

Gill cell line assay for acute fish toxicity prediction: background, FAQs and the FACT database



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Content:

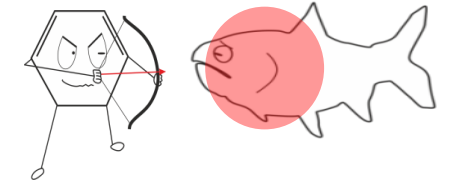
- Where we come from: The fish acute toxicity test (*in vivo*)
- Cells in culture to replace fish?
- From CEFIC-LRI Eco.8 to ISO Standard & OECD Test Guideline (TG)
- OECD TG249-compliant assay procedure
- Validation studies
- Frequently Asked Questions
- FACT db and the fish invitrome



Acute fish toxicity test

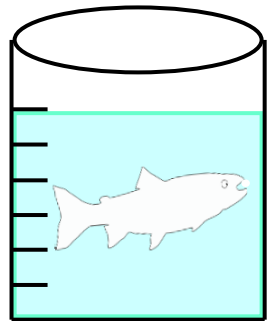
e.g. according to OECD TG203

highest severity
degree (death as
endpoint)

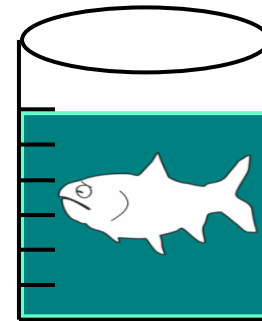
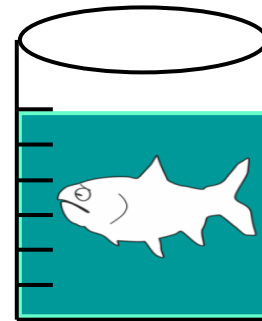
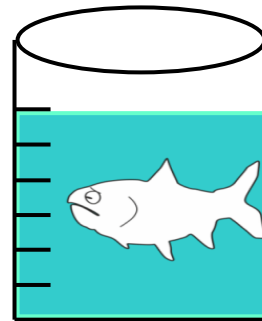
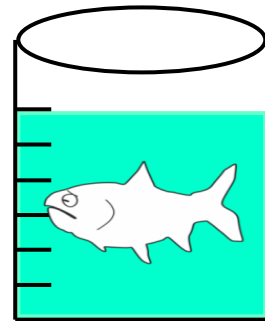
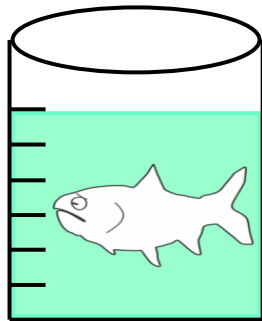


Evans. 1987. Environmental
Health Perspectives 71: 47-58

7-10 fish per aquarium (min: 42 fish)



control



increasing concentrations ($\geq 5x$)



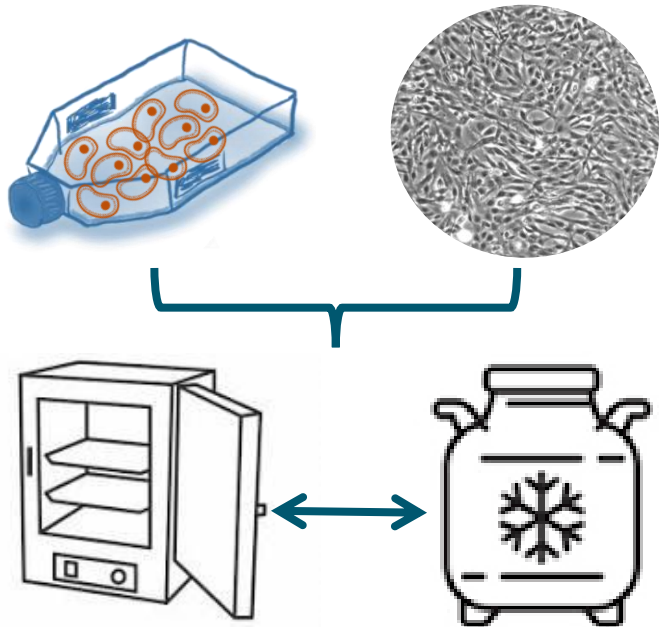
96 hours

Cells as primary site of interaction with chemicals

RTgill-W1

(Rainbow Trout gill cell line)

Bols et al., 1994, J. Fish Dis 17, 601-611



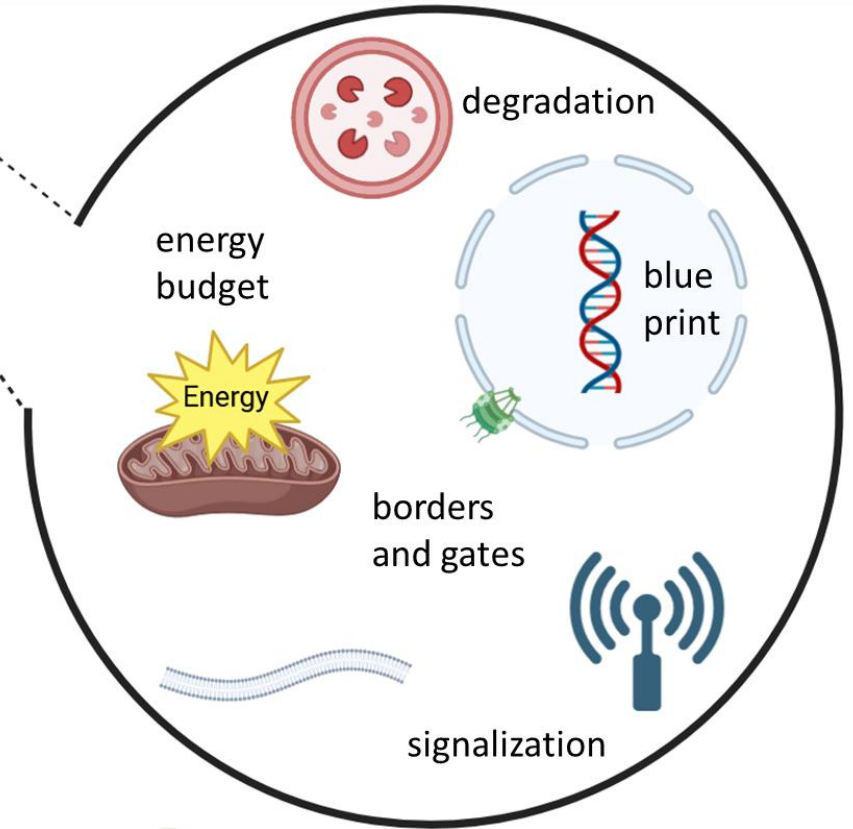
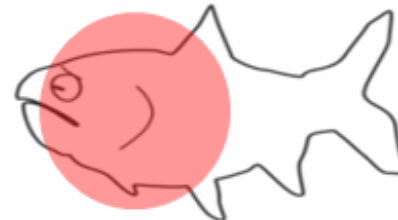
Cell



Gill epithelium



Organism

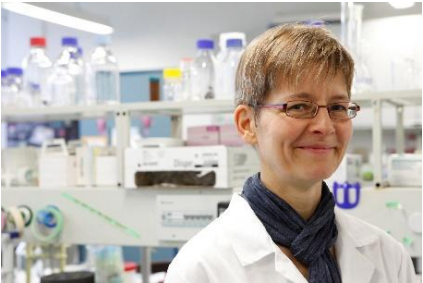


From CEFIC-LRI Eco.8 to ISO Standard & OECD TG

2008



CEFIC-LRI Eco.8



Katrin Tanneberger Melanie Fischer

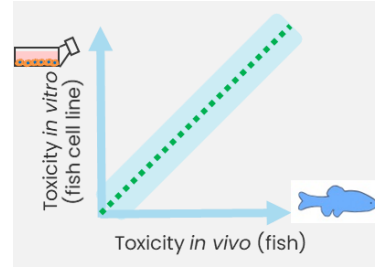
From CEFIC-LRI Eco.8 to ISO Standard & OECD TG

2008



CEFIC-LRI Eco 8

2013



Proof-of-Concept

Tanneberger et al., 2013,
ES&T 47: 1110–1119

[and six publications on the
way of development]



Katrin Tanneberger Melanie Fischer

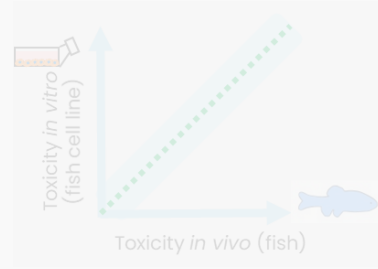
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Tanneberger et al., 2013,
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[and six publications on the
way of development]

~2014-2017



International
Round-Robin

Givaudan[®]

P&G



Partly funded by:



Fischer et al. ToxSci 2019,
169(2), 353–364.



Katrin Tanneberger



Melanie Fischer

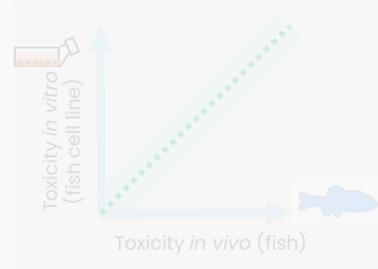
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CEFIC-LRI Eco 8

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Proof-of-Concept

~2014-2017



International Round-Robin



Katrin Tanneberger



Melanie Fischer

Tanneberger et al., 2013,
ES&T 47: 1110–1119

[and six publications on the way of development]



Partly funded by:



Fischer et al. ToxSci 2019,
169(2), 353–364.

2019



ISO Standard 21115

2021



OECD TG249

RTgill-W1 cell line for acute fish toxicity prediction



published in April 2019

Standards catalogue | Publications and products


Home > Store > Standards catalogue > Browse by ICS > 13 > 13.060 > 13.060.70 > ISO 21115:2019

ISO 21115:2019 [Preview](#)

Water quality -- Determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1)



published in June 2021



Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay

The RTgill-W1 cell line assay describes a 24-well plate format fish cell line acute toxicity test using the permanent cell line from rainbow trout (*Oncorhynchus mykiss*) gill, RTgill-W1. After 24 h of exposure to the test chemical, cell viability is assessed based on three fluorescent cell viability indicator dyes, measured on the same set of cells. Resazurin enters the cells in its non-fluorescent form and is converted to the fluorescent product, resorufin, by mitochondrial, microsomal or cytoplasmic oxidoreductases. A reduction in the fluorescence of resorufin indicates a decline in cellular metabolic activity. [More](#)

Published on June 18, 2021 Also available in: French

In series: [OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems](#) ([view more titles](#))

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OECD TG249: Two fully available resources

Section 2
Effects on Biotic Systems

Test Guideline No. 249
Fish Cell Line Acute Toxicity:
The RTgill-W1 cell line assay

14 June 2021

OECD Guidelines for the
Testing of Chemicals

OECD
BETTER POLICIES FOR BETTER LIVES

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)6

Unclassified English or English
8 July 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Validation report for the Test Guideline 249 on Fish cell line acute toxicity - the RTgill-W1 cell line assay

Series on Testing and Assessment,
No. 334

JT03479322

This document, as well as any data and map included herein, are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.

Snap-shot of content – validation report

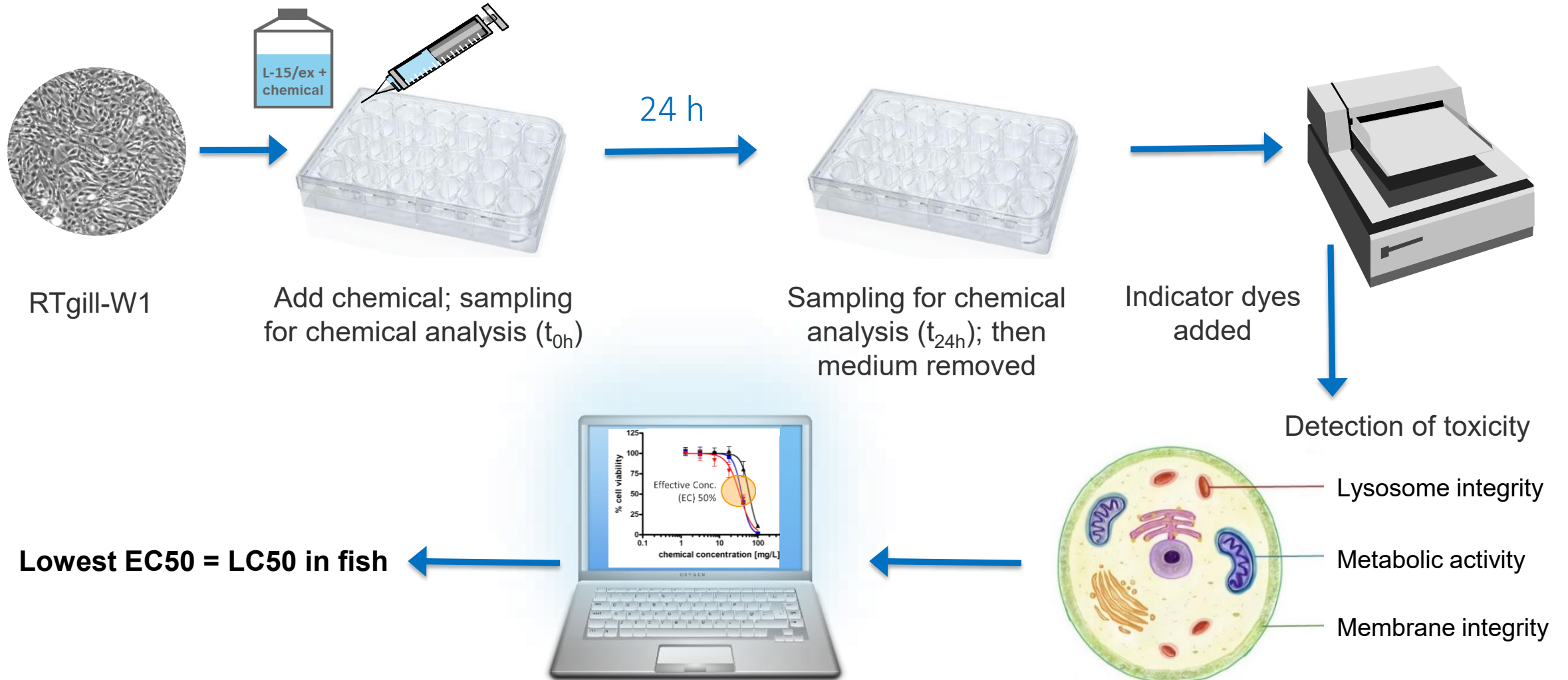
Validation report	6
1. Executive summary	9
1.1 The assay presented in this report	9
1.2. Applicability of the assay	9
1.3. Development and maturity of the assay	9
1.4. Fields of use	10
2. Definitions and abbreviations	11
3. Background and Rational	12
3.1. Scientific Basis	12
3.2. The RTgill-W1 cell line	12
3.2.1. Rational for cell line selection	12
3.2.2. Availability of the cell line	12
3.3. Specific aspects considered during assay development	12
3.3.1. Exposure is performed on monolayers of cells	12
3.3.2. Role of solvent and dosing procedure	12
3.3.3. Plate format	13
3.3.4. Plate cover	13
3.3.5. Verification of exposure concentrations	13
3.3.6. Time of exposure	14
3.3.7. Using a set of cell viability indicator dyes	14
3.3.8. Controls and acceptance criteria	15
3.3.9. Biological replication	16
3.4. Deriving effective concentrations	17
3.5. Predicting fish acute toxicity	18
3.6. Improvements compared to existing methods	20
3.6.1. Throughput	20
3.6.2. Comparison with fish acute toxicity test	20
3.6.3. Comparison with fish embryo acute toxicity test	20
3.6.4. Comparison with other vertebrate cell-line based tests	20
3.7. Compliance with relevant guidance documents: difficult to test chemicals and good <i>in vitro</i> methods practice	21
3.7.1. Difficult to test chemicals	21
3.7.2. Good <i>in vitro</i> method practices	21
4. Studies for assessing method validity	23
4.1. Original description of the application of the assay (Tanneberger et al., 2013)	23
4.1.1. Participating laboratories	23

Excerpt from OECD TG249

This Test Guideline (TG) describes a 24-well plate format fish cell line acute toxicity test using the permanent cell line from rainbow trout (*Oncorhynchus mykiss*) gill, RTgill-W1. After 24 h of exposure to the respective test chemical, cell viability is assessed based on three fluorescent cell viability indicator dyes, measured on the same set of cells. One plate is required per one test chemical and one biological replicate. The test is designed to:

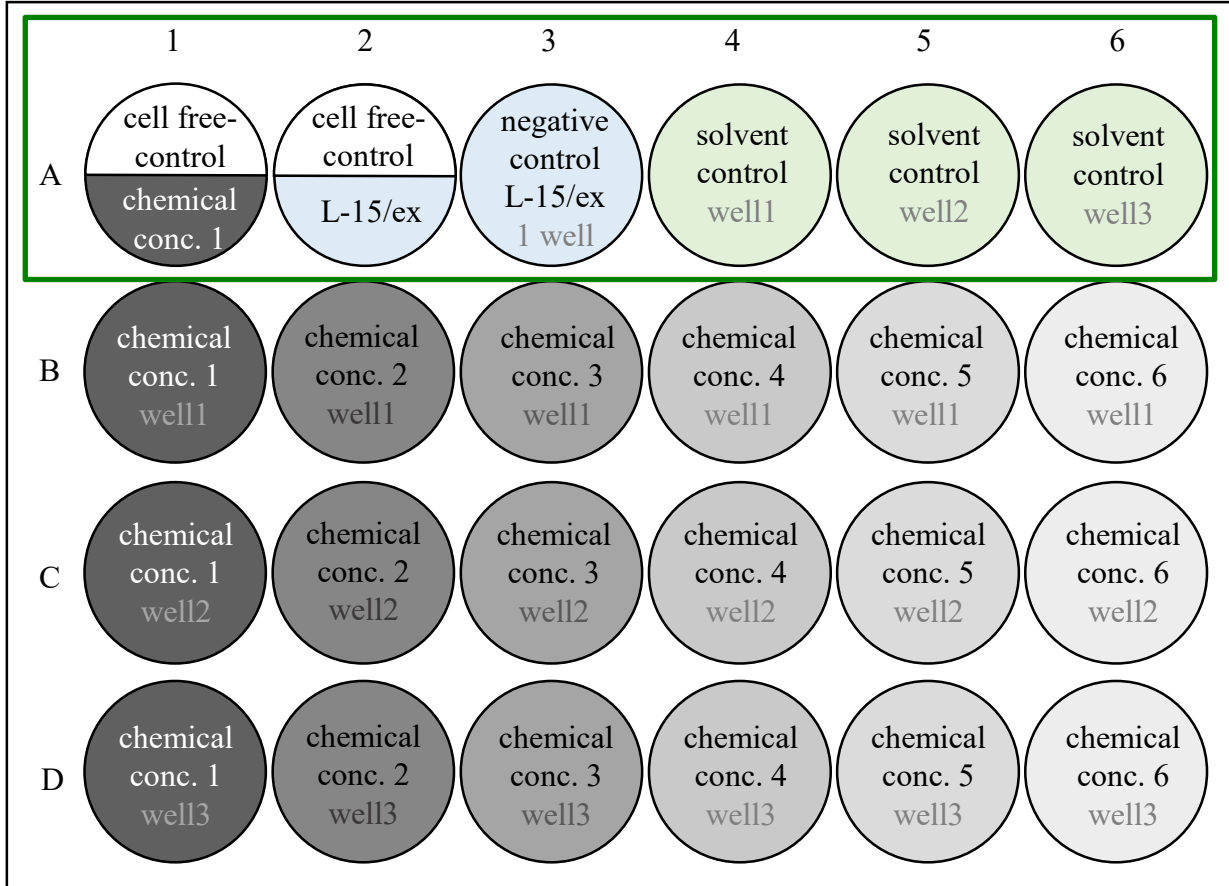
- (i) predict fish acute toxicity in product testing;
- (ii) range-finding and pre-screening before conducting a full fish acute or other fish-based toxicity test;
- (iii) generation of toxicity information to be used for hazard assessment in combination with other lines of evidences (e.g., Quantitative Structure Activity Relationships (QSAR), weight of evidence (WoE)) within Integrated Testing Strategy (ITS)/Integrated Approach to Testing and Assessment (IATA).

Assay procedure



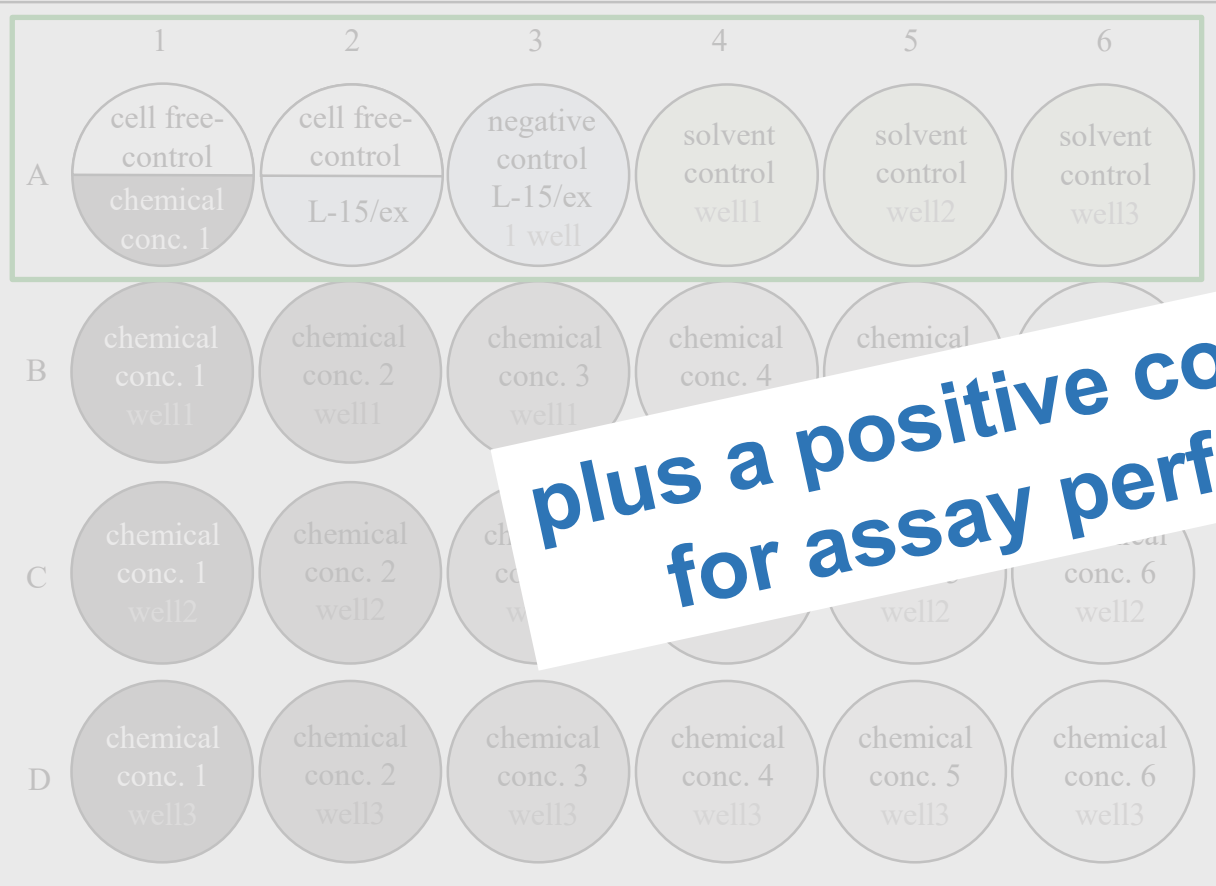
1 plate = 1 chemical = 1 biological replicate

delivery via organic solvent ($\leq 0.5\%$)



1 plate = 1 chemical = 1 biological replicate

delivery via organic solvent ($\leq 0.5\%$)



delivery via L-15/ex

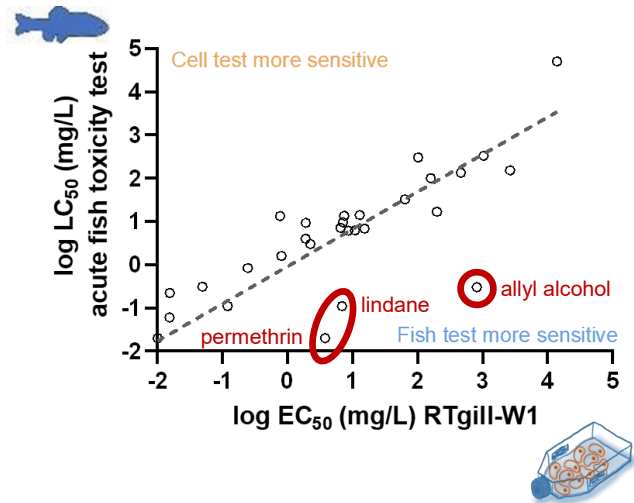


**plus a positive control plate per run
for assay performance control**

Validation – organic chemicals

see OECD 249 validation report

wide range of chemicals

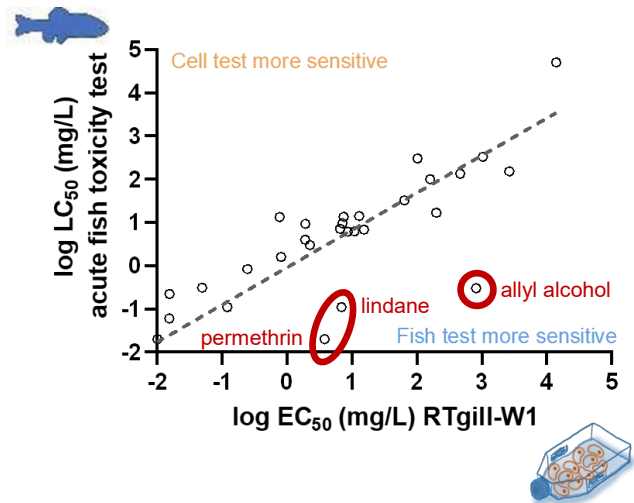


Tanneberger et al., 2013, ES&T 47: 1110–1119

Validation – organic chemicals

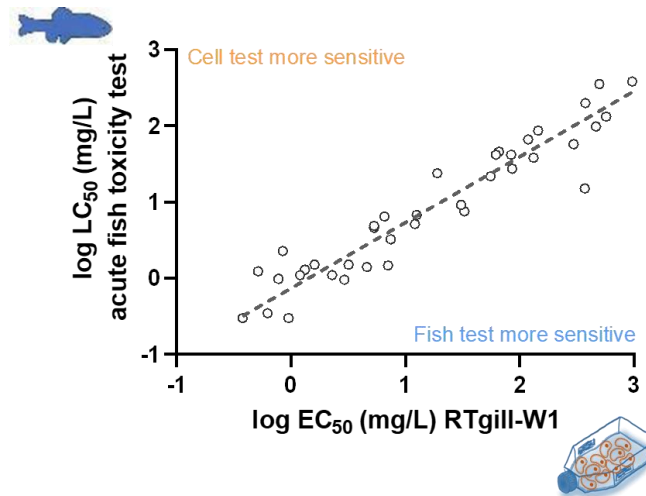
see OECD 249 validation report

wide range of chemicals



Tanneberger et al., 2013, ES&T 47: 1110–1119

fragrances



Natsch et al., 2018, ET&C 37(3): 931-941

Collaboration: aQuaTox-Solutions/
BASF [SETAC Europe 2024]

pesticides

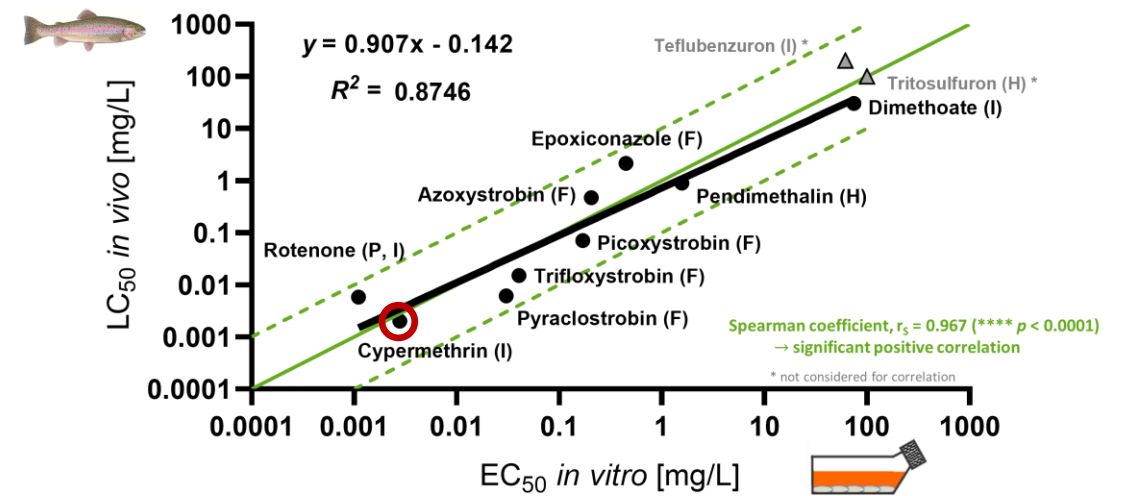
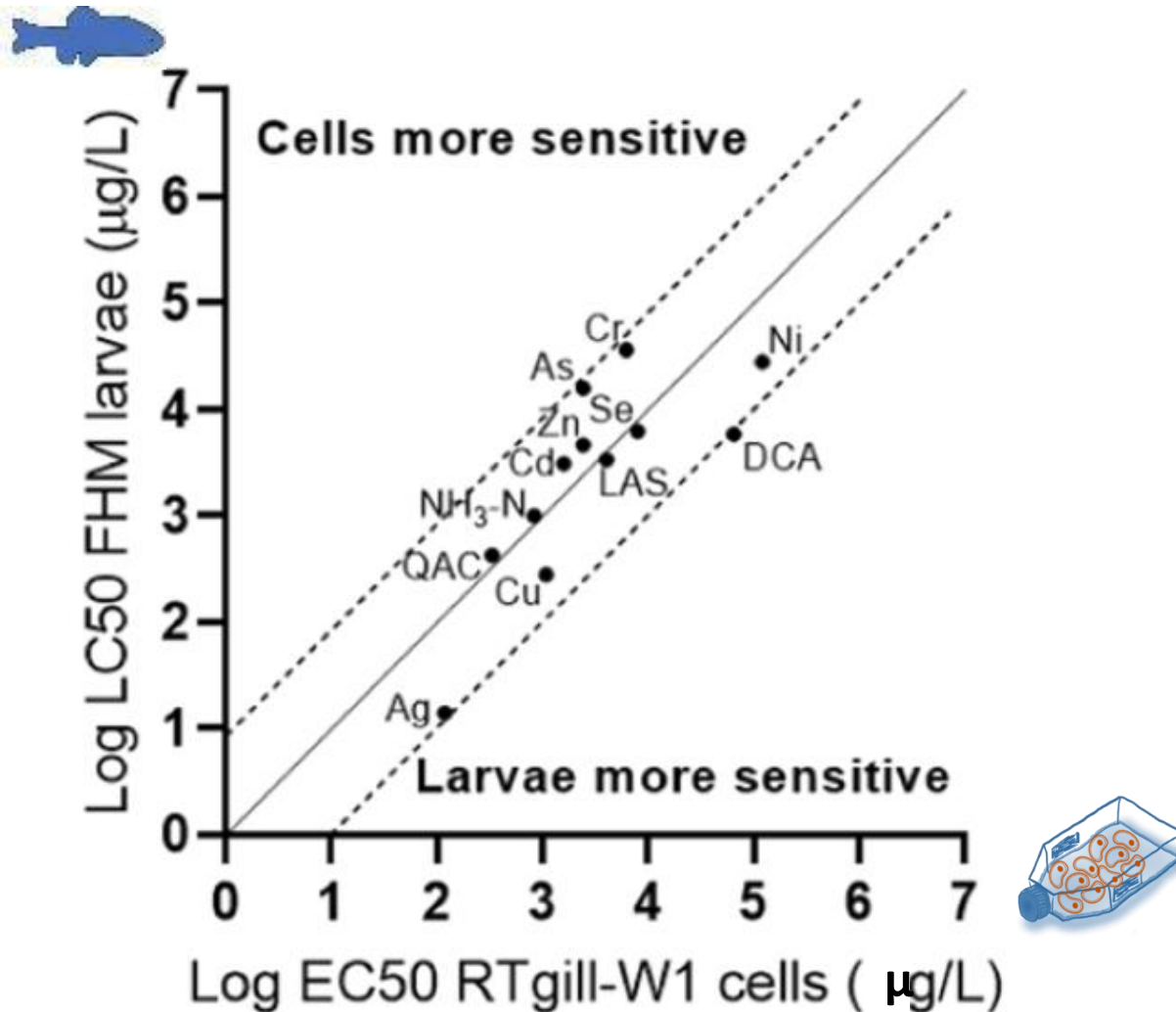


Figure 2: Linear correlation of *in vivo* LC₅₀ (rainbow trout) and *in vitro* EC₅₀ (RTgill-W1 cell line) values. A significant positive linear relationship was found for a set of substances with various MoA and over a broad concentration range.

Validation – inorganics/metals



Comparison fathead minnow larvae vs. RTgill-W1 cell line assay; see Scott et al., 2022. ET&C 41:2721–2731.

Frequently Asked Questions



- (1) Chemical analysis possible?
- (2) Biotransformation capacity?
- (3) Applicability domain?
- (4) What about the outliers?

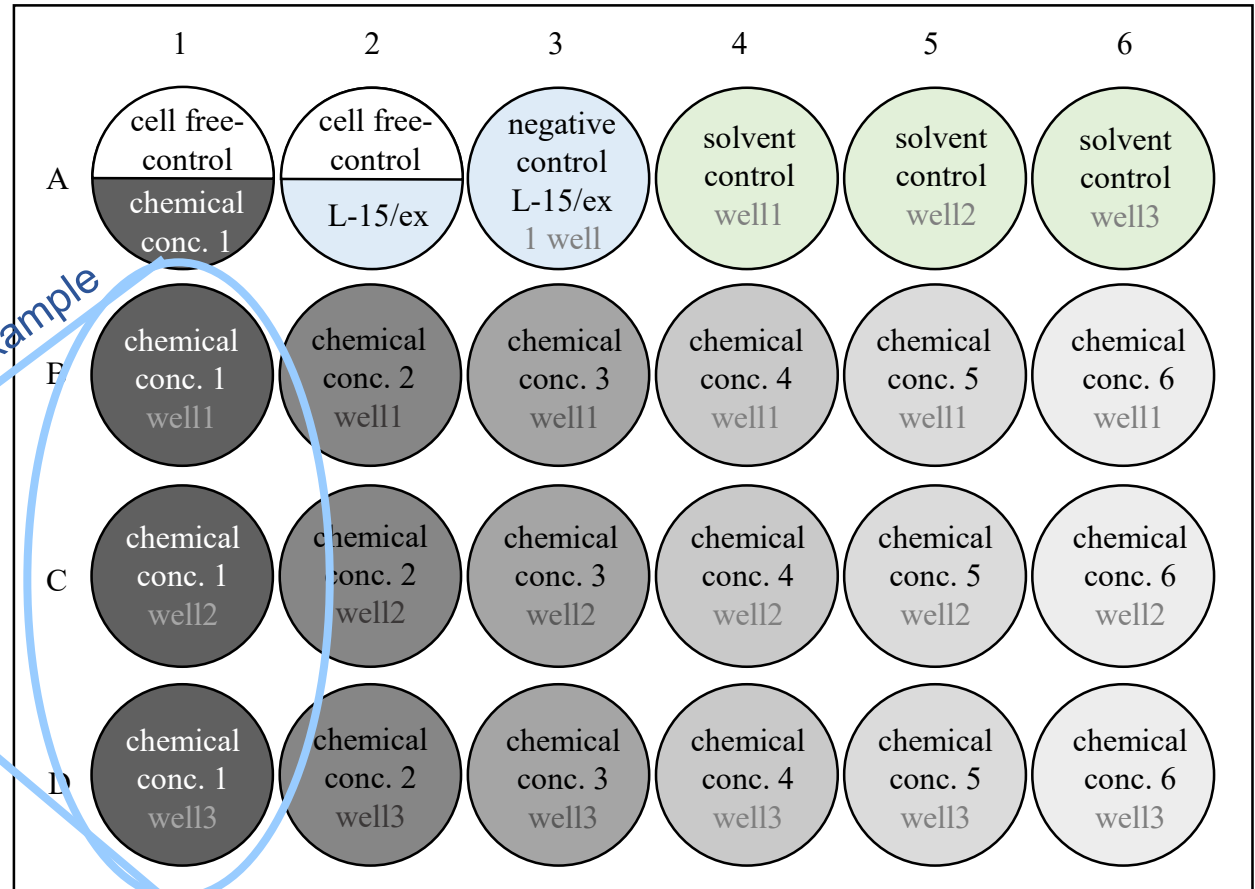
FAQ (1) – Verification of exposure concentrations

Is there sufficient volume to quantify?

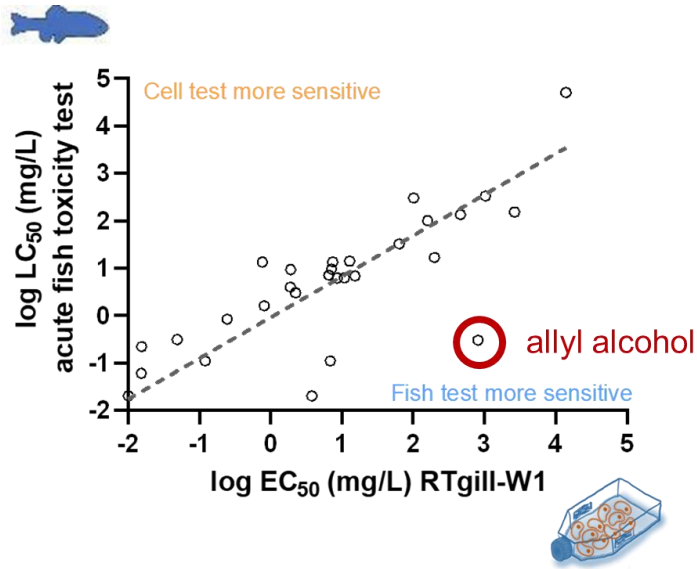
YES

	Time 0 h	Time 24 h
regular sample volume	3 x 0.5 mL (pooled and split into 2)	3 x 0.5 mL (pooled and split into 2)

for example



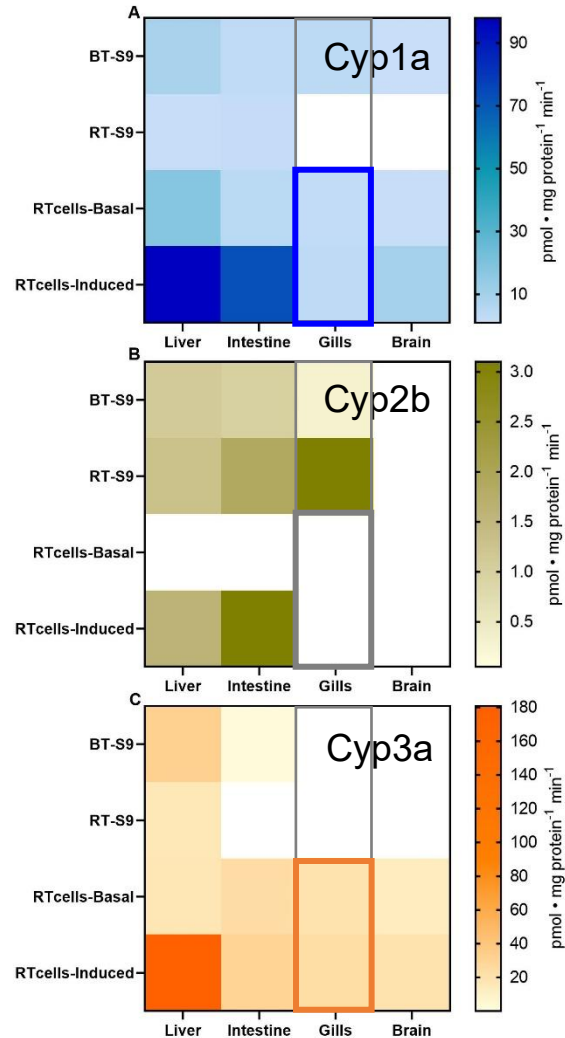
FAQ (2) – Bio(de)activation



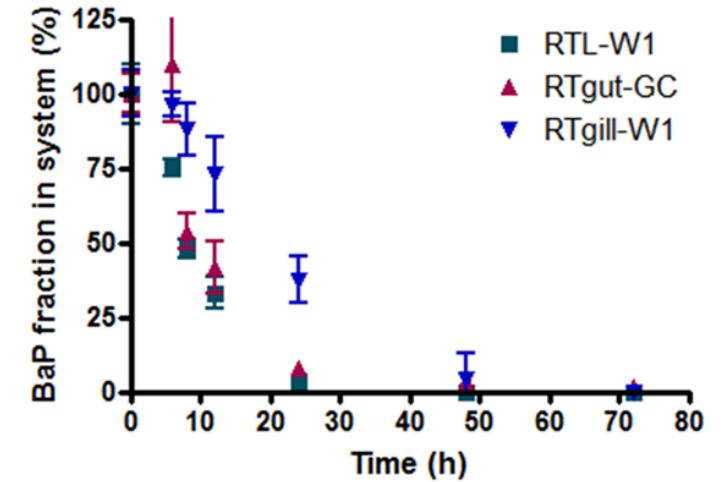
Tanneberger et al., 2013, ES&T 47: 1110-1119

Martin et al., 2025, Cell Biol Toxicol 28, 41: 37

Example:
max. reaction rates



Example:
biotransformation of BaP



Stadnicka-Michalak et al., 2018, ES&T 52: 3091-3100

→ RTgill-W1 possess
biotransformation activity

FAQ (3) – Applicability domain

Does the assay work for difficult to test substances?

YES

Does it work for mixtures?

YES

Validation report, section 3.7.1: Difficult to test chemicals.

Property	Value	Tanneberger et al / # of chemicals out of total	Natsch et al / # of chemicals out of total
logK _{ow}	> 4	8/35	13/38
Water solubility (@ 25 °C)	< 100 mg/L	12/35	19/38
Henry' Law Constant	> 0.1 Pa m ³ /mol ^s	18/35	31/38

To date, e.g.

- logK_{ow} from -4 up to 7.6 (*even up to an estimated logK_{ow} of 20*)
- logHLC from -13 to 0 (atm m³/mol)
- Chemicals of various size, complexity and charge (e.g. MW up to 700'000, anionic, cationic...)
- Surfactants
- Fragrances
- Pesticides
- Industrial chemicals
- Polymers
- Pharmaceuticals
- Particles
- Inorganics/Metals

FAQ (4) – What about the outliers?

- Allyl alcohol → insufficiently bioactivated to Acrolein?
 - Permethrin/Lindane → specifically acting neurotoxic chemicals?
- Make use of multiple lines of evidence: “you’ll never walk alone”



Special Series

Daphnids can safeguard the use of alternative bioassays to the acute fish toxicity test: a focus on neurotoxicity

Christoph Schür^{1,2}, Martin Paparella^{3,*}, Christopher Faßbender⁴, Gilly Stoddart⁴, Marco Baitj Jesi², and Kristin Schirmer^{1,5}

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²Department of Systems Analysis, Integrated Assessment and Modelling (SIAM), Swiss Federal Institute of Aquatic Science and Technology, Eawag, Switzerland

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C.S. and M.P. shared the majority of the work and are ranked according to their contributions.

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Advance access publication: January 21, 2025

Original Article



Conclusions

- The RTgill-W1 cell line assay allows fish acute toxicity predictions without fish.
- High predictivity has been demonstrated for a wide range of chemicals.
- Uptake by various actors provides for a continually growing knowledge base.



*It marks the start of a journey
towards fish cell line-based toxicity predictions
for chemical risk assessment and other fields of application.*

OECD TG249 – the bigger picture

Fish Acute Cell Toxicity data base



FACTdb About About eawag

Search by name, CAS-Number, ...

All Chemicals
All Particles
 OECD compliant

All Cell Lines
RTgill-W1 (O. mykiss)
RTgutGC (O. mykiss)

All Endpoints
alamarBlue
Neutral Red

All Conc. Determination
nominal
measured

All timepoints
1
2

1192 entries found. Previous 1 2 3 4 ... 118 119 120 Next 10 entries per page CSV Excel

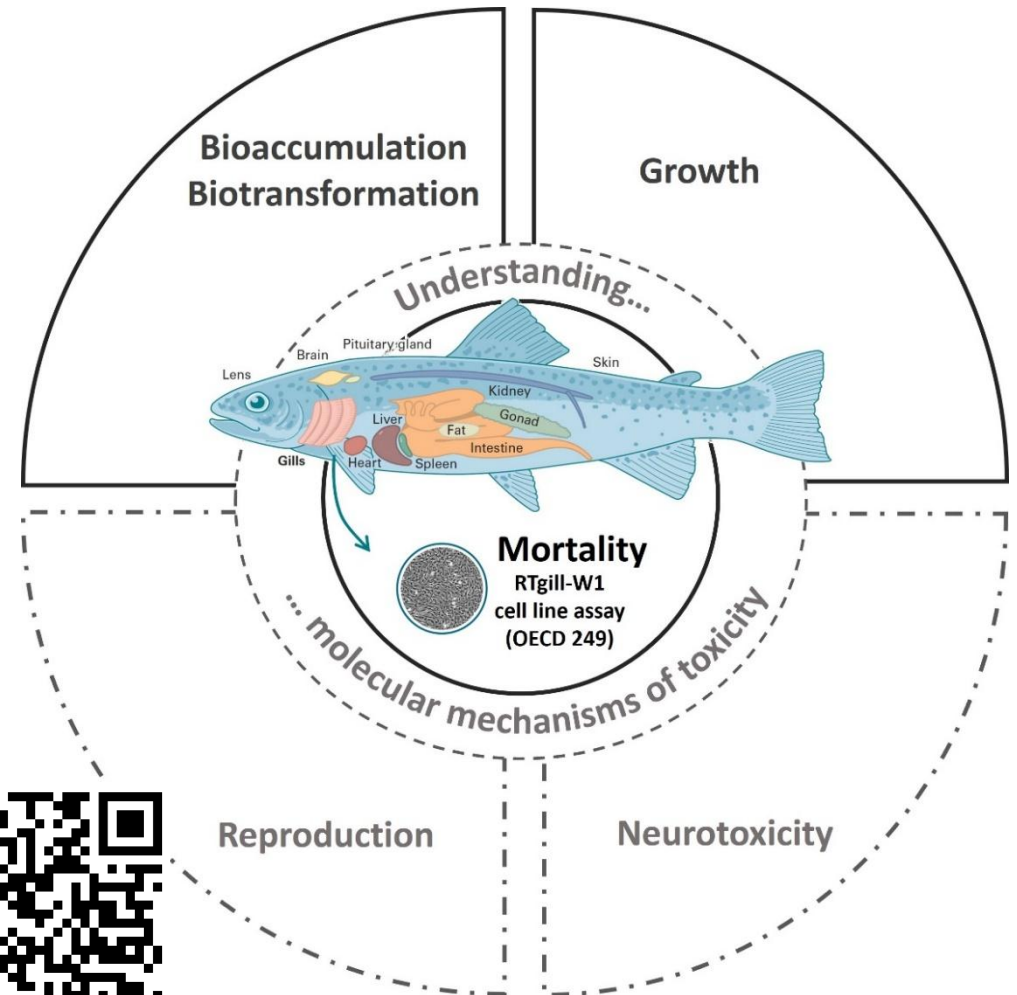
Chemical/Particle/UVCB/Mixture	Cell Line	Endpoint	Timepoint [h]	Conc. determination	EC50 [mg/L]	Details	Data Source
1,2,4-Trichlorobenzene	RTgill-W1 (O. mykiss)	CFDA-AM	24	nominal	7.278 (5.674-9.335)	Details DOI	
1,2,4-Trichlorobenzene	RTgill-W1 (O. mykiss)	alamarBlue	24	nominal	2.185 (1.864-2.561)	Details DOI	



Learn more:

<https://www.eawag.ch/en/departement/utox/projects/fact-fish-acute-cell-toxicity-database/>

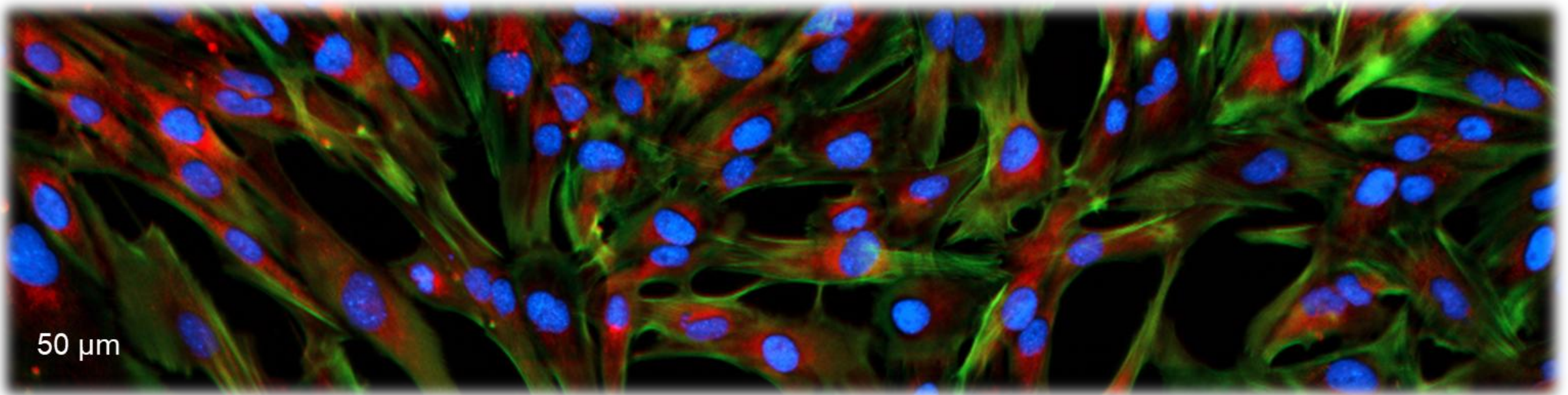
Fish invitrome for chemical risk assessment



Thank you!

Contact:

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Eawag, Swiss Federal Institute of Aquatic Science and Technology
Dübendorf, Switzerland
Email: Kristin.Schirmer@eawag.ch



Stained RTgill-W1 cells: **Nuclei (blue)**, **F-actine (green)**, **Endoplasmic reticulum (red)** Photo: Barbara Jozef, Eawag