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Towards predicting chronic fish toxicity

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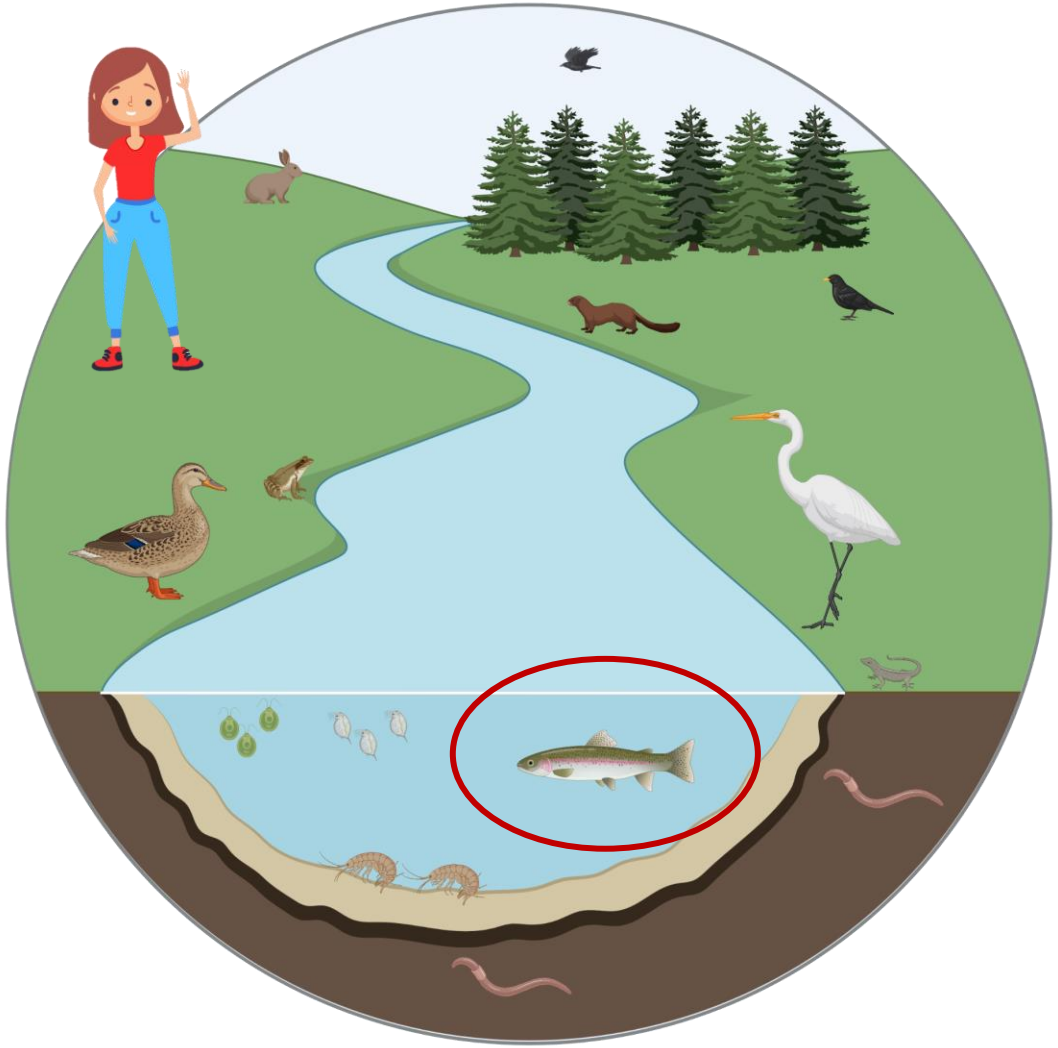
One environment – one health



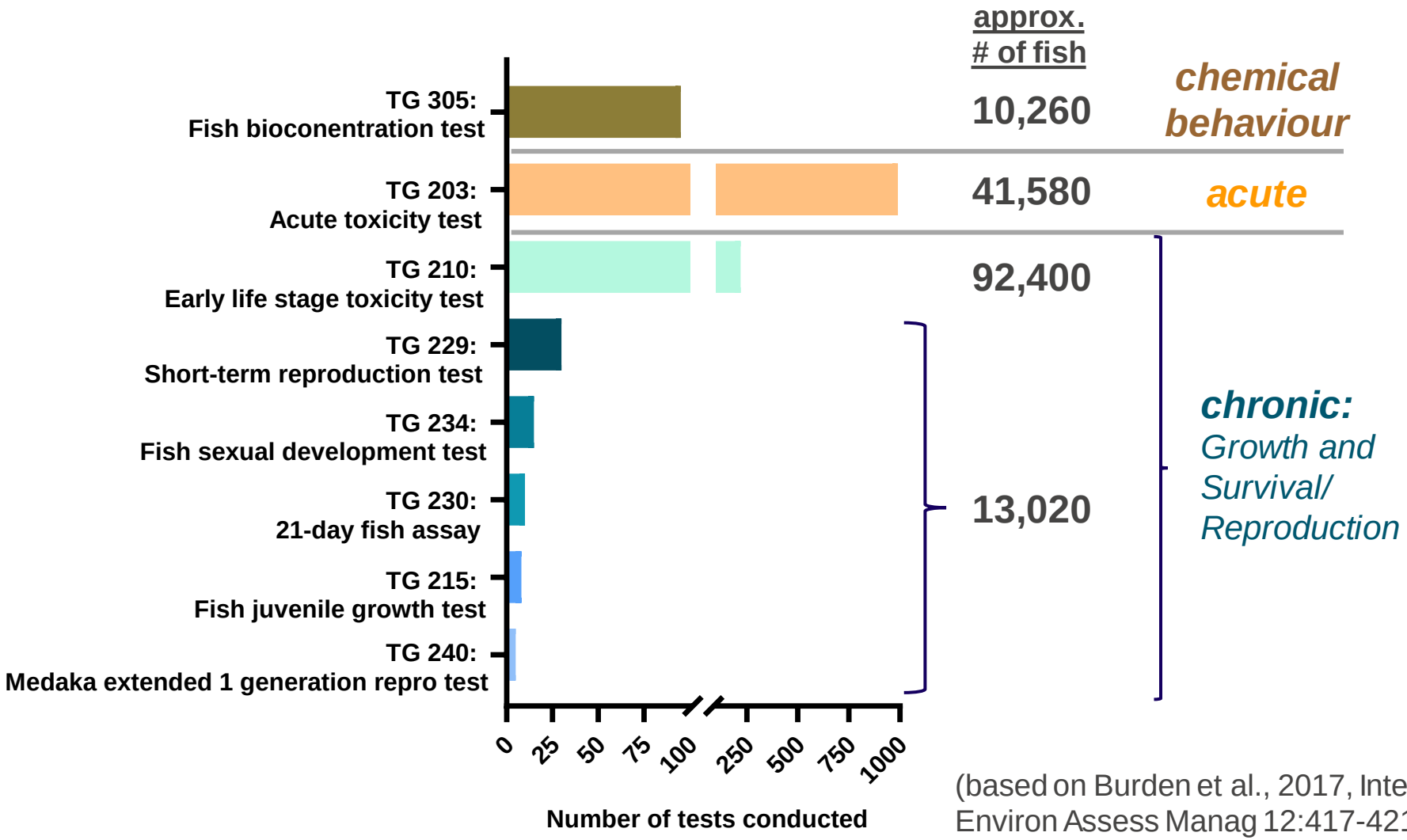
Arts by Fabian Balk

- Regulate food web dynamics
- Provide ecosystem services
- Comprise important source of food

Survive, grow and reproduce …including ability to detoxify

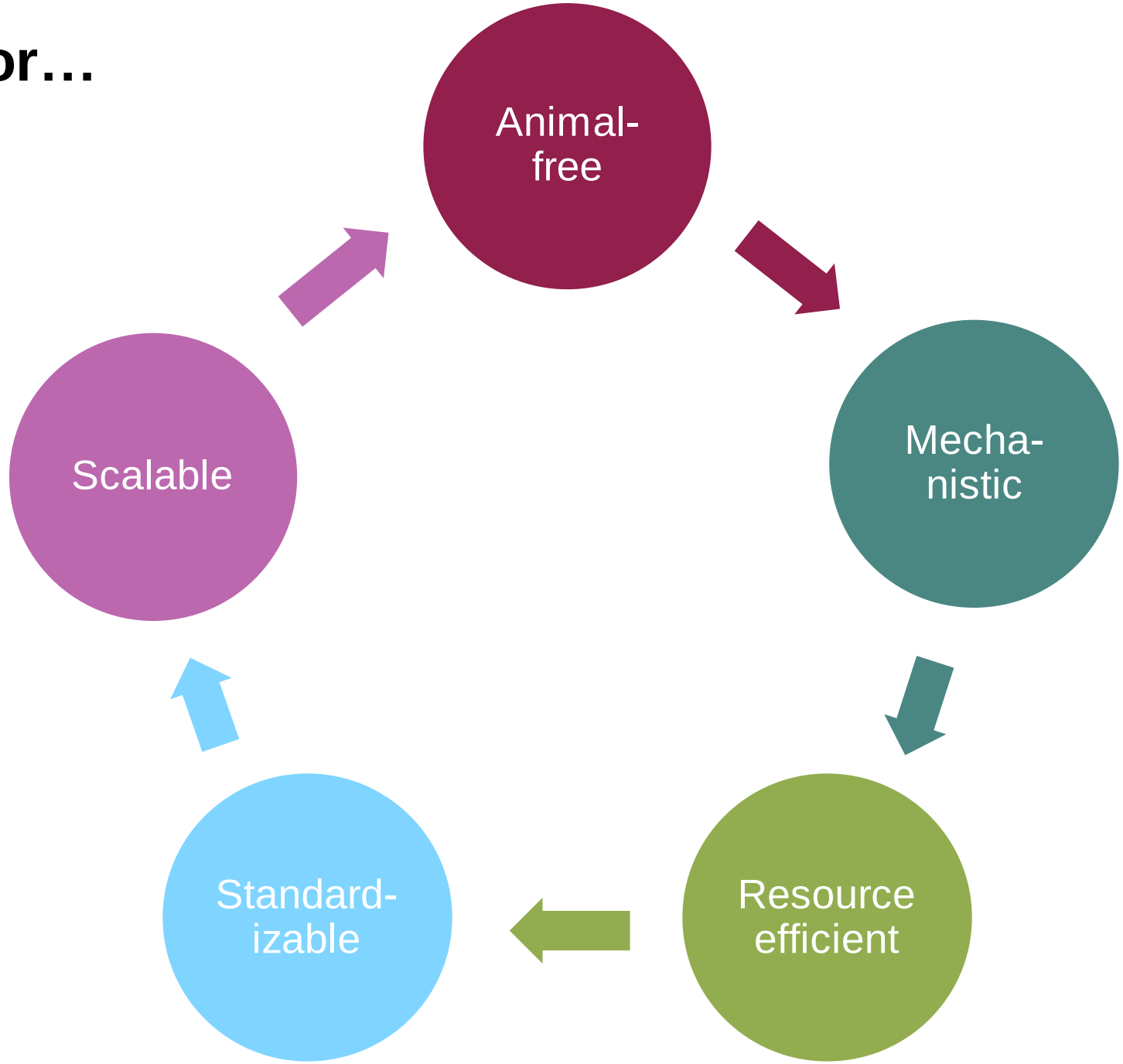
(i.e. not bioaccumulate)

Number of conventional fish tests conducted in 15 CROs (2014-2017)

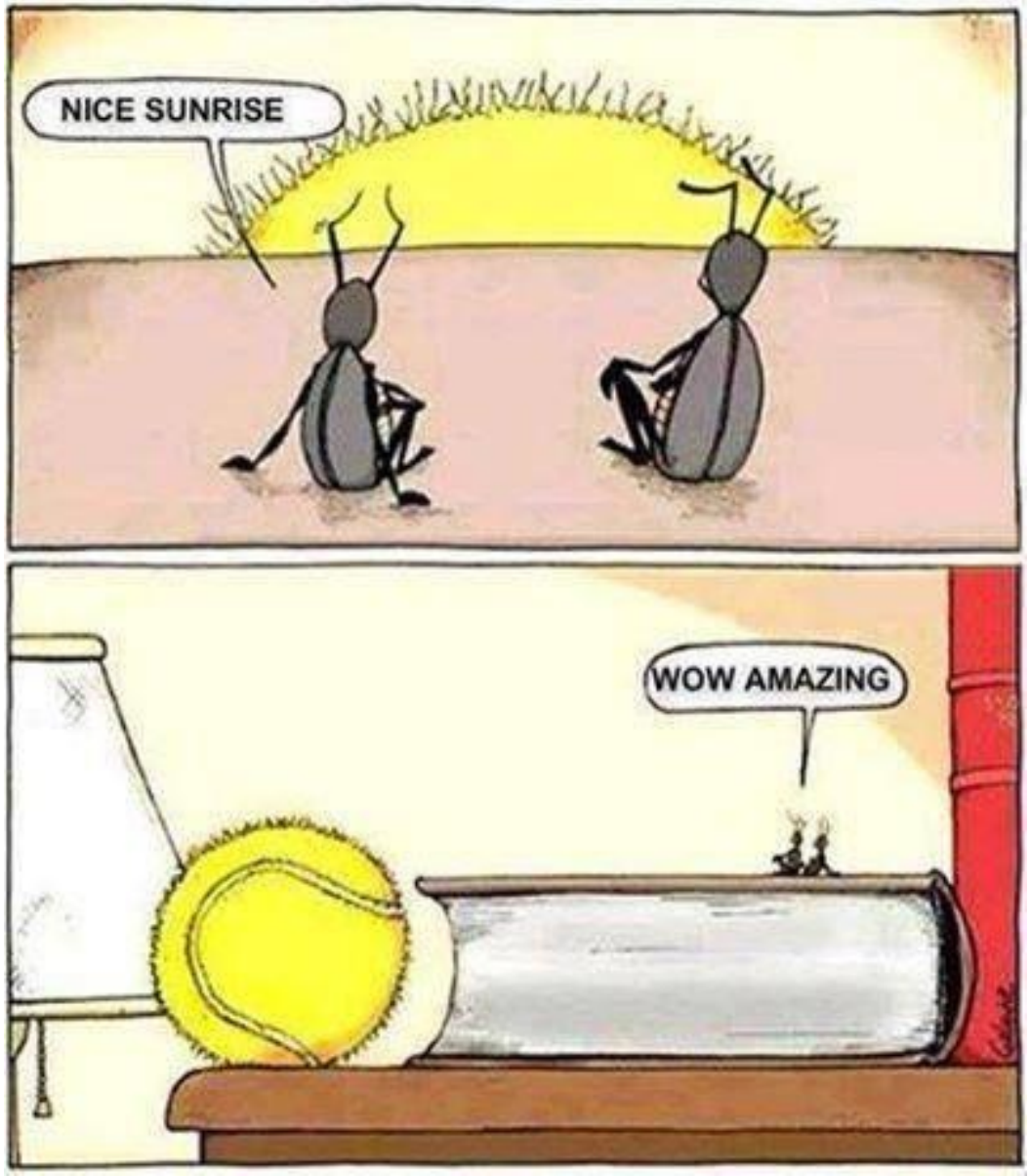


(based on Burden et al., 2017, Integr Environ Assess Manag 12:417-421)

We aim for…


Animal-free
Mecha-nistic
Resource efficient
Standard-izable
Scalable

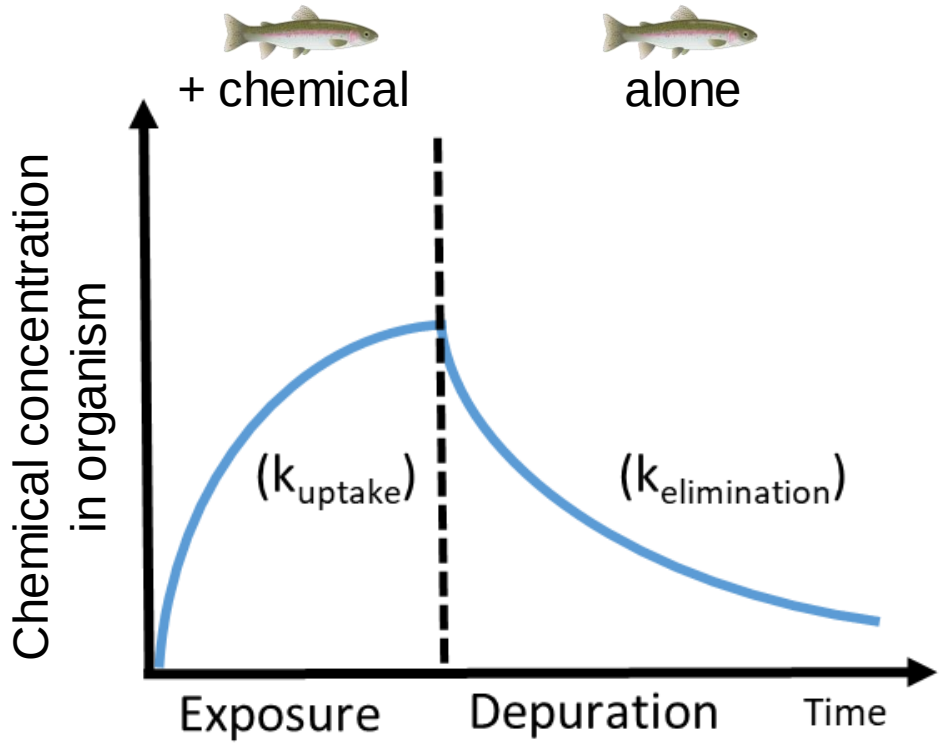
Let’s get ready for a perspective shift



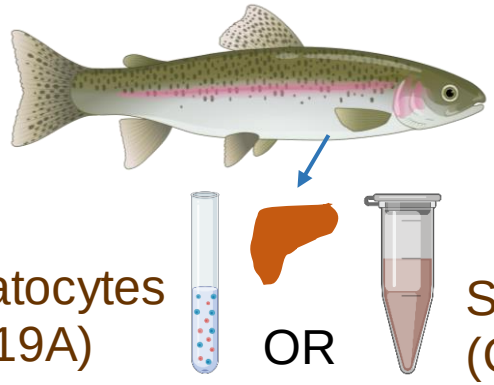
Let's get ready for a perspective shift

Take bioconcentration assessment (OECD TG305)

- ≥ 108 fish
- ~ 28 - 42 days



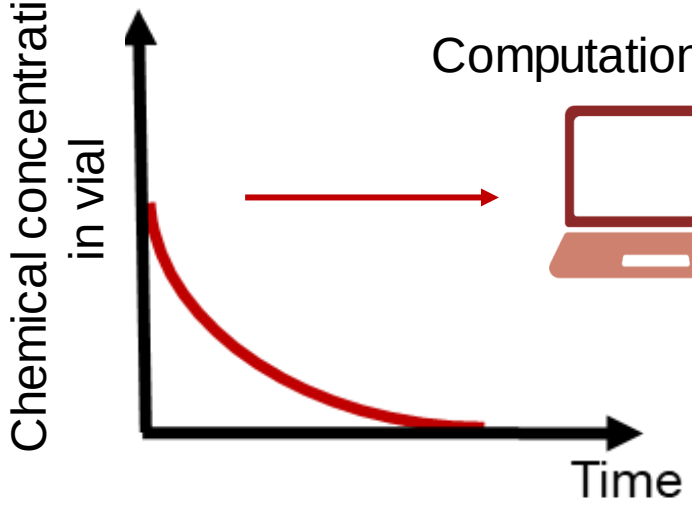
Take instead:



Primary hepatocytes (OECD TG319A)

OR

S9 sub-cellular fractions (OECD TG319B)



Computational model

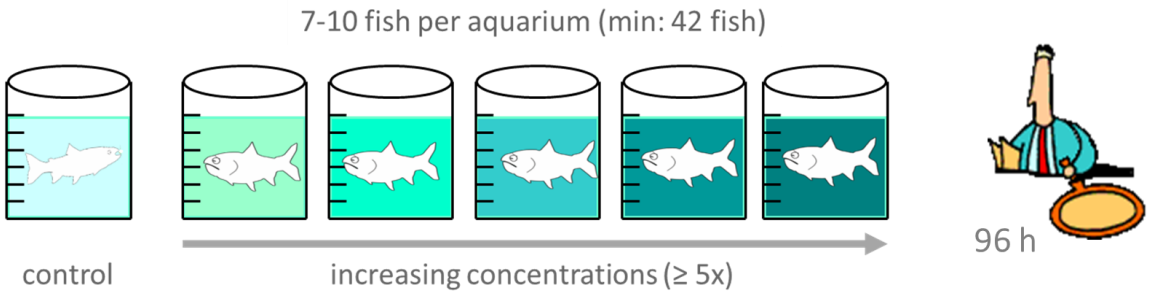
In vitro-derived BCF

See additional information at end of presentation

Let's get ready for a perspective shift

Take acute fish toxicity (survival) (OECD TG203)

- Death as sole endpoint
- ≥ 42 fish
- 4 days



Take instead:

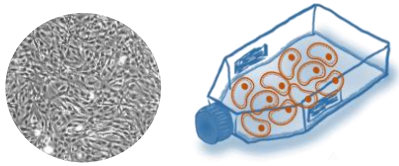
OECD TG236: Fish Embryo Acute Toxicity (FET) Test

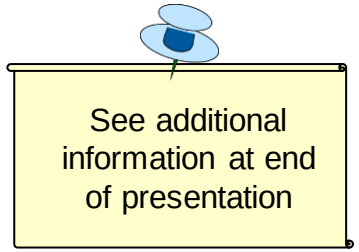
in vivo (non-protected stage)

OR: *in vitro*

RTgill-W1 – a rainbow trout gill cell line

OECD TG249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay

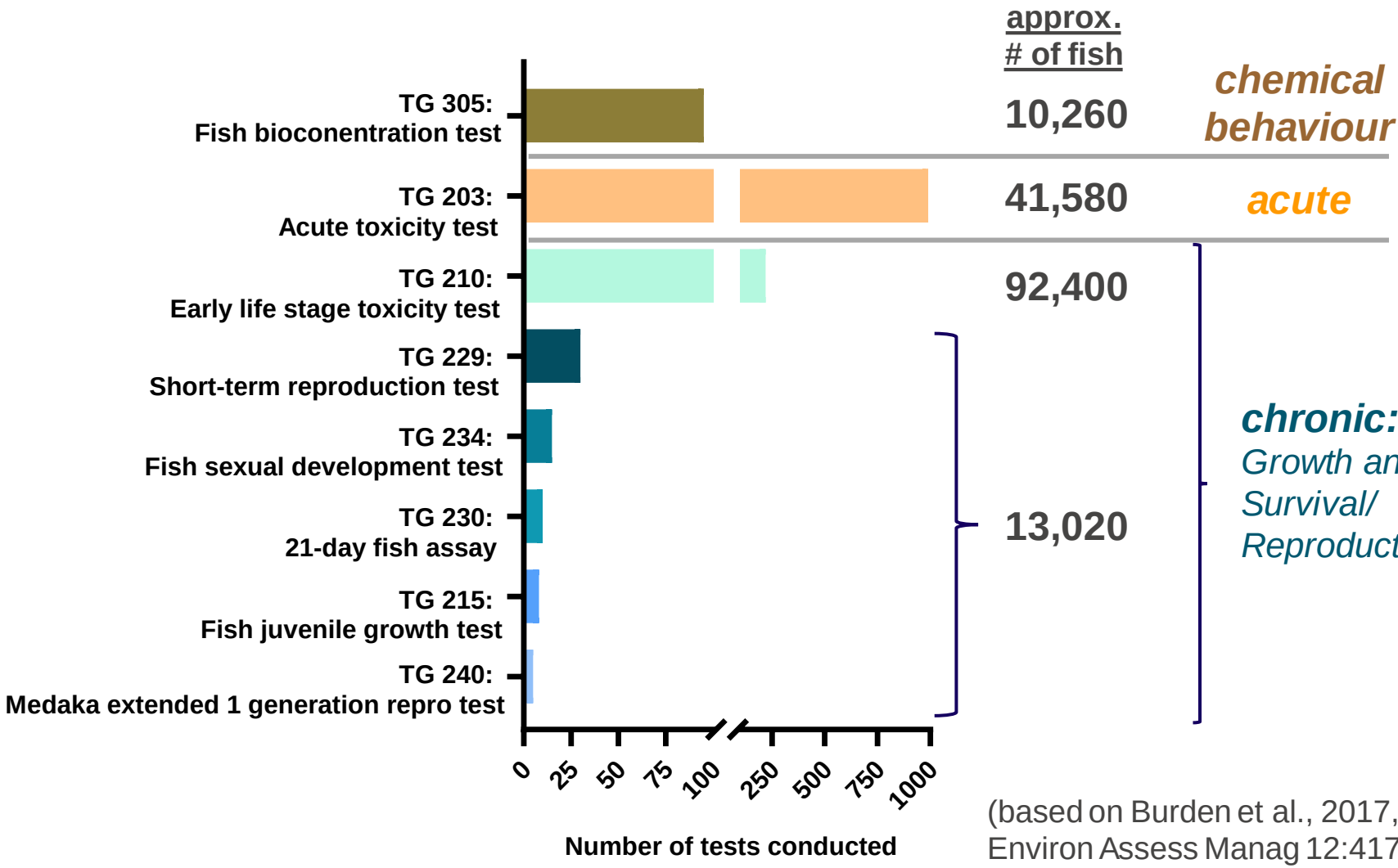




Survive, grow and reproduce …including ability to detoxify

(i.e. not bioaccumulate)

Number of fish tests conducted in 15 CROs (2014-2017)



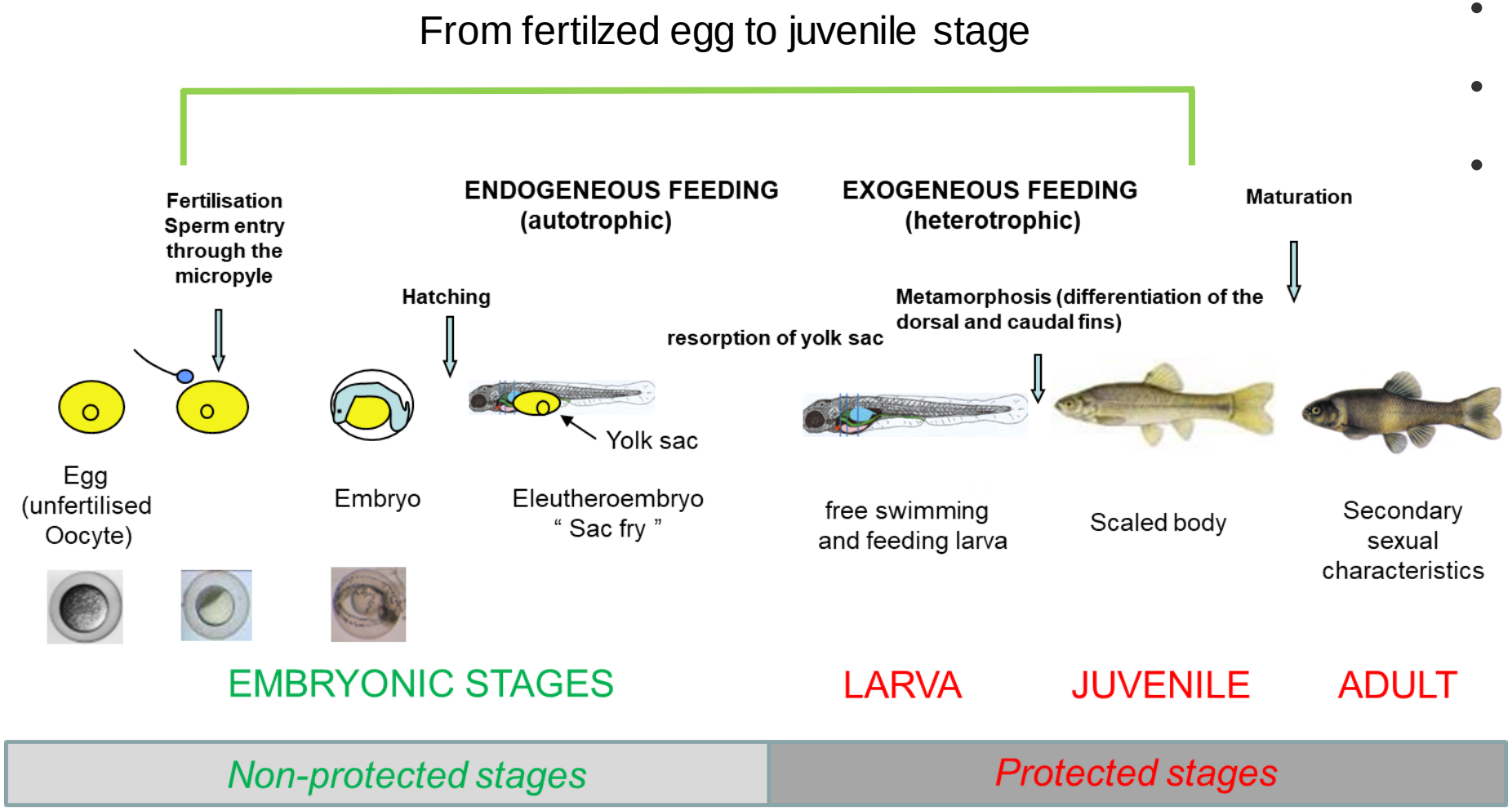
alternatives ready?

✓

✓

(based on Burden et al., 2017, Integr Environ Assess Manag 12:417-421)

Early life stage toxicity — growth (OECD TG210)

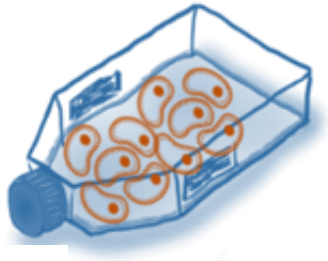


- altered growth/survival
- ≥ 400 fish
- ~ 28 - 90 days

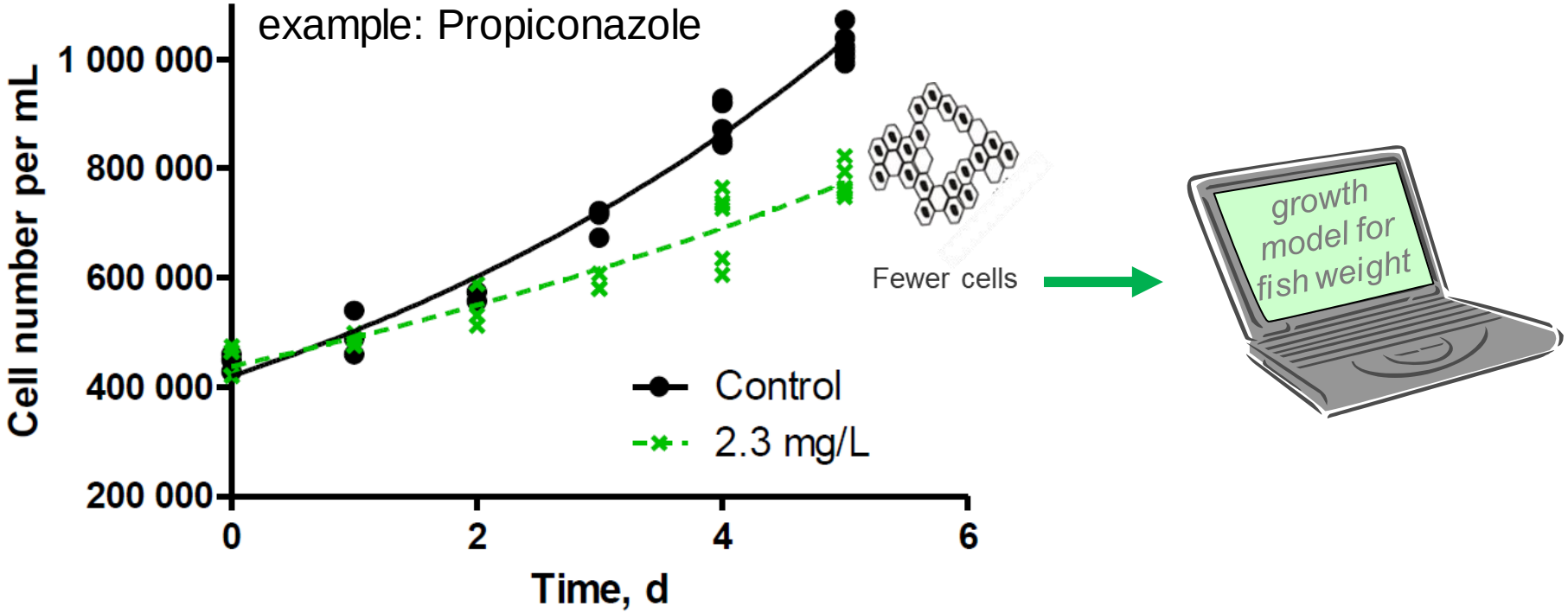
(Embry et al. Aquatic Toxicology 97 (2010) 79–87)

Alternatives: chronic toxicity — growth

Hypothesis: Less growth means fewer cells

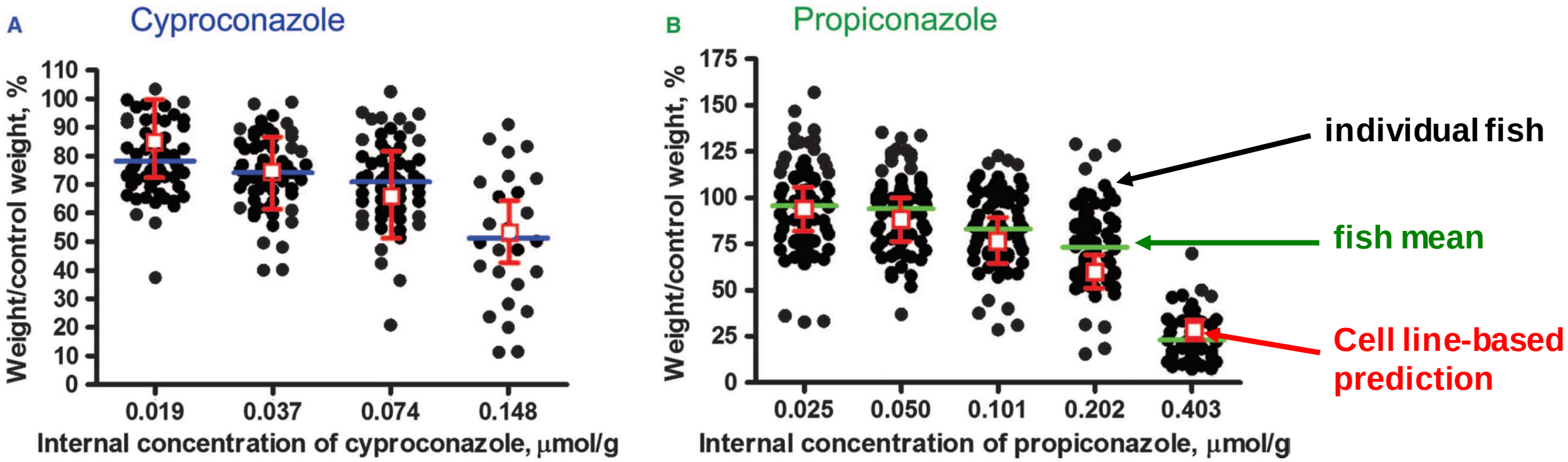


RTgill-W1 cell line as model of richly perfused tissue



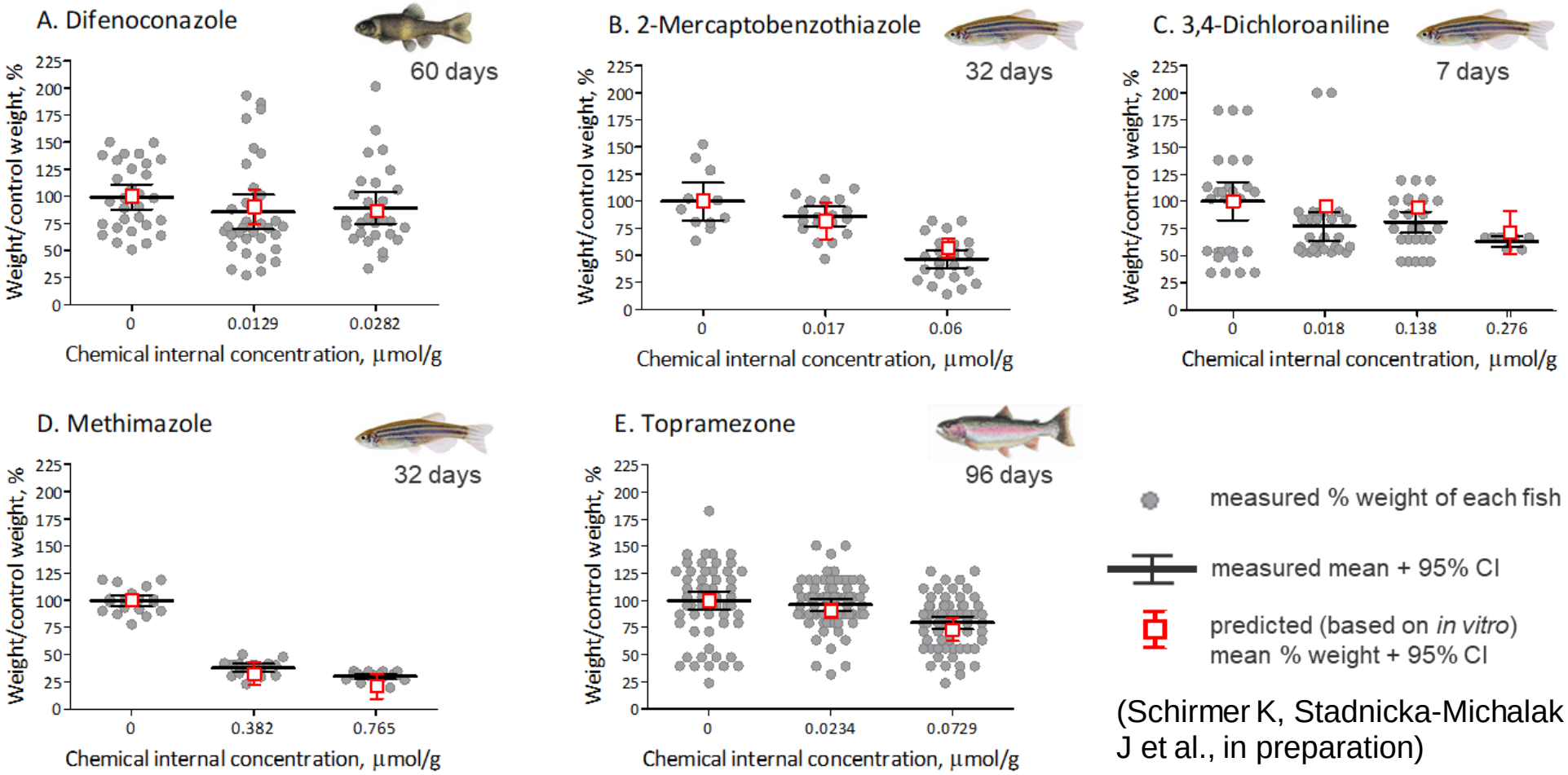
Alternatives: chronic toxicity — growth

Hypothesis: Less growth means fewer cells (proof-of-concept)



(Stadnicka-Michalak J et al., 2015, Science Advances, Vol. 1, no. 7, e1500302.)

Alternatives: chronic toxicity — growth



(Schirmer K, Stadnicka-Michalak J et al., in preparation)

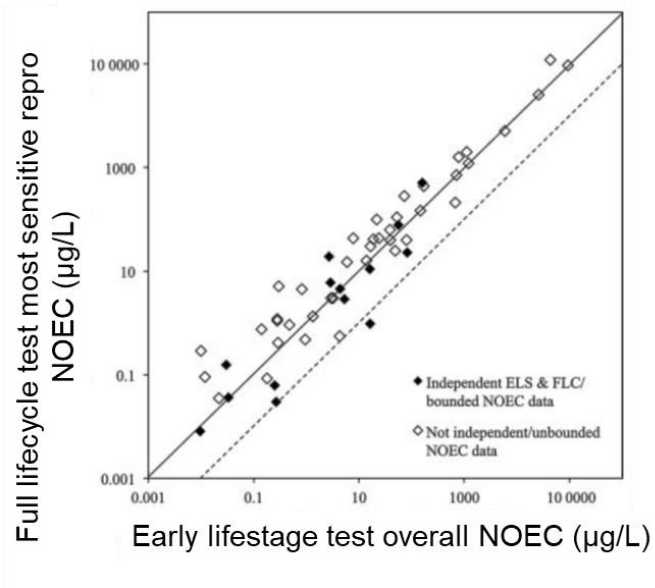
→ Ongoing study to shed light on molecular mechanisms: "Resolving molecular mechanisms underlying reduced fish cell population growth upon chemical exposure" lead by Schirmer K and Zupanic A (NIB, Slovenia).

Reproductive (incl. endocrine) toxicity — several OECD TG → Alternatives?

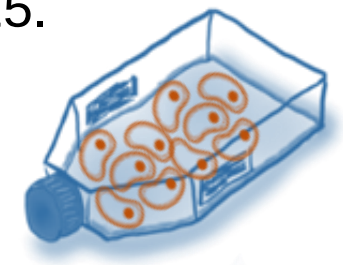
Refinements of existing tests/models:

- Fish early life stage toxicity appears predictive of full life cycle tests.

Wheeler et al., 2014, Environ Toxicol Chem 33(8):1874-8.



- Could it be expanded to include reproductive toxicity measures?
- Opportunity to expand the gill cell line (see previous two slides) or other cell line assay to include markers of reproductive toxicity? → see also slides 15.



Reproductive (incl. endocrine) toxicity — several OECD TG → Alternatives?

Refinements of existing tests/models:

- Same model – additional capabilities, e.g. for zebrafish embryo:
  - ❖ OECD TG250: EASZY assay - Detection of Endocrine Active Substances, acting through estrogen receptors, using transgenic tg(cyp19a1b:GFP) Zebrafish embrYos

- Existing assay procedure but additional analysis:
  - ❖ Thyroid disruption in fish embryo test → https://ergo-project.eu/
  - ❖ Molecular mechanism analyses (e.g. qPCR of selected genes or transcriptome)

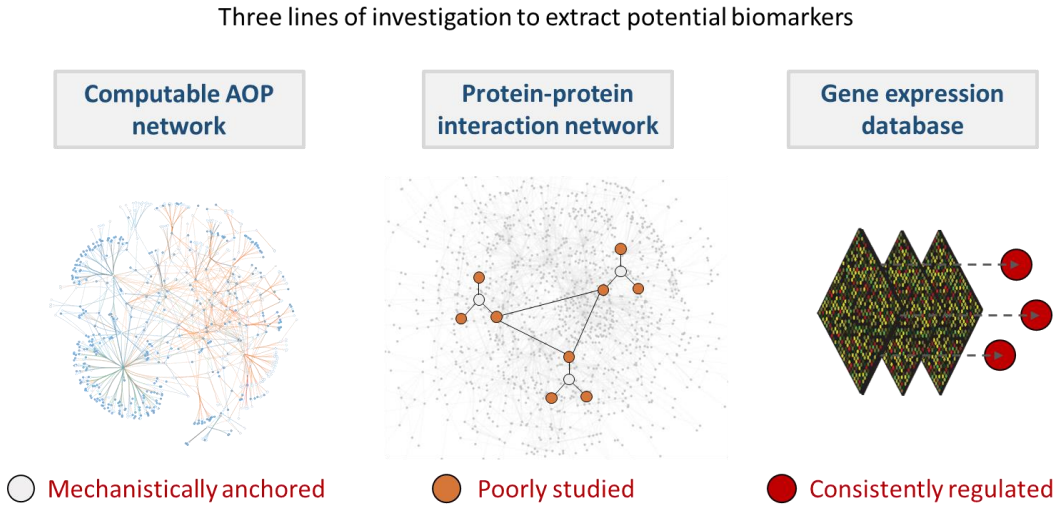
(see also Burden et al., 2021, 18(2): 442-458).

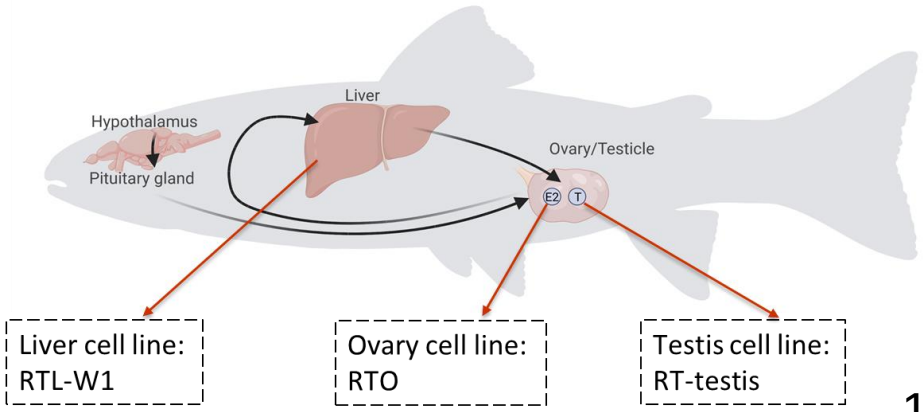
Reproductive (incl. endocrine) toxicity — what about *in vitro* models?

Adverse outcome pathway-based biomarkers:

- Knowledge derived from conserved pathway: e.g. mammalian cell lines → ToxCast

- Biomarkers measured in fish cell lines
  - ❖ Aim of CRACK IT project "SAFE — Innovative Safety Assessment of Fish adverse Effects"





Vision: The fish invitrome for risk assessment

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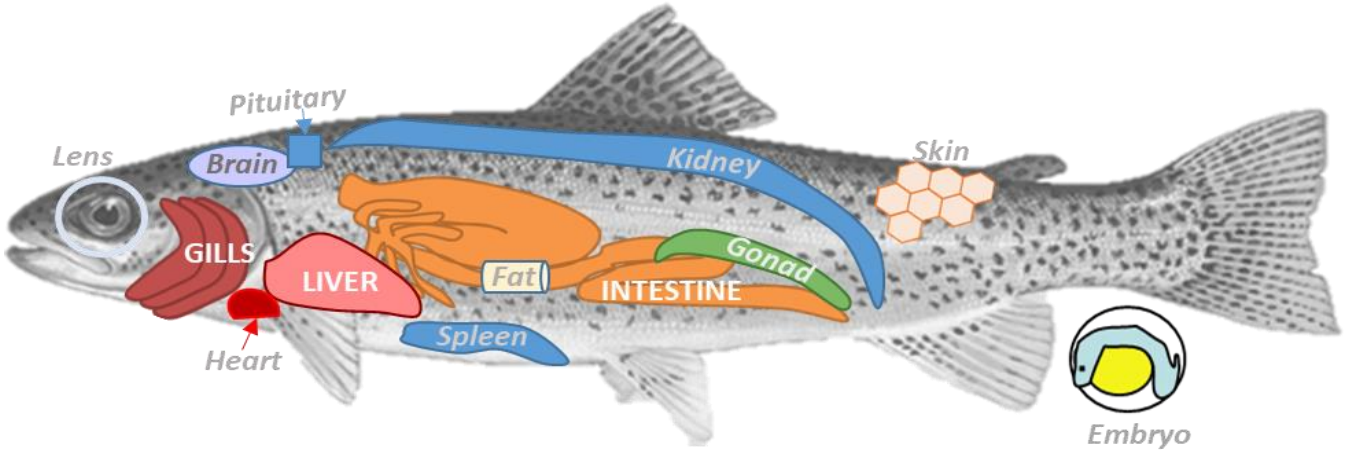
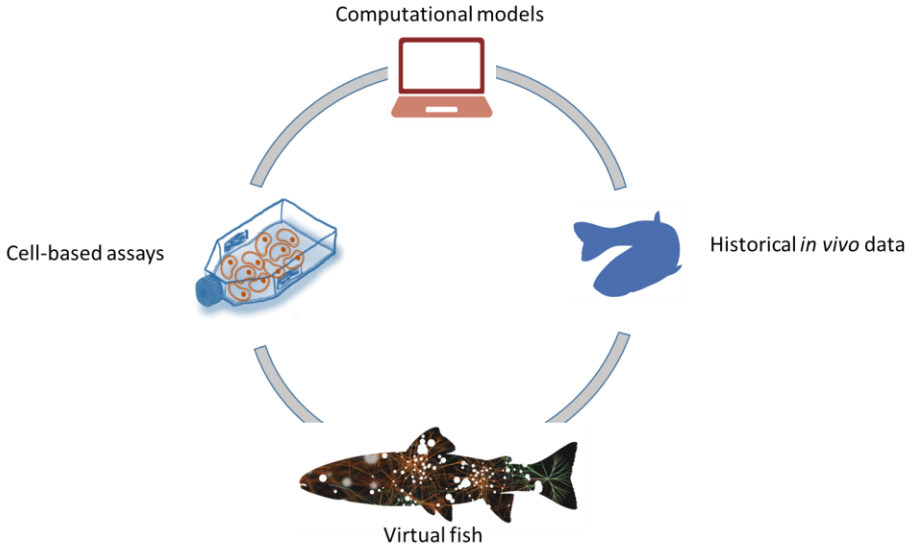
A coherent framework based on fish cell lines



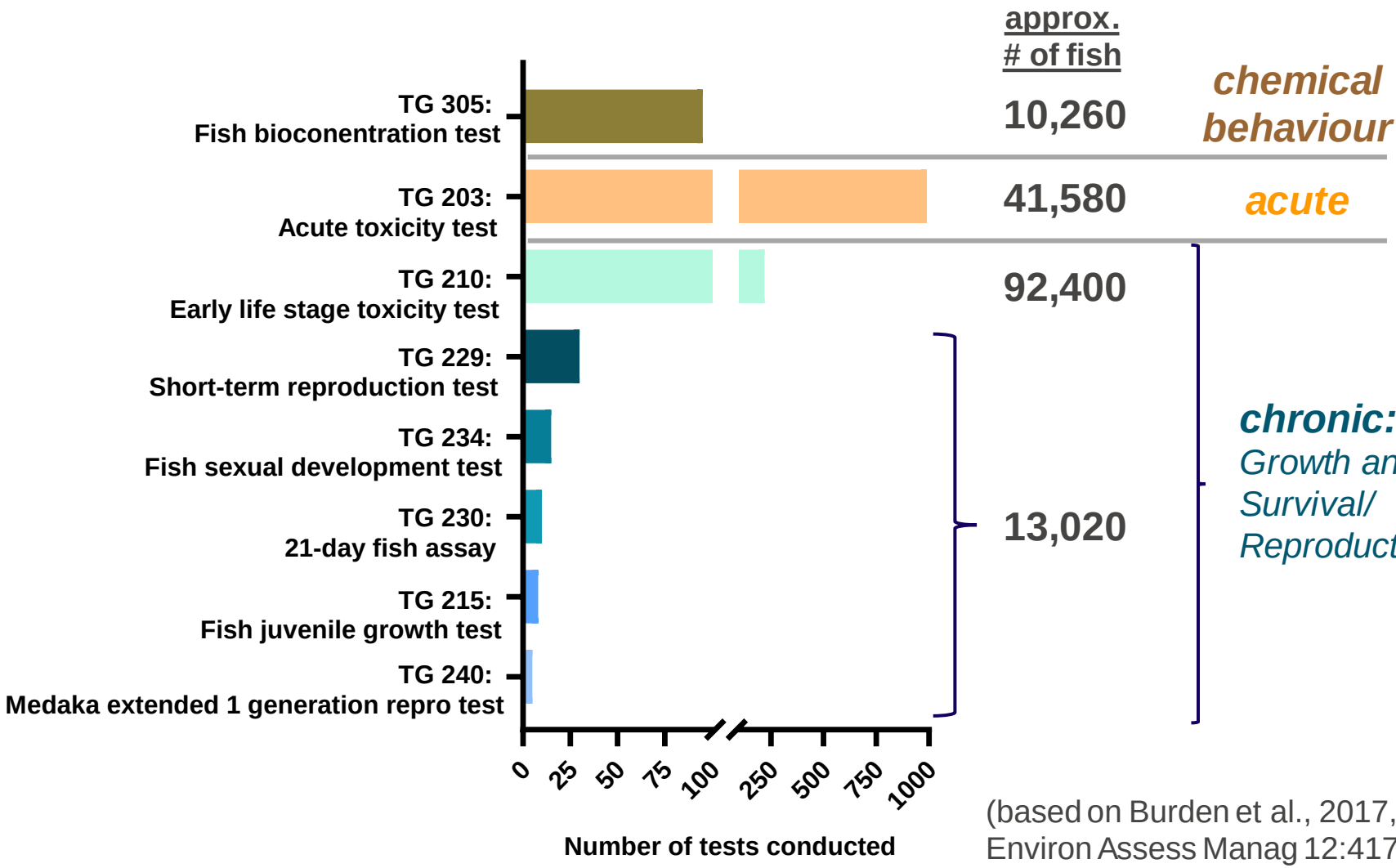


CRACK IT

Survive, grow and reproduce …including ability to detoxify

(i.e. not bioaccumulate)

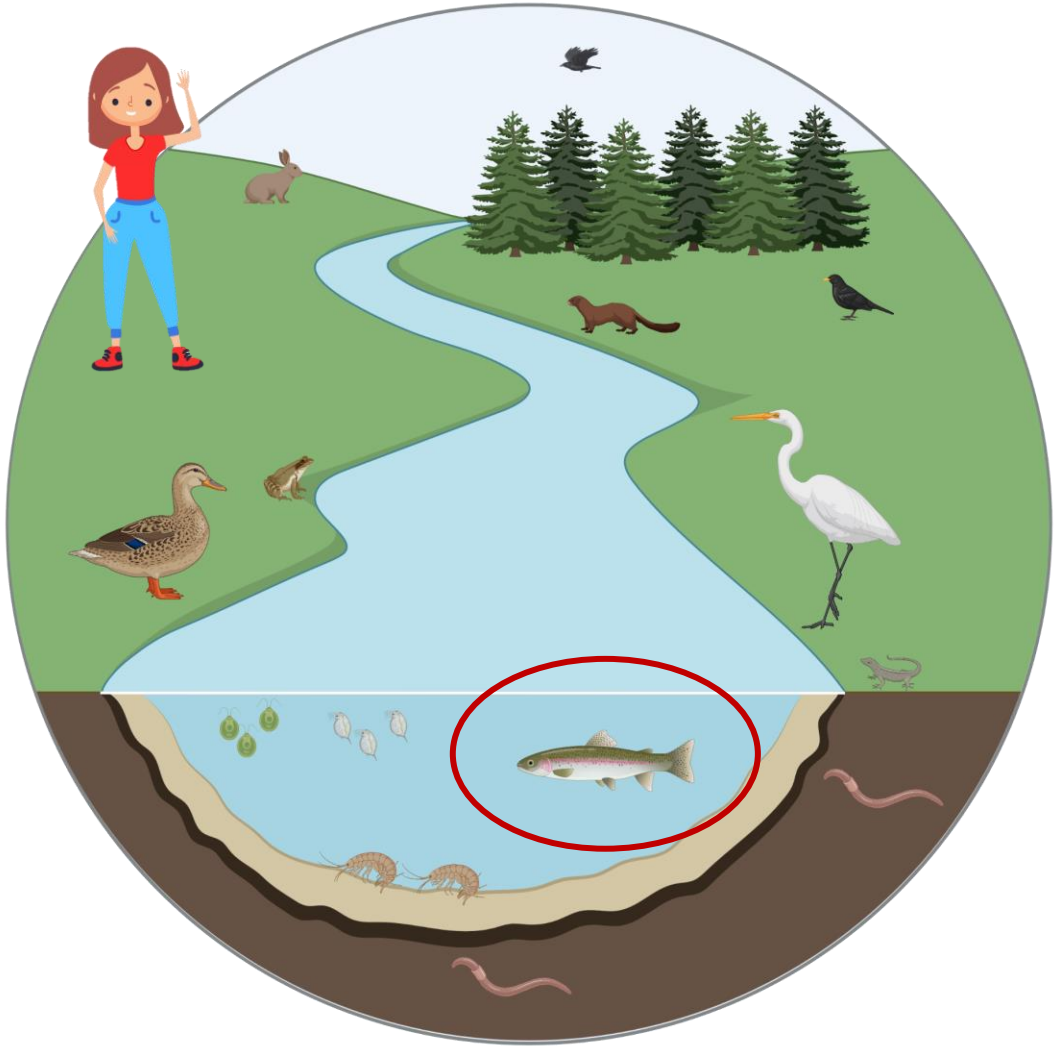
Number of fish tests conducted in 15 CROs (2014-2017)



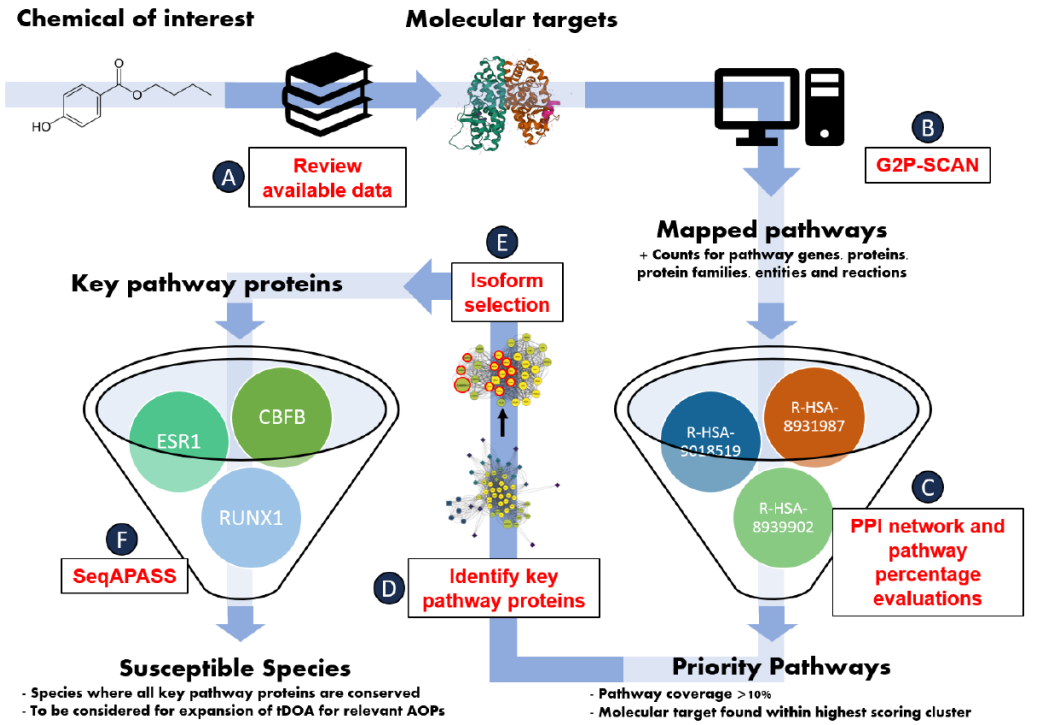
alternatives ready?

(based on Burden et al., 2017, Integr Environ Assess Manag 12:417-421)

One environment – one health



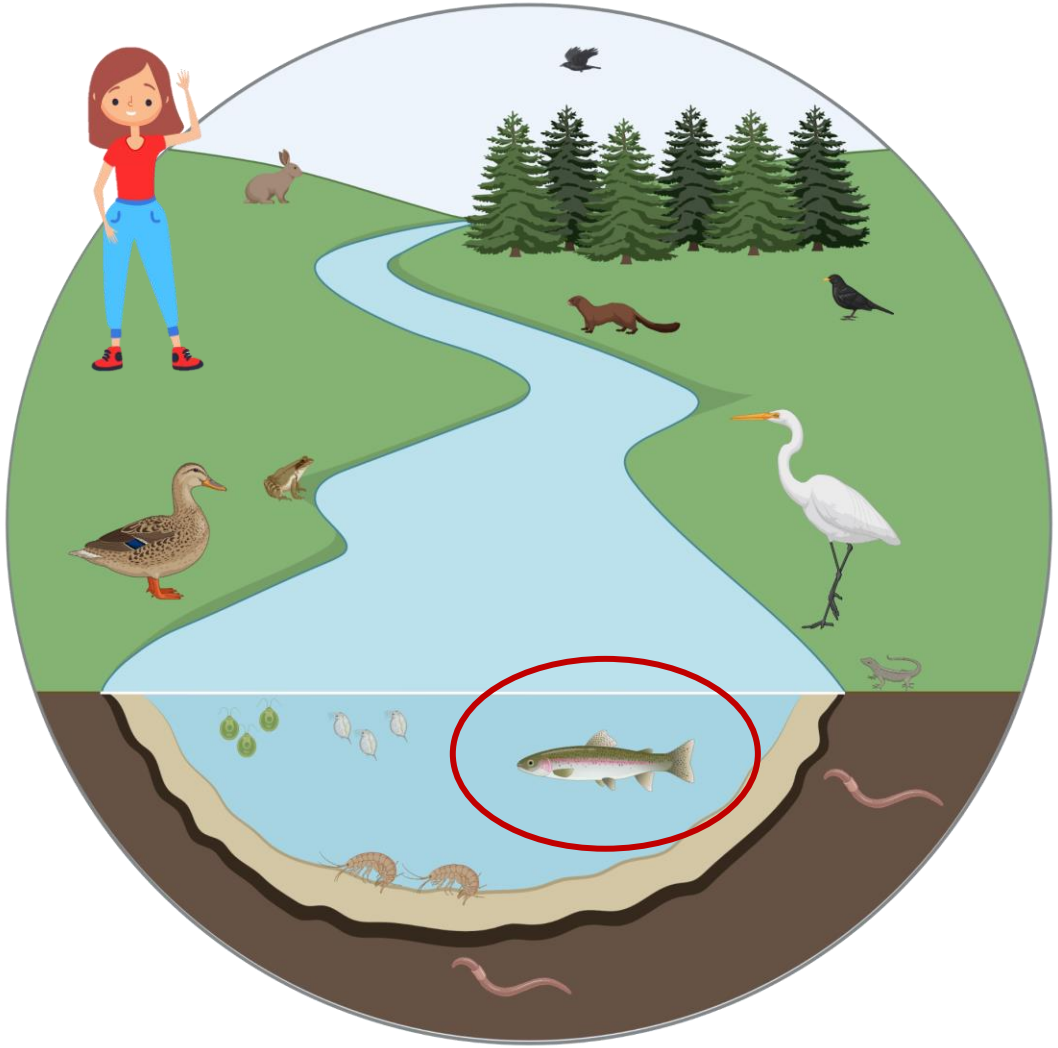
Consortium led by Carlie Lalone and Geoff Hodges:
Combination of Computational New Approach Methodologies for Enhancing Evidence of Biological Pathway Conservation Across Species



https://epa.figshare.com/articles/poster/Combination_of_Computational_New_Approach_Methodologies_for_Enhancing_Evidence_of_Biological_Pathway_Conservation_Across_Species/22589047

- Diagram showing the approach of combining the use of SeqAPASS and G2P-SCAN tools to support cross-species predictions of chemical susceptibility though inferences of pathway conservation.
- Abbreviations: taxonomic domain of applicability (tDOA); adverse outcome pathways (AOPs); molecular complex detection (MCODE); protein-protein interaction (PPI).

One environment – one health



Arts by Fabian Balk

- Regulate food web dynamics
- Provide ecosystem services
- Comprise important source of food

Additional material

Alternatives — Bioconcentration

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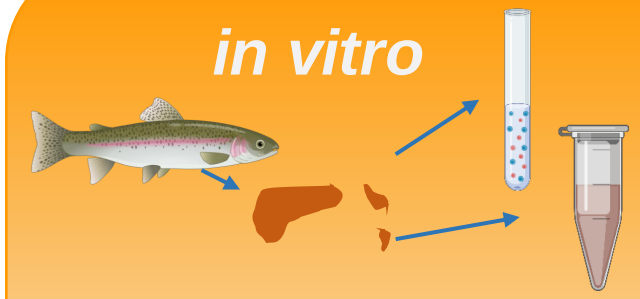
Computational (in silico)

- Quantitative Structure Activity Relationships (QSAR)
- One- and multi-compartment physiology-based models

in vitro



RTL-W1

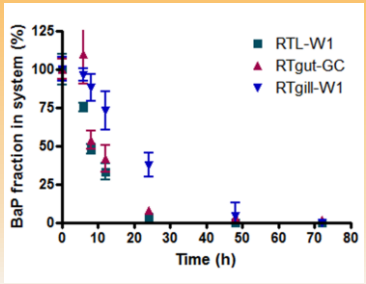
RTgill-W1

RTgutGC

Gill

Gut

- OECD TG319A: Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)
- OECD TG319B: Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)
- Rainbow trout cell lines as models:



(Stadnicka-Michalak et al., 2018, Environmental Science & Techology 52(5), 3091-3100.)

(see also Balk at el., 2023, Environment International Volume 174, April 2023, 107798)

Integrated Approach to Testing and Assessment

- Bioconcentration Assessment Tool (BAT)
- Weight of evidence (WoE) approach for both aquatic and terrestrial environments to support various decision contexts

Alternatives: acute toxicity — survival

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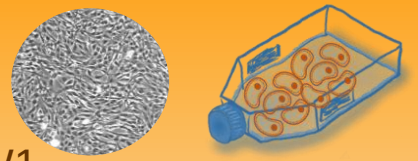
Computational (in silico)

- Quantitative Structure Activity Relationships (QSAR)
- Ecological Threshold of Toxicological Concern (ecoTTC)
- Bayesian network, Machine learning algorithms

in vitro



RTgill-W1

- OECD TG249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay
- As well ISO21115: Water quality — Determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1)

in vivo (non-protected stage)

Fish embryo

- OECD TG236: Fish Embryo Acute Toxicity (FET) Test

Integrated Approach to Testing and Assessment

- Tiered testing based on above
- Including information from algae and daphnia