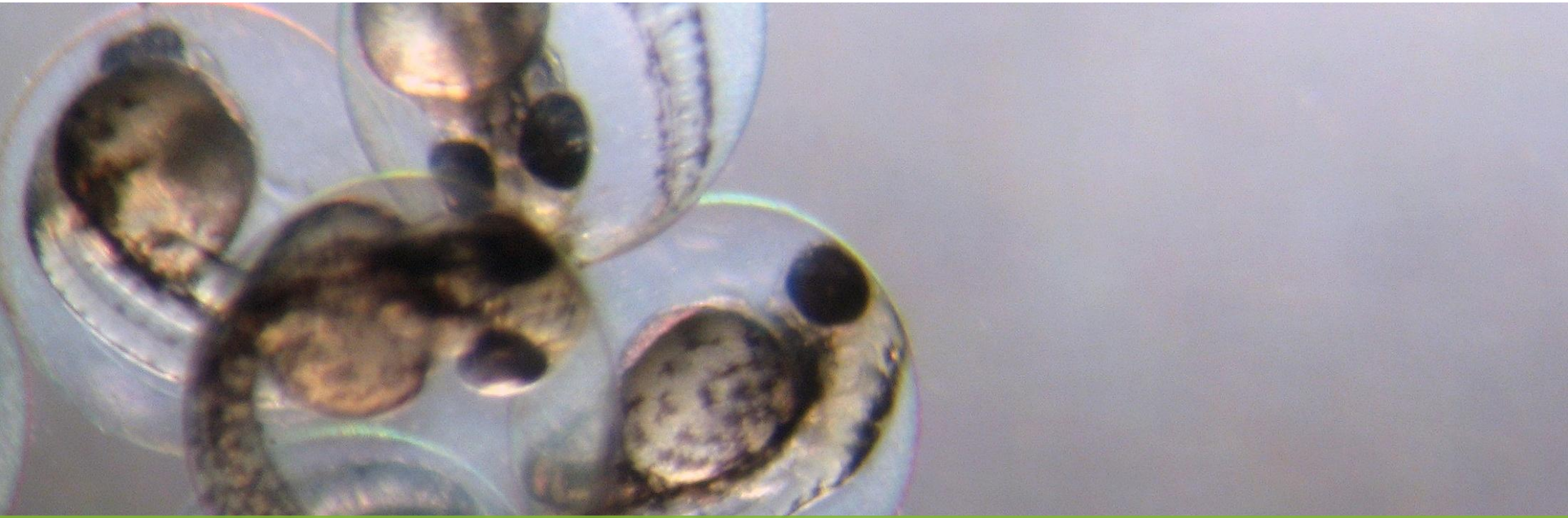
VU University Amsterdam



Zebrafish as a tool to study mechanisms of developmental toxicology of environmental chemicals

Jessica Legradi, P. Cenijn, R. Carvalho, J. Legler

IVM Institute for Environmental Studies

Overview

- Introduce zebrafish as model organism
- Zebrafish embryo toxicity test (ZFET)
- Expanding ZFET
  - Angiogenesis
  - Neurodevelopment
  - Energy metabolism
  - EDA

Zebrafish are a valuable system to study mechanisms of developmental toxicology of environmental chemicals

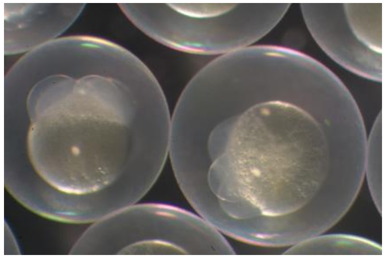
The Zebrafish (Danio Rerio)

- Freshwater fish
- Native to southeastern Himalaya region
- Inhabits streams, canals, ponds, and slow moving water bodies, including rice fields
- Length of the adults 3-4 cm

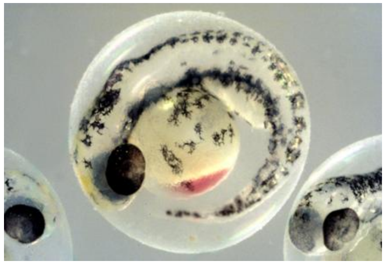
Adult Zebrafish (4-5 month)

Zebrafish are much more then a “tool”

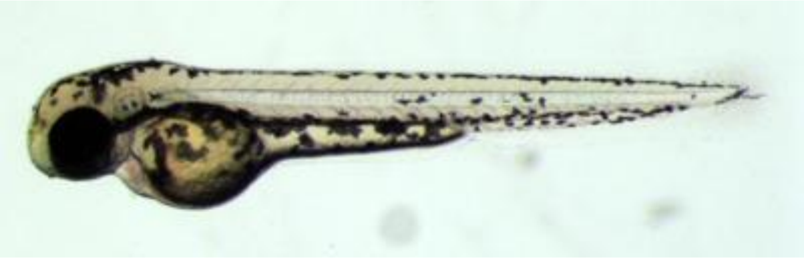
- One female can lay 200-500 eggs per week
- Egg-diameter is around 1 mm
- Clear chorion allows to monitor the early development
- Quick development (hatch at day 3)
- Survive in 96 well plates till day 5
- Till the end of the larva stage they are no „animals“ (replace animal tests)
- Complex organism



1 hour

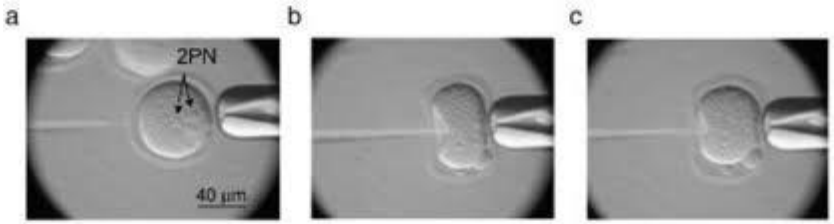


Embryo (2 days)



Larva (3 days)

But we have many “tools” to work with

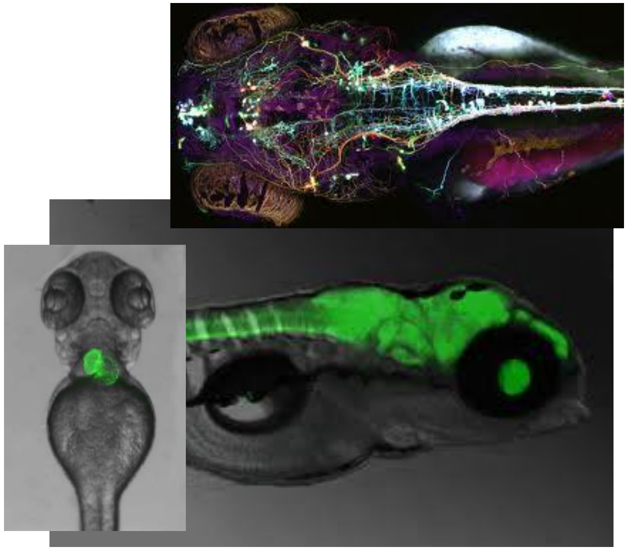


Gene Knock outs

In situ hybridization

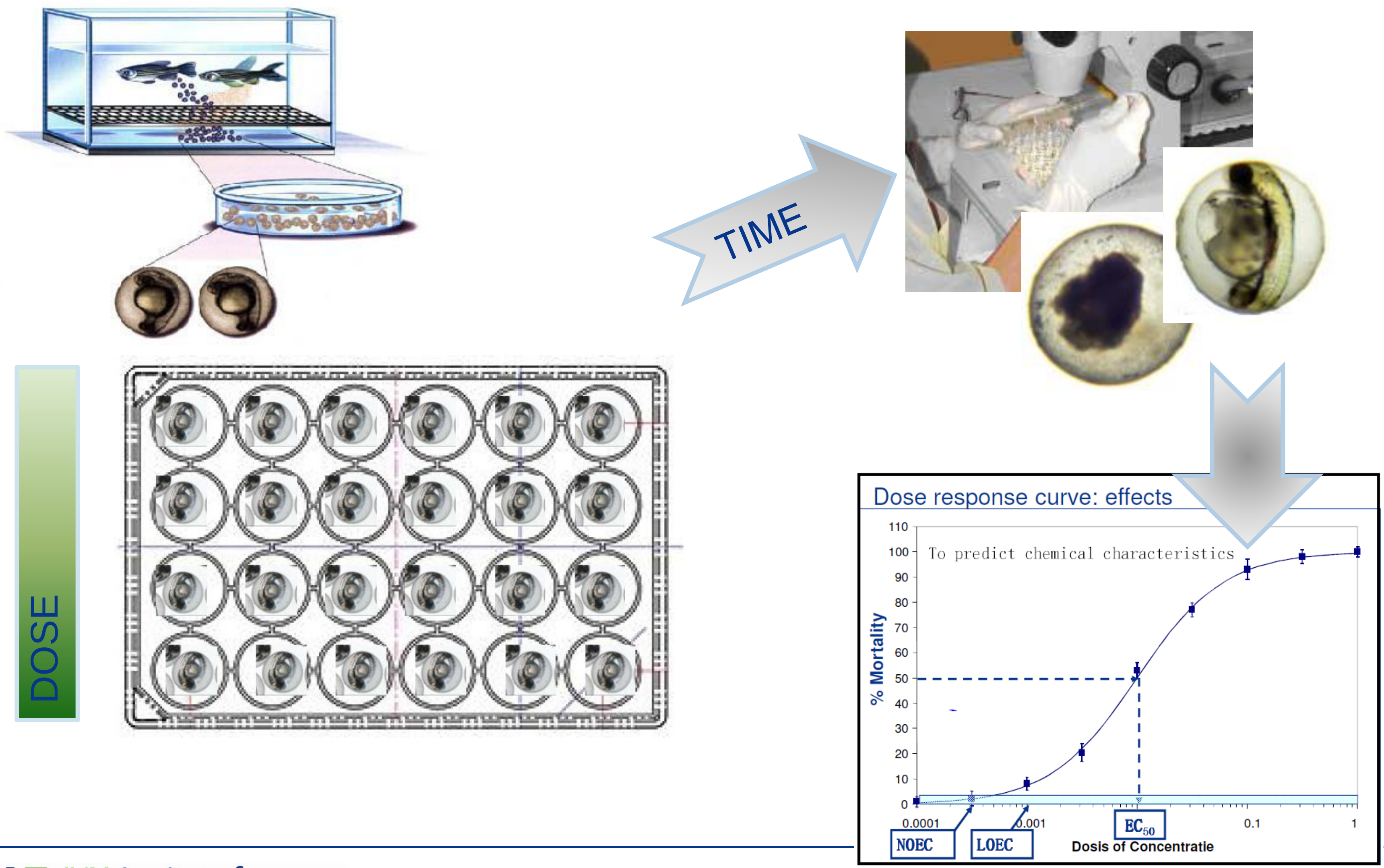
Mutagenic zebrafish

Transcriptomics/ Metabolomics

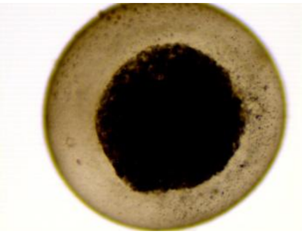


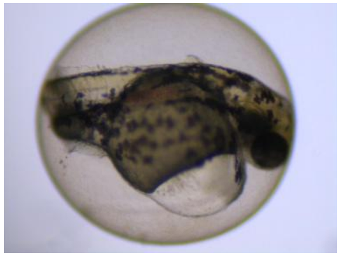
Transgenic zebrafish

Zebrafish embryo toxicity test (ZFET)



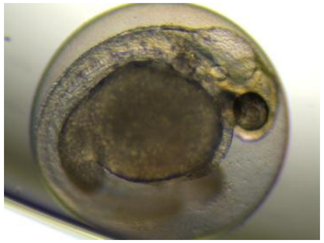
ZFET (Zebrafish embryo toxicity – test)

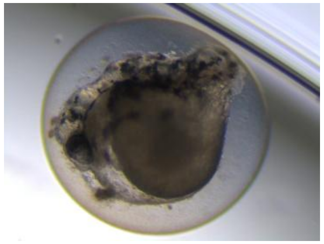


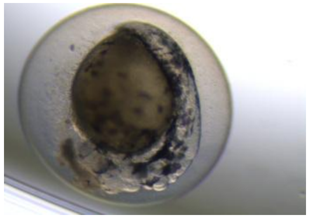


Toxicological endpoints	Exposure time (h)			
Lethal*	**8**	**24**	**48**	**120**
Coagulation	•	•	•	
Tail not detached		•	•	
No somites		•	•	
No heart-beat			•	
Sublethal/Development				
Completion of gastrula	•			
Formation of somites		•		
Development of eyes		•	•	
Spontane		•	•	
Heart bea				
Pigmenta				
Oedema				
Teratogenic				
Malformation of head		•	•	
sacculi/otoliths		•	•	
tail		•	•	
heart		•	•	
modified structure of the corda		•	•	
scoliosis		•	•	
rachischisis		•	•	
deformity of yolk		•	•	
growth-retardation		•	•	
Length of tail**				•

Focus **is not** on Modes of Action
or Adverse Outcome Pathways





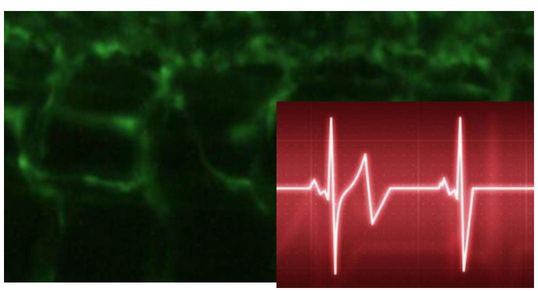


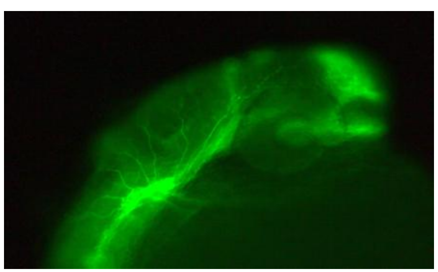
From Nagel *et al*

Expand the ZFET to better understand mechanisms of toxicity

SMART ZFET

- Angiogenesis
- Neurodevelopment
- Energy metabolism





Phenotypic effects of anti-cancer drugs

- ZFET (4hpf-6dpf)

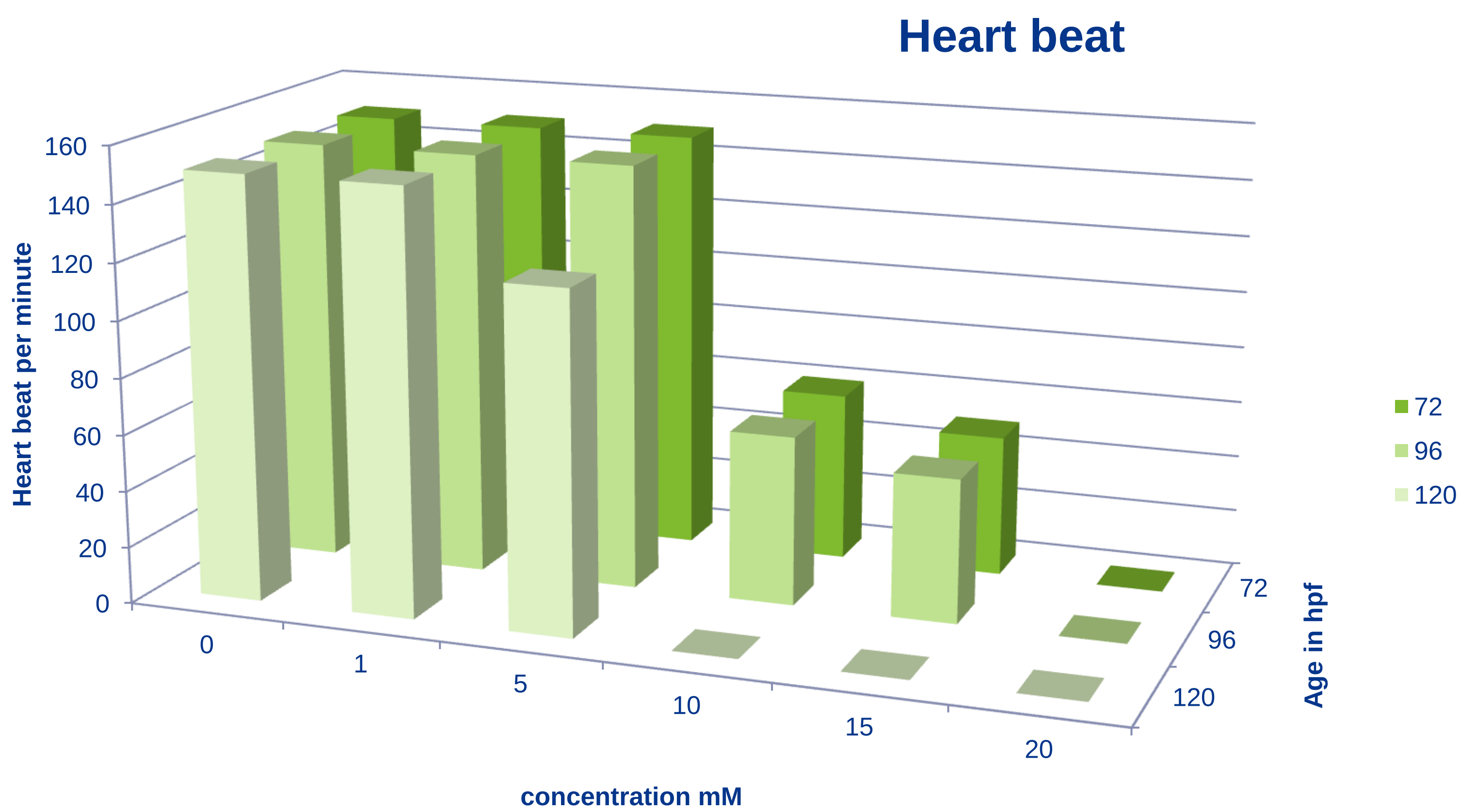
Compound X

mM	24	48	72	96	120	144
0	normal					
1						
5				malformed		
10						
15						
20						dead

Heartbeat

Cardiac edema 4dpf, y mM Compound X

Heartbeat



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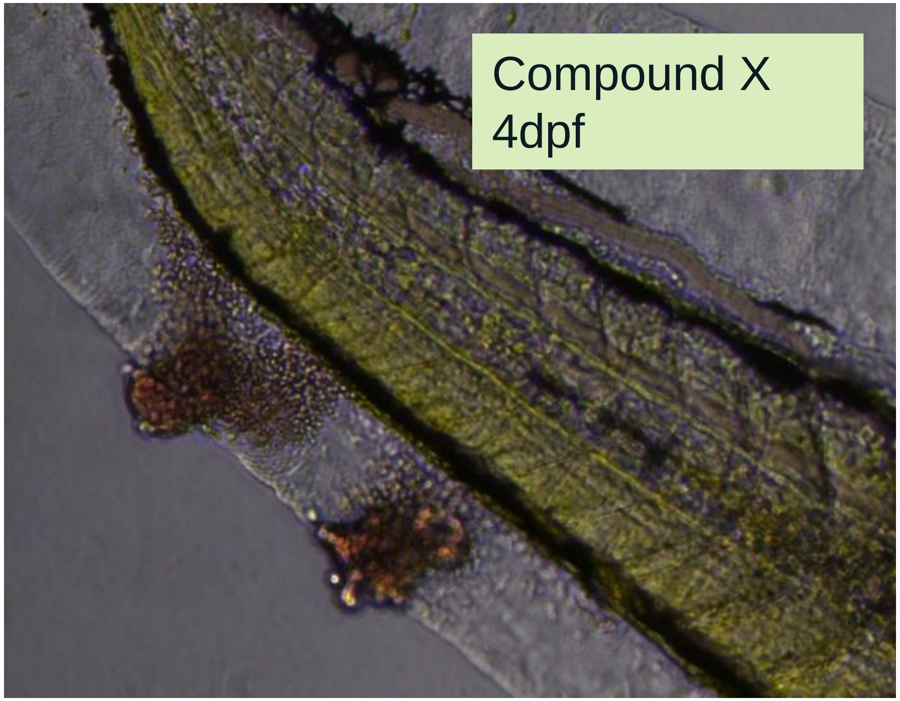
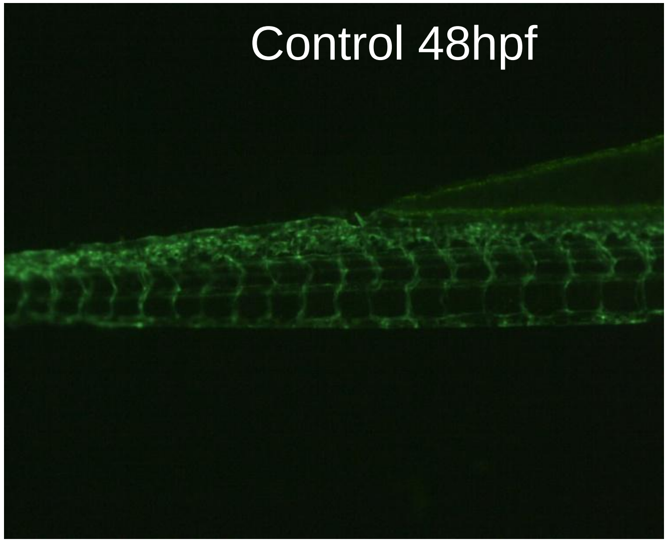
Blood vessel growth

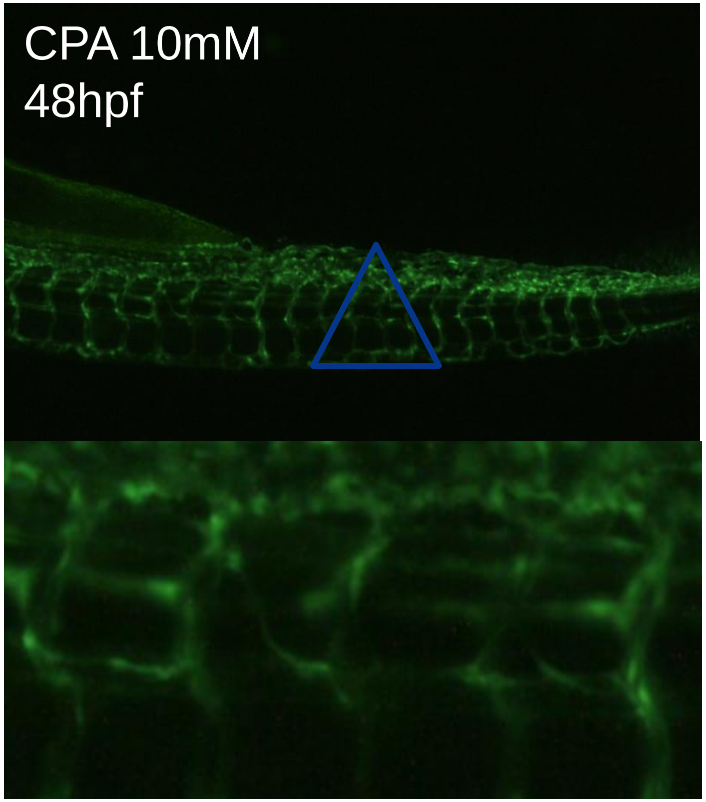
- Cardiovascular toxicity (Casper::FLI)



Blood vessel growth

- Cardiovascular toxicity (Casper::FLI)

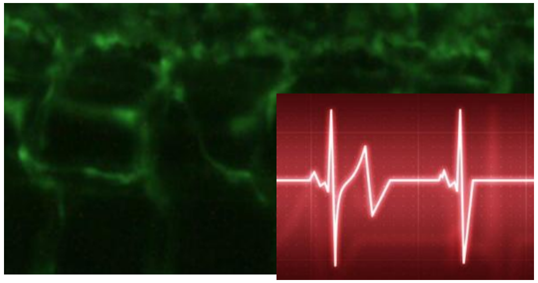


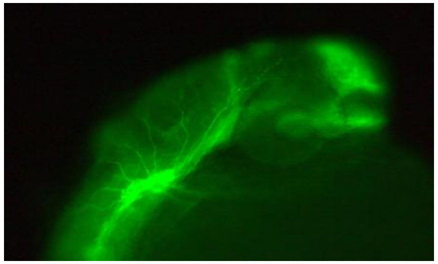


Expand the ZFET to better understand mechanisms of toxicity

SMART ZFET

- Angiogenesis
- Neurodevelopment
- Energy metabolism



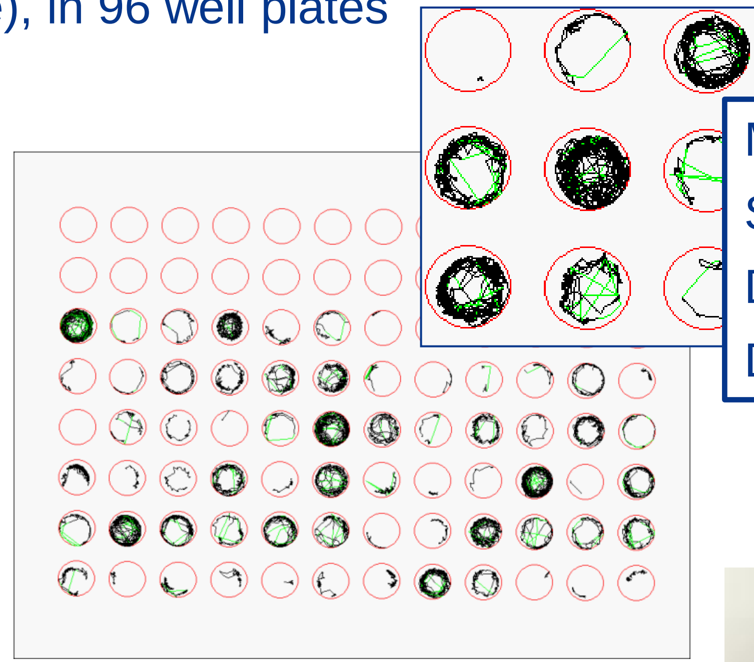


Phenotypic effects of carbamates

	MOA	24 hpf	48hpf	72 hpf	144 hpf	behavior	
aldicarb	AChE inhibitor					hyperactive	
pirimicarb	AChE inhibitor						
methomyl	AChE inhibitor						
carbaryl	AChE inhibitor					hyperactive	

Viewpoint Zebrabox Behavior screen

a startle reflex assay (light response), in 96 well plates



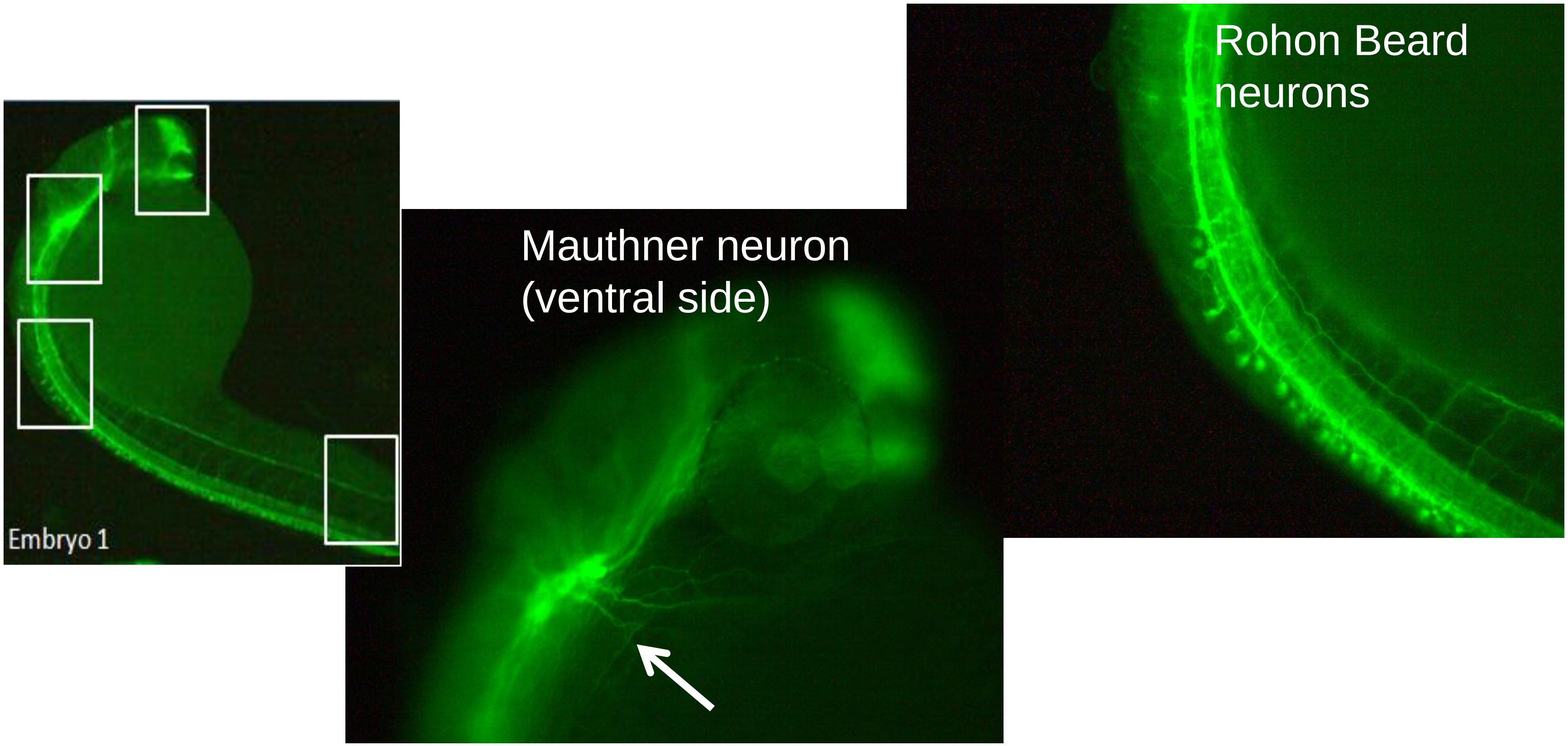
Measure :

Speed

Distance

Duration

Neurite outgrowth

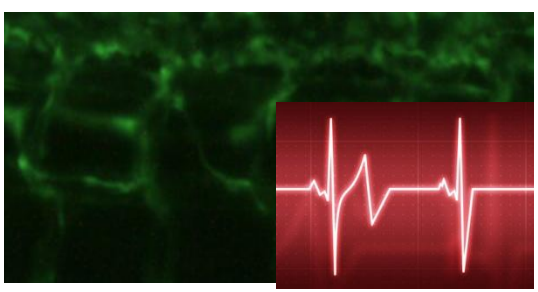


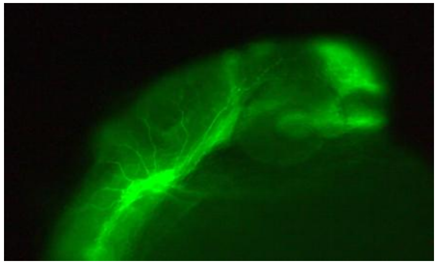
Acetylated alpha-Tubulin Antibody

Expand the ZFET to better understand mechanisms of toxicity

SMART ZFET

- Angiogenesis
- Neurodevelopment
- Energy metabolism



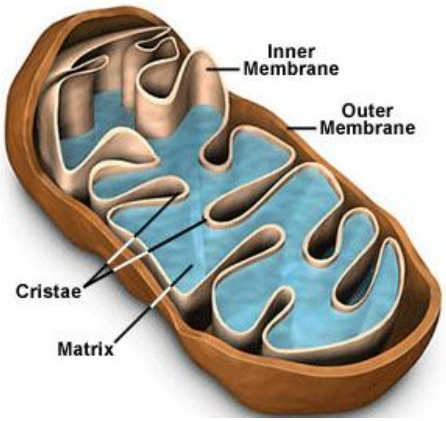


Phenotypic effects of 19 OH-PBDEs

OXPHOSTOX

Aerobic energy metabolism -oxidative phosphorylation (OXPHOS)

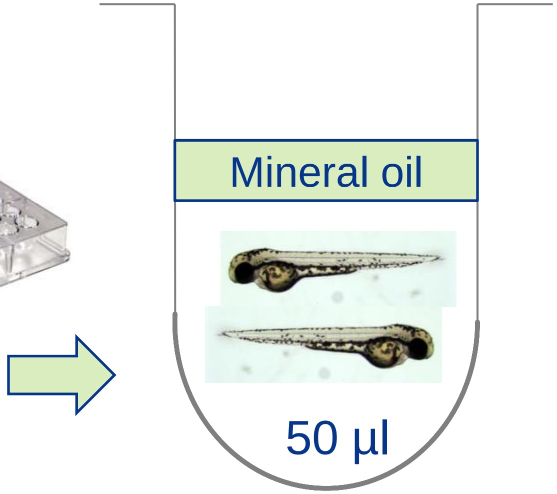
OXPHOSTOX



Energy demand	up
Oxygen consumption	up
CO2 production	up

New in vivo assays for OXPHOS disruption

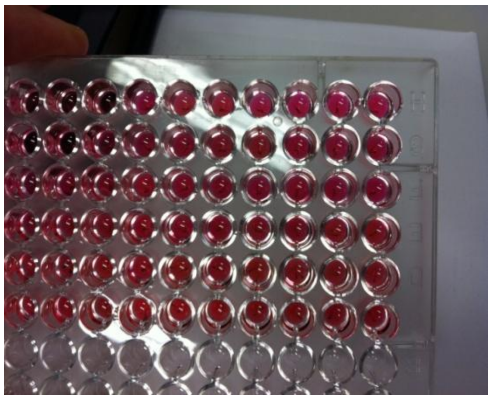
Metabolic rate measurement in humans



Larva 3 days old

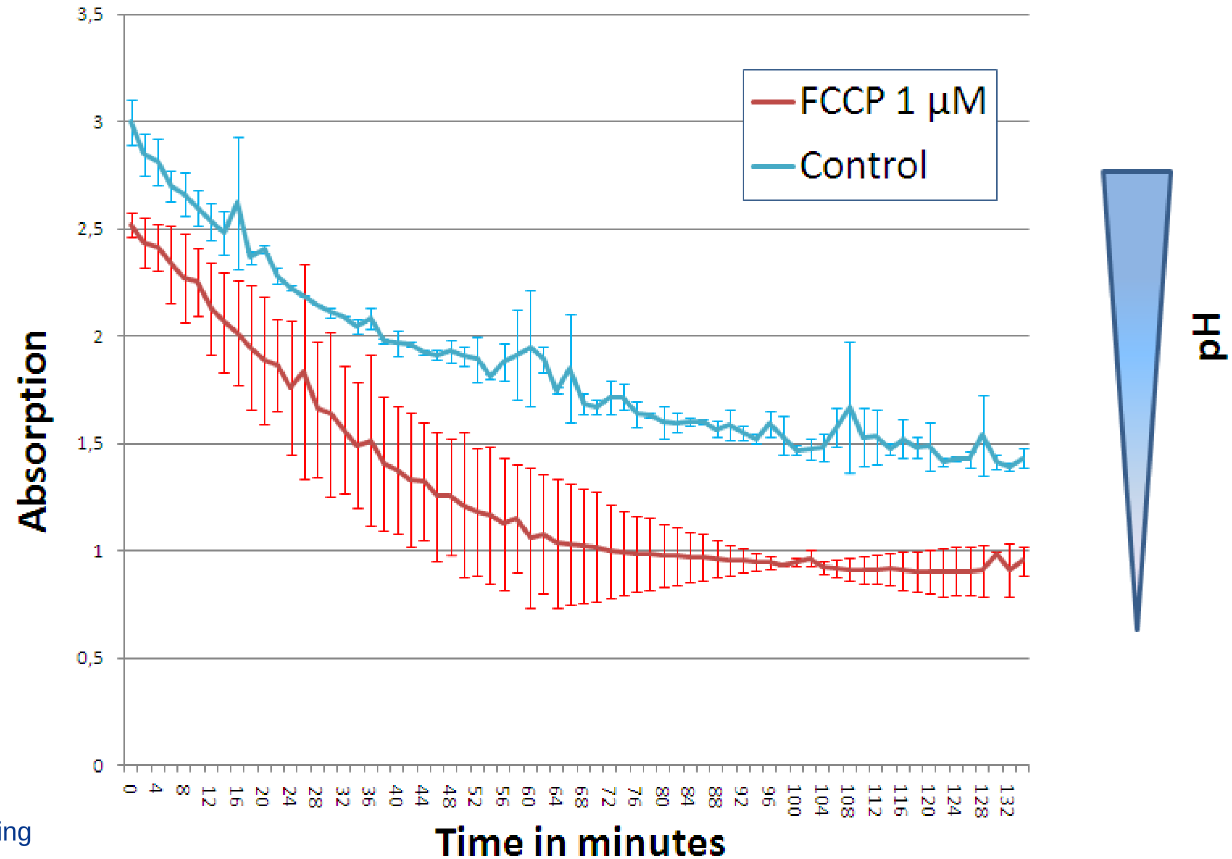
In vivo measurement of CO_2 production

Measure the CO_2 production as acid (H_2CO_3) in the medium via a pH-sensitive dye (phenol red)

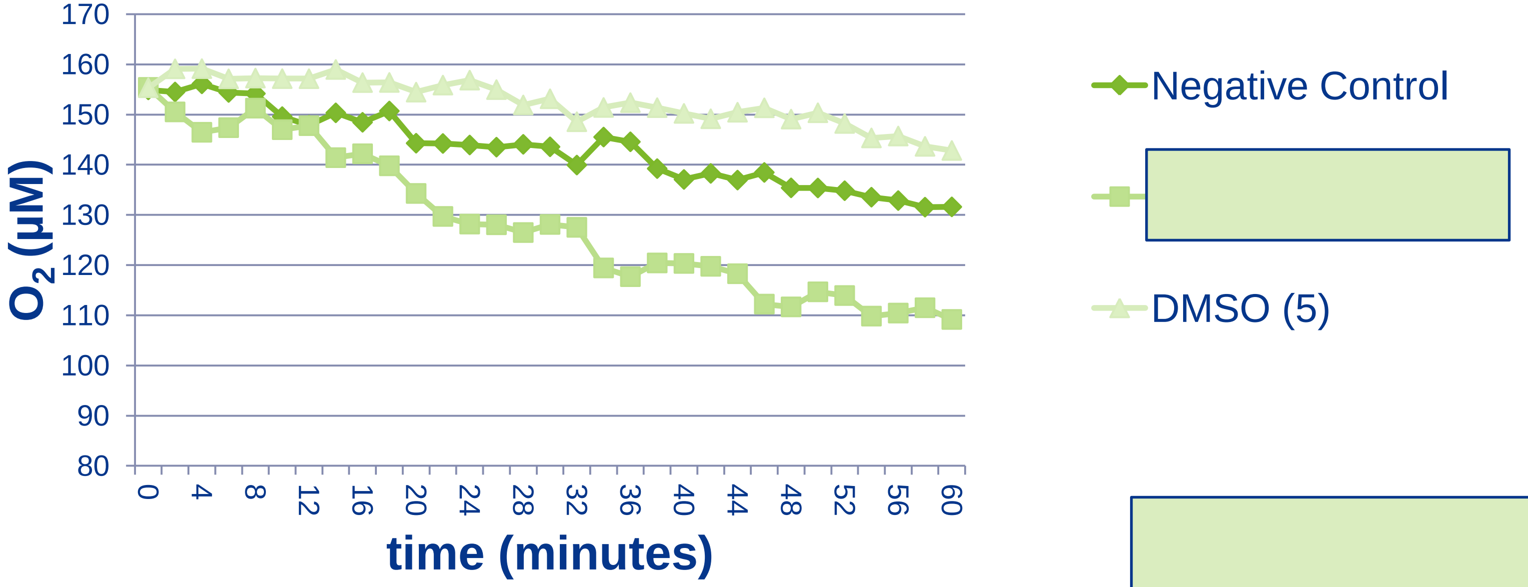


Metabolic rate assay in a 96 well plate

Makky K. (2008), Journal of Biomolecular Screening



In vivo measurement of oxygen consumption



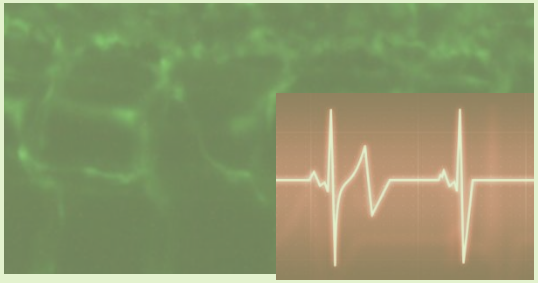
Expand the ZFET to better understand mechanisms of toxicity

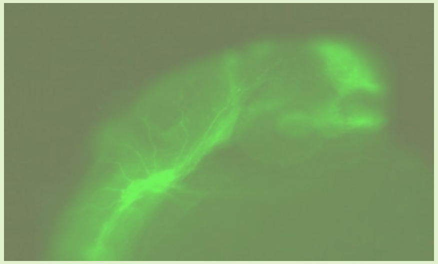
SMART ZFET

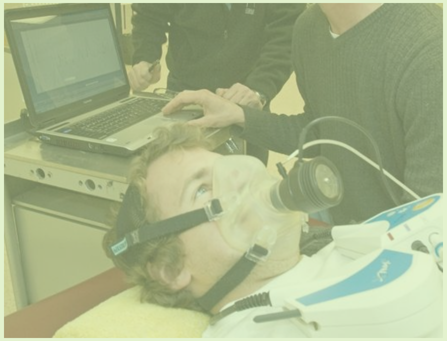
- Angiogenesis

Use SMART ZFET to expand the possibility of chemical analysis—

Effect Directed Analysis (EDA)







Effect directed analysis

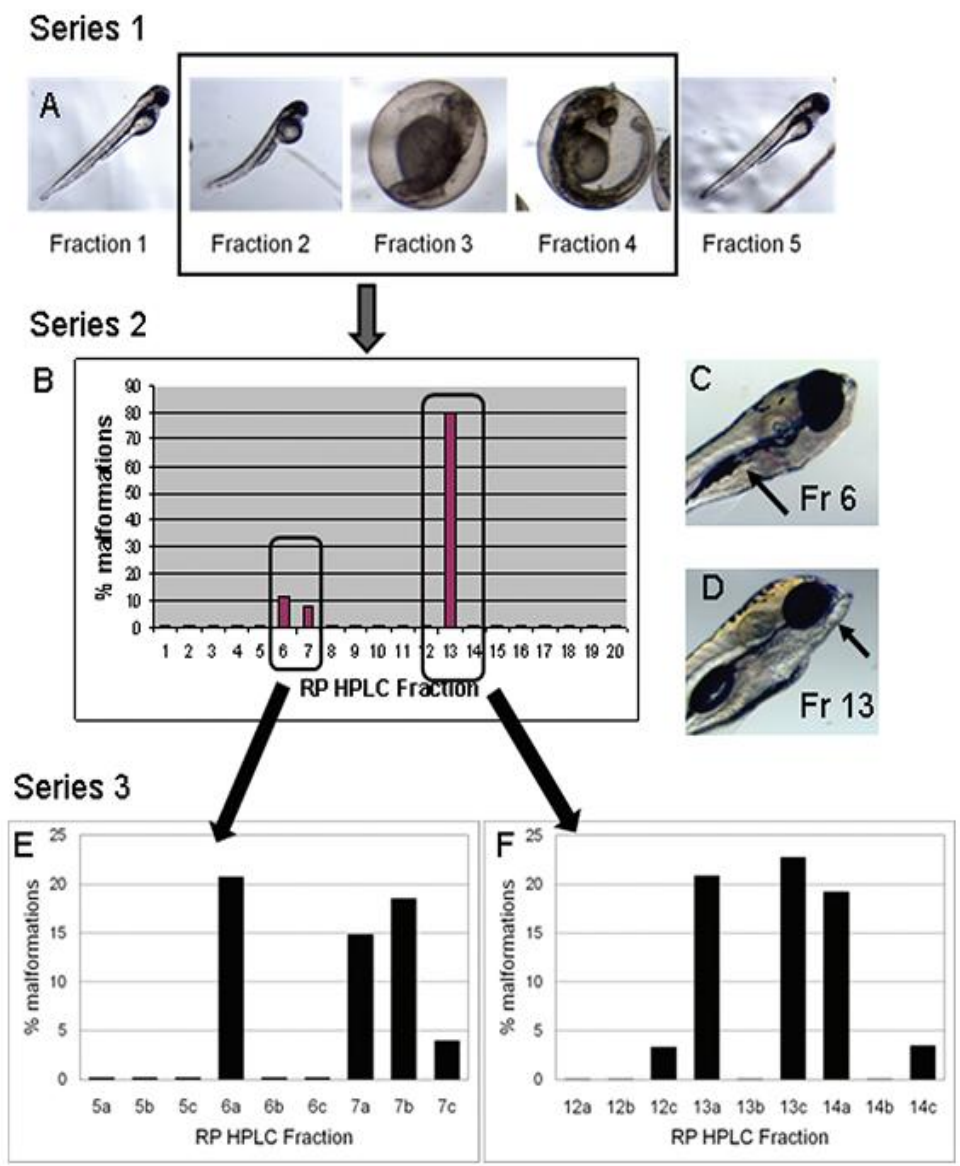
Extraction (ASE DCM/Ac)
Clean up (GPC)

Fractionation
(RP-HPLC)

embryotoxicity

Chemical screen
(GC-MSD + LC Orbitrap MSD

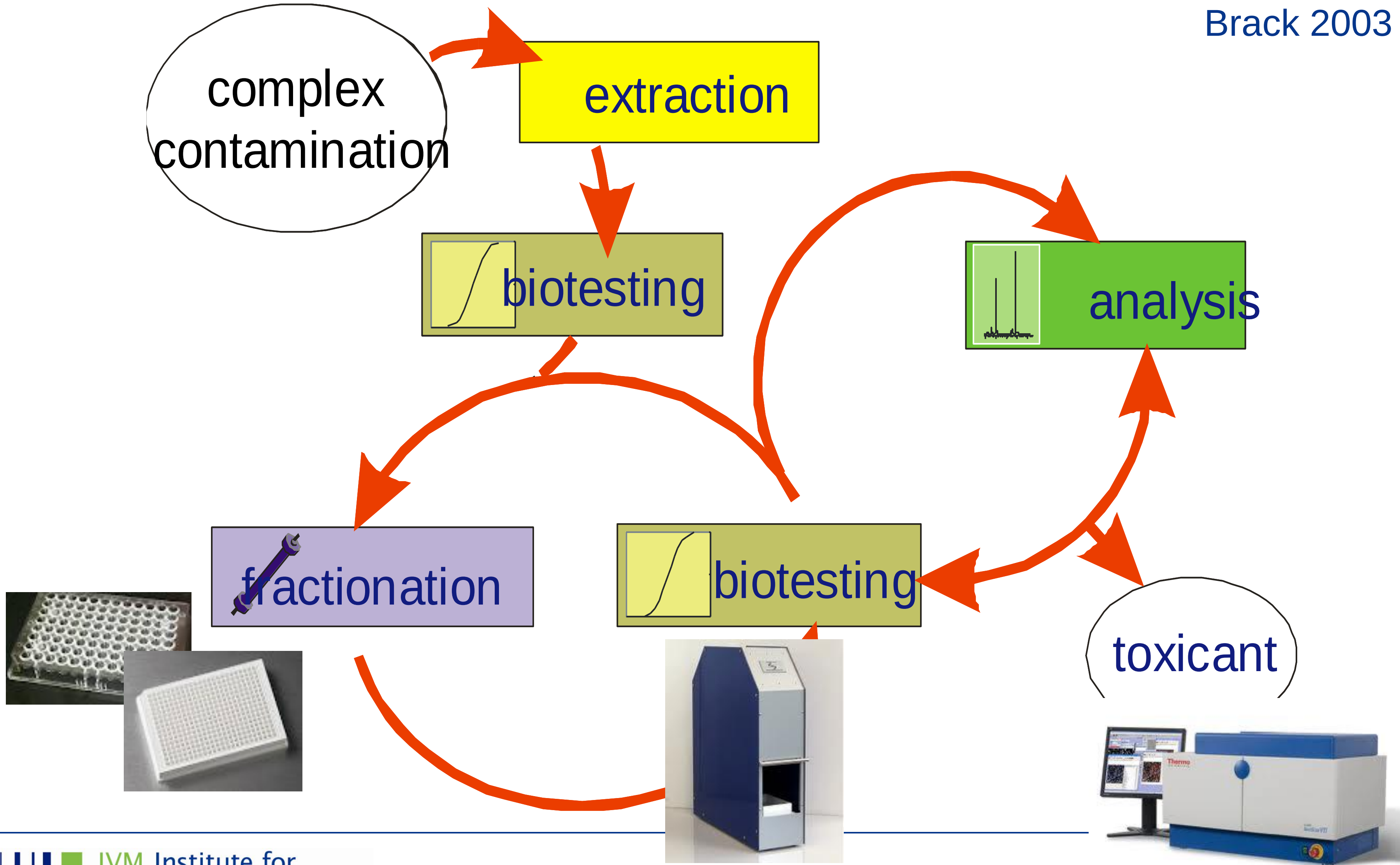
embryotoxicity



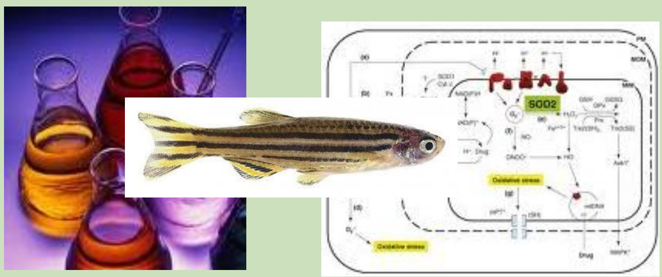
Legler 2011

HT-EDA

Brack 2003

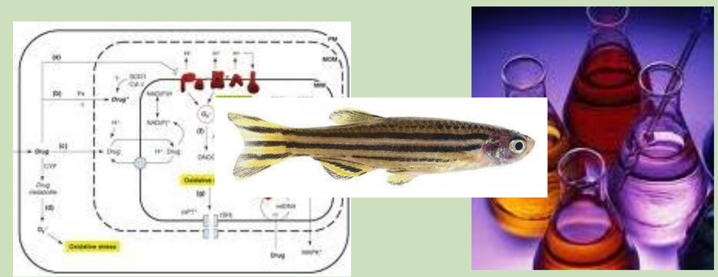


Conclusion



We could link chemicals to mechanisms

- Pharmaceuticals-> Cardiotoxicity
- Pesticides -> Neurotoxicity
- Metabolites of Flameretardents -> Disruption of Energy metabolism



We could also link mechanisms to chemicals

- EDA using mechanistic bioassays in vivo

Acknowledgement

Project coordinator

- John Sumter , Brunel University, UK
- Benoit Roig, Advanced School of Public Health, France

- Pim Leonards, IVM, Netherlands
- Milou Dingemanns, Institute for Risk Assessment Sciences , Netherlands

- Ake Bergman, University of Stockholm, Sweden

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Acknowledgement

- Juliette Legler
- Peter Cenijn
- Ralph Carvalho
- IVM

- Marija Lamoree
- Jana Weiss