For our Environment

Umwelt Bundesamt

NAMs and fish long-term toxicity testing

a Regulator's Perspective

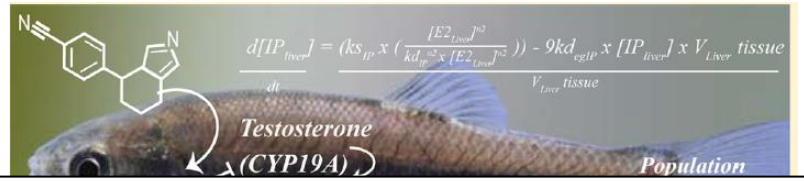
Gerd Maack
Department of Pharmaceuticals

Workshop on the Commission roadmap towards phasing out animal testing for chemical safety assessments,
11/12 December 2023

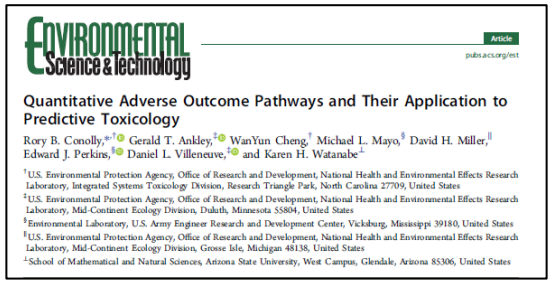
OECD - Chronic aquatic vertebrate tests - an overview

Fish, Early-life Stage Toxicity Test	OECD 210
Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	OECD 212
Fish, Juvenile Growth Test	OECD 215
Fish Short Term Reproduction Assay	OECD 229
21 Day Fish Screening Assay	OECD 230
Fish Sexual Development Test	**OECD 234**
Medaka Extended One Generation Reproduction Test (MEOGRT)	OECD 240
Zebrafish Extended One Generation Reproduction Test (ZEOGRT)	*in preparation*
Bioaccumulation in Fish: Aqueous and Dietary Exposure	OECD 305
Amphibian Metamorphosis Assay (AMA)	OECD 231
The Larval Amphibian Growth and Development Assay (LAGDA)	OECD 241

qAOP - Using In Vitro Aromatase Inhibition Data to Predict Reproductive Outcomes in Fish In Vivo

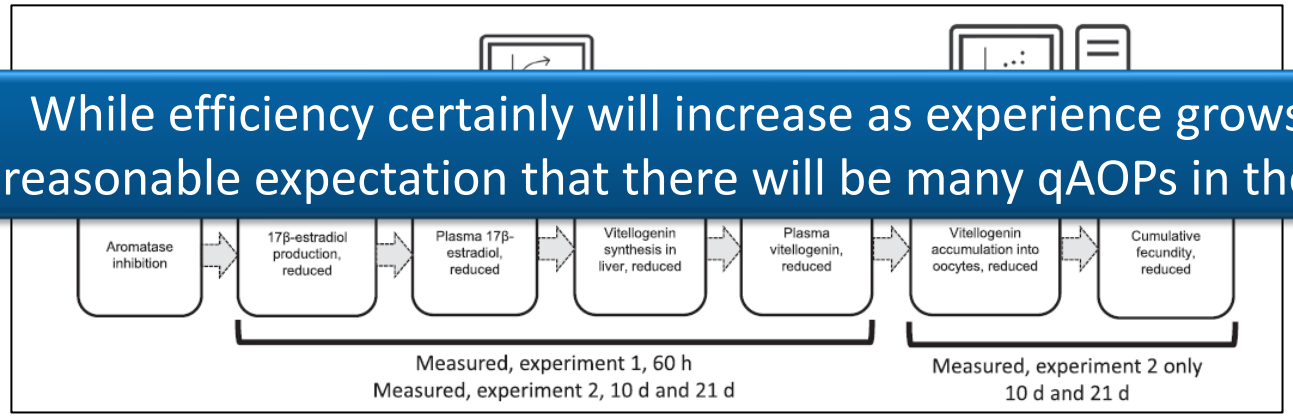


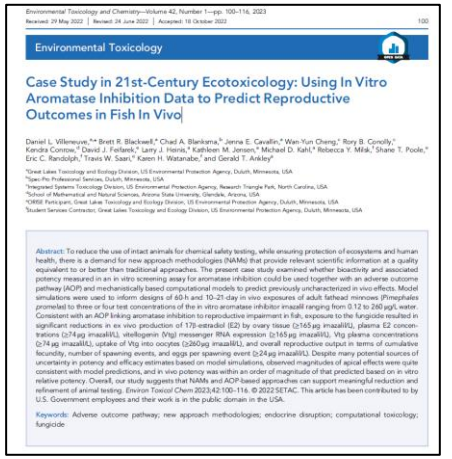
- Initial toxicological work underlying the aromatase inhibition qAOP described herein was published 15 years ago.
- A large amount of additional research critical to the final qAOP.
- Nearly 400 fish were used in the final experiments.



Conolly, R. B., et al. (2017). Environ Sci Technol 51(8): 4661-4672.

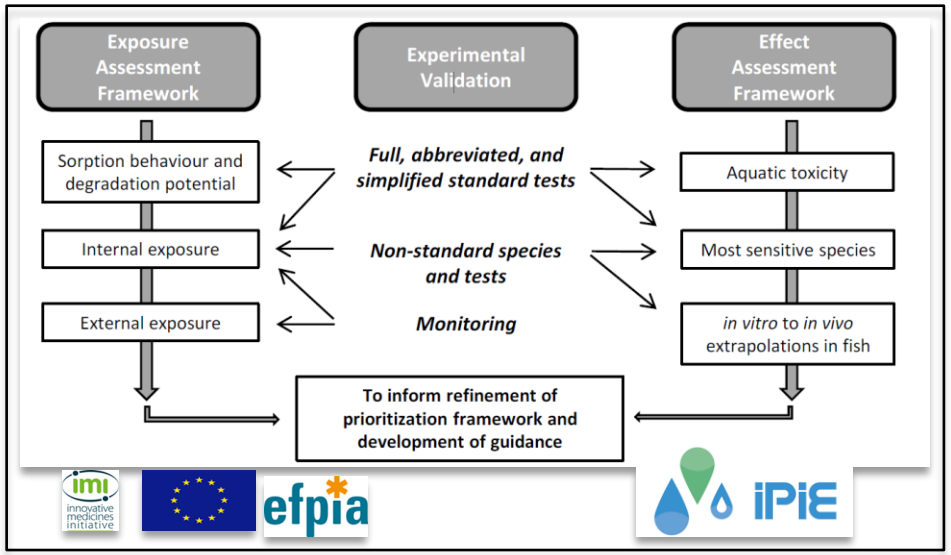
While efficiency certainly will increase as experience grows, it is not a reasonable expectation that there will be many qAOPs in the near term.

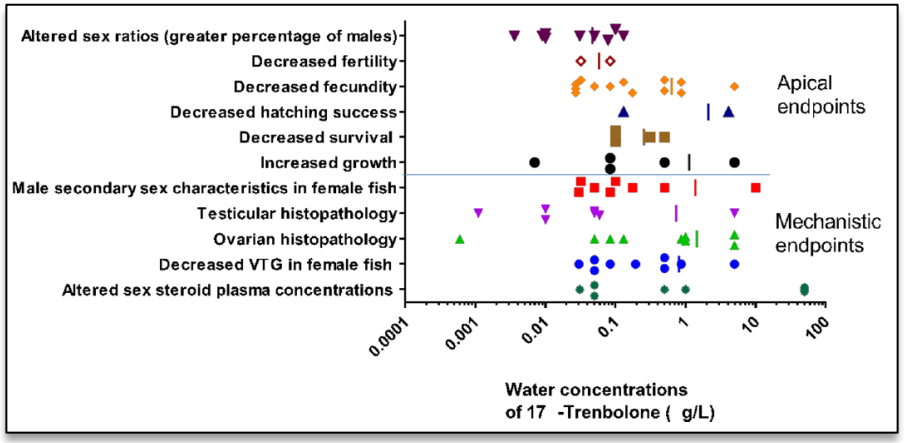




Villeneuve, D. L., et al. (2023). Environmental Toxicology and Chemistry 42(1): 100-116.

Intelligent Testing - Weight of Evidence / Line of Evidence



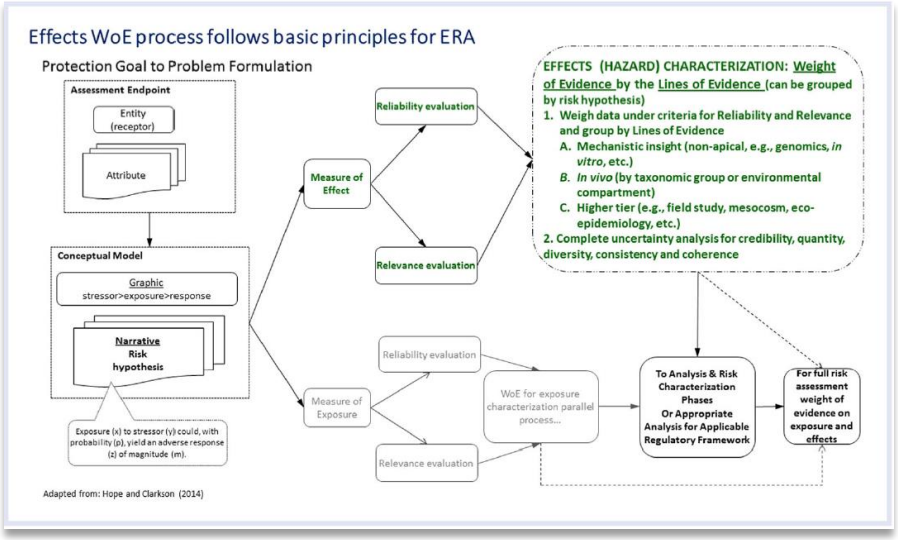


Ankley et al. Environmental Toxicology and Chemistry 37; 2064–2078 (2018)

Recommendations towards evidence-based ecotoxicology

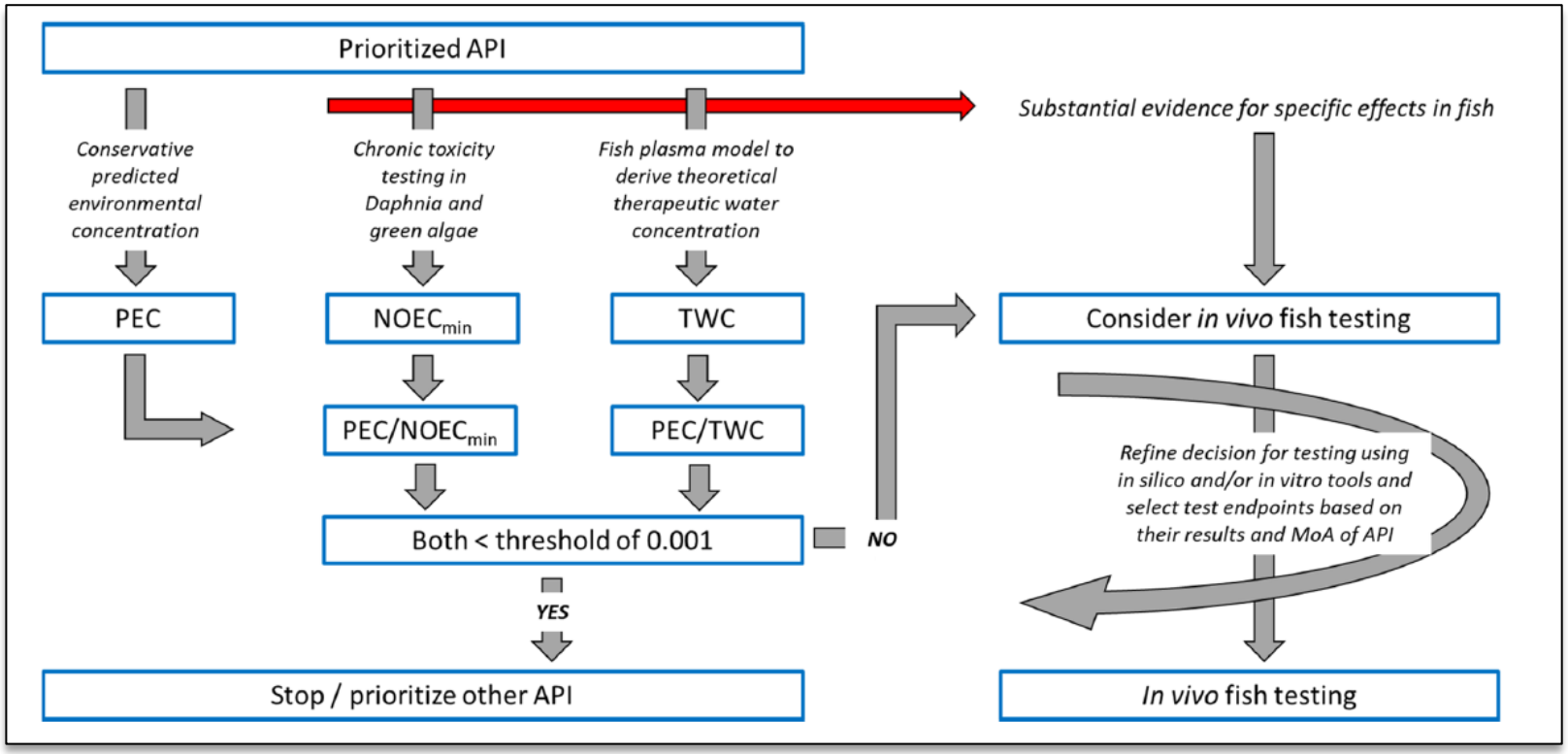
- Consider all applicable studies
- Report all findings of experimental studies
- Make ecotoxicity studies publicly accessible
- Implement reporting guidelines for publication of ecotoxicity studies
- Apply transparent and consistent evaluation criteria to all ecotoxicity studies
- Improve the regulatory guidance for weight-of-evidence evaluations
- Increase collaboration among all stakeholders
- Declare interests
- Improve training and knowledge transfer between all stakeholders

Martin et al. Environment International 128; 210-217 (2019)



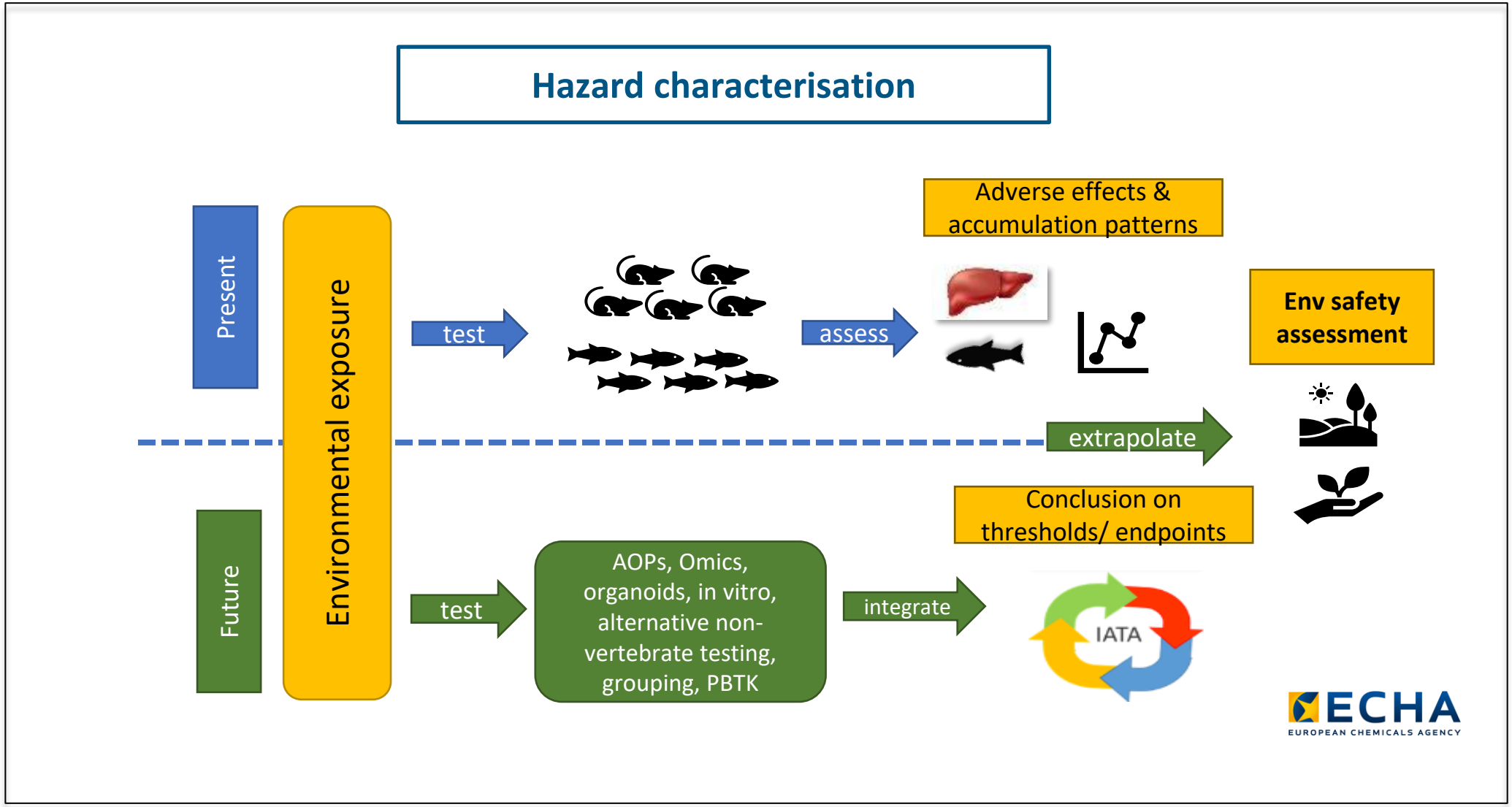
Hall et al. Integrated Environmental Assessment and Management 13; 573–579 (2017)

Minimizing Experimental Testing on Fish for Legacy Pharmaceuticals



34 APIs out of 96 in the verification data set could be excluded from in vivo fish testing

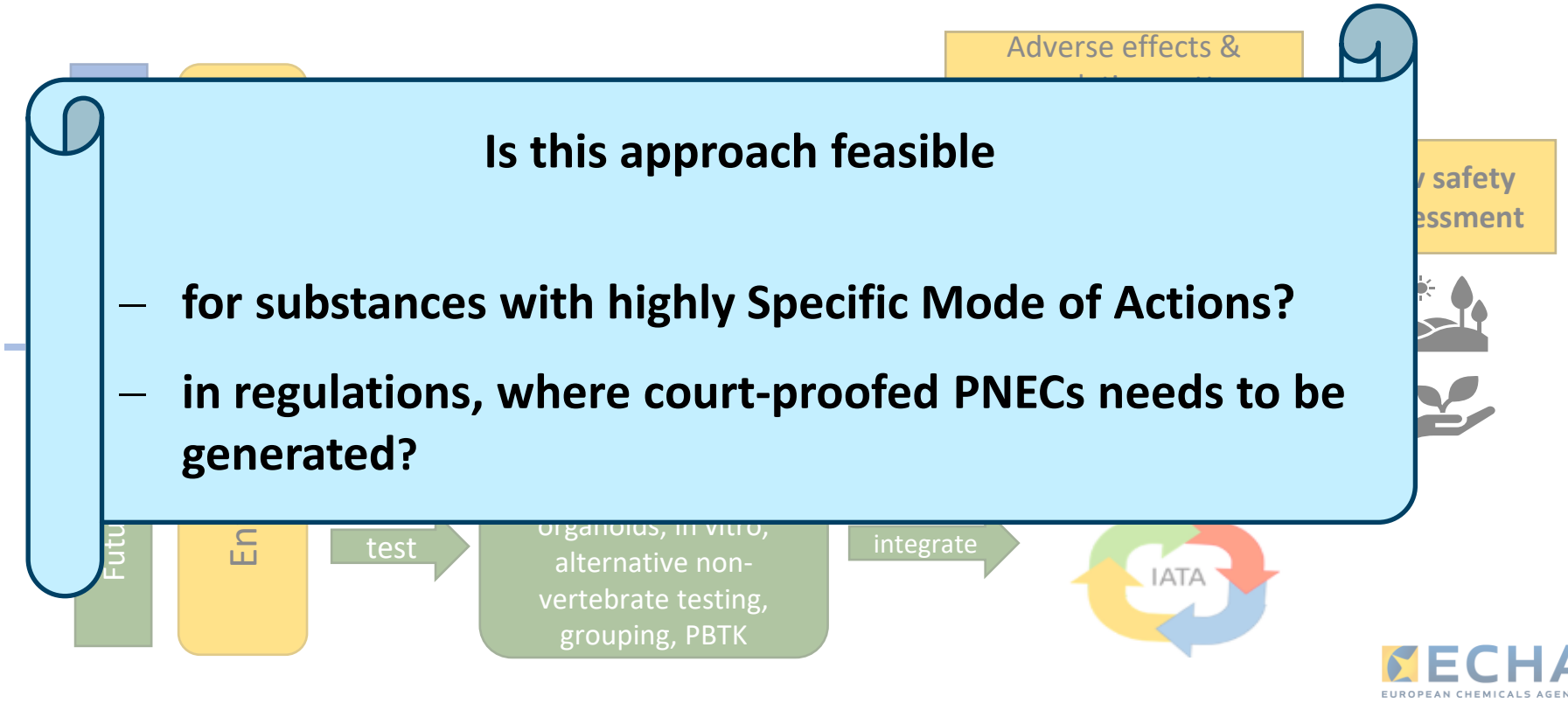
Coors et al. Envrion. Sci. Technol. 2023, 57, 1721-1730



Doris Hirmann et al.: Strengthened role of New Approach Methods (NAMs) in bioaccumulation assessment under REACH

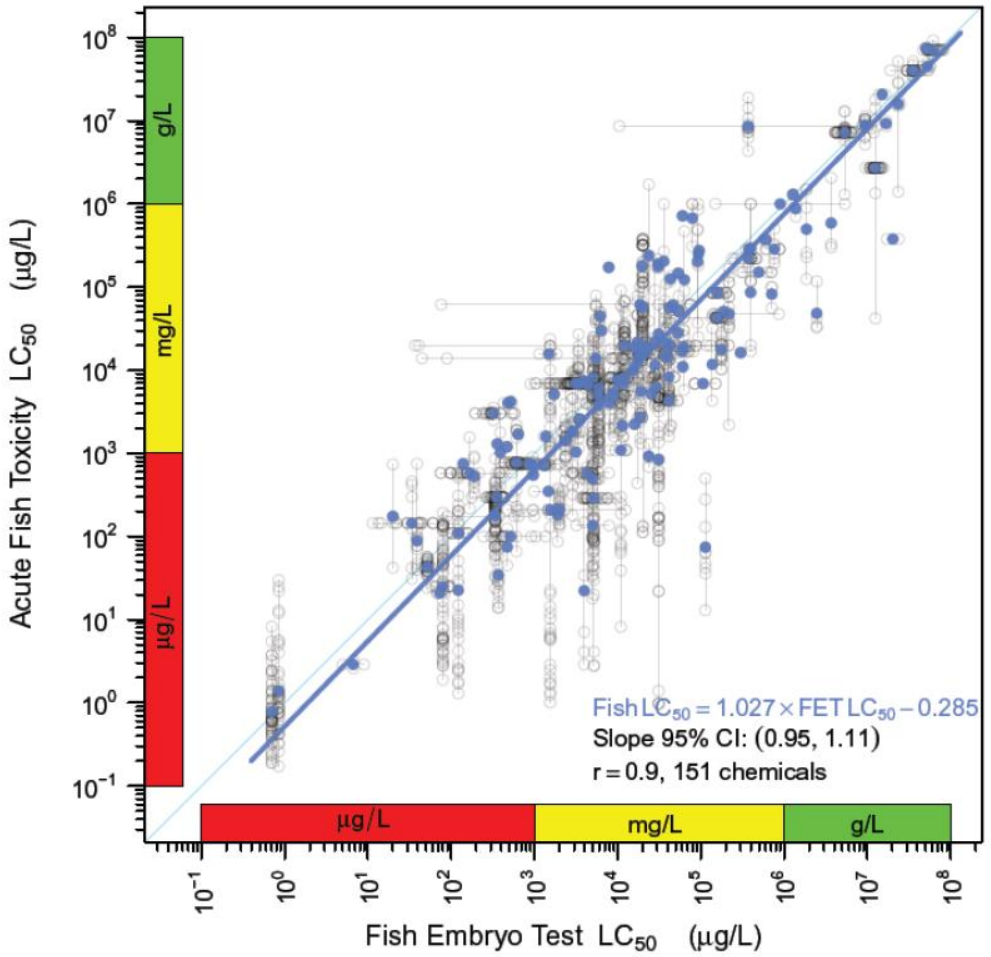
Presentation at SETAC Europe 33rd Annual Meeting 04. May 2023

Quantitative Risk Assessment



An Update to the Fish Embryo Toxicity-Acute Fish Toxicity Relationship and Prospects for Support of the Use of the FET as an Animal Alternative

Scott E. Belanger, Jane M. Rawlings and Gregory J. Carr (2016)



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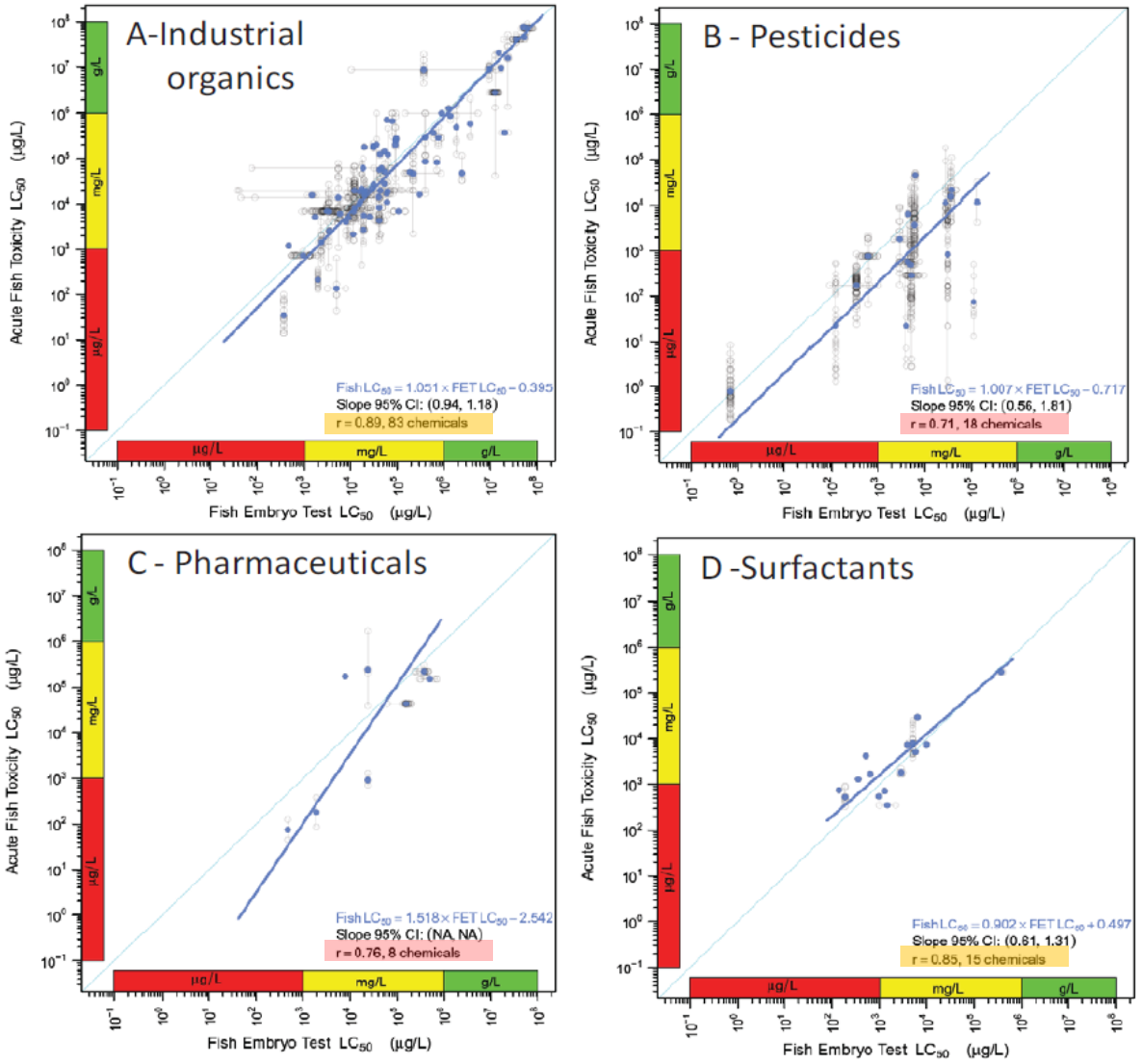
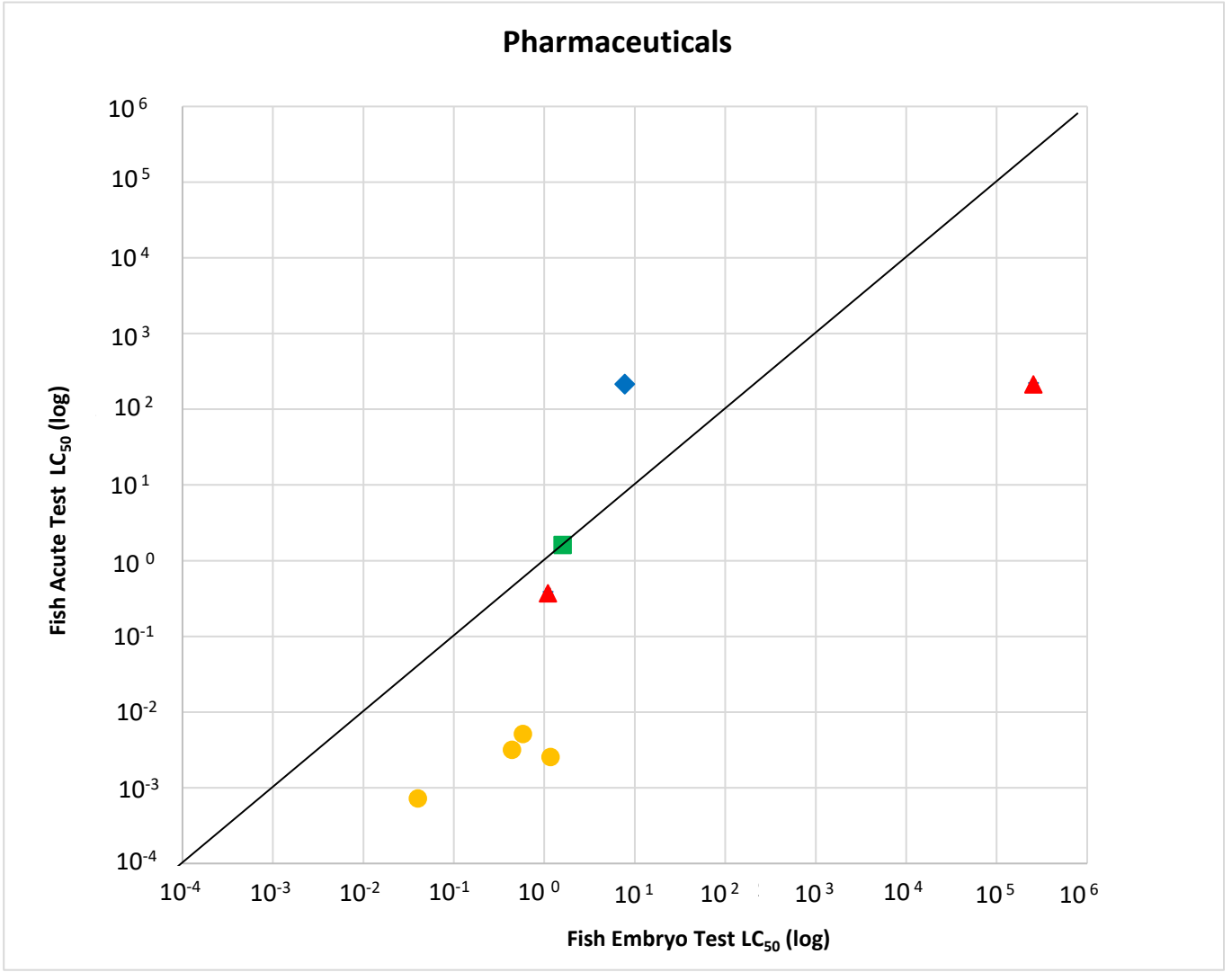


Analysis of the relevance and adequateness of using Fish Embryo Acute Toxicity (FET) Test Guidance (OECD 236) to fulfil the information requirements and addressing concerns under REACH

(Scholz et al. 2016)



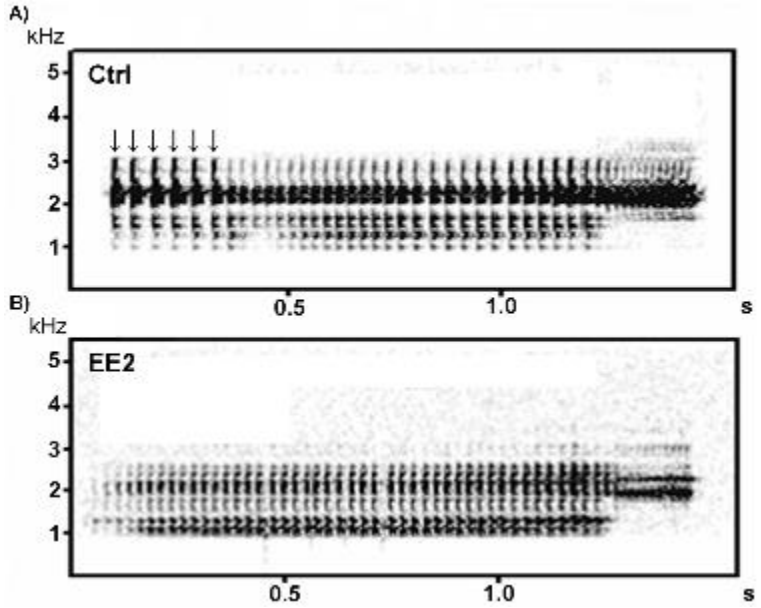
Report ECHA-UFZ contract ECHA/2014/341

Changes in Mating Behaviour

0.3 ng/L EE2 can disrupt amphibian mating behavior

Brian Gratwicke - Flickr: Xenopus laevis, CC BY 2.0, https://commons.wikimedia.org/w/index.php?curid=23752908

Xenopus laevis



Hoffmann, Kloas (2012) Estrogens can disrupt amphibian mating behavior *PLOS ONE, 7(2) e32097*

Matthew Bowden [Wikipedie]
www.digitallyrefreshing.com -
http://www.sxc.hu/photo/103942

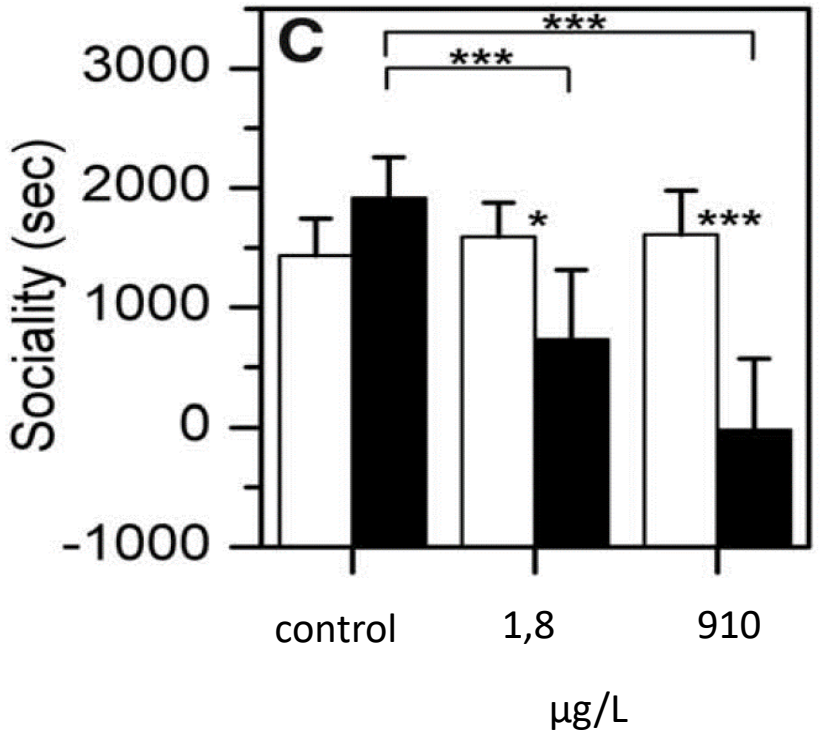
Umwelt Bundesamt

IGB Leibniz-Institute of Freshwater Ecology and Inland Fisheries

Psychiatric Drug Alter Behavior of Fish from Natural Populations

Perca fluviatilis

Time spent close to a group of conspecifics



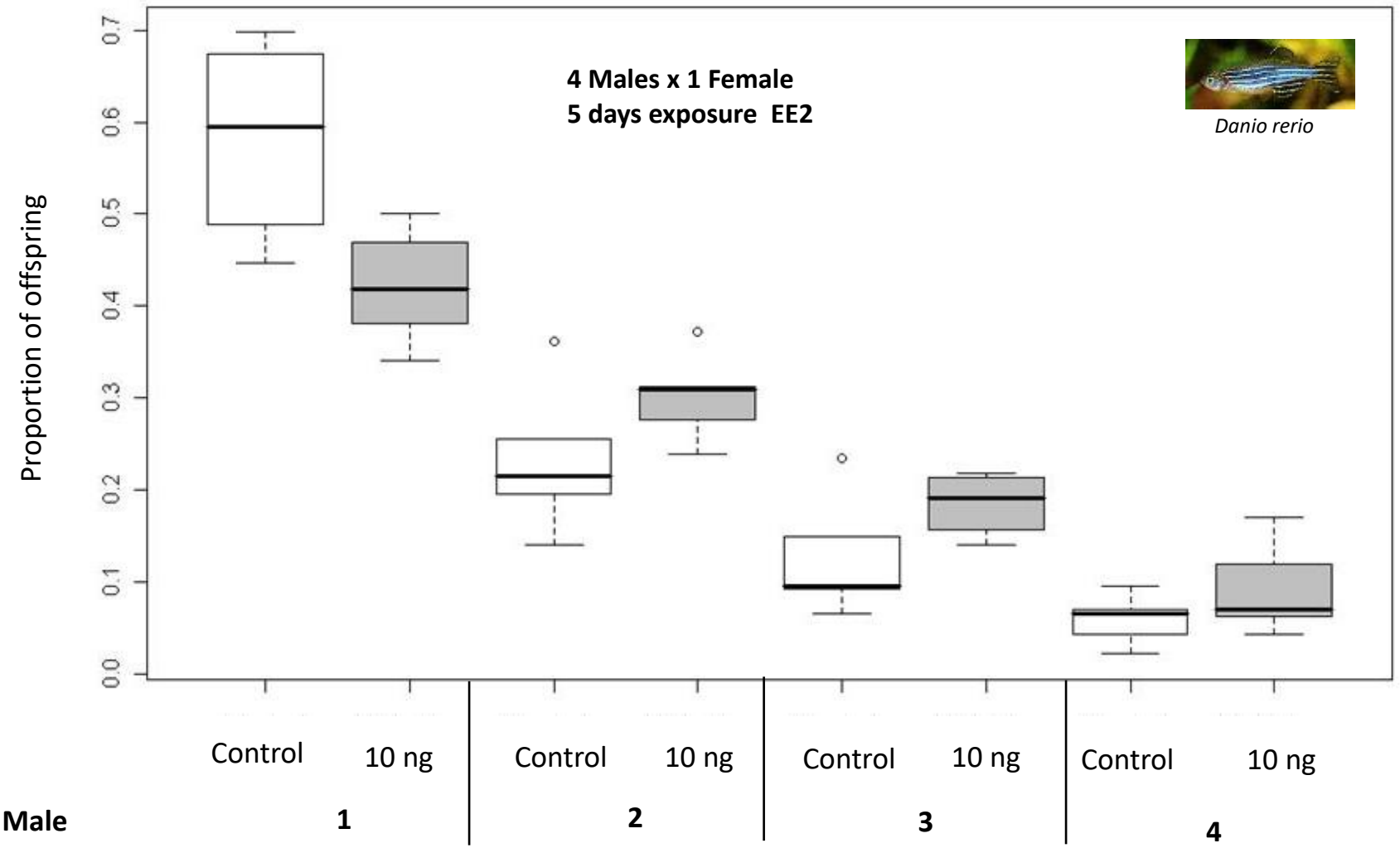
Pre-treatment

Post-treatment

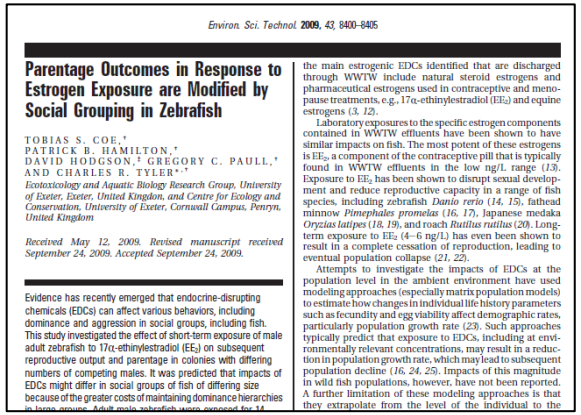
Oxazepam

Brodin et al. 2013: Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations, *SCIENCE, 339 (6121) p 841-845*

Parentage Outcomes in Response to Estrogen Exposure



Danio rerio



Environ. Sci. Technol. 2009, 43, 8400–8405

Parentage Outcomes in Response to Estrogen Exposure are Modified by Social Grouping in Zebrafish

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Ecotoxicology and Aquatic Biology Research Group, University of Exeter, Exeter, United Kingdom, and Centre for Ecology and Conservation, University of Exeter, Cornwall Campus, Penryn, United Kingdom

Received May 12, 2009. Revised manuscript received September 24, 2009. Accepted September 24, 2009.

Evidence has recently emerged that endocrine-disrupting chemicals (EDCs) can affect various behaviors, including dominance and aggression in social groups, including fish. This study investigated the effect of short-term exposure of male adult zebrafish to 17α-ethinylestradiol (EE_2) on subsequent reproductive output and parentage in colonies with differing numbers of competing males. It was predicted that impacts of EDCs might differ in social groups of fish of differing size because of the greater costs of maintaining dominance hierarchies

the main estrogenic EDCs identified that are discharged through WWTW include natural steroid estrogens and pharmaceutical estrogens used in contraceptive and menopause treatments, e.g., 17α-ethinylestradiol (EE_2) and equine estrogens (*3*, *12*).

Laboratory exposures to the specific estrogen components contained in WWTW effluents have been shown to have similar impacts on fish. The most potent of these estrogens is EE_2, a component of the contraceptive pill that is typically found in WWTW effluents in the low ng/L range (*13*). Exposure to EE_2 has been shown to disrupt sexual development and reduce reproductive capacity in a range of fish species, including zebrafish *Danio rerio* (*14*, *15*), fathead minnow *Pimephales promelas* (*16*, *17*), Japanese medaka *Oryzias latipes* (*18*, *19*), and roach *Rutilus rutilus* (*20*). Long-term exposure to EE_2 (4–6 ng/L) has even been shown to result in a complete cessation of reproduction, leading to eventual population collapse (*21*, *22*).

Attempts to investigate the impacts of EDCs at the population level in the ambient environment have used modeling approaches (especially matrix population models) to estimate how changes in individual life-history parameters such as fecundity and egg viability affect demographic rates, particularly population growth rate (*23*). Such approaches typically predict that exposure to EDCs, including at environmentally relevant concentrations, may result in a reduction in population growth rate, which may lead to subsequent population decline (*16*, *24*, *25*). Impacts of this magnitude in wild fish populations, however, have not been reported. A further limitation of these modeling approaches is that they extrapolate from the level of the individual to the

Coe et al. 2008, EST 43, 8400 - 8405

Regulatory Perspective

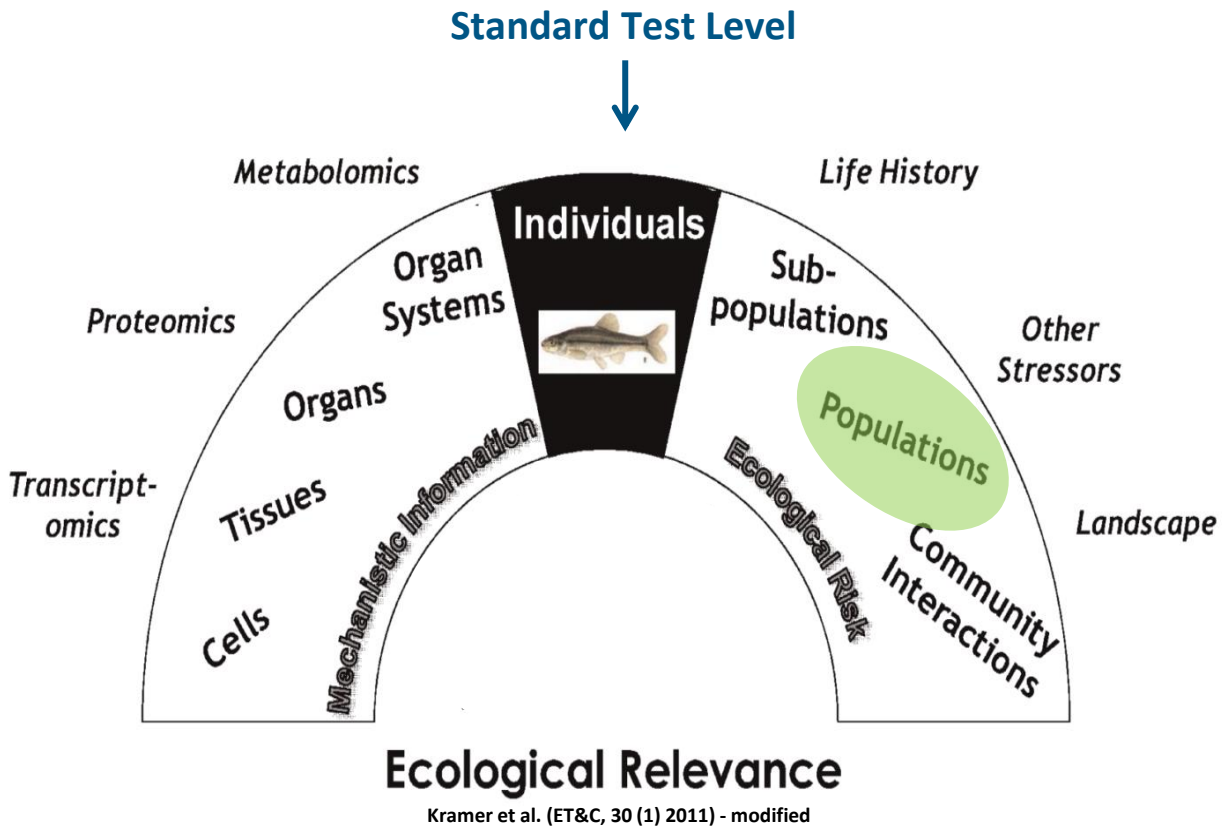
Do we have to distinguish Specific Mode of Actions?

- Extrapolation from General toxicity to Specific Mode of Action?
- Substances with multiple Mode of Actions?
- Substances with Specific but Unknown Mode of Action?

On which level should NAMs be used?

- Replacing study results for a quantitative Risk Assessment?
- Supporting or Guiding a potential Tailored Risk Assessment → **Intelligent Testing**?

Ecologic Perspective



Kramer et al. (ET&C, 30 (1) 2011) - modified

We are testing model organisms

- Algae (Lemna) for *all* Aquatic autotrophs
- Daphnia for *all* aquatic invertebrates
- 2 – 3 fish species (Cyprinids) for *all* aquatic vertebrates
- PNEC for all aquatic organisms ???

How far can we leave the population level and still be confident to protect the Structure and the Function of a population?

Animal Alternatives in the Environmental Risk Assessment - The Regulatory Perspective

Umwelt Bundes Amt
for our Environment

My very personal conclusion:

Do we really need every new product?

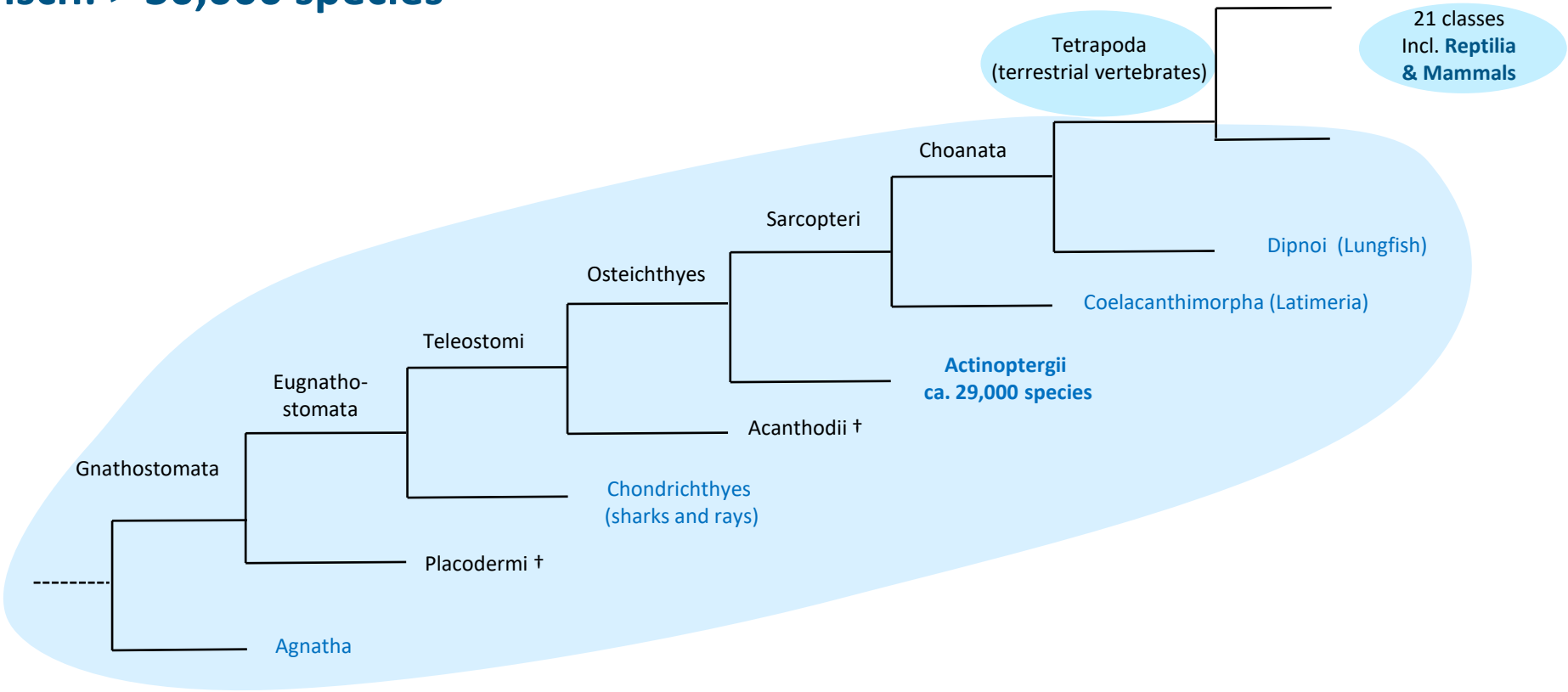
Thank you very much for your attention !

Gerd.Maack@uba.de

The above represents the opinion of the author and is not necessarily an official position of the German Environment Agency

Diversity of fish

- **Mammals: ca. 4,000 species**
- **Fisch: > 30,000 species**



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