

Hazard assessment of fragrances using fish cell lines

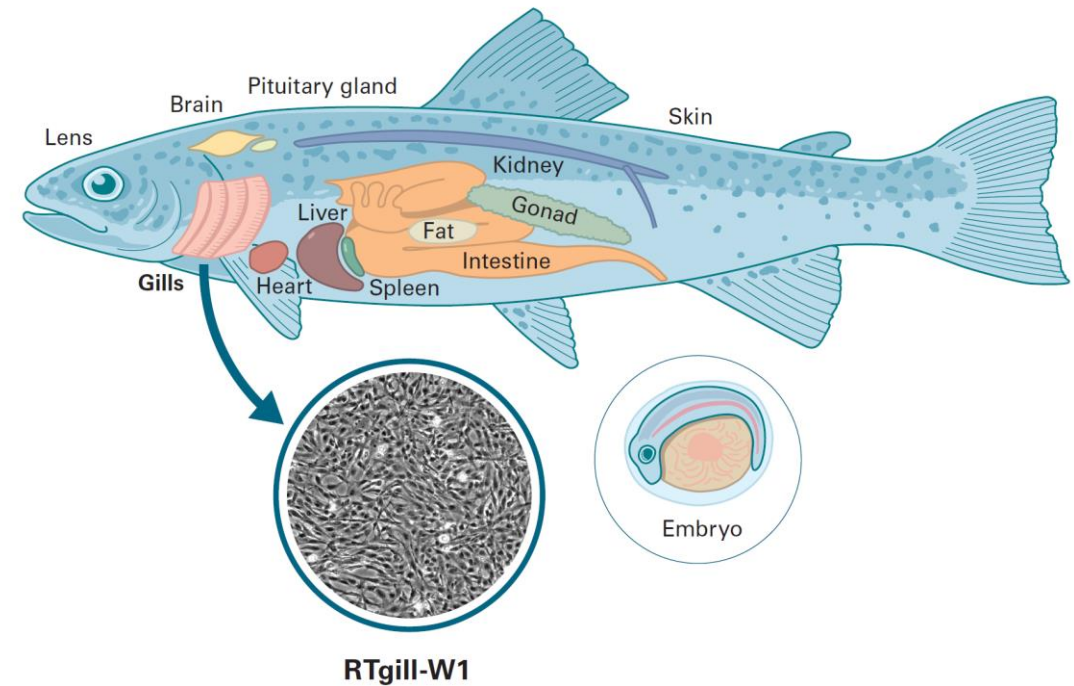
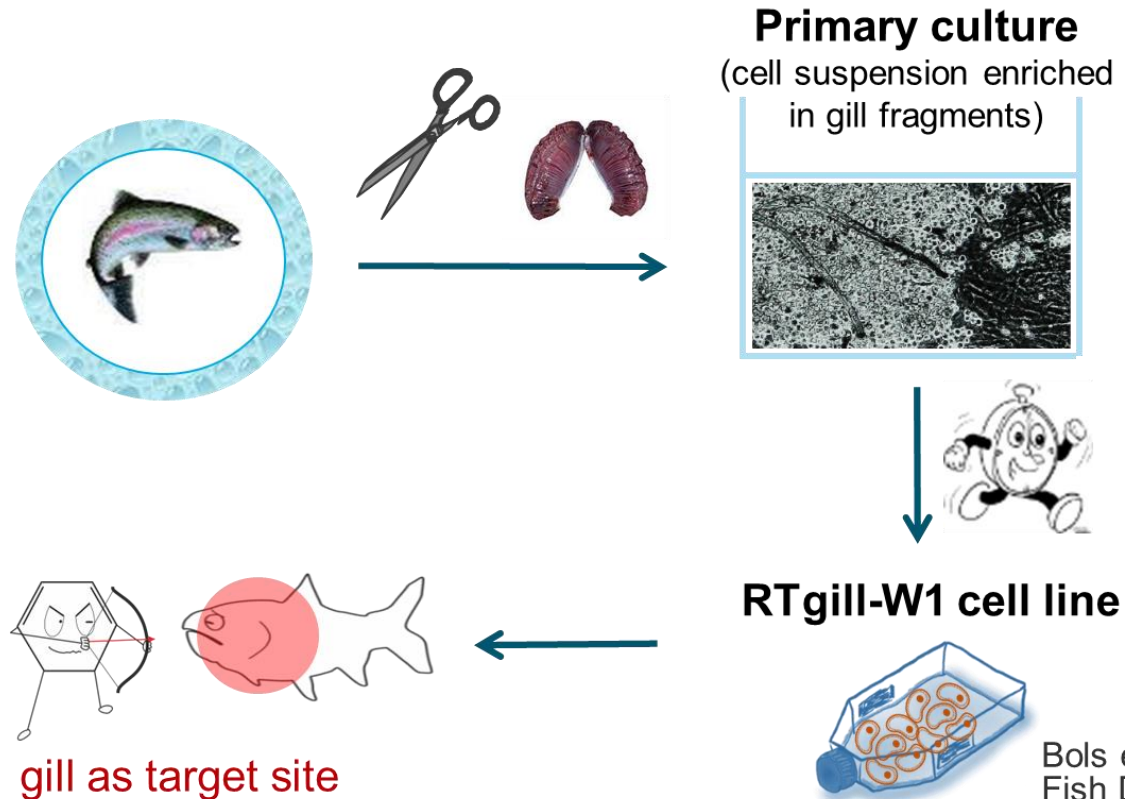


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Fish cell lines as non-animal alternatives

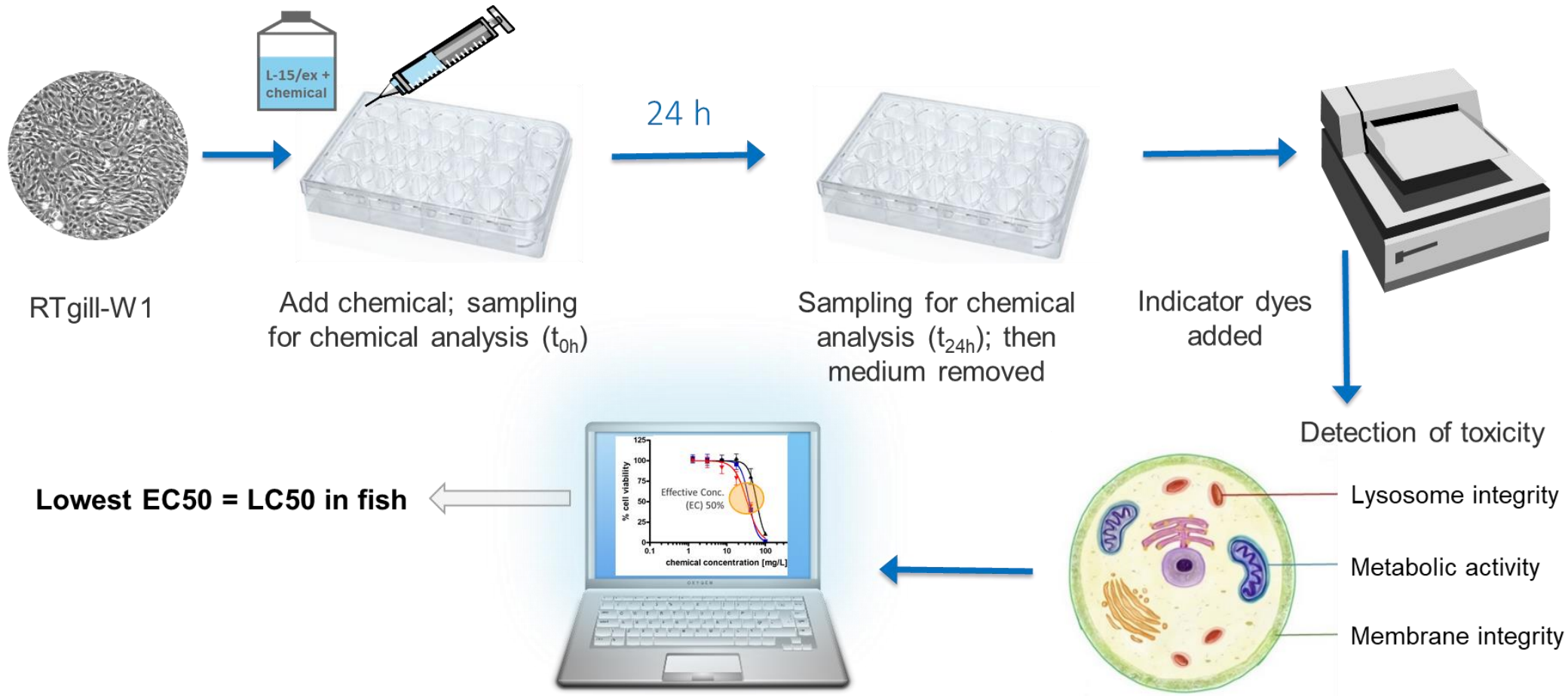


Fish invitrom for environmental risk assessment

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

RTgill-W1 cell line to predict acute fish toxicity (*instead of OECD TG203*)

Hypothesis: Gill as primary target for acute toxicity



RTgill-W1 cell line to predict acute fish toxicity (*instead of OECD TG203*)



published in April 2019

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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

The cover of the OECD Test Guideline No. 249 features a blue and white design. It includes the OECD logo, the title "Test Guideline No. 249 Fish Cell Line Acute Toxicity: The RTgill-W1 cell line assay", and a date "14 June 2021". There is also a small illustration of a fish.

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

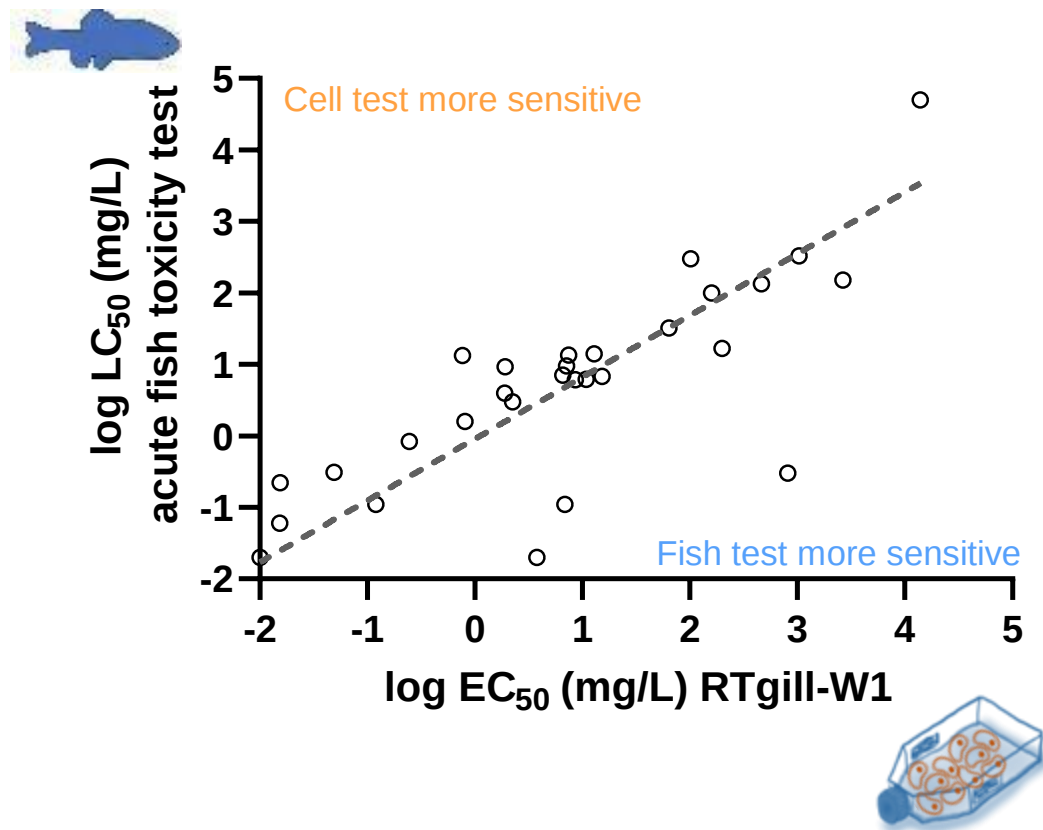
The RTgill-W1 cell line assay describes a 24-well plate format fish cell line acute toxicity test using the permanent cell line from rainbow trout (*Oncorhynchus mykiss*) gill, RTgill-W1. After 24 h of exposure to the test chemical, cell viability is assessed based on three fluorescent cell viability 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](#)

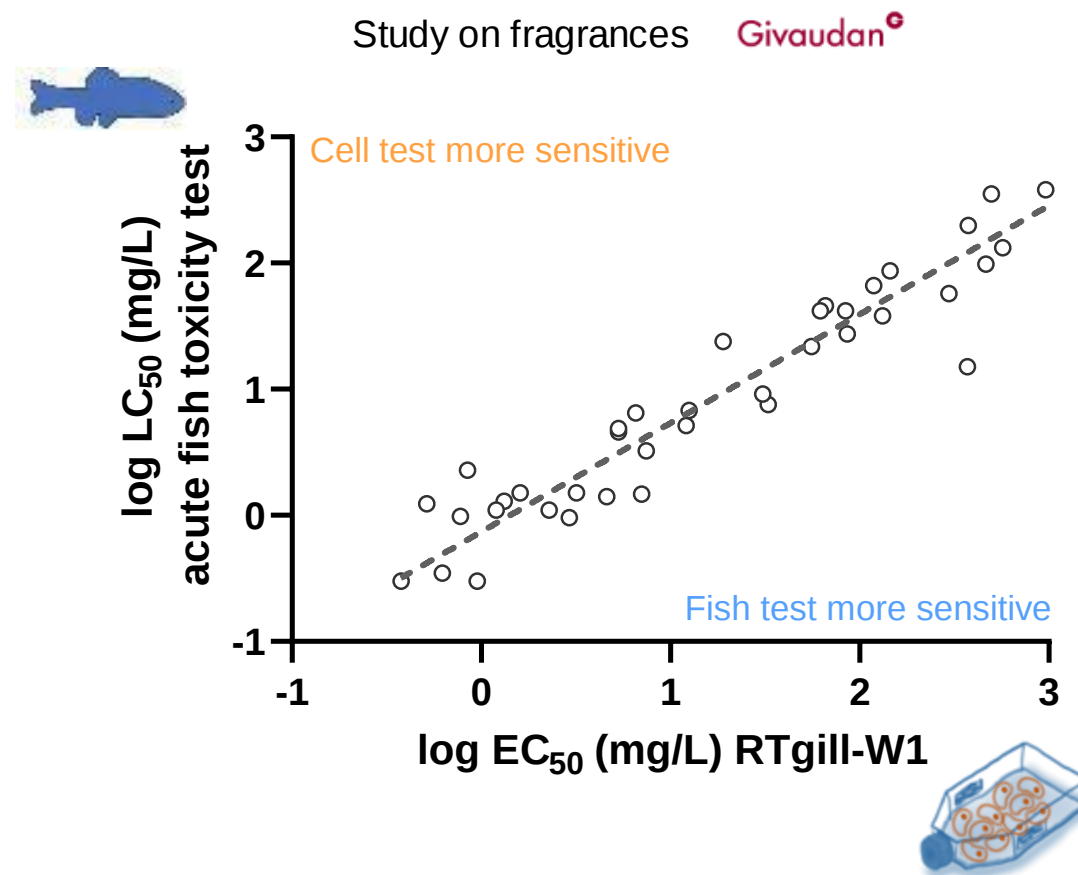
In series: [OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems](#) ([view more titles](#))

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RTgill-W1 cell line assay: Validation studies



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

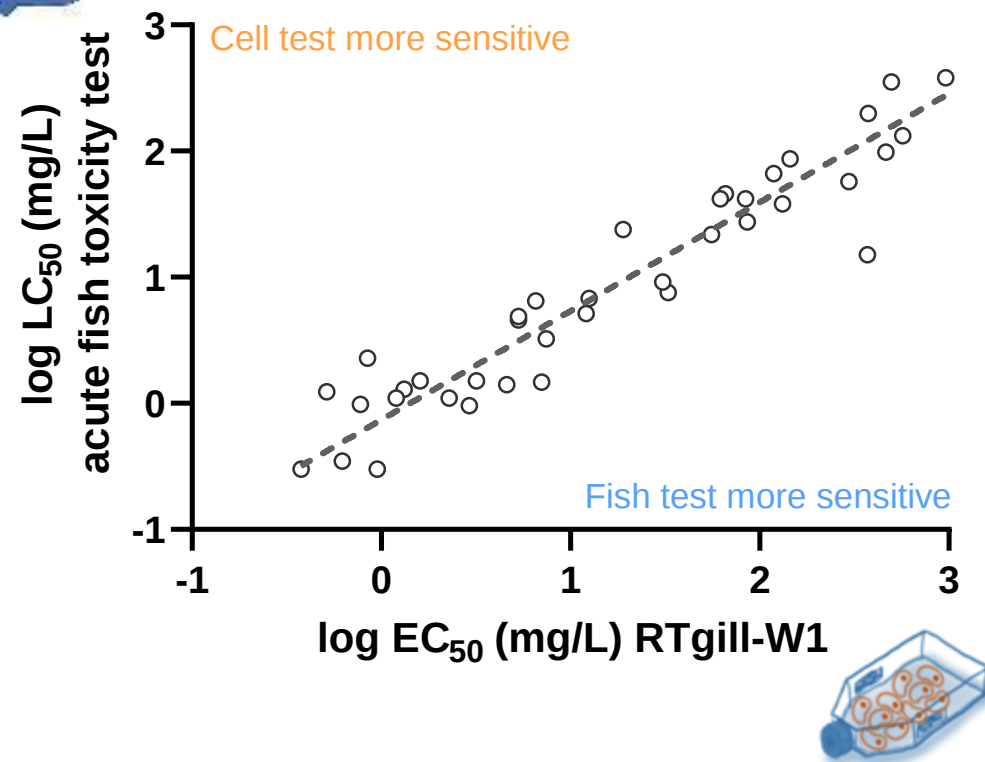


Natsch et al., 2018, ET&C 37(3): 931-941

Predict acute fish toxicity of fragrances: RTgill-W1 & RTgutGC

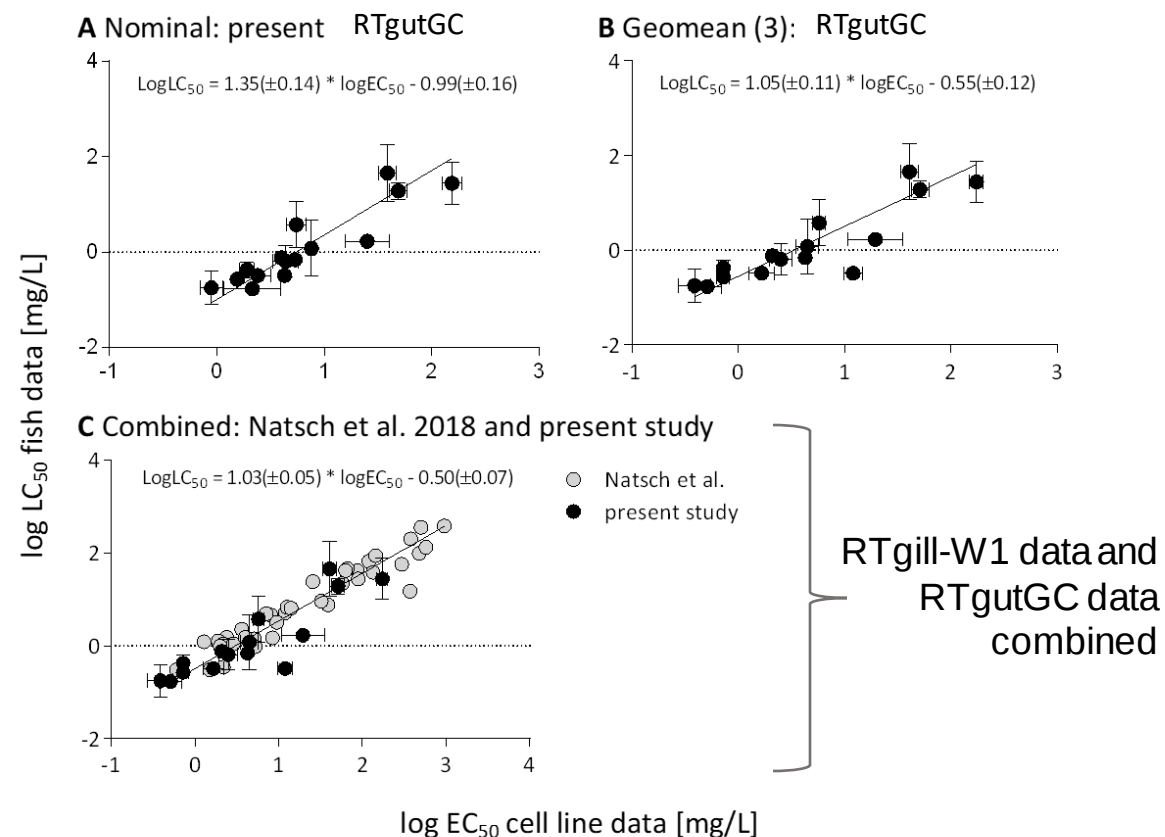
RTgill-W1: 38 fragrances

Study on fragrances **Givaudan[®]**



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

RTgutGC: 16 *OTHER* fragrances



Schug et al., 2020, ALTEX 37(1).
Collaboration with Firmenich

Offering animal-free alternative testing methods for fish,
focusing on fish cells and fish embryos as test models.



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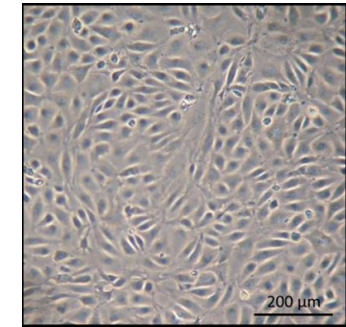
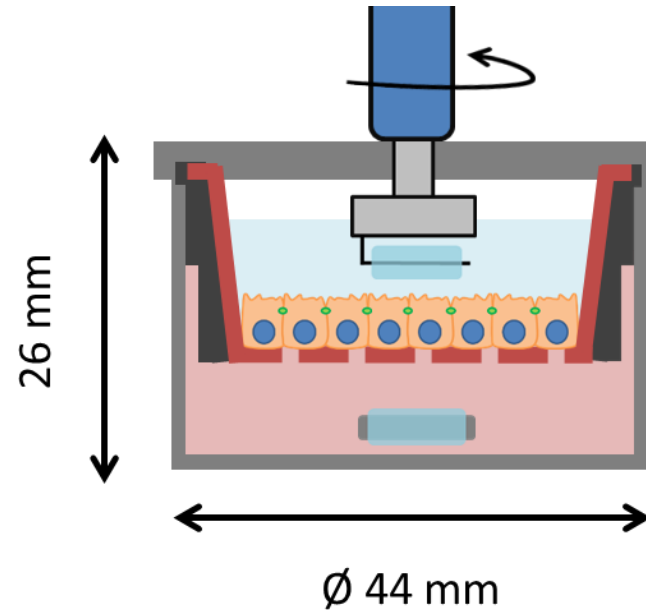
Since February 2023:

GLP:
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Praxis

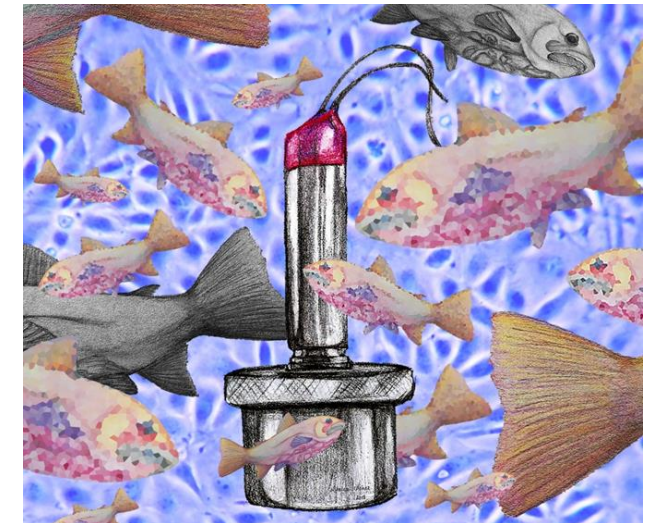
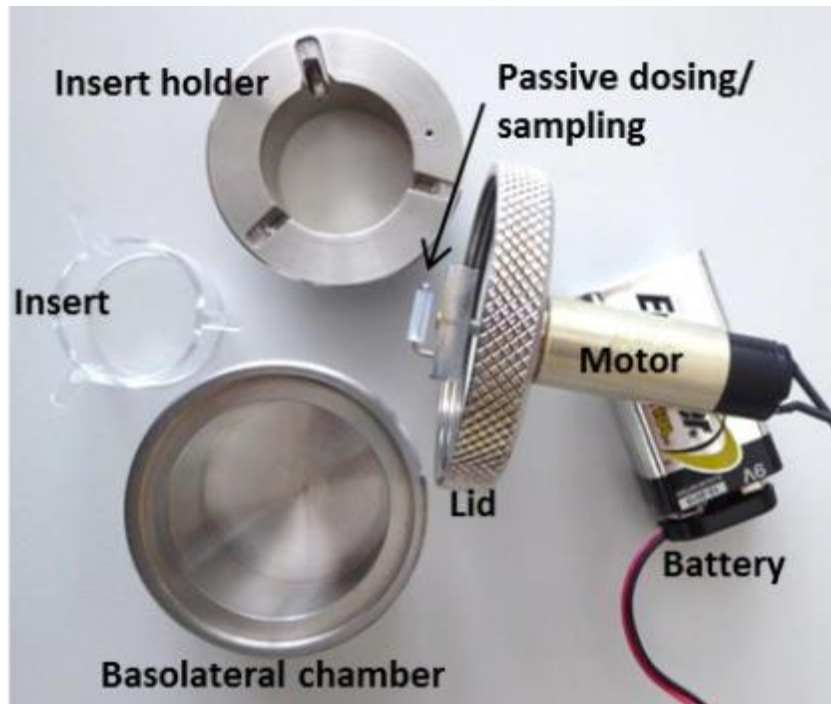
Intestinal accumulation and transfer model - RTgutGC

Different difficulties for *in vitro* testing

1. Sorption to plastic
2. Evaporation
3. Solubility – Chemical dosing

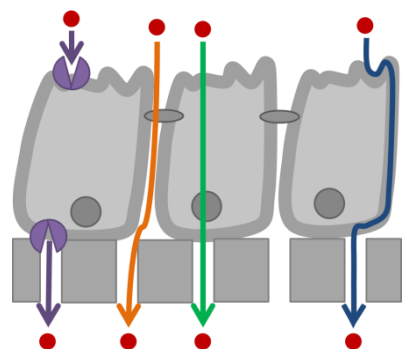


TransFER* chamber
*FE stands for Firmenich - Eawag

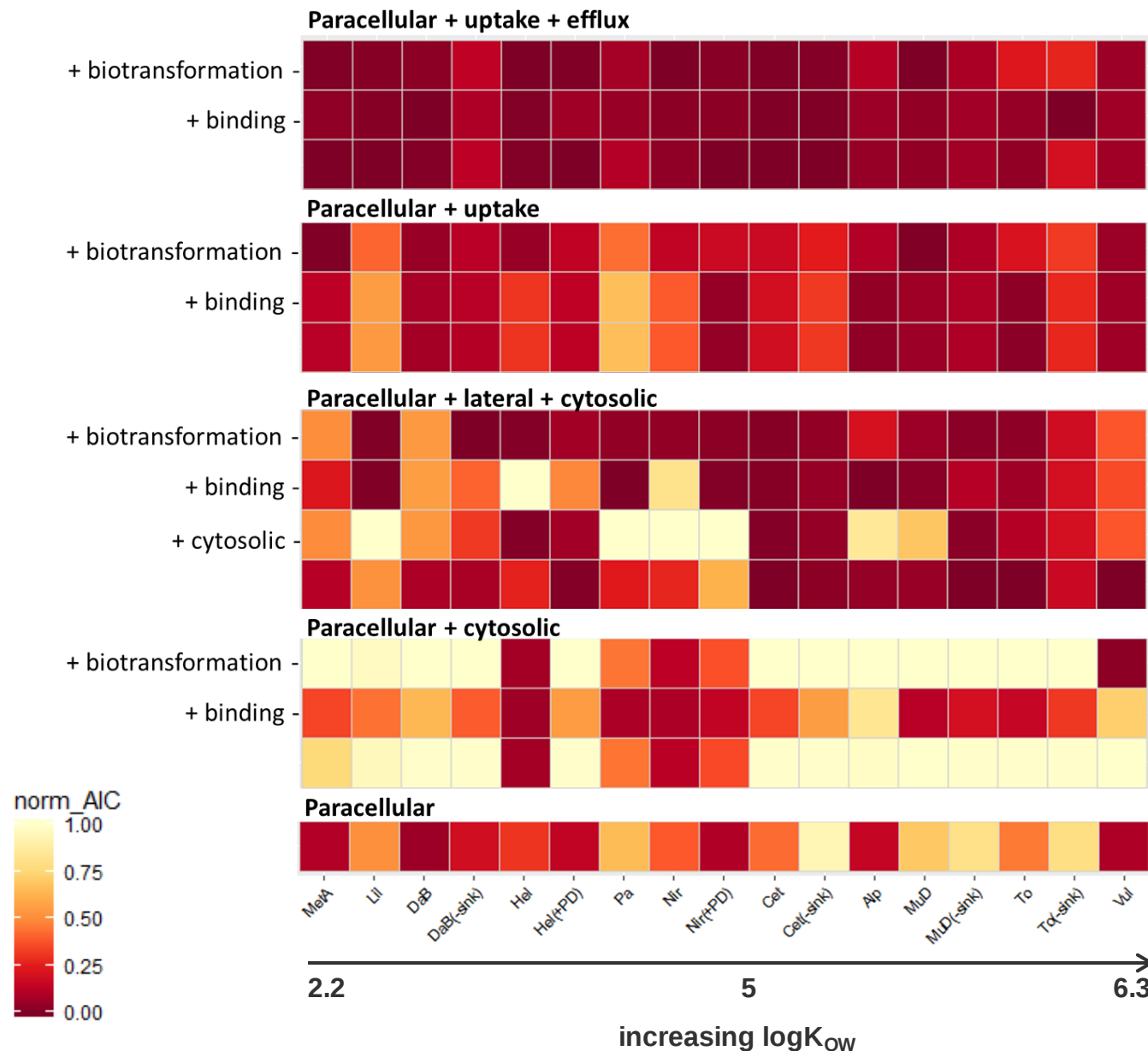


Schug et al., 2018, Analytical
Methods 10(36), 4353–4476.

Intestinal accumulation and transfer model - RTgutGC



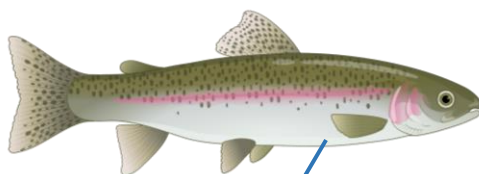
Paracellular
Cytosolic
Lateral
Uptake/efflux
Binding
Biotransformation



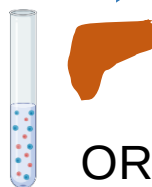
Schug et al., Environ. Sci. Technol.
2019, 53: 12062–12070.

Bioconcentration assessment *(instead of OECD TG305)*

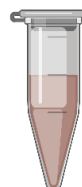
OECD TG319



Primary hepatocytes
(OECD TG319A)

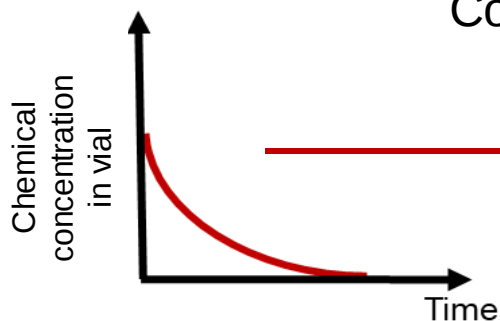


OR



S9 sub-cellular fractions
(OECD TG319B)

Computational model

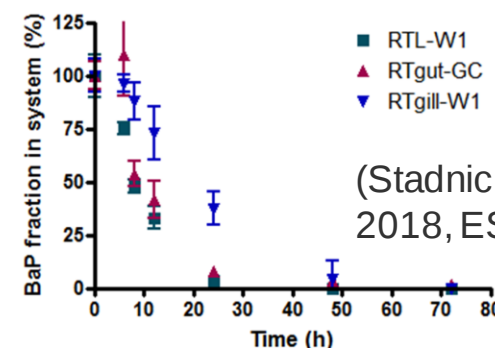


In vitro-
derived BCF

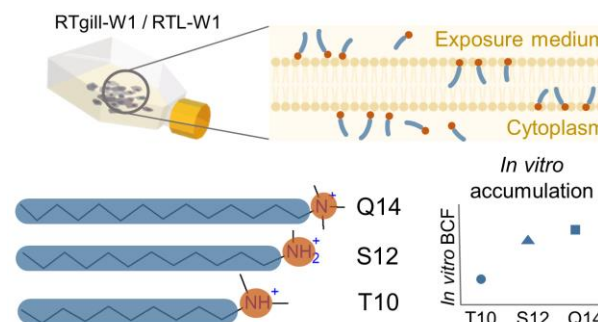
Or: Use of cell lines



RTL-W1 Liver
RTgill-W1 Gill
RTgutGC Gut



(Stadnicka-Michalak et al.,
2018, ES&T 52(5), 3091-3100.)



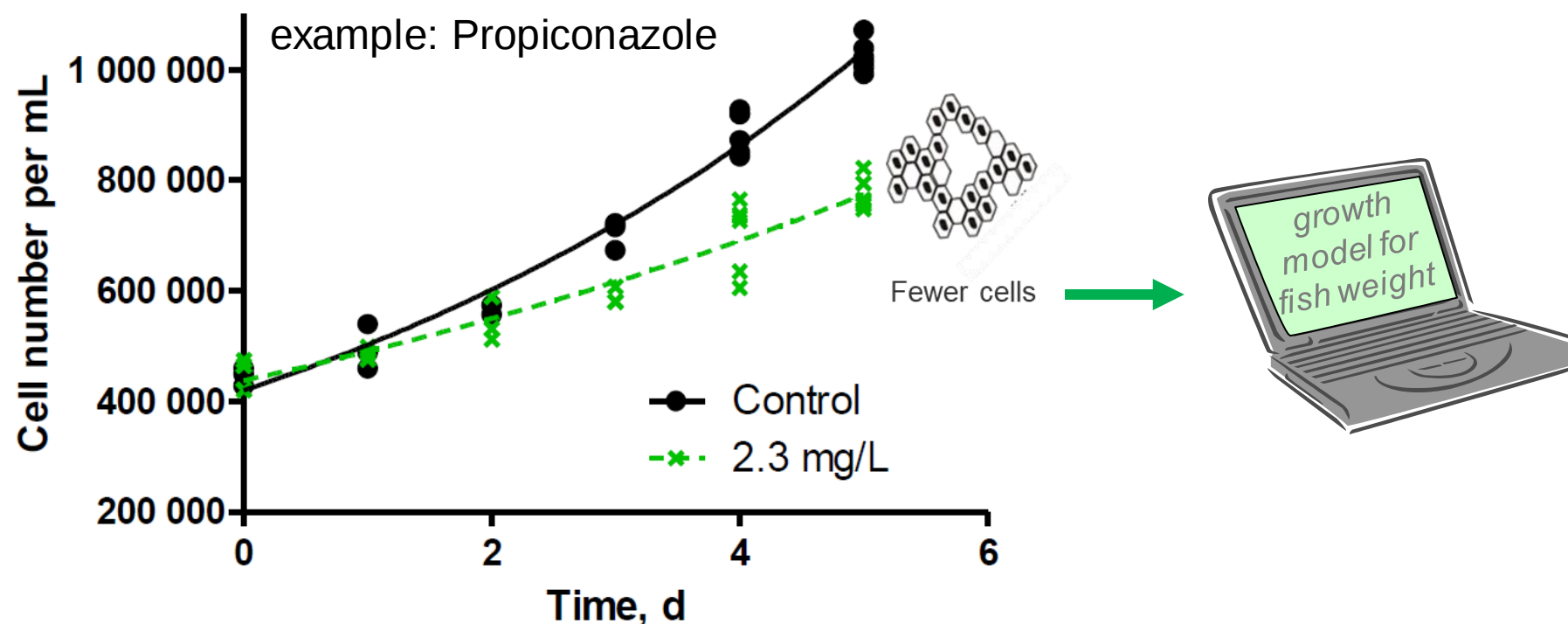
(Balk et al., 2023,
Environment International
174, 107798;
Balk et al., 2024 ES&T
58(3), 1452–1461)

Predicting reduced fish growth

Hypothesis: Less growth means fewer cells

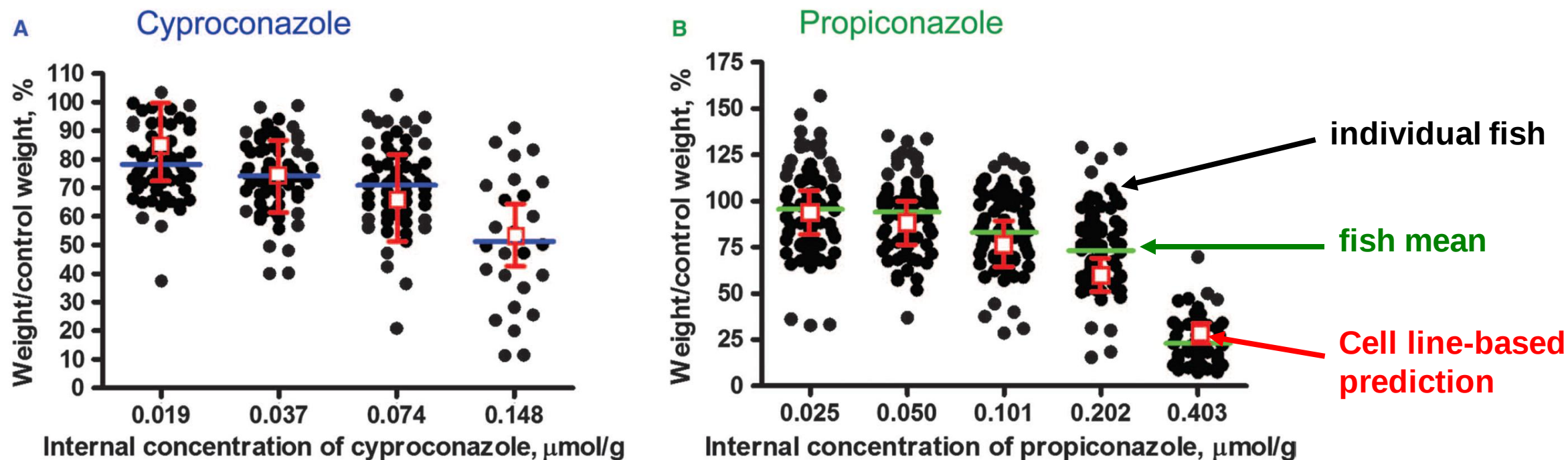


RTgill-W1 cell line as model
of richly perfused tissue



Predicting reduced fish growth

Hypothesis: Less growth means fewer cells (proof-of-concept)



Hazard assessment of fragrances using fish cell lines

Kristin Schirmer
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Fish cell lines as non-animal alternatives

Primary culture (cell suspension enriched in gill fragments)

RTgill-W1 cell line

RTgutGC

Fish in vitro for environmental risk assessment

RTgill-W1 cell line to predict acute fish toxicity (instead of OECD TG203)

Hypothesis: Gill as primary target for acute toxicity

RTgill-W1

Add chemical, sampling for chemical analysis ($t_{0.5}$)

24 h

Sampling for chemical analysis ($t_{0.5}$), then medium removed

Indicator dyes added

Detection of toxicity

Lowest EC50 = LC50 in fish

Lysosome integrity

Metabolic activity

Membrane integrity

RTgill-W1 cell line to predict acute fish toxicity (instead of OECD TG203)

ISO 21115:2019

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

published in April 2019

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

published in June 2021

RTgill-W1 cell line assay: Validation studies

log EC₅₀ (mg/L) RTgill-W1

log EC₅₀ (mg/L) RTgill-W1

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

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

Predict acute fish toxicity of fragrances: RTgill-W1 & RTgutGC

RTgill-W1: 38 fragrances

RTgutGC: 16 OTHER fragrances

log EC₅₀ (mg/L) RTgill-W1

log EC₅₀ (mg/L) RTgutGC

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

Schug et al., 2020, ALTEX 37(1): Collaboration with Firmenich

Offering animal-free alternative testing methods for fish, focusing on fish cells and fish embryos as test models.

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Intestinal accumulation and transfer model - RTgutGC

Different difficulties for in vitro testing

1. Sorption to plastic
2. Evaporation
3. Solubility – Chemical dosing

TransFer[®] chamber
*FE stands for Firmenich – Eawag

Insert holder

Passive dosing / sampling

Insert

Lid

Basolateral chamber

Motor

Battery

Schug et al., 2018, Analytical Methods 10(30): 4335–4413

Intestinal accumulation and transfer model - RTgutGC

Paracellular + uptake + efflux

Paracellular + uptake

Paracellular + lateral + cytosolic

Paracellular + cytosolic

Paracellular

Schug et al., Environ. Sci. Technol. 2019, 53: 12062–12070.

Bioconcentration assessment (instead of OECD TG305)

OECD TG319

Or: Use of cell lines

RTL-W1

RTgill-W1

RTgutGC

Primary hepatocytes (OECD TG319A) OR S9 sub-cellular fractions (OECD TG319B)

Computational model

In vitro-derived BCF

(Balk et al., 2023, Environment International 174: 107760)

(Balk et al., 2024 ES&T 50(3): 1452–1461)

Predicting reduced fish growth

Hypothesis: Less growth means fewer cells

RTgill-W1 cell line as model of richly perfused tissue

example: Propiconazole

Control

2.3 mg/L

Fewer cells

growth model for fish weight

(Stadnicka-Michalak J et al., 2015, Science Advances, Vol. 1, no. 7, e1500302)

Predicting reduced fish growth

Hypothesis: Less growth means fewer cells (proof-of-concept)

Cyproconazole

Propiconazole

Weight control weight, %

Internal concentration of cyproconazole, μ mol/g

Internal concentration of propiconazole, μ mol/g

individual fish

fish mean

Cell line-based prediction

(Stadnicka-Michalak J et al., 2015, Science Advances, Vol. 1, no. 7, e1500302)