ALTERNATIVES TO THE FISH EARLY LIFE-STAGE TEST: A RESEARCH STRATEGY FOR DISCOVERING AND ANNOTATING ADVERSE OUTCOME PATHWAYS FOR EARLY FISH DEVELOPMENT

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13 November 2019

PETA ISC – US EPA – PCRM Webinar Series

Acknowledgements

- Funding
  - HESI's Animal Alternatives in Environmental Risk Assessment Technical Committee
- HESI-sponsored scientific workshops
  - Workshop #1
    - Held at sanofi-aventis in Paris from June 7-9, 2010
  - Workshop #2
    - Held at US EPA in Duluth from May 15-16, 2012
- Cross-sector and global participation
  - Academia, government, industry, and NGOs
  - North America, Europe, and Asia

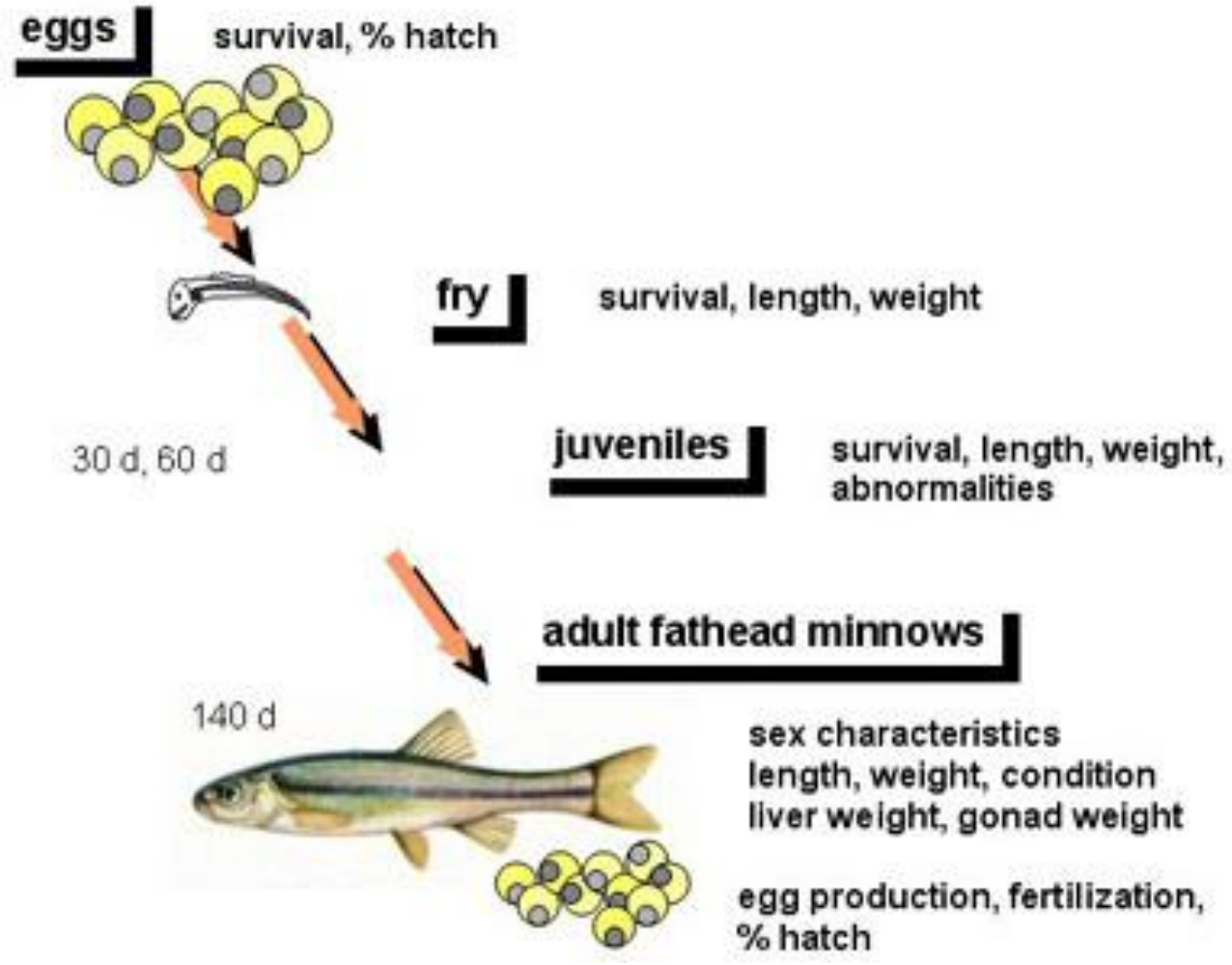
Overview

- FELS test and need for an alternative strategy
- Focus and output of 2010 and 2012 workshops
- Research strategy for FELS AOP development
- Stimulation of RFPs within Europe and the US

Fathead minnow full life-cycle test

Study duration:
5 to 6 months

Typical CRO cost per test:
>350K USD



Source: Environment Canada (www.ec.gc.ca/)

Fish early life-stage (FELS) test

- Introduced >30 years ago as an alternative to FFLC
  - OECD TG 210 or OCSPP Guideline No. 850.1400
- Primary guideline test for estimating chronic toxicity
- Frequently used to support ERAs and chemical management programs around the world

FRESHWATER

SALTWATER

Fathead minnow
(*Pimephales promelas*)

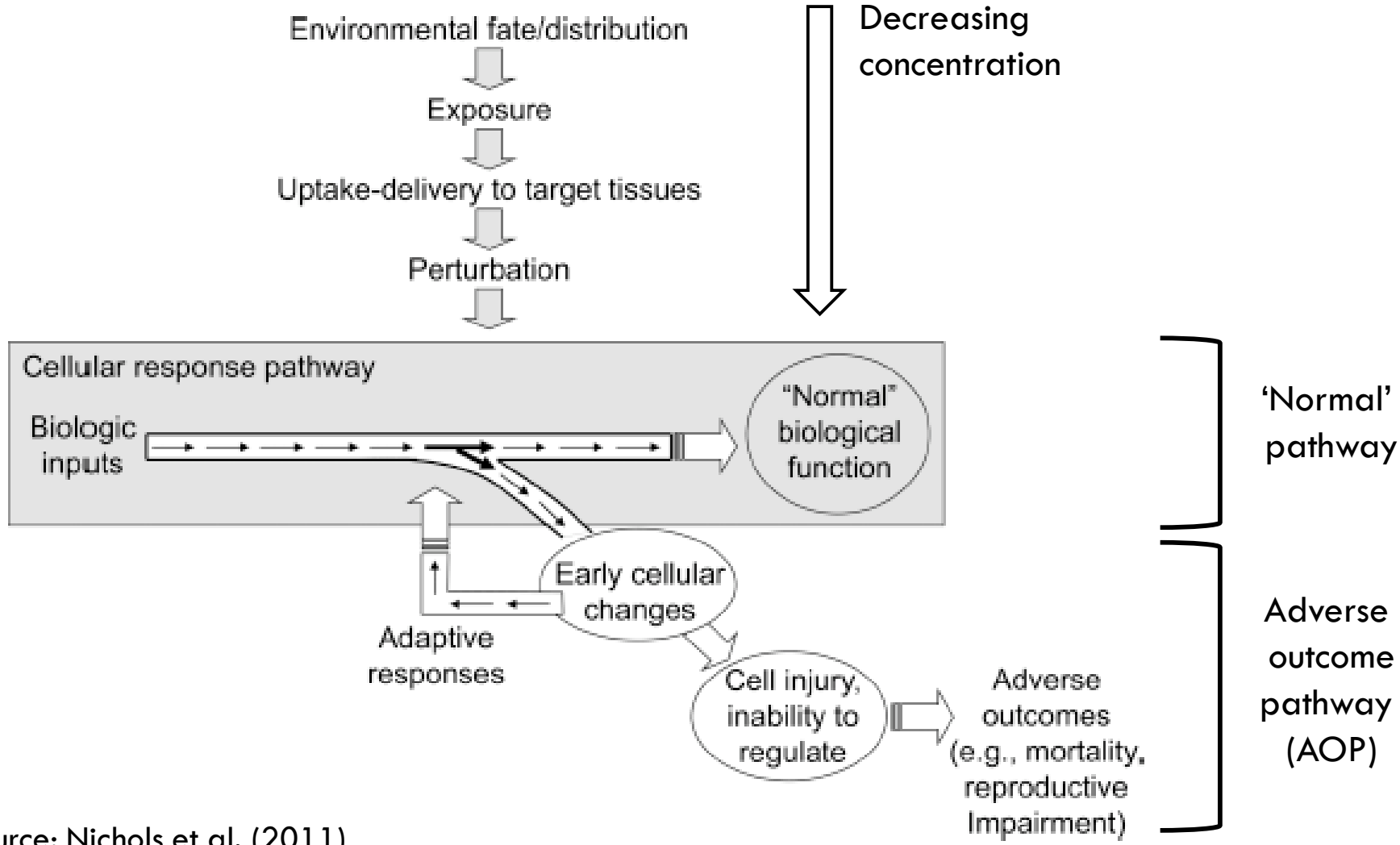
Rainbow trout
(*Oncorhynchus mykiss*)

Sheepshead minnow
(*Cyprinodon variegatus*)

Need for an alternative testing strategy

- FELS test design is labor and resource intensive
  - Study duration is one to three months
  - Requires at least 360 fish, but usually >700 fish
  - Typical CRO cost per test is 50-125K USD
- FELS test endpoints provide little MOA information
  - Narrow focus on gross morphologic endpoints
    - i.e., survival, percent hatch, body length, etc.
  - Chronic NOEC and/or EC_{10} thresholds not helpful for categorizing chemicals by MOA

Adverse outcomes following toxicant exposure



Source: Nichols et al. (2011)

Focus of 2010 workshop

- Overall objective
  - Identify research gaps and strategies related to development of alternatives for chronic fish and amphibian toxicity testing
- First half of workshop focused on the FELS test
  - Explored potential alternatives to a representative, commonly used chronic (long-term) ecotoxicity test
  - Two session topics
    1) FELS data availability and endpoint evaluation
    2) Use of FELS-specific AOPs to identify potential assays for an alternative tiered testing strategy

Key findings and recommendations from 2010

FORUM

Adverse Outcome Pathways during Early Fish Development: A Conceptual Framework for Identification of Chemical Screening and Prioritization Strategies

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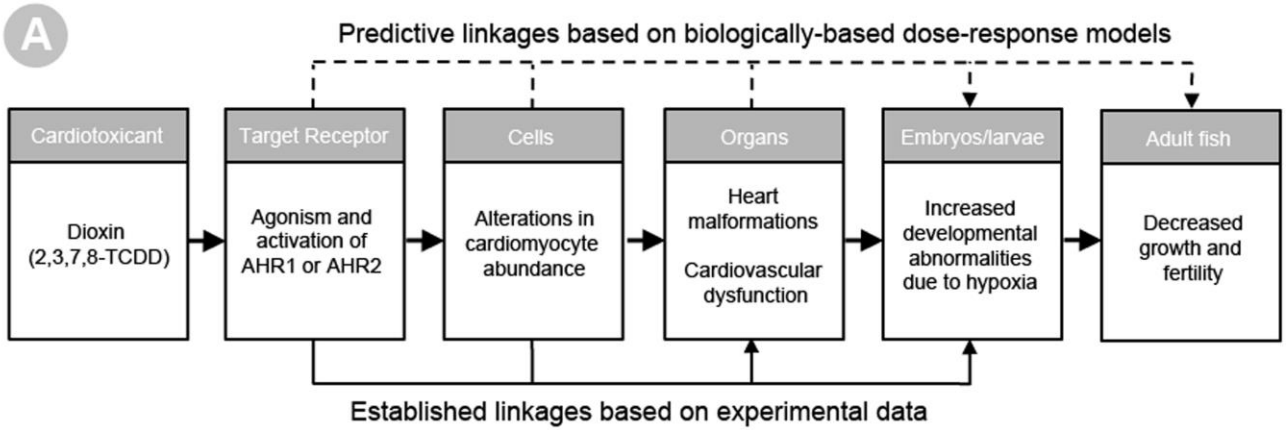
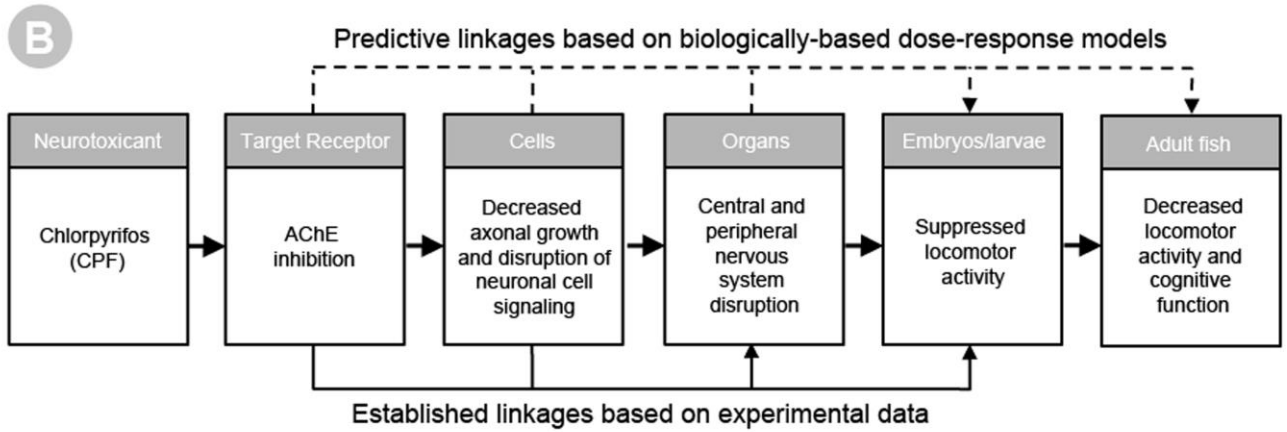
Received May 23, 2011; accepted June 29, 2011

Toxicological Sciences **123(2)**: 349-358 (2011)

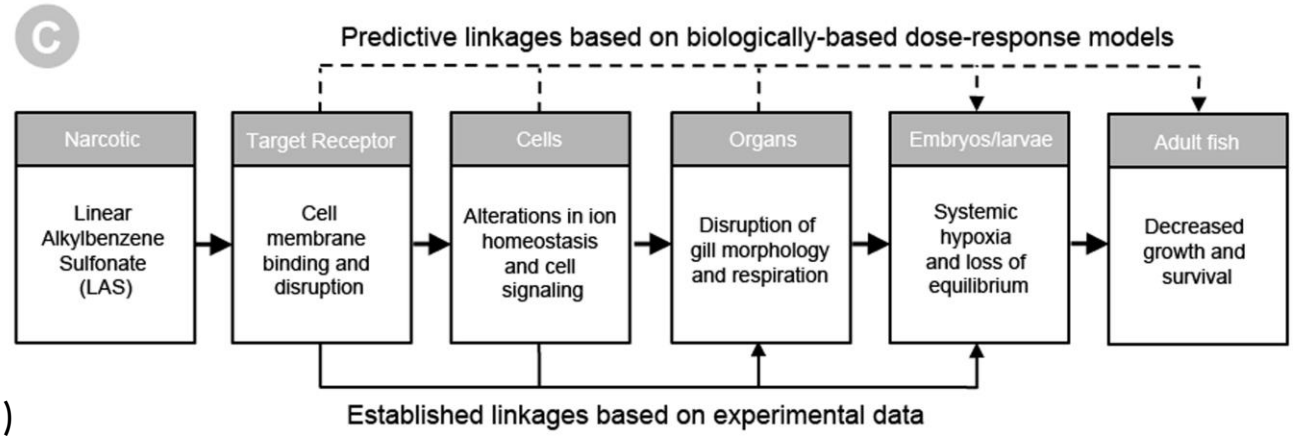
Case Study #1:
TCDD-induced cardiotoxicity



Case Study #2:
CPFO-induced neurotoxicity

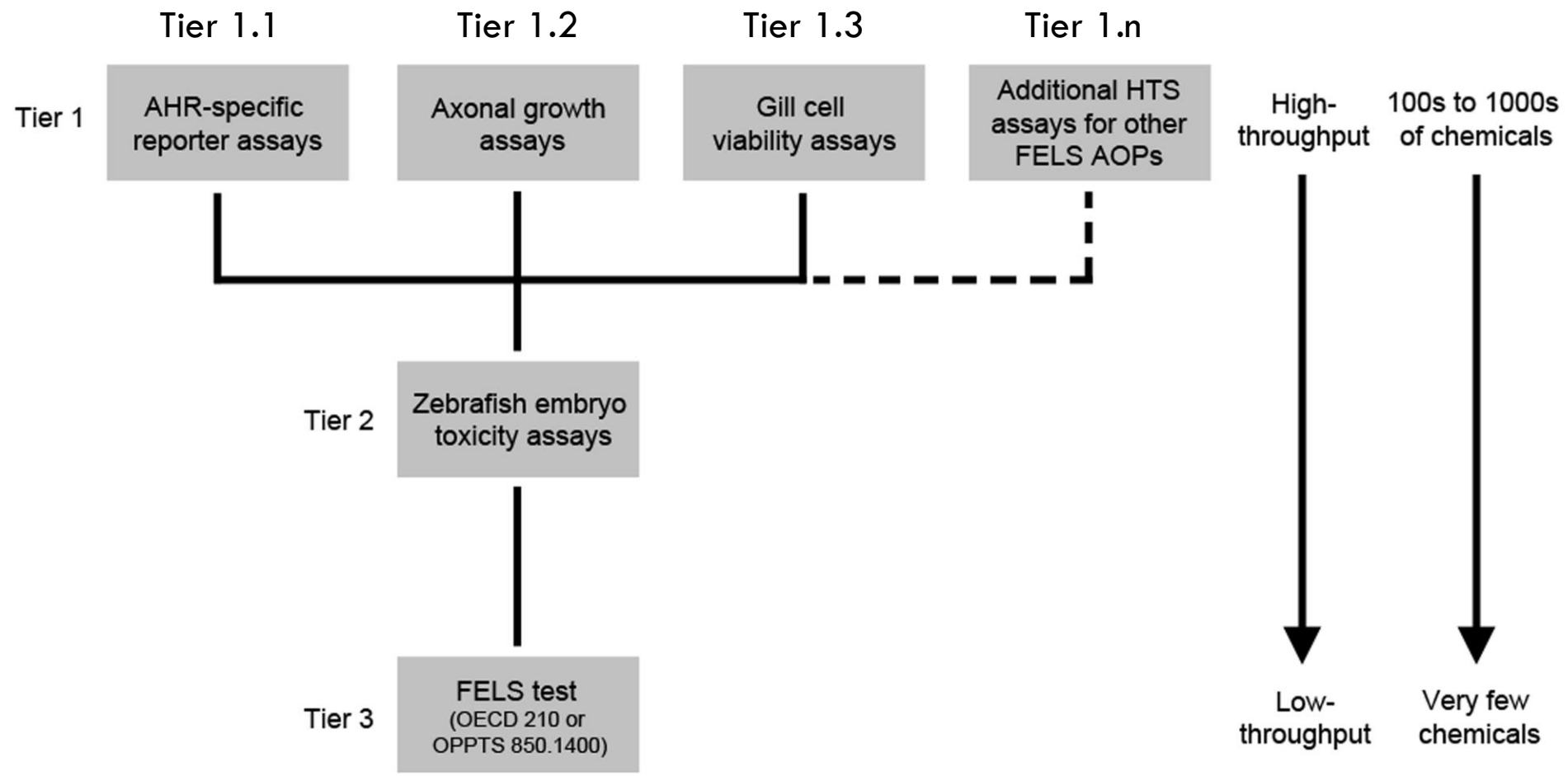


Case Study #3:
LAS-induced gill toxicity



Source: Volz et al. (2011)

Three-tiered testing strategy proposed in 2011



Source: Volz et al. (2011)

Focus of follow-up workshop in 2012

- Key conclusion from our 2011 paper
  - Initial screening tier must be expanded to a broad range, or battery, of toxicologically relevant AOPs
- Entire workshop focused on FELS AOPs
  - Primary objective
    - Identify and discuss the scope and breadth of potential AOPs during early fish development
  - Expected outcome
    - Provide the first critical step for development of an alternative testing strategy for the FELS test

Key findings and recommendations from 2012

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 33, No. 1, pp. 158–169, 2014

Printed in the USA

INVESTIGATING ALTERNATIVES TO THE FISH EARLY-LIFE STAGE TEST: A STRATEGY FOR DISCOVERING AND ANNOTATING ADVERSE OUTCOME PATHWAYS FOR EARLY FISH DEVELOPMENT

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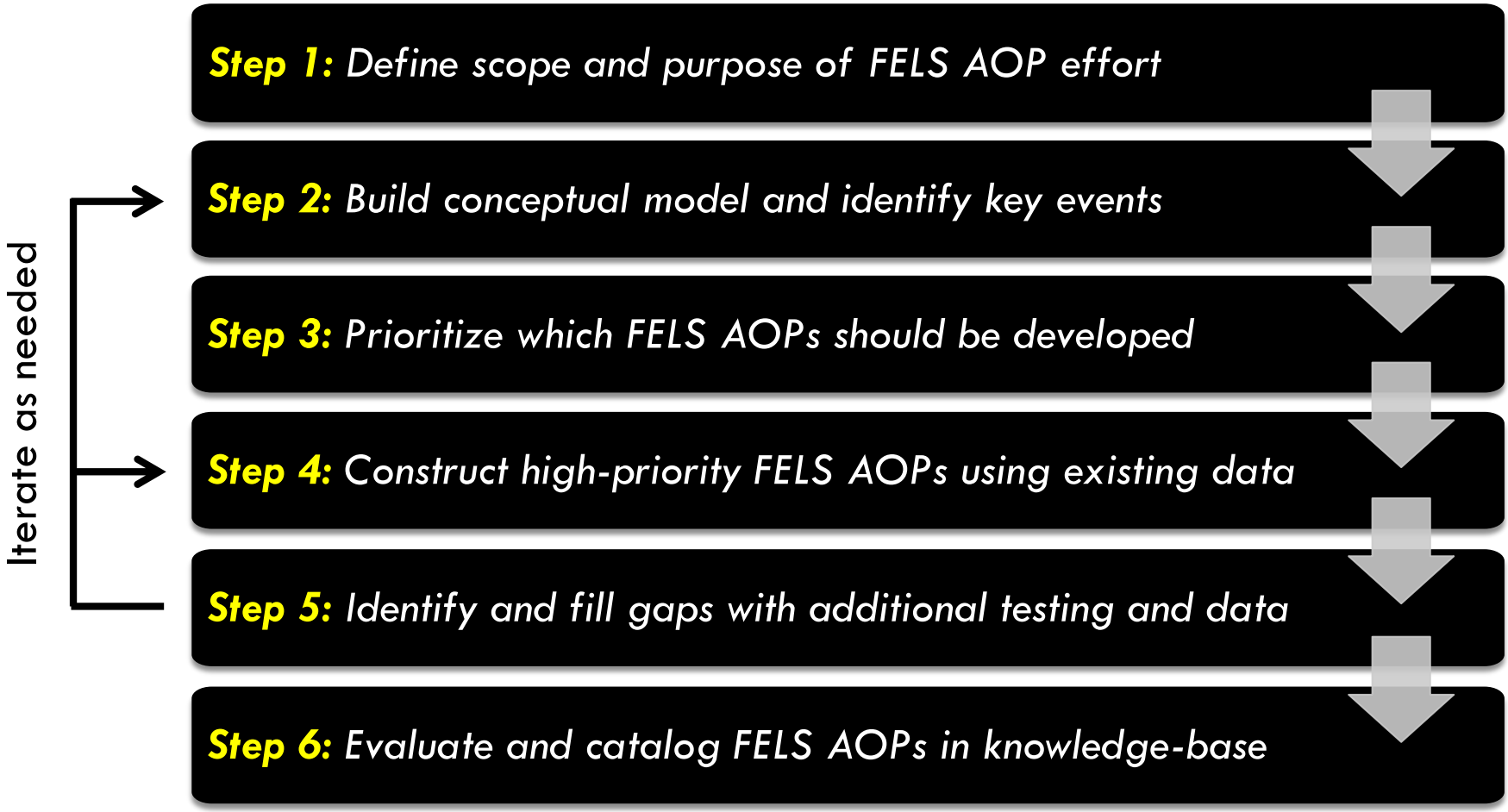
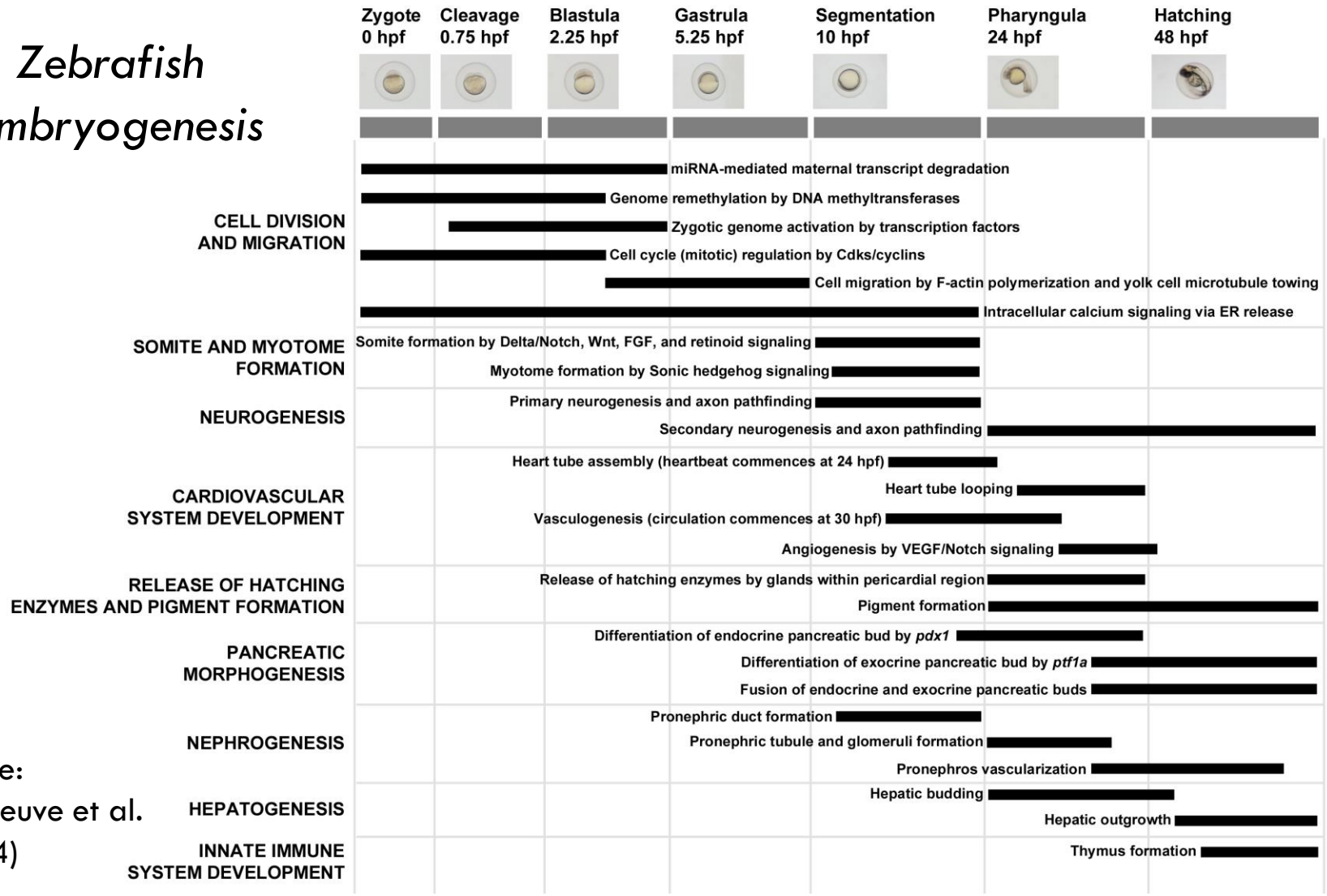
(Submitted 23 July 2013; Returned for Revision 6 September 2013; Accepted 23 September 2013)

Research strategy for FELS AOP development



Step 2.1 – Build conceptual model

Zebrafish embryogenesis

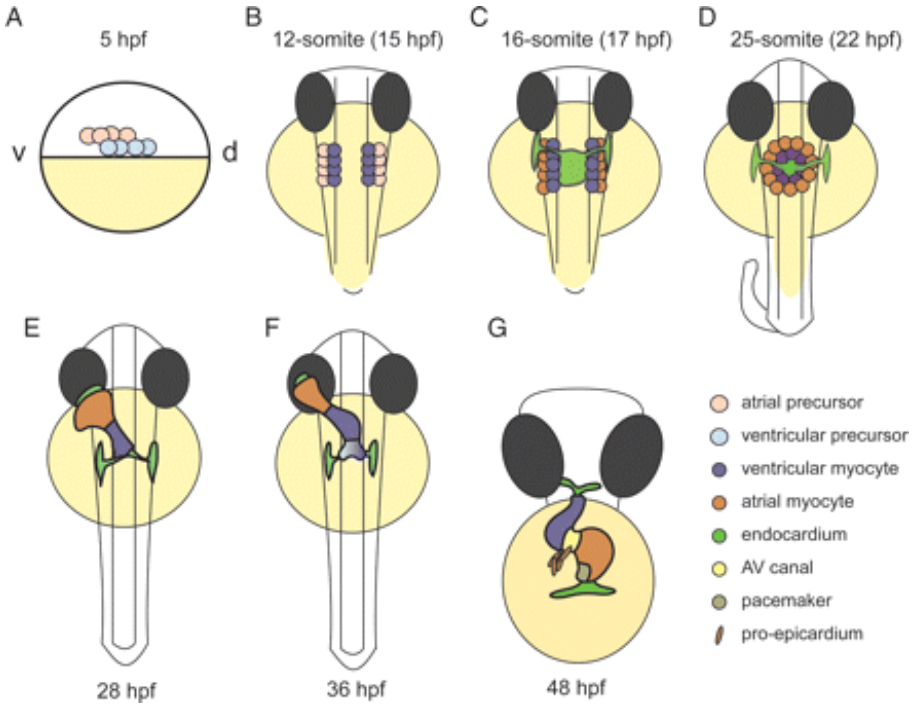


Source: Villeneuve et al. (2014)

Step 2.2 – Identify key biological events

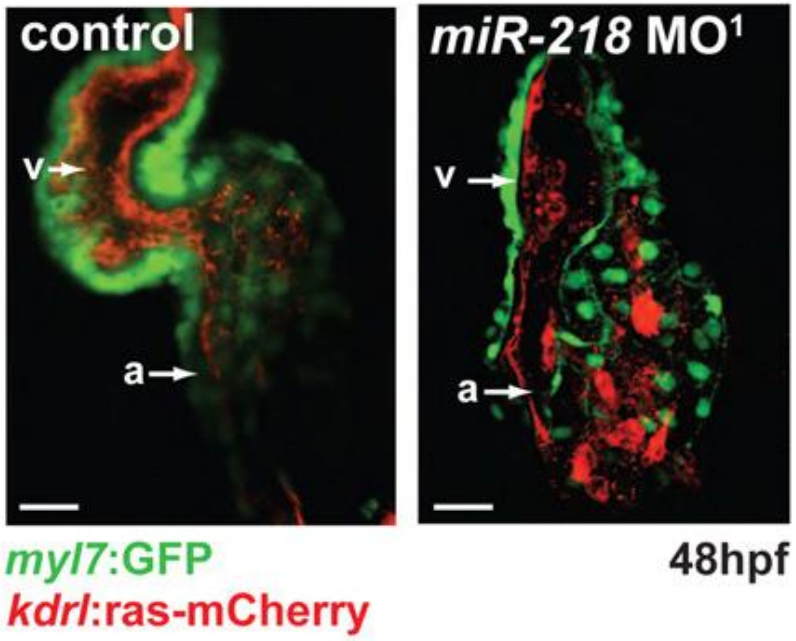
Criteria for identification of a 'key event' (e.g., cardiac looping)

Criterion #1:
Observable and measurable



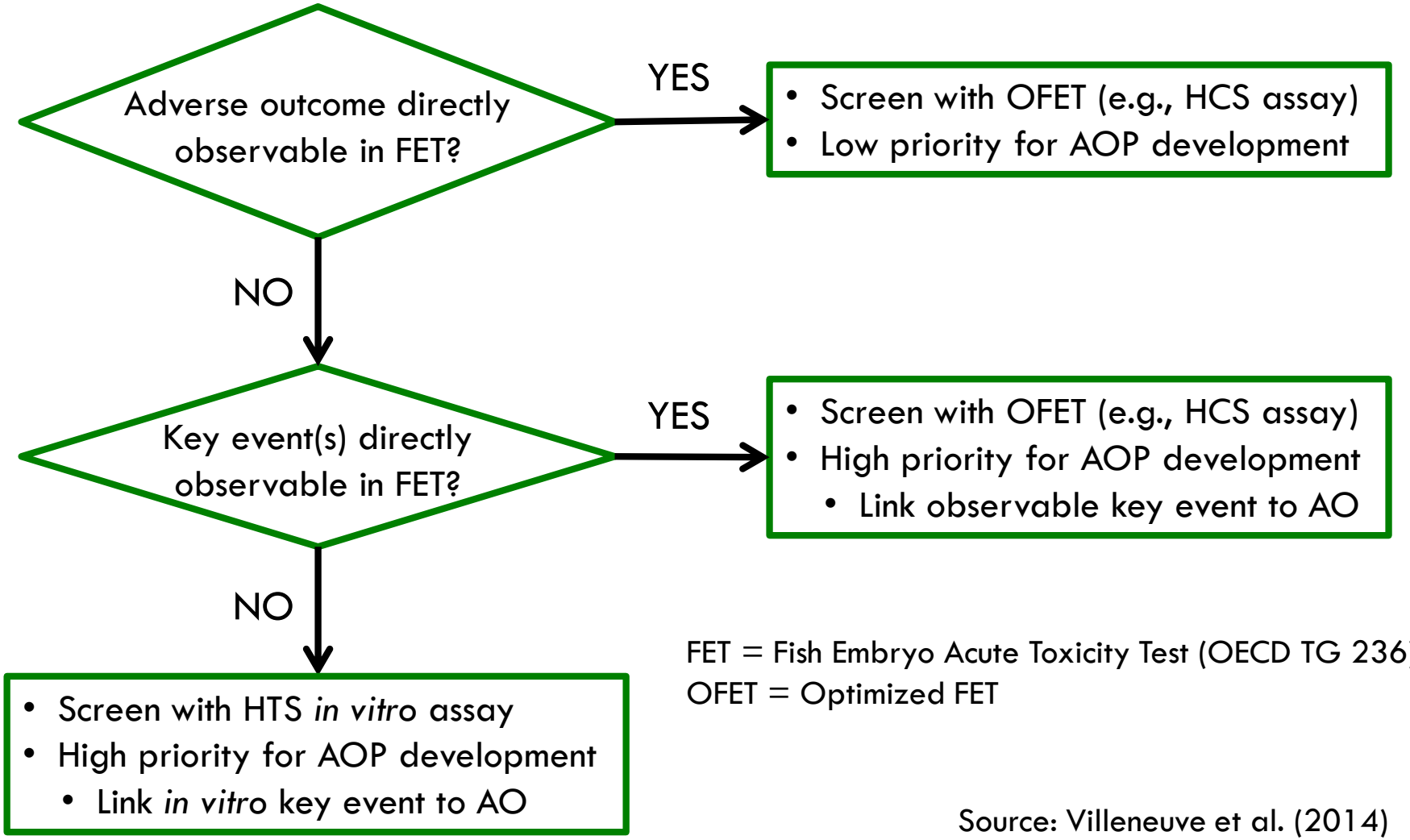
Source: Bakkers (2011)

Criterion #2:
Required for normal growth and survival



Source: Fish et al. (2011)

Step 3 – Prioritize FELS AOPs



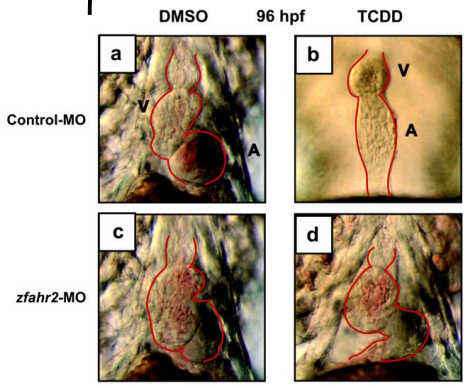
FET = Fish Embryo Acute Toxicity Test (OECD TG 236)
OFET = Optimized FET

Source: Villeneuve et al. (2014)

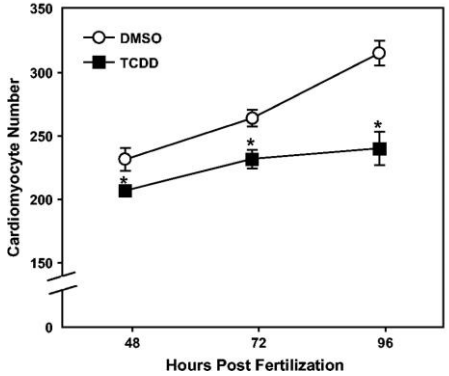
Step 4 – Construct FELS AOPs using existing data

FELS AOP for AHR activation

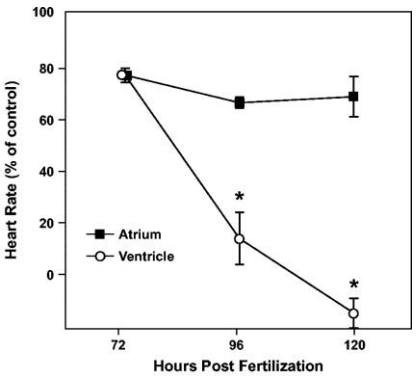
Target Receptor	Cells	Organs	Embryos/larvae
Agonism and activation of AHR1 or AHR2	Alterations in cardiomyocyte abundance	Heart malformations Cardiovascular dysfunction	Increased developmental abnormalities due to hypoxia



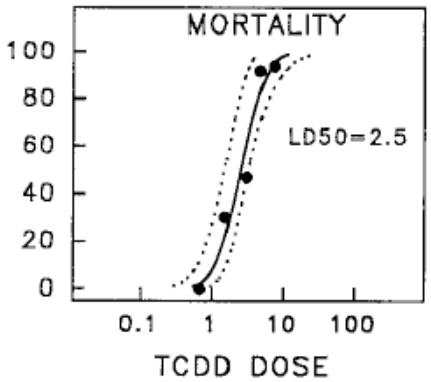
Source: Antkiewicz et al. (2006)



Source: Antkiewicz et al. (2005)

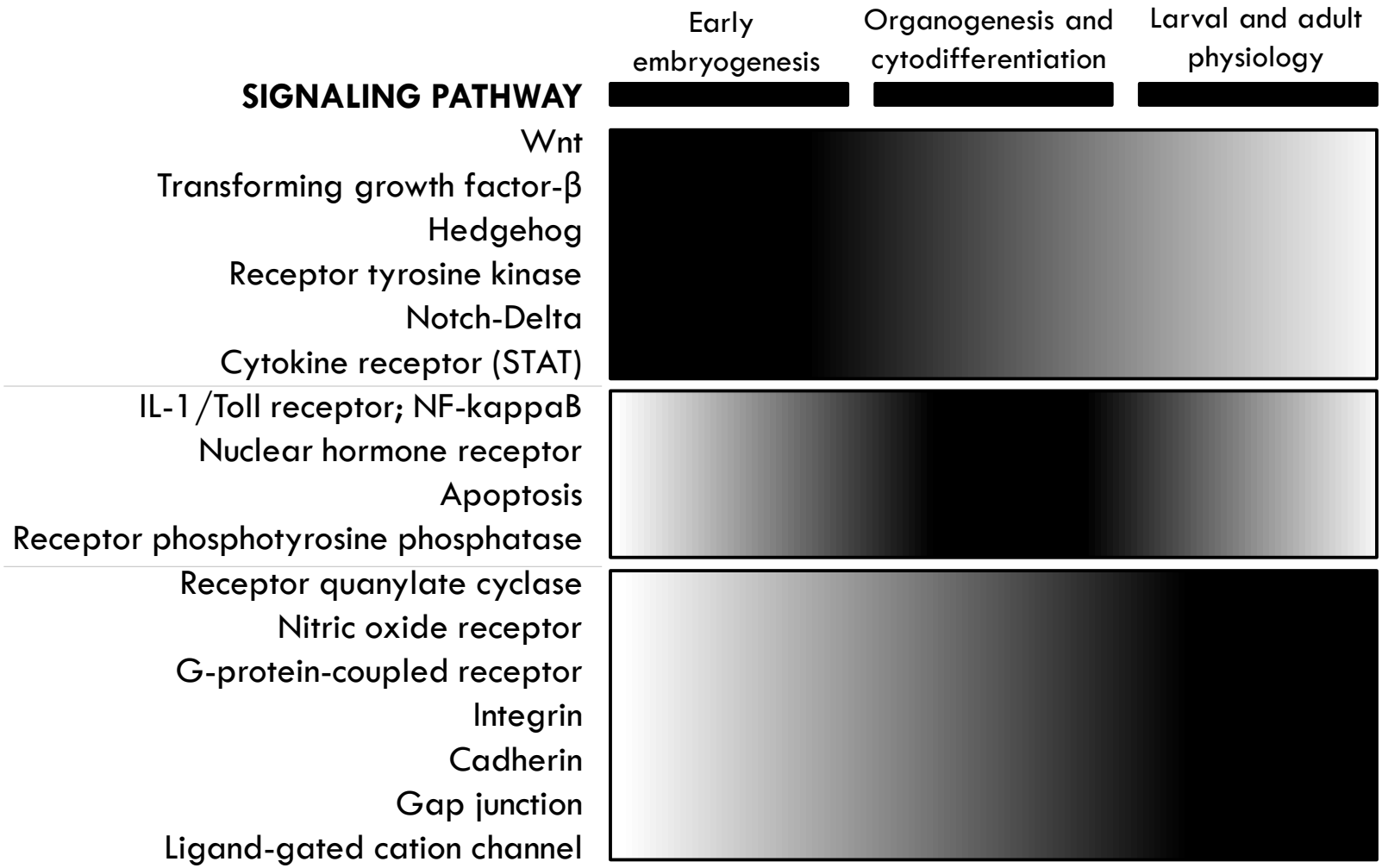


Source: Antkiewicz et al. (2005)



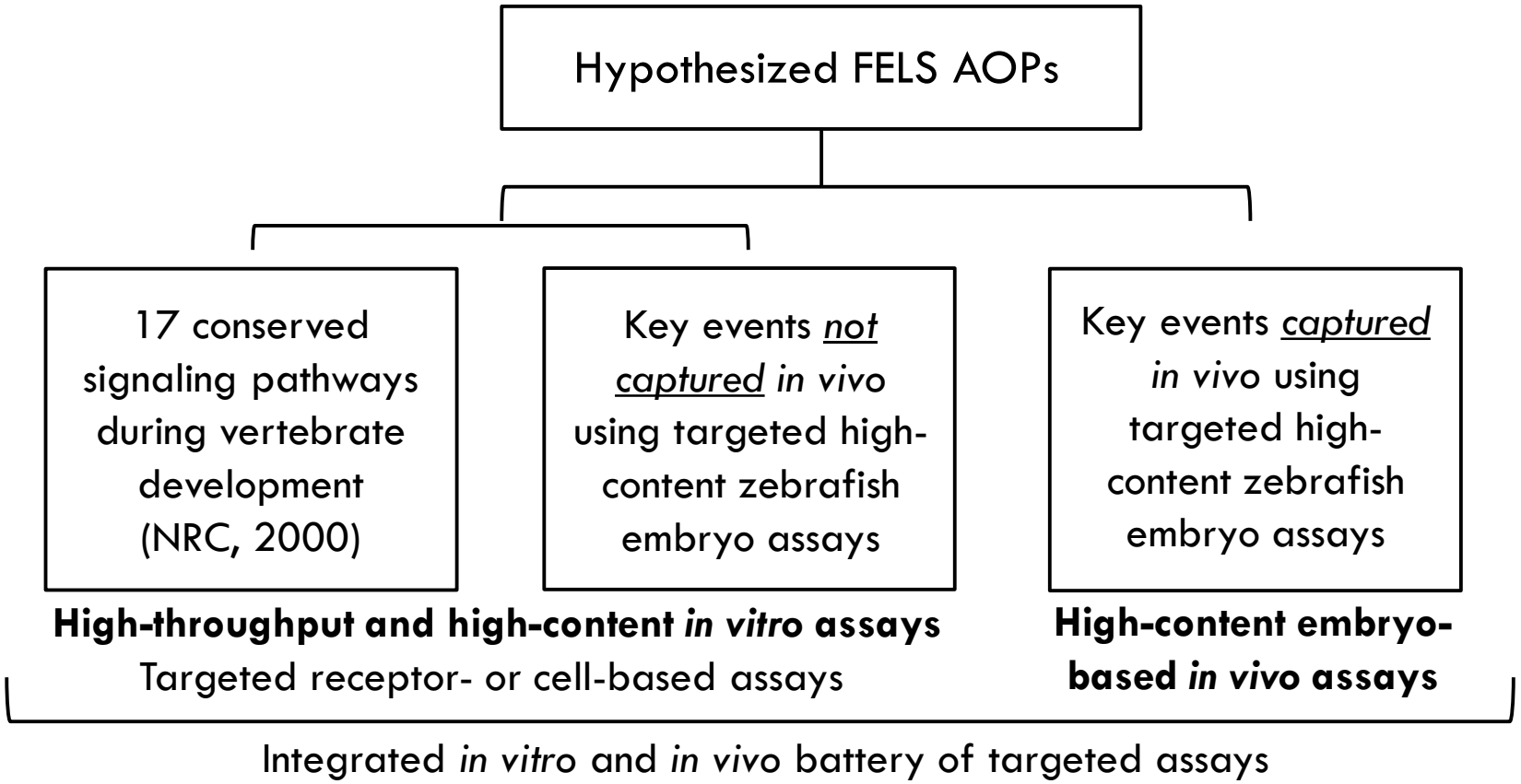
Source: Henry et al. (1997)

Conserved signaling pathways during development



Source: Villeneuve et al. (2014)

Step 5 – Identify/fill gaps with additional data



Source: Villeneuve et al. (2014)

Research strategy moving forward

- Short-term (3-5 years)
  1) Expand and disseminate a conceptual model of normal fish development
  2) Identify 'low-hanging fruit' based on toxicological relevance and immediate regulatory needs
  3) Optimize targeted assays for these high-priority AOPs
  4) Characterize Phase I/II biotransformation in fish embryos
- Long-term (5-10 years)
  1) Optimize targeted assays for lower-priority AOPs
  2) Develop quantitative FELS AOPs using reference chemicals

Search ...

FUNDING OPPORTUNITIES

- Apply for a grant
- Cefic-LRI Award 2019
- All RfP's

LRI-ECO20 DEVELOPMENT OF AN ALTERNATIVE TESTING STRATEGY FOR THE FISH EARLY LIFE-STAGE (FELS) TEST (OECD 210)

Background

The use of conventional whole-organism (vertebrate) bioassays for estimating chronic ecological hazards has been limited due to low efficiency, high cost, extensive animal use, and, for aquatic toxicity studies, generation of large volumes of contaminated water. Moreover, most animal guideline tests provide little or no informative mechanistic toxicity data due to the principal focus on apical endpoints such as survival, growth, and reproduction. Due to these limitations, resource-efficient alternatives to conventional toxicity testing - including high-throughput *in vitro* and *in silico* screening assays - have been proposed as key components of a future testing paradigm for mechanism-based regulatory toxicology and ecotoxicology (Bradbury et al. 2004; NRC 2007; Villeneuve and Garcia-Reyero 2011). However, the predictive power of molecular or cellular perturbations (e.g., modeled or detected *in vitro*) for apical endpoints relevant to ecological risk assessment (i.e., survival, growth, and reproduction) must be sufficiently high to minimize uncertainties and provide meaningful data for regulatory decision-making.

Publications

Events calendar

November 2019

SU	MO	TU	WE	TH	FR	SA
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23

ECO20-UA: DEVELOPMENT OF AN ALTERNATIVE TESTING STRATEGY FOR THE FISH EARLY LIFE-STAGE TEST FOR PREDICTING CHRONIC TOXICITY

ECO20.2: DEVELOPMENT OF AN ALTERNATIVE TESTING STRATEGY FOR THE FISH EARLY LIFE-STAGE TEST FOR PREDICTING CHRONIC TOXICITY: ASSAY VALIDATION

Principal Investigator

Prof. Dr. Dries Knapen
University of Antwerp
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Tel: +32 3 265 27 24

ECO20-UA:
March 2013-February 2016
€499,842

ECO20.2:
March 2016-February 2018
€239,967

Total output: 15 peer-reviewed papers

Project flyer: http://cefic-lri.org/wp-content/uploads/2018/06/LRI-ECO20.2-UA-outcome-flyer.pdf

U.S. Environmental Protection Agency
National Center for Environmental Research
Science to Achieve Results (STAR) Program

CLOSED – FOR REFERENCES PURPOSES ONLY

ADVANCING ACTIONABLE ALTERNATIVES TO VERTEBRATE ANIMAL TESTING FOR CHEMICAL SAFETY ASSESSMENT

This is the initial announcement of this funding opportunity.

Funding Opportunity Number: EPA-G2018-STAR-C1

Catalog of Federal Domestic Assistance (CFDA) Number: 66.509

Solicitation Opening Date: August 8, 2018
Solicitation Closing Date: September 25, 2018: 11:59:59 pm Eastern Time

Related Information

- Research Grants
- P3: Student Design Competition
- Research Fellowships
- Small Business Innovation Research (SBIR)
- Grantee Research Project Results Search

Ecological risk assessments also employ vertebrate tests for which alternative test methods and strategies are desired (e.g., Volz et al. 2011). As noted earlier, structure-activity relationship-based approaches have long been utilized for predicting so called “baseline” acute and chronic toxicity to aquatic organisms. However, for pathways of toxicity other than narcosis, ecological hazard assessment typically still relies on vertebrate ecotoxicity tests to evaluate chemicals for specific chemical modes of action (e.g., neurotoxicity, immune modulations, teratogenesis, endocrine disruption). Stinckens et al. (2018) recently showed the utility of cell-free biochemical assays, anchored to an AOP, for predicting adverse effects on swim bladder development in fish. However, this is just one example of an alternative test method that might be integrated into an overall strategy for refining and reducing the need for vertebrate ecotoxicity tests. Examples of tests for which alternatives are lacking include; fish early life stage test (OECD TG 210), fish juvenile growth test (OECD TG 215), 21-d fish short term reproduction assay (OECD TG 229), fish sexual development test (OECD TG 234), avian reproduction test (OECD TG 206), and avian dietary test (OECD 205).

Reducing the reliance on early-life stage testing with relevance to euryhaline fishes: Development and implementation of in-vitro assays predictive of early life stage toxicity and population-level effects in Menidia beryllina

Related Information

- Research Grants
- P3: Student Design Competition
- Research Fellowships
- Small Business Innovation Research (SBIR)
- Grantee Research Project Results Search

EPA Grant Number: R839503
Title: Reducing the reliance on early-life stage testing with relevance to euryhaline fishes: Development and implementation of in-vitro assays predictive of early life stage toxicity and population-level effects in Menidia beryllina
Investigators: Brander, Susanne M , Armbrust, Kevin , Chappell, Patrick , White, Wilson
Institution: Oregon State University , Louisiana State University , Oregon State University , Oregon State University
EPA Project Officer: Lasat, Mitch
Project Period: August 1, 2019 through July 31, 2022
Project Amount: $849,988
RFA: Advancing Actionable Alternatives to Vertebrate Animal Testing for Chemical Safety Assessment (2018) RFA Text | Recipients Lists
Research Category: Safer Chemicals

Description:

Although the Tox21 directive has been in place for over a decade, toxicity assessment of aquatic pollutants, particularly for estuarine and marine ecosystems, still requires large numbers of live fishes. A handful of *in vitro* assays have been developed for euryhaline species, however, many are not in EPA-approved models. Further complicating euryhaline *in vitro* model development is the documented alteration in uptake and bioavailability of many compounds at higher salinities, which is difficult to account for with cell lines.