Toxico-Kinetic (TK) modelling

Theoretical and practical aspects

Illustration for aquatic ecosystems

MOSAIC

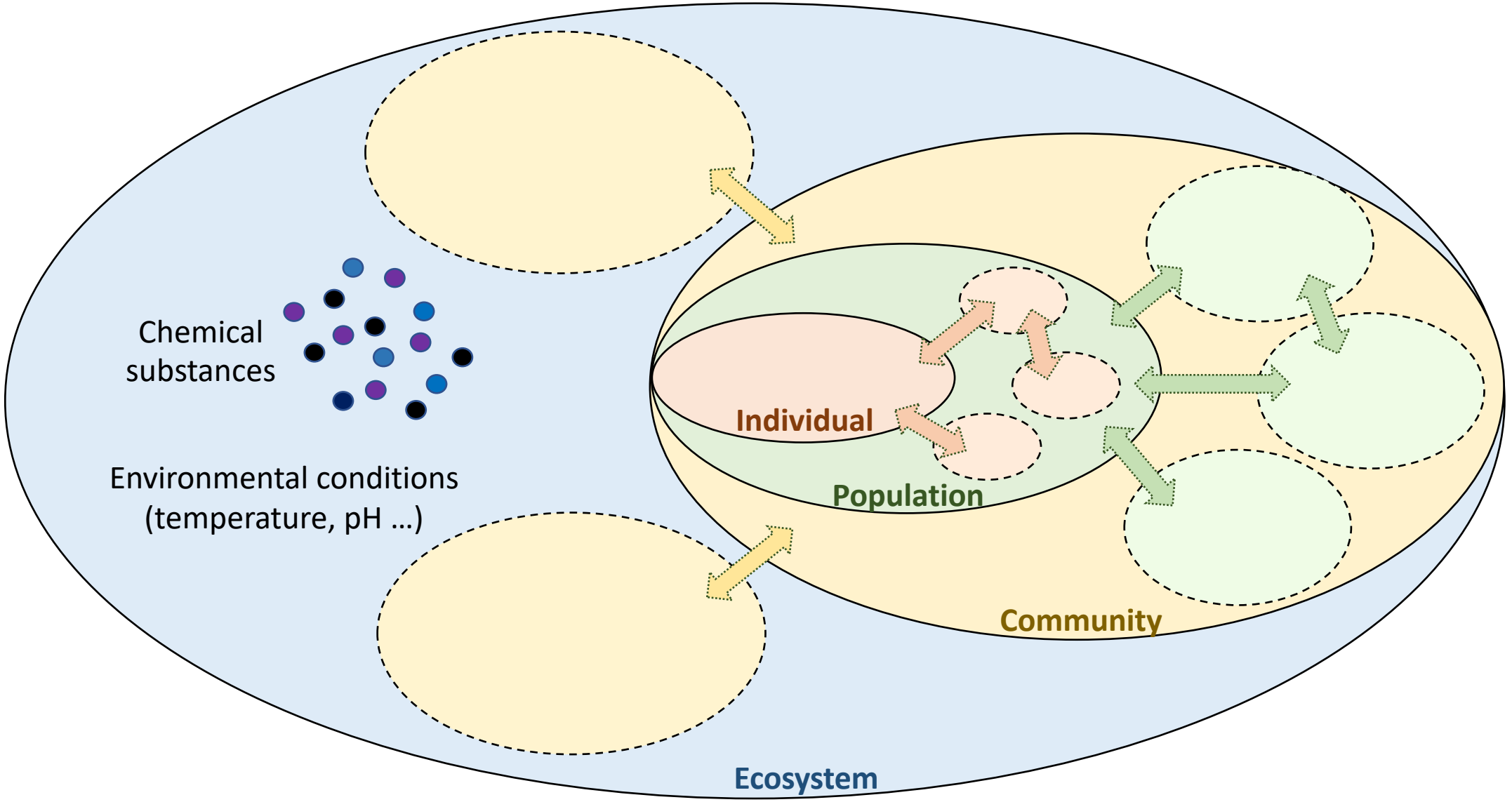
LBBE

LABORATOIRE DE BIOMÉTRIE ET BIOLOGIE ÉVOLUTIVE

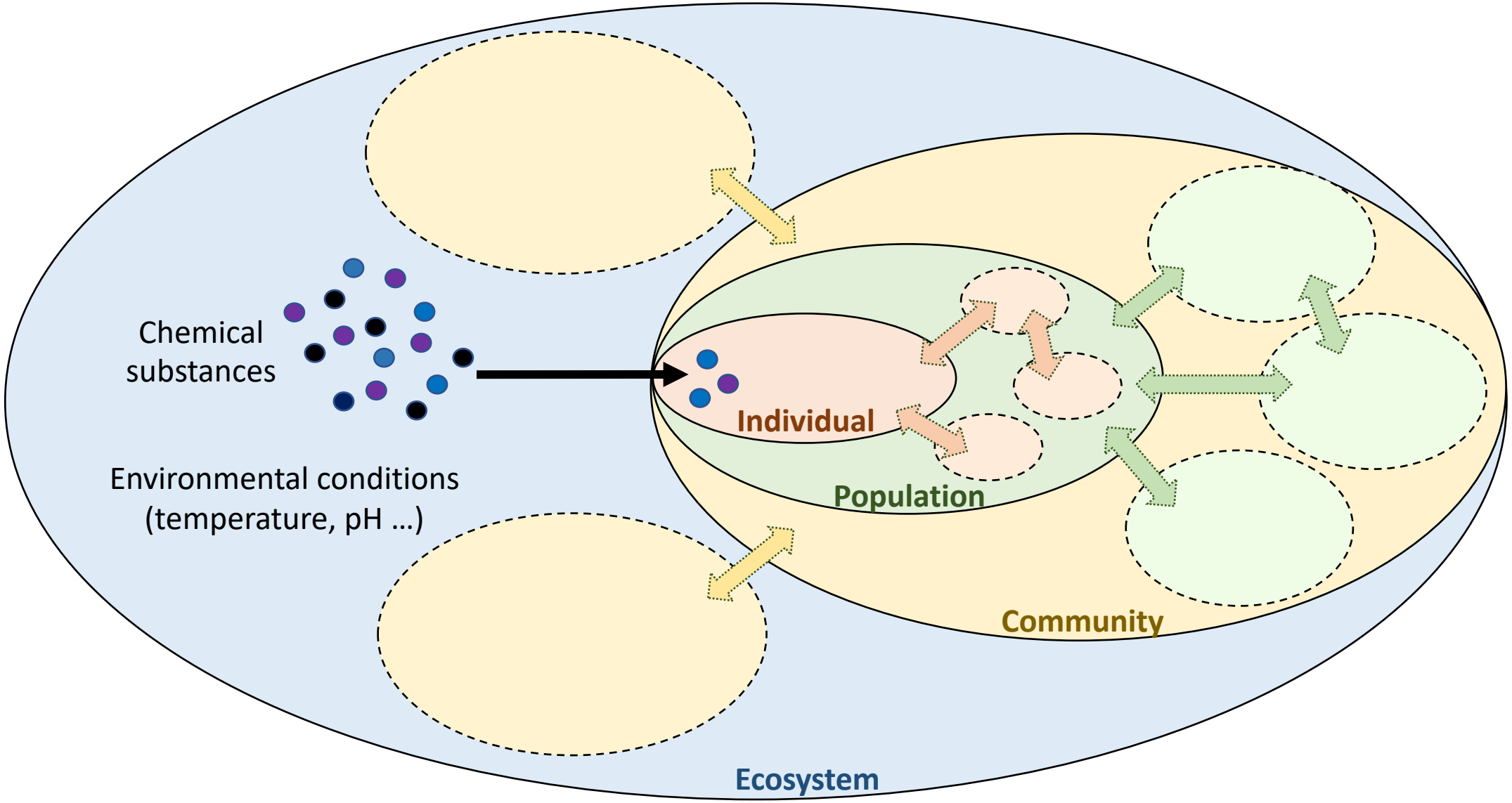
UNIVERSITÉ DE LYON

Lyon 1

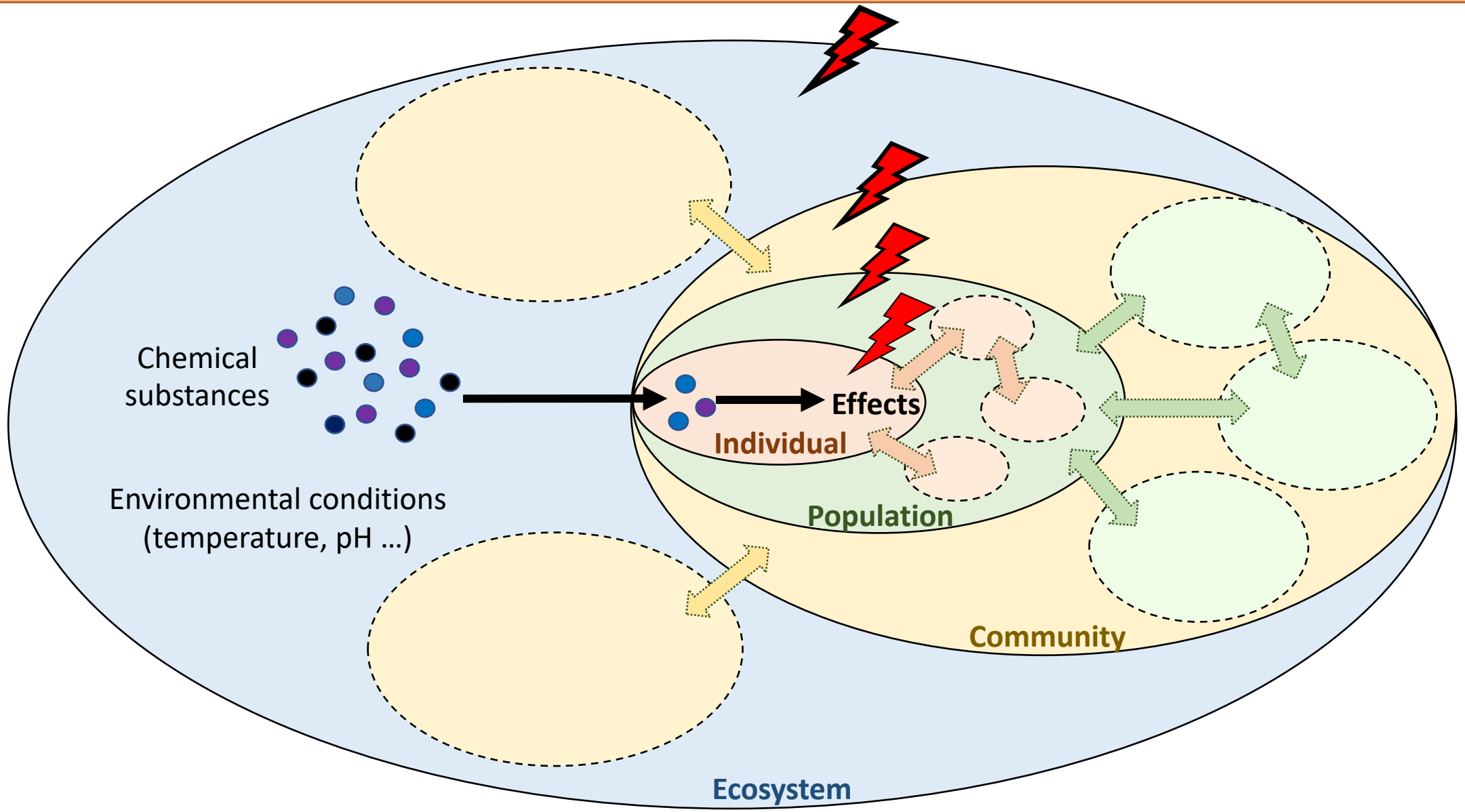
Aquatic ecosystems, made of several communities



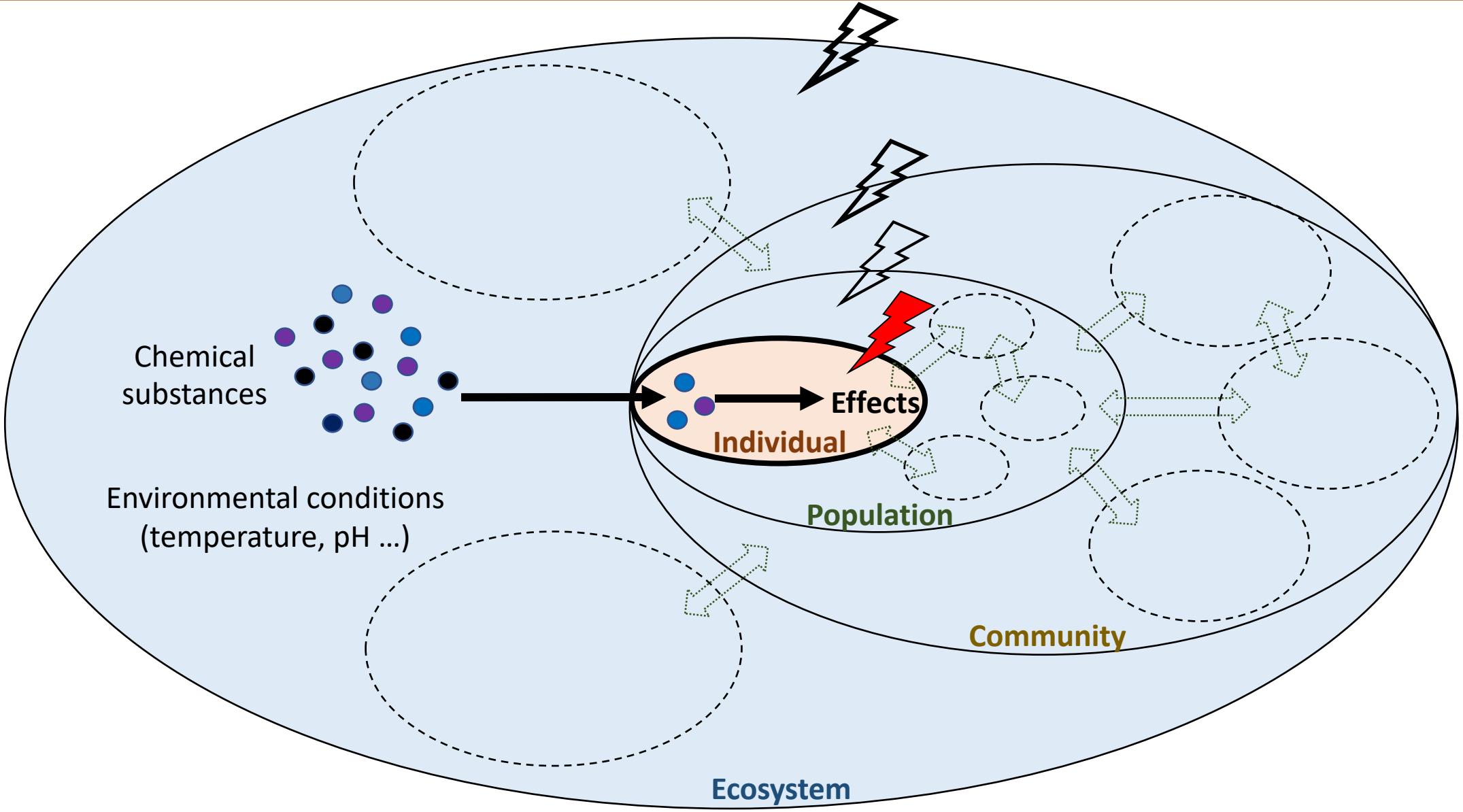
Communities are exposed to chemicals via individuals



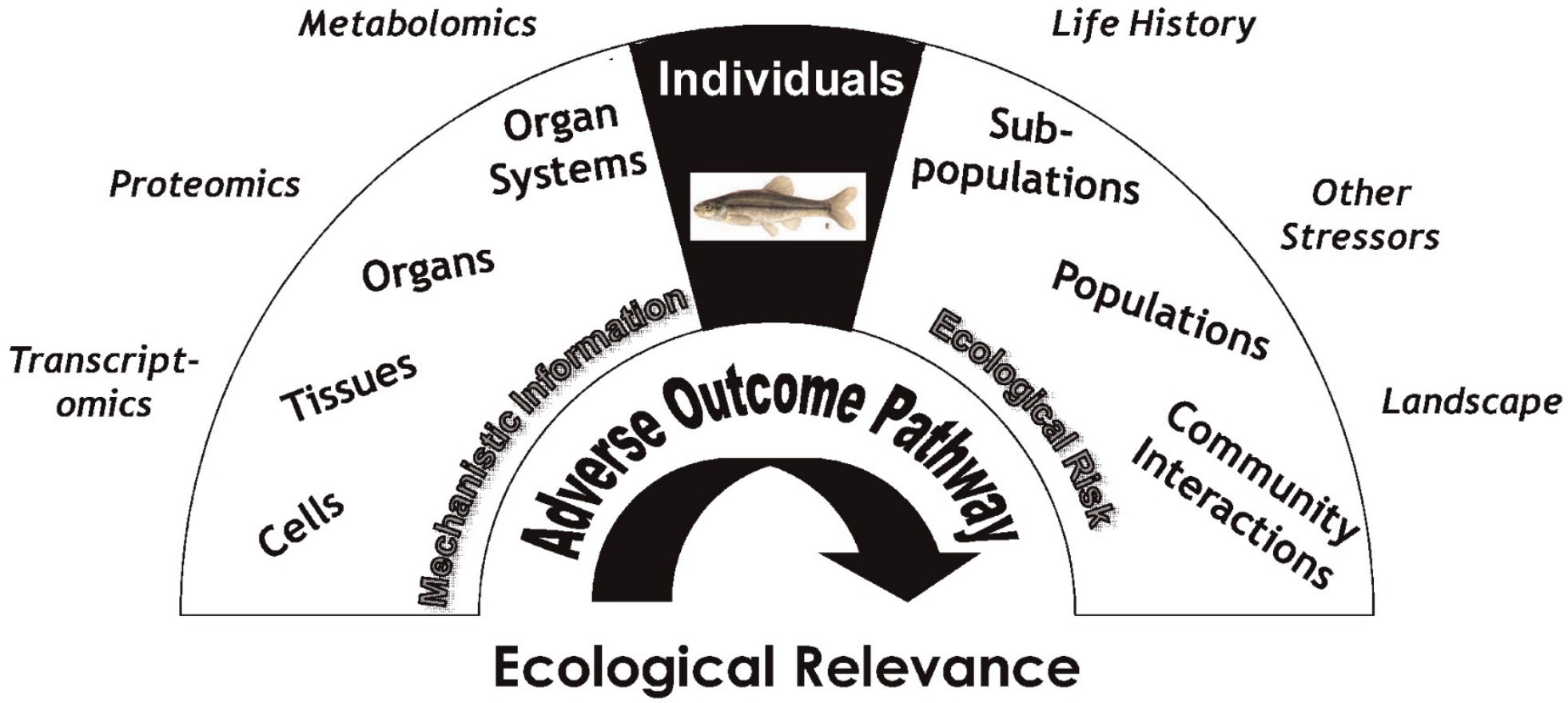
Evaluation of chemical effects at different biological levels



Central role of the individual level



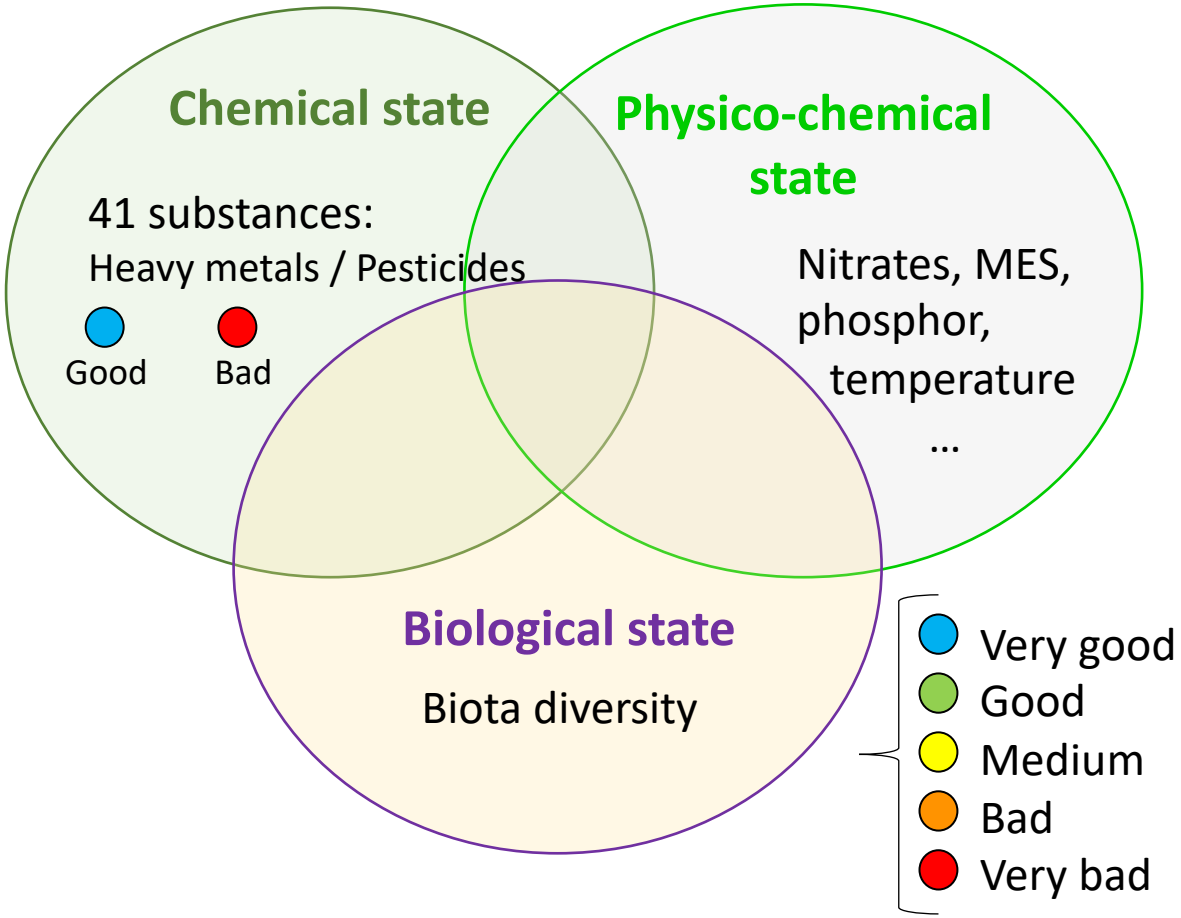
Adverse Outcome Pathways



Kramer et al., 2011

Importance of TK in environmental risk assessment (ERA)

1. Characterize chemical state of aquatic ecosystem (European WFD)
   - EU requires good ecological status



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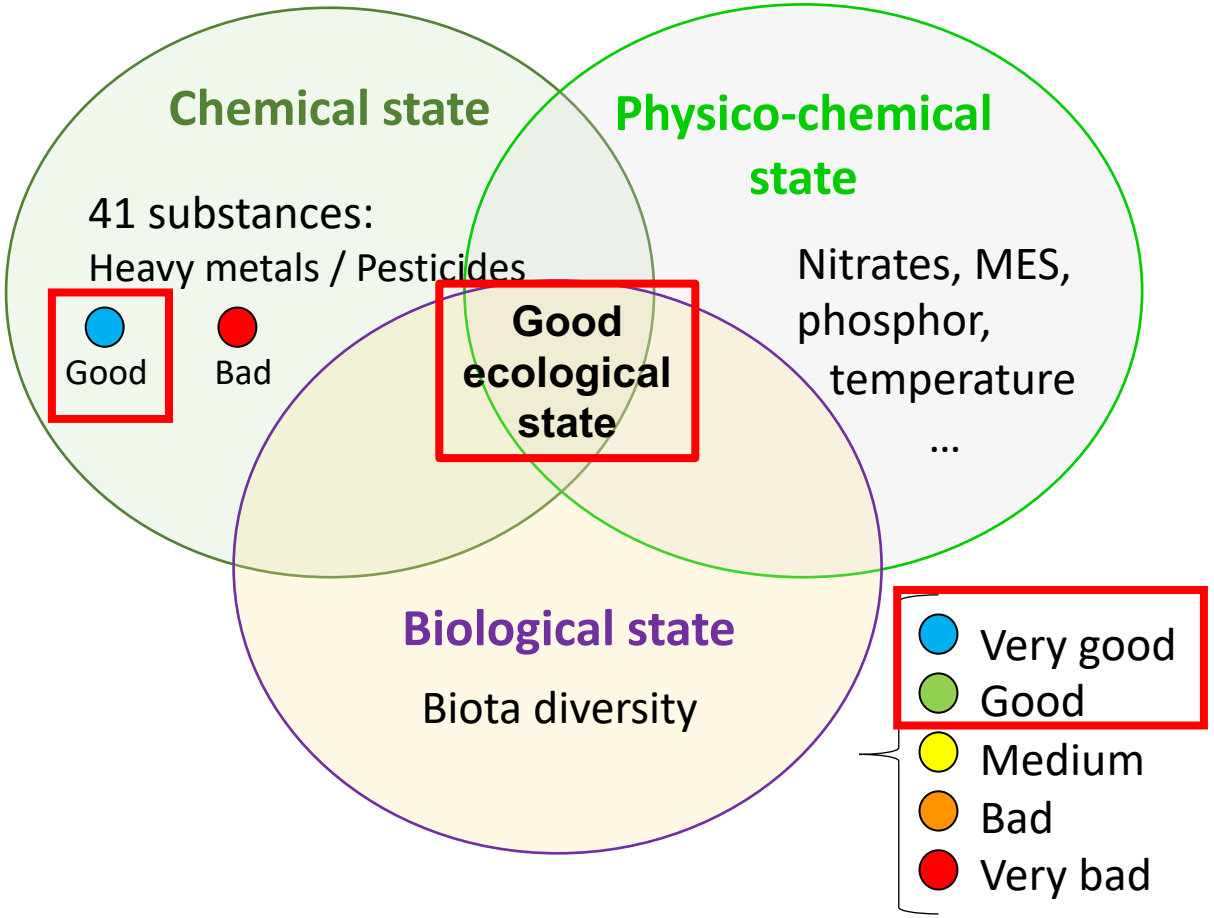

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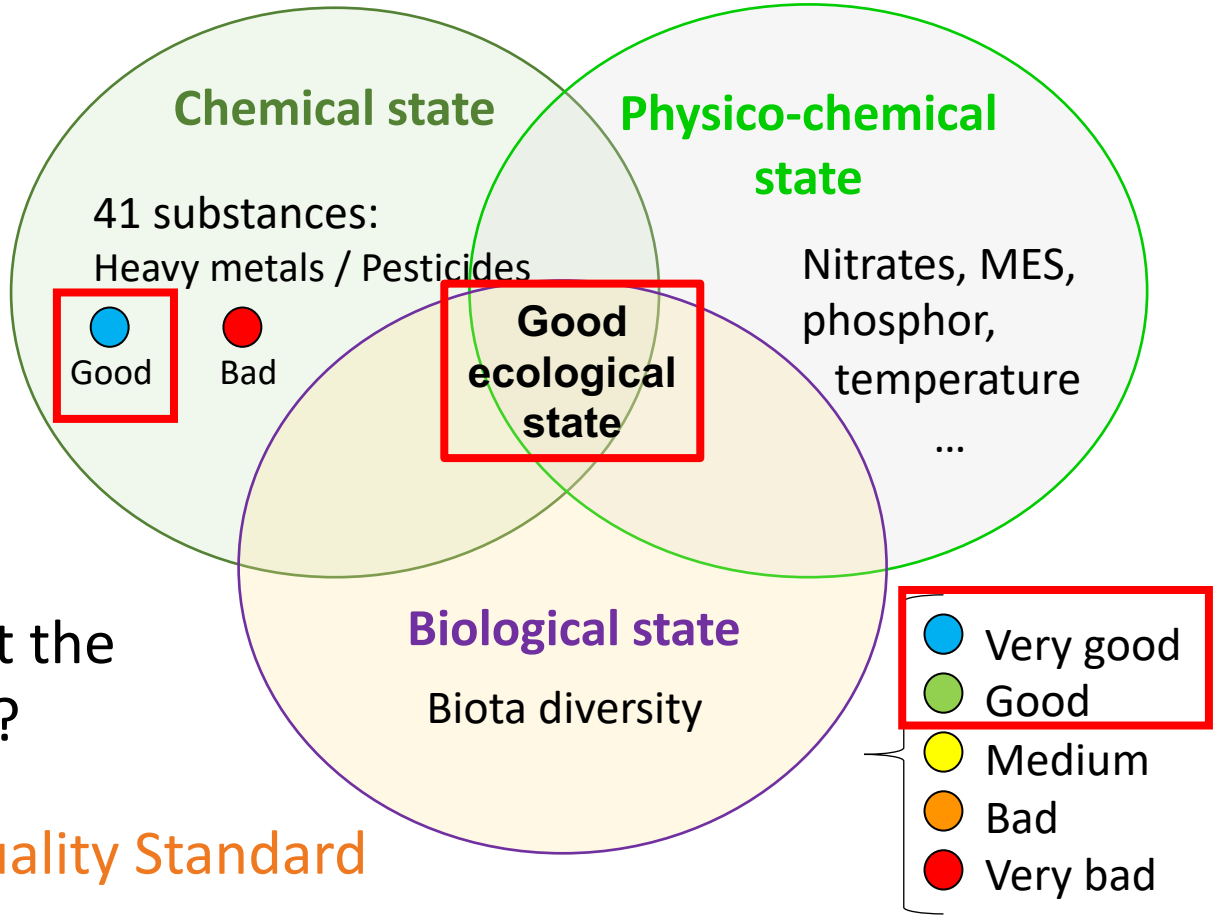

How to determine that the chemical state is good?

Environmental Quality Standard

Environmental Quality Standard (EQS)

« *Concentration of a pollutant or group of pollutants which must not be exceeded, in order to protect human and environmental health* »

- EQS for water
- EQS for sediment
- EQS for biota

↪ Derived from extrapolation of effects, not from bioaccumulation capacity of the chemicals within the body of organisms

Gammarids

- Sentinel species *Gammarus fossarum*

- Freshwater crustaceans
- Representative of European aquatic ecosystems
- Easy to manipulate in situ or under lab conditions
- Sensitive to most of contaminants

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➢ Sub-lethal chronic toxicity test: measure of life-history traits related to reproductive success (duration of molt stages, fecundity, feeding rate...) [Geffard *et al.*, 2010; Coulaud *et al.*, 2011]

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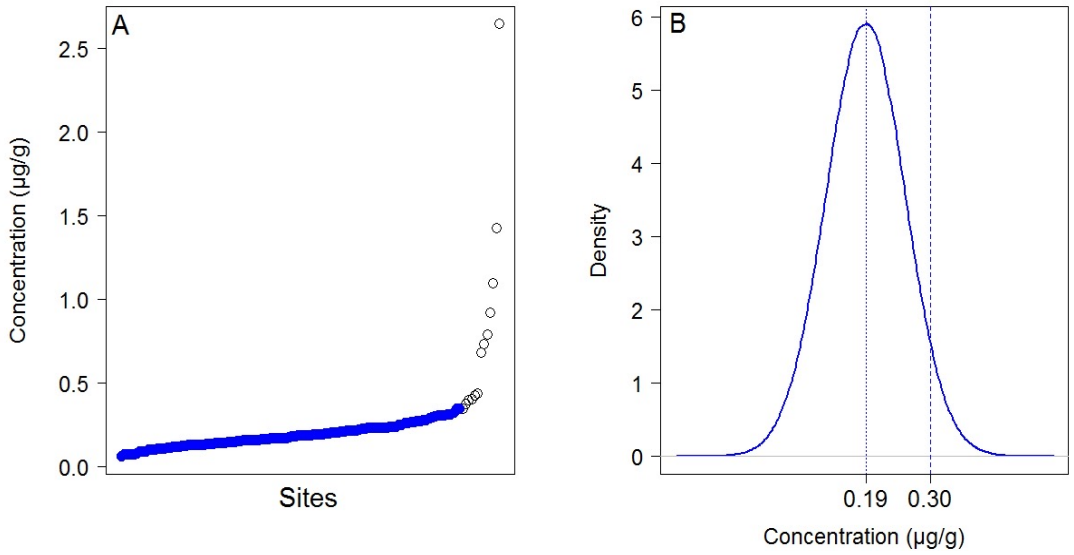
- *In situ* experimental approach [Coulaud et al., 2011]

- Bioavailable Background Assessment Concentration (BBAC) [Besse *et al.*, 2013]

Bioavailable Background Assessment Concentration (BBAC)

- *In situ* exposure within a lot of geographical sites during 7 days
  => measure of internal concentrations for priority substances

- Development of a statistical procedure to estimate a BBAC for each substance: concentration above which bioavailability is significantly greater than the « background »

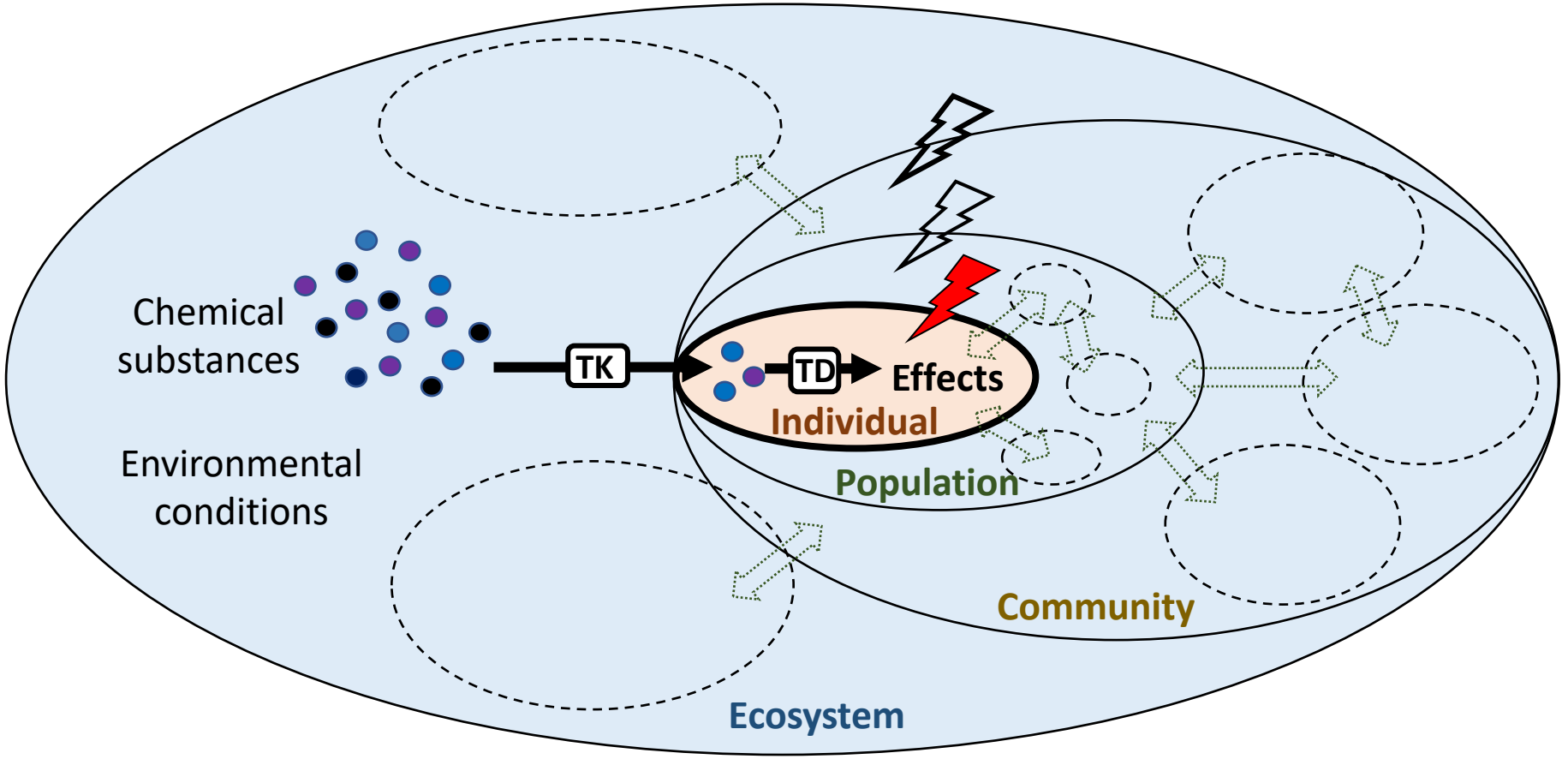


Not interpretated in terms of impact on organisms

Importance of TK in ERA

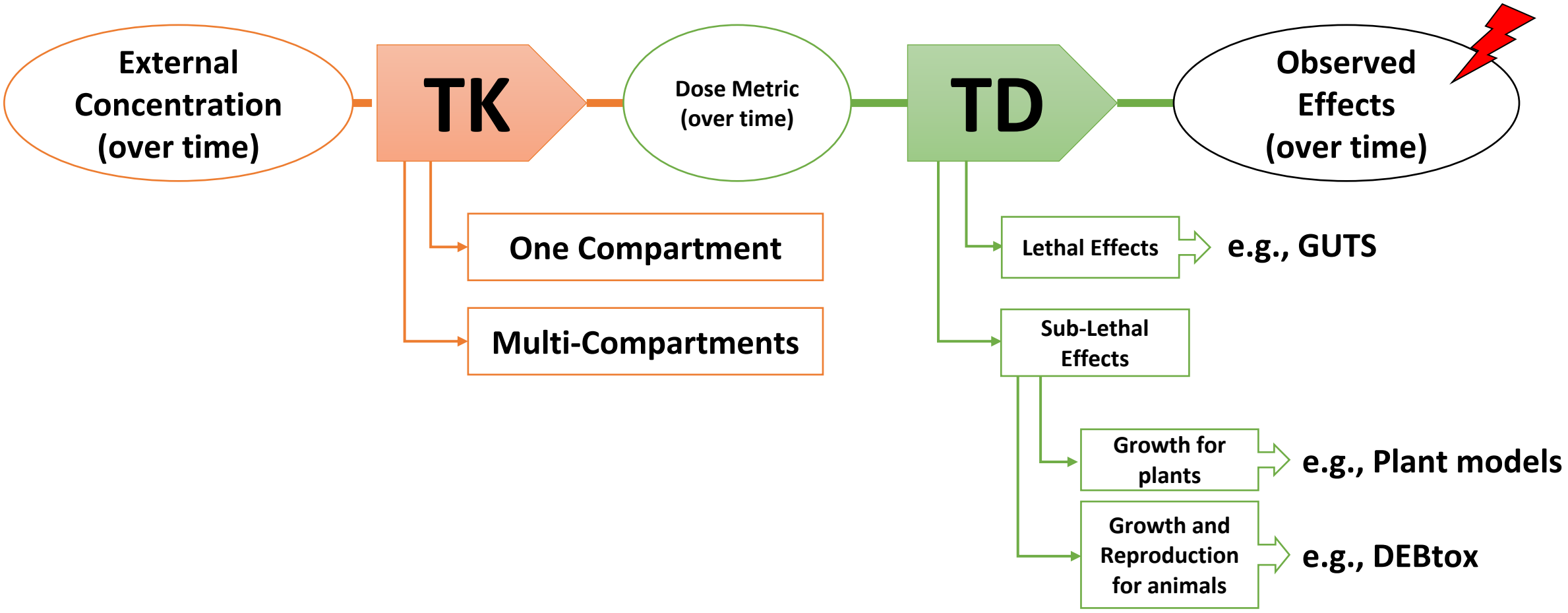
1. Characterize chemical state of aquatic ecosystem (European WFD)

2. Make the link between exposure concentration and individual effects



Toxico-Kinetic and Toxico-Dynamic framework

- Translate (time-varying) external concentrations to time patterns of effects.

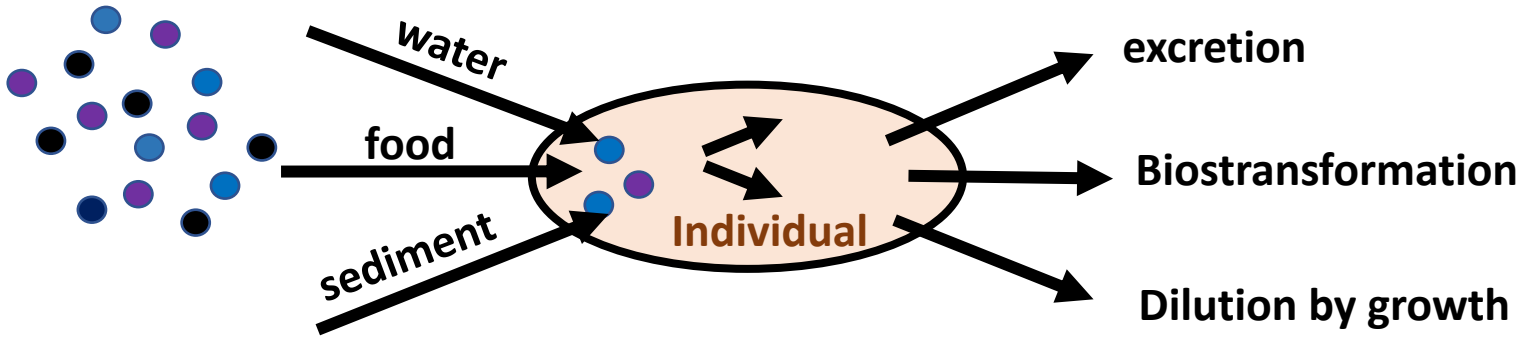


Importance of TK in environmental risk assessment

1. Characterize chemical state of aquatic ecosystem (European WFD)

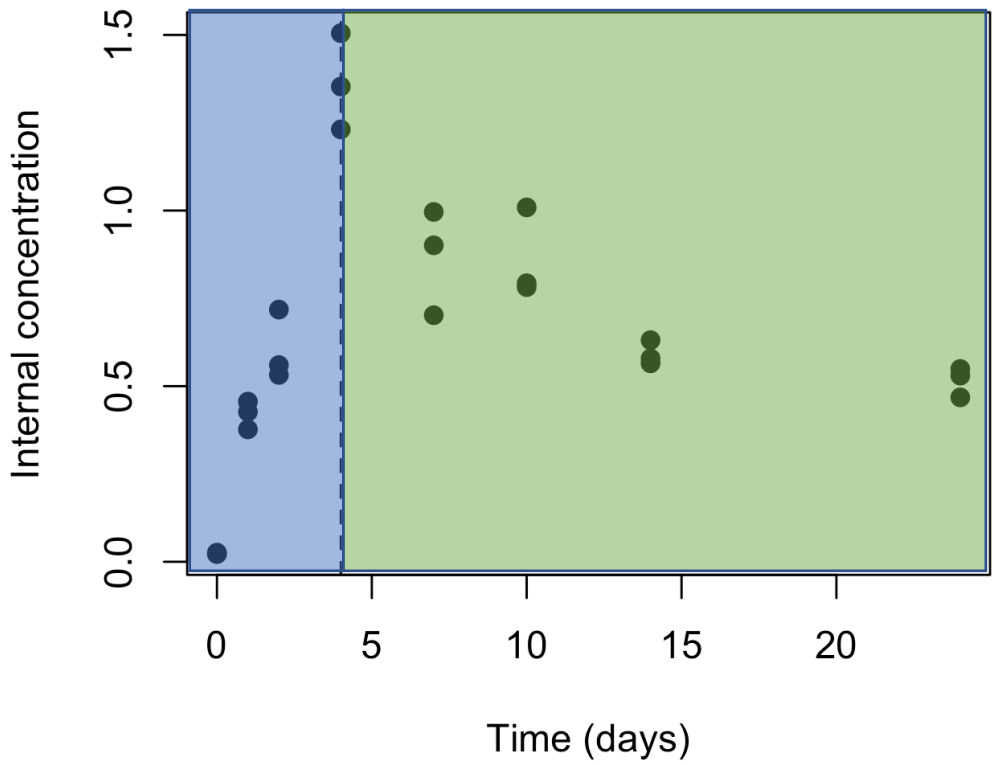
2. Make the link between exposure concentration and individual effects

3. Understand and describe bioaccumulation processes
   → ADME = Absorption, Distribution, Metabolism, Excretion



TK data

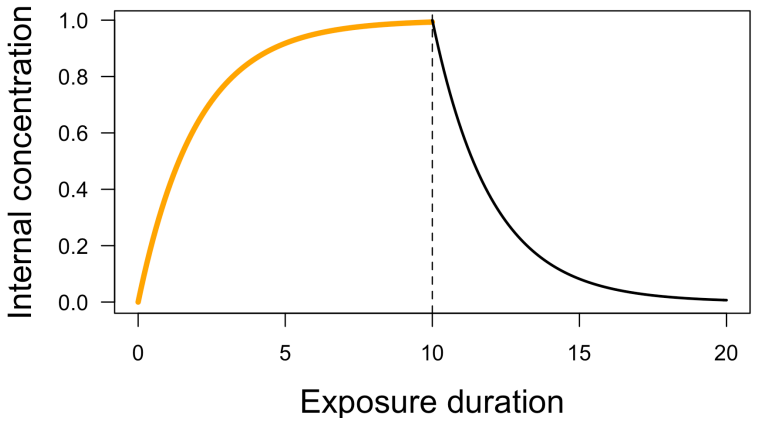
- **TK data**: accumulation and depuration phases
  - **Accumulation phase**: individuals exposed at a given (*constant*) concentration (all ADME processes may occur) during a pre-defined time
  - **Depuration phase**: individuals in clean medium (*without toxicant*) (only depuration processes occur)



TK data and TK models

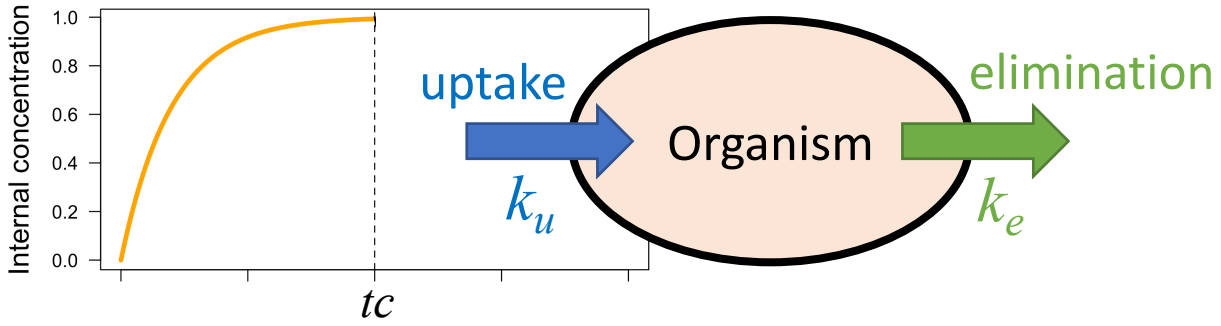
- TK data: accumulation and depuration phases
  - Accumulation phase: individuals exposed at a given (constant) concentration (all ADME processes occur) during a defined time
  - Depuration phase: individuals in clean medium (without toxicant) (only elimination processes occur)

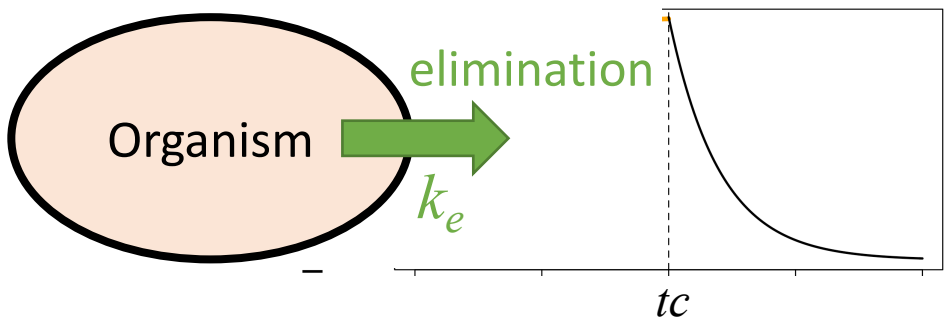
- **TK models**: compartment models; the chemical is assumed to be evenly distributed within the compartment(s).
  - One-compartment models
  - Multi-compartments models



One-compartment models: Theory

Very simplistic and general one-compartment model





- The organism is reduced to a single well-mixed compartment
- There is one single homogeneous **internal** concentration: $C_i(t)$
- The uptake flux is proportional to the external concentration: $k_u \; C_w(t)$
- The elimination flux is proportional to the internal concentration: $k_e \; C_i(t)$

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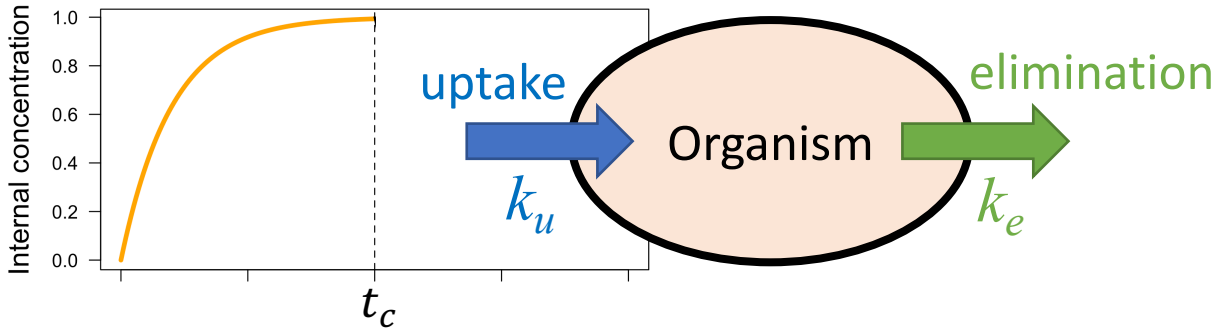

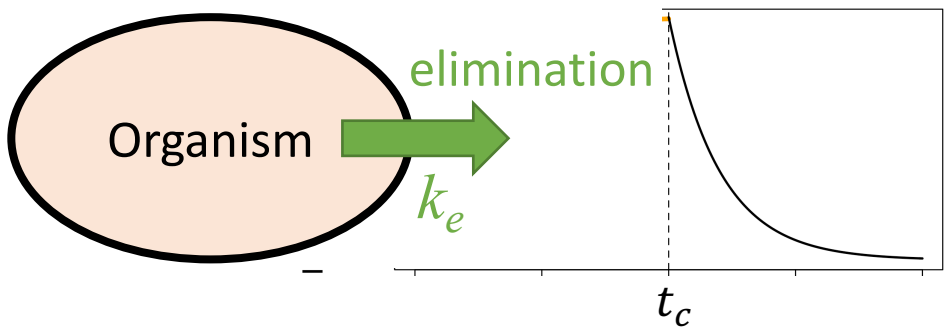

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$$\begin{cases} \dfrac{dC_i(t)}{dt} = k_u \times C_w(t) - k_e \times C_i(t) & \text{if } 0 \le t \le t_c \\ \dfrac{dC_i(t)}{dt} = -k_e \times C_i(t) & \text{if } t > t_c \end{cases}$$

t_c : duration of the accumulation phase

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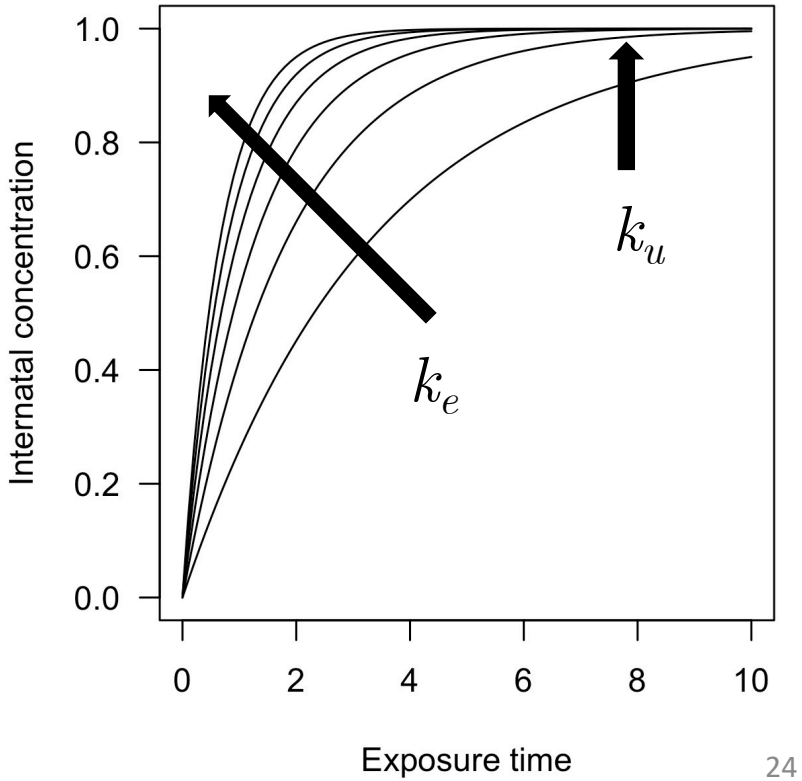
- k_e [time]$^{-1}$ influences the shape of the curve, and the time to reach x % of the steady-state
- k_u [time]$^{-1}$ influences the height of the curve, that is the level of the steady-state



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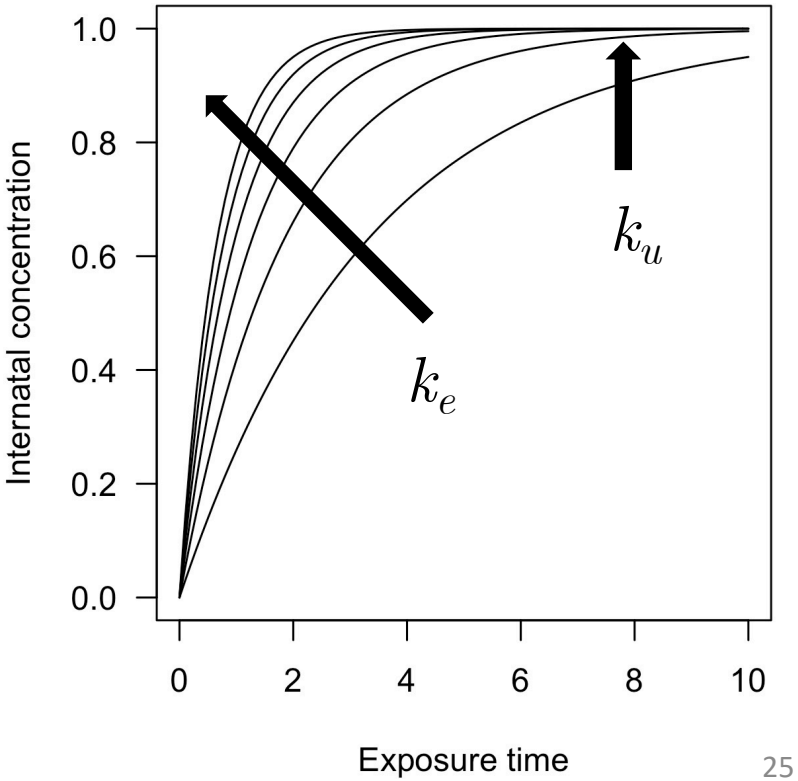

- If the steady state is rapidly achieved, the chemical effects will appear soon after the exposure to the chemical starts.
- If the accumulation process is slow, the chemical effects will only appear after a more prolonged exposure.

Very simplistic and general one-compartment model

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- Analytical solution if C_w is **constant** over time

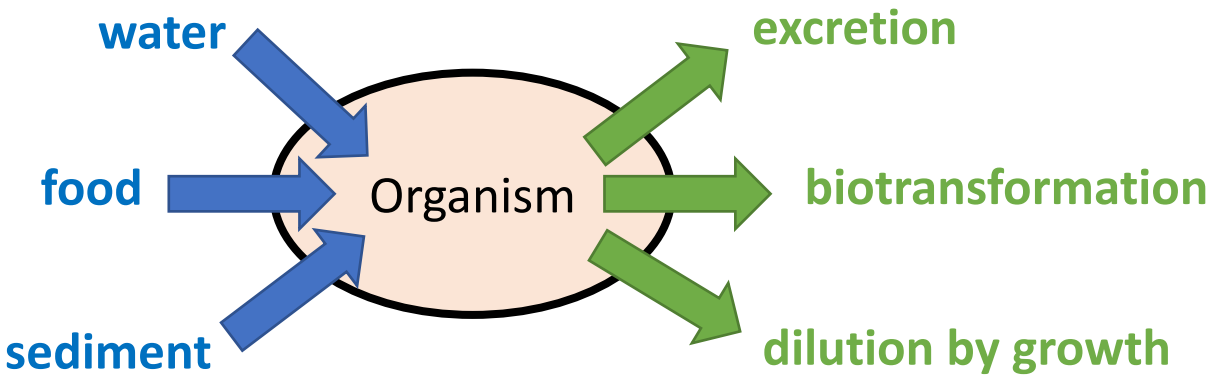
$$\begin{cases} C_i(t) = \frac{k_u \times C_w}{k_e} + \left(C_0 - \frac{k_u \times C_w}{k_e}\right) \times e^{-k_e \times t} & \text{if } 0 \le t \le t_c \\ C_i(t) = \frac{k_u \times C_w}{k_e} \times e^{-k_e \times (t - t_c)} + \left(C_0 - \frac{k_u \times C_w}{k_e}\right) \times e^{-k_e \times t} & \text{if } t > t_c \end{cases}$$

Ratier et al., 2019

Charles et al., 2021

More complex one-compartment models

- Several uptake routes and eliminations processes



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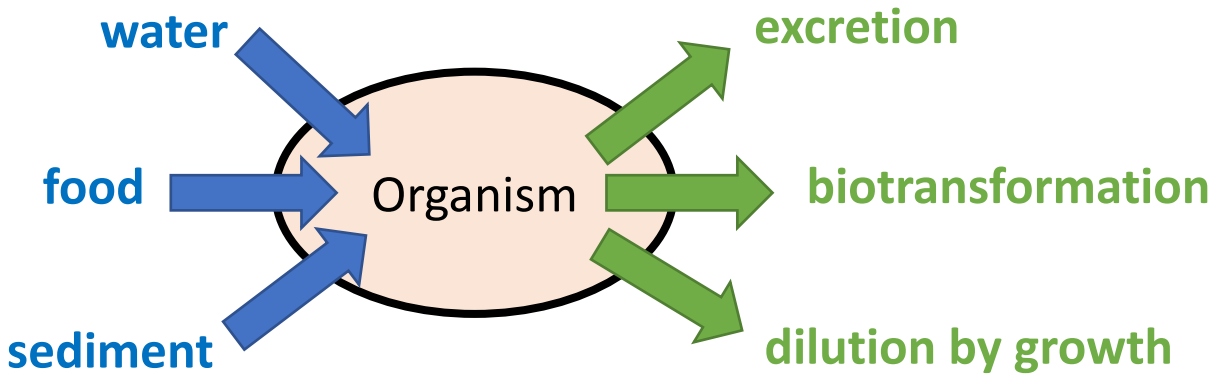

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n uptake routes (k_u)
- k_w : water
- k_s : sediment
- k_l : leaves
- ...

m elimination processes (k_j)
- k_e : excretion
- k_g : growth
- k_m : biotransformation
- ...

Ratier et al. 2019.

More complex one-compartment models

- Choice according to experimental data

Constant exposure by **water** and **sediment**

Excretion and **Growth**

$$\begin{cases} \dfrac{dC_i(t)}{dt} = (k_w \times C_w) + (\boldsymbol{k_s} \times \boldsymbol{C_s}) - (k_e + k_g) \times C_i(t) \\ \dfrac{dL(t)}{dt} = k_g \times (L_\infty - L(t)) \end{cases}$$

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C_i: internal concentration
L: growth variable

Constant exposure by **water**
Excretion and **biotransformation**
(1 metabolite)

$$\begin{cases} \dfrac{dC_p(t)}{dt} = k_w \times C_w - (k_{e,p} + \boldsymbol{k_m}) \times C_p(t) \\ \dfrac{dC_m(t)}{dt} = \boldsymbol{k_m} \times C_p(t) - k_{e,m} \times C_m(t) \end{cases}$$

C_p: internal parent concentration
C_m: internal metabolite concentration

Derivation of Bioaccumulation metrics

- To evaluate the bioaccumulation potential of chemical substances
- Three metrics according to the source of exposure
  - BCF: Bio-Concentration Factor (exposure via water)
  - BSAF: Biota-Sediment Accumulation Factor (exposure via sediment)
  - BMF: Bio-Magnification Factor (exposure via food)

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- For each metric, two types of calculation according to the "shape" of the data during the accumulation phase
  - If the internal concentration reaches a plateau at the end of the accumulation phase

    => Steady-state bioaccumulation metric
  - If not

    => Kinetic bioaccumulation metric

Derivation of Bioaccumulation metrics

	Steady-state bioaccumulation metric	Kinetic bioaccumulation metric
➢ BCF	$BCF_{ss} = \frac{C_i(t_c)}{C_w}$	$BCF_k = \frac{k_w}{\sum_{j=1}^{m} k_j}$

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➢ BSAF	$BSAF_{ss} = \frac{C_i(t_c)}{C_s}$	$BSAF_k = \frac{k_s}{\sum_{j=1}^{m} k_j}$

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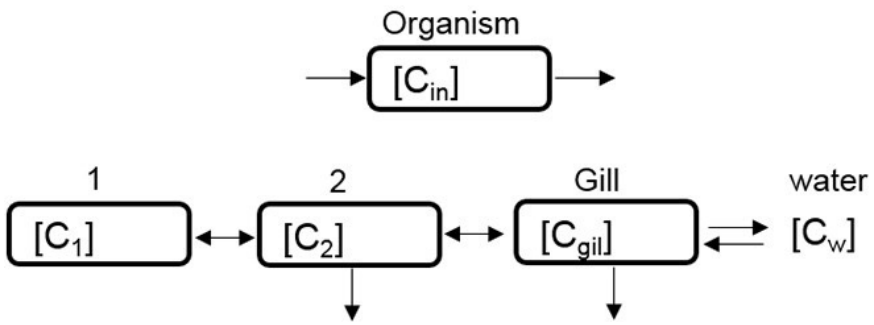
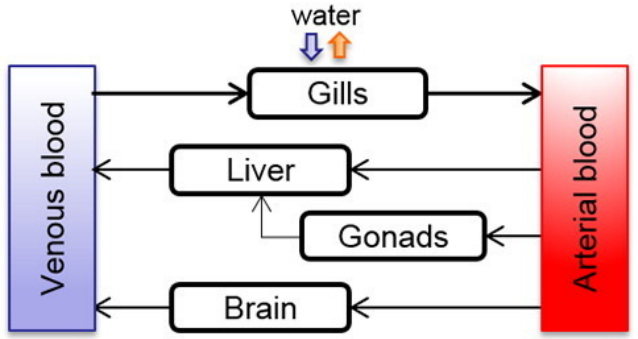
Multi-compartment models: Theory

Multi-compartment models

- The organism is divided into different compartments
- There is one single homogeneous internal concentration for each compartment: $C_x(t)$
- The compartments are linked to each others and with the environment

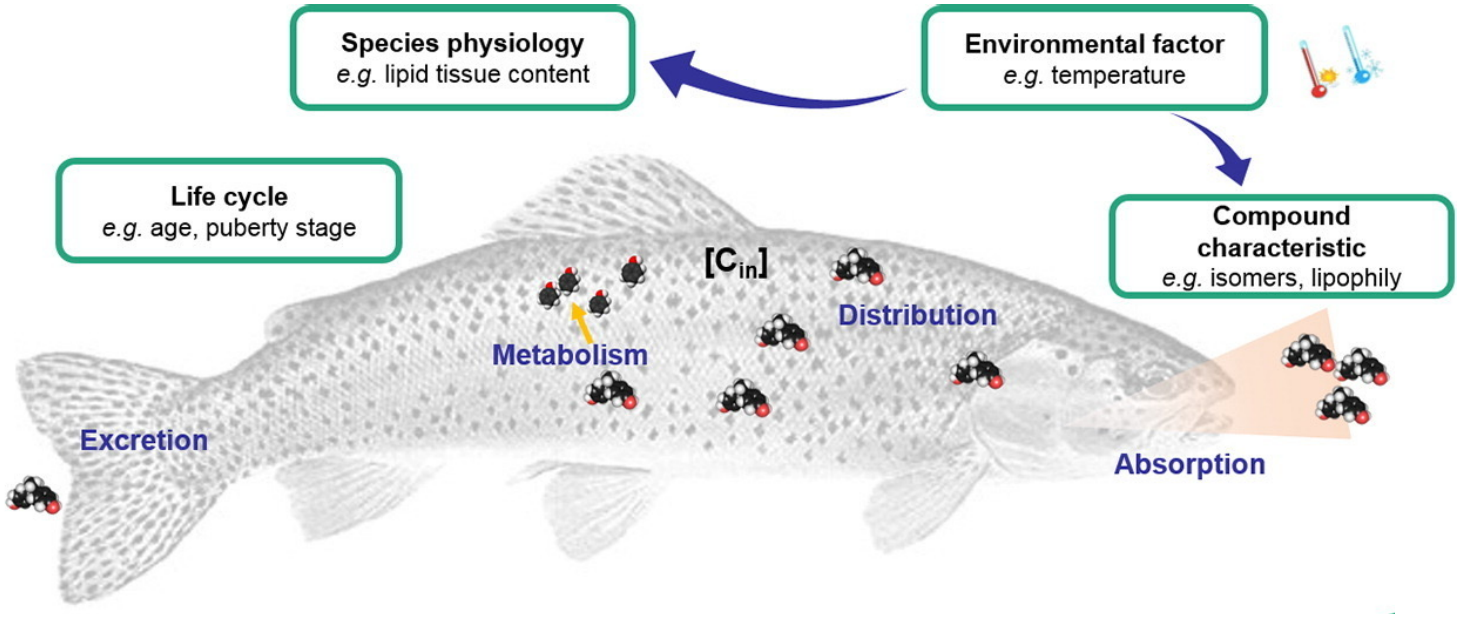


Compartmental models



Mechanistic models

Multi-compartments models



Generally restricted to large-bodied organisms (e.g., fish or mammals)

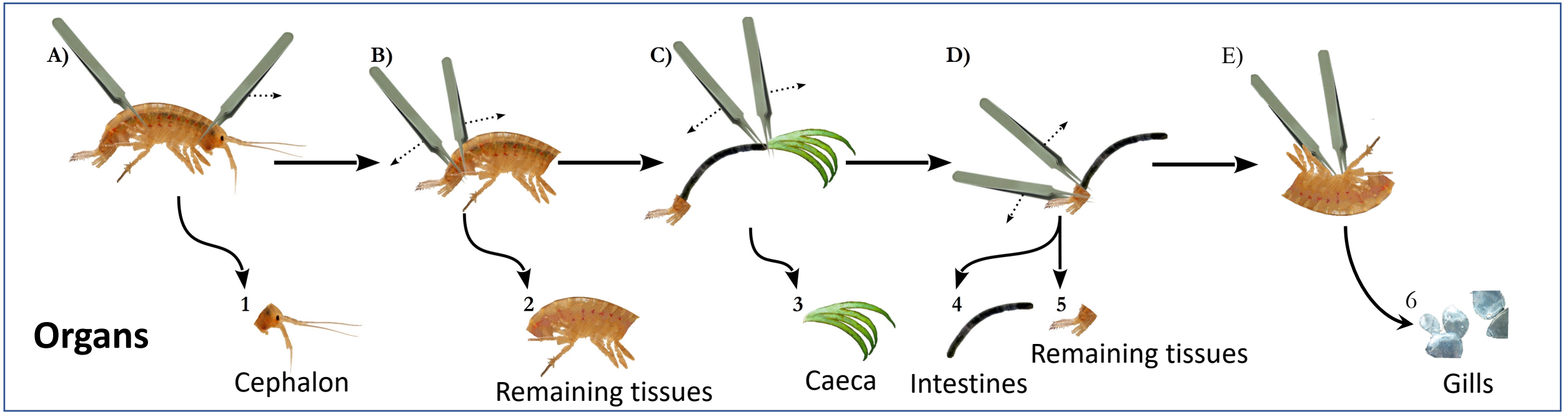
Grech A, Brochot C, Dorne J-L, Quignot N, Bois FY, Beaudouin R. 2016. Toxicokineticmodels and related tools in environmental risk assessment of chemicals. *Sci. Total Environ*.

Multi-compartments models: case study for *Gammarus fossarum*

- Gammarids exposed during 7 days, then placed in clean water during 21 days

Multi-compartments models: case study for *Gammarus fossarum*

- Dissection at each time step : 4 pools of 5 organs



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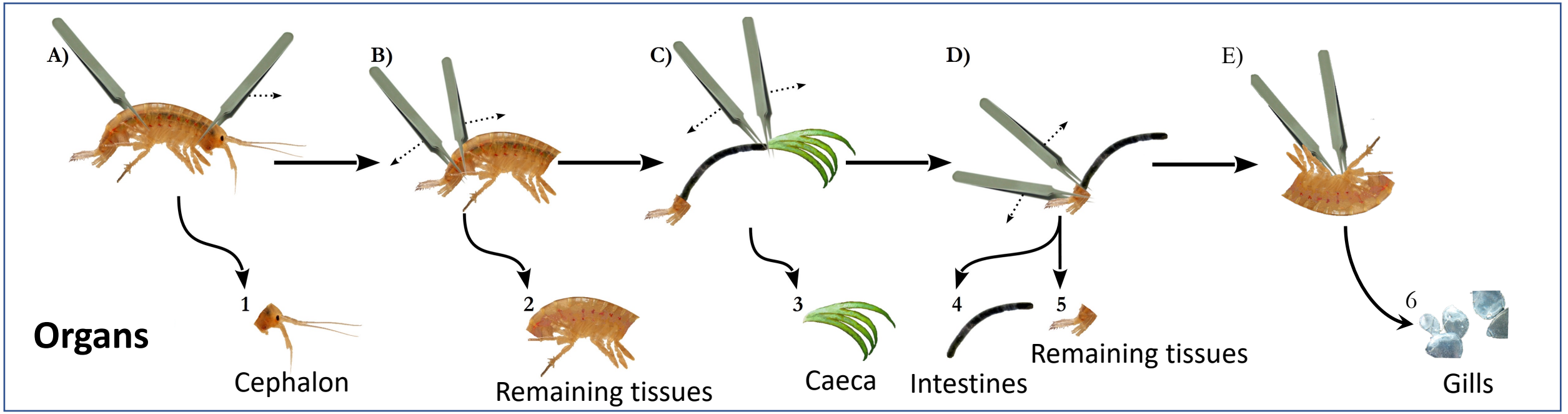
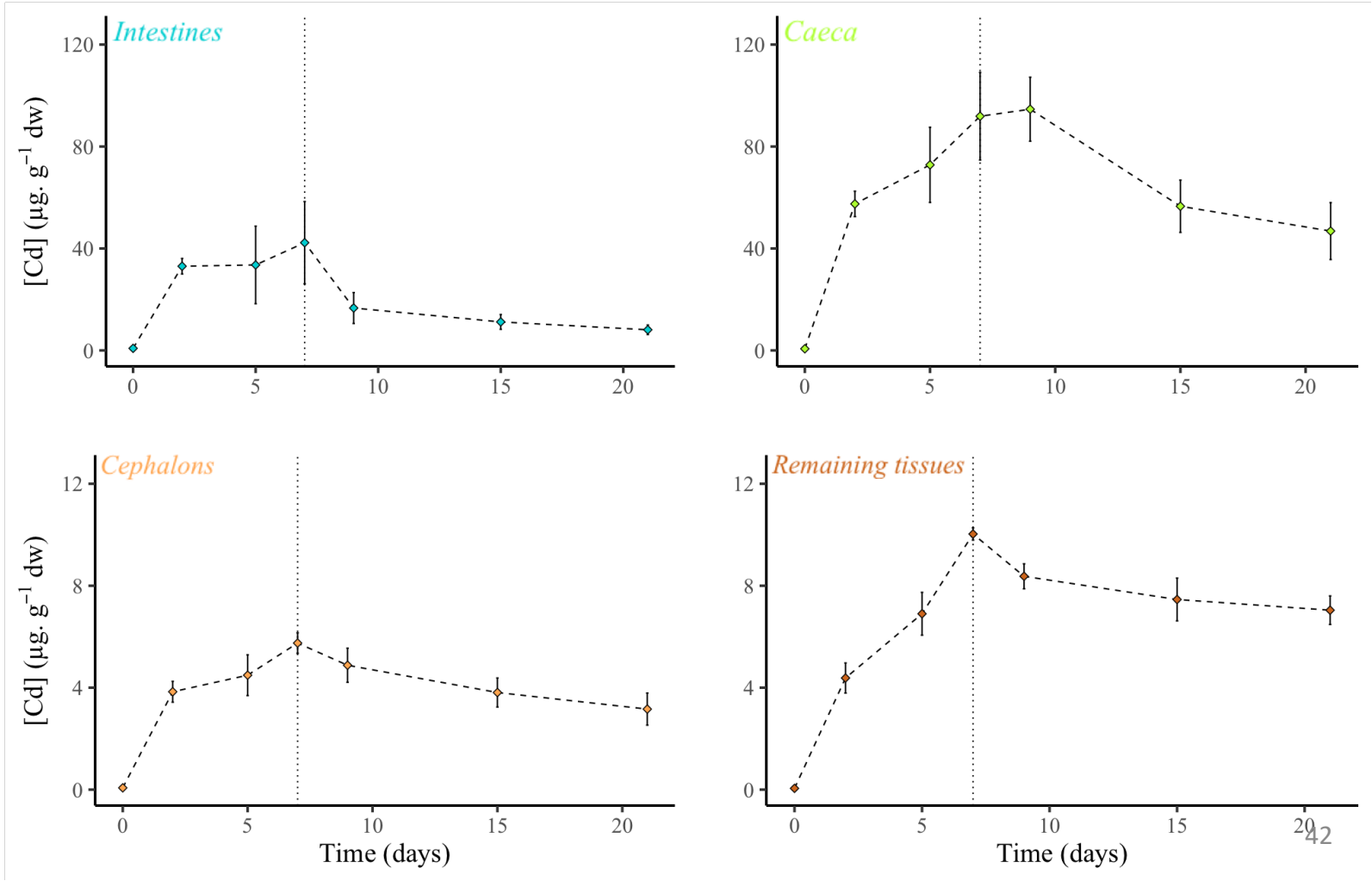


15% of total weight | **77%** | **5%** | **1%** | **2%**

An adult ≈ 1 cm (6 mg)

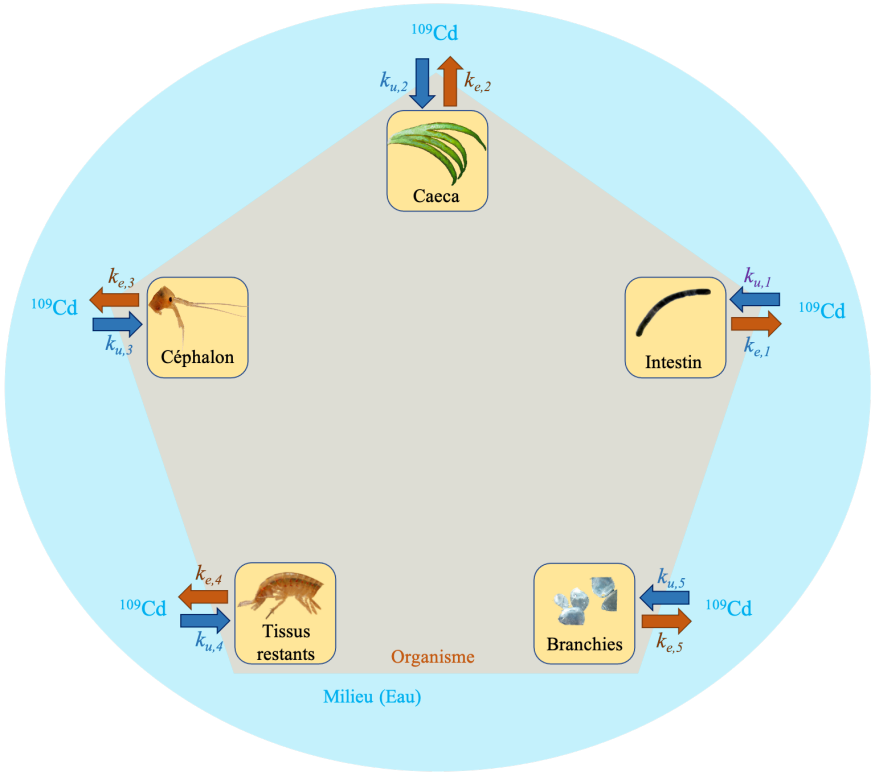
Multi-compartments models: case study for *Gammarus fossarum*

- Dissection at each time step : 4 pools of 5 organs
- Kinetic for each compartment



Multi-compartments models: case study for *Gammarus fossarum*

- One compartment model for each organ
  with the aim to compare accumulation kinetic between organs

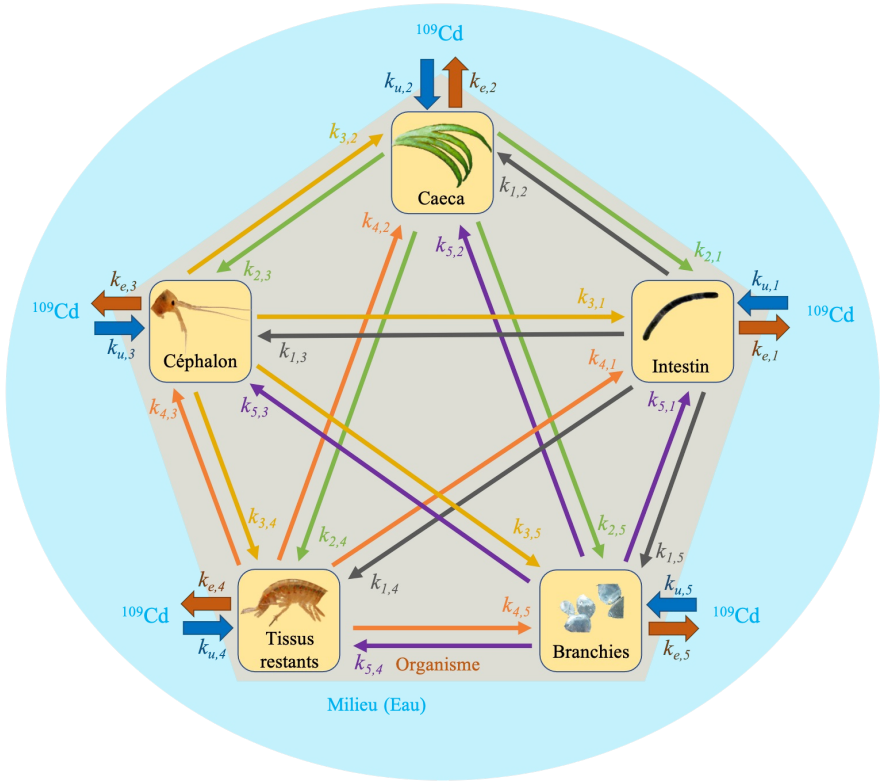


$$\begin{cases} \dfrac{dC_x(t)}{dt} = k_{u,x} \times C_w - k_{e,x} \times C_{x(t)} & \text{if } t \le t_c \\ \dfrac{dC_x(t)}{dt} = -k_{e,x} \times C_{x(t)} & else \end{cases}$$

x=1..5 :
x=1 for intestine, x=2 for caeca, x=3 for cephalon,
x=4 for remaining tissues and x=5 for gills

Multi-compartment models: case study for *Gammarus fossarum*

➢ **Multi-compartment model** :
with the aim to describe distribution and fate of contaminant within the organism and to identify the role of the different organs



10 inter-dependent ODE : for each compartment, one equation for the accumulation phase and another one for the depuration phase

30 parameters

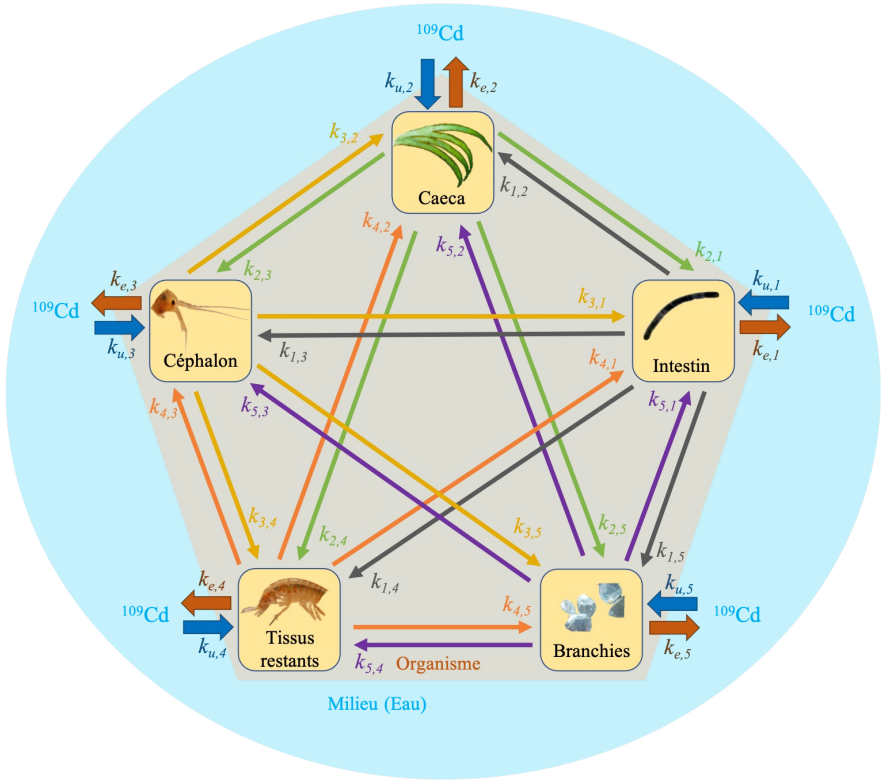
Multi-compartments models: case study for *Gammarus fossarum*

➢ Example for compartment 1:

if $t \leq t_c$ $\quad \frac{dC_1(t)}{dt} = k_{u,1} \times C_w(t) - k_{e,1} \times C_1(t)$

$$+k_{21} \times C_2(t) + k_{31} \times C_3(t) + k_{41} \times C_4(t) + k_{51} \times C_5(t)$$

$$-(k_{12} + k_{13} + k_{14} + k_{15}) \times C_1(t)$$



Multi-compartments models: case study for *Gammarus fossarum*

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if $t > t_c$ $\quad \frac{dC_1(t)}{dt} = -k_{e,1} \times C_1(t)$

$$+k_{21} \times C_2(t) + k_{31} \times C_3(t) + k_{41} \times C_4(t) + k_{51} \times C_5(t)$$

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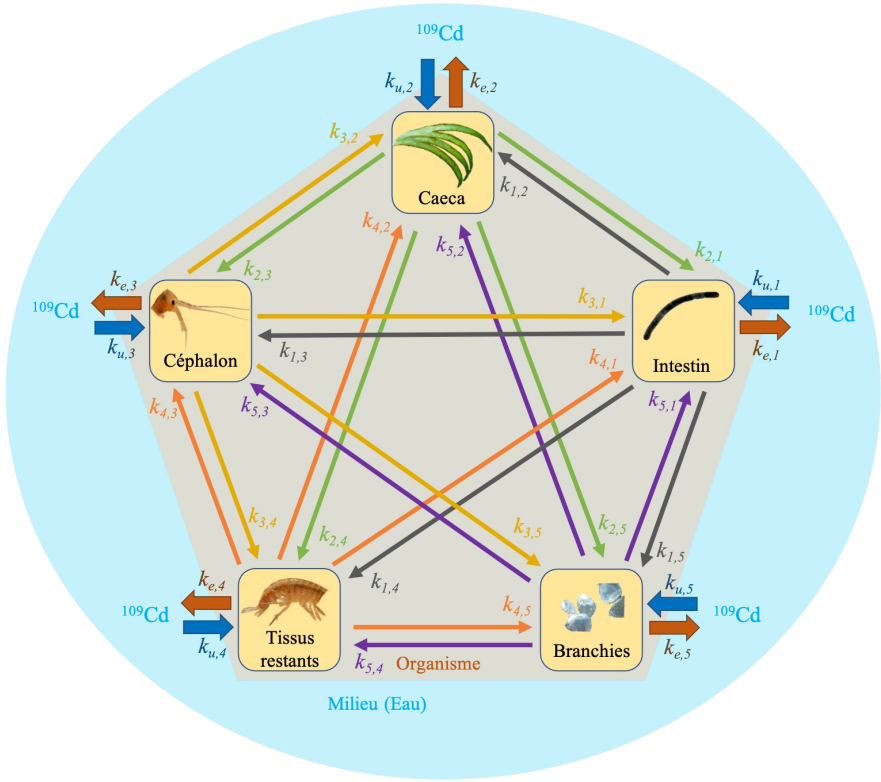

Multi-compartments models: case study for *Gammarus fossarum*

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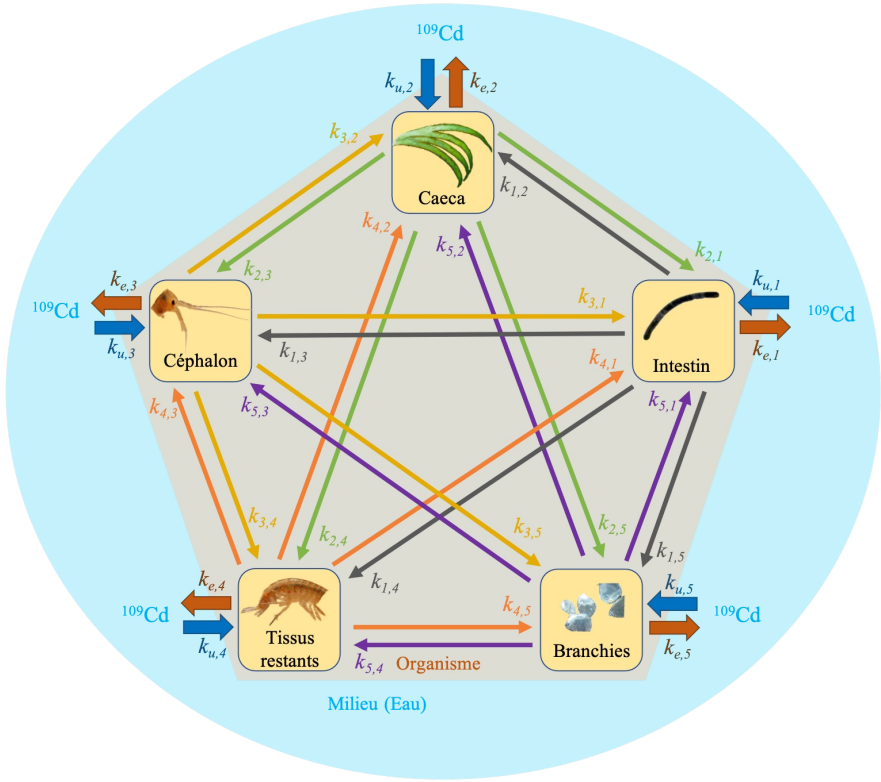
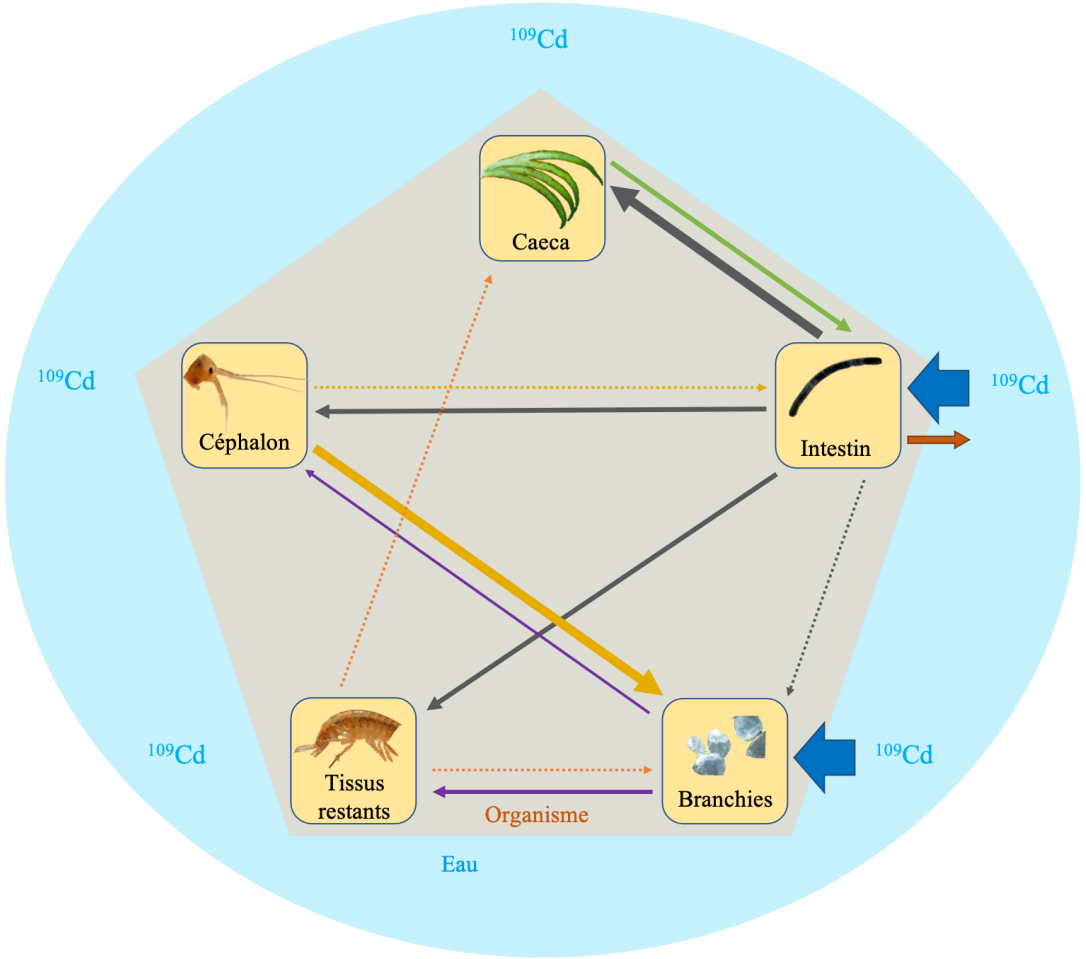


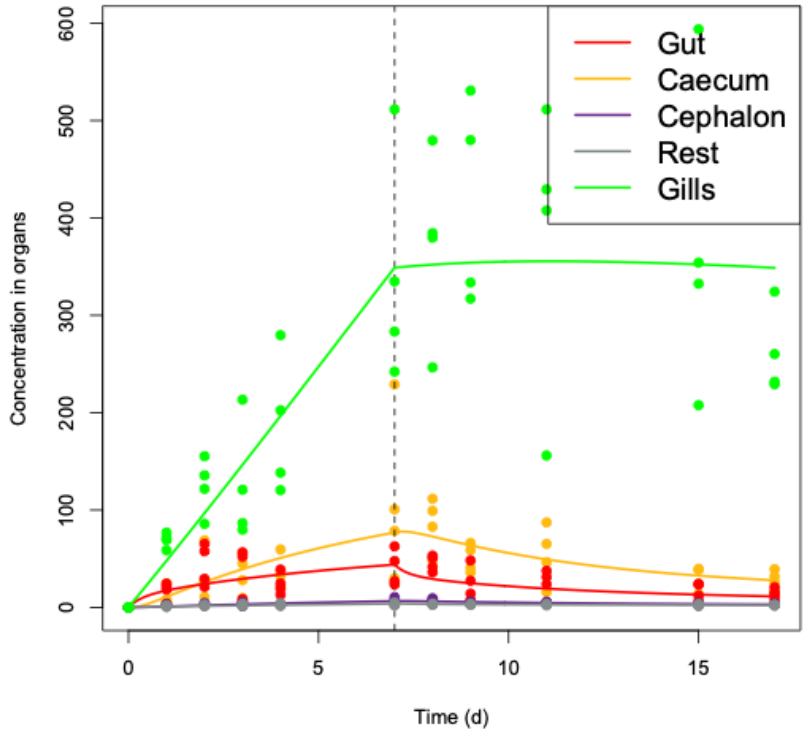
➢ Analytical solution exist

Charles et al., 2022

Multi-compartments models: case study for *Gammarus fossarum*

- Objective: find the more parcimonious model to simultaneously describe all organs' data





Fit one-compartment models: practice

Stochastic part of a model

- All models are composed of a deterministic part and a stochastic part
- Deterministic part describes the mean tendency as deduced from the data, while the stochastic part describes the variability around this mean tendency
- Here internal concentration = quantitative continuous data => Gaussian stochastic part

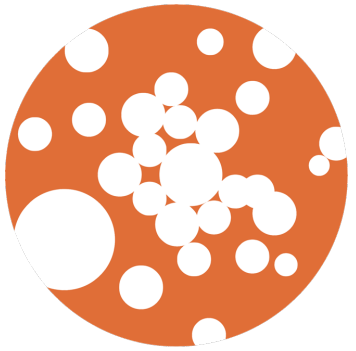
$$C_{obs}(t) \sim \mathcal{N}(C_i(t), \sigma)$$

MOSAIC$_{bioacc}$

- All-in-one web platform to fit one-compartment models to uptake and elimination experimental data

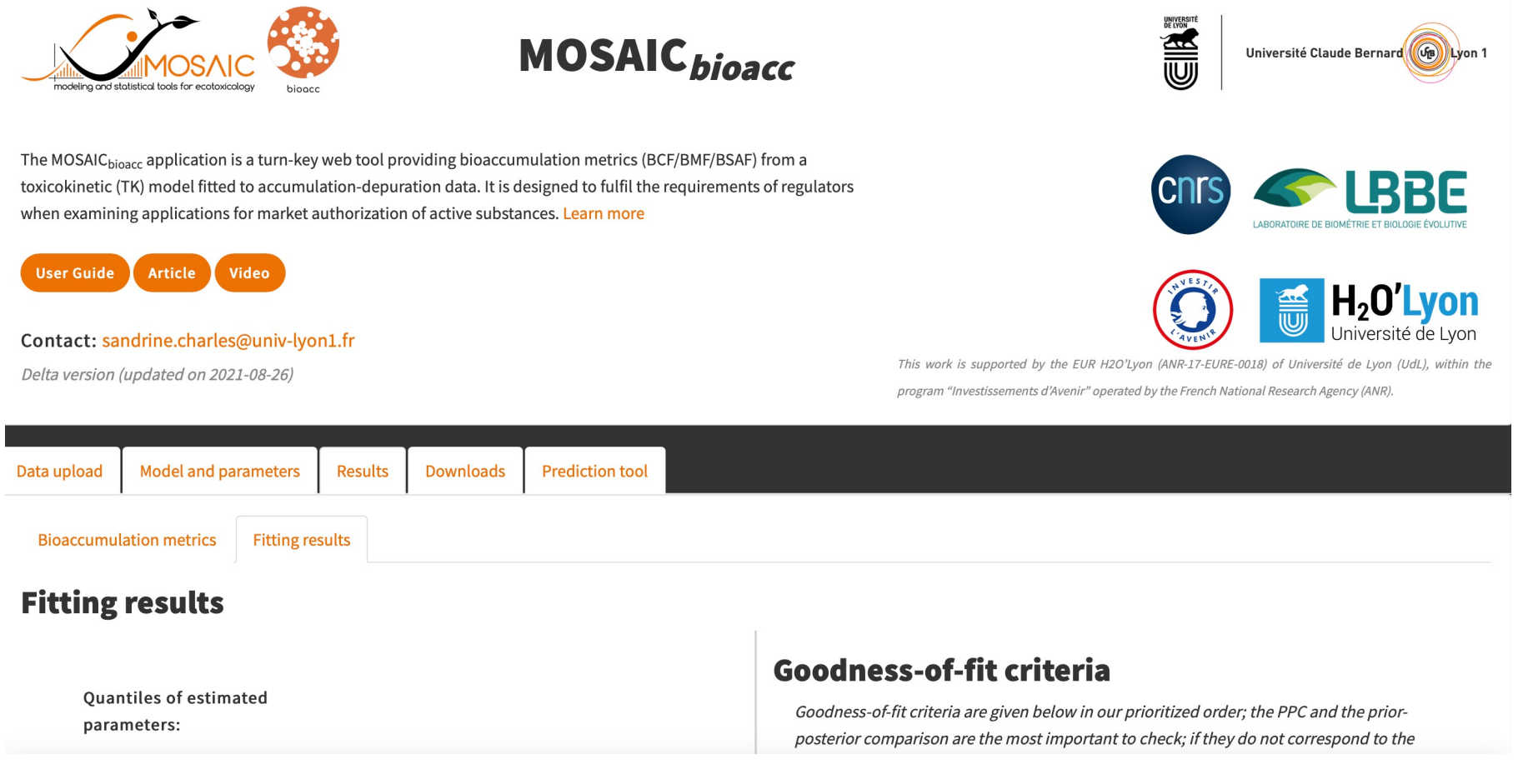
- All implemented models may combine
  4 exposure routes (water, sediment, food, pore water)
  3 elimination processes (excretion, dilution by growth and biotransformation for phase I metabolites)

- Automatic fit of the one-compartment model corresponding to the input experimental data



$MOSAIC_{bioacc}$: demonstration

http://lbbe-shiny.univ-lyon1.fr/mosaic-bioacc/



$MOSAIC_{bioacc}$: output

- Bioaccumulation metrics
- Parameters estimates: median and bounds of the 95% CI (quantiles of marginal posterior distributions)
- Fit of the median prediction with its uncertainty band superimposed to observed data
- Goodness-of-fit criteria

Goodness-of-fit criteria

- PPC
- Prior / posterior distributions
- Correlation between parameters
- Convergence criteria (Gelman and Rubin)
- WAIC
- MCMC traces

$MOSAIC_{bioacc}$: database

http://lbbe-shiny.univ-lyon1.fr/mosaic-bioacc/data/database/TK_database.html

- Accumulation – depuration data collection in support to TK modelling integrated in $MOSAIC_{bioacc}$

Ratier & Charles, 2022

- Wide variety of datasets:
  - Wide variety of species: freshwater and marine invertebrates, fishes, insects, terrestrial worms, vertebrates...
    => wide variety of contaminant uptake routes
  - Wide variety of contaminants: metals, organic compounds, insecticide, pharmaceuticals...
    => wide variety of bioavailability
  - Wide variety of accumulation durations: acute and chronic exposure

Package `rbioacc`

https://CRAN.R-project.org/package=rbioacc

rbioacc: Inference and Prediction of ToxicoKinetic (TK) Models

The MOSAICbioacc application is a turnkey package providing bioaccumulation factors (BCF/BMF/BSAF) from a toxicokinetic (TK) model fitted to accumulation-depuration data. It is designed to fulfil the requirements of regulators when examining applications for market authorization of active substances. See Ratier et al. (2021) <doi:10.1101/2021.09.08.459421 >.

Version:	1.1-0
Depends:	R (≥ 3.5.0)
Imports:	ggplot2, methods, Rcpp, rstan (≥ 2.18.1), rstantools (≥ 2.1.1), ggmcmc, GGally, loo, stringr, stats, zoo
LinkingTo:	BH (≥ 1.66.0), Rcpp, RcppEigen (≥ 0.3.3.3.0), RcppParallel (≥ 5.0.1), rstan (≥ 2.18.1), StanHeaders (≥ 2.18.0)
Suggests:	knitr, rmarkdown, testthat
Published:	2022-01-12
Author:	Virgile Baudrot [aut], Sandrine Charles [aut], Ophélia Gestin [ctb], Mélina Kaag [aut], Christelle Lopes [ctb], Gauthier Multari [ctb], Alain Pavé [ctb], Aude Ratier [aut], Aurélie Siberchicot [aut, cre]
Maintainer:	Aurélie Siberchicot <aurelie.siberchicot at univ-lyon1.fr>
BugReports:	https://github.com/aursiber/rbioacc/issues
License:	MIT + file LICENSE
URL:	https://github.com/aursiber/rbioacc

Fit multi-compartment models: practice

Package `rPBK`

https://CRAN.R-project.org/package=rPBK

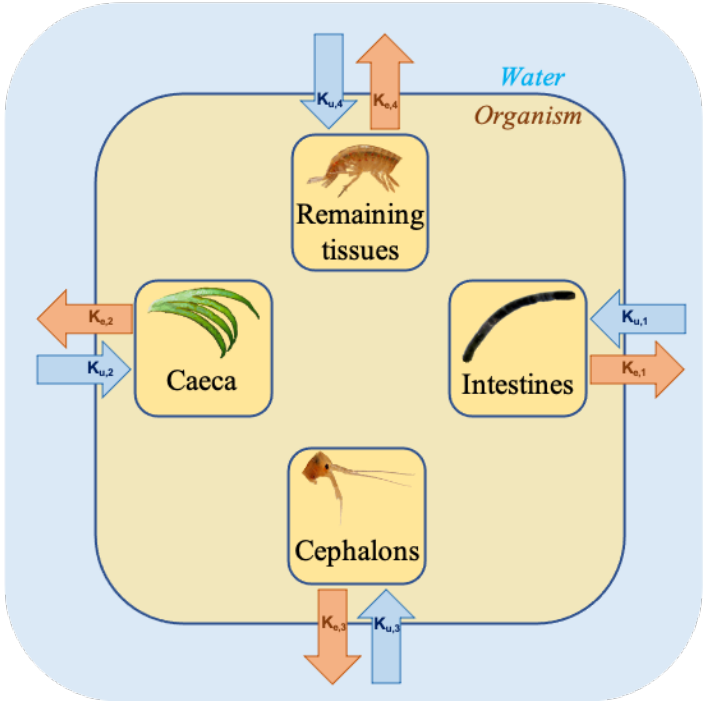
rPBK: Inference and Prediction of Generic Physiologically-Based Kinetic Models

Fit and simulate any kind of physiologically-based kinetic ('PBK') models whatever the number of compartments. Moreover, it allows to account for any link between pairs of compartments, as well as any link of each of the compartments with the external medium. Such generic PBK models have today applications in pharmacology (PBPK models) to describe drug effects, in toxicology and ecotoxicology (PBTK models) to describe chemical substance effects. In case of exposure to a parent compound (drug or chemical) the 'rPBK' package allows to consider metabolites, whatever their number and their phase (I, II, ...). Last but not least, package 'rPBK' can also be used for dynamic flux balance analysis (dFBA) to deal with metabolic networks. See also Charles et al. (2022) <doi:10.1101/2022.04.29.490045 >.

Version:	0.2.0
Depends:	R (≥ 3.4.0)
Imports:	ggplot2, methods, Rcpp (≥ 0.12.0), rstan (≥ 2.18.1)
LinkingTo:	BH (≥ 1.66.0), Rcpp (≥ 0.12.0), RcppEigen (≥ 0.3.3.3.0), RcppParallel (≥ 5.0.1), rstan (≥ 2.18.1), StanHeaders (≥ 2.18.0)
Suggests:	knitr, loo, rmarkdown, testthat
Published:	2022-09-01
Author:	Virgile Baudrot [aut, cre], Sandrine Charles [aut], Christelle Lopes [aut], Ophélia Gestin [ctb], Dominique Lamonica [ctb], Aurélie Siberchicot [ctb]
Maintainer:	Virgile Baudrot <virgile.baudrot at qonfluens.com>
License:	MIT + file LICENSE

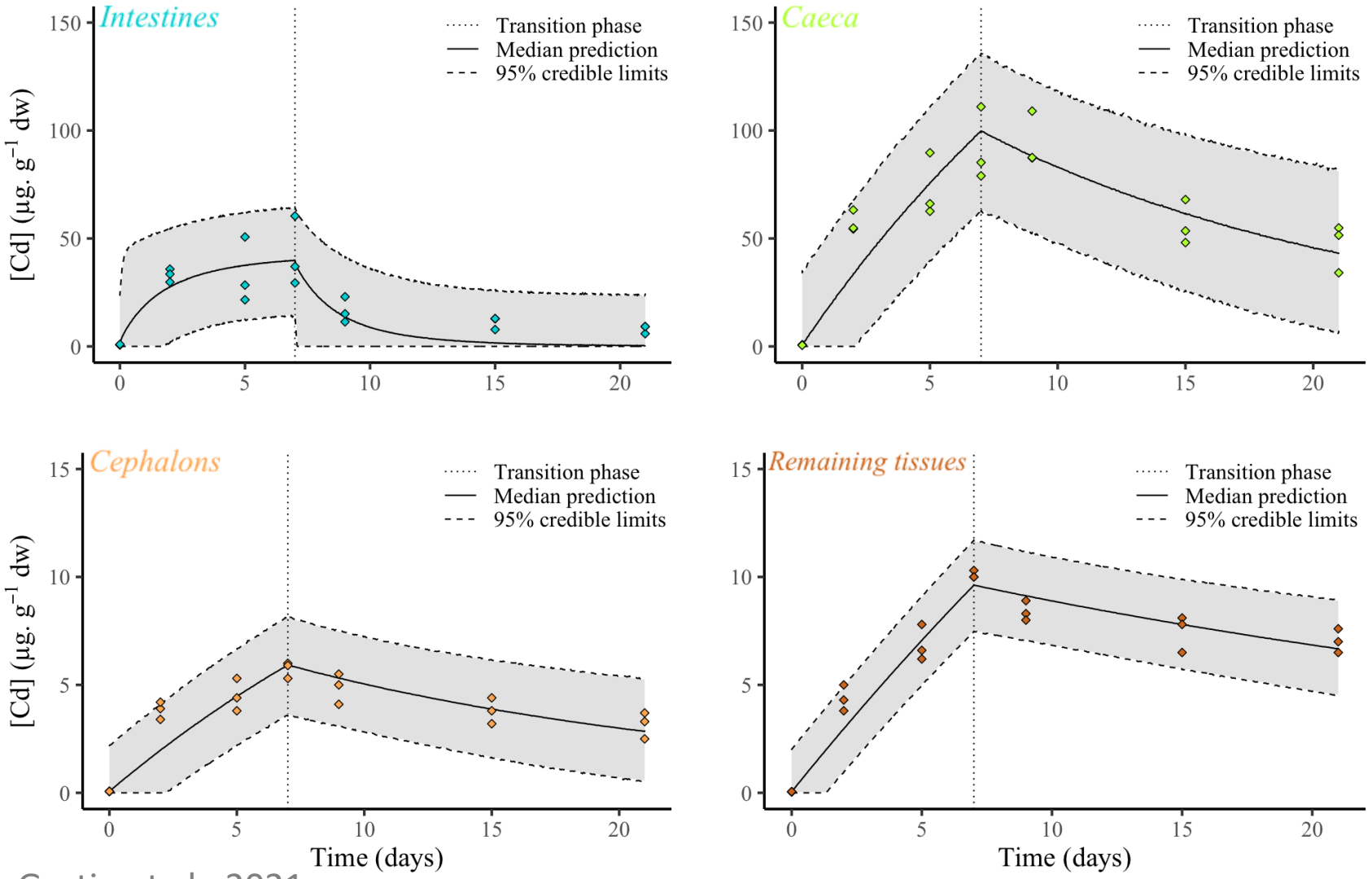
Added-value of multi-compartment models: a case study

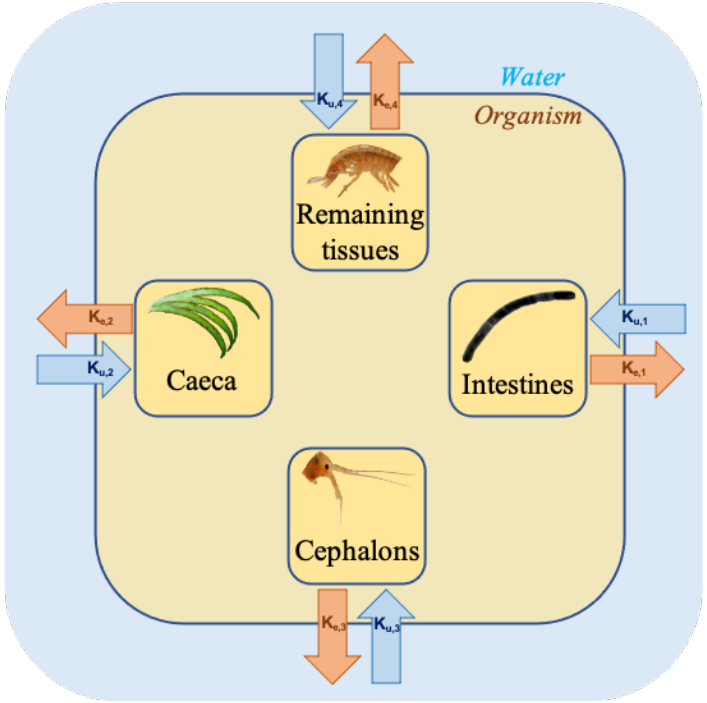
- Gammarids exposed to Cadmium at 11 µg/L during 7 days followed by 14 days of depuration
- Measure of Cd concentrations in 4 organs (cephalon, intestines, caeca and remaining tissues)
- Fit of a one-compartment model to each organ TK data



Gestin et al., 2021

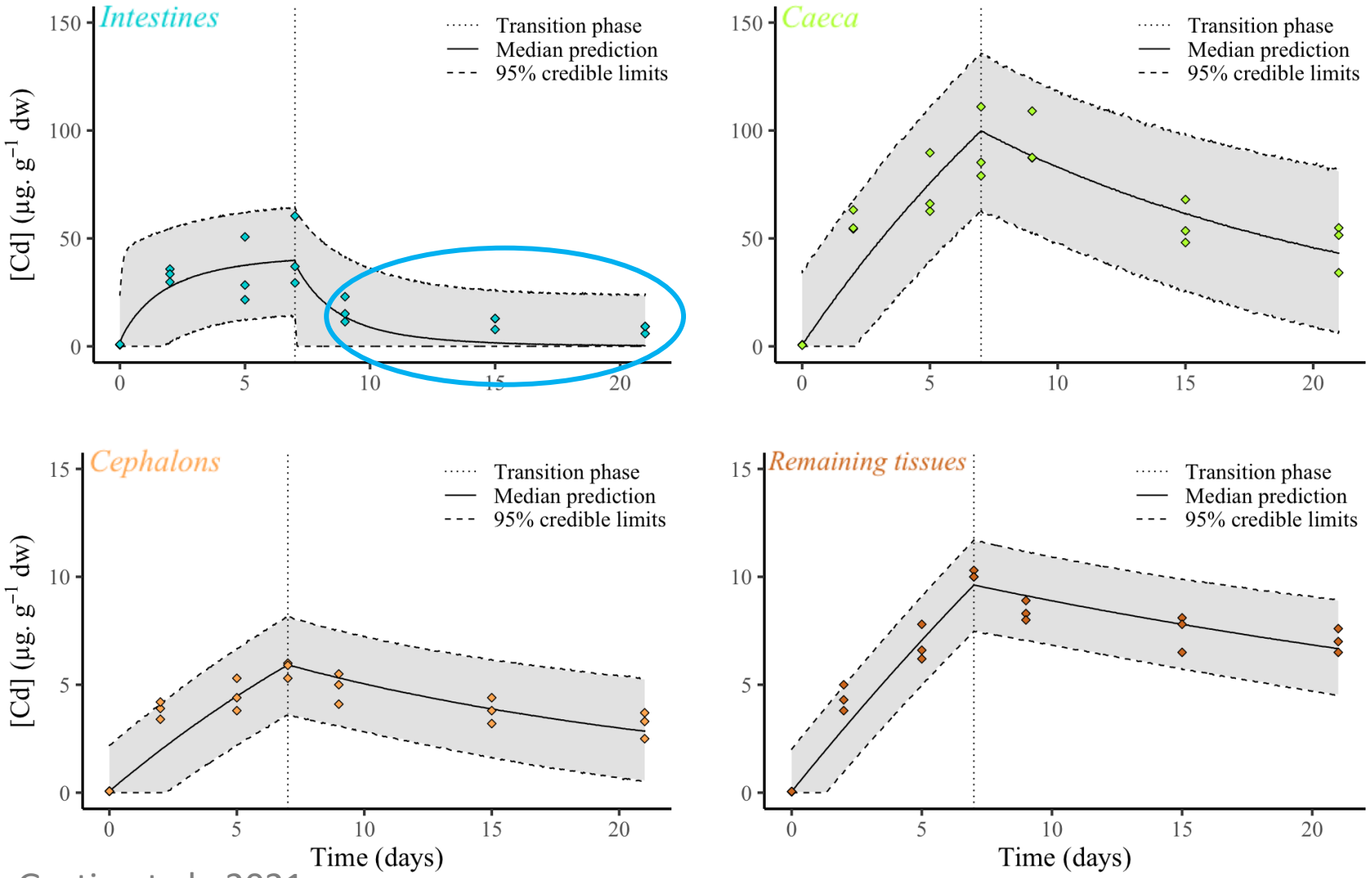
Added-value of multi-compartment models: a case study

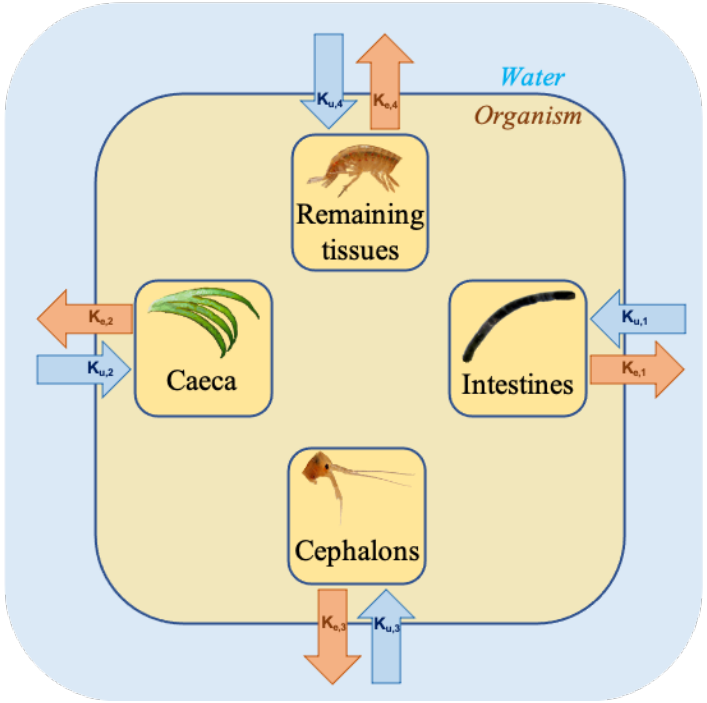




Gestin et al., 2021

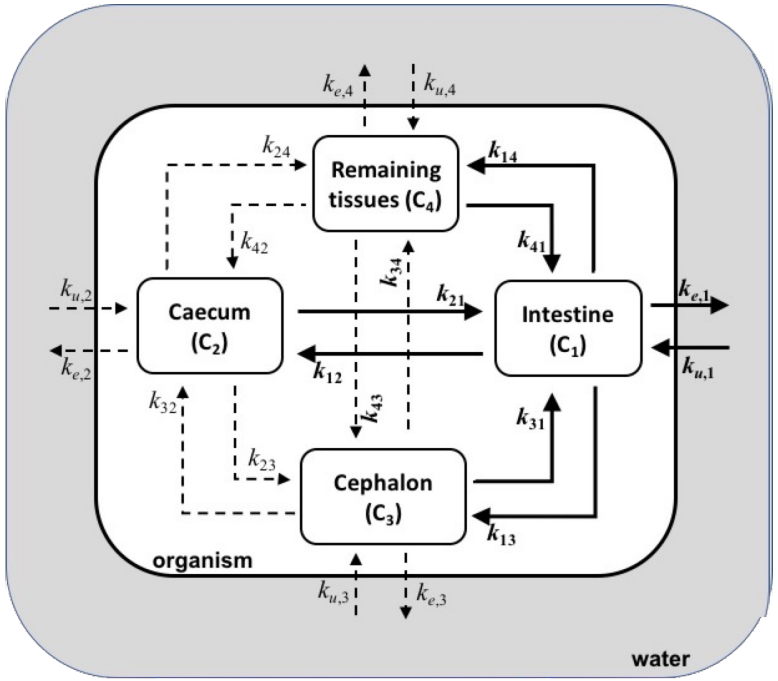
Added-value of multi-compartments models: a case study



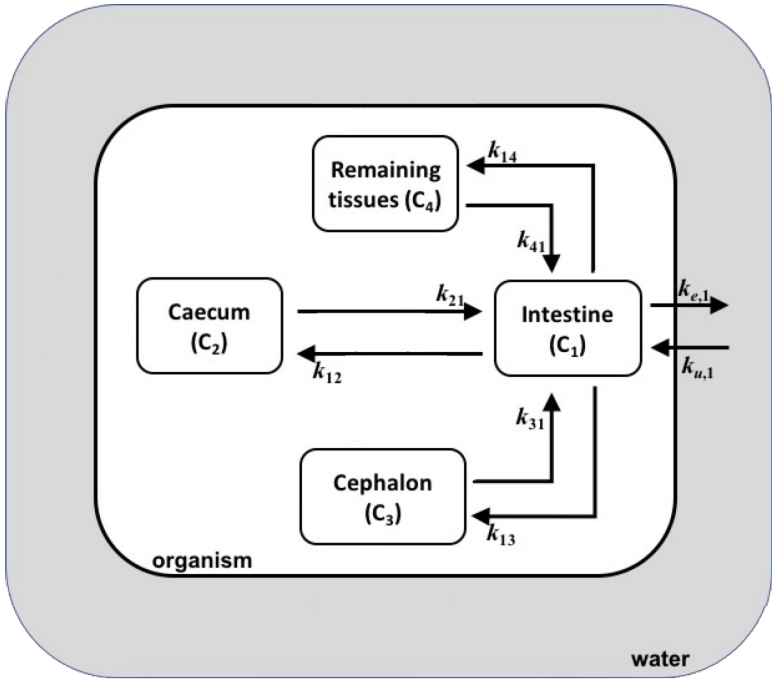


Gestin et al., 2021

Added-value of multi-compartment models: a case study

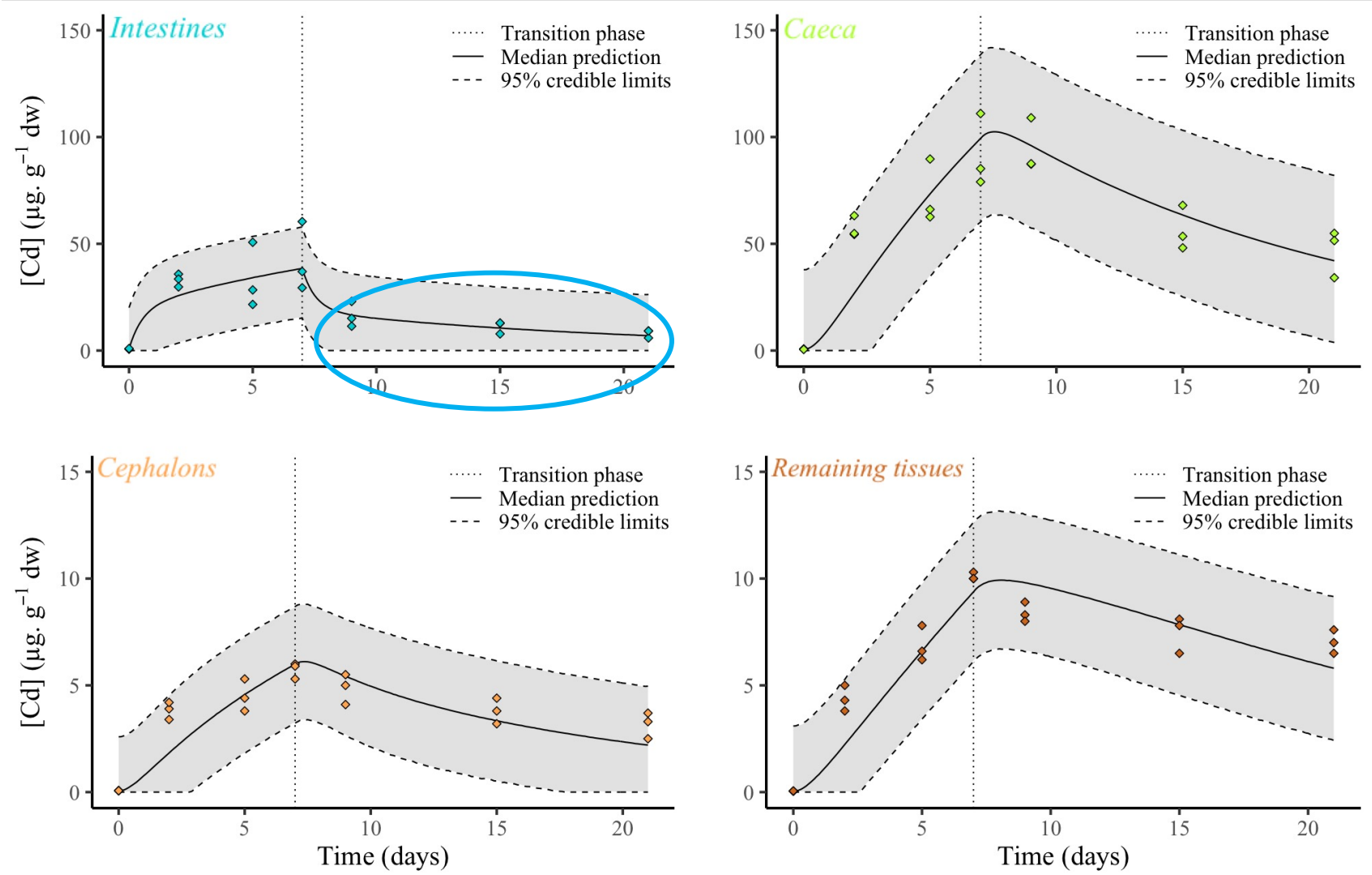


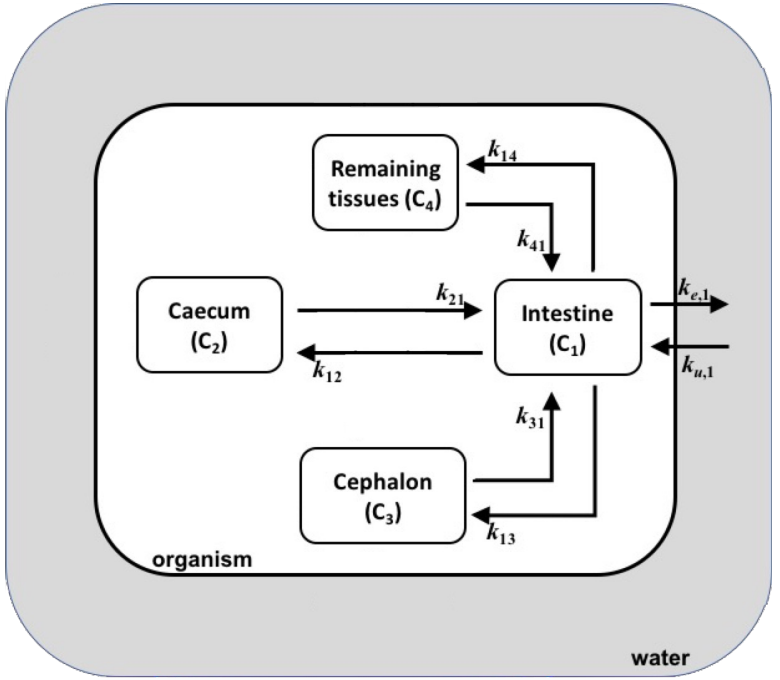
Interactive procedure



Gestin et al., 2021

Added-value of multi-compartment models: a case study

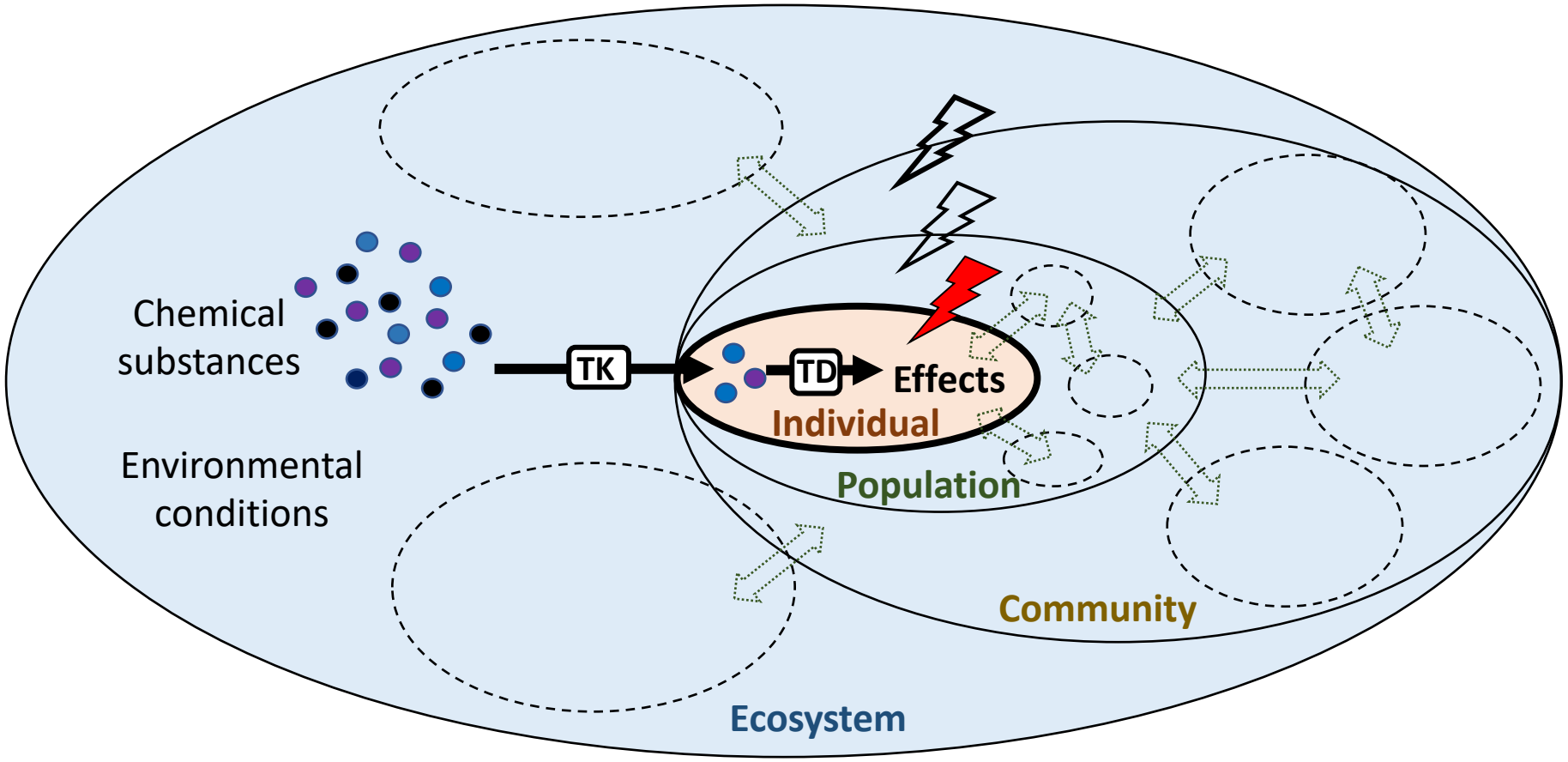




Gestin et al., 2021

Link of TK to TD

- Better description of effects with PBTK models ?



Toxico-Kinetic and Toxico-Dynamic framework

- Translate (time-varying) external concentrations to time patterns of effects.