Physiological modes of action: the key to (almost) everything?

Ecotoxicology & Models

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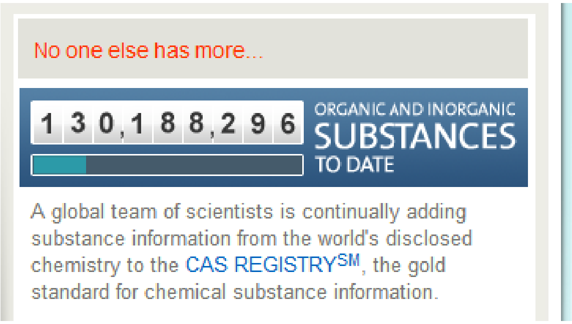
Ecotoxicology: the rift

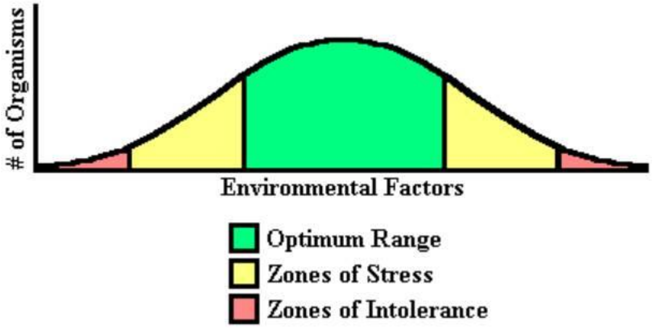
High throughput testing vs ecology?

Ecotoxicology

Tox21 inspired

Stress ecology inspired





Ecotoxicology: the rift

High throughput testing vs ecology?

Tox21 inspired

- Ankley et al. 2010, ET&C: **Adverse outcome pathways**: A conceptual framework to support ecotoxicology research and risk assessment.
- Huang et al. 2016, Nat Commun: Modelling the **Tox21 10K chemical profiles** for in vivo toxicity prediction and mechanism characterization
- Zhang et al. 2010 , JToxEnvHealth: **Computational Systems Biology** and Dose-Response Modeling in Relation to **New Directions in Toxicity Testing**
- Gunnarsson et al. 2008, ES&T: **Evolutionary conservation of** human drug **targets** in organisms used for environmental risk assessments.
- LaLone et al. 2013, AquatToxicol: **Molecular target sequence similarity** as a basis for species extrapolation to assess the ecological risk of chemicals with known modes of action.

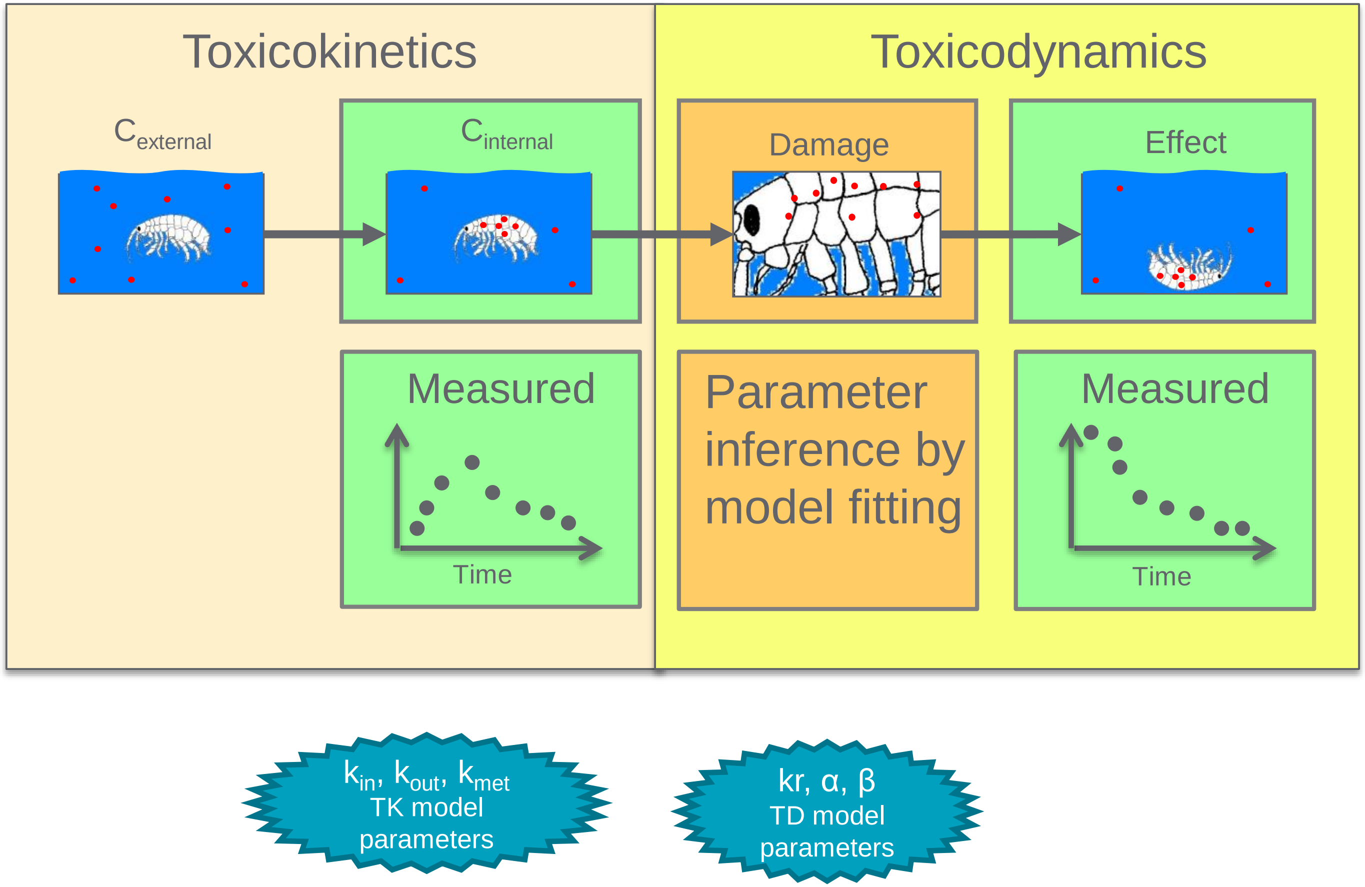
Stress ecology inspired

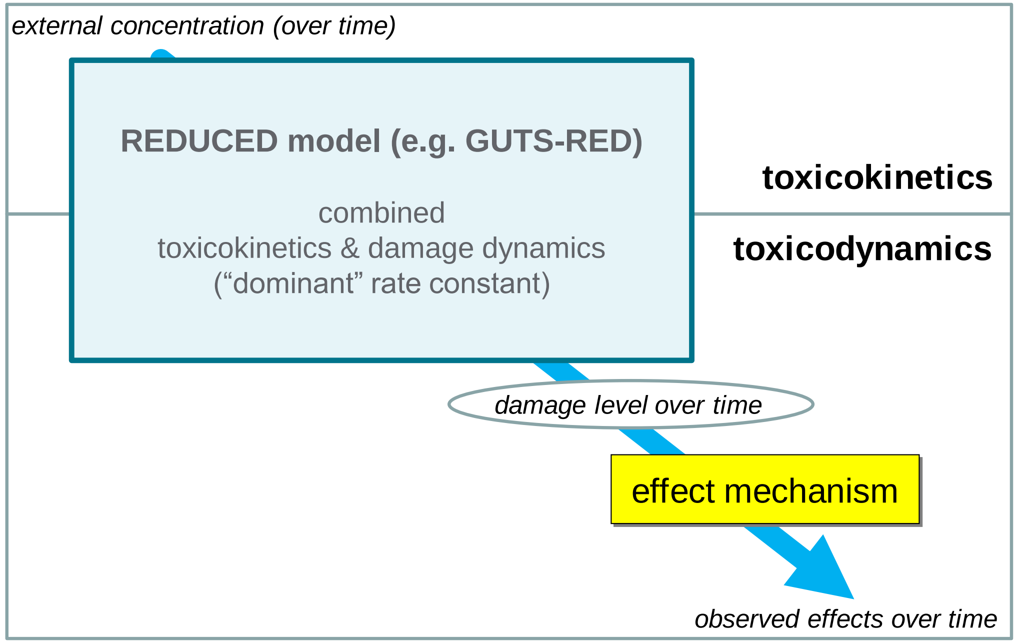
- Van den Brink 2008, ES&T: Ecological risk assessment: From book-keeping to **chemical stress ecology**
- Relyea & Hoverman 2006, EcolLett: Assessing the **ecology in ecotoxicology**: a review and synthesis in freshwater systems.
- Rohr et al. 2006, TREE: **Community ecology** as a framework for predicting contaminant effects.
- Rubach et al. 2011, IEAM: Framework for **traits**-based assessment in ecotoxicology.
- Schäfer et al. 2016, FreshwBiol: Contribution of organic toxicants to **multiple stress** in river ecosystems.
- SCENIHR, SCHER & SCCS [**EFSA**] 2012: Addressing the New Challenges for Risk Assessment.

 Classification: PUBLIC

Toxicokinetic-toxicodynamic models

DEBtox and the General Unified Threshold model of Survival GUTS are both TKTD models





Effects: survival

→ GUTS

Effects: growth & reproduction

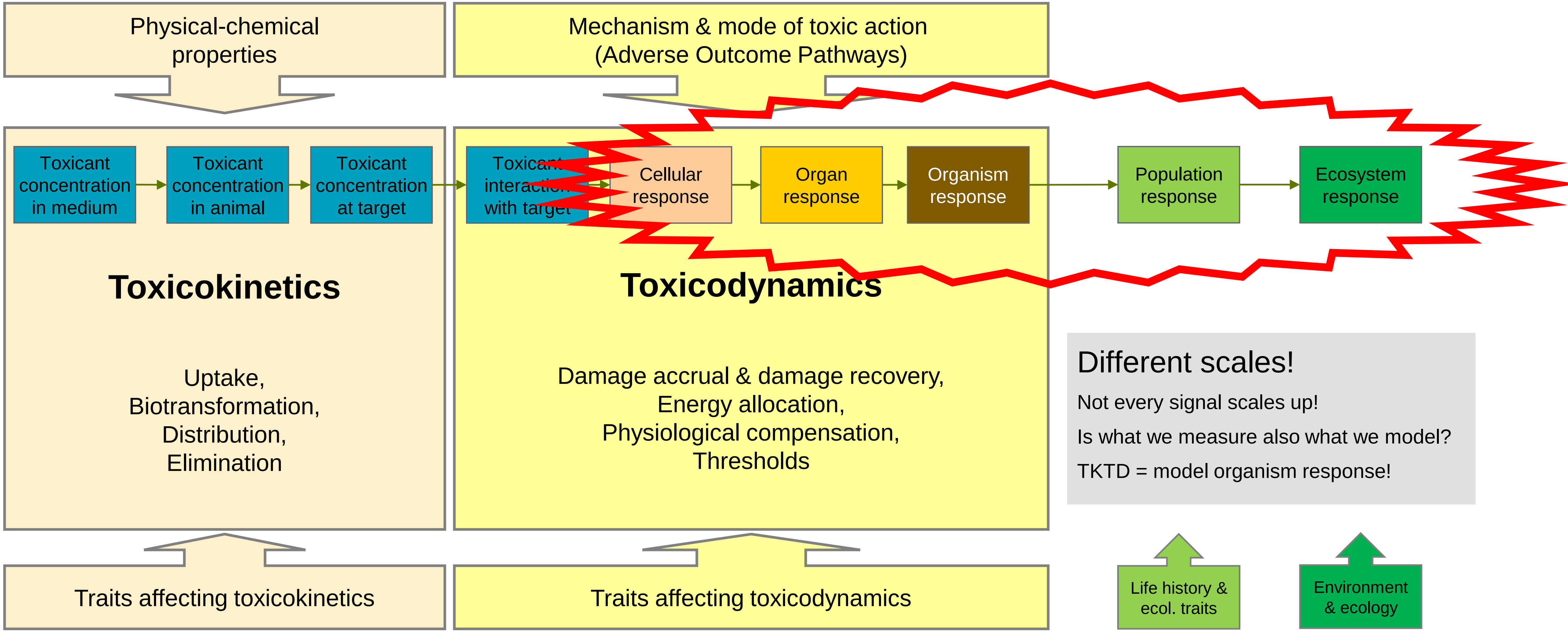
→ DEBtox

https://leanpub.com/guts_book

https://leanpub.com/debtox_book

Toxicokinetic-toxicodynamic models

Which level of biological organisation?



Different scales!

Not every signal scales up!

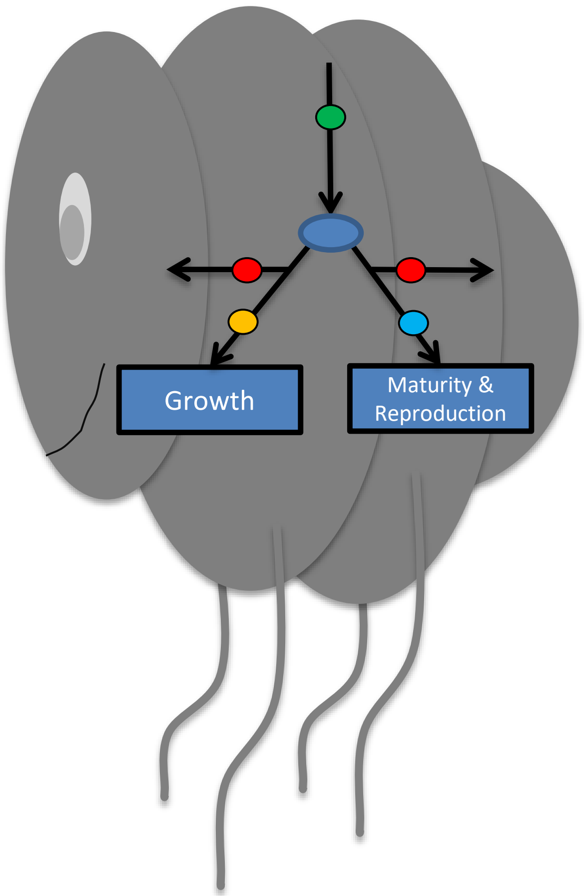
Is what we measure also what we model?

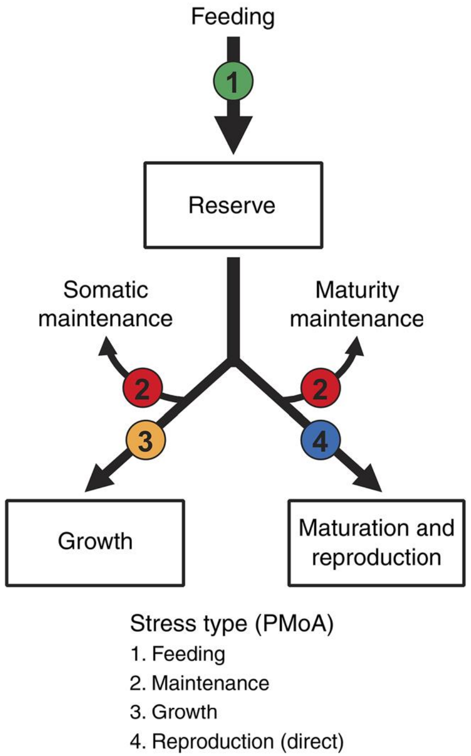
TKTD = model organism response!

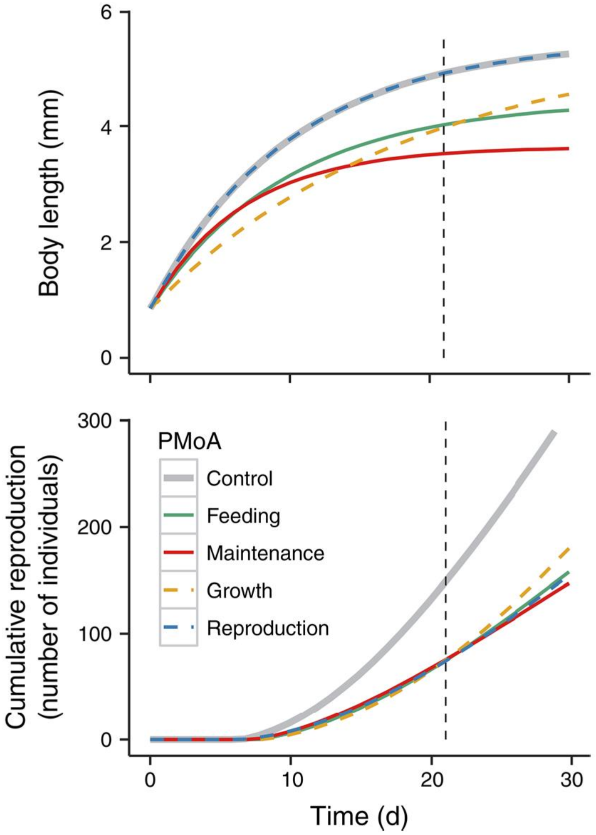
Ashauer & Escher (2010) *JEM.*, Rubach *et al.* (2011) *IEAM*

DEBtox physiological models of action

How can we identify the pMoA?







Identfying pMoA is difficult:

- Growth curves similar shape
- Reproduction curves similar
- Variability in data

Way forward?

- Automate measurements
- Additional information (?)
- New ideas needed!

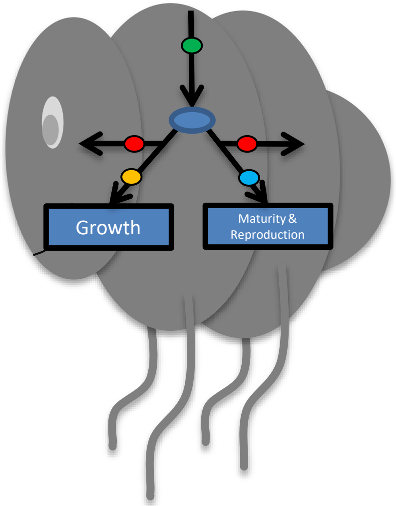
Martin, B., et al., *Limitations of extrapolating toxic effects on reproduction to the population level.* Ecological Applications, 2014. **24**(8): p. 1972-1983.

Ashauer, R. and T. Jager, *Physiological modes of action across species and toxicants: the key to predictive ecotoxicology.* Environ Sci Process Impacts, 2018. **20**(1): p. 48-57.

Duckworth, J., T. Jager, and R. Ashauer, *Automated, high-throughput measurement of size and growth curves of small organisms in well plates.* Scientific Reports, 2019. **9**(1): p. 10.

Physiological mode of action (pMoA) is key

Predicitive ecotox across species & compounds



1. Feeding & assimilation [A]
2. Maintenance [M]
3. Growth [G]
4. Reproduction [R]

			Acrobeloides nanus	Caenorhabditis elegans	Dendrobaena octaedra	Lumbricus rubellus	Capitella teleta	Folsomia candida	Daphnia magna	Moina micrura	Mytilus californianus	Mytilus galloprovincialis	Mytilus edulis	Crassostrea gigas	Lymnaea stagnalis	Danio rerio	Strongylocentrotus droebachiensis
Baseline toxicants	Neutral organics	PAH mixture														A	
Baseline toxicants	Neutral organics	Benzo(k)-fluoranthene														A	
Baseline toxicants	Neutral organics	Fluoranthene		G+R, G+R					R								
Baseline toxicants	Neutral organics	Pyrene							R								
Baseline toxicants	Neutral organics	Pyridine							M								
Baseline toxicants	Neutral organics	Acetone													A		
Baseline toxicants	Neutral organics	Diquat													A/M		
Baseline toxicants	Neutral organics	Pentachlorobenzene	A	G+R													
Baseline toxicants	Anilines	3,4-dichloroaniline							R/H								
Baseline toxicants	Aromatic triazine	Atrazine		M													
Baseline toxicants	Phenols	Pentachlorophenol											A+M				
Specific toxicity	Imidazoles, carbamate esters	Carbendazim	A	A													
Specific toxicity	Oxime carbamate ester	Aldicarb		M													
Specific toxicity	Monothiophosphate ester, halopyrdines	Chlorpyrifos						R									
Specific toxicity	Esters, Benzyl Nitriles, Pyrethroids	Fenvalerate							A								
Specific toxicity	Neutral organics[1]	Tetradifon							A								
Specific toxicity	Phenols	Nonylphenol					G+R										
Specific toxicity	n.a.	Tributyltin													A		
Specific toxicity	n.a.	Triphenyltin						M									
Metals	Metals[2]	Cadmium	G	A, A, A, A/M				A, A	A								
Metals	Metals[2]	Copper		A	A	M+R, A			G								
Metals	Metals[2]	Uranium		A, A/M					A							M+G	
Metals	Metals[2]	Zinc				M			A/M								
Metals	Metals[2]	Mercury										A		A/M			
Metals	n.a.	Zinc-oxide nanoparticles										A+M					
Others	n.a.	Toxic cyanobacteria								A/M							
Others	n.a.	pH (ocean acidification)															M
Others	n.a.	Produced water									A+M	A+M					

<u>What do we know?</u>

- Limited data coverage
- Lab methods bottleneck

<u>What do we not know?</u>

- How do pMoAs vary across species & compounds?
- Are there patterns?

<u>What do we need to do?</u>

- Automate lab methods
- Find patterns & leverage them for predictive tools
- Link pMoAs to phylogeny, traits & molecular properties

Ashauer, R. and T. Jager, *Physiological modes of action across species and toxicants: the key to predictive ecotoxicology.* Environ Sci Process Impacts, 2018. **20**(1): p. 48-57.

Adverse Outcome Pathways (AOPs) vs Physiological Mode of Action (pMoA)

Use pMoAs to test central assumption of AOPs? Can we learn about conserved pathways?

1) Are AOPs ‘chemical agnostic’?
   a) If yes, then same MIE → same AO (i.e. pMoA)
   b) If no, then what?

2) How do we find out if an AOP differs between species?
   a) Quantitatively?
   b) Where do annotations come from?

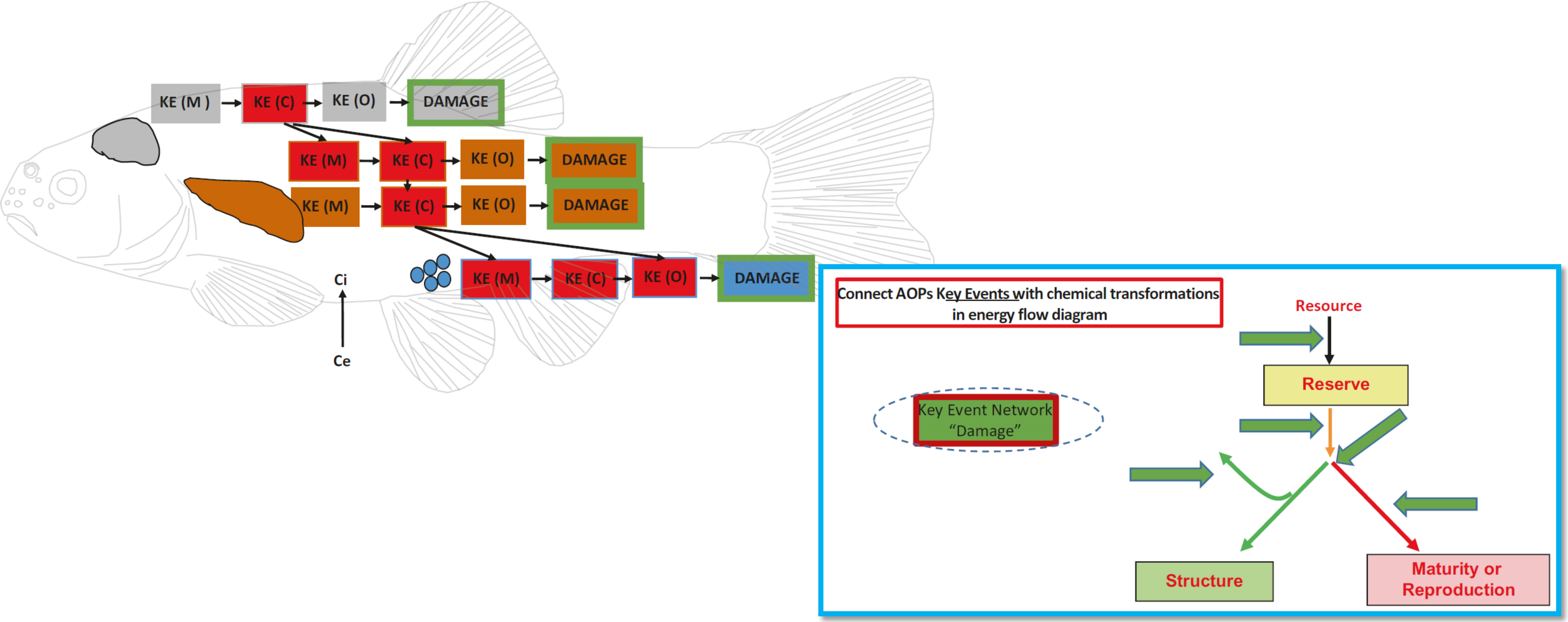
3) Are we interested in the cases where pathways are conserved or when not?
   a) Rule or exception?
   b) What’s more important for chemical ERA?
   c) How can we know?

			Acrobeloides nanus	Caenorhabditis elegans	Dendrobaena octaedra	Lumbricus rubellus	Capitella teleta	Folsomia candida	Daphnia magna	[illegible]
Baseline toxicants	Neutral organics	PAH mixture								
Baseline toxicants	Neutral organics	Benzo(k)-fluoranthene								
Baseline toxicants	Neutral organics	Fluoranthene		G+R, G+R					R	
Baseline toxicants	Neutral organics	Pyrene							R	
Baseline toxicants	Neutral organics	Pyridine							M	
Baseline toxicants	Neutral organics	Acetone								
Baseline toxicants	Neutral organics	Diquat								
Baseline toxicants	Neutral organics	Pentachlorobenzene	A	G+R						
Baseline toxicants	Anilines	3,4-dichloroaniline							R/H	
Baseline toxicants	Aromatic triazine	Atrazine		M						
Baseline toxicants	Phenols	Pentachlorophenol								
Specific toxicity	Imidazoles, carbamate esters	Carbendazim	A	A						
Specific toxicity	Oxime carbamate ester	Aldicarb		M						
Specific toxicity	Monothiophosphate ester, halopyrdines	Chlorpyrifos						R		
Specific toxicity	Esters, Benzyl Nitriles, Pyrethroids	Fenvalerate							A	
Specific toxicity	Neutral organics[1]	Tetradifon							A	
Specific toxicity	Phenols	Nonylphenol					G+R			
Specific toxicity	n.a.	Tributyltin								
Specific toxicity	n.a.	Triphenyltin						M		
Metals	Metals[2]	Cadmium	G	A, A, A, A/M				A, A	A	
Metals	Metals[2]	Copper		A	A	M+R, A			G	
Metals	Metals[2]	Uranium		A, A/M					A	
Metals	Metals[2]	Zinc				M			A/M	
Metals	Metals[2]	Mercury								
Metals	n.a.	Zinc-oxide nanoparticles								
Others	n.a.	Toxic cyanobacteria								A/
Others	n.a.	pH (ocean acidification)								
Others	n.a.	Produced water								

Classification: PUBLIC

Linking qAOPs & DEBtox via *damage*?

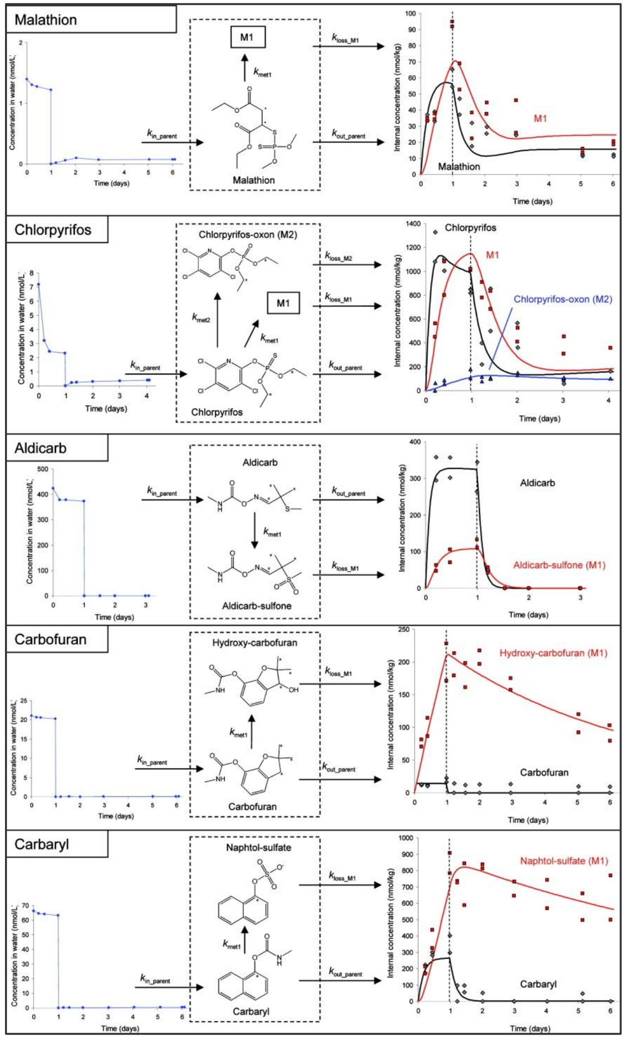
Conceptual diagram...

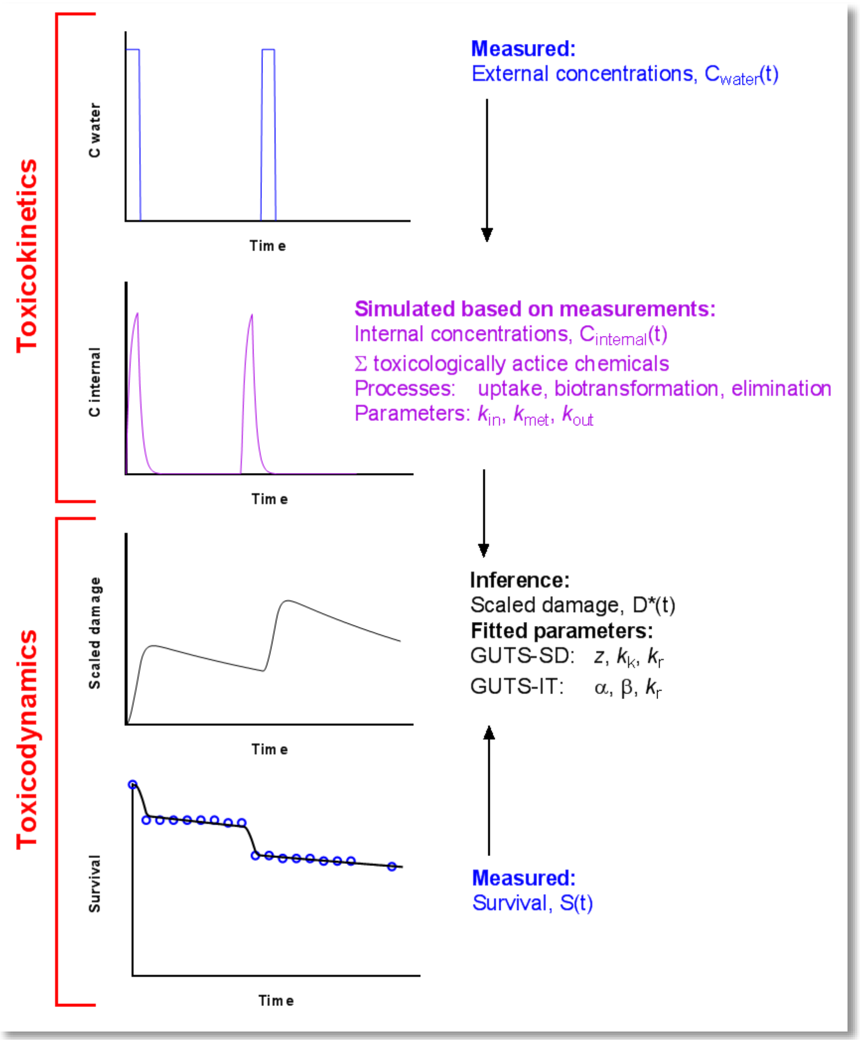


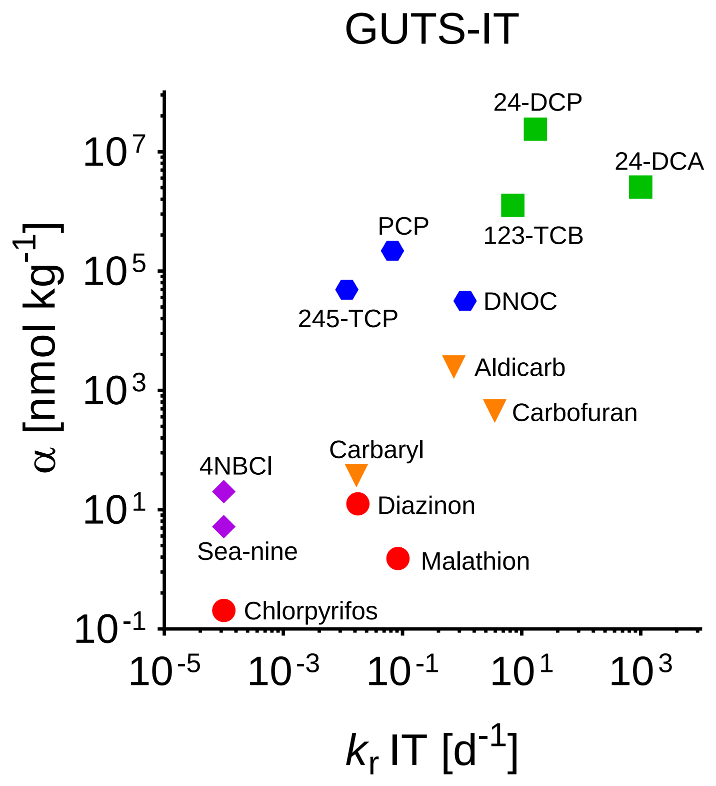
Murphy, C.A., et al., *Linking adverse outcome pathways to dynamic energy budgets: A conceptual model*, in *A Systems Biology Approach to Advancing Adverse Outcome Pathways for Risk Assessment*. 2018, Springer International Publishing. p. 281-302.

Linking qAOPs & DEBtox via *damage*?

How can we quantify toxicodynamic *damage*? Example with the General Unified Threshold model of Survival GUTS





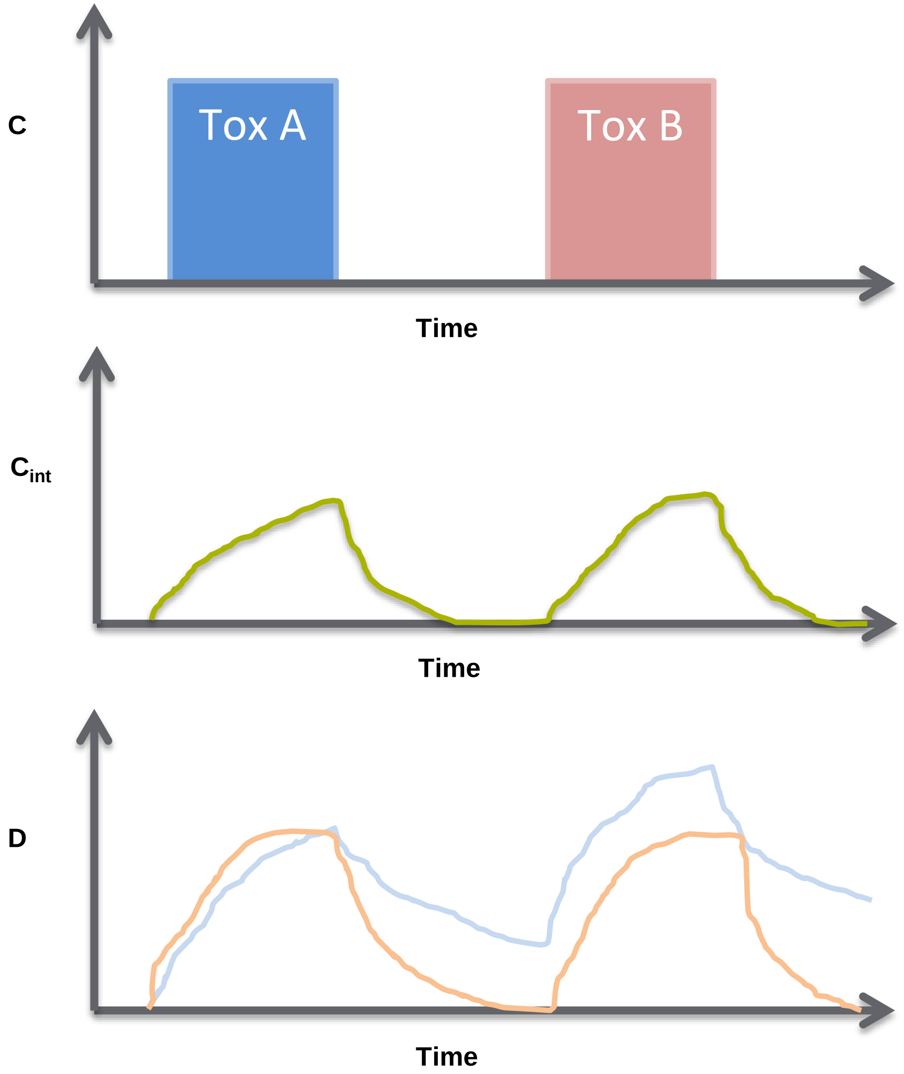


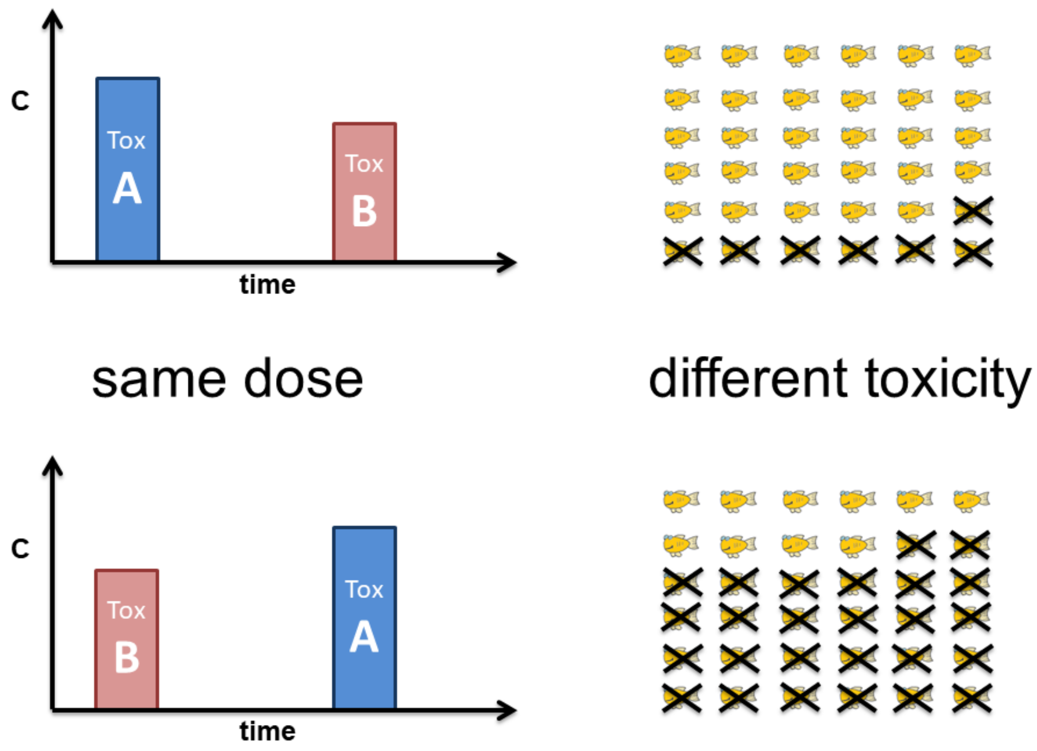
Ashauer, et al. (2012): Significance of Xenobiotic Metabolism for Bioaccumulation Kinetics of Organic Chemicals in *Gammarus pulex*. *Environ. Sci. Technol.* 46(6).

Ashauer, O'Connor, Hintermeister, Escher (2015): Death Dilemma and Organism Recovery in Ecotoxicology. *Environ. Sci. Technol. 49*, (16).

Linking qAOPs & DEBtox via *damage*?

How do we know a toxicodynamic *damage* state variable is meaningful?

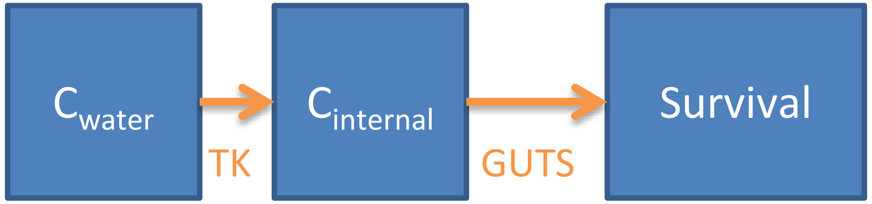


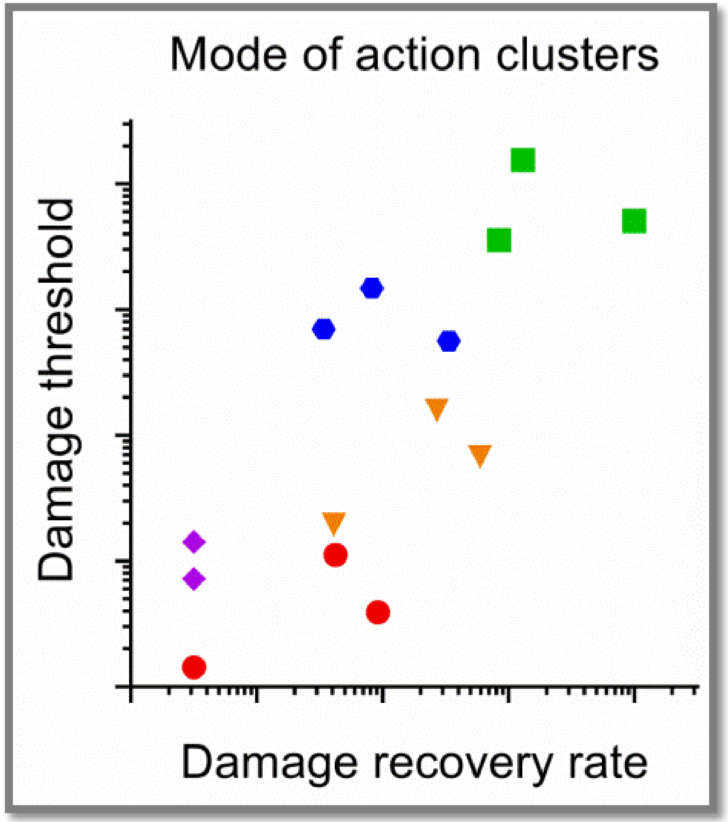


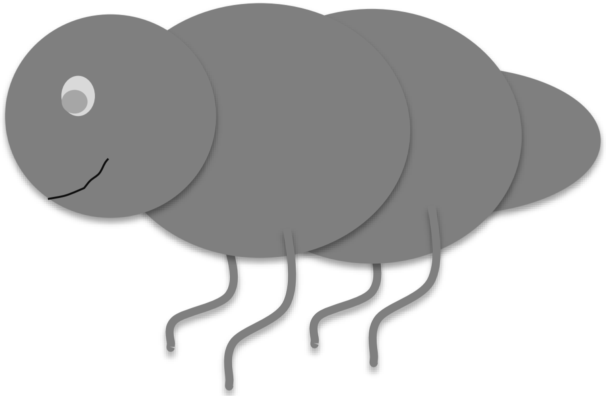
Ashauer R, O'Connor I, & Escher BI (2017):
Toxic Mixtures in Time — The Sequence Makes the Poison.
Environ. Sci. Technol. 51(5):3084-3092.

Linking qAOPs & DEBtox via *damage*?

How do we know toxicodynamic *damage* is related to mode of action?

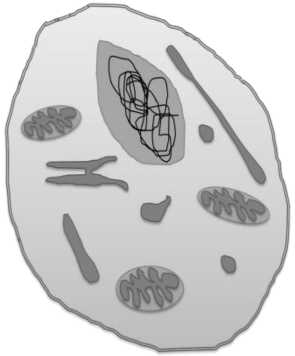






Toxicodynamic parameters cluster according to mode of action!

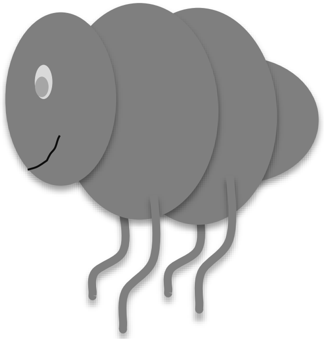
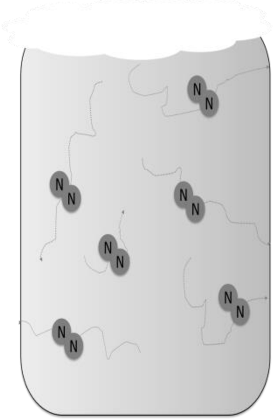
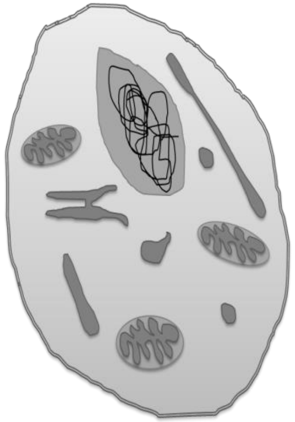
→ Biochemistry (MoA) is reflected at organism level (TD parameters)!



Ashauer, O'Connor, Hintermeister, Escher (2015): Death Dilemma and Organism Recovery in Ecotoxicology. *Environ. Sci. Technol. 49*, (16).

Ecotoxicology: towards a predictive science

Missing theory at the core...

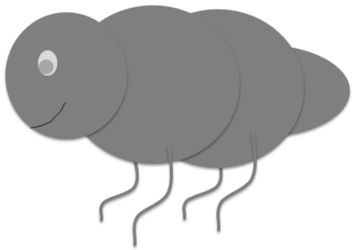
	Physical chemistry		(Eco)toxicology	
Macroscopic scale		Perfect gas $p \times V = \text{constant}$ (Boyle's law)		Generic organism $\text{stress} = 1/C_T \times \max(0, C_V - C_0)$ (DEBtox)
		↑ Emerges		↑ Missing theory
Molecular scale		Kinetic model of gases • Random motion • Size negligible • Elastic collisions only		Cellular pathways • Reaction networks • -omics • Pathway models

Ashauer, R. and T. Jager, *Physiological modes of action across species and toxicants: the key to predictive ecotoxicology.* Environ Sci Process Impacts, 2018. **20**(1): p. 48-57.

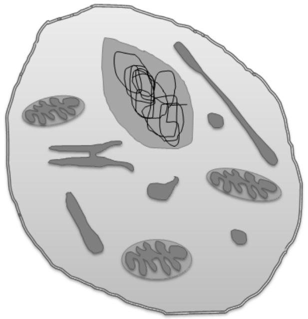
Ecotoxicology: towards a predictive science

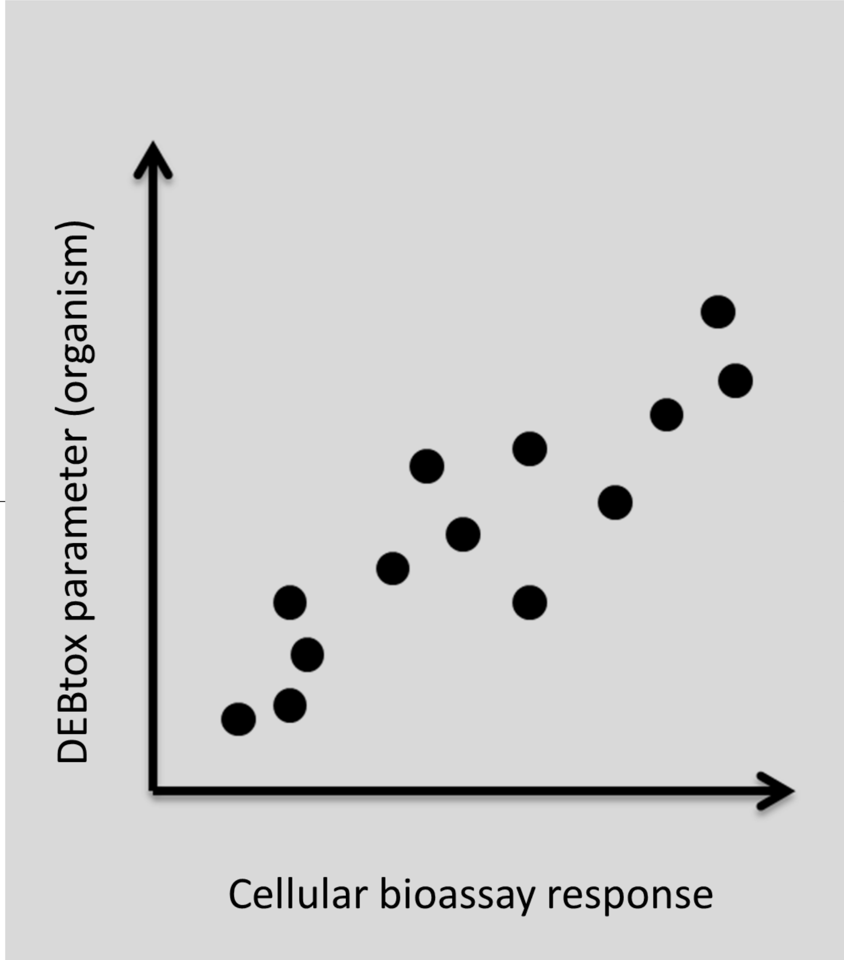
There must be a link between what´s going on in the cells and the whole organism, ***the trick is to find it*** ! (Tjalling Jager)

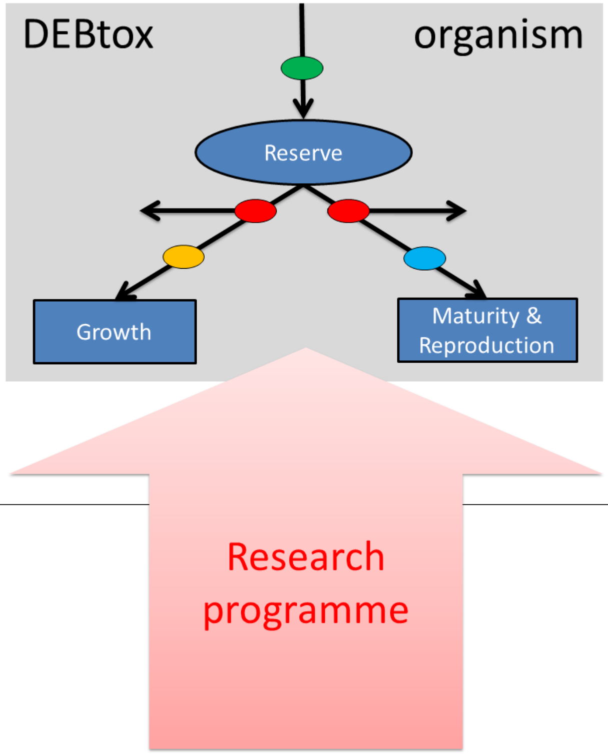
Macroscopic scale



Molecular scale







Cellular bioassays

- In-vitro, cell based toxicity tests
- High-throughput

Ashauer, R. and T. Jager, *Physiological modes of action across species and toxicants: the key to predictive ecotoxicology.* Environ Sci Process Impacts, 2018. **20**(1): p. 48-57.

EFSA Scientific Opinion on TKTD models

Models for sub-lethal effects: DEBtox

Ockleford, C., et al., **Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms**. EFSA Journal, 2018. 16(8): p. e05377.

EFSA Journal

SCIENTIFIC OPINION

ADOPTED: 27 June 2018

doi: 10.2903/j.efsa.2018.5377

Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms

“The **GUTS** model and the *Lemna* model are considered **ready to be used** in risk assessment.”

“…the **DEBtox modelling** approach is currently limited to research applications. However, its **great potential for future use** in prospective ERA for pesticides is recognised.”

Jager, T., et al., **General Unified Threshold Model of Survival - a Toxicokinetic-Toxicodynamic Framework for Ecotoxicology**. Environmental Science & Technology, 2011. 45(7): p. 2529-2540.

General Unified Threshold model of Survival (GUTS)

A toxicokinetic-toxicodynamic model

leanpub.com/guts_book

Why GUTS? Because of time variable exposure...

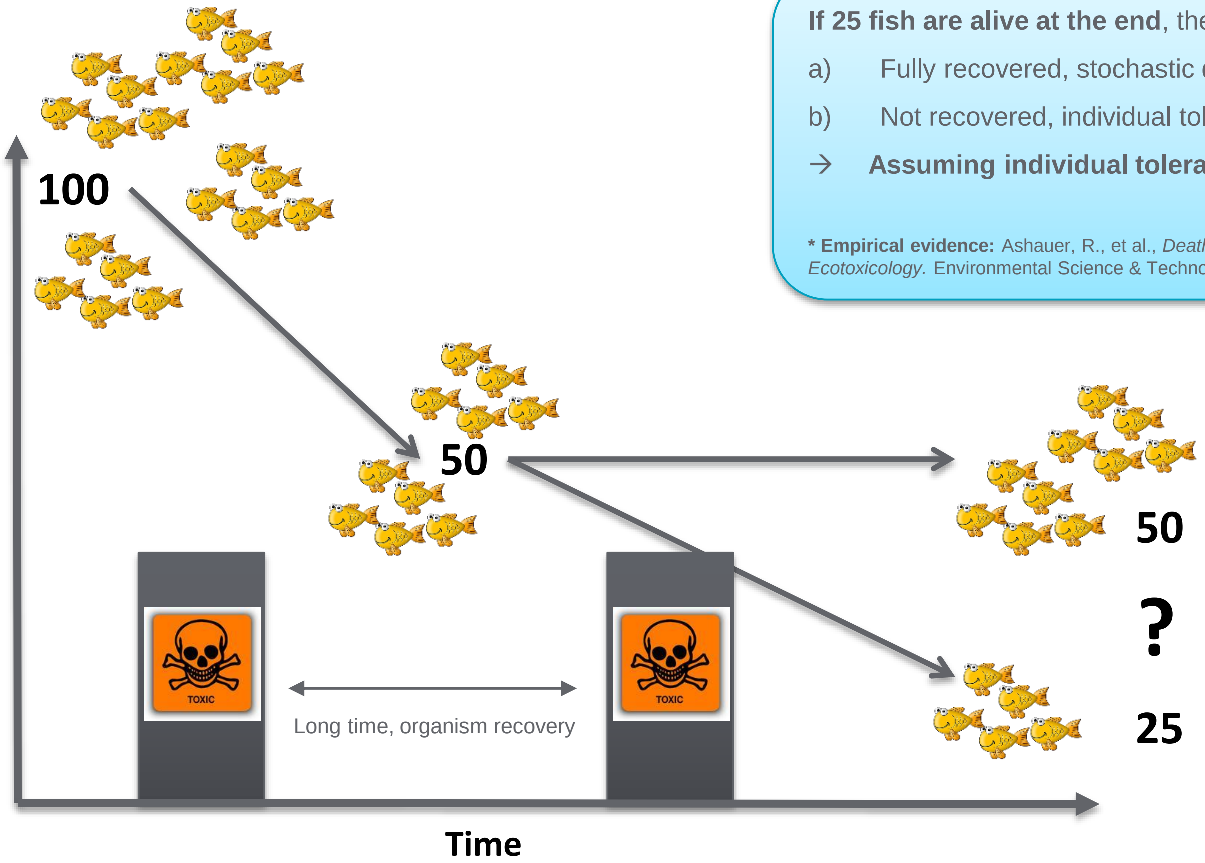
If 25 fish are alive at the end, there are two explanations:

a) Fully recovered, stochastic death

b) Not recovered, individual tolerance

→ **Assuming individual tolerance implies slower recovery! ***

* **Empirical evidence:** Ashauer, R., et al., *Death Dilemma and Organism Recovery in Ecotoxicology.* Environmental Science & Technology, 2015. **49**(16): p. 10136-10146.



"Individual Tolerance"

Each fish has different "tolerance", when this is exceeded = dead.

"Stochastic Death"

All fish have same "tolerance", death is a chance process.

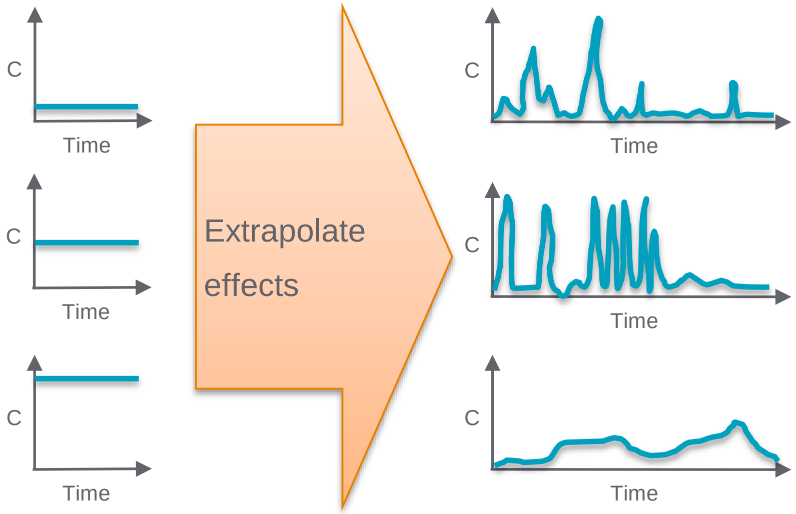
Risk assessment question

Standard laboratory tests:

Constant exposure

Realistic environment:

Time variable exposure



Time variable exposure drivers

- Agronomy, landscape, ...
- Rainfall, degradation, distribution, ...
- Feeding habits, movement, ecology, ...

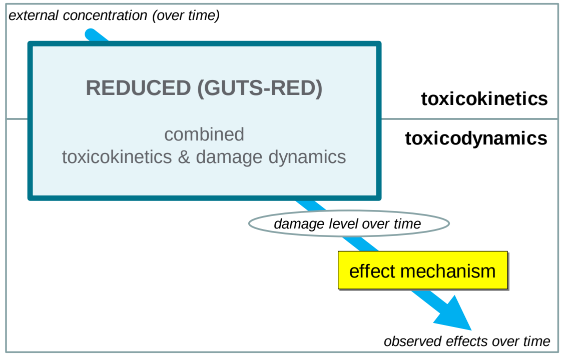
General Unified Threshold model of Survival (GUTS)

A toxicokinetic-toxicodynamic model

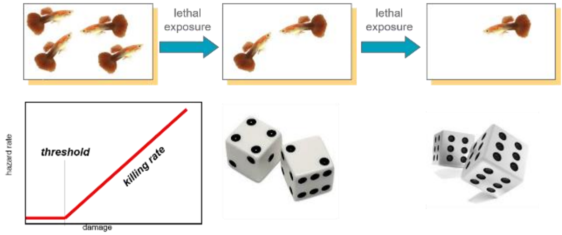
leanpub.com/guts_book

Ashauer, Thorbek, Warinton, Wheeler & Maund (2013): **A method to predict and understand fish survival under dynamic chemical stress using standard ecotoxicity data**. *ET&C*

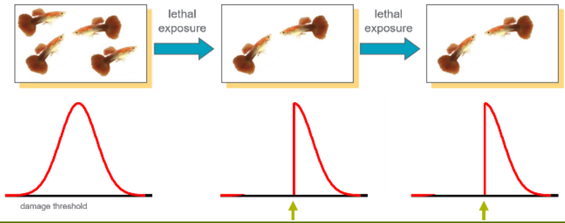
Ockleford, C., et al., **Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms**. EFSA Journal, 2018. 16(8): p. e05377.



Stochastic Death (GUTS-SD)



Individual Tolerance (GUTS-IT)

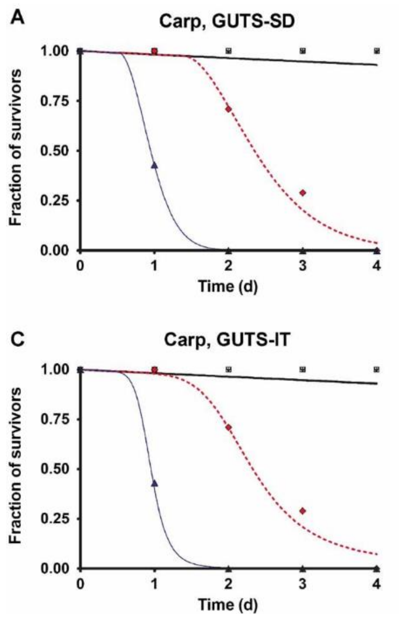


1) Calibration

- Acute tox (raw data, #, times)
- Preliminary predictions
- Calculate DRT_{95}

2) Validation

- Pulsed exposures
- Intervals vary (DRT_{95})



3) Calculate LP50 value

- Concentration time series as input
- Multiply until 50% dead at end
- Multiplication factor = Lethal Profile 50 (LP50)

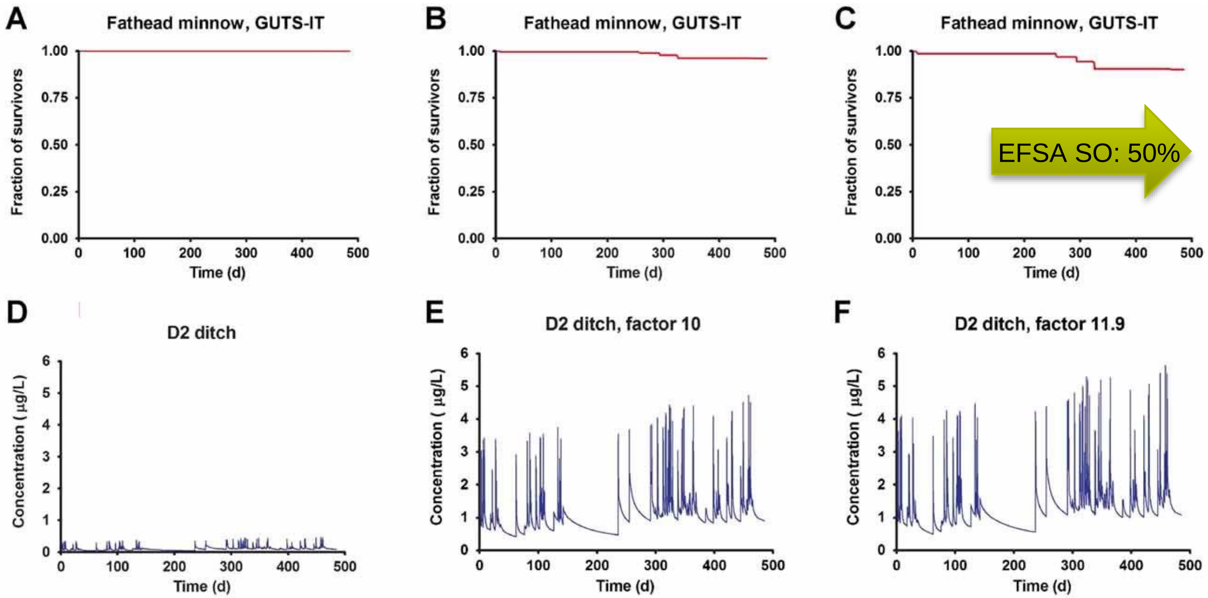
4) Risk assessment scheme in EFSA Scientific Opinion

- Do for most sensitive species
- Do for multiple species (SSD like or geomean)

Time-variable exposure

- Birds & mammals
- Terrestrial organisms
- Any acute tox issue...

No guidance, only SO



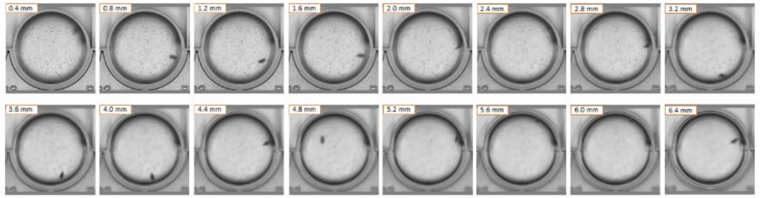
 Classification: public

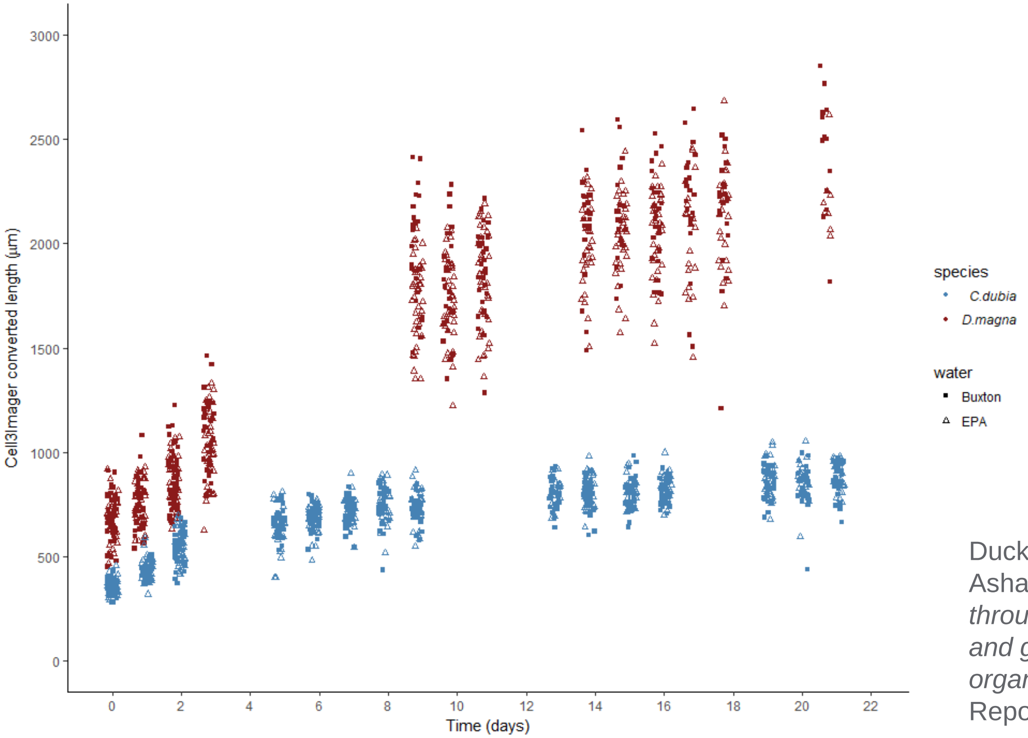
syngenta

Physiological mode of action – the key to (almost) everything?

What next? 1st priority: increase throughput of pMoA research & try different measurements?

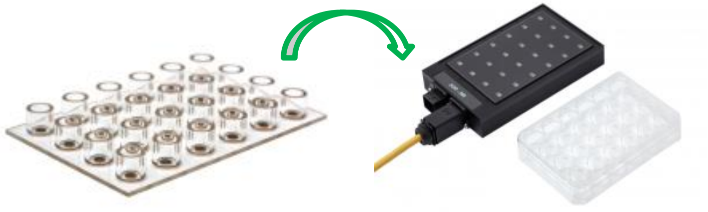
Automated scanning of well-plates (Cell³imager)





Duckworth, J., T. Jager, and R. Ashauer, *Automated, high-throughput measurement of size and growth curves of small organisms in well plates.* Scientific Reports, 2019. **9**(1): p. 10..

Measure respiration (Loligo Systems)



- 24-well glass microplate with individual oxygen sensor spots at the base of each well.
- Well volume is 600 or 1700 µl.

- Fluorescence based O_2-measurement
- Good temporal resolution (e.g. every 30 sec)
- Can upscale

How to increase throughput?

- Select species strategically
- Leverage technology (automation, image analysis, sensors, ...)
- Collaborate with experimentalists

Physiological mode of action – the key to (almost) everything?

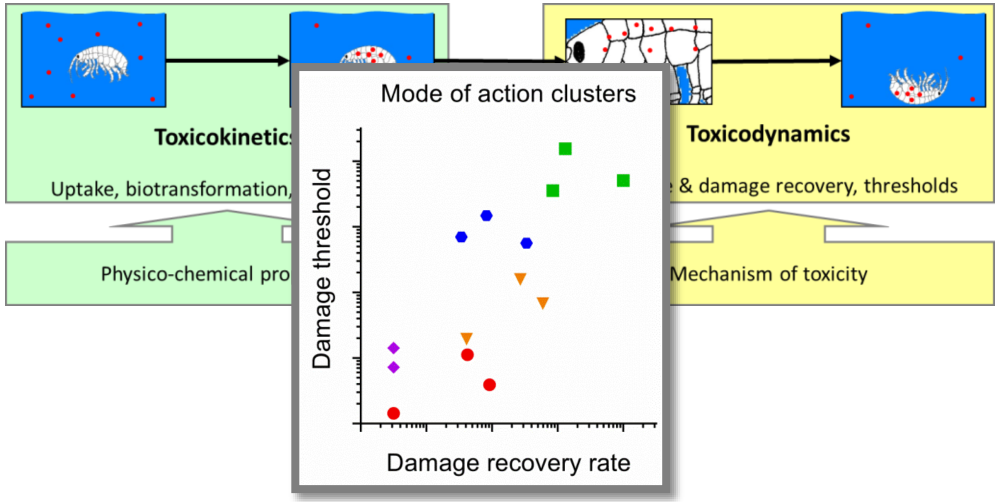
What next? 2nd priority: find patterns?

Compare across sprecies

Group	Chemical class	Toxicant	Acrobeloides nanus	Caenorhabditis elegans	Dendrobaena octaedra	Lumbricus rubellus	Capitella teleta	Folsomia candida	Daphnia magna	Moina micrura	Mytilus californianus	Mytilus galloprovincialis	Mytilus edulis	Crassostrea gigas	Lymnaea stagnalis	Danio rerio	Strongylocentrotus droebachiensis
Baseline toxicants	Neutral organics	PAH mixture														A	
Baseline toxicants	Neutral organics	Benzo(k)-fluoranthene														A	
Baseline toxicants	Neutral organics	Fluoranthene		G+R, G+R					R								
Baseline toxicants	Neutral organics	Pyrene							R								
Baseline toxicants	Neutral organics	Pyridine							M								
Baseline toxicants	Neutral organics	Acetone													A		
Baseline toxicants	Neutral organics	Diquat													A/M		
Baseline toxicants	Neutral organics	Pentachlorobenzene	A	G+R													
Baseline toxicants	Anilines	3,4-dichloroaniline							R/H								
Baseline toxicants	Aromatic triazine	Atrazine		M													
Baseline toxicants	Phenols	Pentachlorophenol											A+M				
Specific toxicity	Imidazoles, carbamate esters	Carbendazim	A	A													
Specific toxicity	Oxime carbamate ester	Aldicarb		M													
Specific toxicity	Monothiophosphate ester, halopyrdines	Chlorpyrifos						R									
Specific toxicity	Esters, Benzyl Nitriles, Pyrethroids	Fenvalerate							A								
Specific toxicity	Neutral organics[1]	Tetradifon							A								
Specific toxicity	Phenols	Nonylphenol					G+R										
Specific toxicity	n.a.	Tributyltin													A		
Specific toxicity	n.a.	Triphenyltin						M									
Metals	Metals[2]	Cadmium	G	A, A, A, A/M				A, A	A								
Metals	Metals[2]	Copper		A	A	M+R, A			G								
Metals	Metals[2]	Uranium		A, A/M					A							M+G	
Metals	Metals[2]	Zinc				M			A/M								
Metals	Metals[2]	Mercury										A		A/M			
Metals	n.a.	Zinc-oxide nanoparticles										A+M					
Others	n.a.	Toxic cyanobacteria								A/M							
Others	n.a.	pH (ocean acidification)															M
Others	n.a.	Produced water									A+M	A+M					

Ashauer, R. and T. Jager, *Physiological modes of action across species and toxicants: the key to predictive ecotoxicology.* Environ Sci Process Impacts, 2018. **20**(1): p. 48-57.

Compare across chemicals

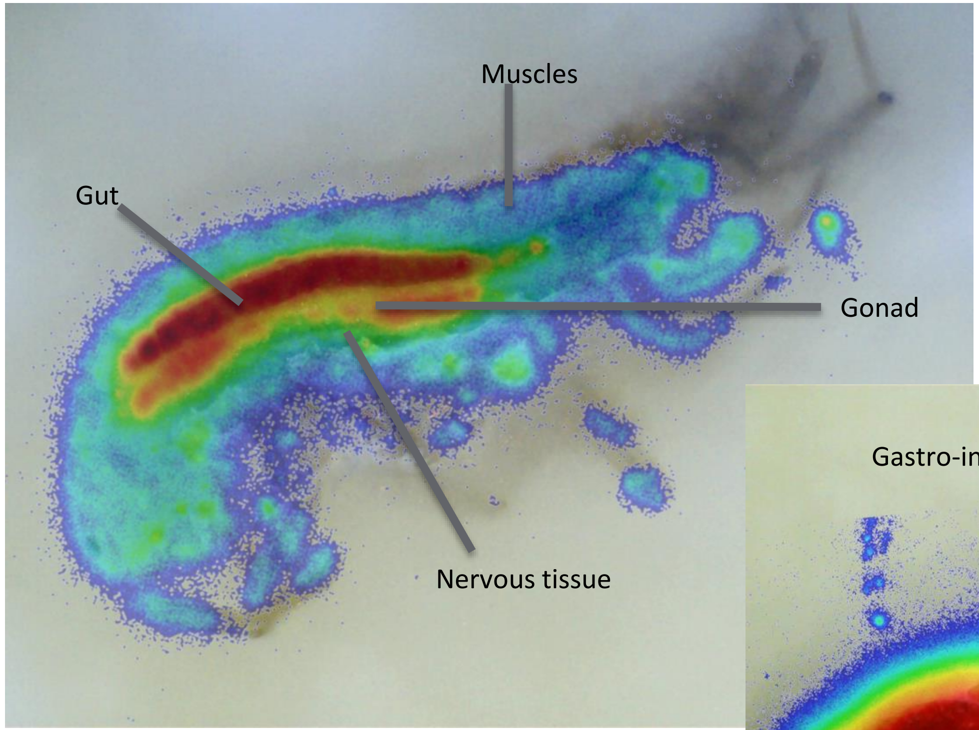


<u>How to look for patterns and why?</u>

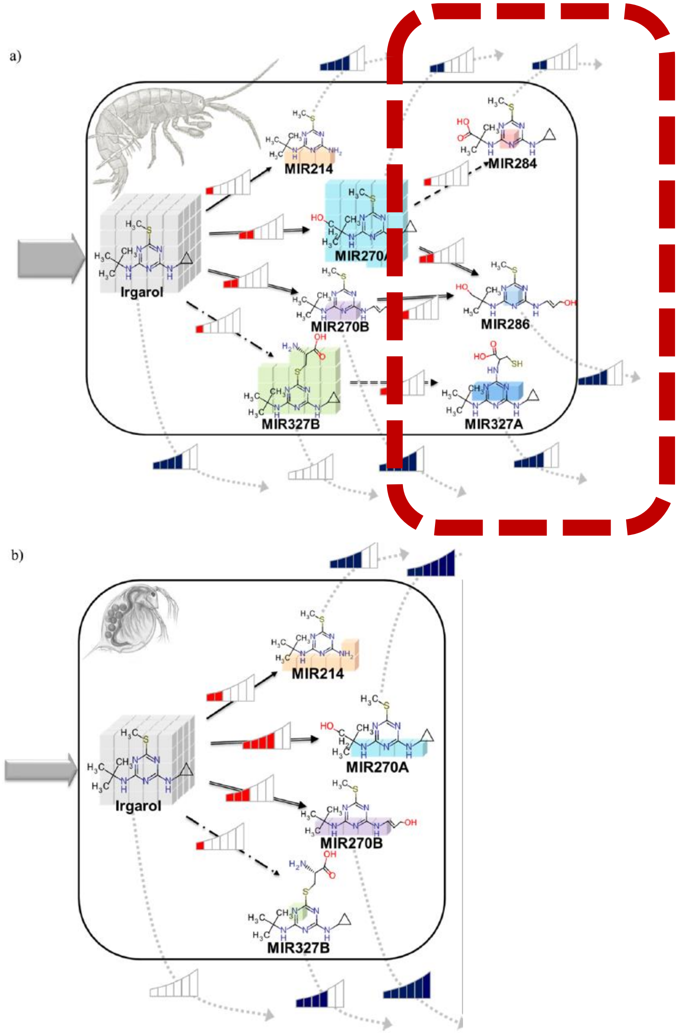
- Study multiple species
- Study strategically selected groups of chemicals
- Patterns can be turned into predictive relationships
- Build prediction tools

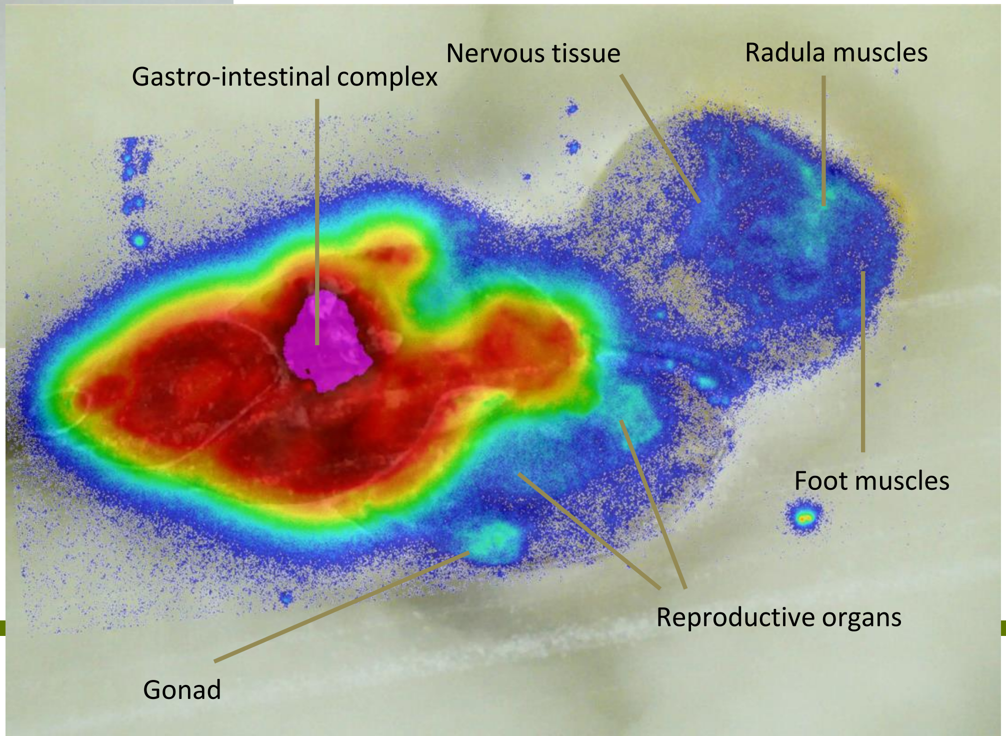
Physiological mode of action – the key to (almost) everything?

What next? 3rd priority: what is the concentration at the target site? A tough challenge...



Jeon J, Kurth D, Ashauer R, Hollender J (2013) Comparative toxicokinetics of organic micropollutants in freshwater crustaceans. ES & T, 47: 8809-8817.

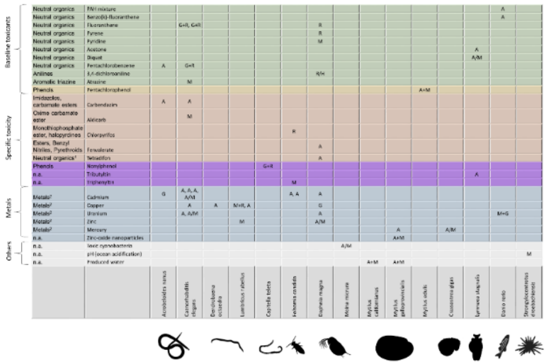




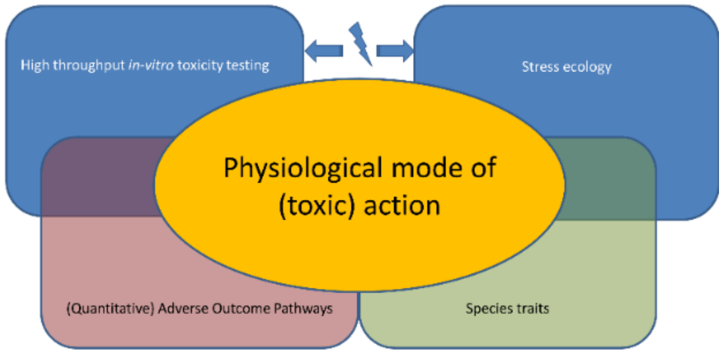
Nyman, Schirmer, Ashauer (2014): The importance of toxicokinetics for interspecies variation in sensitivity to chemicals. *Environmental Science & Technology*, 48(10)

Thank you for your attention!

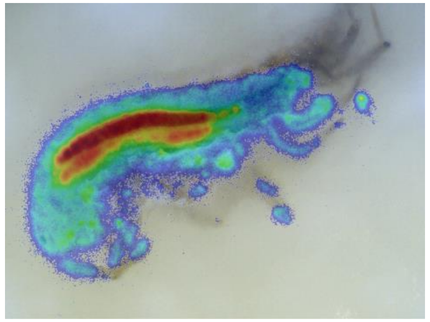
1) Increase throughput, find patterns



2) Build theory, make predictions



3) Models & experiments



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