TerAmphiTox: Designing a strategy based on toxicity evaluation to improve pesticide risk assessment for terrestrial amphibians

PERIAMAR

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Project info

Funding body

Consortium

Duration: **autumn 2020 – autumn 2023**

Regulatory questions

1. Which properties of an active substance are most likely to cause toxicity via dermal exposure?
2. Is there a correlation between the properties of a chemical substance and its toxicity to amphibians that researchers have already discovered? Can we identify gaps in today's knowledge?
3. Can we establish a hypothesis about the toxicity of a chemical to amphibians, and what do we need to know about the properties of the substance to make a prediction?

Project objectives

1. To investigate the toxicity of different pesticides, including active ingredients and formulations, on amphibian terrestrial stages and establish patterns of toxicity based on the properties of the substances.
2. To determine the role that effects on skin, because of direct exposure, play on the toxicity of the different substances to terrestrial amphibians.
3. To establish criteria for identification of those types of pesticides whose toxicity to amphibians can be extrapolated from surrogate taxa, and those pesticides for which there could be a need of specific toxicity testing on amphibians.
4. To calculate assessment factors for those pesticides whose toxicity on amphibians can be extrapolated from surrogates.

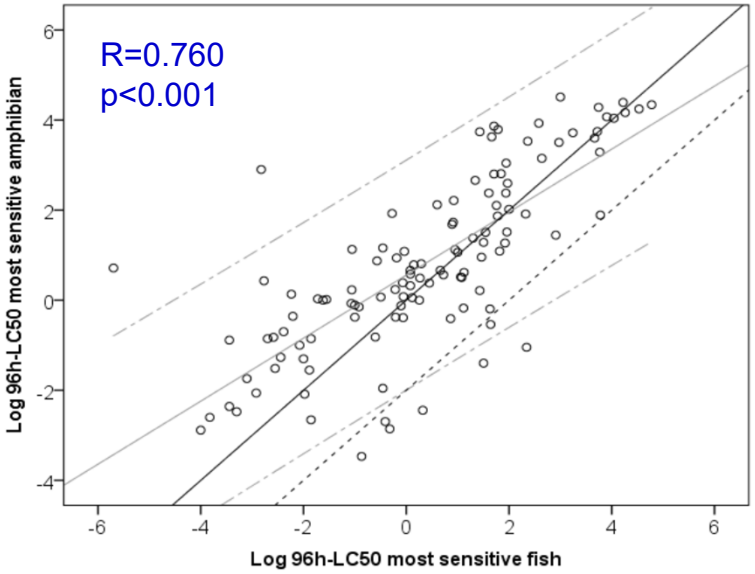
Background

Ecotoxicology (2018) 27:819–833
https://doi.org/10.1007/s10646-018-1911-y

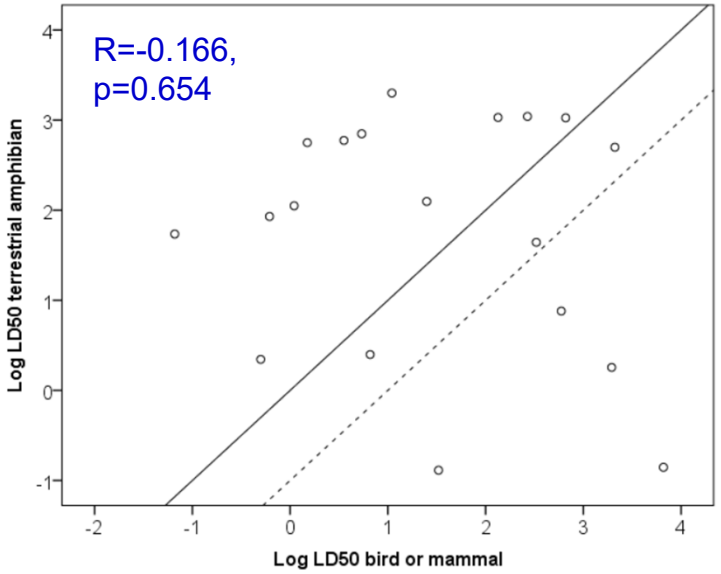
Validity of fish, birds and mammals as surrogates for amphibians and reptiles in pesticide toxicity assessment

Manuel E. Ortiz-Santaliestra[1] · Joao P. Maia[2] · Andrés Egea-Serrano[1] · Isabel Lopes[2]

Aquatic stages vs. fish



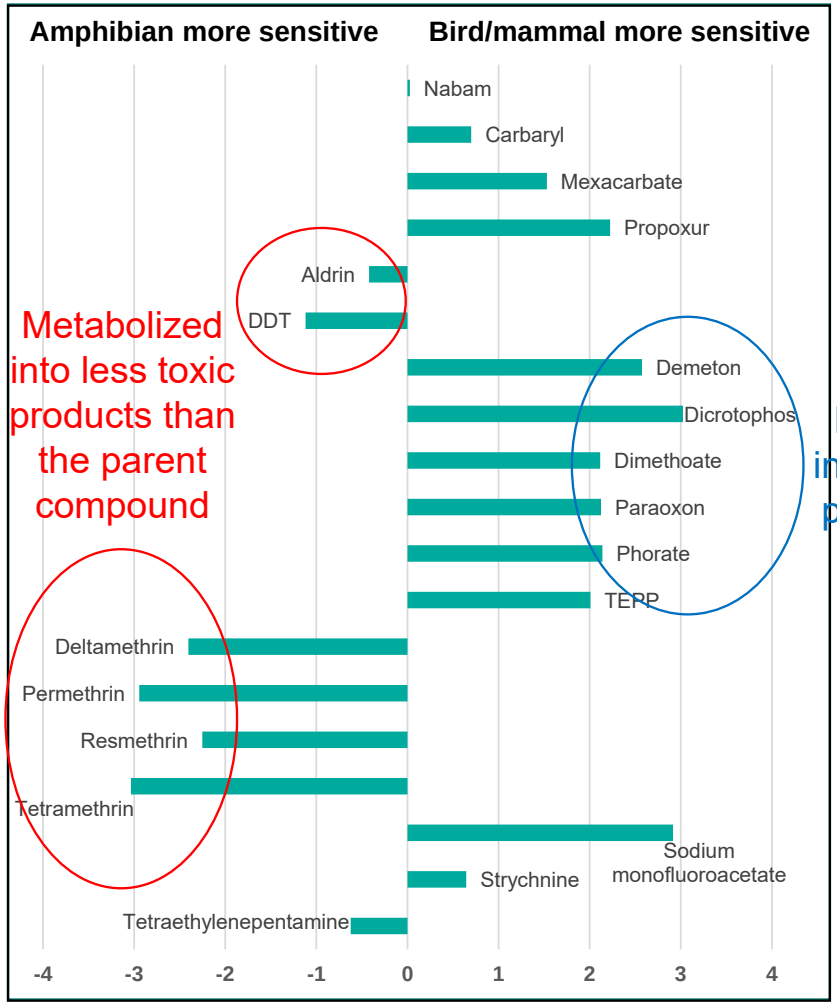
Terrestrial stages vs. birds / mammals



Good correlations with surrogates for aquatic stages, not so good for terrestrial ones

Background

Can toxicity be predicted on the basis of substance properties or modes of action?



Metabolized into less toxic products than the parent compound

Metabolized into more toxic products than the parent compound

Product selection

Database with all currently approved a.i. with at least a formulation registered in Germany or Spain, including:

- Chemical properties: molecular weight, K_{OW}, water solubility, dissociation constant, DT_{50} in soil, water and water-sediment phases, RL_{50} in plant matrices, K_{OC} and chemical classifications
- (Eco) toxicological properties: LC_{50} / LD_{50} (birds, mammals, fish, Daphnia, sediment dwellers, bees, earthworms), NOEC (Repro) for birds, dermal and inhalation LC_{50} in mammals, classification of modes of action (Verhaar, Kienzler et al.), BCF

Selection of **16 active ingredients** covering the variability of all of the above properties

+

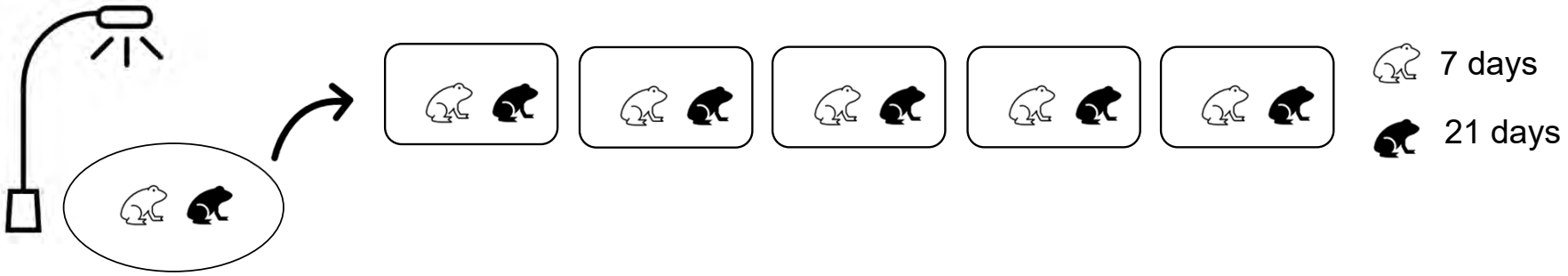
6 formulations, each containing one of the above a.i.

+

2 co-formulants from those formulation causing higher differential toxicity compared to a.i.

Experimental designs

Pelophylax perezi juveniles (worst-case, maximized surface-to-volumen ratio)



One-time exposure by overspray, contact to sprayed soil, or both

		Overspray			
		No pesticide	*0.1x A.R.*	*1x A.R.*	*10x A.R.*
Soil treatment	*No pesticide*	10 frogs	5 frogs	5 frogs	5 frogs
	1x A.R.	5 frogs	-	5 frogs	-

A.R.: Application rate.

Preliminary results

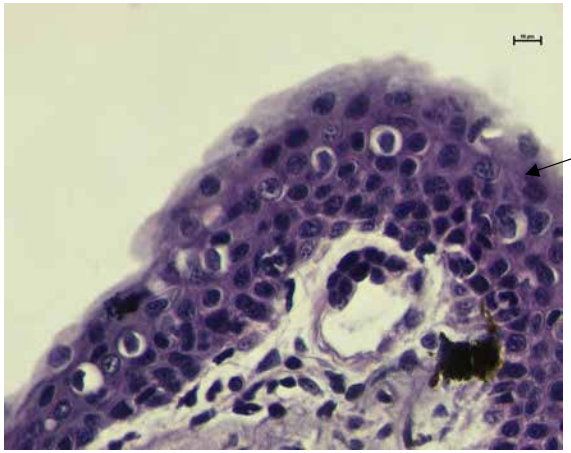
Acute mortality caused by formulations, but not by a.i.

No effects on growth or activity of surviving frogs

The majority of the tested products so far cause histological effects:

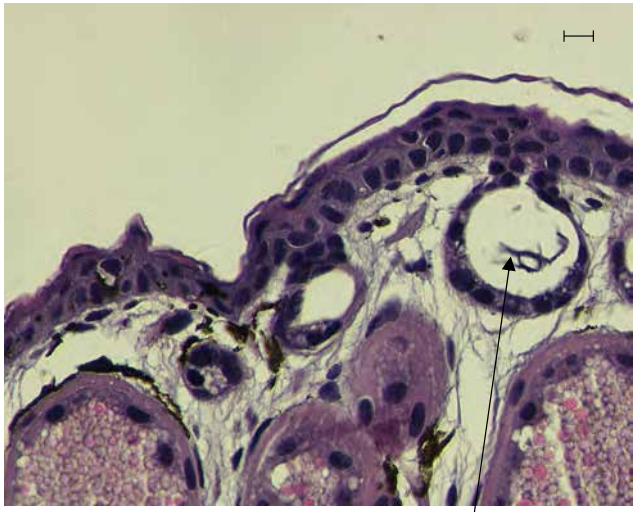
- Increased stratification of the stratum corneum (SC)
- Increased cellular disorganization
- Swelling of keratinocytes

Alpha-cypermethrin (a. i.)



Swelling of keratinocytes

Control

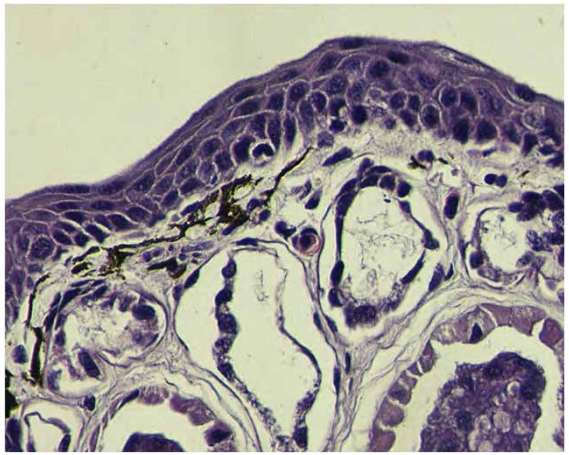


Stratum corneum

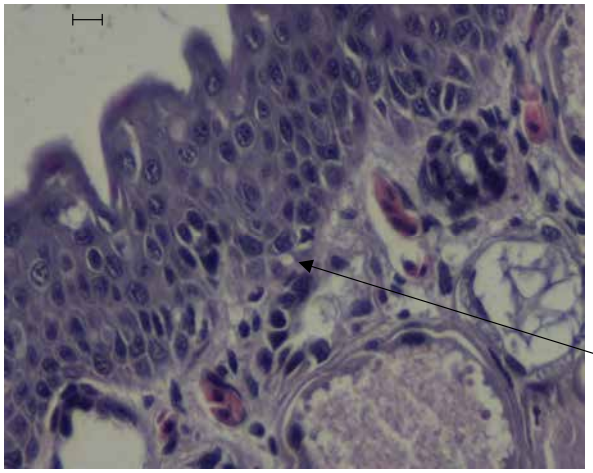
Granular gland

Serous gland

Metsulfuron-methyl



Fasthrin 10 EC



Cellular disorganization

Upcoming analyses

Pesticide body residues

Skin functionality:

- Secretion and antimicrobial activity of cutaneous peptides

- Skin microbiome diversity

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AMPHIDEB: Development of biologically-based models in environmental risk assessment to assess the impact of chemicals and pathogenic fungi on amphibian and reptile populations

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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Project info

Funding body

Consortium

Duration: **January 2022 – July 2025**

Objectives

1. **Structured data collection for amphibian and reptiles and development of open source biologically-based models for European amphibians and reptiles**

2. Field monitoring of Bd, Bsal in wild and captive amphibian populations and Ophidiomycosis in wild and captive snakes

3. **Calibration and validation of the biologically-based models for the risk assessment of single chemicals, multiple chemicals, chytrid fungi and multiple stressors in amphibians and reptiles**

Data collection for model development

Source	Biometry / life cycle	Physiology	Toxicity	Toxicokinetic
EFSA procurement	6 species	6 species	Yes, no restrictions	No
PERIAMAR	European amphibians, Saurians and Amphisbaenians	European amphibians, Saurians and Amphisbaenians	No	No
Add-my-pet	Yes, no restrictions	Yes, no restrictions	No	No
US EPA Ecotox	No	No	Yes, no restrictions	Yes, restricted to simple sum parameters (BCF)

Data collection for model development

Additional searches for missing data:

- Life history traits on non-EU species particularly well studied in ecotoxicology (e.g. Xenopus, North American ranids and bufonids…)
- Data sources for developing TKTD models (concentration- and time-dependent responses)
- Data to support PBTK models: bioaccumulation, blood perfussion rate and organ weights
- Mode of action of pathogens, to be incoporated to effect models.

Resources and data collection – Biologically-based models

- **Open-source program platforms**.
  - Resources: GitHub, R packages (CRAN), US-EPA Comptox chemicals dashboard, Add-my-pet models.
  - Search identificators: DEB, DEB-TOX, DEB-Kiss, DEB-TKTD, GUTS, PB-K, PBK-D, PBTK, TKTD, QSAR and all respective variants.

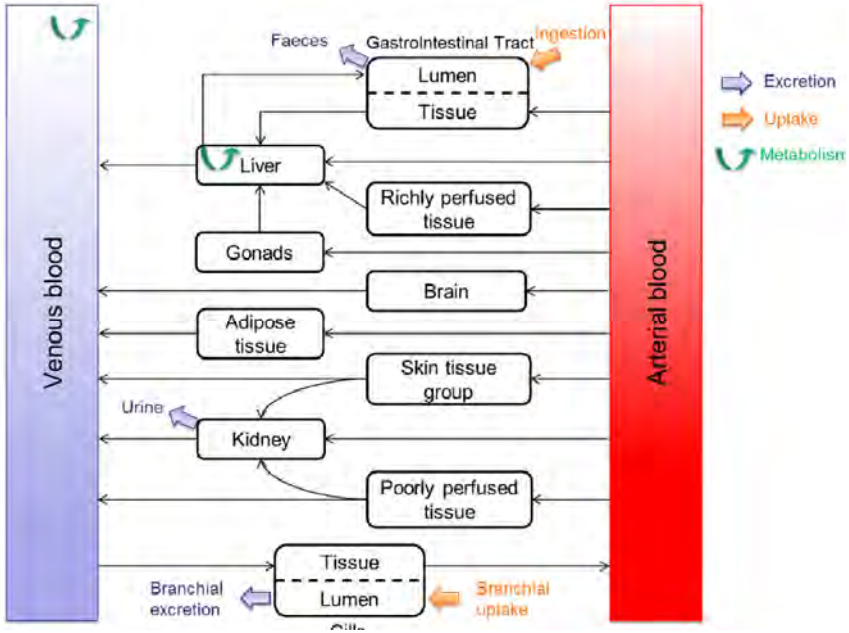
- **Peer-reviewed scientific literature**.
  - Similar strategy as for previous data types.

Evaluation of models for amphibians and reptiles

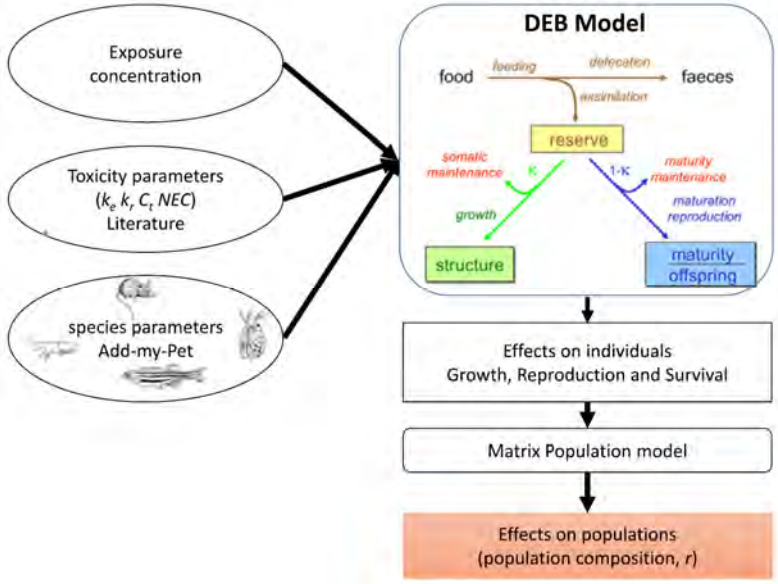
- Biologically-based models as identified during database and literature search will be analysed
- Each model will be evaluated concerning :
  - Relevance for modelling individual development of reptiles & amphibians
  - Incorporation of ecotoxicological processes/toxicokinetics/toxicodynamics
  - Model and source code availability
  - Status of model validation
  - Status of model documentation
  - Programming language/environment (R, C++, STAN, Matlab, Python)
- Subsequent discussion with EFSA and selection of the most relevant PBTK and single- and multiple-chemical effect models

Model implementation and development: chemicals

Adaptation and/or development of selected, most relevant PBTK and single- and multiple-chemical effect models in a relevant language (R, C++, Matlab, Python), future integration into the EFSA TKplate environment will be considered



PB-TK models for fish (Grech et al., 2019)



Dynamic energy budget models in ecological risk assessments (Baas et al., 2018)

Model implementation and development: chemicals

- Adaptation and/or development of selected, most relevant PBTK and single- and multiple-chemical effect models in a relevant language (R, C++, Matlab, Python), future integration into the EFSA TKplate environment will be considered
- Refined mechanistic equations will expand the applicability of generic PBTK models for fish as e.g. published by Grech et al. to amphibians and reptiles, both concerning physiological and TK processes.
- Parameters will be collected from publicly available data sources as far as available.
- Physiological parameters: the AmP database contains entries for 31.5% (n=30) of the amphibian and 15.9% (n=33) of the reptile species
- Toxicokinetic parameters: next to using (possibly limited) available datasets, estimation based on chemical and physiological properties (QSAR/ML) will be applied to allow the prediction of uptake, internal distribution and elimination of chemicals for a maximum range of amphibian and reptile species.
- Projections of population growth rates using, e.g., matrix models could be a useful connecting element for individual- to population level (e.g. Kooi and Koijman 2020).

Model implementation and development: biological stressors and multiple stressors

- Development of biologically-based models for the assessment of biological stressors and multiple stressors, will be explored with focus on infectious diseases; specifically, fungal infections, and on the influence of temperature.
- Accounting for temperature as a stressor is conceptually simple (Arrhenius and variants)
- Consideration of infectious diseases as biological stressor in a PB-TK-TD modelling context will need basic research and developmental work, and is to our knowledge completely new.
- Difficult to identify and/or quantify metrics for the impact of pathogen infections on the host organisms
- Potentially relevant aspects:
  - Infectious diseases might enhance exposure/sensitivity of an organism towards chemical stressors.
  - Chemical exposure might lower general resilience or immunocompetence and so enhance the susceptibility towards infections or outbreaks of infectious diseases.
  - The outbreak of infectious diseases such as Bd, Bsal and others might affect the physiological status of the infected organisms, and can result in reduced growth, reduced reproduction rates and, finally, in reduced survival.
- Models accounting for the effect of infectious disease outbreak as biological stressor will be developed at different levels of complexity, from simple approaches that link an infection immediately to reduced survival over time, towards more complex DEB-related models that assume infectious diseases to impact energy flows, and in consequence to reduced growth, reproduction or survival.

Calibration and validation of models

- ❑ 10 case studies, single chemicals (task 3.1)
- ❑ 10 case studies, chemical mixtures (task 3.2)

Probably achievable with existing information (although strongly biased towards certain species and chemicals)

- ❑ 5 case studies, diseases (task 3.3)
- ❑ 5 case studies, interaction chemicals-biological stressors (task 3.4)

Unlikely to be achievable with existing information (even after objective 1)

Experiments → ad hoc design to obtain useful data for model calibration and validation, to be completed, if possible, with **field data**.

Calibration and validation of models
Experimental designs

Exp	Goal	Treatment(s)	Candiate species	Life stage(s)	Endpoints	Other data
1	Single chemical	Pyrethroid	*Bombina variegata Discoglossus pictus* *Alytes obstetricans* *(Epidalea calamita, Rana temporaria, Pelophlyax* sp.)	Tadpoles and metamorphs	Survival Growth Development	Internal residues over time
2	Chemical mixture	Pyrethroid Widely studied pesticide (e.g. glyphosate)	Same as #1	Tadpoles and metamorphs	Survival Growth Development	Internal residues over time
3	Bd	Bd	*Discoglossus pictus* *Bombina variegata*	Tadpoles and metamorphs	Survival Growth Development	Bd infection loads
4	Bd + pesticide	Bd Pyrethroid (if no effect on Bd)	Same as #3	Tadpoles and metamorphs	Survival Growth Development	Bd infection loads
5	Bsal + pesticide	Bsal Pyrethroid (if no effect on Bsal)	*Ichthyosaura alpestris* *Triturus carnifex*	Larvae and metamorphs	Survival Growth Development	Bsal infection loads
6	Multiple stressors	RV, Bd Pyerthroid (no effect on pathogens) Temperature	*Alytes obstetricans*	Tadpoles, metamorphs and juveniles	Survival Growth Development	Bd+RV infection loads

Calibration and validation of models Methodology

- Chemical-specific calibration of the multi-parameter models: Both Bayesian and Frequentist approaches will be evaluated for their suitability, based on R packages (morse, rDEBtktd; Baudrot and Charles 2021, Trijau et al. 2021) and Matlab libraries (BYOM, Jager 2021).
- Available toxicokinetic data will be used to calibrate the PBTK, while effect observation over time (e.g. time series of survival, growth or reproduction) will be used to calibrate the PB-TK-TD models.
- For potential sublethal effects on growth, expressed via impairment of energy dynamics, possible mechanistic modes of action (EFSA PPR Panel 2018b, Ashauer and Jager 2018) will be analysed.
- Next, suitable validation steps will be proposed to and adjusted with EFSA, ideally using independent data sets; if this is not applicable due to lack of data, methods such as leave-one-out cross validation will be discussed with EFSA.
- All calibration and validation results will be reported as case studies, and included in appropriate documentation formats (e.g., TRACE).

Calibration and validation of models
Case studies

Task 3.1. Single chemicals

Bombina variegata
Discoglossus pictus
Alytes obstetricans

calibration ✕ Pyrethroid (Exp.1)
Other pesticides from literature

validation →

Task 3.2. Multiple chemicals (mixtures, Exp.2)

Bombina variegata
Discoglossus pictus
Alytes obstetricans

✕ Pyrethroid
Other pesticides from literature

Task 3.3. Fungal diseases

Bombina variegata
Discoglossus pictus

calibration ✕ Bd (**Exp. 3**)

validation →

Task 3.4. Multiple stressors

Bombina variegata
Discoglossus pictus

Exp. 4 ✕ Bd
Pyrethroid

validation →

Alytes obstetricans

Exp. 6 ✕ RV, Bd
Pyrethroid
Temperature

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PPP exposure of terrestrial stages of amphibians and possible management measures: a progress report

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in colllaboration with

Annette Aldrich (BAFU, Switzerland), Erich Szerencsits (Agroscope, Switzerland), Greg Churko (Agroscope, Switzerland)

WP 1: management options

- Survey among Agroscope collaborators: how could PPP exposure of amphibians in the terrestrial environment be reduced?

- Answers 1: The usual suspects, no novel ideas which could be implemented at the farm scale without harming amphibians (e.g., fences around fields).

- Answers 2: «Why are amphibians using agricultural fields?», suggesting a lac of awareness of the ecology and habitats of amphibians.

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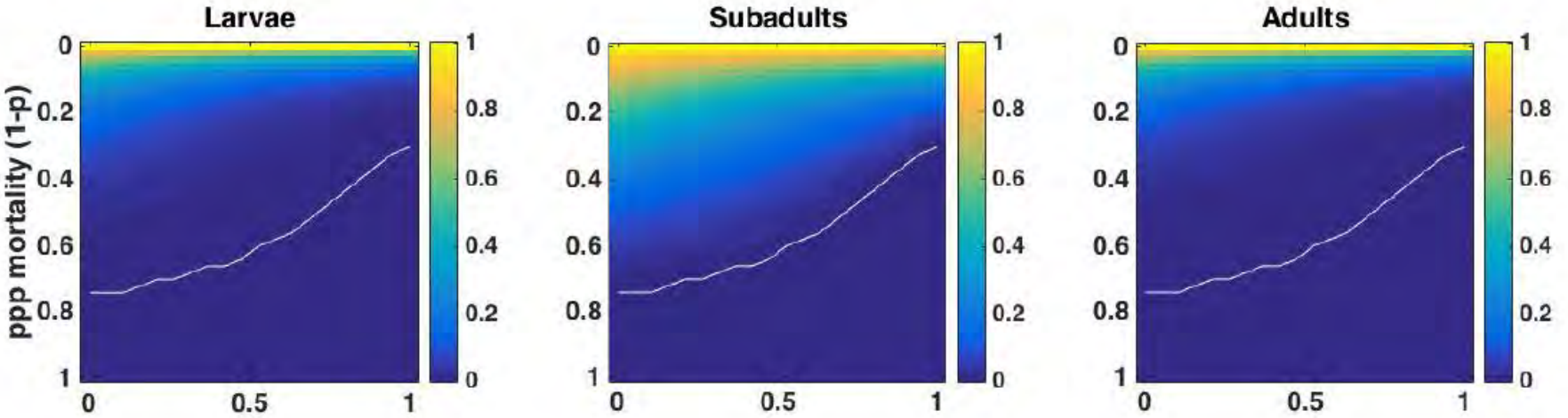
WP 2: population modelling

- Modelling was done by Claudio Bozzuto, Wildlife Analysis GmbH
- Single population 1: PPP-induced mortality reduces population size strongly



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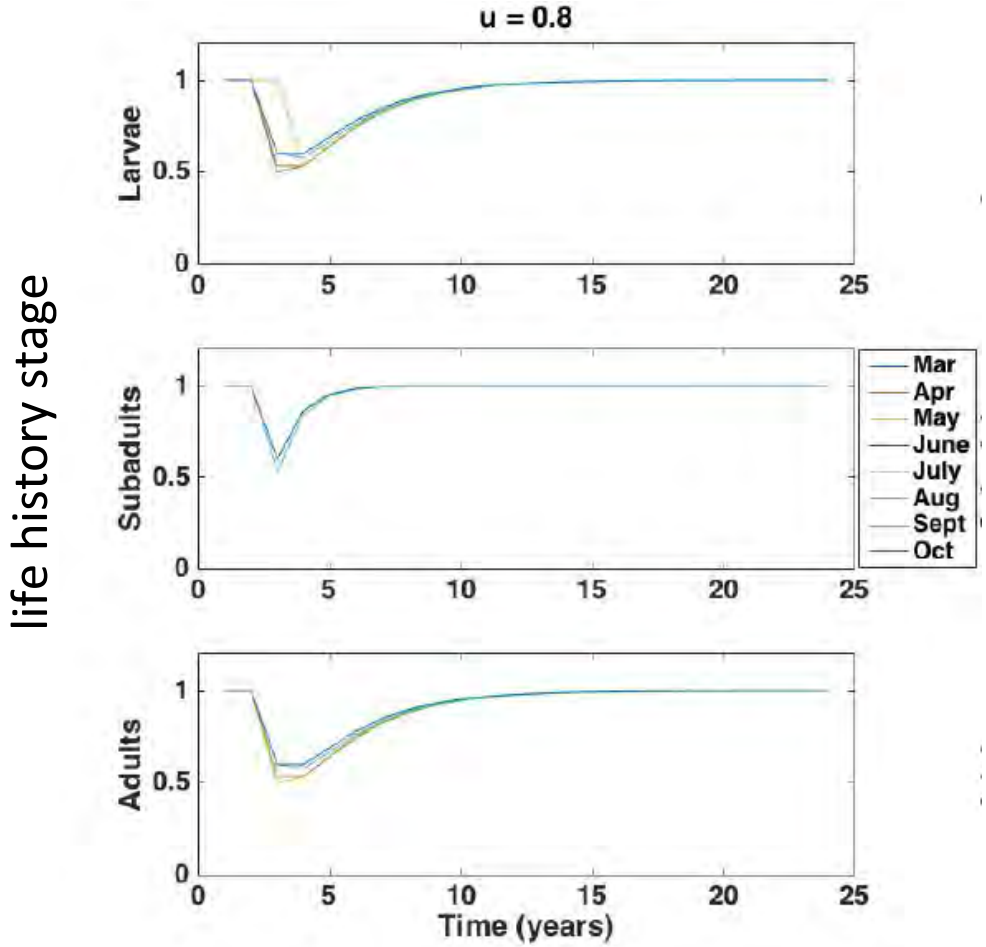
WP 2: population modelling

- Single population 3: recovery happens but takes time



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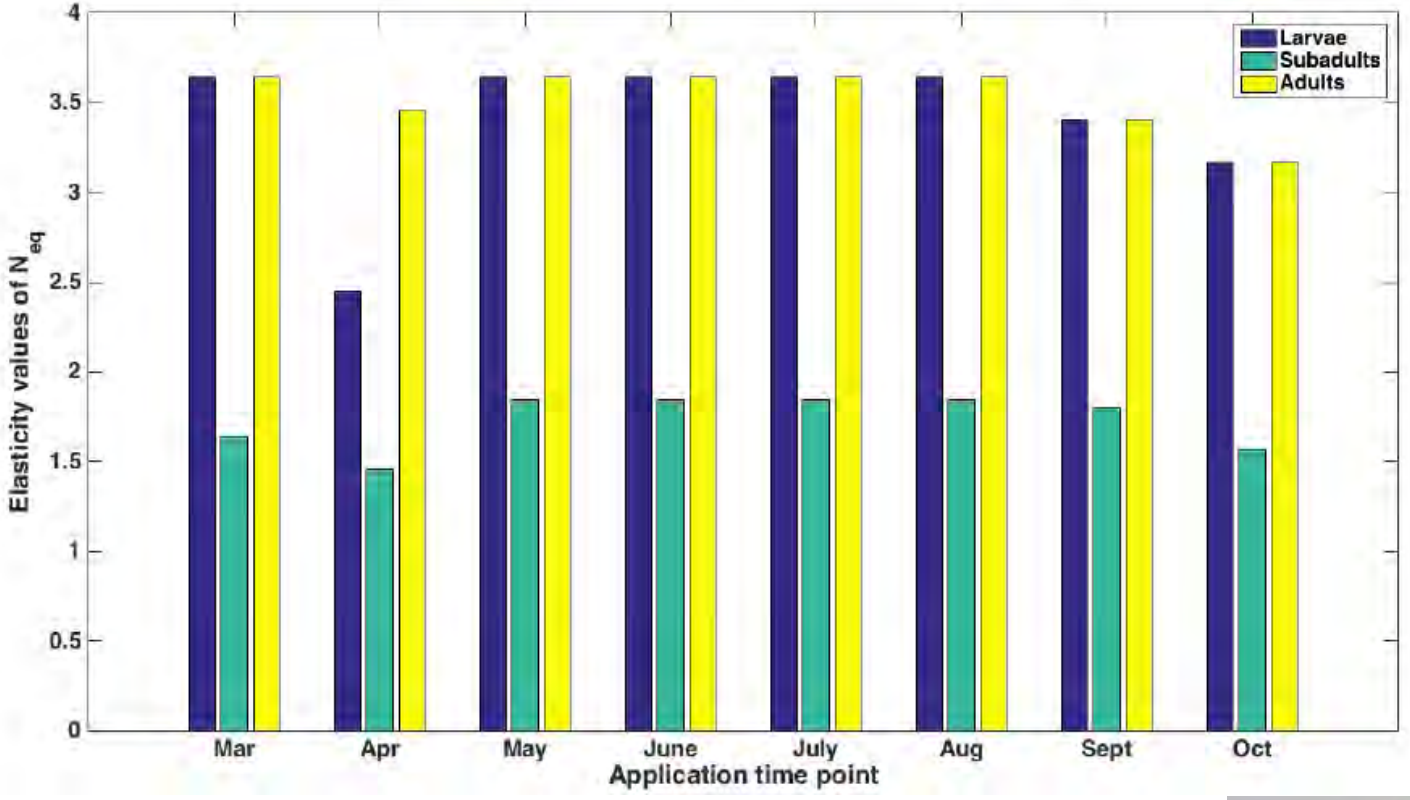
WP 2: population modelling

- Single population 3: the effects of PPP are the same in all months (and therefore all life history stages)



elasticity

month

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WP 2: population modelling

- Metapopulation model

- How to increase the viability of a metapopulation?

  - Reduce PPP-induced mortality in the aquatic habitat
  - Reduce PPP-induced mortality in the terrestrial habitat
  - A subset of the populations is not exposed to PPP
  - increase metapopulation size (add ponds & terrestrial habitat)

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WP 2: population modelling

- increase metapopulation size

  = or >

  subset of the populations is not exposed to PPP

  > Reduce PPP-induced mortality in the aquatic habitat,
  Reduce PPP-induced mortality in the terrestrial habitat

- More modelling on this topic underway

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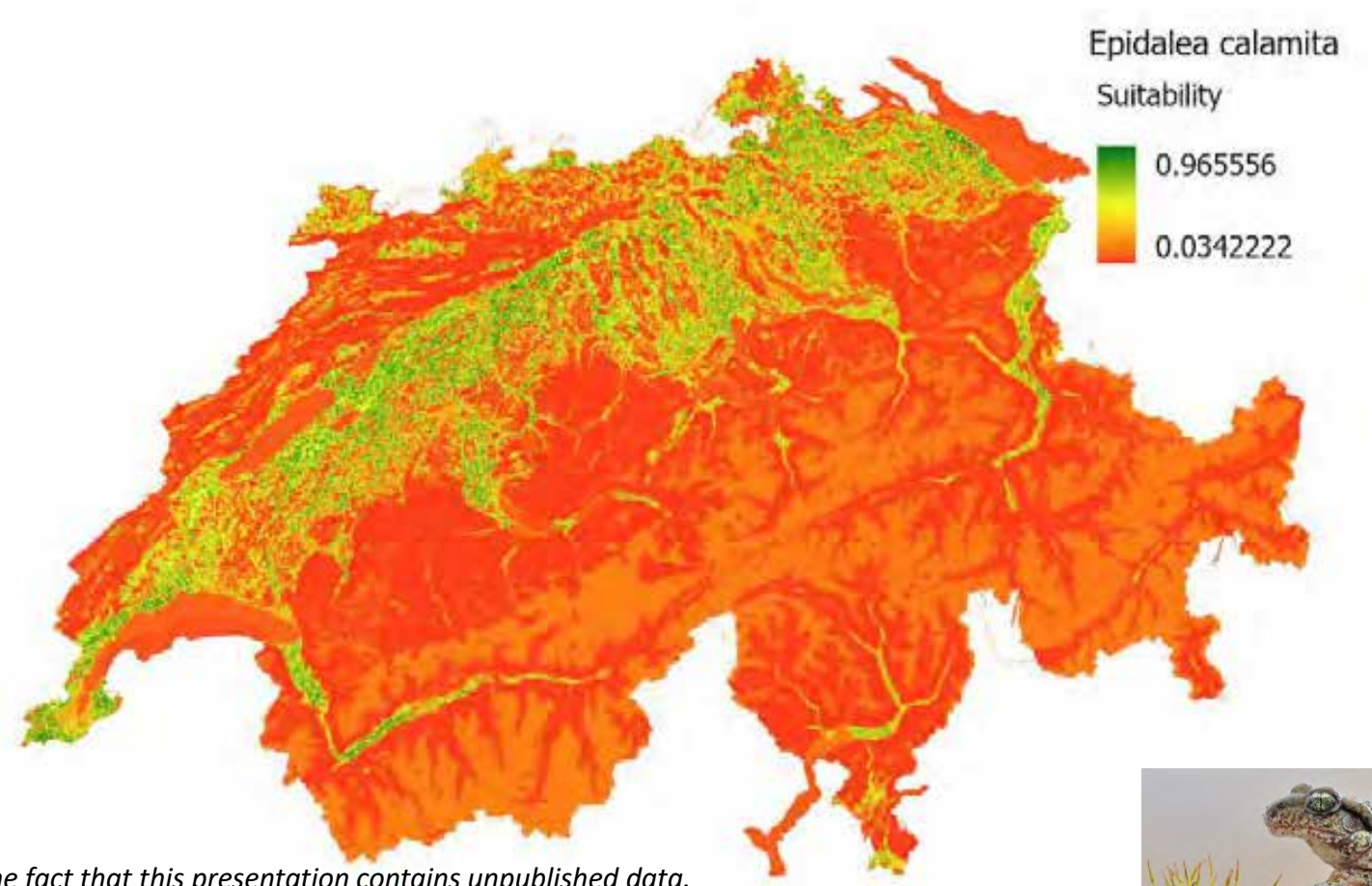
WP 3: landscape scale analyses

- Habitat suitability maps based on observations in the terrestrial habitat



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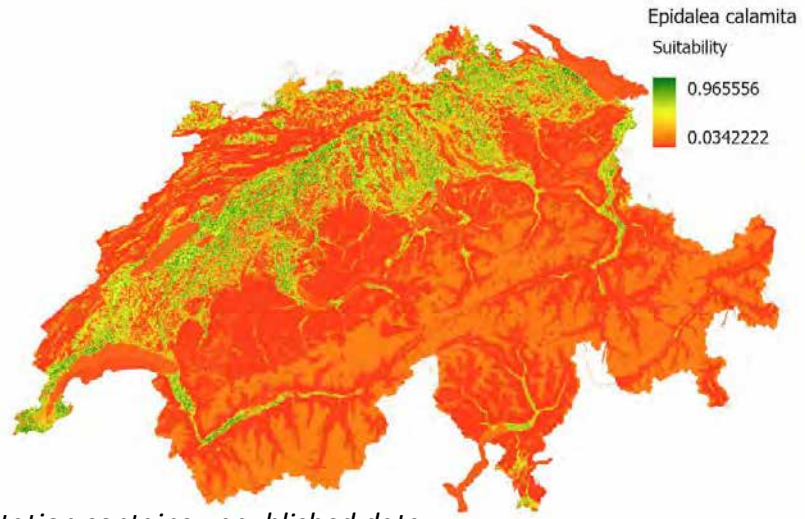
WP 3: landscape scale analyses

- Overlay habitat suitability with maps of PPP agriculture
- Use the maps to calculate corridors for dispersal among populations

-> Identify locations where management measures can mitigate the PSM-exposure to amphibians within their home ranges and while dispersing



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WP 4: use of agricultural fields

- Cover boards were placed in different habitats in study areas with large *Epidalea calamita* populations

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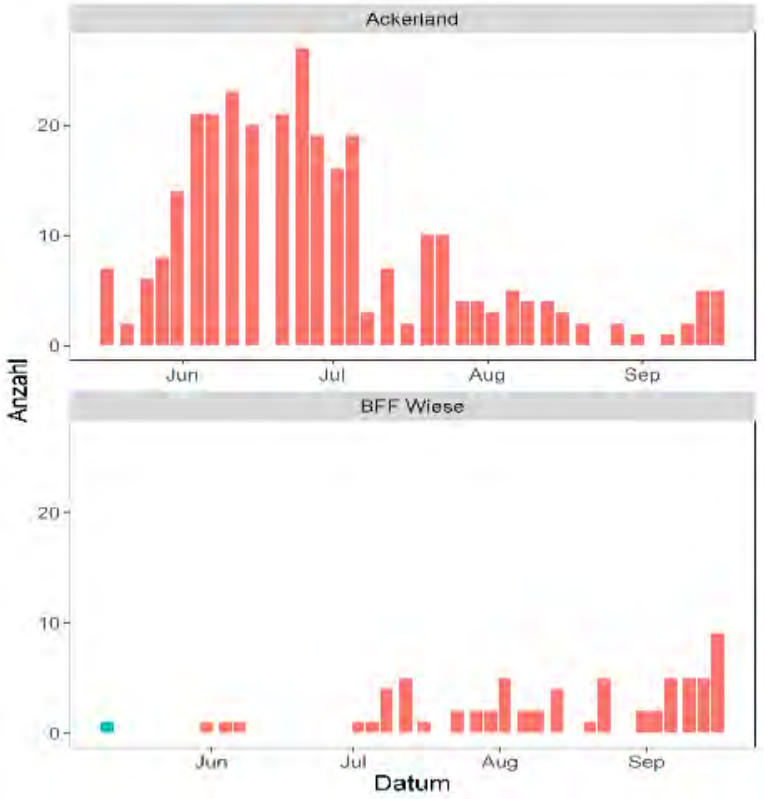
WP 4: use of agricultural fields

- Toads are found in the fields all summer long
- They prefer fields over grassland
- But individuals stay in one habitat type and don't switch habitat types much
- Some toads appear to be sedentary while others appear to be nomadic



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Modelling pollution exposure, accumulation and effects in reptile species

PERIAMAR project: Lifecycle energetics of reptiles under pollution stress
https://periamar.com/projects/ttView/21

STSM: Predicting maternal transfer of pesticides in reptiles based on pollutant molecular structure

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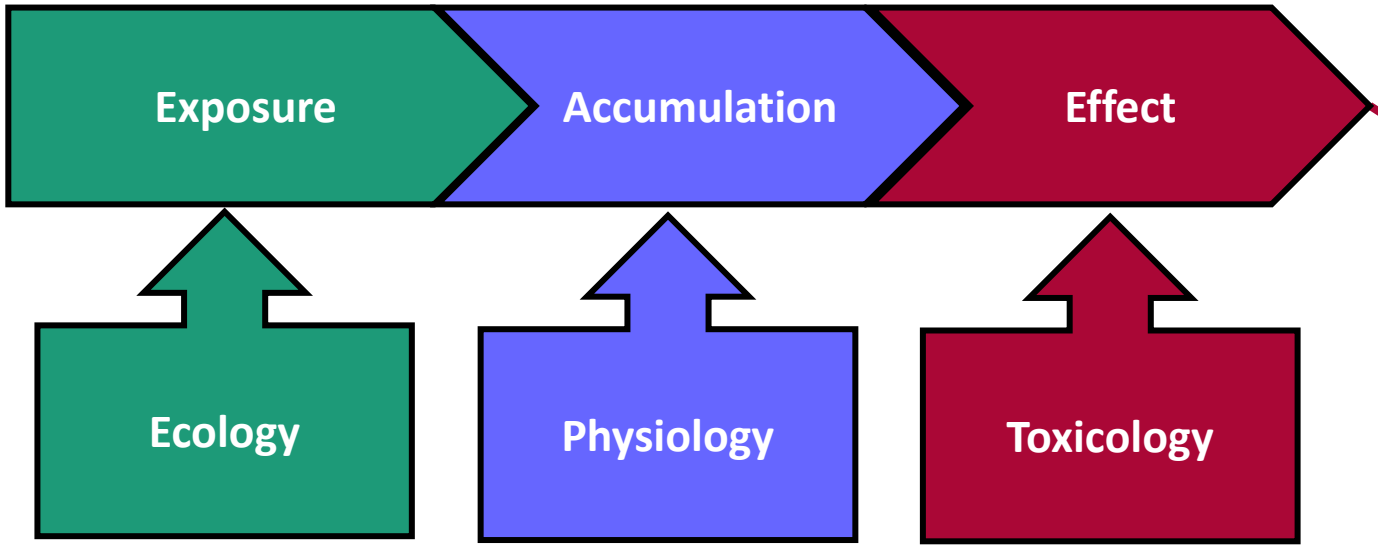
How do populations of reptiles respond to pollution in their natural environment?



✓ Individual variation

✓ Chemical mixture

✓ Multiple stressors

Alligator mississippiensis

Approach: Develop a mechanistic model to simulate population-level risk for reptile populations under multiple stressors

AlPA: Alligator Pollution bioAccumulation model

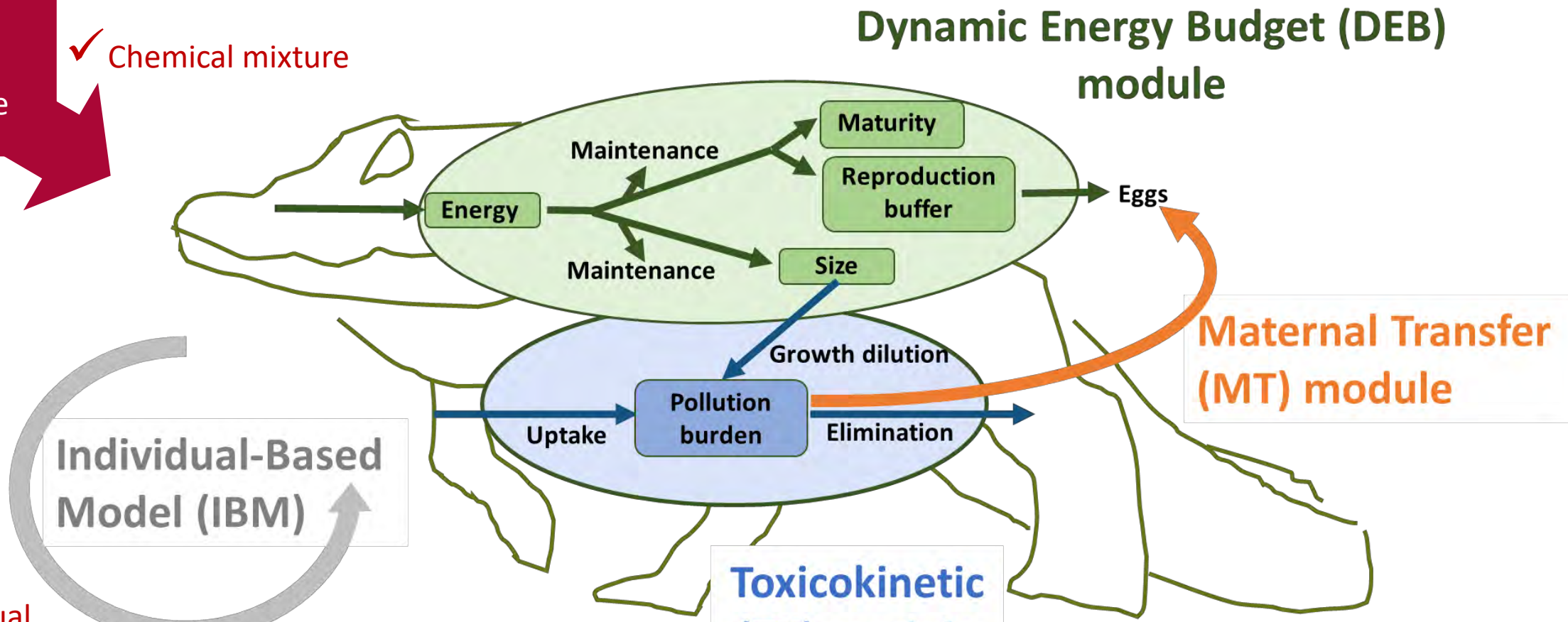
✓ Multiple stressors

Food

Pollutants

Temperature

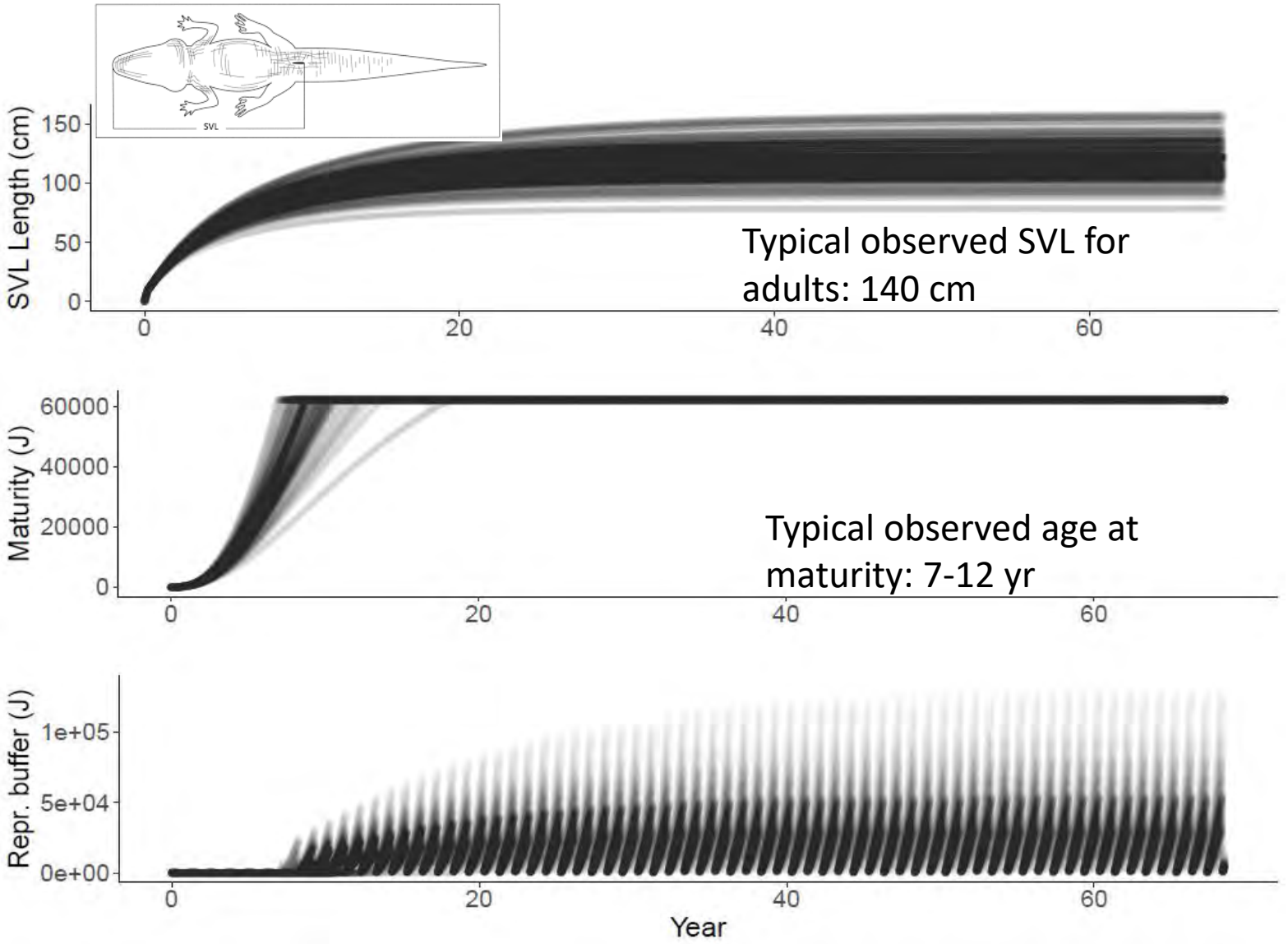
✓ Chemical mixture



✓ Individual variation

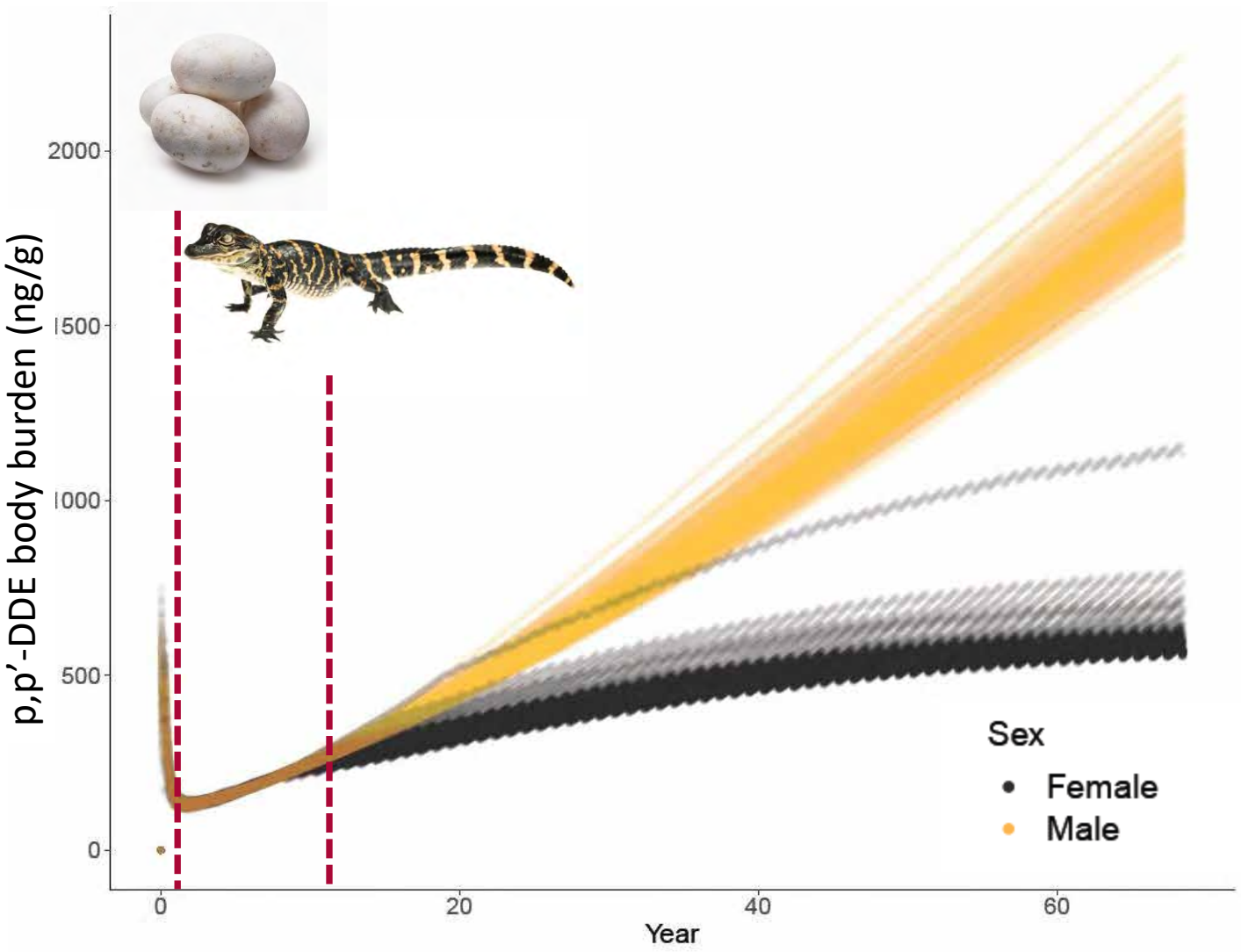
Preliminary results

- ✓ **Simulations**
  - ✓ 70 yr., daily timestep
  - ✓ Preliminary parameter estimates (AmP database)
  - ✓ 100 females + 100 males
- ✓ **Environmental scenario**
  - ✓ Constant temperature (33 deg. C)
  - ✓ Constant pollution exposure
    - ✓ OCP levels in fish 2004-2008
  - ✓ Variable food availability



Vermeiren et al. in prep.

Preliminary results

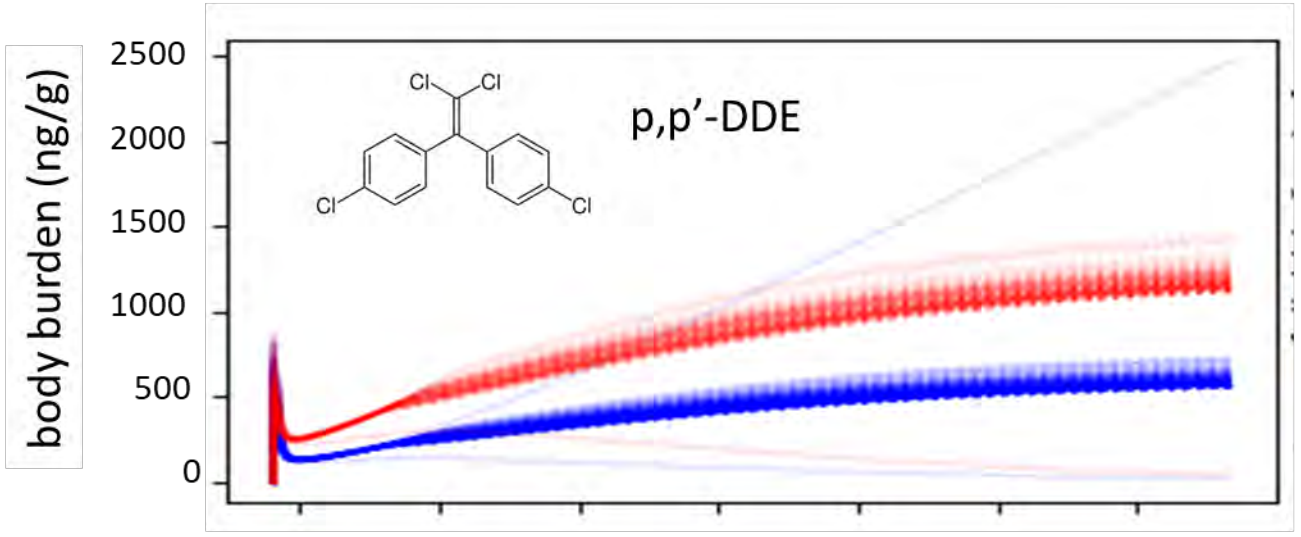


Observed p,p'DDE values
(lake Apopka, FL., USA, 2001)
✓ Liver: 876 ng/g lipid*
✓ Muscle: 2087 ng/g lipid*

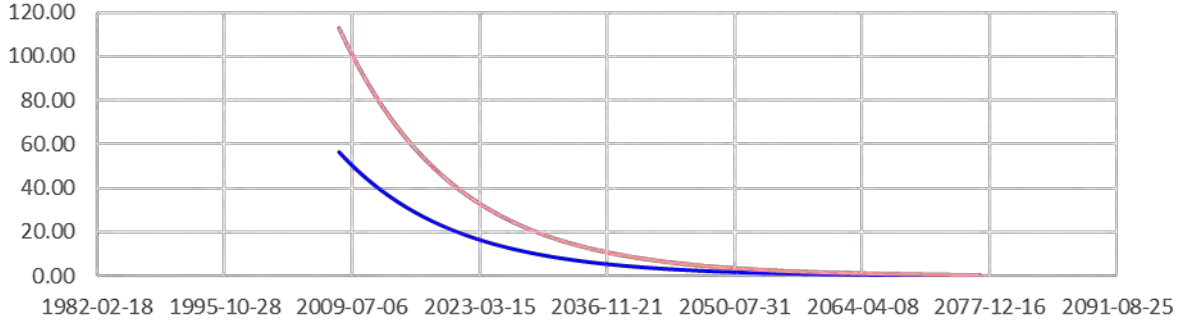
Vermeiren et al. in prep.

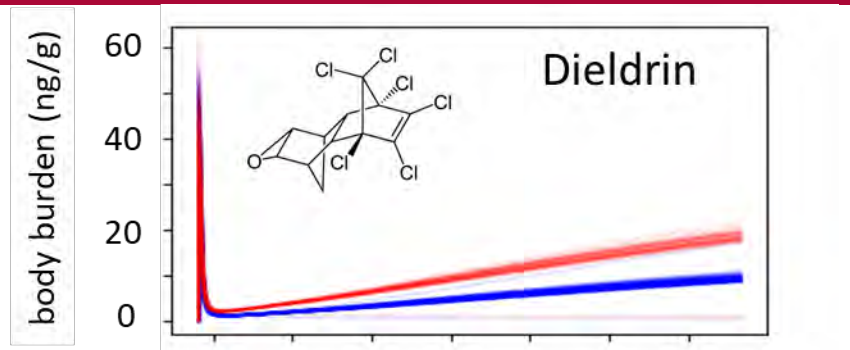
* Preliminary validation data

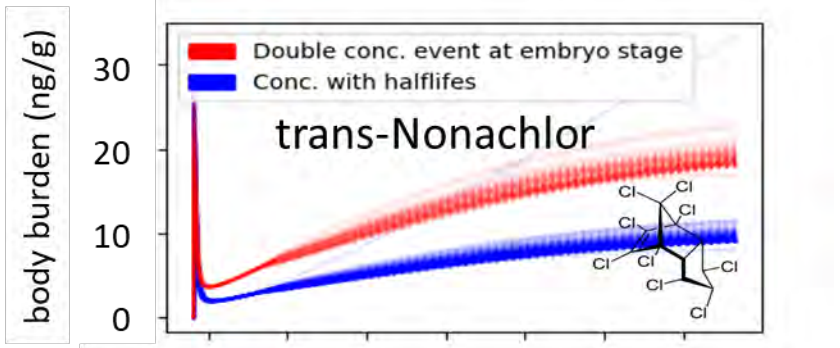
(mixture) scenarios

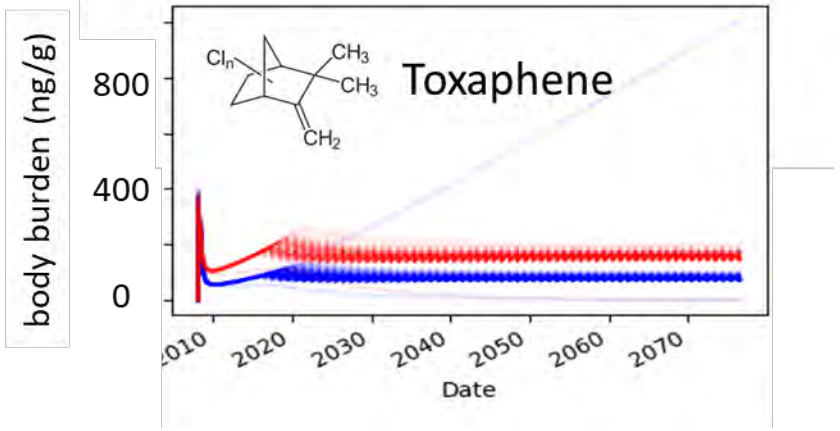


concentration timelines of pp-DDE in gizzard shad



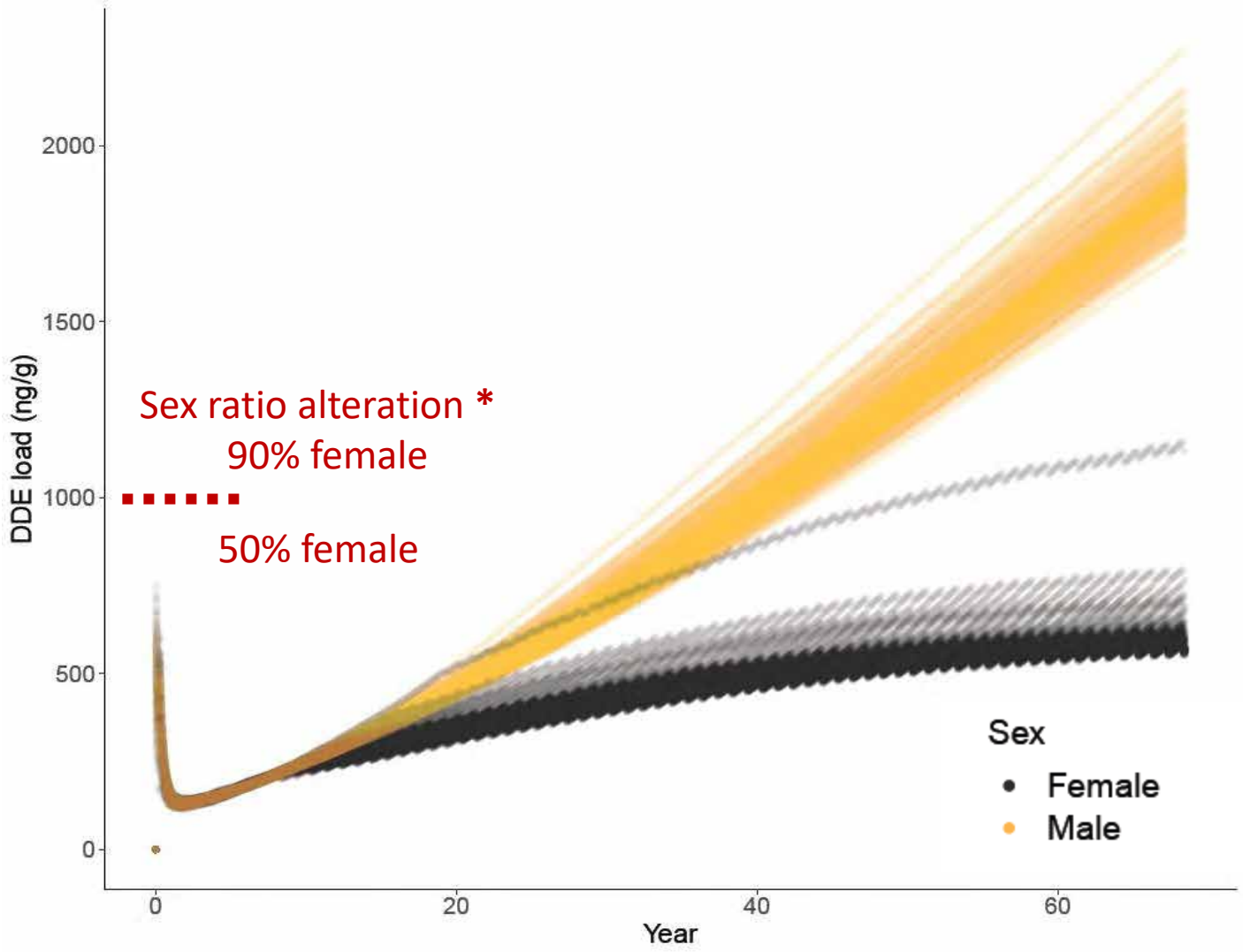






Vermeiren et al. in prep.

Toxic effects



Risk assessment

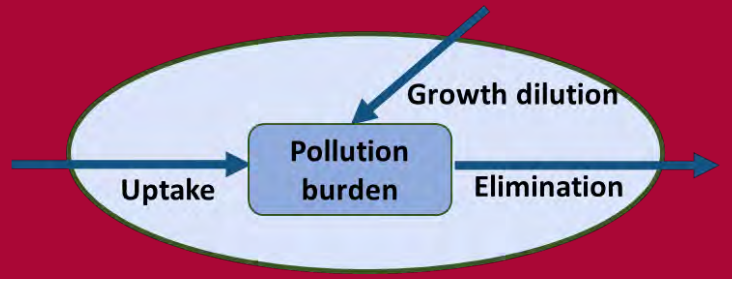
- ✓ Toxic reference values
- ✓ Modelling effects

Toxic effects

- ✓ Hormone levels
- ✓ Sex ratios of hatchlings
- ✓ Morphological alterations
- ✓ Lowered clutch viability (embryo mortality)
- ✓ Increased juvenile mortality

*Matter et al 1998, Milnes et al. 2005

Further development

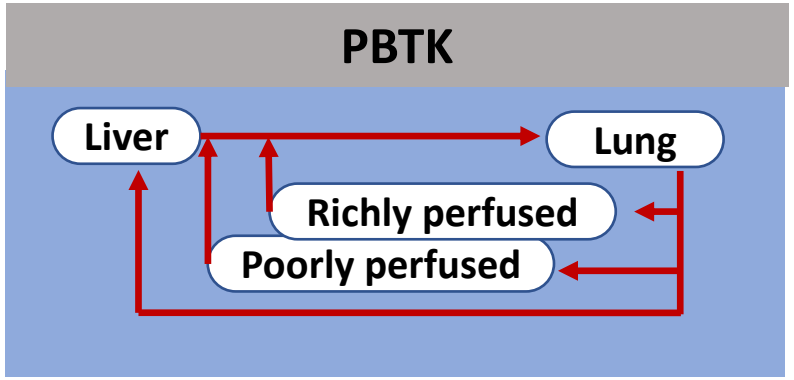


Included

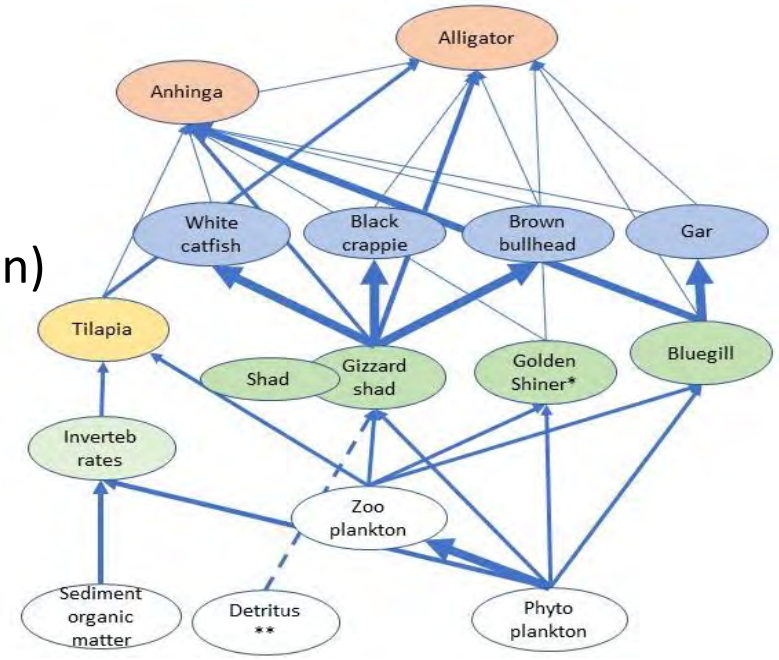
- ✓ **Food** (food web model)
- ✓ **Maternal transfer**
- ✓ Growth dilution

Extensions

- ✓ Respiration, dermal
- ✓ Metabolization (collaboration)
  - ✓ New experimental data
- ✓ **Distribution (PBTK)**

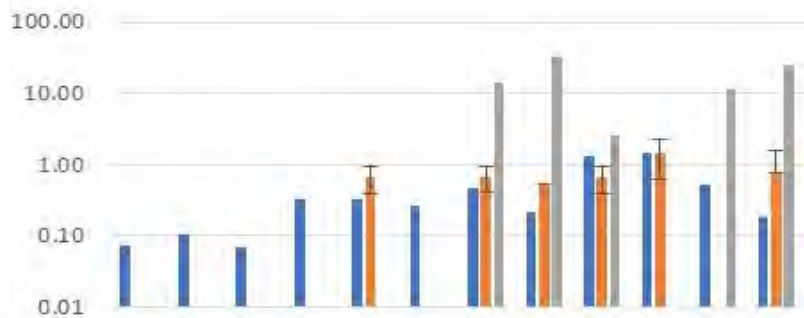


- ✓ Extrapolate to other pollutants / fish
- ✓ Changes in food web

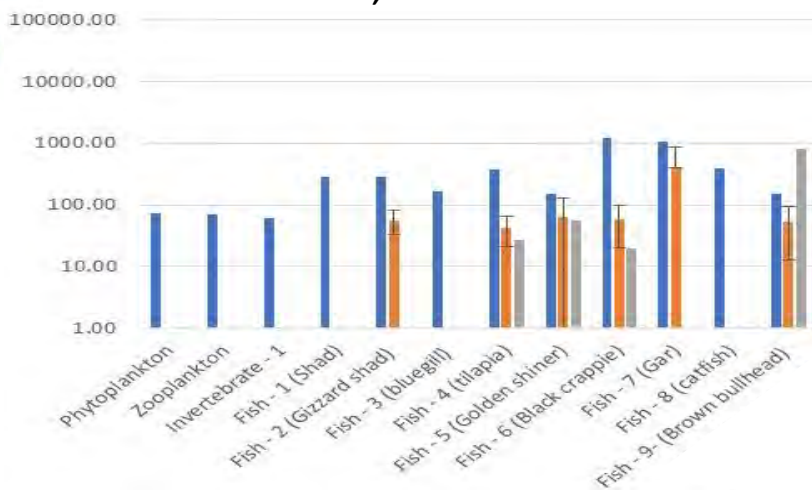


Vermeiren in prep.

4,4´DDT



4,4´DDE



Predicted
Observed 2000s
Observed 1999

Maternal transfer

STSM: Predicting maternal transfer of pesticides in reptiles based on pollutant molecular structure

- ✓ Assess risk to early life stages
- ✓ Use of eggs in biomonitoring

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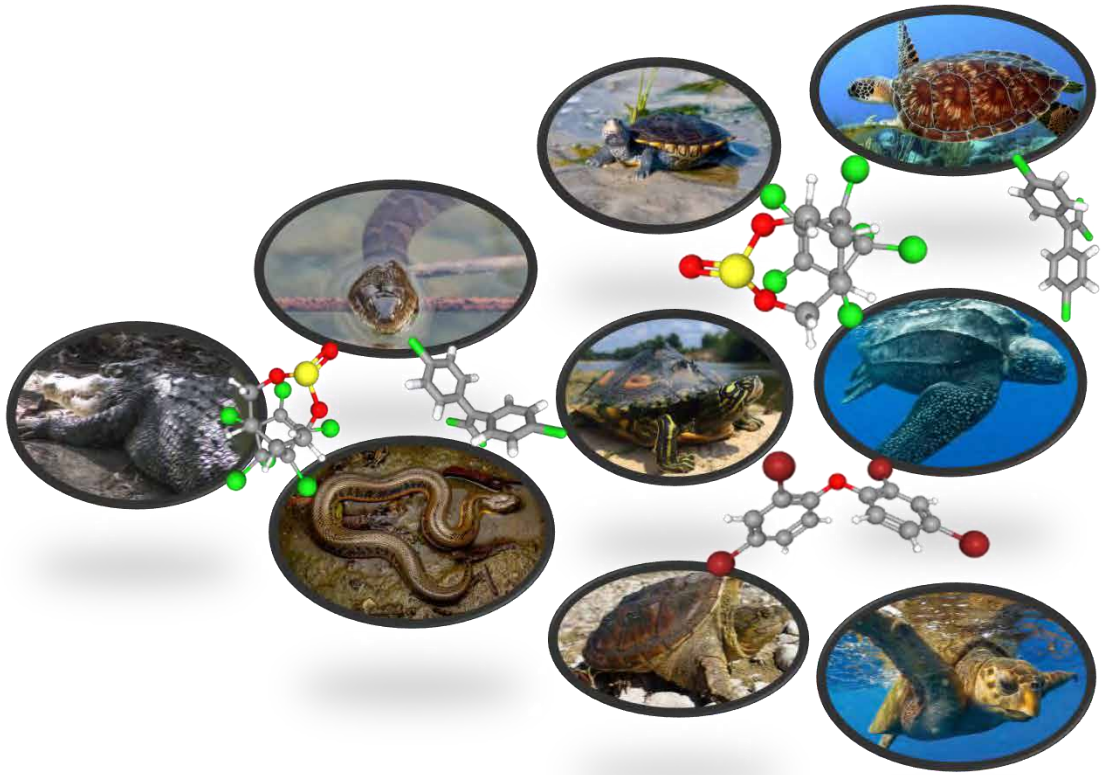


PERIAMAR

Université Claude Bernard Lyon 1

Radboud University
Nijmegen, the Netherlands

Systematic search for data

Studies

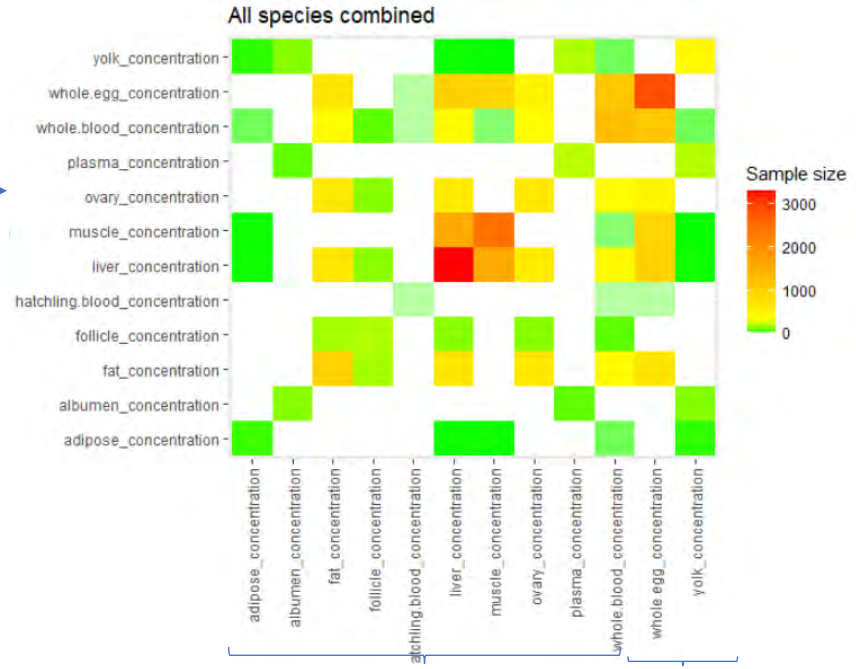
Harmonization

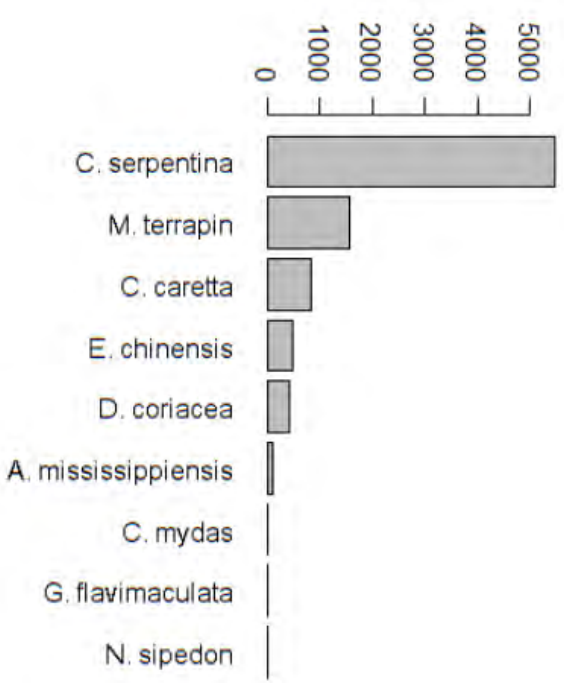
ng/g

lipid weight

< detection limit

* Lipid database



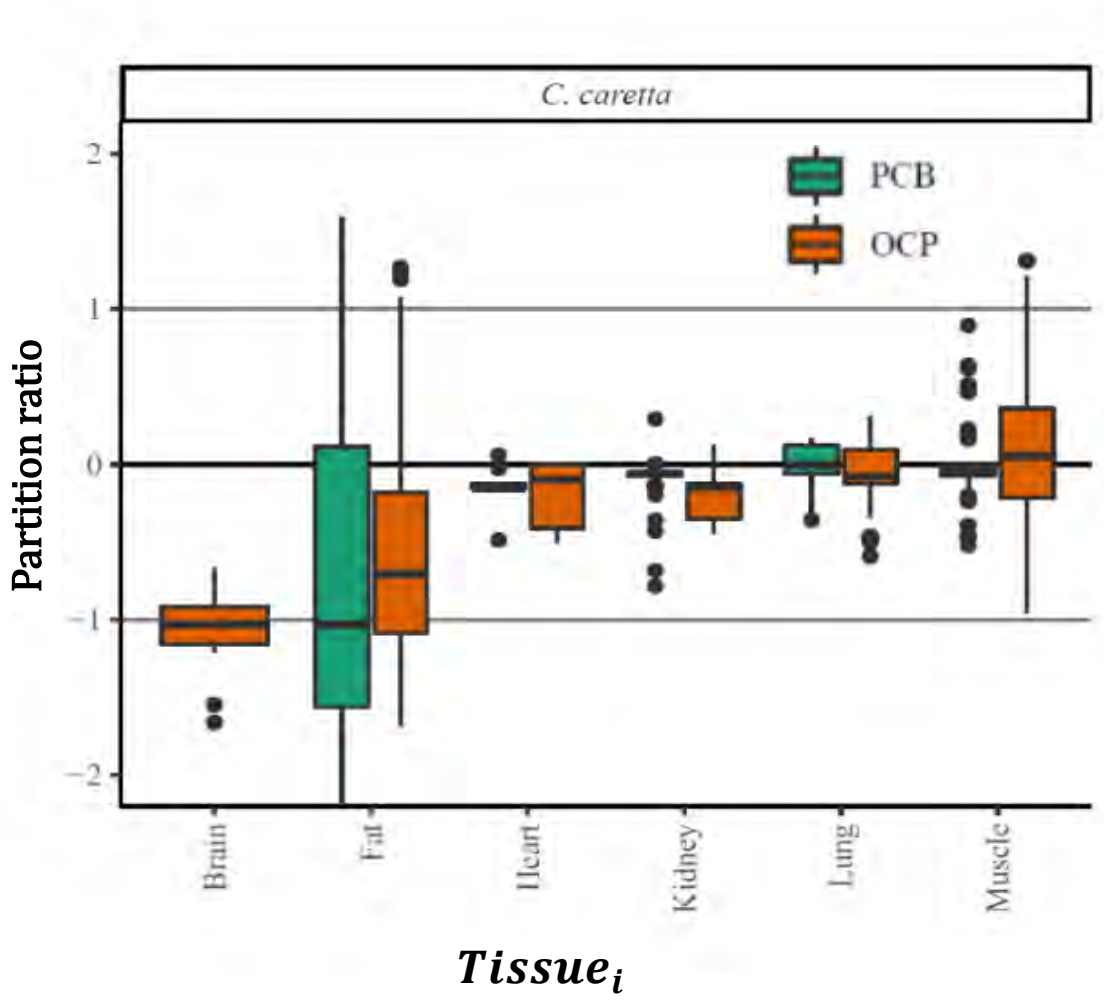


Partitioning ratio: $log_{10}(female/egg)$

Molecular descriptors

Muñoz et al. in prep.

Internal & maternal distribution (sea turtles)



Partitioning ratio:

$$log_{10}(Tissue_i/Liver) = 0$$

Heart, kidney, muscle and lung = equilibrium

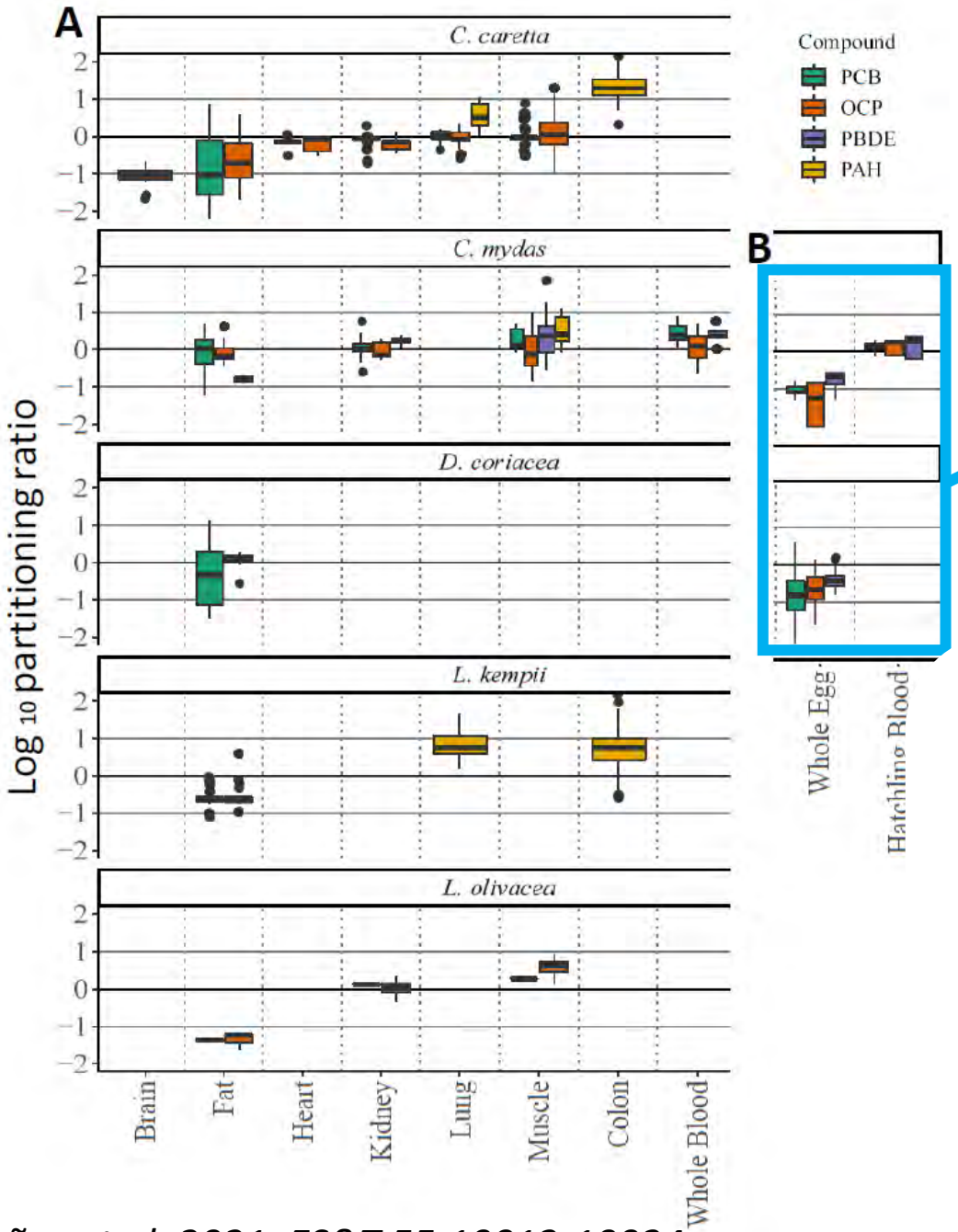
Brain ≠ equilibrium

- The blood-brain barrier (BBB) restricts the transfer of POPs toward the brain

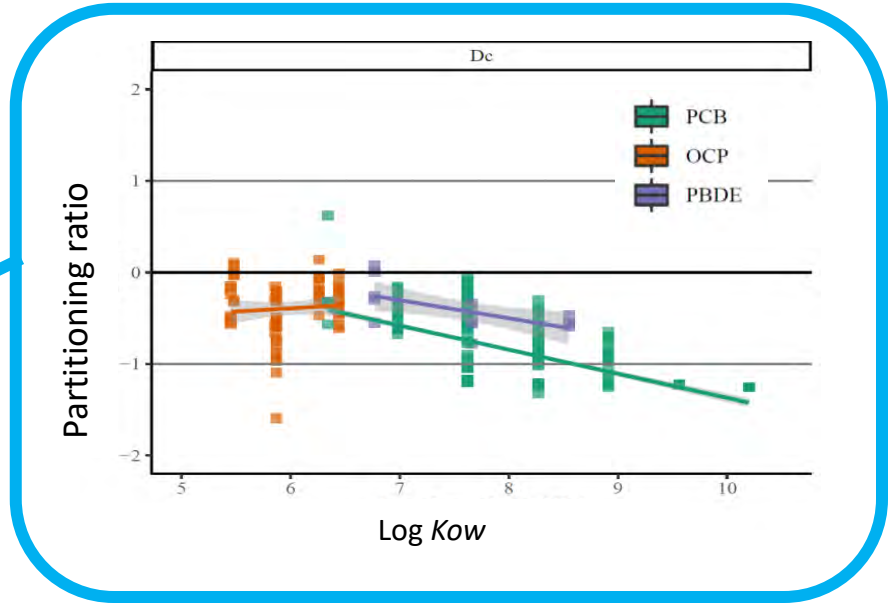
Fat ≠ equilibrium

- Poor blood perfusion (diffusion limitation)

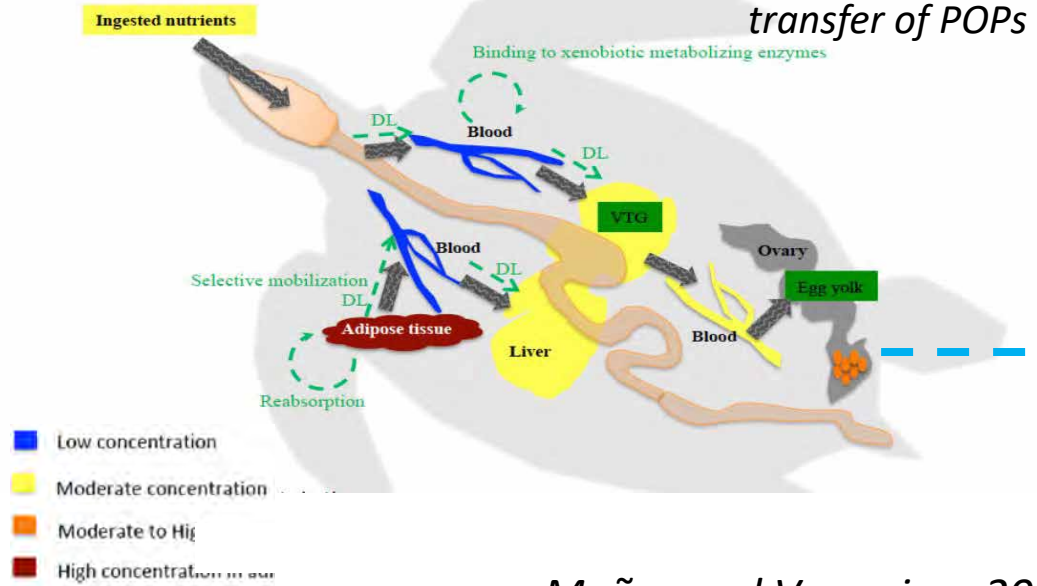
Muñoz et al. 2021, ES&T 55:10012-10024

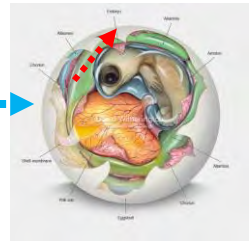


Compound specific maternal transfer



Processes potentially influencing maternal transfer of POPs during vitellogenesis





Muñoz et al. 2021, ES&T 55:10012-10024

Muñoz and Vermeiren 2020, ET&C 39:9-29

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ATTAC-issues

Issue	Best Practice guidelines
Access	• Deposit data into administered repositories (e.g., institutional or governmental repositories, data archives or journal appendices) • Provide public access to data • Provide compound-specific concentrations • Provide data at individual turtle level • Apply data extraction (image analysis) and analysis (weighting) techniques to make best use of presented data
Transferability	• Clearly identify measurement units and bases (dry, wet, lipid) • Provide key physiological data such as water, lipid and protein contents • Develop and extend databases of key physiological data (e.g., Table S5) • Report sample sizes, allowing for weighting in analysis
Transparency	• Report (compound-specific) detection and quantification limits • Document field, lab, and statistical methods used to allow replication • List the identify of chemical compounds analysed, to clarify which compounds were investigated, and which contributed to group summaries
Additional metrics	• Report sex, life stage (cohort), and age if possible • Include health related metrics (e.g., body condition) and presence of visual health status indicators (e.g., fibropapillomas, parasites, or injuries)
Conservation sensitivity	• Design a study to include sufficient replication to produce statistically meaningful results • Contribute to tissue databanks • Integrate existing data to complement analyses • Promote ecotoxicological collaboration with researchers in related fields

Conclusion

Preliminary results

- ✓ Modelling approach: growth, reproduction and pollution accumulation can be recreated.
- ✓ Scenarios indicate high initial accumulation due to maternal transfer, and differences between sexes in the adult stage.

A framework

- ✓ Synthesise & integrate current understanding and data on reptile **ecotoxicology**, **physiology** and **ecology**.
- ✓ Assess risks for **reptiles/larger species** for which laboratory experiments are lacking/unfeasible.
- ✓ Reduce need for animal toxicity testing
  - ✓ Fill gap in reptile ecotoxicology / guide experimental design
  - ✓ *In-silico* scenario analyse: Temperature, food availability, pollution levels

Thank you

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