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# High-throughput ecotoxicology with a rainbow trout gill cell line

Johanna Nyffeler<sup>1,2</sup>, Felix Harris<sup>1,3</sup>, Steven Lasee<sup>1,2</sup>, Clinton Willis<sup>1</sup>, Gabby Byrd<sup>1,3</sup>, Christopher Schaupp<sup>1,2</sup>, Jon Haselman<sup>1</sup>, John Nichols<sup>1</sup>, Brett Blackwell<sup>1</sup>, Kevin Flynn<sup>1</sup>, Dan Villeneuve<sup>1</sup>, Joshua Harrill<sup>1</sup>

<sup>1</sup>US Environmental Protection Agency, Center for Computational Toxicology & Exposure, Office of Research and Development, Research Triangle Park, NC.

<sup>2</sup>ORISE, Oak Ridge, TN. <sup>3</sup>ORAU, Oak Ridge, TN

ORCID 0000-0002-6155-9743

Nyffeler.Johanna@epa.gov

## State of the art

### In vitro methods in ecotoxicology

- In vivo* chemical hazard evaluations are cost and time intensive and low throughput.
- High-throughput *in vitro* methods for ecotoxicology are needed to accelerate the pace of chemical hazard evaluation.
- A lower throughput *in vitro* method using a rainbow trout gill cell line (RTgill-W1) has been developed by Schirmer et al. 1998 and Tanneberger et al. 2013.
  - adopted by the OECD (OECD TG 249).
  - Upon adjustment of assay results for *in vitro* disposition and conducting in vitro-to in vivo-extrapolation (IVIVE), excellent correlation with *in vivo* survival data was found (Tanneberger et al. 2013)

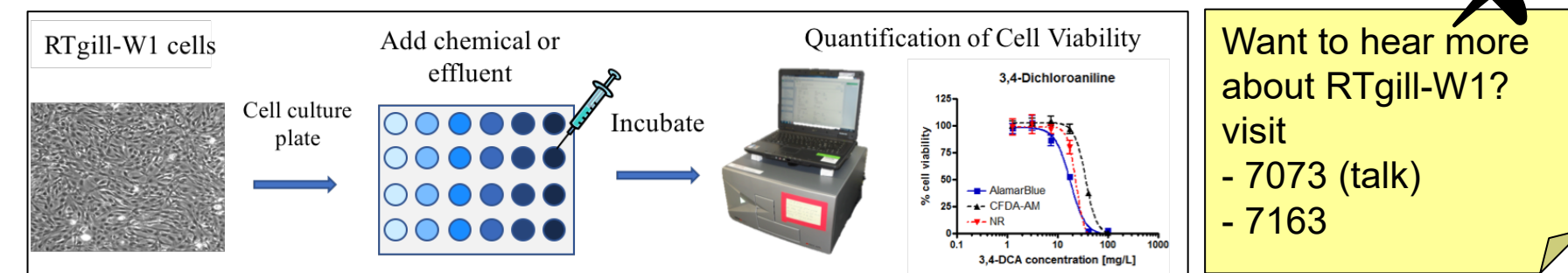


Illustration: www.aquatox-solutions.ch

### High-throughput methods in human toxicology

- The US EPA has implemented two high-throughput profiling methods: transcriptomics and Cell Painting (CP) (Thomas et al. 2019)
- CP is cost-effective and is amenable to any type of adherend cells
- We have screened >1000 chemicals in human osteosarcoma (U-2 OS) cells (Nyffeler et al. 2020, and unpublished data)

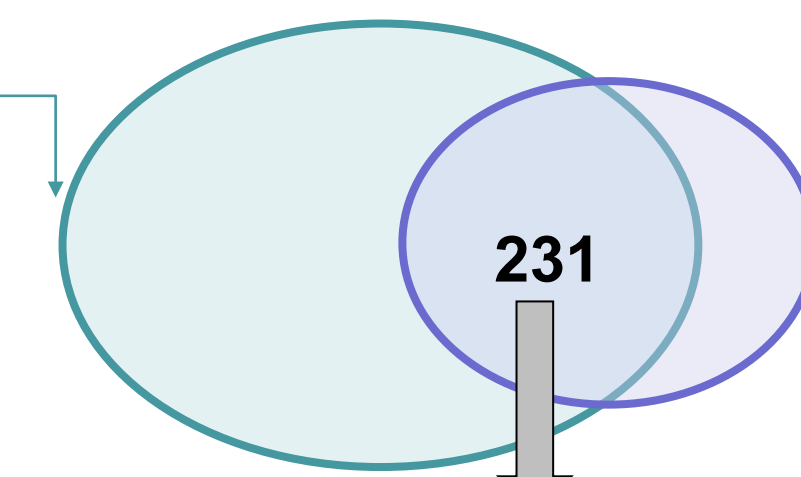
## Chemical selection

### Chemical inventory

- 6000 compounds (cmp) in library
- 27 cmp of interest to the center
- 12 cmp selected for analytical chemistry analysis

### Chemicals of interest

- 202 cmp with in vivo rainbow trout toxicity data in the ECOTOX Knowledgebase
- 27 cmp of interest to the center
- 110 cmp with reported rainbow trout in vitro toxicity data
- known piscicide
- known inert chemical
- detected in Great Lakes water
- nominated by experts



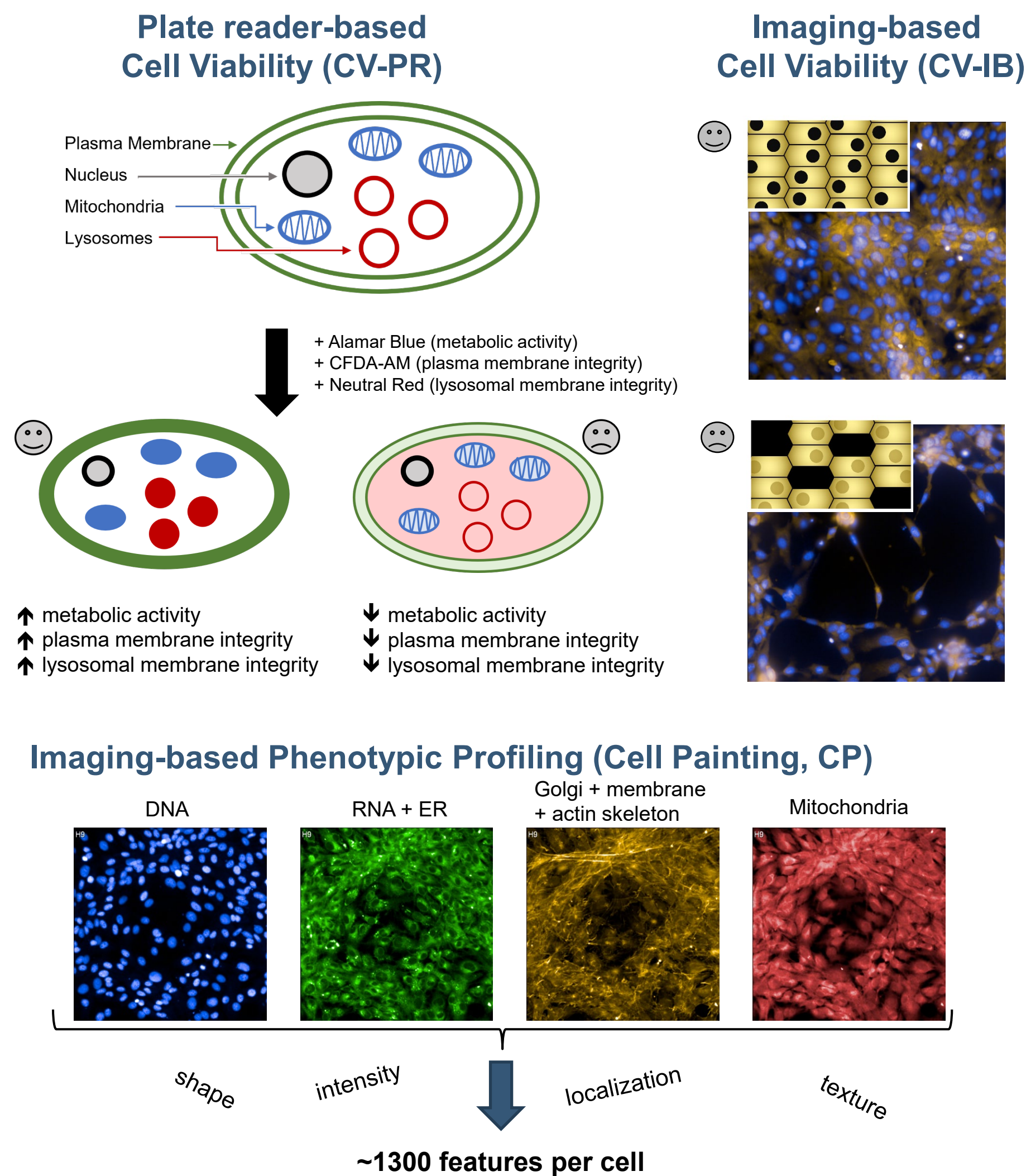
### The 231 chemicals comprise:

- 129 with in vivo rainbow trout data in ECOTOX Knowledgebase
- 27 of interest to the center
- 12 selected for analytical chemistry analysis
- 69 with reported rainbow trout in vitro data
- 5 known piscicides
- 10 known inert chemicals
- 29 detected in Great Lakes water
- 10 nominated by experts
- 110 tested in house in human U-2 OS osteosarcoma cells
- + 9 chemicals screened in duplicate (for quality control purposes)

## Aims

- Miniaturize the existing OECD TG249 (CV-PR) assay to 384-well format.
- Apply imaging-based Cell Viability (CV-IB) and Cell Painting assays in RTgill-W1 cells.
- Test 231 chemicals of interest in all three assays and compare the resulting potency estimates:
  - among the three assays
  - with literature data from RTgill-W1 cells
  - to existing data from a human cell line (U-2 OS cells)

## In vitro assays

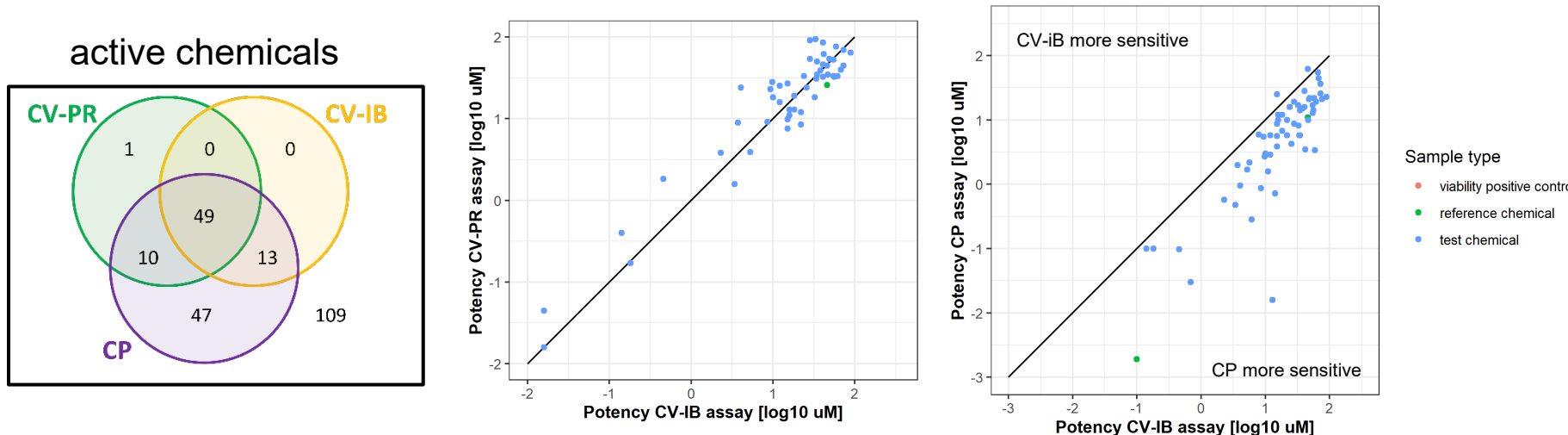


## Conclusions

- With minor modifications, OECD TG249 could be miniaturized.
- RTgill-W1 cells were amenable to the CV-IB and CP assays.
- Approximately half the chemicals were active in at least one assay.
  - The CP assay was more sensitive than the CV assays.
  - There was good correlation with literature data from RTgill-W1 cells.
  - For some chemicals, RTgill-W1 cells were more sensitive than human U-2 OS cells

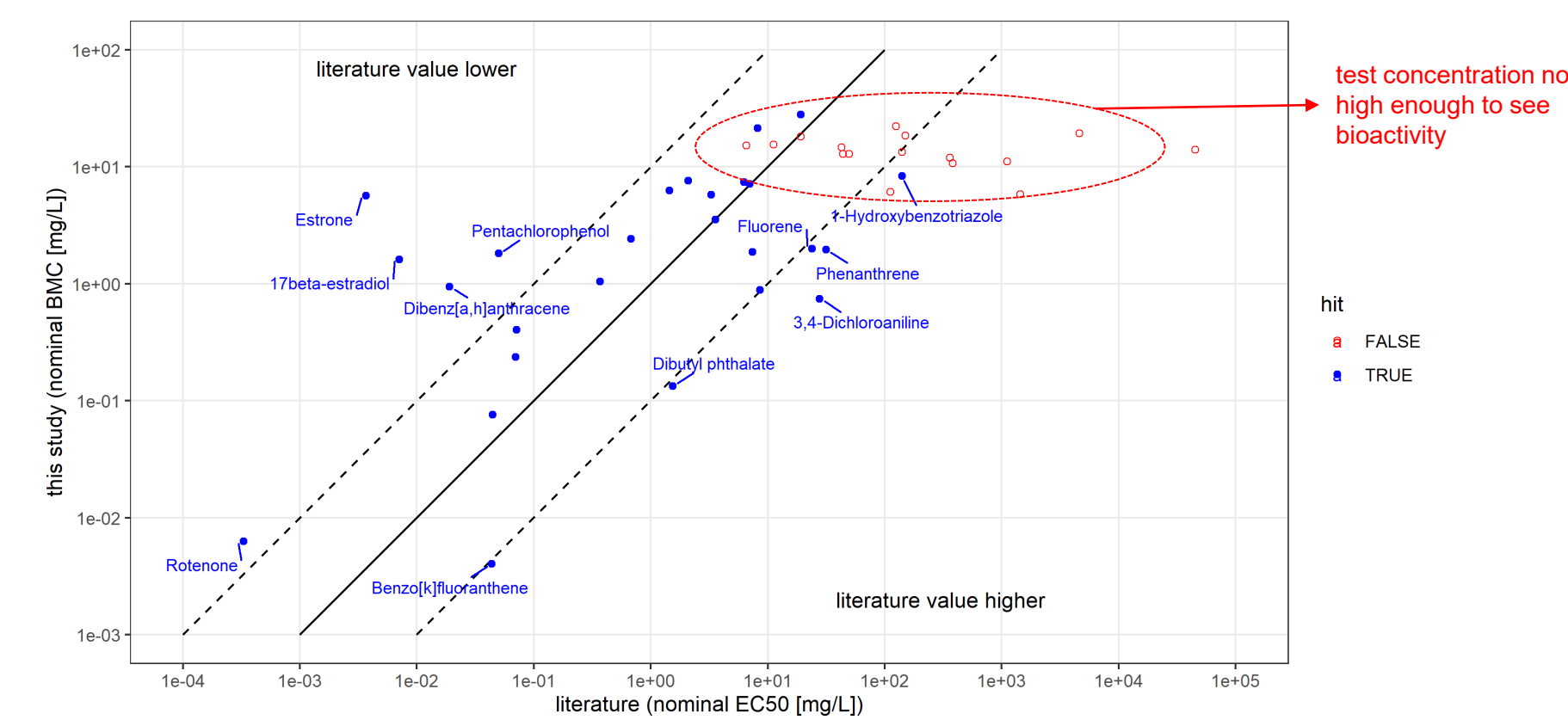
## Preliminary Results I: Assay results and comparison with literature

### Comparison of the three assays



- the two CV assays give very similar results
- CP assay detects more chemicals as active than the CV assays and at a lower concentration

### Comparison with RTgill-W1 data from the literature

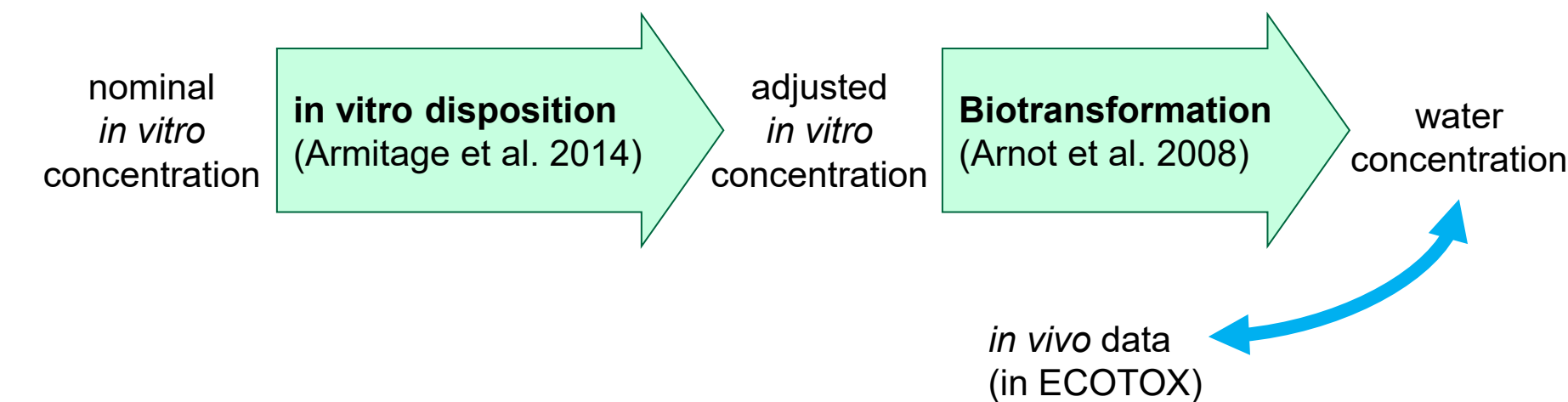


- good correlation with literature data, with the exception for two estrogenic compounds

## Next steps

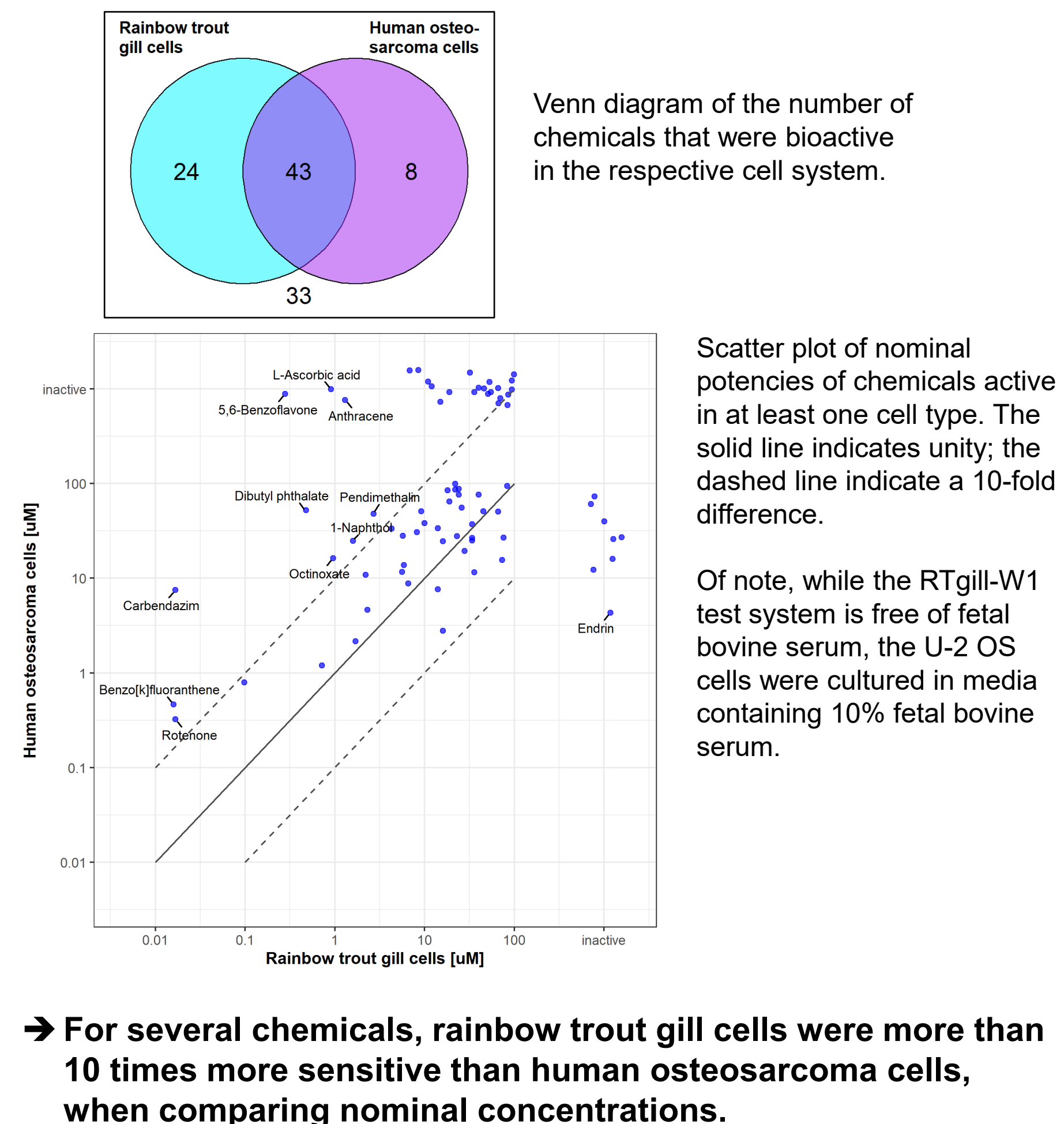
Compare the in vitro potencies with in vivo water concentration data from the ECOTOX Knowledgebase for rainbow trout. To this end:

- The free concentration in the medium is estimated with the Armitage model.
- Concentrations will be analytically measured to verify the model for a subset of chemicals (~12).
- Biotransformation will be taken into account to estimate a water concentration.



## Preliminary Results II: Comparison to a human cell line

Of the tested chemicals, 108 have previously been tested in a screen with human osteosarcoma cells (U-2 OS cells).



Scatter plot of nominal potencies of chemicals active in at least one cell type. The solid line indicates unity; the dashed line indicate a 10-fold difference.

Of note, while the RTgill-W1 test system is free of fetal bovine serum, the U-2 OS cells were cultured in media containing 10% fetal bovine serum.

- For several chemicals, rainbow trout gill cells were more than 10 times more sensitive than human osteosarcoma cells, when comparing nominal concentrations.