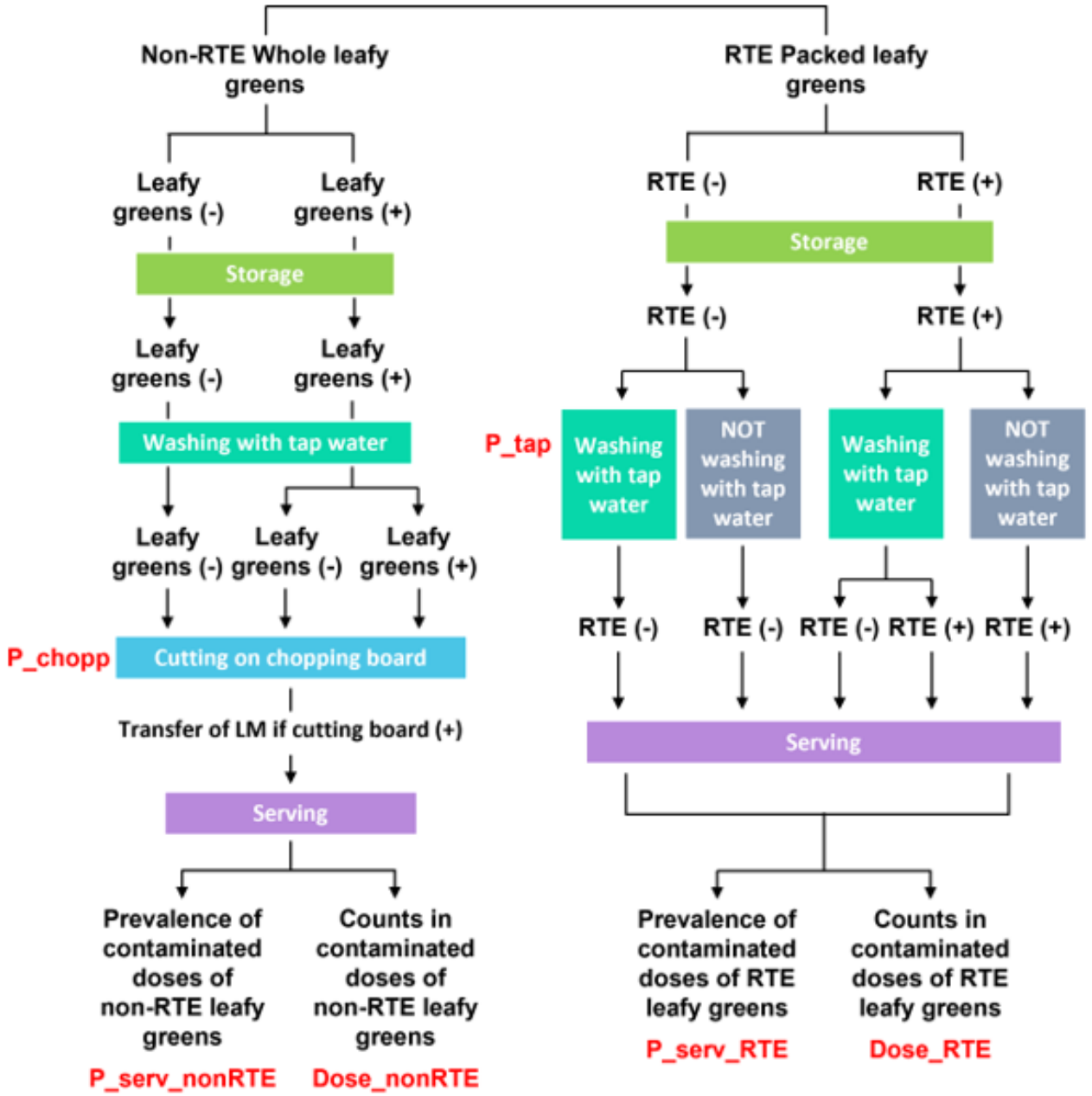
Conceptual model

- An example of **MPRM sketch** for frozen corn/peas to be modelled from processing to consumption



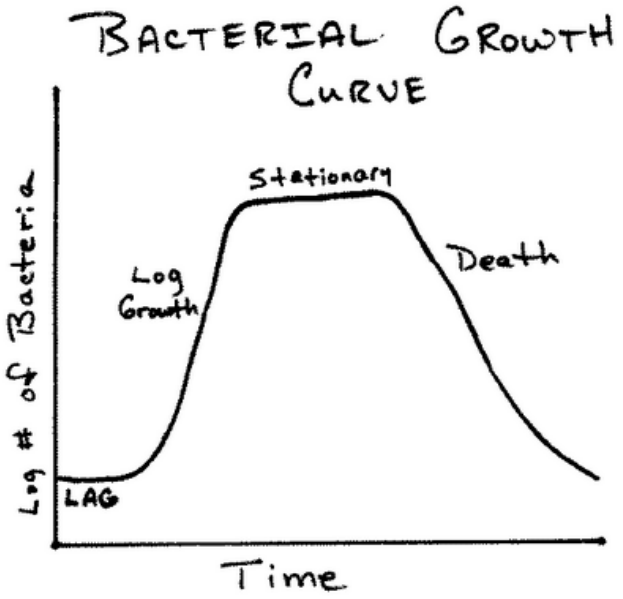
Conceptual model

- An example of home preparation module non-RTE and RTE leafy greens
  - **Event tree sketch**



Microbial growth/inactivation models in exposure assessment

- Critical features
- Use of challenge testing data
- Use of kinetic models/parameters



Application of kinetic models/parameters in exposure assessment

- Some features are critical to the utility of predictive microbiology models

Range of independent variables to which the model applies
(Avoid extrapolation, for example, by trimming extreme temperatures)

Sources of variability and uncertainty
(Identify the microbial parameters subject to variation, and which variation)

Effect of spoilage microbiota on shelf life of the product
(Contaminated foods that become spoiled will not be consumed)

Validated sources!

Use of challenge testing data

- Suppose that we need information on the inactivation of *L. monocytogenes* in raw vegetables, and we found the following "readily extractable" data from the literature

TABLE 1. *Heat resistance of* L. monocytogenes *in different vegetables*

Vegetable	Condition	*D*-value (min) at temperature of: 56°C/133°F	60°C/140°F	62°C/144°F
Onions	Stationary phase	0.8 ± 0.07	0.23 ± 0.09	0.10 ± 0.03
Broccoli	Stationary phase	2.3 ± 0.21	0.62 ± 0.03	0.39 ± 0.05
Peppers	Stationary phase	3.9 ± 0.35	0.92 ± 0.08	0.31 ± 0.01
Mushroom	Stationary phase	5.0 ± 0.43	0.69 ± 0.06	0.30 ± 0.03
Mushroom	Starved	7.0 ± 0.35	1.68 ± 0.11	0.96 ± 0.13
Peas	Stationary phase	5.2 ± 0.28	1.04 ± 0.14	0.41 ± 0.08
Peas	Starved	6.5 ± 0.21	1.13 ± 0.10	0.52 ± 0.06

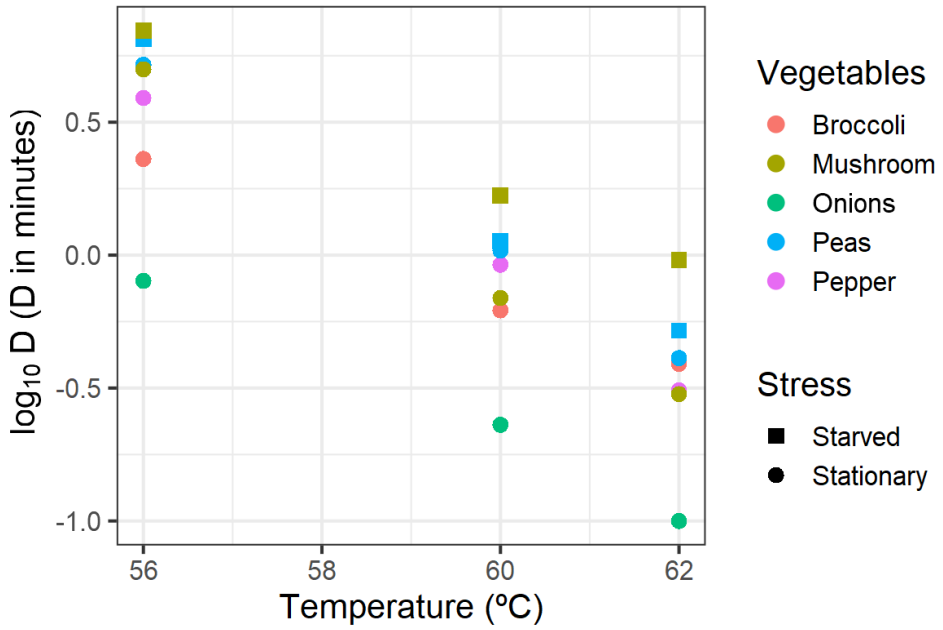
(Mazzotta, 2001)

- How can we use these data to model inactivation parameters for exposure assessment?

Use of challenge testing data

- Decisions to make:

1) Confidence about the data
2) The model



- The Bigelow model can be used

$$\log_{10} D = \log_{10} D_{ref} - \frac{(Temp - T_{ref})}{z_T} \qquad T_{ref} = 70$$

```
41
42  bigelow_vegetables<-function(Temp,Tref,log10Tref,zT)
43  {log10DT<-log10Tref-((Temp-Tref)/zT)
44  return(log10DT)
45  }
```

```
50  ## 2.1 Simple nls fit
51  fitmodel1<- nls(log10D~bigelow_vegetables(Temperature,Tref=70,log10DTref,zT),
52                  start = list(log10DTref=-1.5,zT=10),
53                  upper=c(4,10),
54                  lower=c(-3,0.01),
55                  data = data,
56                  algorithm="port",
57                  trace=TRUE)
58  summary(fitmodel1)
```

Use of challenge testing data

3) Sources of variability:

```
Parameters:
           Estimate Std. Error t value Pr(>|t|)
log10DTref  -1.7940     0.2478  -7.239 2.98e-07 ***
zT           5.8914     0.7852   7.503 1.68e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2765 on 22 degrees of freedom

Algorithm "port", convergence message: relative convergence (4)
```

- Heat resistance parameters:
  - **Between-strain variability?** – mixed effects
  - **Between-vegetable variability?** – mixed
  - **Uncertainty?** – fixed effects

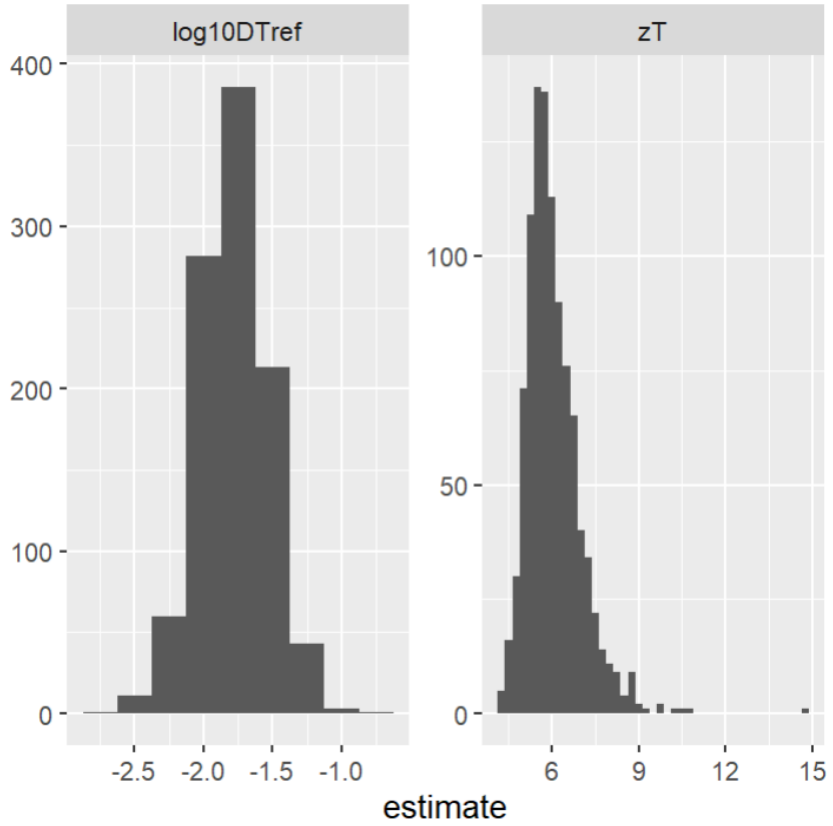
- This decision depends on the amount of data available and the complexity to add to the model
- We will opt for modelling only the uncertainty about the estimate(s)

Use of challenge testing data

3) Sources of variability:

```
## 2.3 Bootstrap using broom package
fit_nls_on_bootstrap <- function(split) {
  nls(log10D~bigelow_vegetables(Temperature,Tref=70,log10DTref,zT),
      analysis(split), start = list(log10DTref=-1.5,zT=6))
}

boot_models <- boots %>%
  mutate(model = map(splits, fit_nls_on_bootstrap),
         coef_info = map(model, tidy))

boot_coefs <- boot_models %>%
  unnest(coef_info)

alpha <- .05
boot_coefs %>%
  group_by(term) %>%
  summarize(low = quantile(estimate, alpha / 2),
            high = quantile(estimate, 1 - alpha / 2))

boot_coefs %>%
  group_by(term) %>%
  summarize(mean_log10 = mean(estimate),
            sd_log10 = sd(estimate))

ggplot(boot_coefs, aes(estimate)) +
  geom_histogram(binwidth = 0.25) +
  facet_wrap(~ term, scales = "free")
```

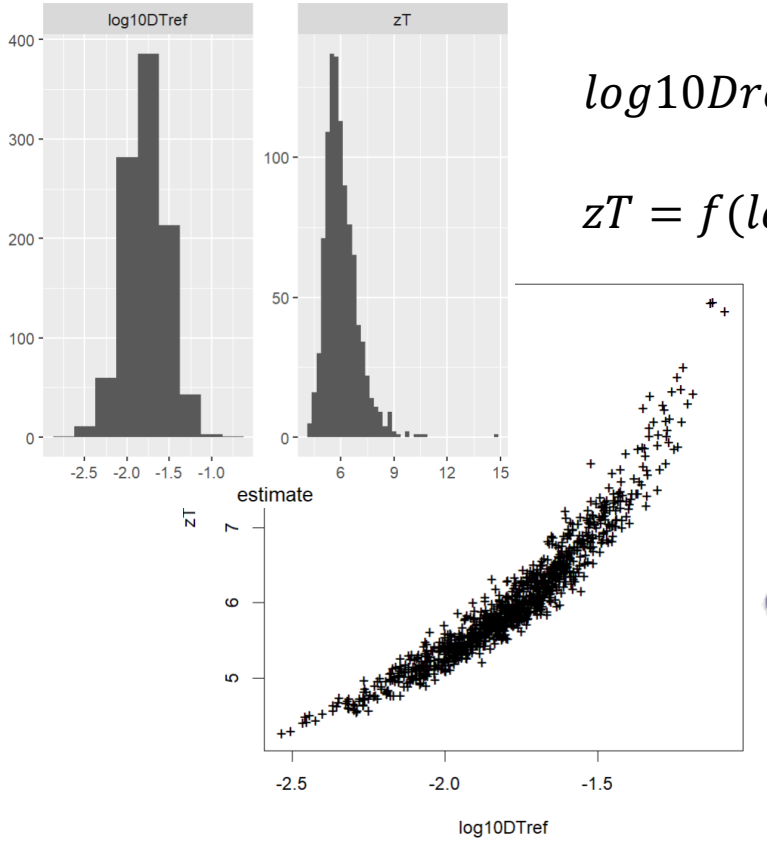
- Bootstrapping techniques allow building an uncertainty distribution about the fitted parameter



Use of challenge testing data

- Two options are possible:

Complex: 2 uncertainties

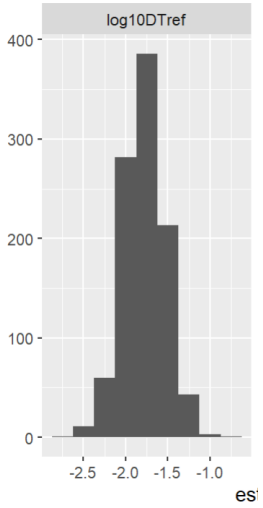


$log10Dref \sim Normal(-1.794, 0.248)$

$zT = f(log10Dref)$

- These parameters are correlated!

Simple: 1 uncertainty



$log10Dref \sim Normal(-1.794, 0.248)$

$zT = 5.8914$

Use of challenge testing data

4) The stochastic function of mild heat treatment MildHeat() for exposure assessment

- Initial concentration

  $C_0 \sim xxx$ log CFU/g

- Heat treatment conditions

  $Time \sim Pert(1, 3, 7)$ min

  $Temp \sim Pert(45, 70, 90)$ ºC

- Hyperparameters and inactivation model

  $log10Dref \sim Normal(-1.794, 0.248)$

  $$\log_{10} D = \log_{10} D_{ref} - \frac{(Temp - 70)}{5.8914}$$

- Output

  $$C_f = C_0 - \frac{Time}{D}$$

Use of challenge testing data

5) Testing the function

- Initial concentration (contaminated fraction)

  $C_0 \sim Normal(0.8, 0.3)$ log CFU/g

- Heat treatment conditions

  $\boldsymbol{Time = 1.5}$ **min** $Temp = 68$ ºC

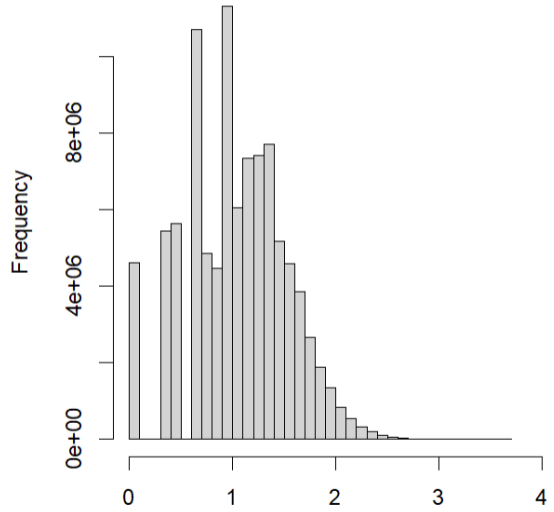
- Initial concentration (contaminated fraction)

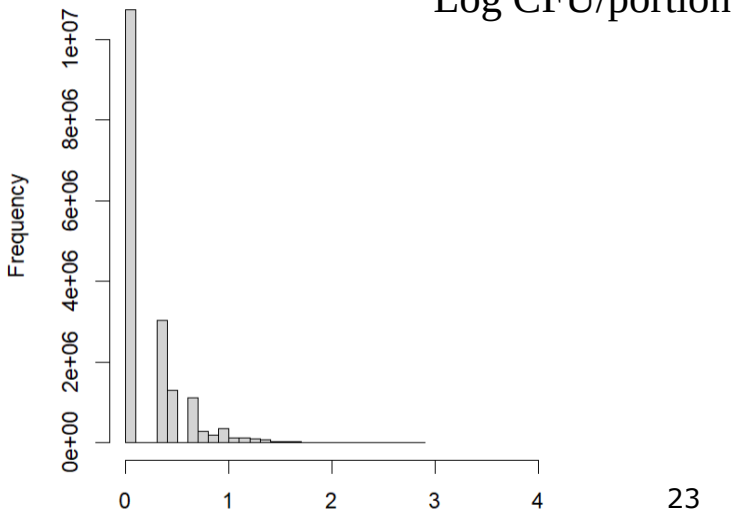
  $C_0 \sim Normal(0.8, 0.3)$ log CFU/g

- Heat treatment conditions

  $\boldsymbol{Time = 3}$ **min** $Temp = 68$ ºC

- Outputs, Cf

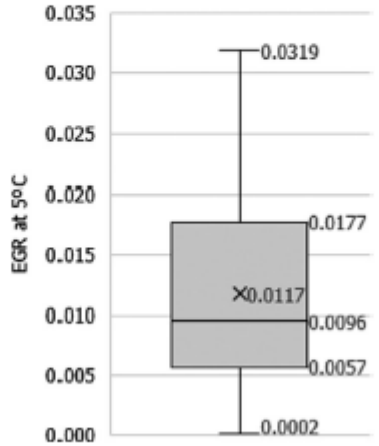




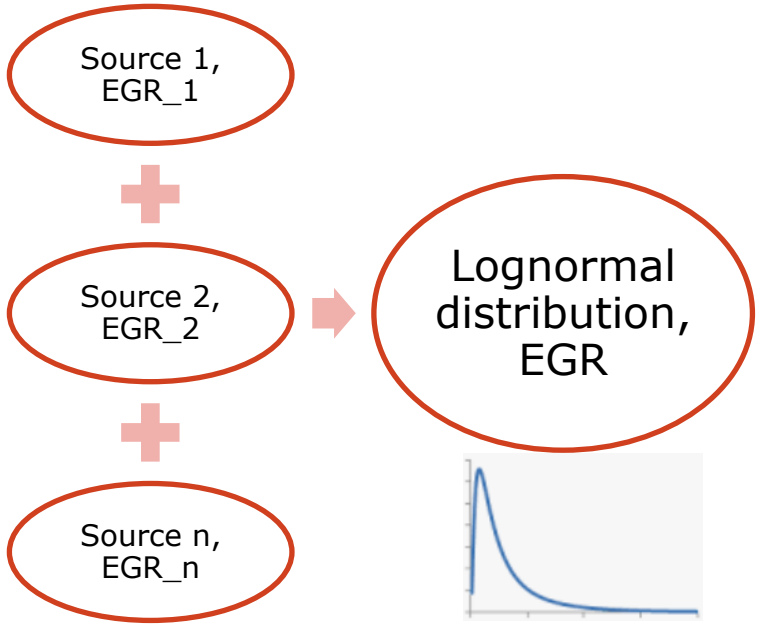
Use of published kinetic models/parameters

- Suppose that we need information on the growth of *L. monocytogenes* in heat-treated vegetables, and we found a synthesis of data collected from the literature. How can we use this information?

Figure 5 shows the distribution of the **exponential growth rate** at 5°C ($EGR_{5°C}$, log_{10}/h) using data collected from scientific literature and Combase records reporting *L. monocytogenes* growth at different temperatures in heat-treated (blanched) vegetables, which has been converted to $EGR_{5°C}$ using the square-root-based secondary model for bacterial growth. The intensity of the heat treatment ranged from 50°C for 60 s up to 90°C for 10 min. The mean, standard deviation and truncated maximum (mean + 2 standard deviations) of the growth rate was used for the mgQMRA (Table 2). The mean value was estimated to be 0.0117 log_{10}/h, meaning that, on average, after 10 h at 5°C the population increases by 0.117 log_{10} units



Data extracted from the literature and ComBase were transformed to $EGR_{5°C}$, using T_{min} = −1.18°C (FDA and FSIS, 2003). (EFSA, 2020)

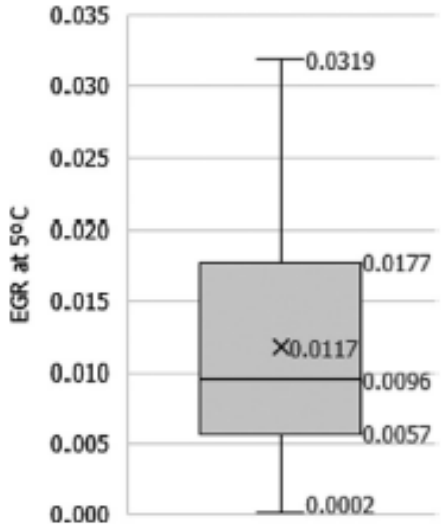


Use of published kinetic models/parameters

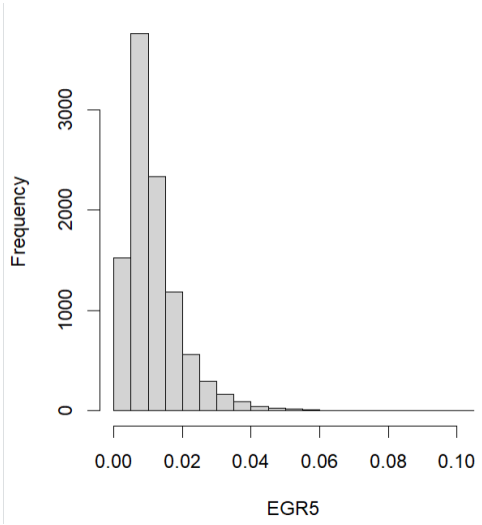
- Decisions to make:

1) Assess the confidence on the compilation work done
2) Do we need to access the compiled data (source data)?

  - We opt for taking the distribution on EGR5 from the publication, as is



$$EGR_5 \sim Lognormal\ (-4.65\ , 0.63)$$



Use of published kinetic models/parameters

3) The stochastic function of growth Storage() for exposure assessment

- Initial concentration

  $C_0 \sim xxx$ log CFU/g

- Storage conditions

  $Time \sim Pert(5, 16, 48)$ h

  $Temp \sim Pert(4, 7, 12)$ ºC

- Hyperparameters and growth model

  $EGR_5 \sim Lognormal\ (-4.65\ , 0.63)$ log/h

  $T_{min} = -1.18$ ºC

  $MPD = 8$ log CFU/g

  $$Growth = EGR_5 \left(\frac{Temp - T_{min}}{5 - T_{min}} \right) Time$$

- Output

  $$C_f = \begin{cases} C_0 + Growth, & C_0 + Growth < MPD \\ MPD, & C_0 + Growth \geq MPD \end{cases}$$

Use of published kinetic models/parameters

4) Testing the function

- Initial concentration (contaminated fraction)

  $C_0 \sim Normal(-0.5, 0.1)$ log CFU/g

- Cold storage conditions

  $Time = 16$ h $\boldsymbol{Temp = 8\ ^oC}$

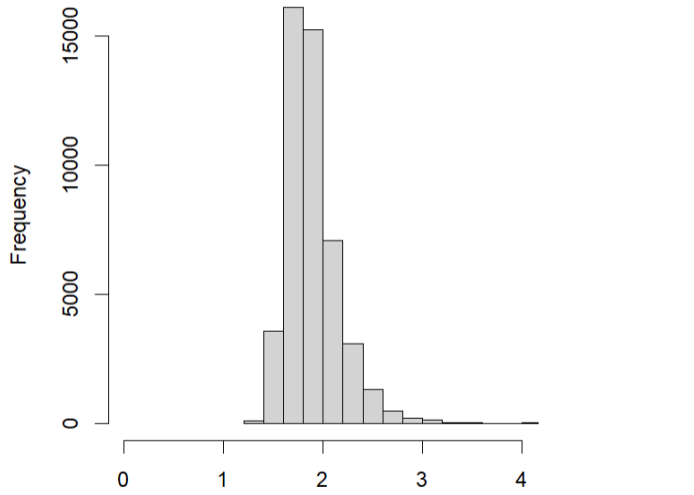
- Initial concentration (contaminated fraction)

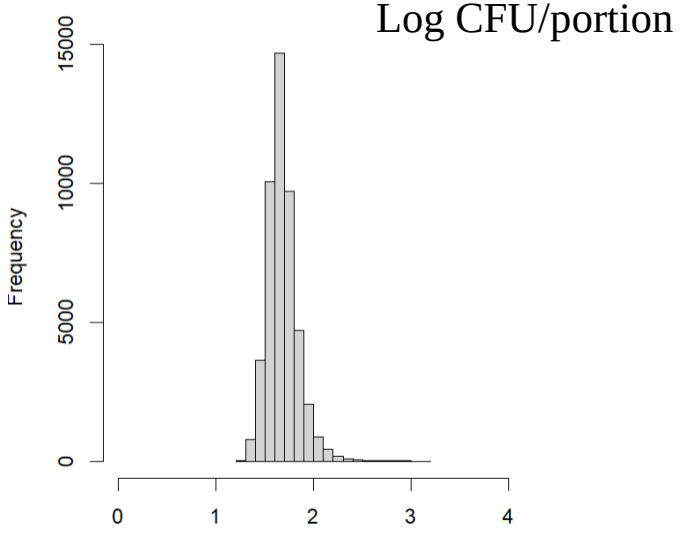
  $C_0 \sim Normal(-0.5, 0.1)$ log CFU/g

- Heat treatment conditions

  $Time = 16$ h $\boldsymbol{Temp = 5\ ^oC}$

- Outputs, Cf

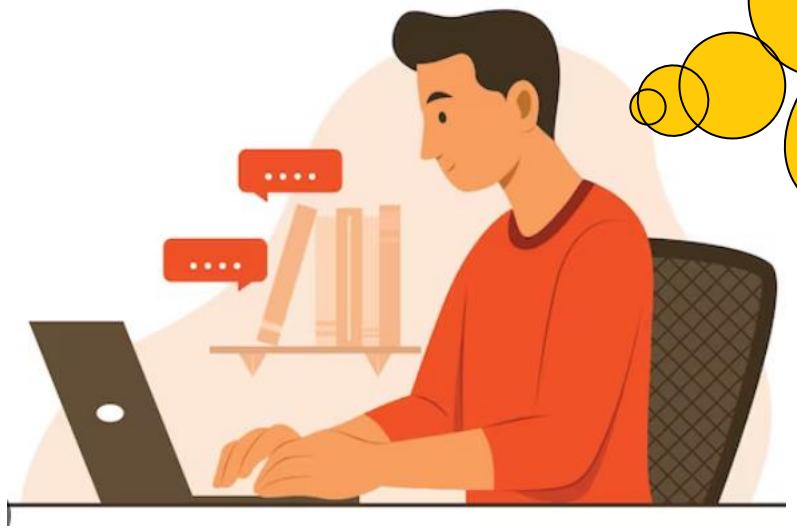




An example of use of growth/inactivation models in exposure assessment

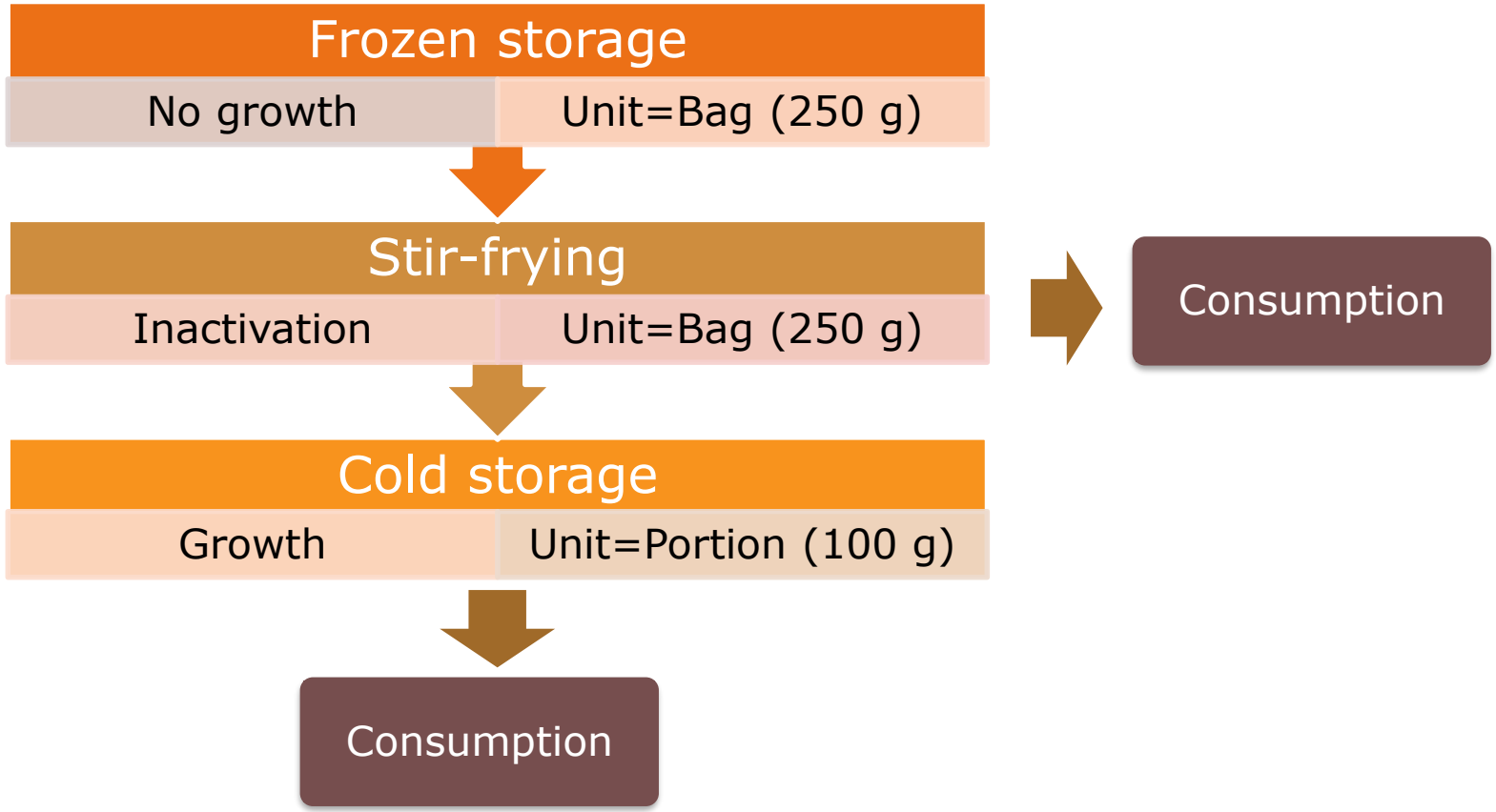
$$Growth = EGR_5 \left(\frac{Temp - T_{min}}{5 - T_{min}} \right) Time$$

$$EGR_5 \sim Lognormal\ (-4.65\ , 0.63)$$



Example

- You wish to build a consumer-phase exposure assessment model of *L. monocytogenes* in frozen stir-fry vegetables to assess the impact of consumer's mishandling on exposure. Make a draft of the conceptual model



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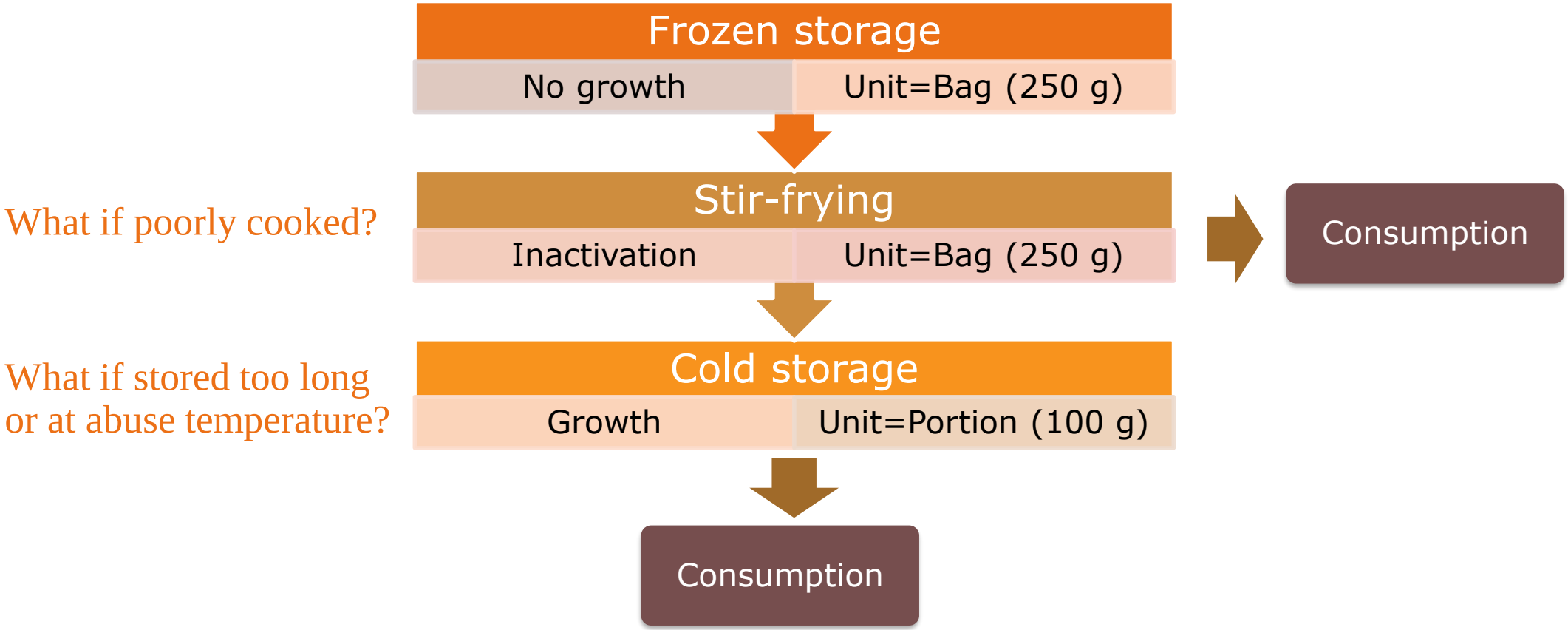
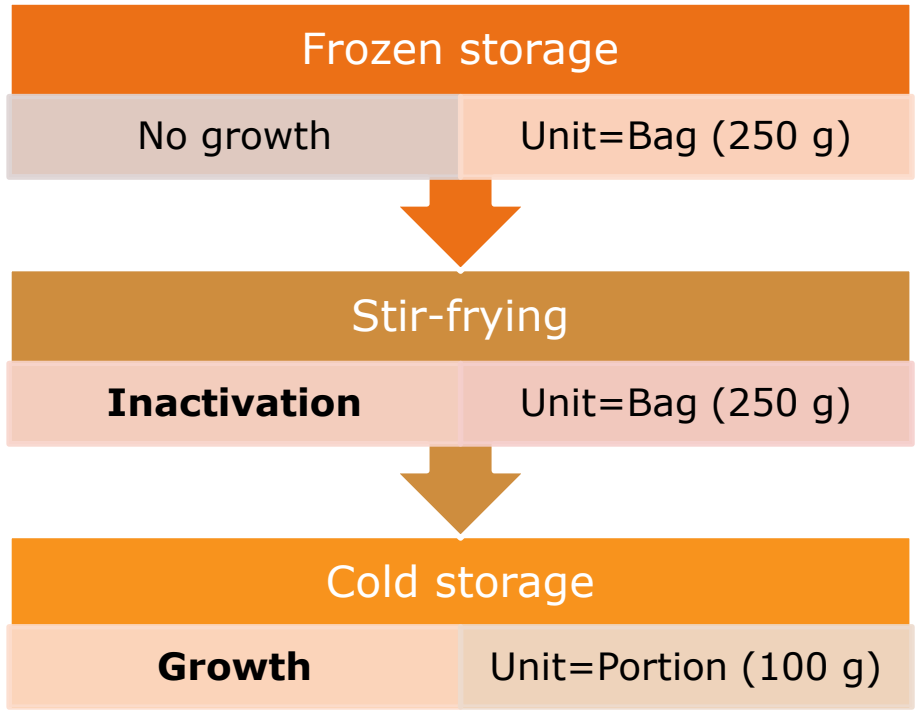


Example

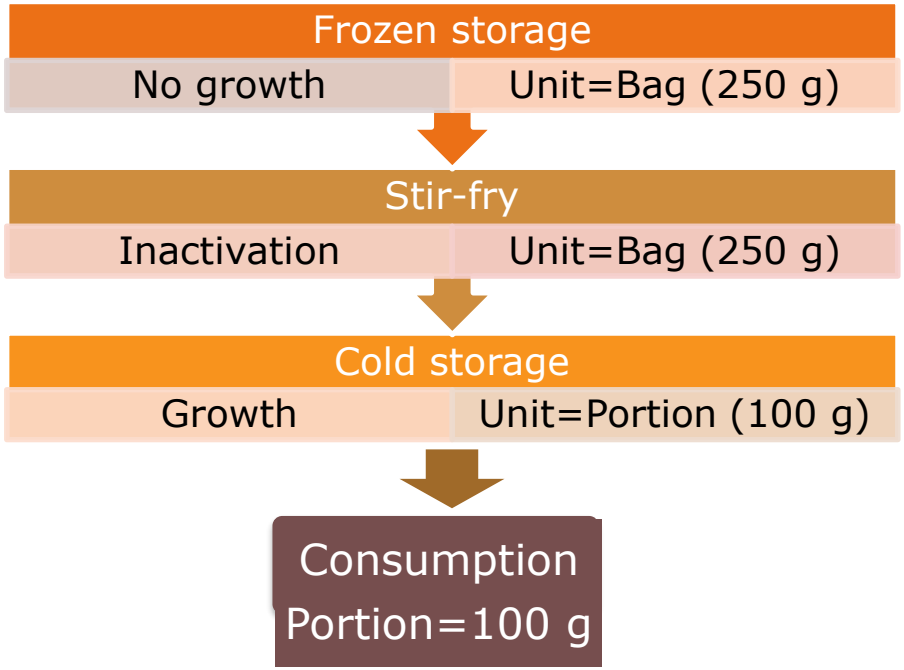


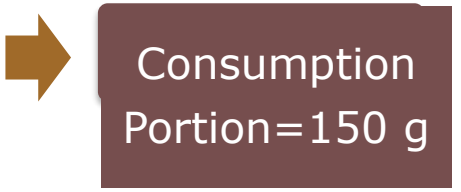
Data collection

- C_0, Concentration of LM in stir-fry frozen vegs at retail
- P, Prevalence of contaminated packs
- Inactivation kinetics of LM in **raw** vegetables
- Growth kinetics of LM in **heat-treated** (cooked) vegetables

Data pre-processing

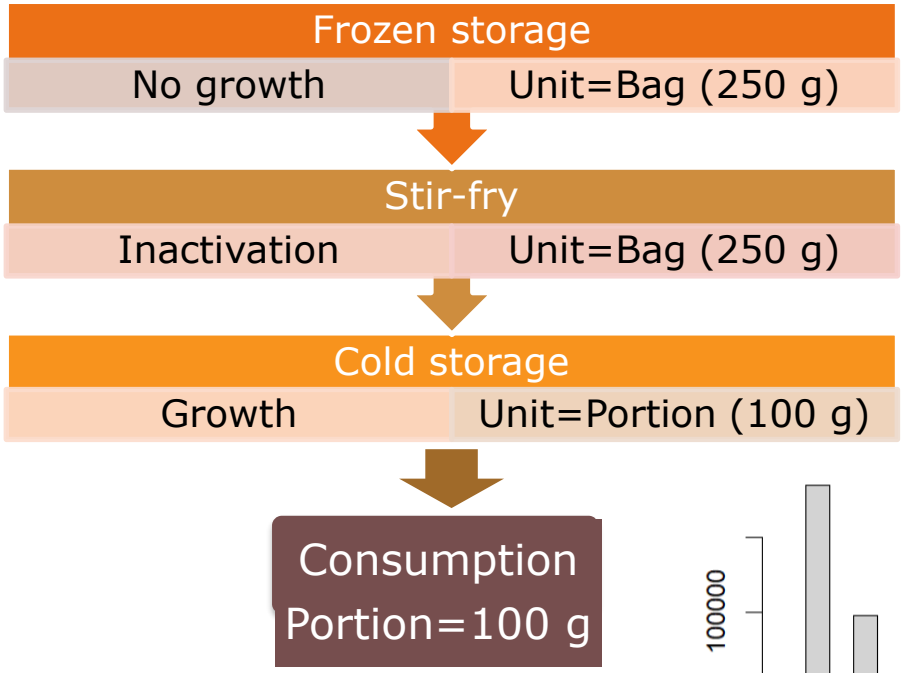
- C_0, Fitting distribution for C_0
- Building the function **MildHeat()**
- Building the function **Storage()**





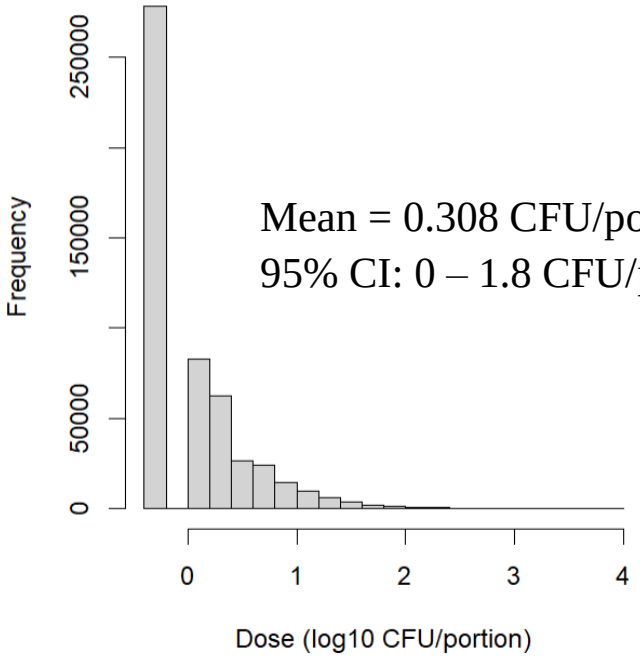
Let us do the job in R studio and estimate exposure

Results

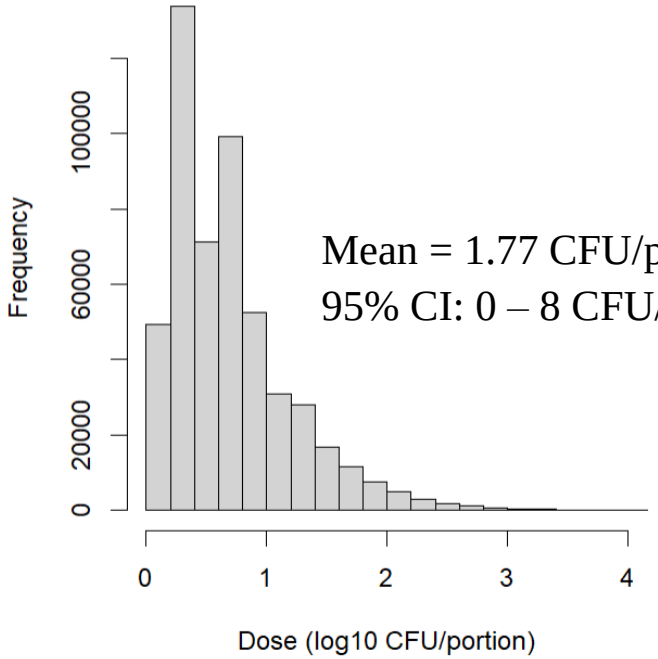


Consumption Portion=100 g

Consumption Portion=150 g



Mean = 0.308 CFU/portion
95% CI: 0 – 1.8 CFU/portion



Mean = 1.77 CFU/portion
95% CI: 0 – 8 CFU/portion

Remember that the analysis applies to the contaminated fraction, which occurs at a probability P

Conclusions

- Knowing the pathogen's kinetics in the target food is a **key requisite** for conducting an exposure assessment

- The conceptual model is a very important step and is ultimately defined by the need for complexity (linked to decision making) and the amount of data available

- Different levels of data pre-processing may be needed when incorporating microbial kinetics into exposure assessments, which go from direct use of validated growth/inactivation parameters to the conduction of mixed-effects models from many published sources

Sources

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Further reading

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Exploitation of challenge testing in exposure assessment of foodborne pathogens

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