

Impact of **uncertainties** and **estimation procedure** inherent to predictive microbiology model construction on compliance with a **food safety objective** within a large range of preservative conditions

Laure Pujol, Sandrine Guillou, Jeanne-Marie Membré





Overview

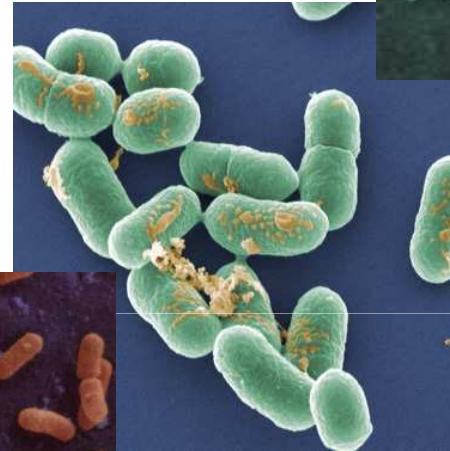
1. Context

2. Aims

3. Data simulation

4. Results : Impact on compliance with a food safety objectives (FSO)

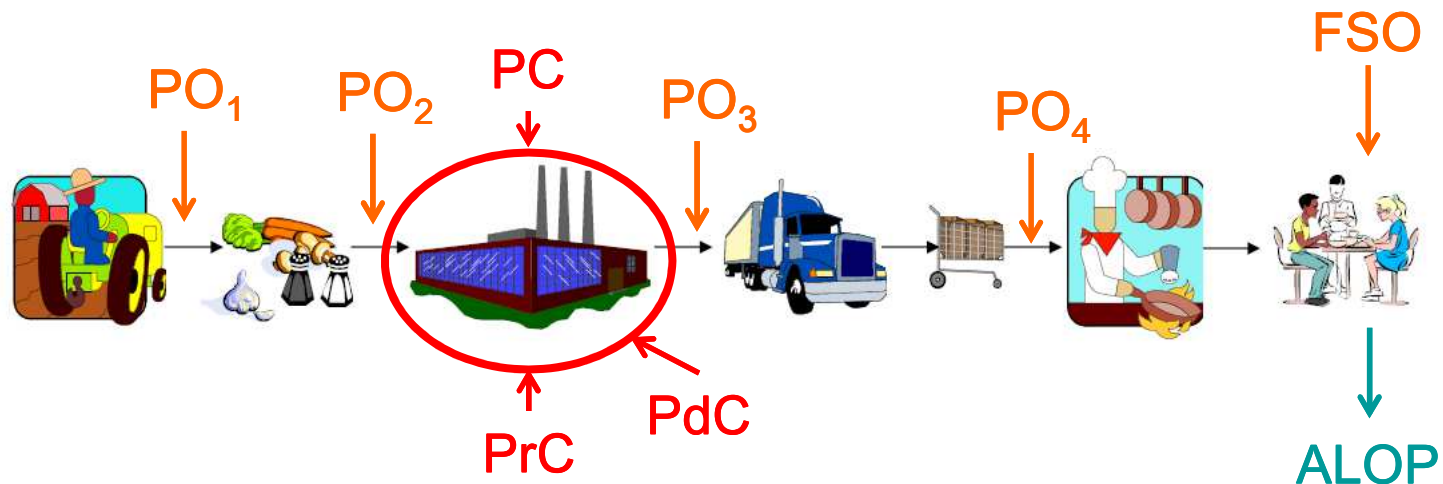
5. Conclusions





1.Context

- Last decade: new food safety management = **risk analysis**
 - New concept :
 - **ALOP**: e.g.: 0.2 cases of Listeriosis per 100 000 people concerned
 - **FSO/PO**: e.g.: < 100cfu/g of Listeria
- Food business operator: compliance with PO/FSO
 - **PC**: e.g. : 3 log reduction
- Operational level → **PrC PdC**
 - PdC: e.g. pH=5
 - PrC: e.g. T<8°C





1. Case study

- **PrC, PdC** → **FSO/PO** : use predictive model
- Ambient stable product (e.g.: “Mayonnaise”)
 - Stable after opening: re-open SL
- Hazard ID: *Listeria monocytogenes*: **FSO=100cfu/g**
- **PdC**: pH, aw, sorbic, acetic and lactic acids
- **PrC**: temperature of storage
- SL = Time to achieve 100cfu/g (FSO)





Overview

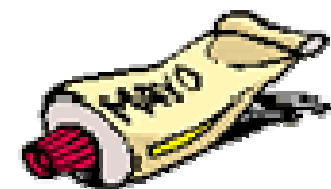
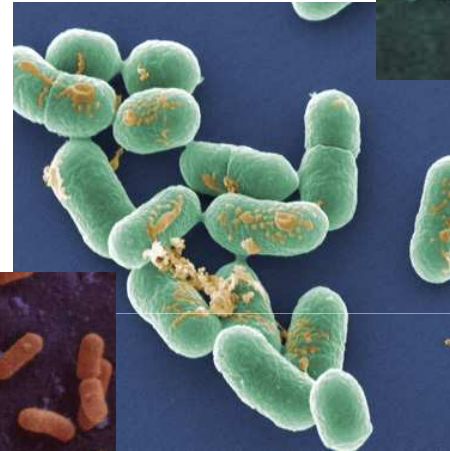
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2. Preliminary study

- *Listeria monocytogenes*: **widely studied** → lots of data generated **BUT** often disparate
- Methodology to **re-use** such data



Establishing Equivalence for Microbial-Growth-Inhibitory Effects (“Iso-Hurdle Rules”) by Analyzing Disparate *Listeria monocytogenes* Data with a Gamma-Type Predictive Model

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INRA, UMR1014 Secalim, Nantes, France^a; LUNAM Université, Oniris, Nantes, France^b; and Unilever Safety and Environmental Assurance Centre, Sharnbrook, United Kingdom^c

- i) developing a predictive model based on existing data
- ii) building experimental design
- iii) validating the model with new data



2. Disparate dataset

T(°C)	pH	a _w	μ _{obs} (h ⁻¹)
20	7.1	0.992	0.049
20	7.1	0.921	0.032
20	7.1	0.929	0.015
6	7.3	0.988	0.037
18	7.3	0.997	0.37
6	7.3	0.997	0.07

$$\mu = \mu_{opt}^1 \times \gamma_T \times \gamma_{pH} \times \gamma_{aw}$$

T, pH, a_w

T(°C)	pH	Acetic acid(%)	μ _{obs} (h ⁻¹)
13	6	0.038	0.050
19	6	0.074	0.078
7	5.6	0.074	0.027
13	5.7	0.074	0.079
3	6.1	0.147	0.016
9	6.1	0.147	0.043

$$\mu = \mu_{opt}^2 \times \gamma_T \times \gamma_{pH} \times \gamma_{acetic}$$

T, pH, acetic acid

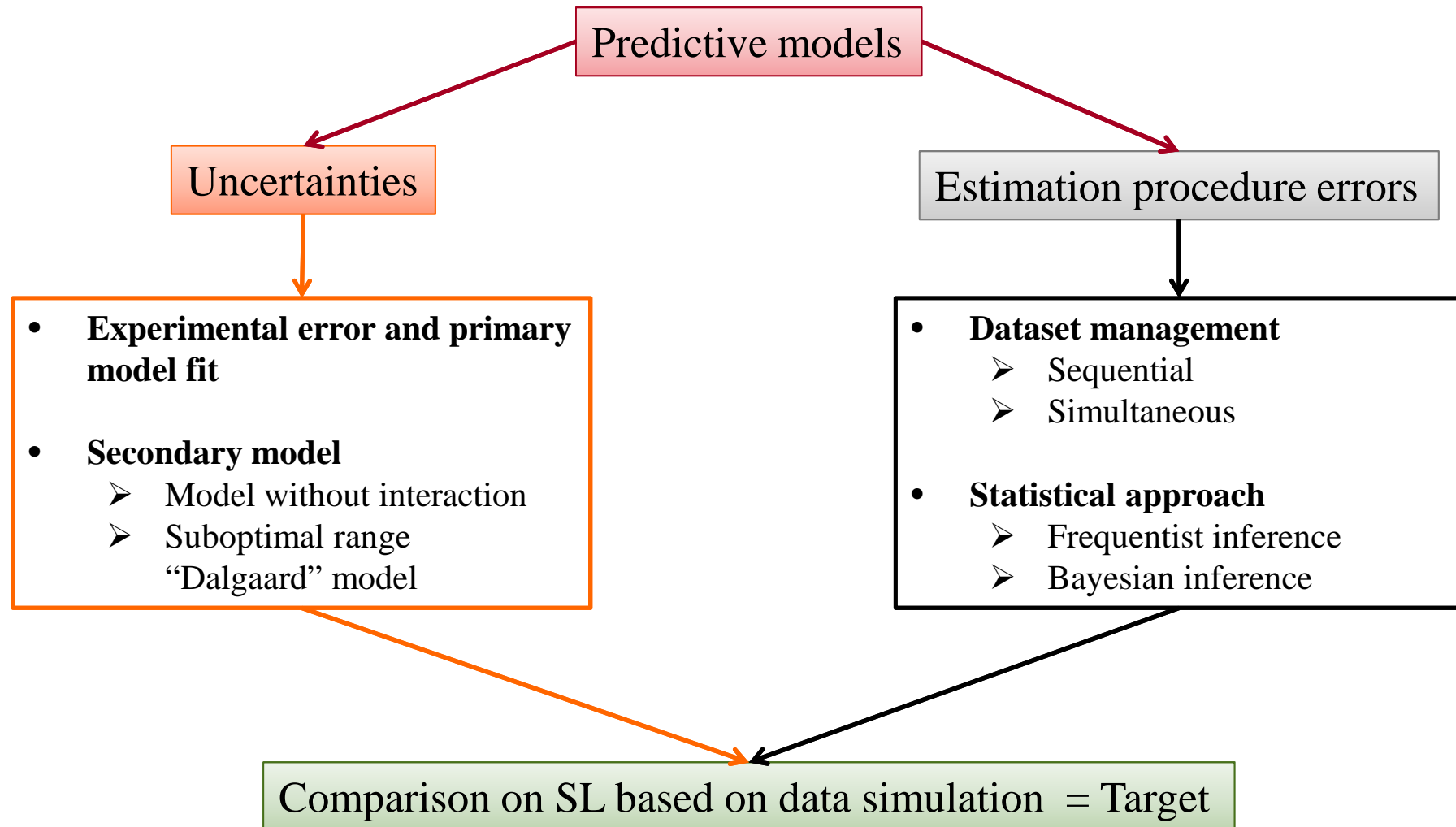
$$\mu = \mu_{opt} \times \gamma_T \times \gamma_{pH} \times \gamma_{aw} \times \gamma_{acetic}$$

Model describing the effect of T,
pH, a_w, and acetic acid

Need to be robust to assess the compliance with FSO/PO



2. Impact of uncertainty and estimation procedure error





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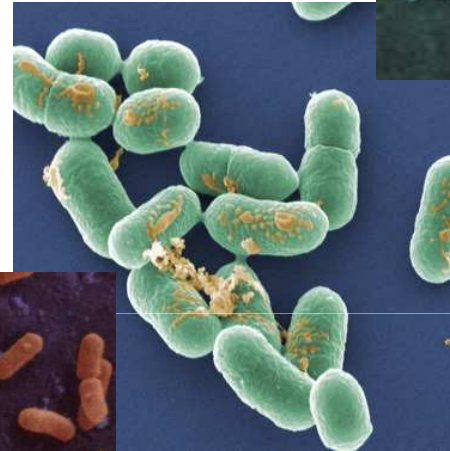
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3. **Data simulation**

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3.Data simulation

- **Rationale:** Analyse only the impact of uncertainty and estimation procedure, whatever the dataset
- **Experimental design :**
 - Factors: T, pH, aw, sorbic, lactic and acetic acid
 - Balanced dataset: full factorial design with repetition for each factor separately
- **Growth rate simulation:**
 - Gamma model of Zwietering et al. 1992 $\sqrt{\mu} = \sqrt{\mu_{opt} \times \gamma(T) \times \gamma(pH) \times \gamma(aw) \times \gamma(acids) \times \xi(T, pH, aw, acids)}$
 - with interaction term of Le Marc et al. 2002
- **Target SL: time to achieve 100cfu/g (t_{100})**

T_{100} for several inhibition:

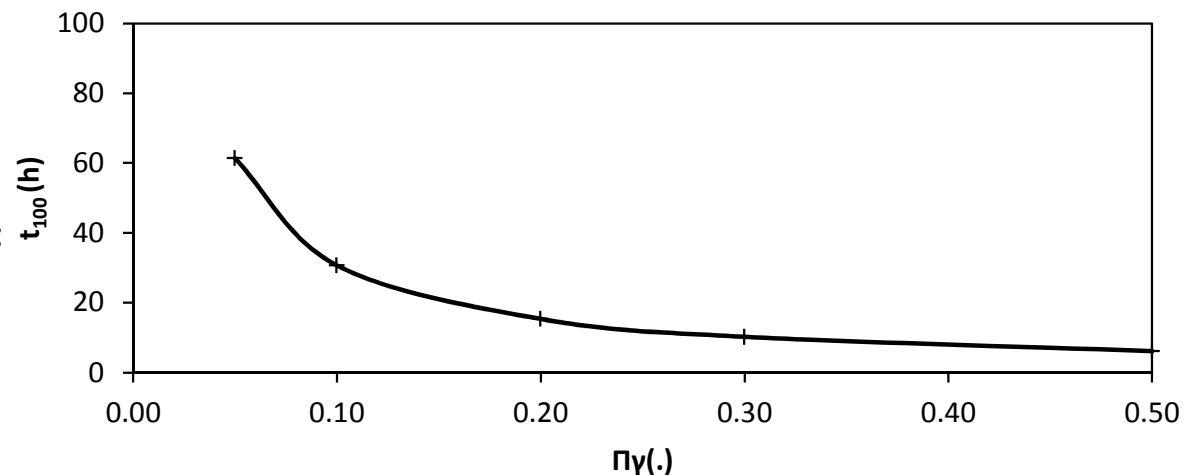
- $N_0 = 1\text{cfu/g}$
- No lag

➤ **From strong inhibitory effect:**

$$\Pi\gamma(.) = 0.05$$

➤ **To mild inhibitory effect:**

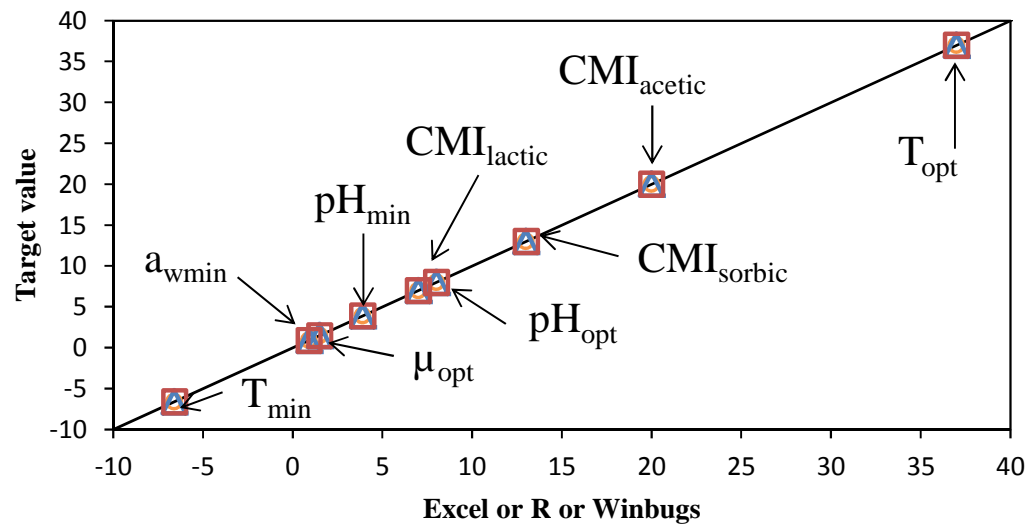
$$\Pi\gamma(.) = 0.5$$





3. Dataset validation

- Model used = Model of the target**

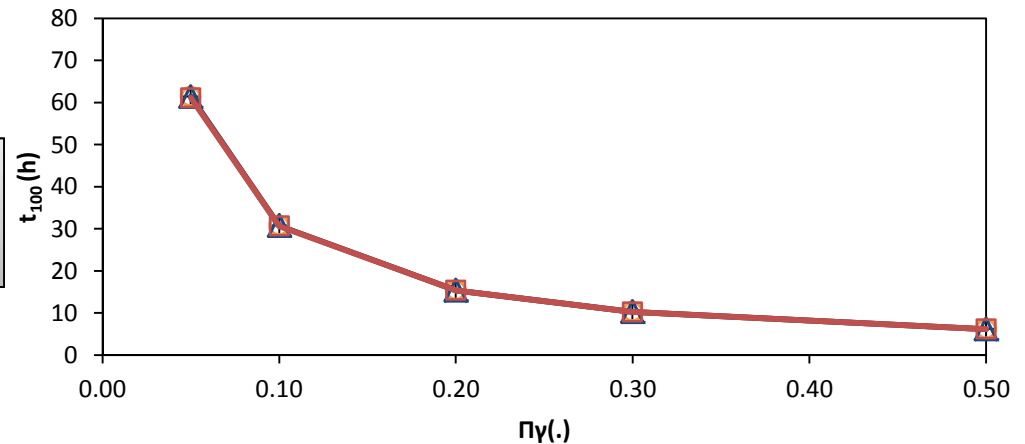


**No difference for the
estimated parameters**

— Excel
— R
— Winbugs

No impact on SL

**Accuracy of the parameter
estimation**





Overview

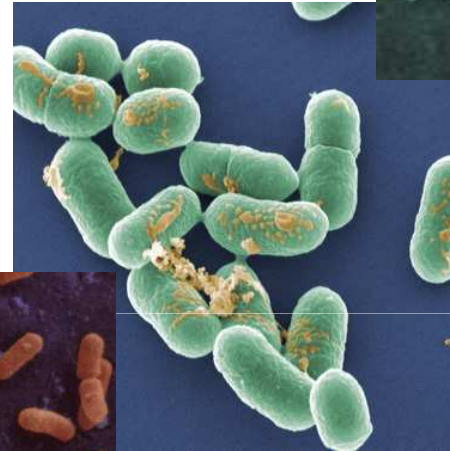
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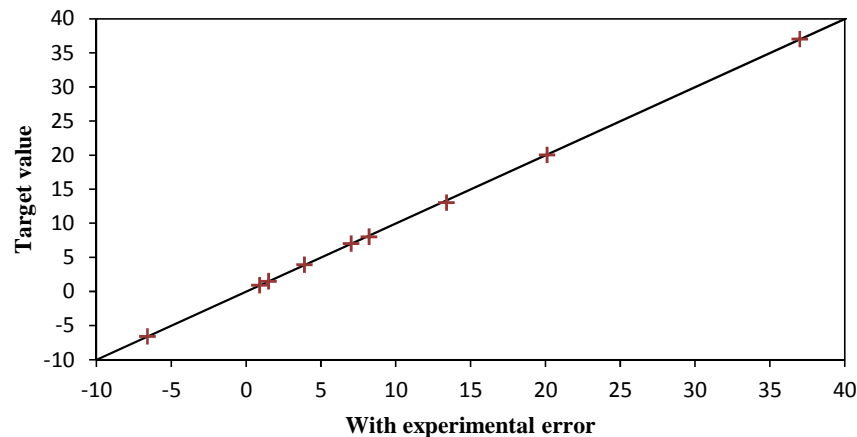




4.1. Uncertainty analysis: experimental error and primary model fit

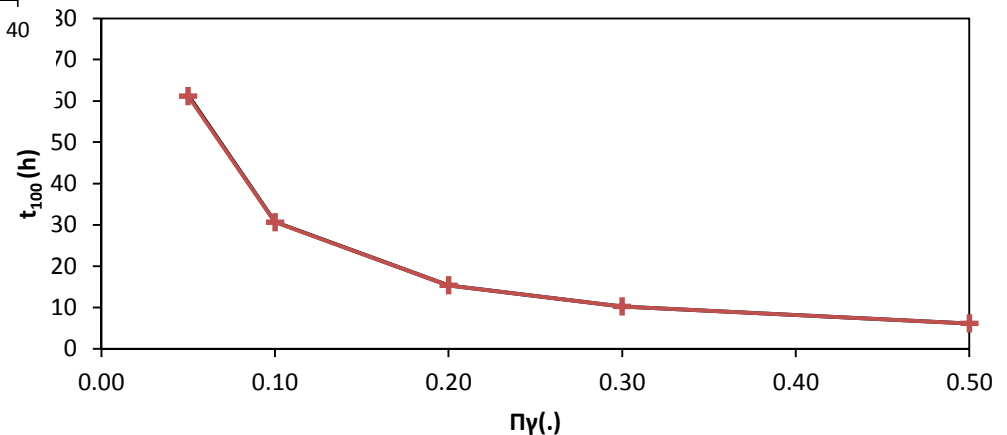
- **Experimental error:** 0.5 log cfu/ml along the logcount curve
- **Primary model:** to fit logcount curve with the experimental error
 - logistic model with delay (Kono 1968)

- **Parameters estimation:**



No difference for the estimated parameters

- **Impact of experimental error and primary model fit on T_{100}**



No impact on the SL



4.2. Uncertainty analysis: secondary model

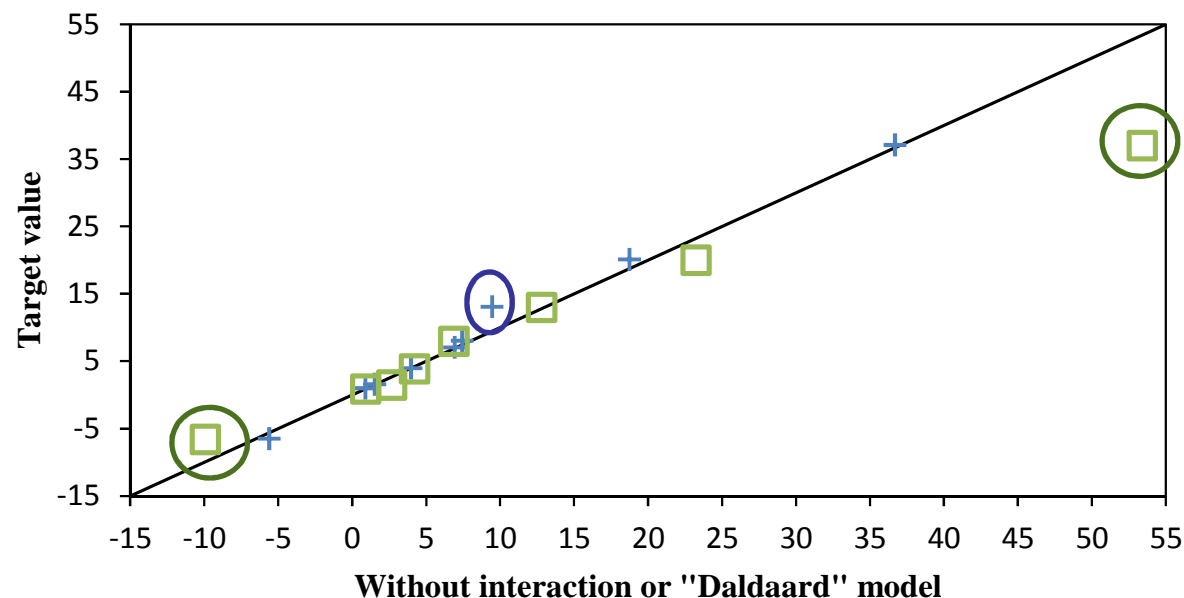
- **2 models tested:**

- **Gamma model without interaction:**

- **Suboptimal range “Dalgaard” model :** simpler form for the cardinal model of γ_T , γ_{pH} and γ_{aw}

$$\sqrt{\mu} = \sqrt{\mu_{opt} \times \gamma(T) \times \gamma(pH) \times \gamma(aw) \times \gamma(acids)}$$

- **Parameters estimation:**



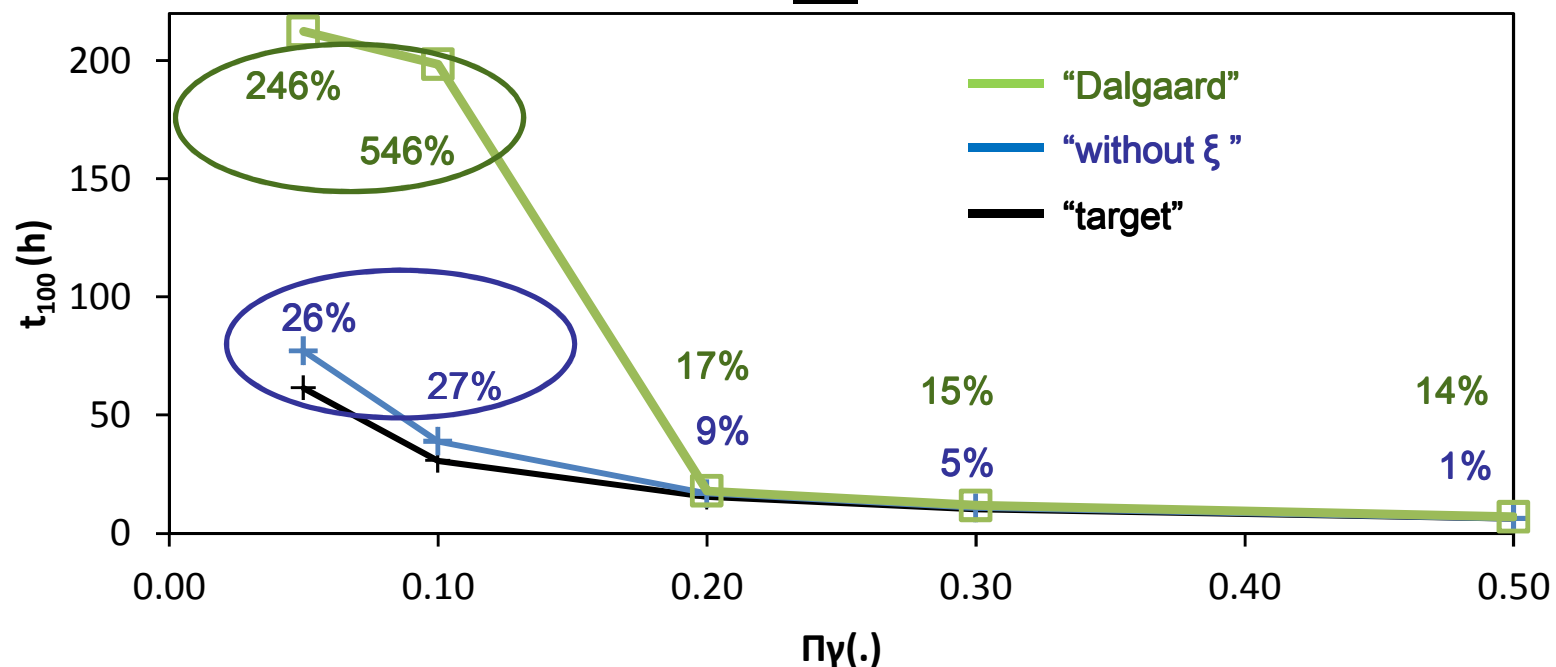
Model without
interaction :
**Slight
difference**

“Dalgaard”
model:
**Huge
difference
(T_{min} , T_{opt})**



4.2. Uncertainty analysis: secondary model

- Impact of secondary model on T_{100}**



- High inhibition $\Pi\gamma(\cdot) < 0.20$: High impact on SL : $\approx 30\%$ of error or $SL \times 2$ or $\times 5$
- Mild inhibition $\Pi\gamma(\cdot) > 0.20$: no big impact

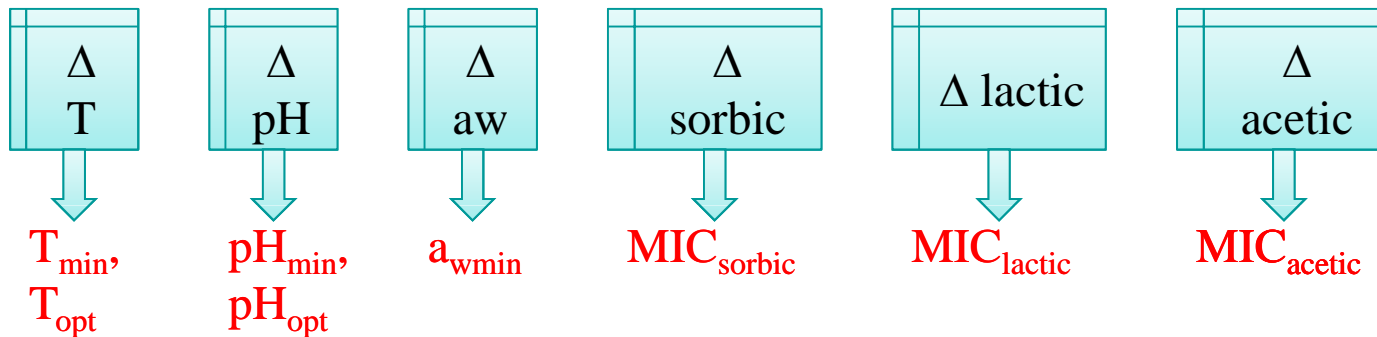
The choice of the secondary model: Important for high inhibition



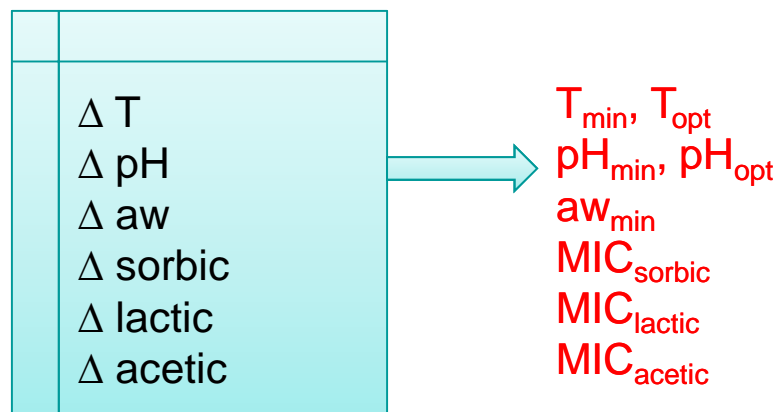
4.3. Estimation procedure analysis: dataset management

- Two dataset management procedure :

– “sequential” :



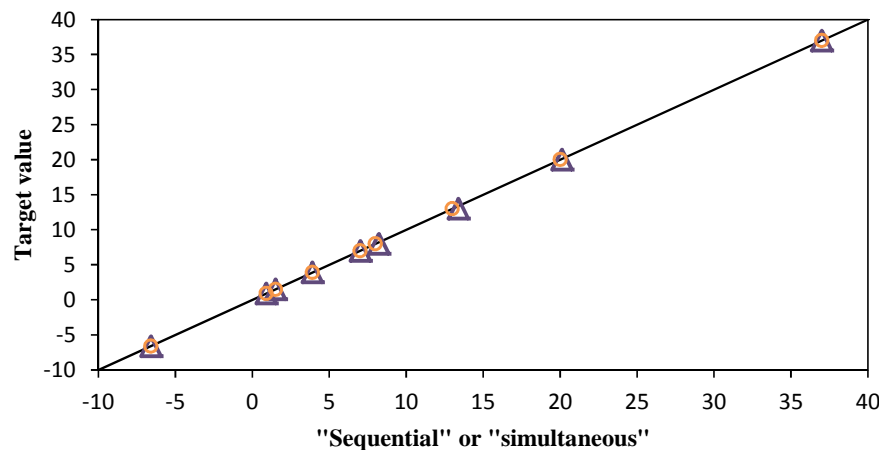
– “simultaneous”:





4.3. Estimation procedure analysis: dataset management

- **Parameter estimation:**

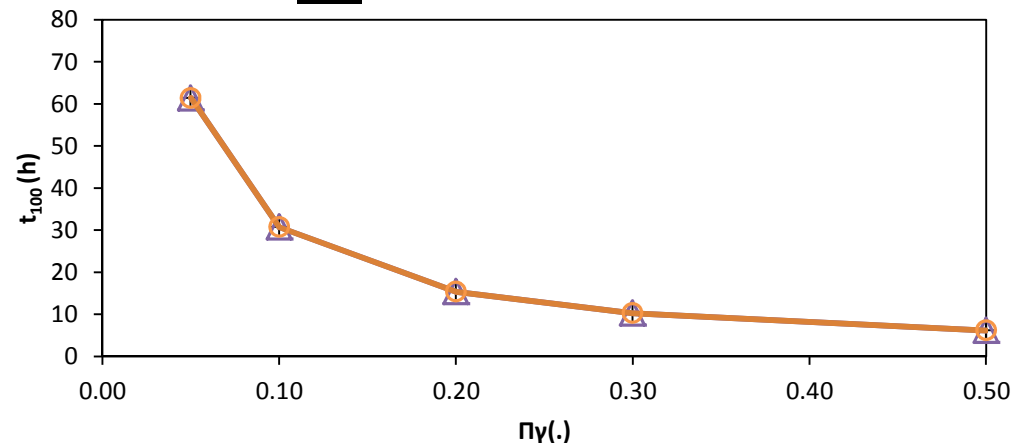


No difference
for the
estimated
parameters

— "sequential"
— "simultaneous"

- **Impact of dataset management on T_{100}**

No impact on the SL



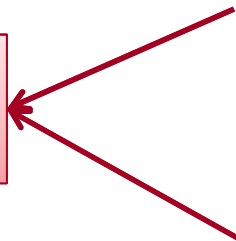


4.4. Estimation procedure: statistical approach

- **Frequentist inference approach:**
 - Least square criterion: **Excel** (add-in Solver)
 - Maximum-likelihood criterion: **R** (nls function and package nlstools)
- **Bayesian inference approach**
 - Markov Chain Monte Carlo method: **WinBugs**
 - Prior :

Parameter	Distribution	Mean	Distribution Percentile		Target value
			2.5%	97.5%	
$\ln\mu_{\text{opt}}$	dnorm(0.01, 0.01)	/	/	/	/
μ_{opt}	exp($\ln\mu_{\text{opt}}$)	1	3.07	3.20×10^8	1,5
T_{min}	dnorm(-1.5, 1)	-1.5	-3.46	-0.46	-6,6
T_{opt}	dnorm(37, 1)	37	35.04	38.96	37
pH_{min}	dnorm(4.2, 10)	4.2	2.24	6.16	3,9
pH_{opt}	dnorm(7, 10)	7	5.04	8.96	7
aw_{min}	dnorm(0.92, 10)	0.92	0.301	1.539	0,9
$\text{MIC}_{\text{sorbic}}$	dnorm(7, 1)	7	5.04	8.96	13
$\text{MIC}_{\text{lactic}}$	dnorm(7, 1)	7	5.04	8.96	8
$\text{MIC}_{\text{acetic}}$	dorm(22, 1)	22	20.04	23.96	20
σ	dunif(0, 10)	5	0.25	9.75	/

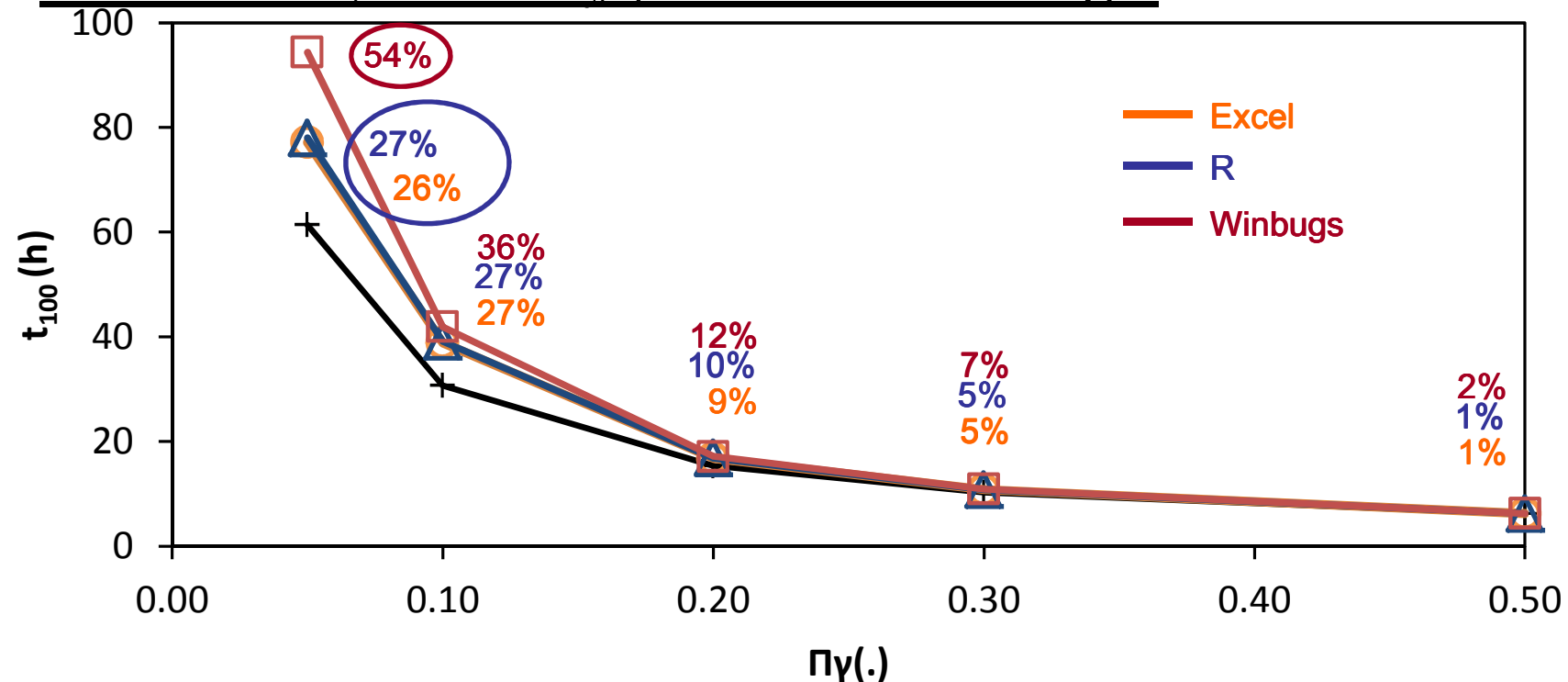
Informative prior





4.4. Estimation procedure: statistical approach

- Model used (without ξ) \neq Model of the target



- 27% due to secondary model choice, **no additional effect** of Frequentist inference approach
- 54% due to secondary model choice **and** the Bayesian inference approach

Bayesian approach: effect of the informative prior



4.4. Estimation procedure: statistical approach

- Prior and estimated parameters for Bayesian inference approach for the model without ξ

Parameter	Prior			Target value	Estimated		
	Mean	Distribution Percentile			Mean	Credibility interval	
		2.5%	97.5%			2.5%	97.5%
μ_{opt}	1	3.0	3.20×10^8	1,5	1,52	1,50	1,55
T_{min}	-1.5	-3.46	-0.46	-6,6	-5,46	-5,88	-5,05
T_{opt}	37	35.04	38.96	37	36,61	36,65	37
pH_{min}	4.2	2.24	6.16	3,9	3,99	3,93	4,04
pH_{opt}	7	5.04	8.96	7	6,93	6,83	7,04
aw_{min}	0.92	0.301	1.539	0,9	0,903	0,900	0,907
$\text{MIC}_{\text{sorbic}}$	7	5.04	8.96	13	8,99	8,14	9,93
$\text{MIC}_{\text{lactic}}$	7	5.04	8.96	8	7,16	6,65	7,45
$\text{MIC}_{\text{acetic}}$	22	20.04	23.96	20	19,3	18,43	20,2
σ	5	0.25	9.75	/	0,031	0,029	0,034

Prior too informative and false → poor estimation (for model \neq target model)



4.4. Estimation procedure: statistical approach

- **Model implementation**

Model	Excel	R	Winbugs
“Experimental error”	😊	😊	😞
Without ξ	😊	😊	😊
“Dalgaard”	😊	😞	😞
“Sequential”	😊	😊	😊
“Simultaneous”	😊	😊	😞

- **Excel**: easy-to-use software **BUT** only parameters value
- **Winbugs**: more information **BUT** difficult to run with a complex model
- **R**: more information (correlation matrix and confidence interval)

R = Good compromise



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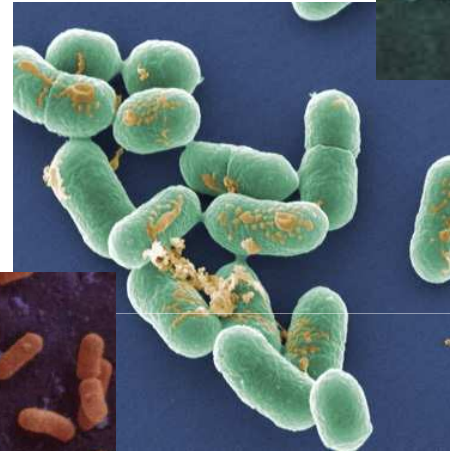
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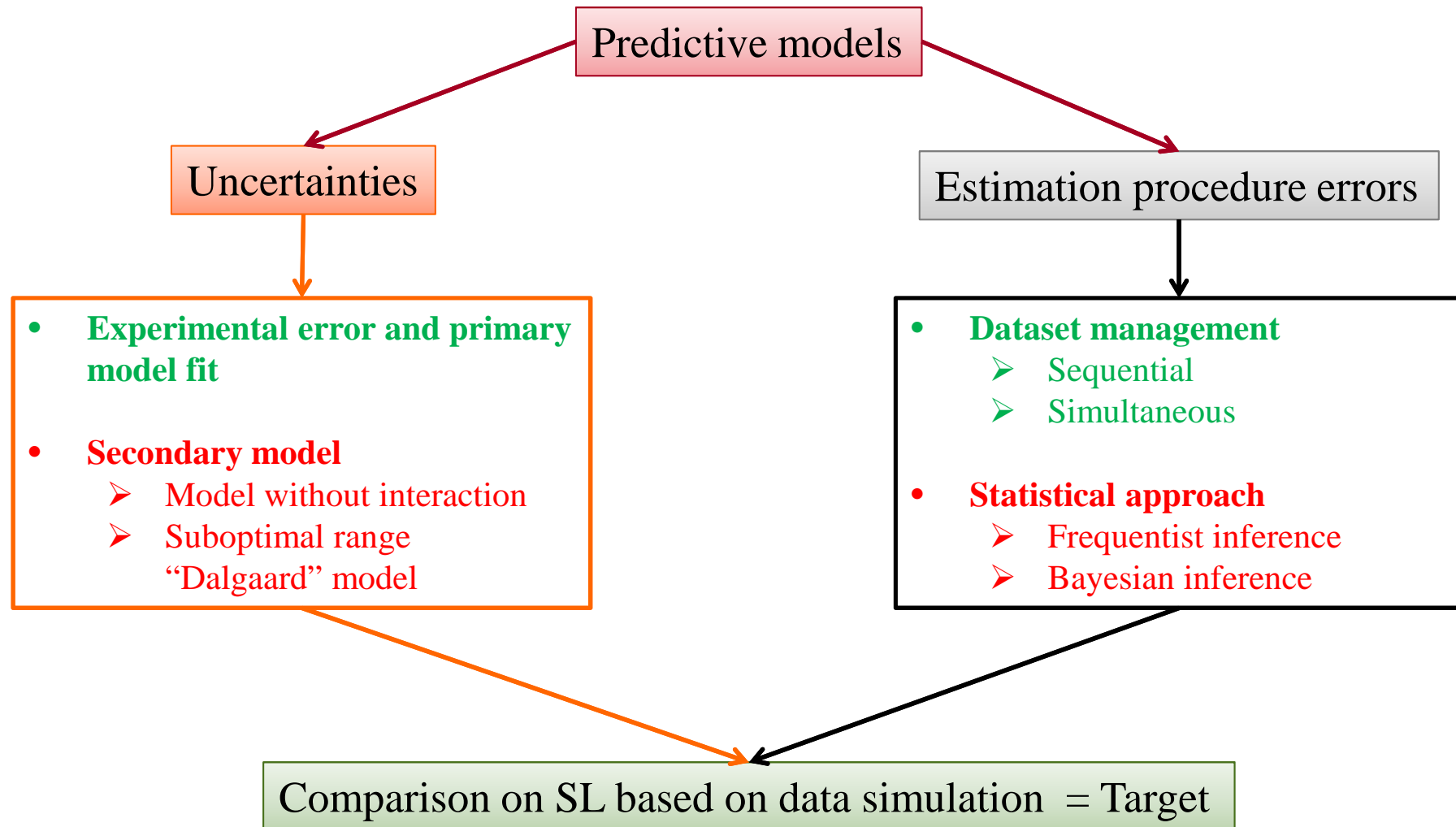
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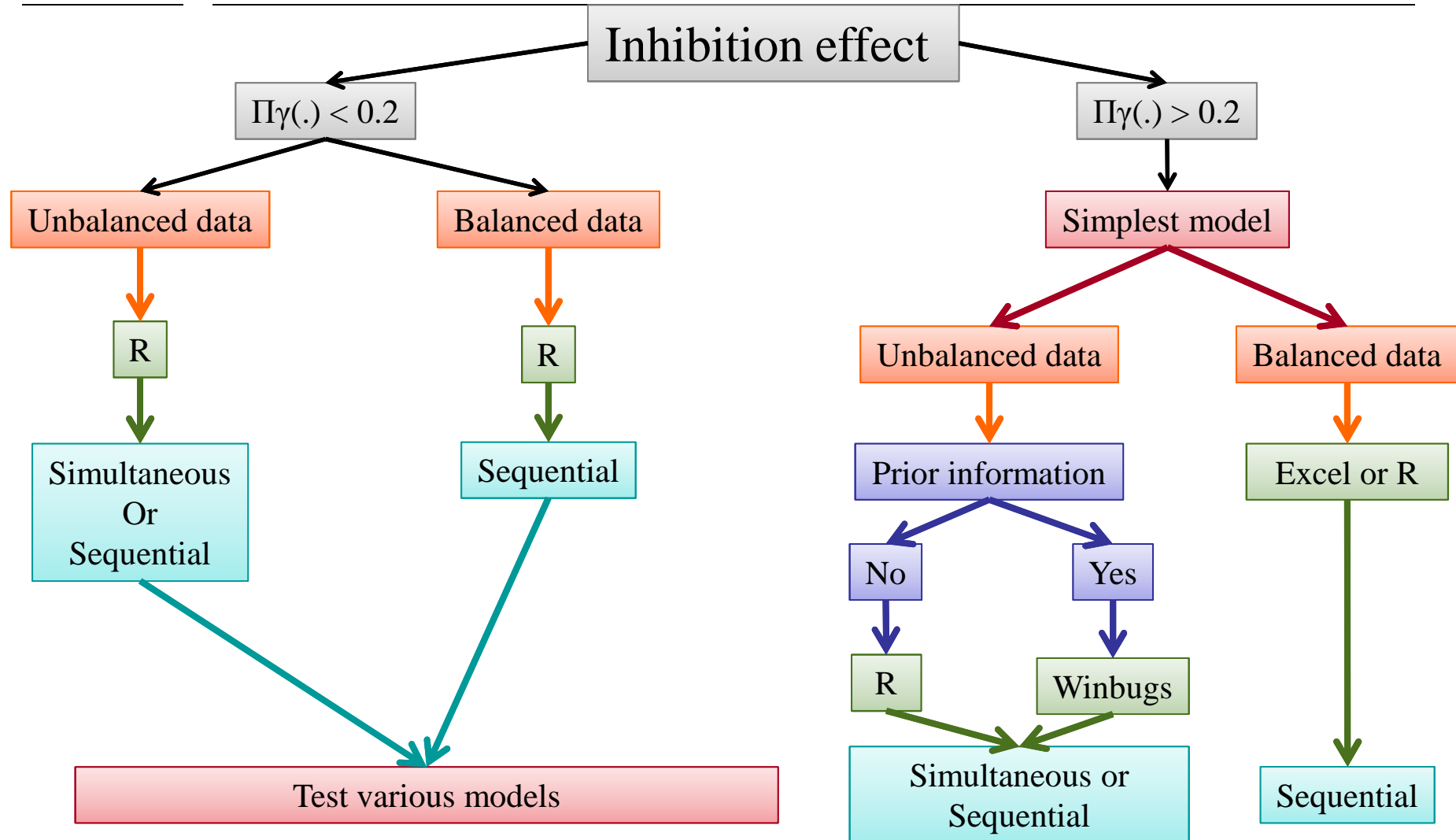


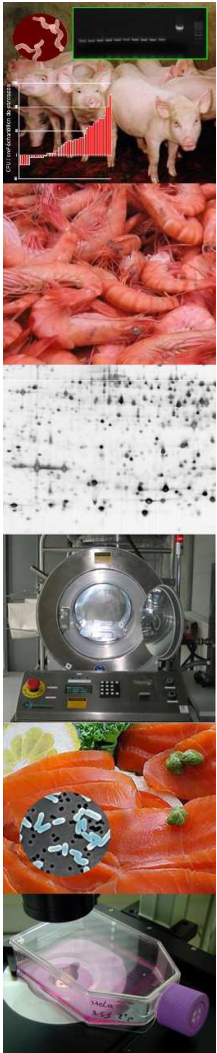
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Thank you for your attention

