

For our Environment

Umwelt 
Bundesamt

NAMs and fish long-term toxicity testing

a Regulator's Perspective

Gerd Maack
Department of Pharmaceuticals

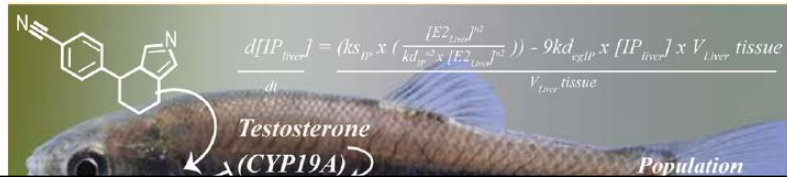
Workshop on the Commission roadmap towards phasing out animal testing for chemical safety assessments,
11/12 December 2023

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

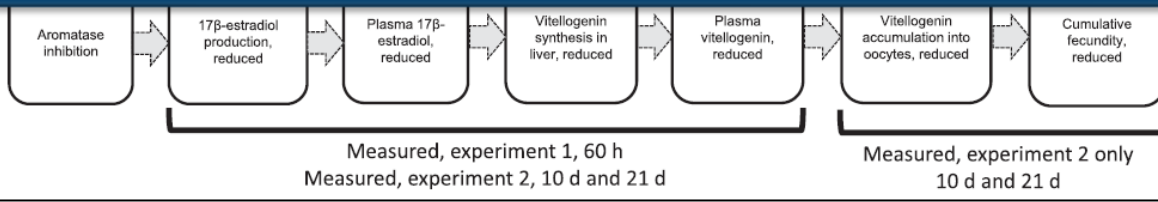
- Amphibian Metamorphosis Assay (AMA) OECD 231
- The Larval Amphibian Growth and Development Assay (LAGDA) OECD 241

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.

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



ENVIRONMENTAL Science & Technology Article
pubs.acs.org/est

Quantitative Adverse Outcome Pathways and Their Application to Predictive Toxicology

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

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Environmental Toxicology

Case Study in 21st-Century Ecotoxicology: Using In Vitro Aromatase Inhibition Data to Predict Reproductive Outcomes in Fish In Vivo

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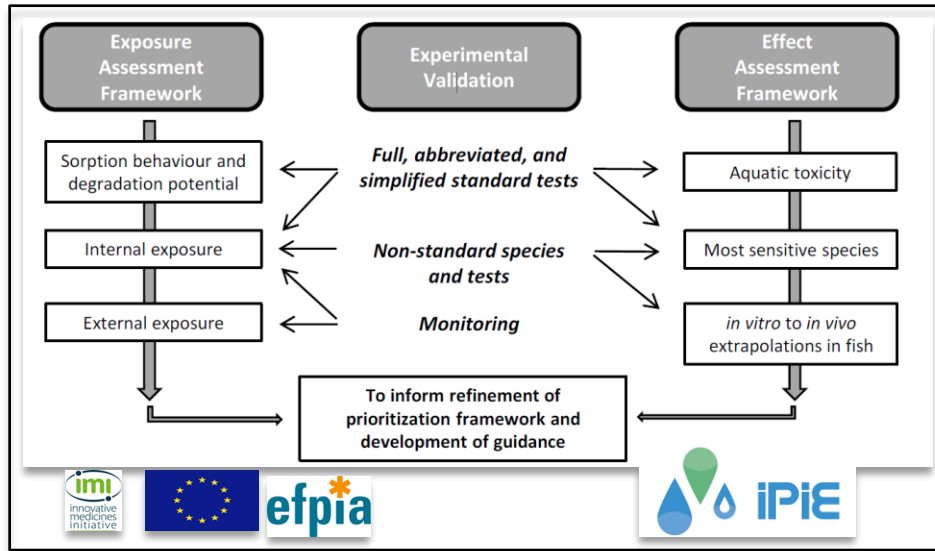
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Abstract: To reduce the use of intact animals for chemical safety testing, while ensuring protection of ecosystems and human health, there is a demand for new approach methodologies (NAMs) that provide relevant scientific information at a quality equivalent to or better than traditional approaches. The present case study examined whether bioactivity and associated potency measured in an in vitro screening assay for aromatase inhibition could be used together with an adverse outcome pathway (AOP) and mechanistically based computational models to predict previously uncharacterized in vivo effects. Model simulations were used to inform designs of 60-h and 10–21-day in vivo exposures of adult female mosquitofish (*Gambusia holbrooki*) to three or four test concentrations of the in vitro aromatase inhibitor inazafurane ranging from 0.12 to 240 µg/L water. Consistent with an AOP linking aromatase inhibition to reproductive impairment in fish, exposure to the fungicide resulted in significant reductions in in vivo production of 17β-estradiol (E2) by ovary tissue (2.16 µg inazafurane/L plasma; E2 concentrations 0.27 µg inazafurane/L), vitellogenin (Vtg) messenger RNA expression (0.165 µg inazafurane/L), Vtg plasma concentrations (0.74 µg inazafurane/L), uptake of Vtg into oocytes (0.26 µg inazafurane/L), and overall reproductive output in terms of cumulative fecundity, number of spawning events, and eggs per spawning event (0.24 µg inazafurane/L). Despite many potential sources of uncertainty in potency and efficacy estimates based on model simulations, observed magnitudes of apical effects were quite consistent with model predictions, and in vivo potency was within an order of magnitude of that predicted based on in vitro relative potency. Overall, our study suggests that NAMs and AOP-based approaches can support meaningful reduction and refinement of animal testing. *Environ Toxicol Chem* 2023, 42(1): 100–116. © 2022 SETAC. This article has been contributed to by U.S. Government employees and their work is in the public domain in the USA.

Keywords: Adverse outcome pathway; new approach methodologies; endocrine disruptor; computational toxicology; fungicide

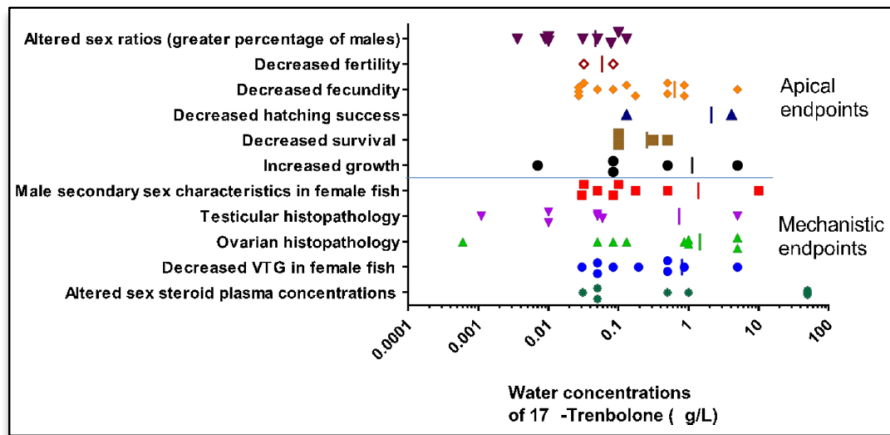
Villeneuve, D. L., et al. (2023). *Environmental Toxicology and Chemistry* 42(1): 100-116.

Intelligent Testing - Weight of Evidence / Line of Evidence

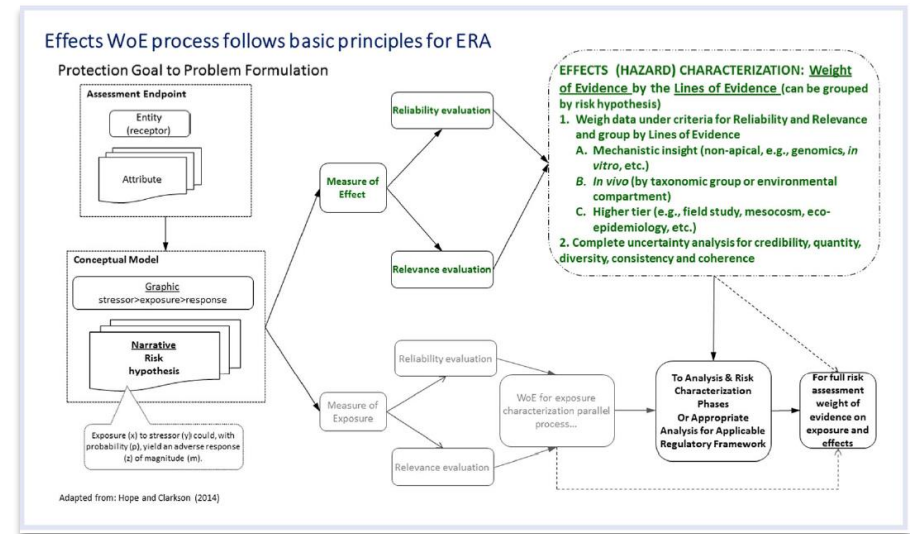


- ### Recommendations towards evidence-based ecotoxicology
- Consider all applicable studies
 - Report all findings of experimental studies
 - Make ecotoxicity studies publicly accessible
 - Implement reporting guidelines for publication of ecotoxicity studies
 - Apply transparent and consistent evaluation 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)

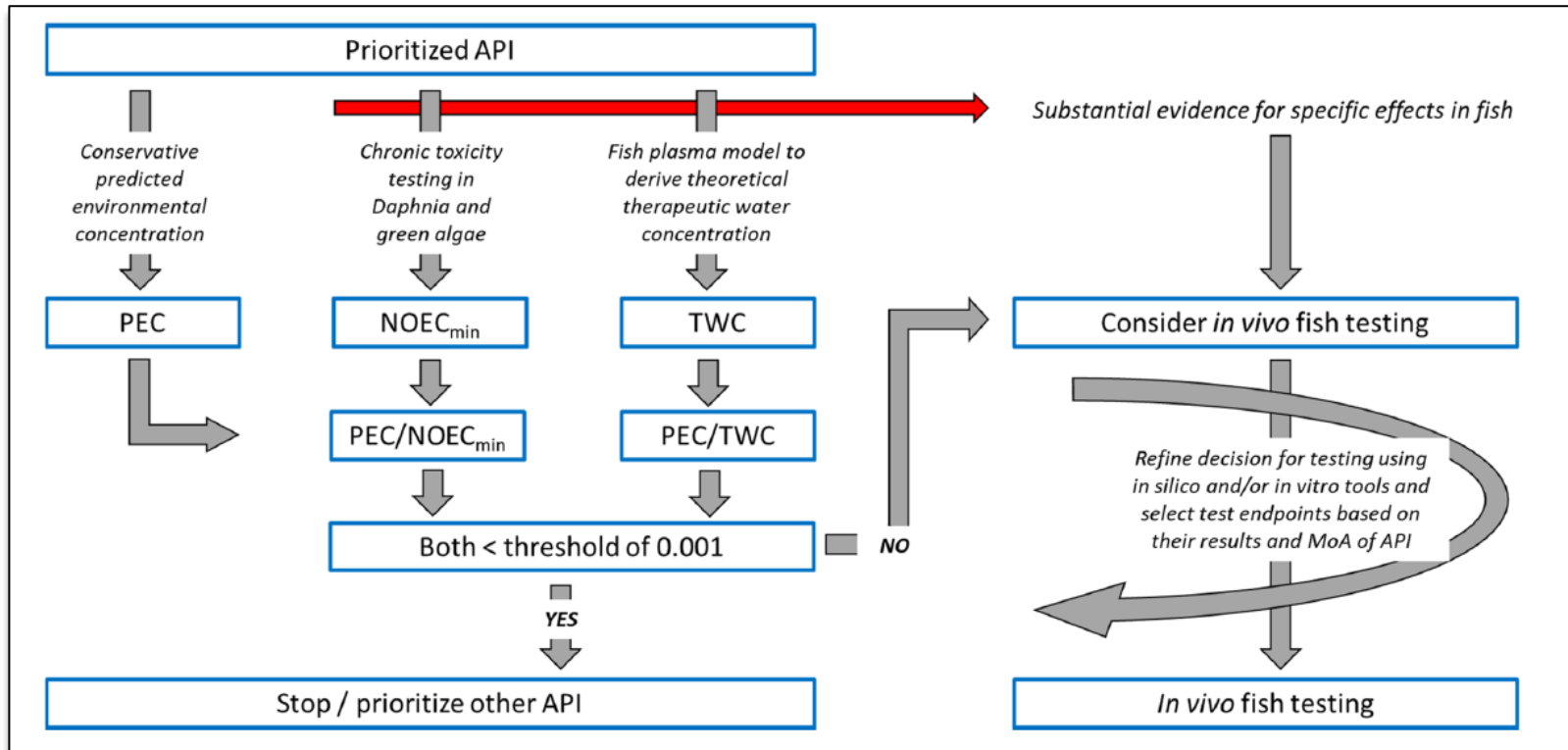


Ankley et al. Environmental Toxicology and Chemistry 37; 2064–2078 (2018)



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

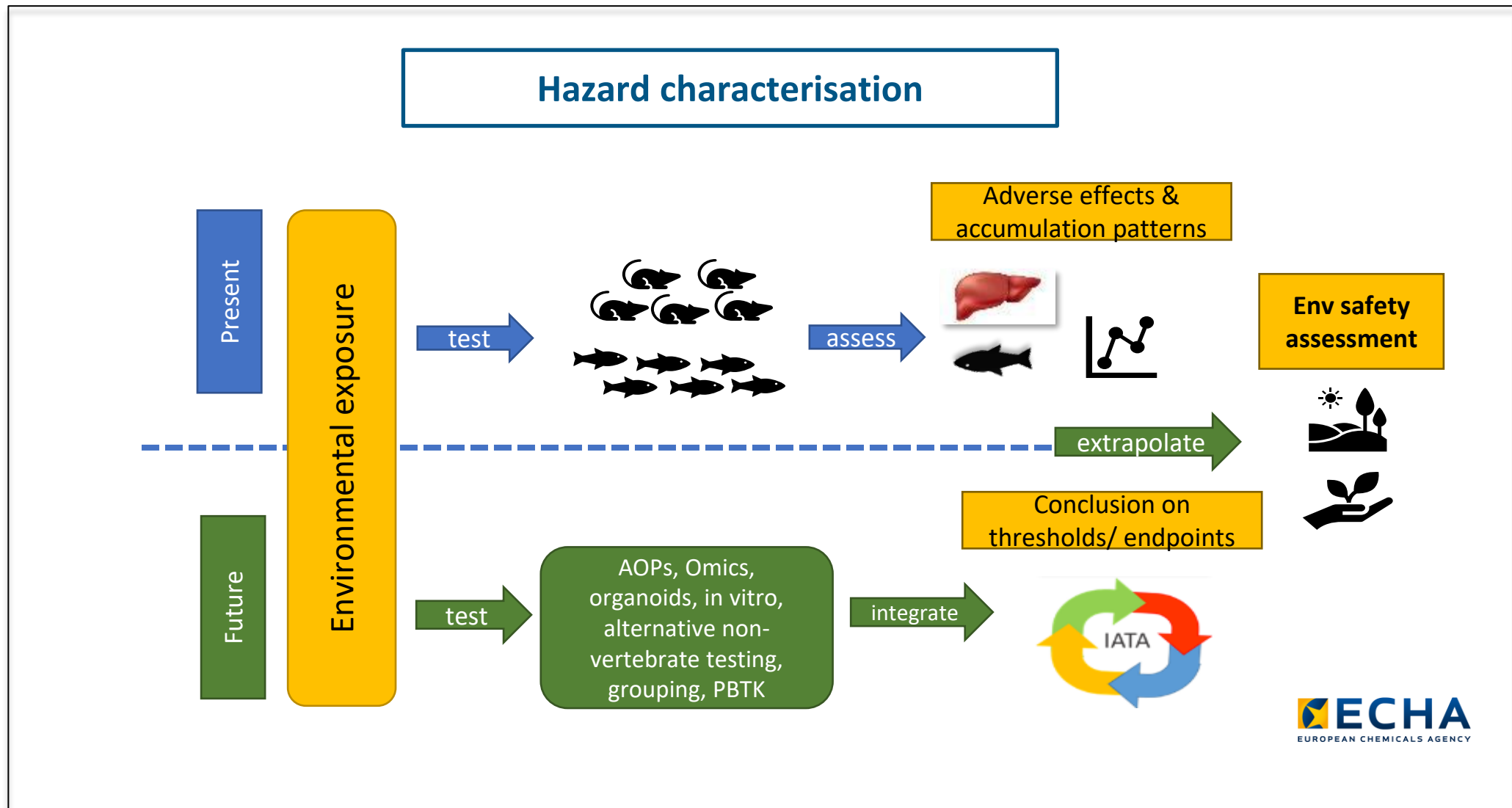
Minimizing Experimental Testing on Fish for Legacy Pharmaceuticals



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



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

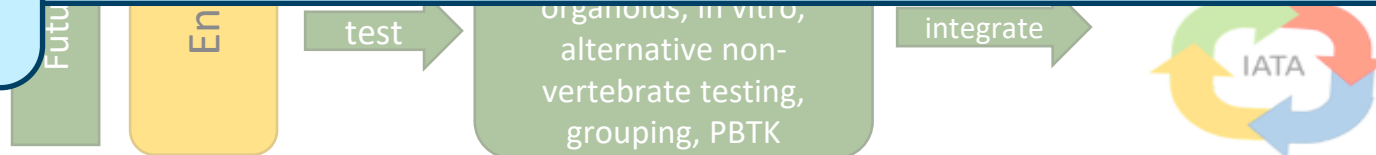


Doris Hirmann et al.: Strengthened role of New Approach Methods (NAMs) in bioaccumulation assessment under REACH
 Presentation at SETAC Europe 33rd Annual Meeting 04. May 2023

Quantitative Risk Assessment

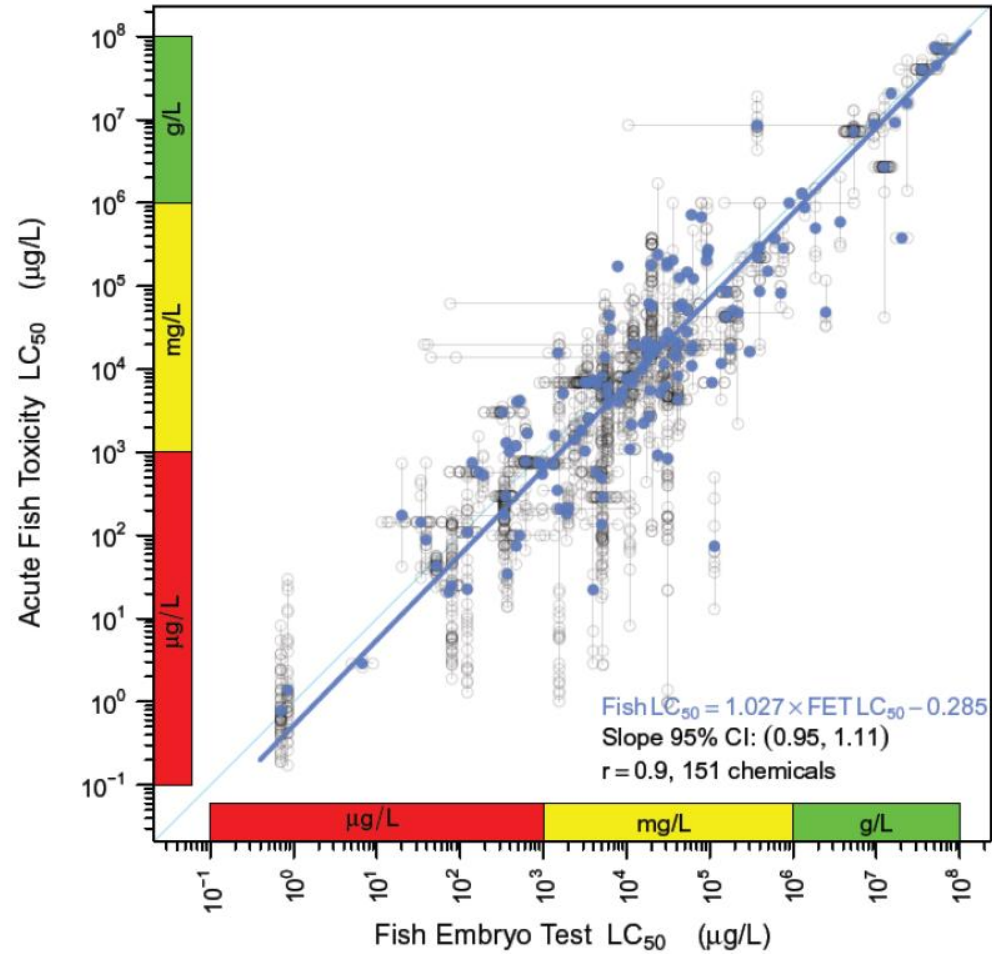
Is this approach feasible

- for substances with highly Specific Mode of Actions?
- in regulations, where court-proofed PNECs needs to be generated?

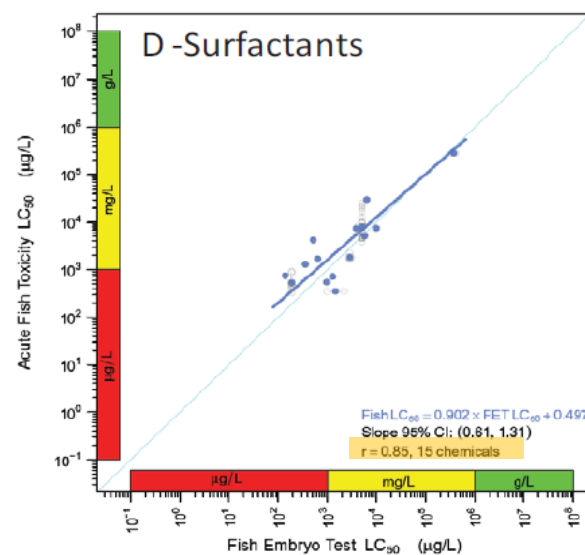
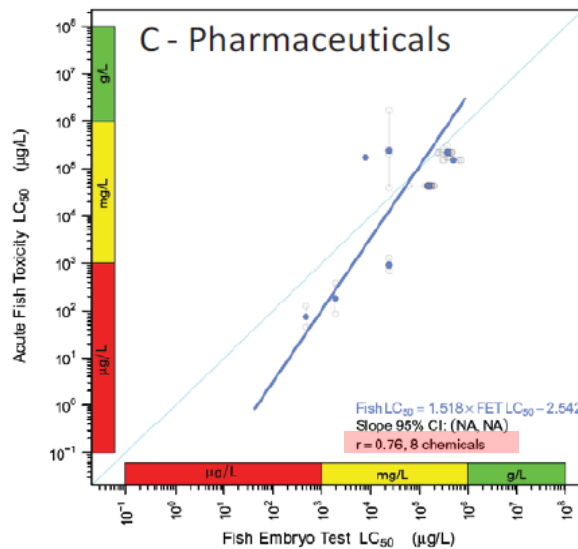
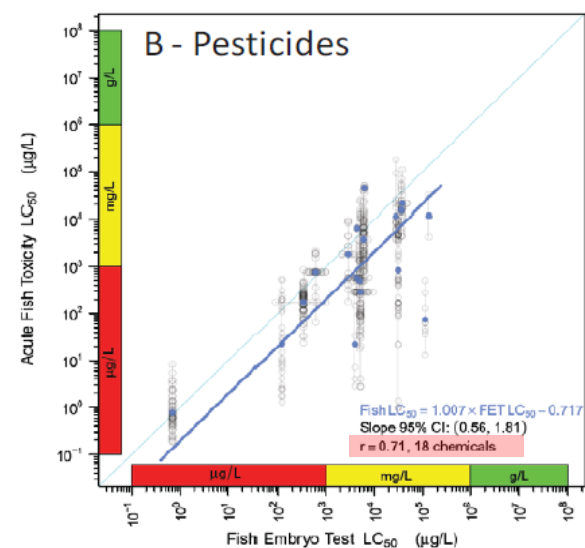
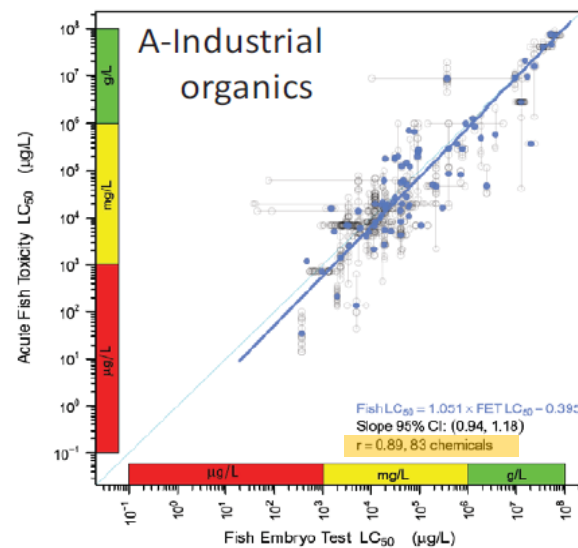


An Update to the Fish Embryo Toxicity-Acute Fish Toxicity Relationship and Prospects for Support of the Use of the FET as an Animal Alternative

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

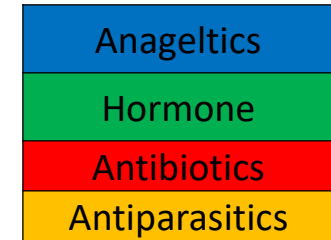
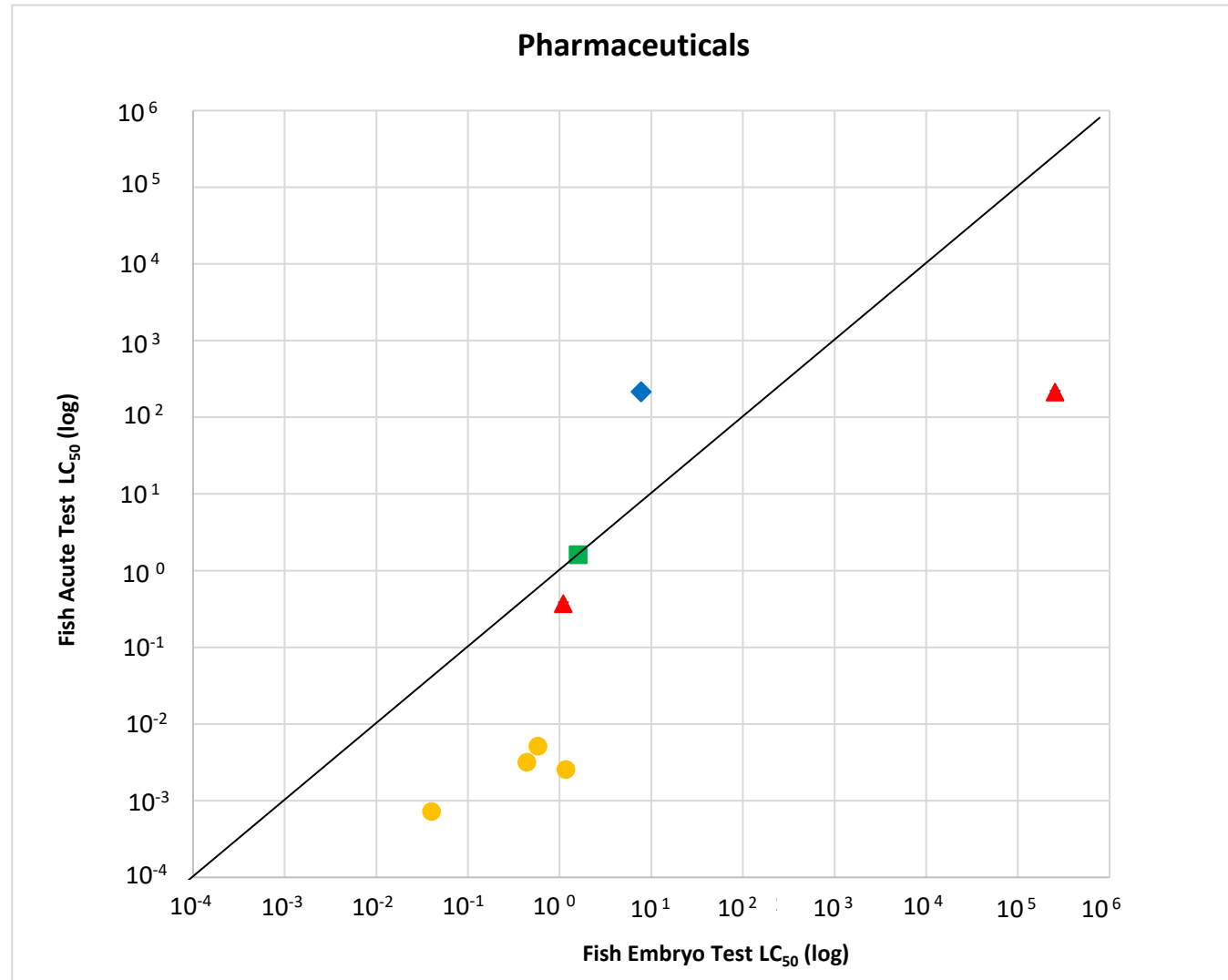


An Update to the Fish Embryo Toxicity-Acute Fish Toxicity Relationship and Prospects for Support of the Use of the FET as an Animal Alternative



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)



Changes in Mating Behaviour

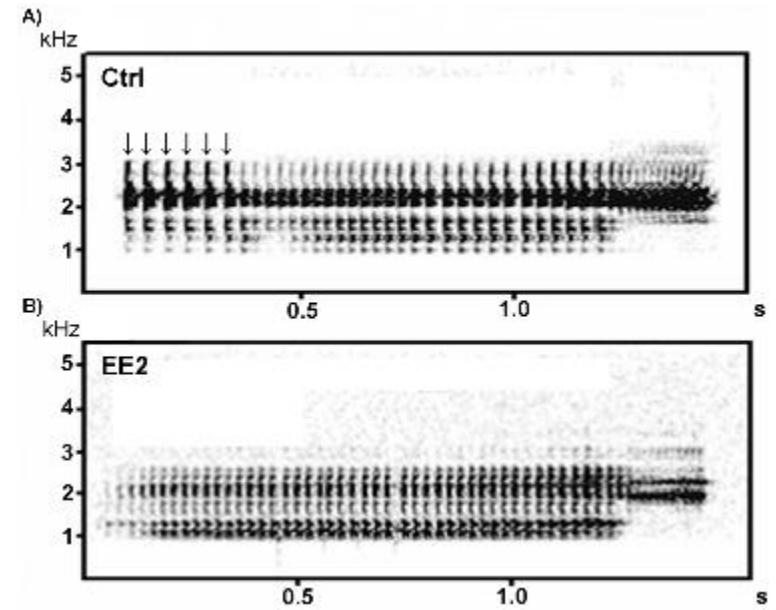
0.3 ng/L EE2 can disrupt amphibian mating behavior



Xenopus laevis



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



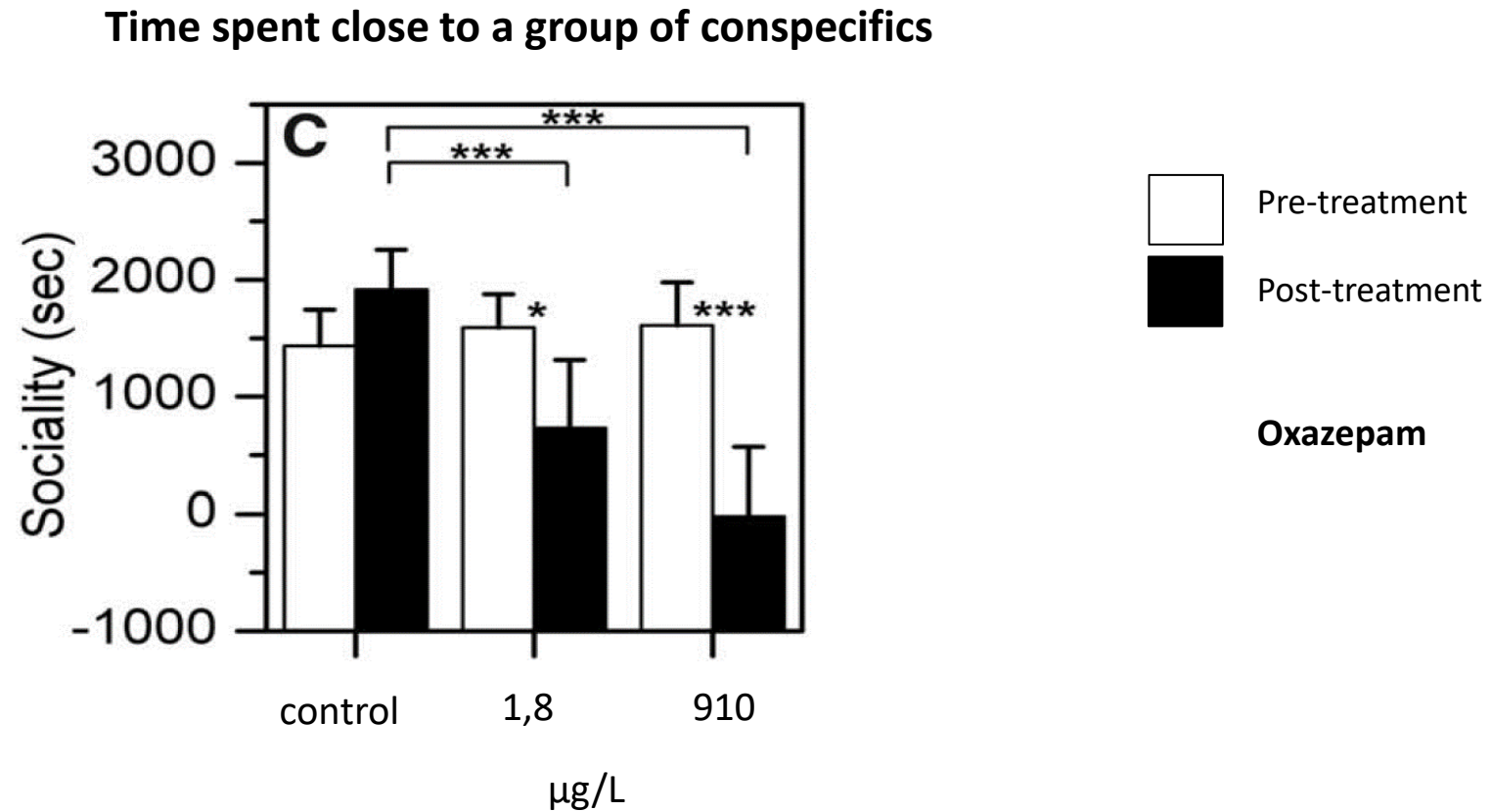
Hoffmann, Kloas (2012) Estrogens can disrupt amphibian mating behavior *PLOS ONE*, 7(2) e32097



Psychiatric Drug Alter Behavior of Fish from Natural Populations

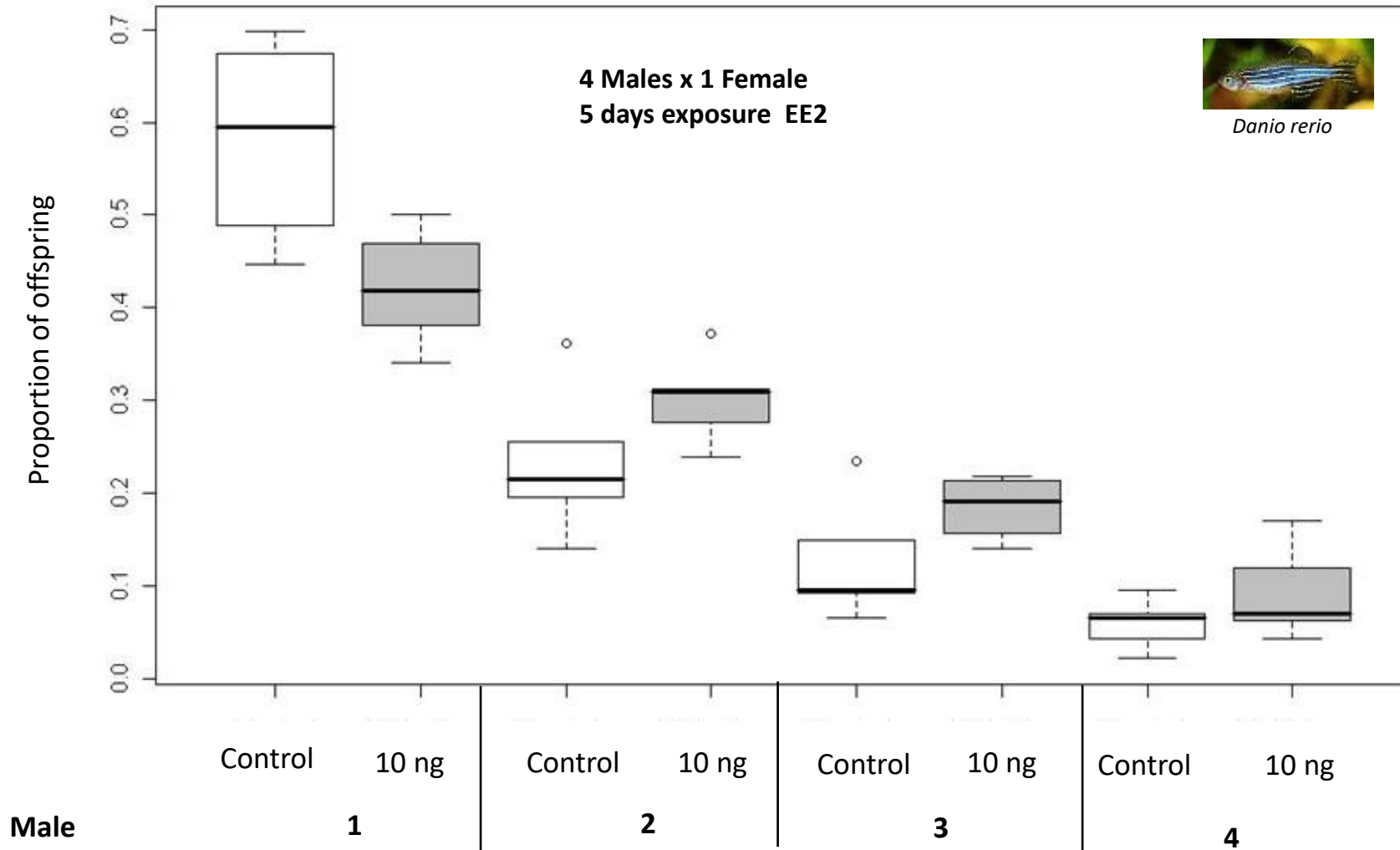


Perca fluviatilis



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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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 17 α -ethinylestradiol (EE₂) 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 in large groups. Adult male zebrafish were exposed for 4

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., 17 α -ethinylestradiol (EE₂) and estrone 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 EE₂, a component of the contraceptive pill that is typically found in WWTW effluents in the low ng/L range (13). Exposure to EE₂ 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 EE₂ (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

Regulatory Perspective

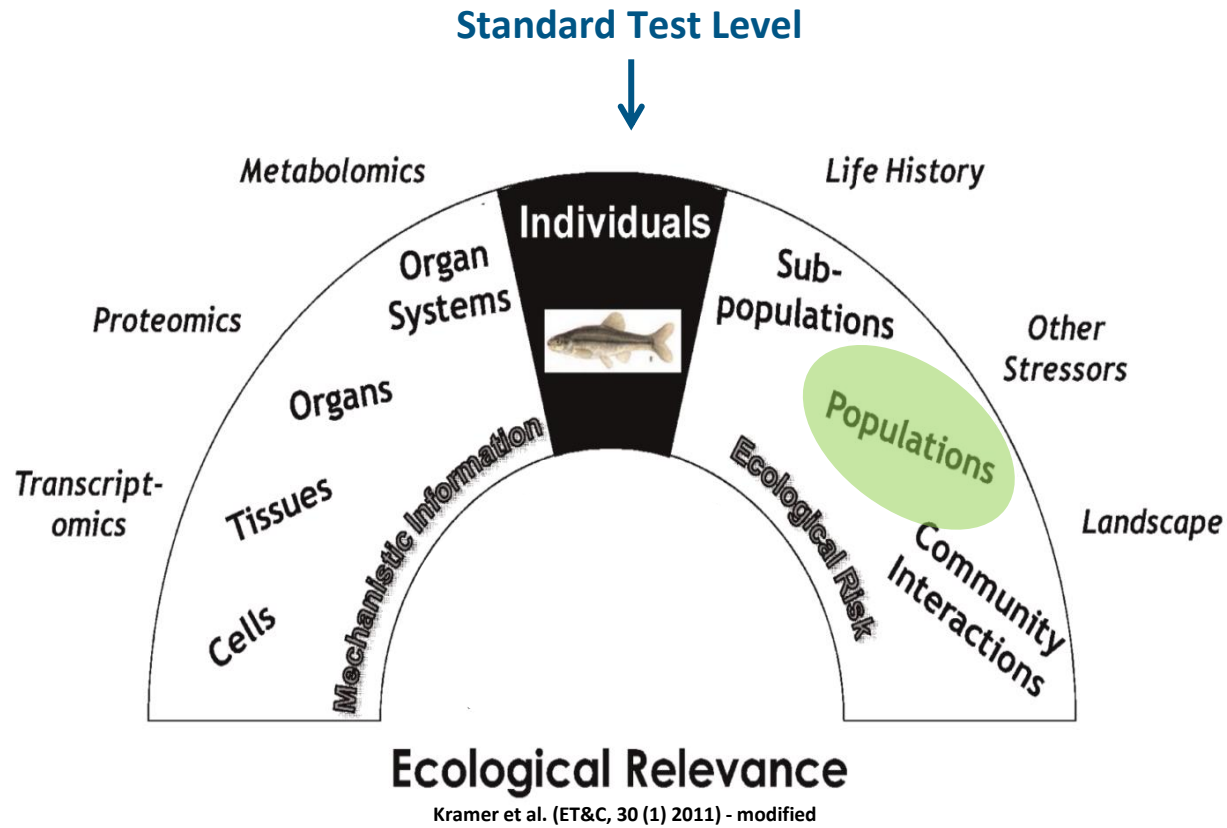
Do we have to distinguish Specific Mode of Actions?

- 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



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 !**

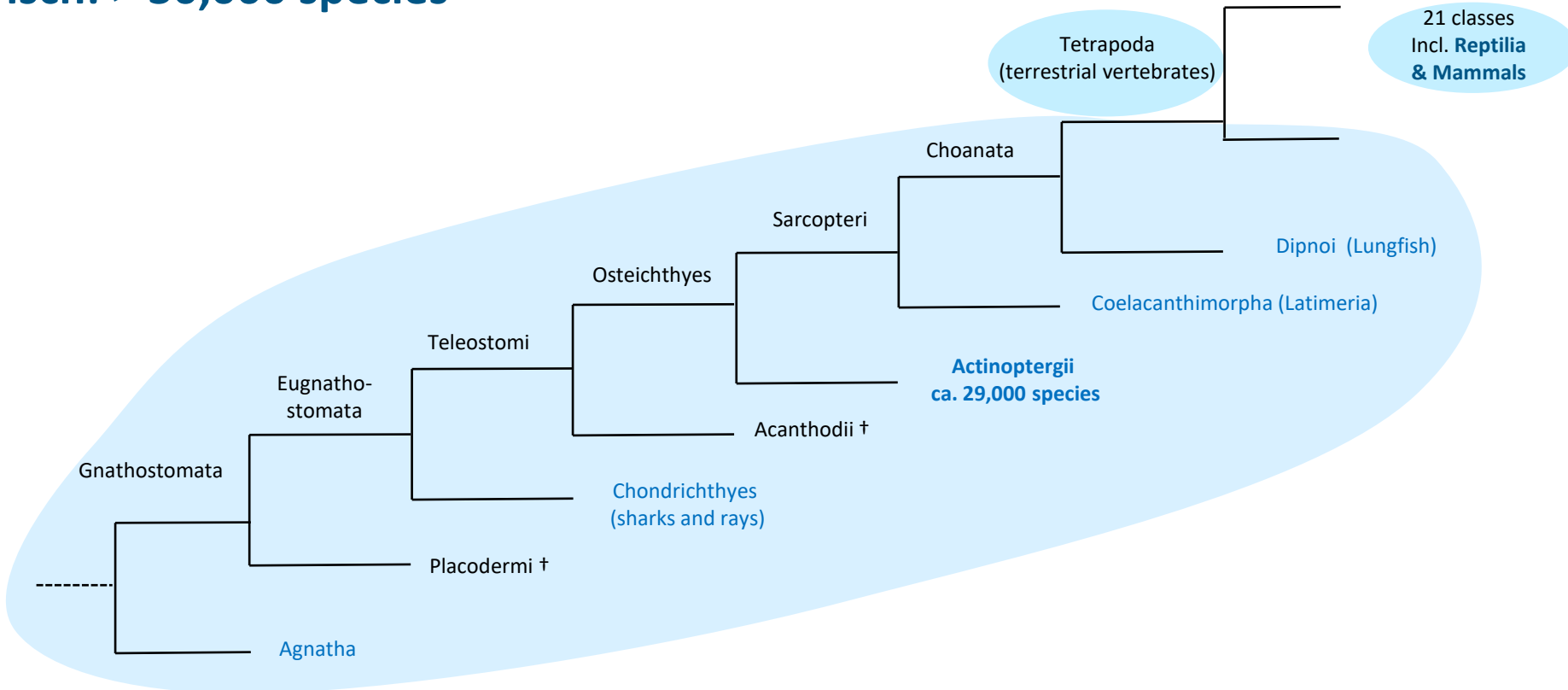
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*The above represents the opinion of the author and is not necessarily an official position of the
German Environment Agency*



Diversity of fish

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



Diversity of fish

- **Mammals: ca. 4,000 species**
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