

**Population PKPD modelling of enrofloxacin for the three major trout pathogens *A. salmonicida*, *F. psychrophilum* and *Y. ruckeri* according to fish ploidy**

Alexis Viel - DVM, PhD, ECVPT Resident  
Anses - France

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- Salmonid production is increasing worldwide
  - Rainbow trout : the most frequent (freshwater)
  - Triploidy → sterility, increase weight/growth...



*Oncorhynchus mykiss*

- Main bacterial diseases → economic losses
  - G- : *Yersinia ruckeri* ; *Aeromonas salmonicida* ; *Flavobacterium psychrophilum*
- Enrofloxacin (ENRO) in trout → off-label used (« the cascade »)
  - Dosing regimen based without PKPD concept, nor epidemiological data.

**« Standard dose » : 10 mg/kg/day for 5-10 days, oral route  
(metaphylaxis)**

*(Bowser et al, 1990,1992; Hsu et al., 1995)*

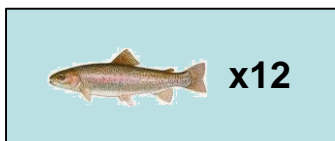
- **Revise the "standard" dose of ENRO thanks to a PKPD approach:**
  - (i) develop a popPK model in rainbow trout (new PK data); identification of relevant physiological covariates (ploidy, weight)
  - (ii) collection of MIC data for *Y.ruckeri*, *A. salmonicida* and *F. psychrophilum* and calculate provisional epidemiological cut-off (COwt)
  - (iii) PKPD integration and Monte Carlo simulations (MCS) to calculate optimal dosing regimens (PTA)

## PK experiment

T~11° C  
Open system

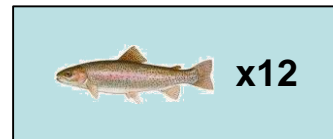
Oral route  
(gavage)

5 mg/kg

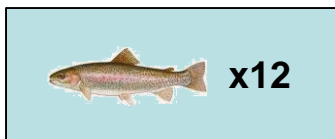


IV bolus

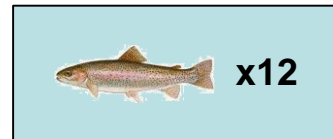
5 mg/kg



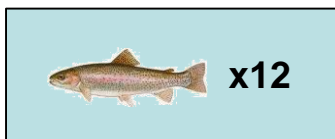
10 mg/kg



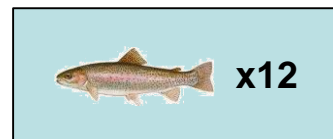
10 mg/kg



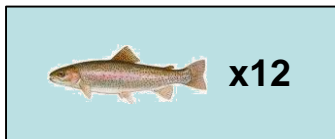
20 mg/kg



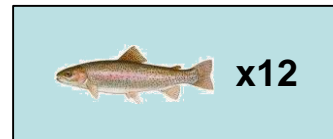
30 mg/kg



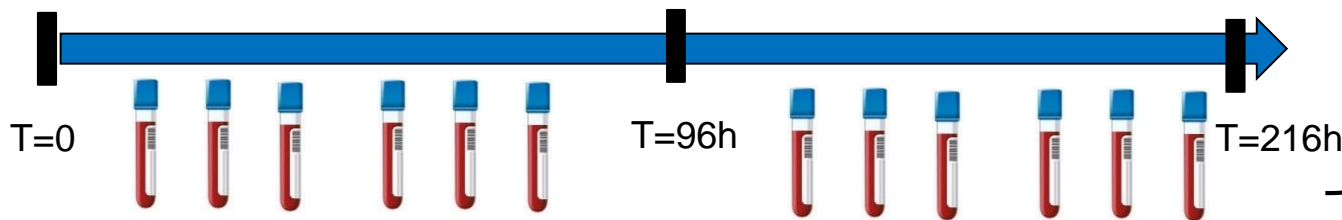
40 mg/kg



60 mg/kg



Same design  
for diploid  
and triploid  
( → 2 x 48  
juvenile trout)



Individual rich blood sampling → HPLC method

## Enrofloxacin MIC data

- MIC data from Anses and Oniris laboratories
- +
- MIC data from literature



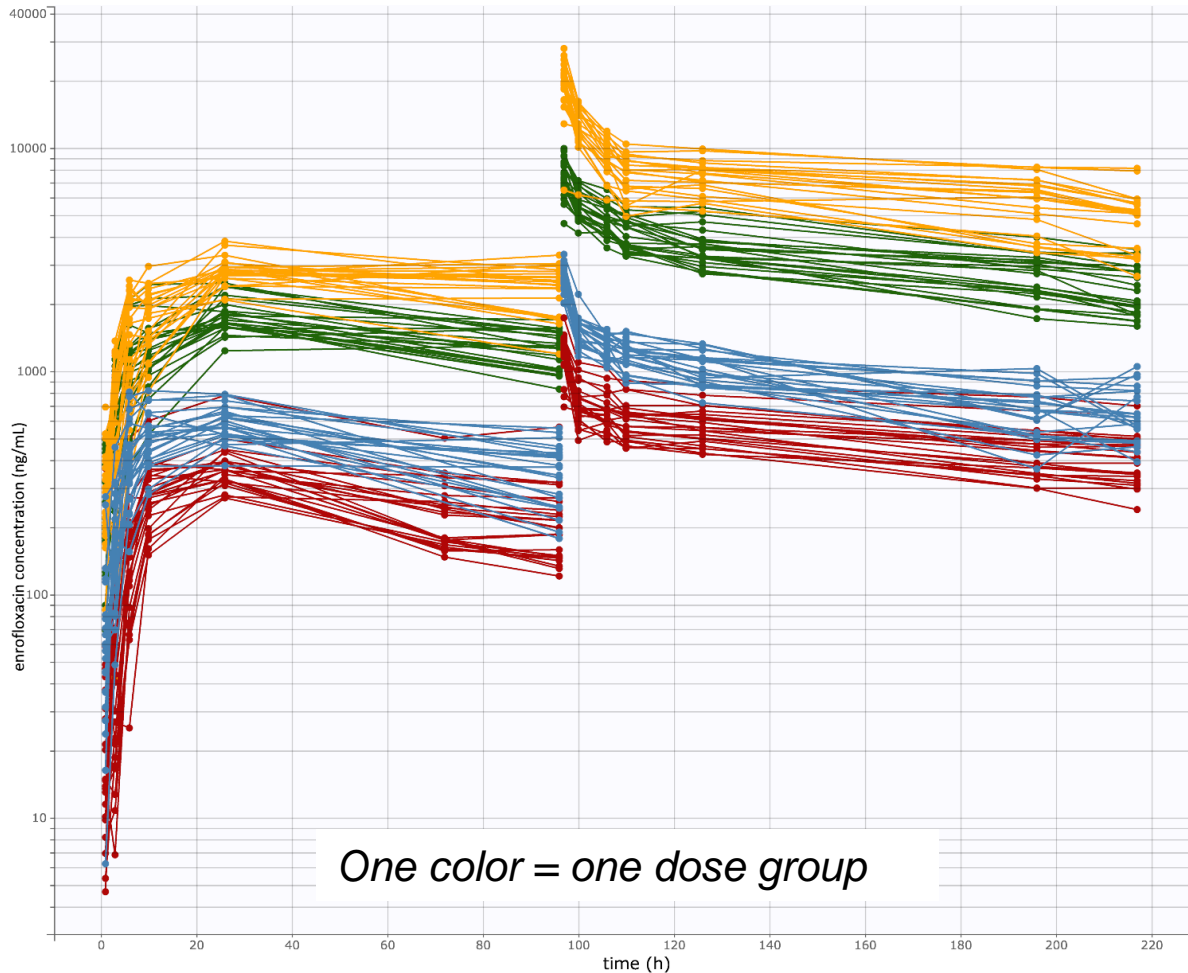
Only broth  
microdilution method +  
NRI analysis → **COwt**  
*(Kronvall, 2010)*

## Modeling approach

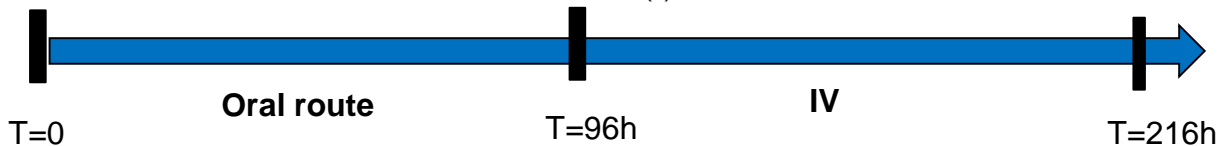
- Population PK model (NLME) using Monolix
- PKPD integration/MCS using simuX (R)



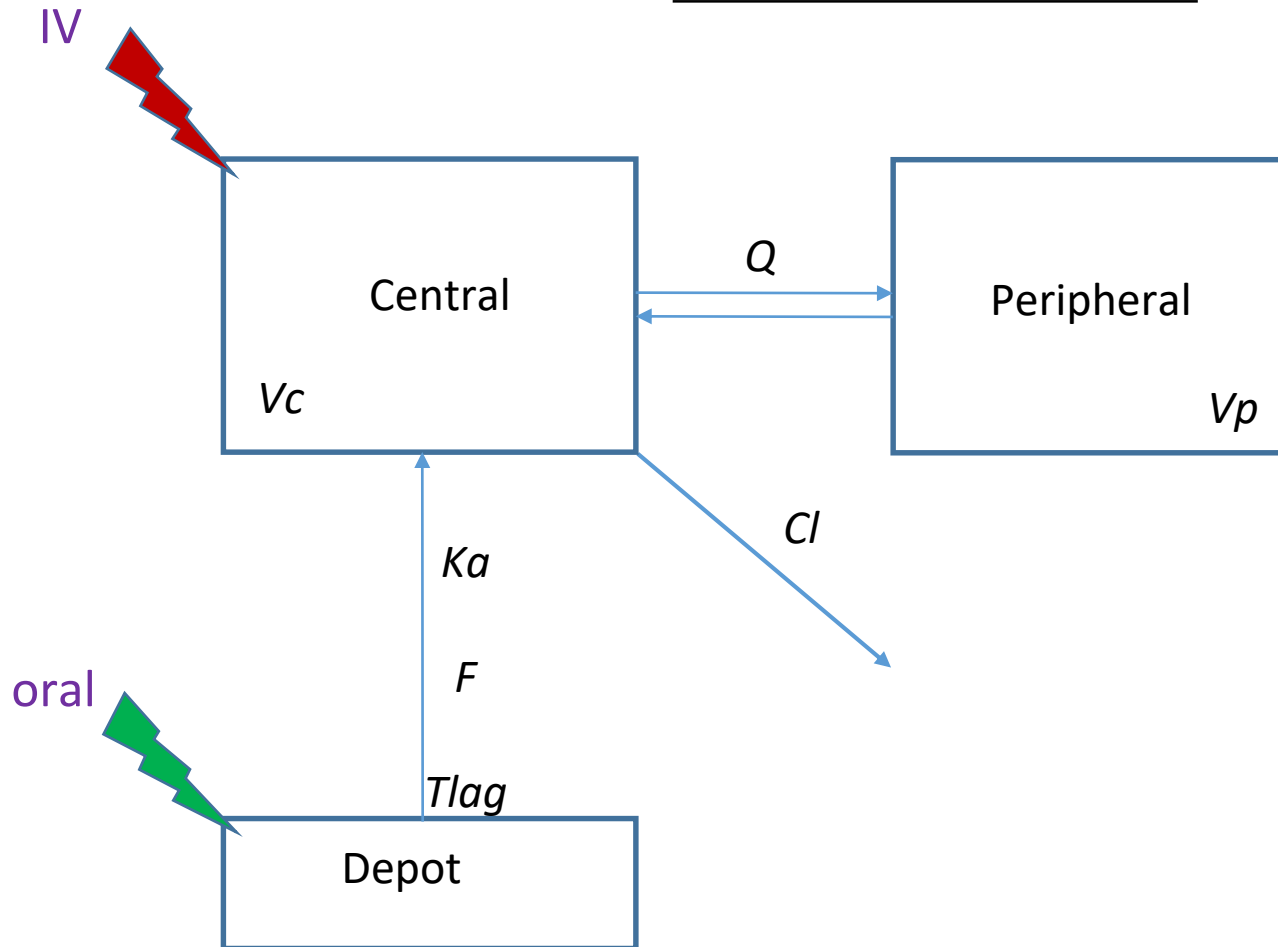
## PK raw data



- ➔ Long Half-life
- ➔ No « flip-flop »
- ➔ Dose proportionality
- ➔ Bi-compartmental

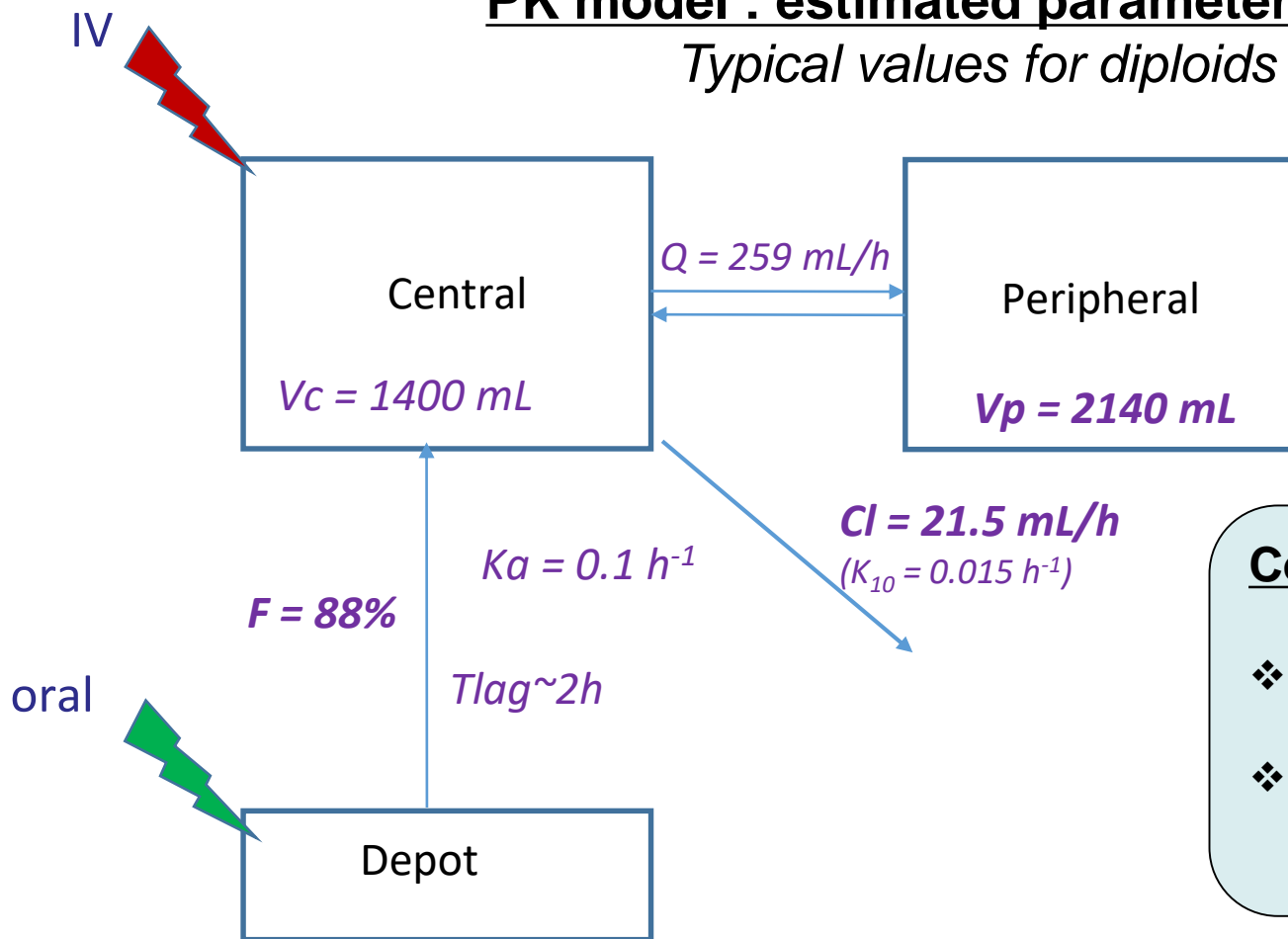


## PK model : structure



## PK model : estimated parameters

*Typical values for diploids*



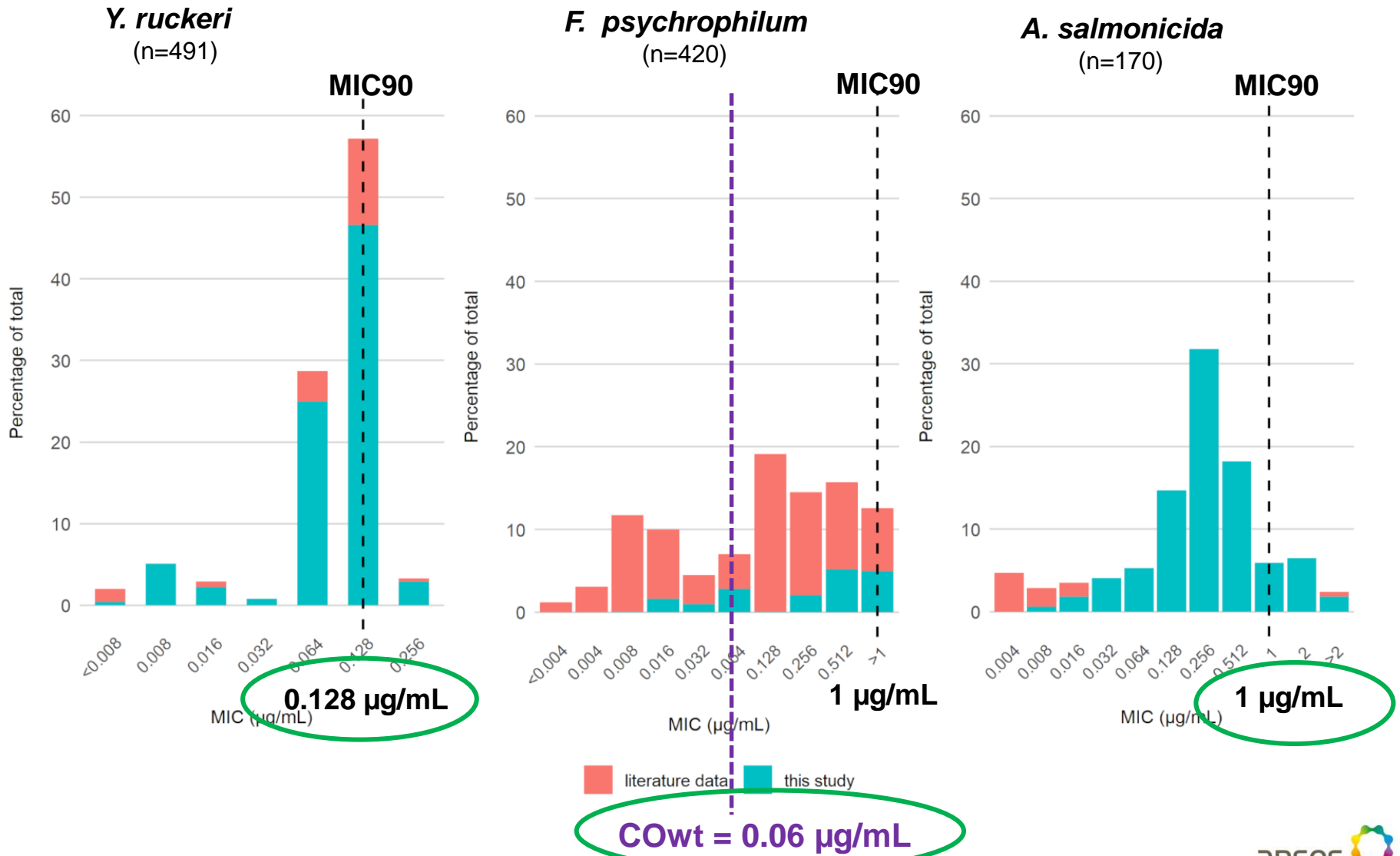
### Covariate analysis :

- ❖ BW  $\rightarrow$   $V_c$ ,  $V_p$ ,  $Cl$
- ❖ Ploidy = triploid  
 $\downarrow$ 30%  $Cl$  and  $\downarrow$ 40%  $K_a$ ,  
 $\uparrow$   $T_{lag}$

**$\rightarrow$  Triploid fish absorb and eliminate ENR more slowly vs diploid fish ( $T_{1/2}$  : 115h dip vs 150h tri)**



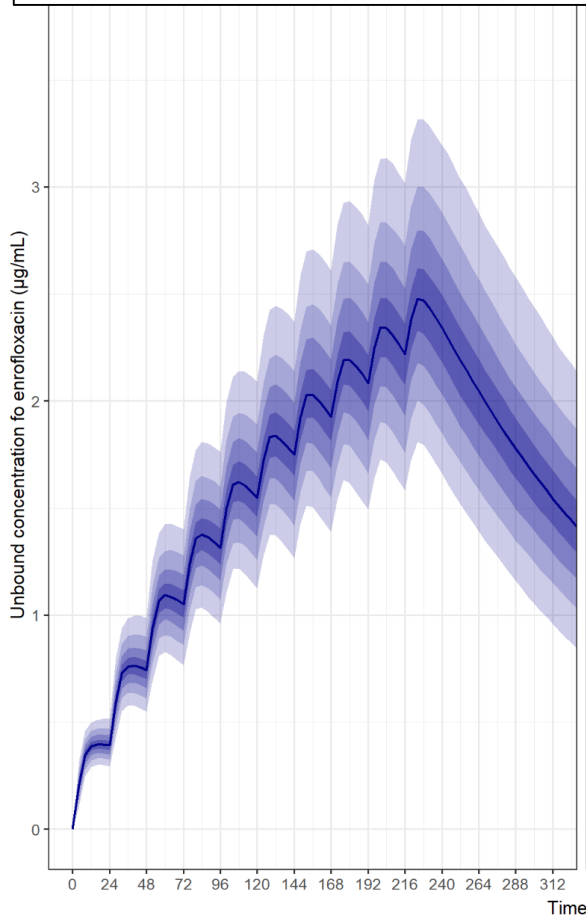
## MIC data and PKPD breakpoint



## PKPD integration (MCS) for the standard dose

FluoroQ → PKPD index =  $fAUC/MIC > 100-125 \text{ h}$  (*steady-state, over 24h*) but pathogen-specific  
 → Range from 50 to 125h

Simulated plasma concentrations  
(10mg/kg/d, diploid)



Time to reach PKPD breakpoint value

		Diploid			
		Value of fAUC/MIC			
		50h	75h	100h	125h
MIC (µg/ml)	≤0.03	24h	24h	24h	24h
	0.06	24h	24h	48h	48h
	0.12	48h	48h	48h	72h
	0.25	48h	72h	120h	144h
	0.5	120h	168h	>192h	NA
	1	>192h	NA	NA	NA
	2	>192h	NA	NA	NA
	≥4	NA	NA	NA	NA

→ ENRO behaves as a long-acting drug in trout

## PKPD integration (MCS) – Optimal doses

$$\text{Loading/Single Dose}_{\tau} = R_{\tau} \times \frac{CL \times \tau \times SF \times MIC}{f_u \times F} \quad (\text{Toutain et al., 2004})$$

with: -  $\tau$  = 4 or 5 days (96-120h)

- PKPD index as SF = fAUC/MIC/ $\tau$  (Toutain et al., 2007)

- MIC : *MIC distribution* < *COwt* \* or *Fixed MIC90* #

### Calculated dose by MCS (mg/kg)

	Bacteria specie	Duration of activity							
		96h				120h			
		Value of SF (PKPD index)				Value of SF (PKPD index)			
		2	3	4	5	2	3	4	5
Diploid	<i>F. psychrophilum</i> *	2.0	3.0	4.0	5.0	2.3	3.4	4.6	5.7
	<i>Y. ruckeri</i> #	5.7	8.6	11.5	14.3	6.6	9.9	13.2	16.5
	<i>A. salmonicida</i> #	47.8	71.7	95.6	119.5	55	82.5	110	137.5
Tripliod	<i>F. psychrophilum</i> *	1.7	2.6	3.4	4.3	1.9	2.9	3.8	4.8
	<i>Y. ruckeri</i> #	4.8	7.2	9.6	12	5.3	8	10.7	13.3
	<i>A. salmonicida</i> #	40.1	60.1	80.2	100.2	44.4	66.6	88.8	111

<< standard dose



<< standard dose



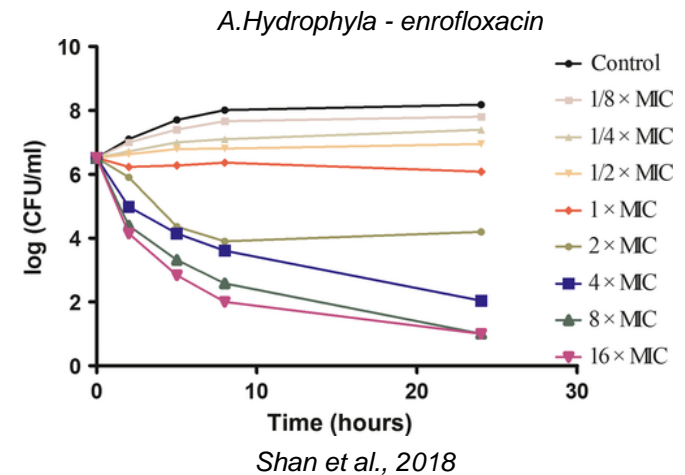
## PK

- NLME approach → Ploidy status affects the ENRO PK.  
→ **Adapt the dose to the ploidy status ?**
  - Hyp: differences in the intestinal transporters expression and/or in the proteome(metabolic protein) ? (To, *et al.*, 2019; Babaheydari, *et al.*, 2017)
  - Other relevant covariate : water temperature
- Long  $t_{1/2}$  → daily administrations not justified
  - One administration for 4-5 days of sufficient exposure
  - Huge decrease of the amount of ATB to be used  
**except for *A. salmonicida***

**Decrease the risk of ATB-R**

## PD / MIC

- Lack of epidemiological data for the 3 bacteria
  - No clinical BP nor ECOFF.
- Need to refine the PKPD index value (fAUC/MIC)
  - ➔ TK assay at relevant temperature + PKPD modelling
  - ➔ magnitude of bacterial killing for each bacteria specie.



➤ **Calculated dose from this work need to be clinically confirmed**

- **One Health approach :**
  - Use vaccines (ex: *Y. ruckeri*)
  - Use other route of administration (IM, IP) to ensure dose compliance

## **TAKE home messages**

- **Ploidy status could affect PK of antibiotic in trout**
- **Need of a proper PKPD approach for old molecule in fish production**
- **Need to increase the collection of epidemiological data for fish pathogenic bacteria**

# Thanks for your attention

## Acknowledgment

- ONIRIS : A. Rostang and S. Calvez
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