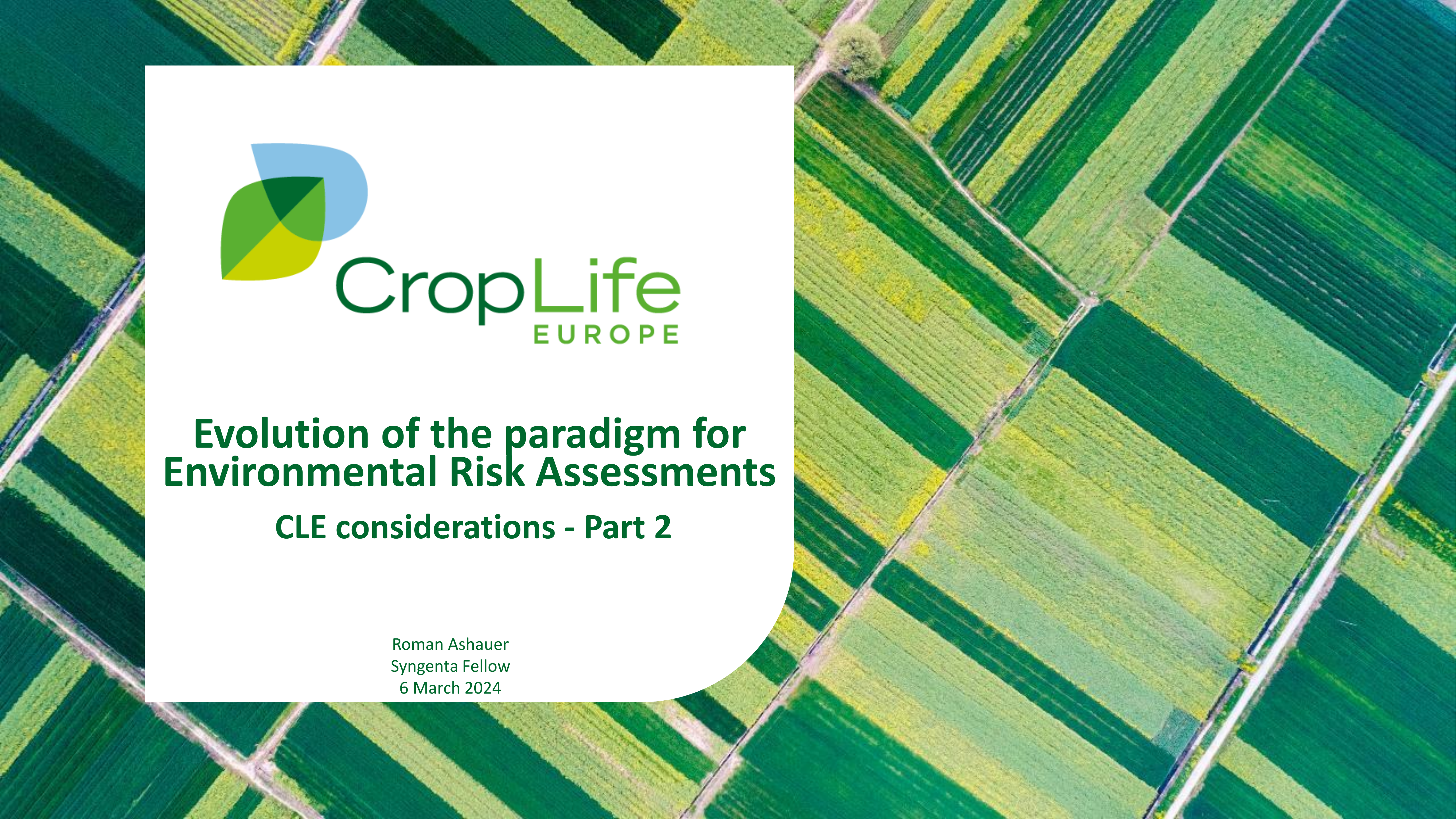
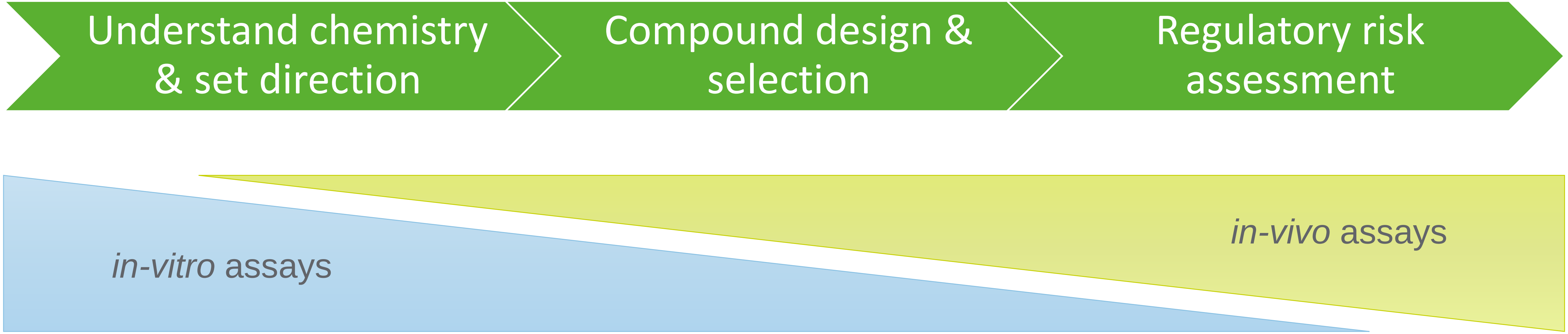


Evolution of the paradigm for Environmental Risk Assessments

CLE considerations - Part 2

Roman Ashauer
Syngenta Fellow
6 March 2024

Early or late in the pipeline makes a difference



<u>Early stage drivers for *in-vitro* assays:</u>

- Less test item needed
- Cheaper & faster
- Automation & throughput

<u>Late stage drivers for *in-vivo* assays:</u>

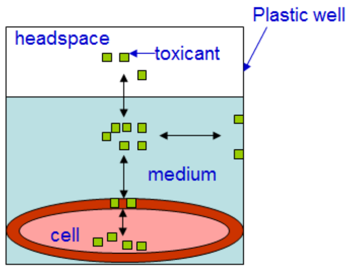
- Definite regulatory endpoint (acceptance)
- No *in-vitro* to *in-vivo* extrapolation needed
- Species sensitivity differences & coverage

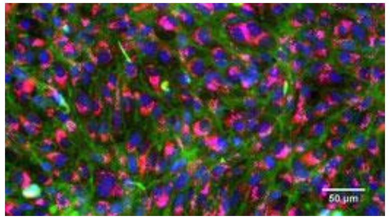
Intra-species *in-vitro* to *in-vivo* toxicity extrapolation (IVIVE)

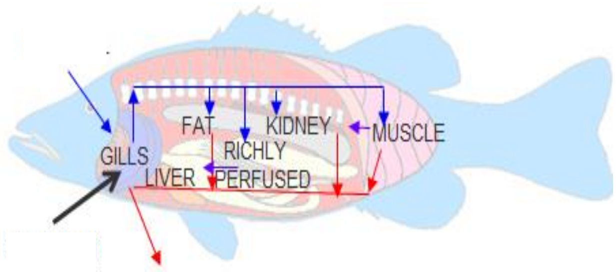
- **Intra-species** extrapolation, e.g. *in-vitro* to *in-vivo*
  - Typical approach to replace a certain standard test
  - Often involves *in-vitro* assays from the species of interest (e.g. fish cell lines to predict fish endpoints)
  - Combined with modelling to account for partitioning, toxicokinetics & toxicodynamics

C_{medium} → TK → $C_{internal,\ gill\ cell}$ → von Bertalanffy → Reduced growth (cell population)

C_{water} → PBTK → $C_{internal,\ gill\ cell}$ → von Bertalanffy → Reduced growth (fish)




Rainbow trout gill cells.
Photo credit: Vivian Lu Tan/Eawag

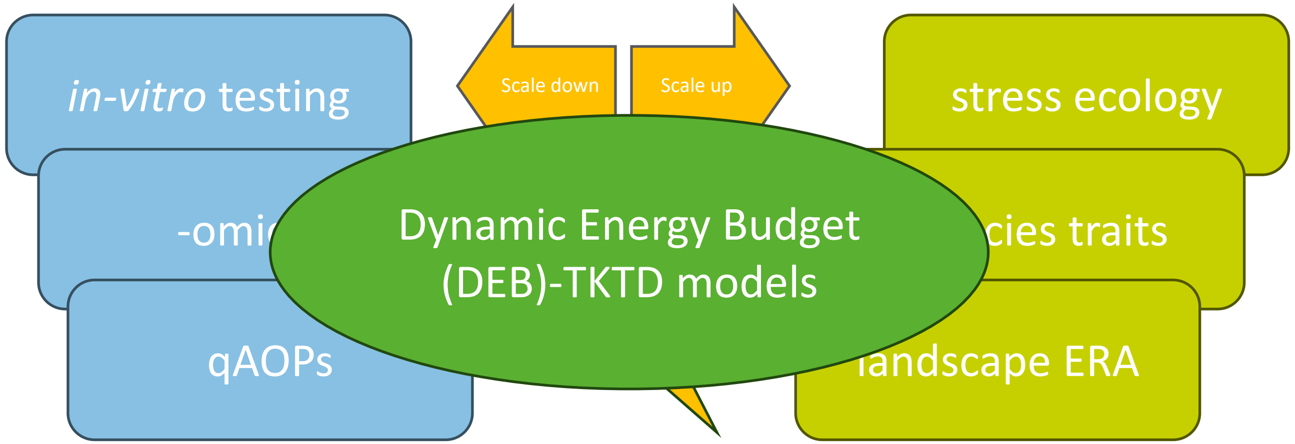



Rainbow trout.
http://underwater-fish.blogspot.co.uk

Stadnicka-Michalak, Tanneberger, Schirmer, Ashauer (2014): Measured and Modeled Toxicokinetics in Cultured Fish Cells and Application to *In Vitro-In Vivo* Toxicity Extrapolation. *PLoS ONE* 9: e92303.
Stadnicka-Michalak, J., K. Schirmer and R. Ashauer, *Toxicology across scales: Cell population growth in vitro predicts reduced fish growth. Science Advances, 2015. 1(7): p. 1-8.*

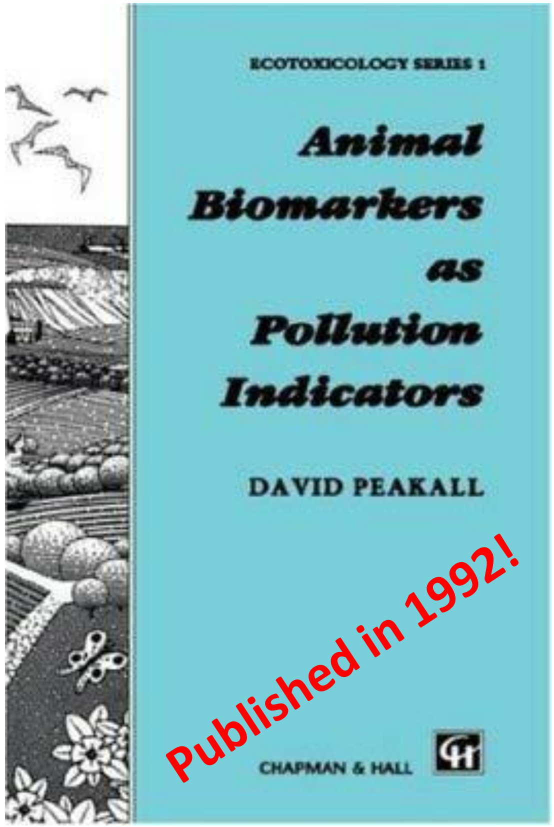
Inter-species toxicity extrapolation & ecology

- **Inter-species** extrapolation (e.g. from standard test species to untested or endangered species) & ecology
  - Modelling for toxicokinetics & toxicodynamics, species traits, physiology, ecology, populations, landscapes
  - Can benefit from AOPs, but is conservation of MIE & pathways known in both species (tested & predicted)?
  - Are we more interested in cases where pathways are conserved or when they are not?!



Critical review: 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.*

How to build a model (e.g. qAOP): scales



220 *The role of biomarkers in environmental assessment*

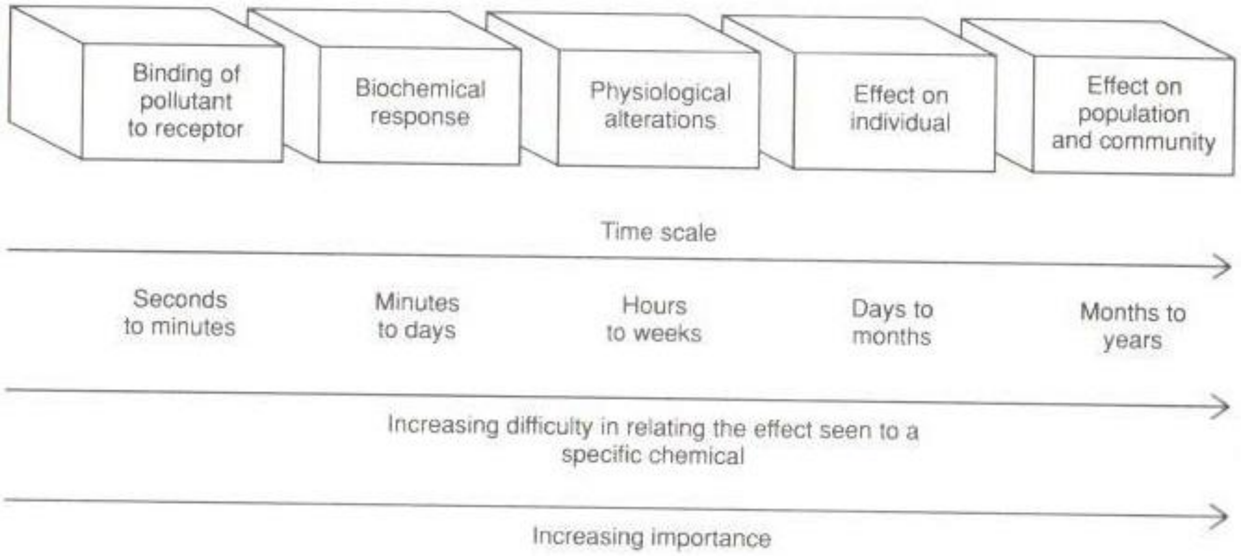


Figure 10.5 Linkages between biochemical, physiological, individual and population responses to pollutants.

We need better understanding of scale transitions
&
Carefully decide what to include in qAOPs

- Different time scales and different system sizes matter!
- Scale transitions are inherently difficult to model

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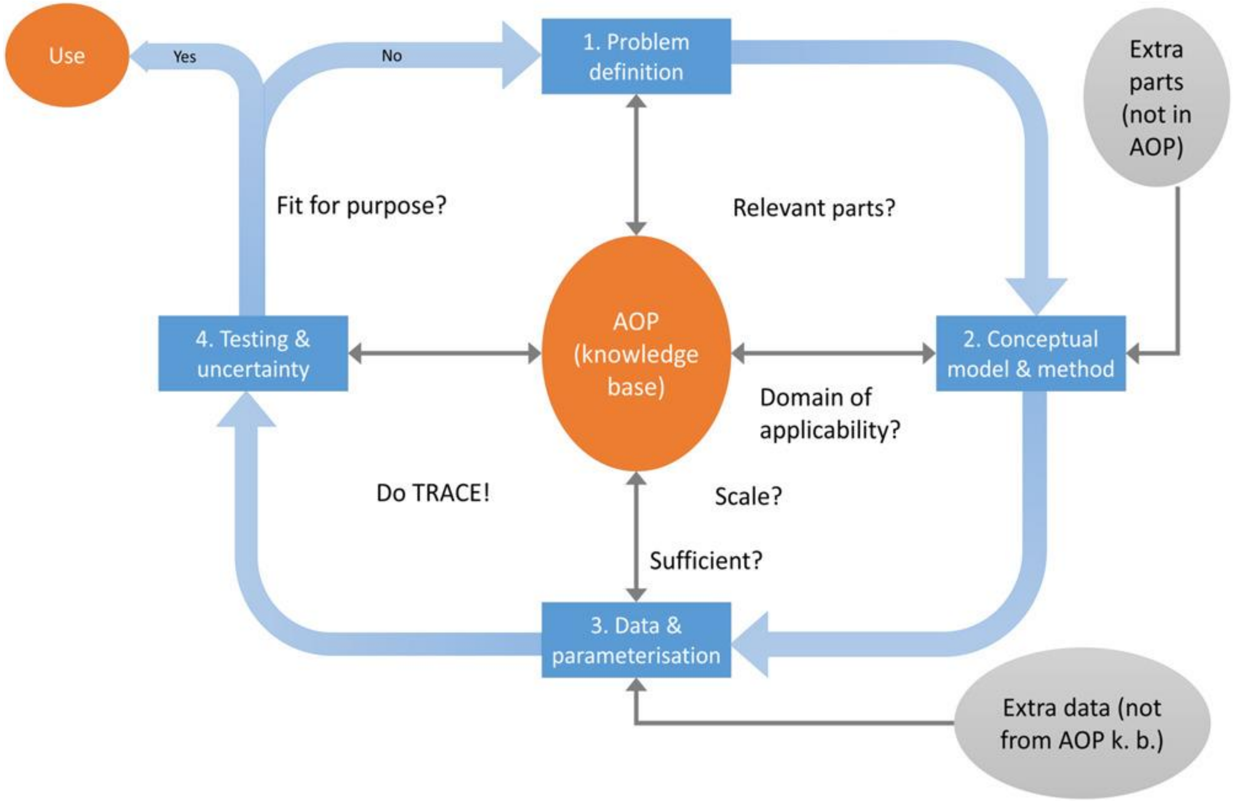
How to build a model (e.g. qAOP): complexity

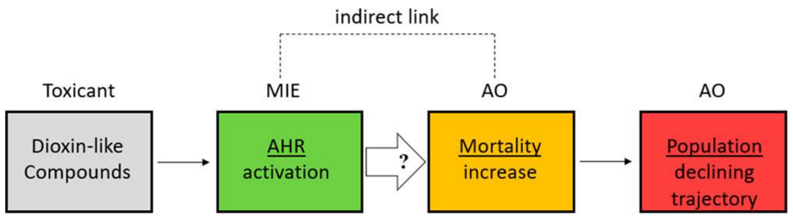


As simple as possible
&
As complex as needed!

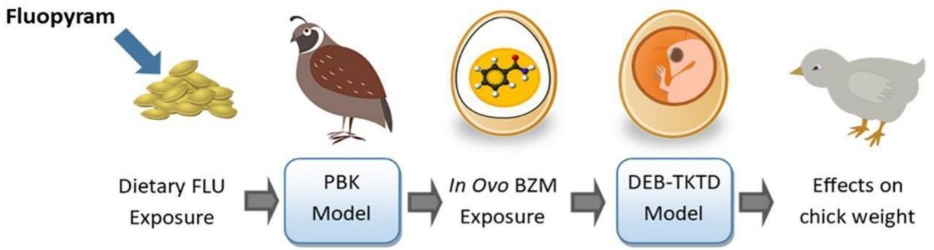
<u>Guiding principles for building qAOPs:</u> Perkins, E.J., et al., *Building and Applying Quantitative Adverse Outcome Pathway Models for Chemical Hazard and Risk Assessment. Environmental Toxicology and Chemistry, 2019. 38(9): p. 1850-1865.*

Examples of very simple & successful «1 step» qAOPs

Species	Measured *in-vitro*	Predicted *in-vivo*	Modelling	Reference
Fish	Gill cell proliferation	Fish growth	Partitioning, PBK & van Bertalanffy growth	Stadnicka-Michalak, J., K. Schirmer and R. Ashauer, *Toxicology across scales: Cell population growth in vitro predicts reduced fish growth. Science Advances, 2015. 1(7): p. 1-8.*
Fish & birds	AHR activation	Early life-stage mortality	Exposure normalised to concentrations in eggs	Doering, J.A., S. Wiseman, J.P. Giesy and M. Hecker, *A Cross-species Quantitative Adverse Outcome Pathway for Activation of the Aryl Hydrocarbon Receptor Leading to Early Life Stage Mortality in Birds and Fishes. Environmental Science & Technology, 2018. 52(13): p. 7524-7533.*
Birds	Embryo weight & length in egg injection study	Effects on hatchling and 14-day chick weight	PBK & DEB-TKTD	Martin, T., et al., *Reproductive toxicity in birds predicted by physiologically-based kinetics and bioenergetics modelling. Science of The Total Environment, 2024. 912: p. 169096.*



qAOP in Doering et al. 2018



qAOP in Martin et al. 2024

Critical success factors for NG ERA

Ecotox differs from human tox. → Create ERA specific problem definition.

The question dictates the model. → Include modellers from the start.

The model dictates the data needs. → Ensure data addresses modelling needs.

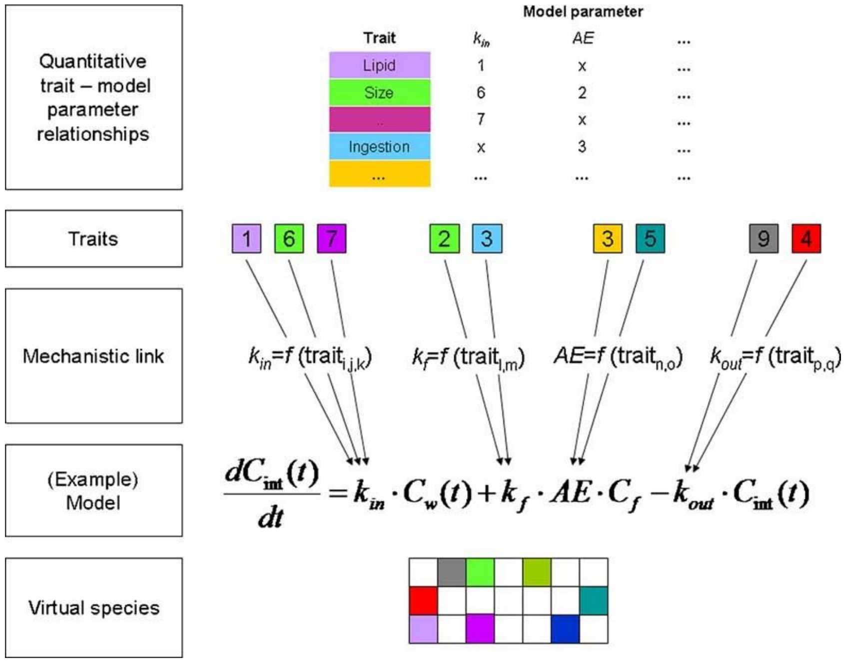
Bonus slides

Inter-species toxicity extrapolation

- **Inter-species** extrapolation, e.g. from standard test species to untested or endangered species
  - Combined with modelling to account for toxicokinetics & toxicodynamics, species traits, physiology
  - Can benefit from AOPs, but is conservation of MIE & pathways known in both species (tested & predicted)?
  - Are we more interested in cases where pathways are conserved or when they are not?!

			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	
	Neutral organics	Benzo(k)-fluoranthene														A	
	Neutral organics	Fluoranthene		G+R, G+R					R								
	Neutral organics	Pyrene							R								
	Neutral organics	Pyridine							M								
	Neutral organics	Acetone													A		
	Neutral organics	Diquat													A/M		
	Neutral organics	Pentachlorobenzene	A	G+R													
	Anilines	3,4-dichloroaniline							R/H								
	Aromatic triazine	Atrazine		M													
	Phenols	Pentachlorophenol											A+M				
Specific toxicity	Imidazoles, carbamate esters	Carbendazim	A	A													
	Oxime carbamate ester	Aldicarb		M													
	Monothiophosphate ester, halopyrdines	Chlorpyrifos						R									
	Esters, Benzyl Nitriles, Pyrethroids	Fenvalerate							A								
	Neutral organics[1]	Tetradifon							A								
	Phenols	Nonylphenol					G+R										
	n.a.	Tributyltin													A		
	n.a.	Triphenyltin						M									
Metals	Metals[2]	Cadmium	G	A, A, A, A/M				A, A	A								
	Metals[2]	Copper		A	A	M+R, A			G								
	Metals[2]	Uranium		A, A/M					A							M+G	
	Metals[2]	Zinc				M			A/M								
	Metals[2]	Mercury										A		A/M			
	n.a.	Zinc-oxide nanoparticles										A+M					
Others	n.a.	Toxic cyanobacteria								A/M							
	n.a.	pH (ocean acidification)															M
	n.a.	Produced water									A+M	A+M					

Critical review: 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.*



Conceptual paper: Rubach, M.N., et al., *Framework for traits-based assessment in ecotoxicology. IEAM, 2011. 7(2): p. 172-186.*

Will ecotoxicology become a truly predictive science?

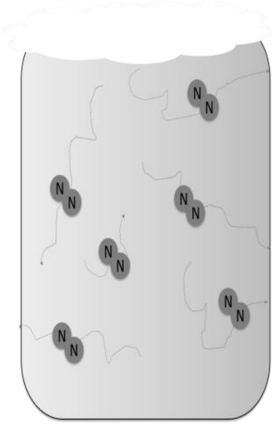
Physical chemistry

Macroscopic scale

Perfect gas
$p \times V = \text{constant}$
(Boyle's law)

Emerges

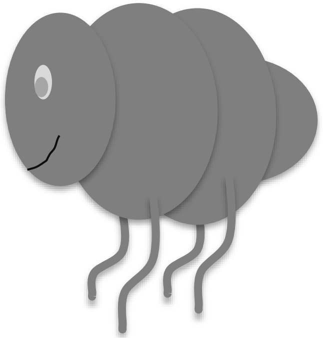
Molecular scale



Kinetic model of gases
- Random motion
- Ignore size
- Elastic collisions only

(Eco)toxicology

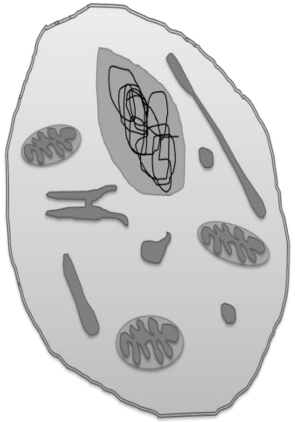
Macroscopic scale



Generic organism
$\text{stress} = 1/C_T \times \max(0, C_V - C_0)$
(DEBtox)

Missing theory

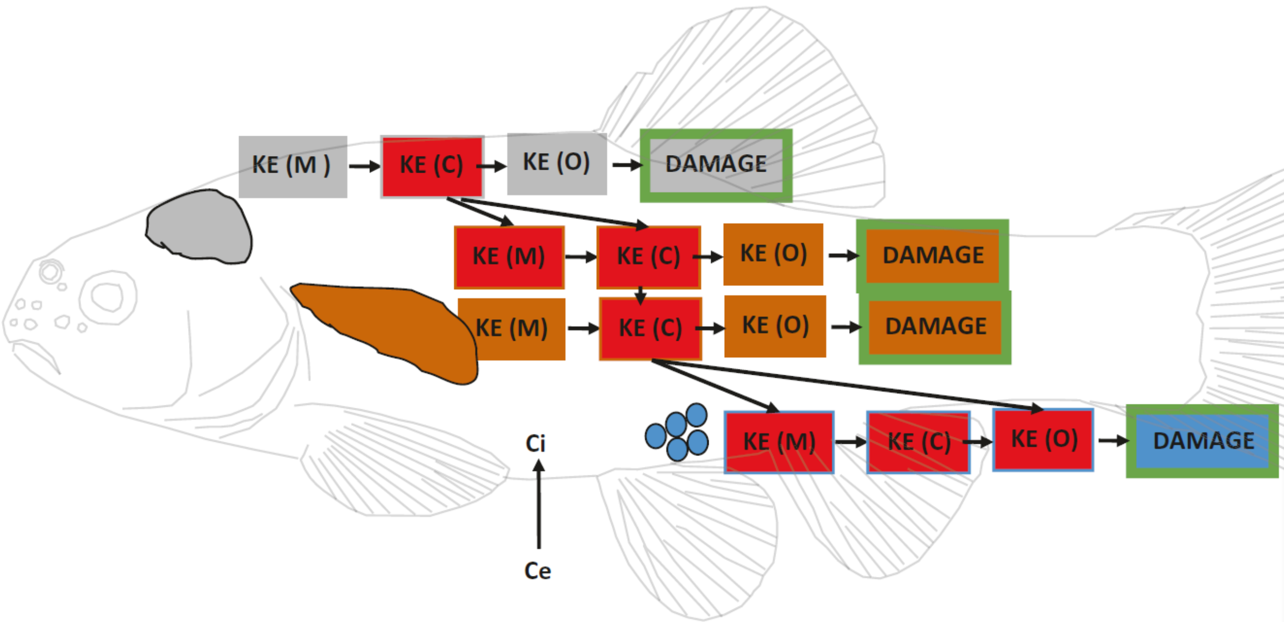
Molecular scale

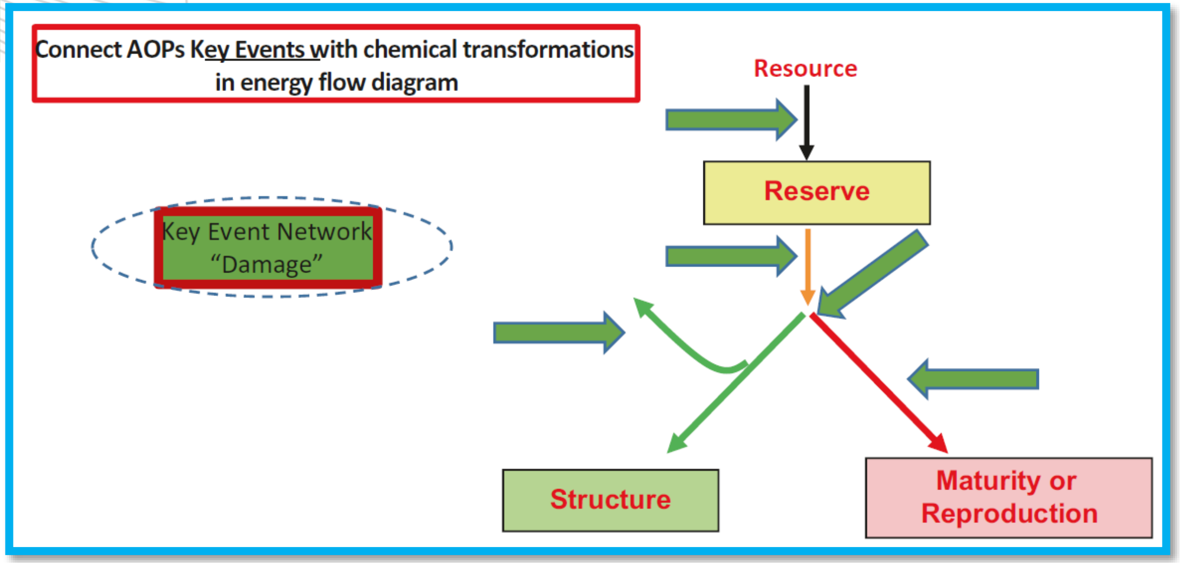


Cellular pathways
- Reaction networks
- -omics
- Pathway models

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

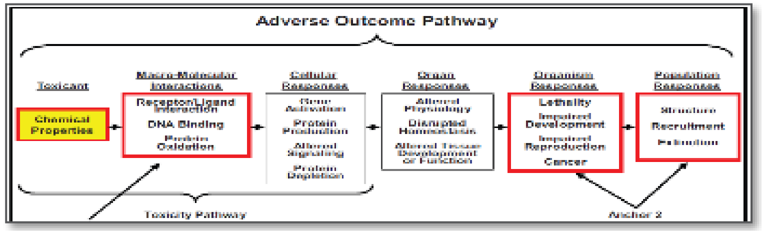
DEB-TKTD as link between AOPs & ERA?



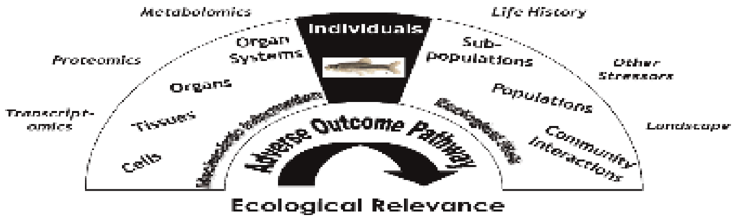


Murphy, C.A., et al., *Linking adverse outcome pathways to dynamic energy budgets: A conceptual model*, in *A Systems Biology Approach to Advancing Adverse Outcome Pathways for Risk Assessment*. 2018, Springer International Publishing. p. 281-302.

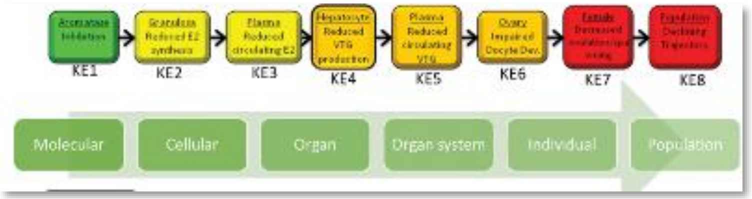
How to build a model (e.g. qAOP): scales



Ankley et al. AOPs … *Environ. Toxicol. Chem.* **2010,** *29*



Kramer et al. AOPs & ERA … *ET&C* **2011,** *30*



Wittwehr et al. … *Toxicol. Sci.* **2016,** *155*

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CropLife EUROPE

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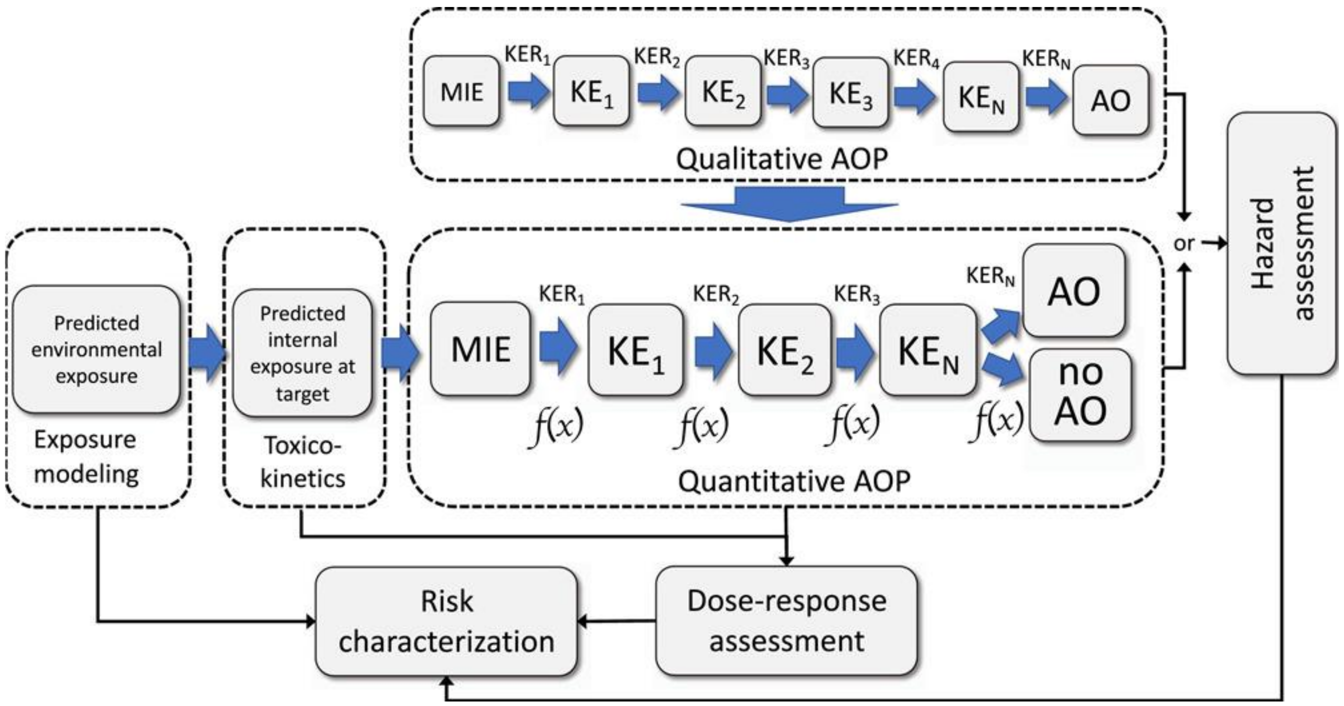

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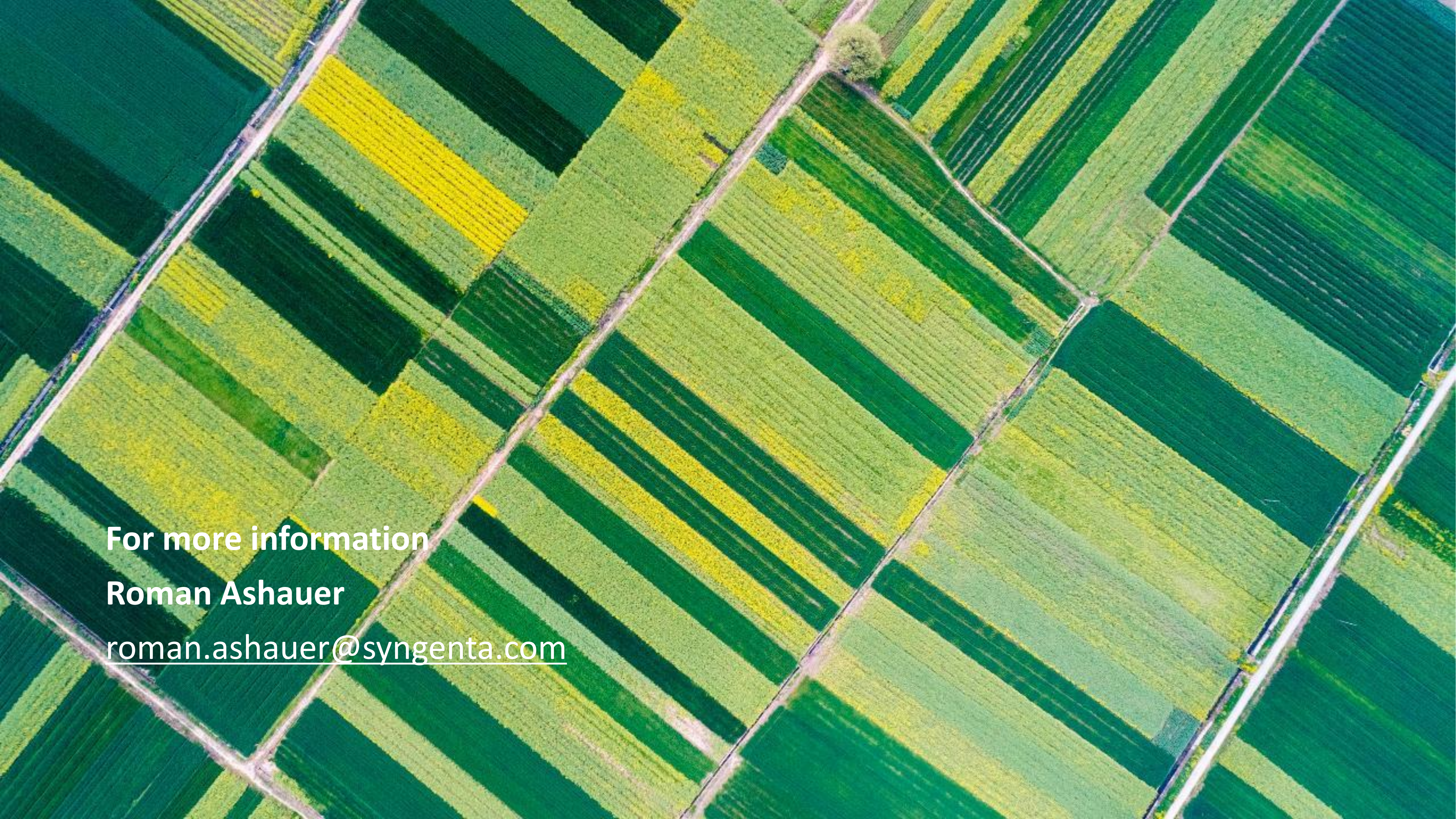

For more information

Roman Ashauer

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