High-throughput phenotypic profiling within the NAMs-based, tiered hazard evaluation strategy at the United States Environmental Protection Agency
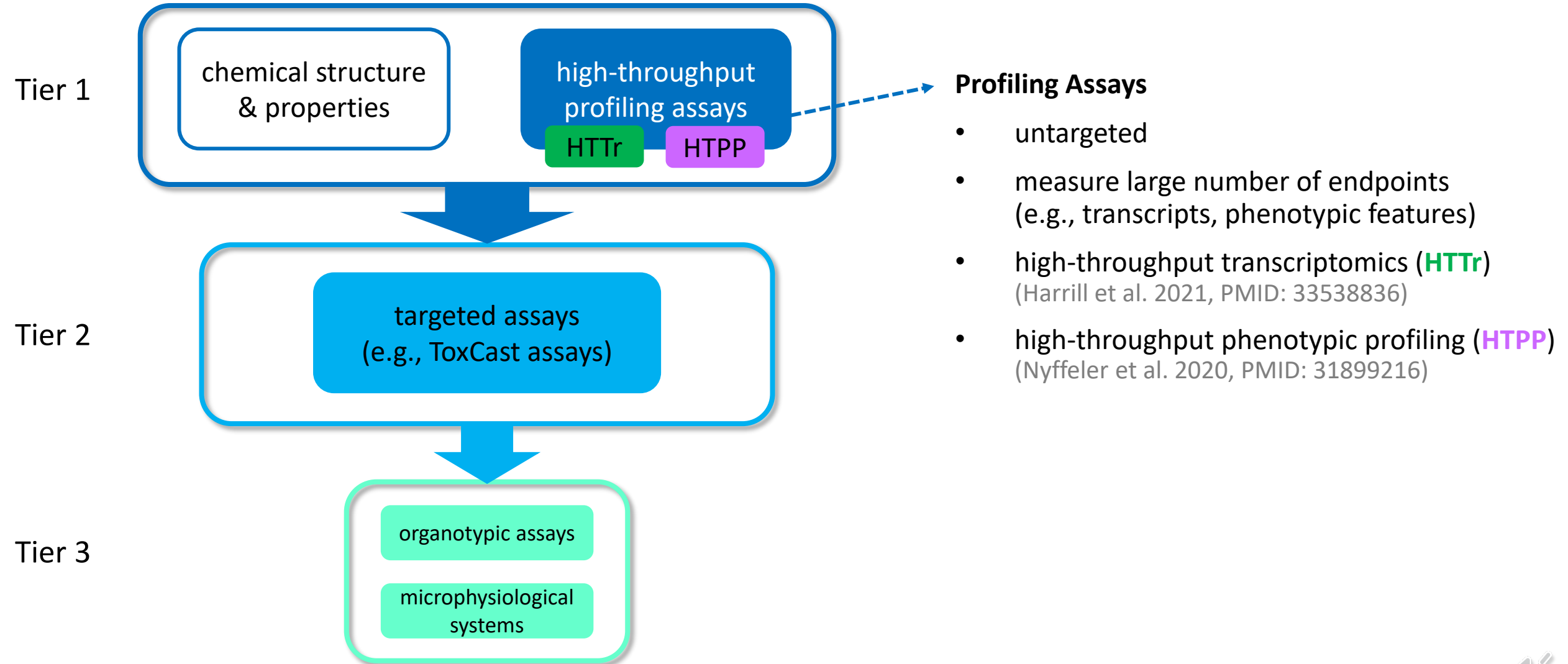
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The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

Tiered Hazard Evaluation Strategy based on New Approach Methods (NAMs)

Tier 1

chemical structure & properties

high-throughput profiling assays

HTTr HTPP

Tier 2

targeted assays (e.g., ToxCast assays)

Tier 3

organotypic assays

microphysiological systems

Profiling Assays

- untargeted

- measure large number of endpoints (e.g., transcripts, phenotypic features)

- high-throughput transcriptomics (**HTTr**) (Harrill et al. 2021, PMID: 33538836)

- high-throughput phenotypic profiling (**HTPP**) (Nyffeler et al. 2020, PMID: 31899216)

adapted from "The Next Generation Blueprint of Computational Toxicology at the U.S. EPA", Tox. Sci. 2019; 169(2):317-322. PMID: 30835285
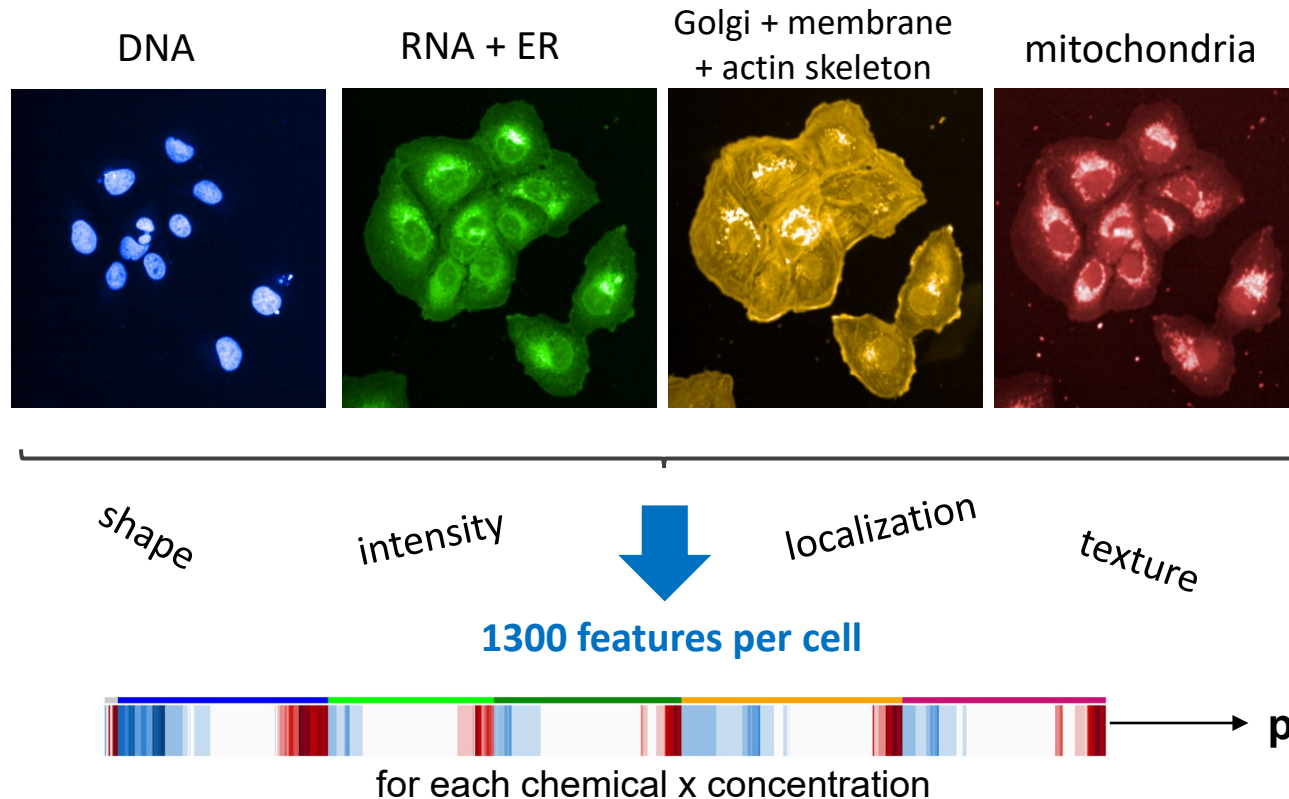
High-Throughput Phenotypic Profiling (HTPP)

- labeling of various cell organelles with fluorescent probes in *in vitro* cultures

- assessing a large variety of morphological features on individual cells via imaging

'Cell Painting' assay
Gustafsdottir *et al.* 2013, PMID: 24312513

Bray *et al.* 2016, PMID: 27560178

Nyffeler *et al.* 2020

DNA   RNA + ER   Golgi + membrane + actin skeleton   mitochondria

shape   intensity   localization   texture

1300 features per cell

→ **profile**

for each chemical x concentration

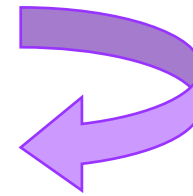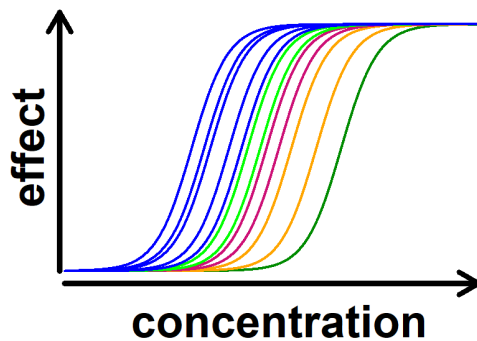| **Fluorescent labels** |
|---|
| **DNA**: H-33342 |
| **RNA**: SYTO14 |
| **ER**: Concanavalin A-488 |
| **Actin**: Phalloidin-568 |
| **Golgi + Membrane**: wheat germ agglutinin (WGA) -555 |
| **Mitochondria**: MitoTracker |

HTPP: Two Applications
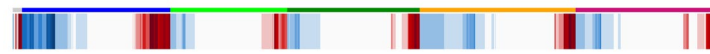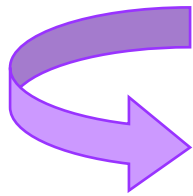
profile

for each chemical x concentration

Application 1

concentration-response modelling

Potency estimation:
→ *in vitro* point-of-departure (POD)

- Nyffeler *et al.* (2020) Toxicol Appl Pharmacol. PMID: 31899216
- Willis *et al.* (2020). SLAS Discov. PMID: 32546035
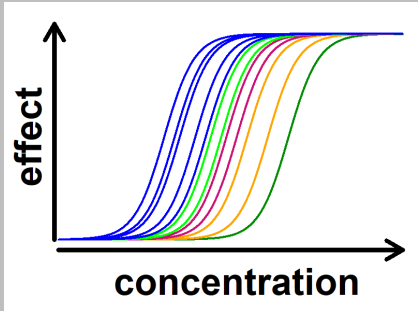- Nyffeler *et al.* (2021). SLAS Discov. PMID: 32862757

Application 2
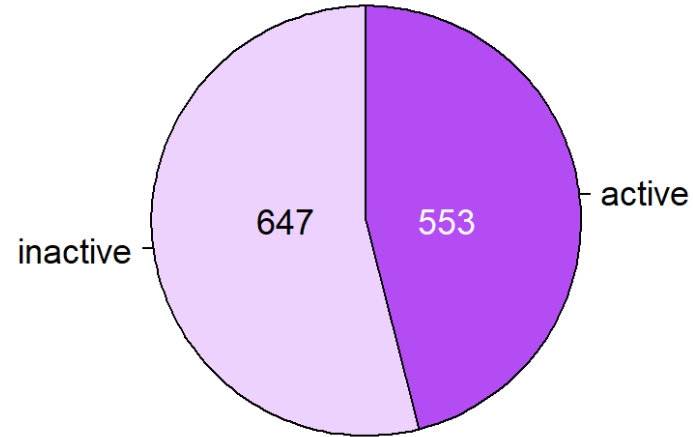
Chemical A

0	1.80	0	9.69	5.73	0	6.47	-12.84	0	0

Biological similarity

Chemical B

0	0	0	10.00	6.00	1.60	6.47	-15.00	0	0

Compare profiles with annotated reference chemicals
→ putative mechanisms

work in progress

HTPP Screening Results

Application 1:

Potency estimation

Active chemicals:

inactive 647 | 553 active

⇨ **~ 40% of chemicals were active**

⇨ **Most activity is > 10 µM**

Comparison with ToxCast screening results:

HTPP more potent

ToxCast more potent

ToxCast POD $\log_{10}$ (µM)

HTPP POD $\log_{10}$ (µM)
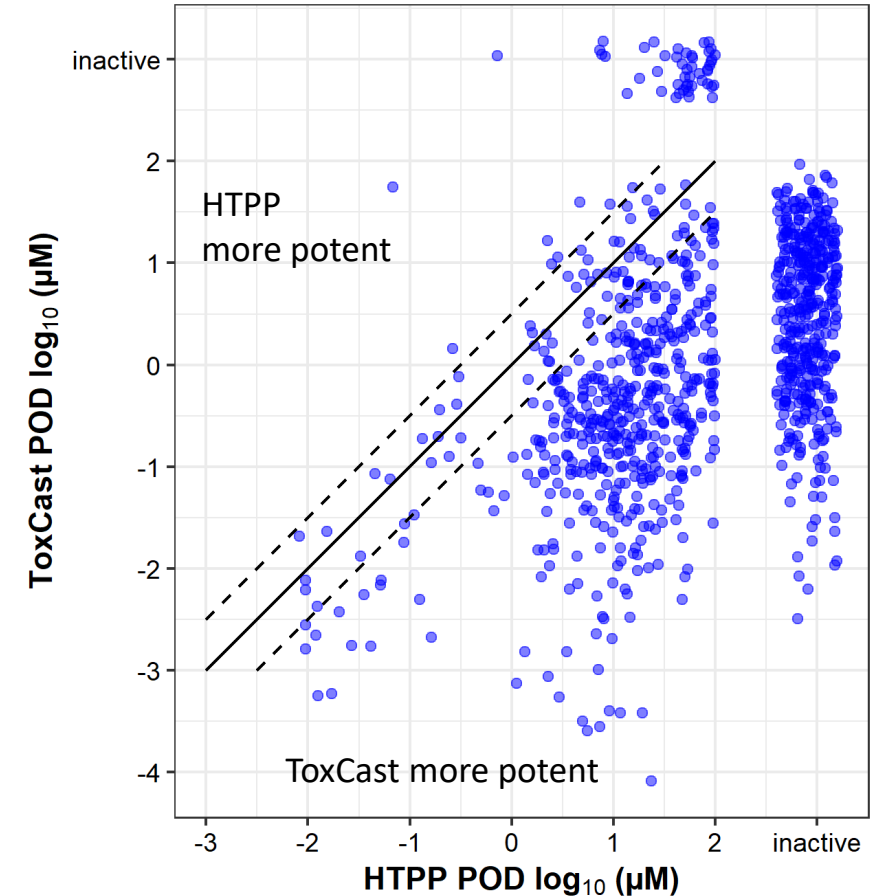
⇨ **Less potent than ToxCast POD**

Preliminary results. Do not cite or quote.

Application 1:

Potency estimation

effect

concentration

POD: point-of-departure
AED: administered equivalent dose
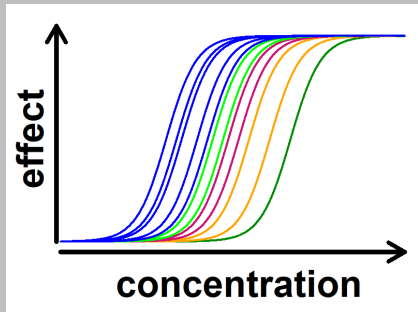
- 303 chemicals were active and had pharmacokinetic (PK) information

HTPP POD (µM)

In vitro-to-*in vivo* extrapolation (IVIVE)

HTPP AED (mg/kg bw/day)

in vivo point-of-departure

5%   50%   95%

5%

$\log_{10}$(mg/kg/day)

78%

43%

NAM < *in vivo*

NAM > *in vivo*

Number of chemicals

0.2

$\log_{10}$ (HTPP AED 50th / POD_{trad})
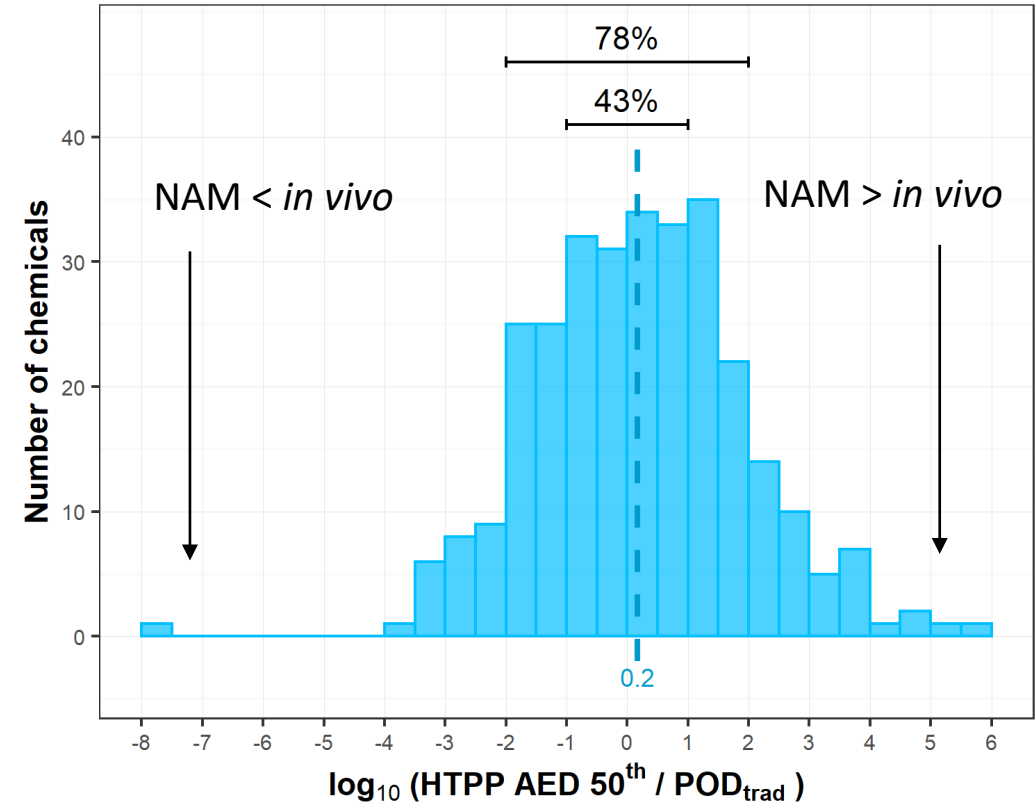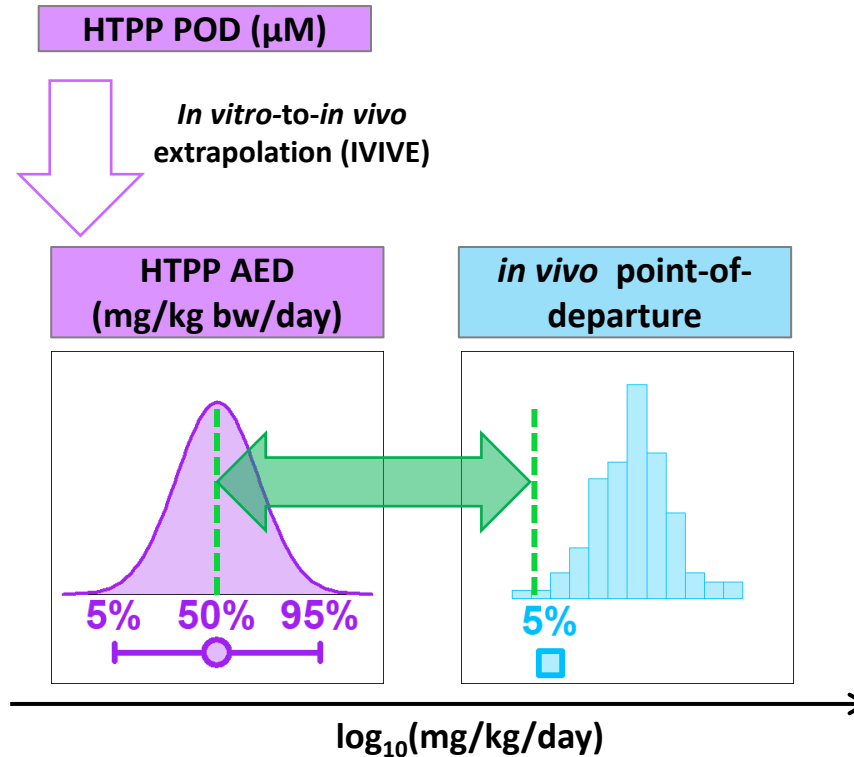
⇨ **78% of HTPP AED are within 2 orders of magnitude of the *in vivo* POD**

Application 1:

Potency estimation

HTPP POD (μM)

In vitro-to-*in vivo* extrapolation (IVIVE)

HTPP AED (mg/kg bw/day)

Predicted exposure

95%

5% 50% 95%

log$_{10}$(mg/kg/day)

POD: point-of-departure
AED: administered equivalent dose

Potential for humans to be exposed to bioactive concentrations

49%

chemicals of lesser concern

2.9

Number of chemicals
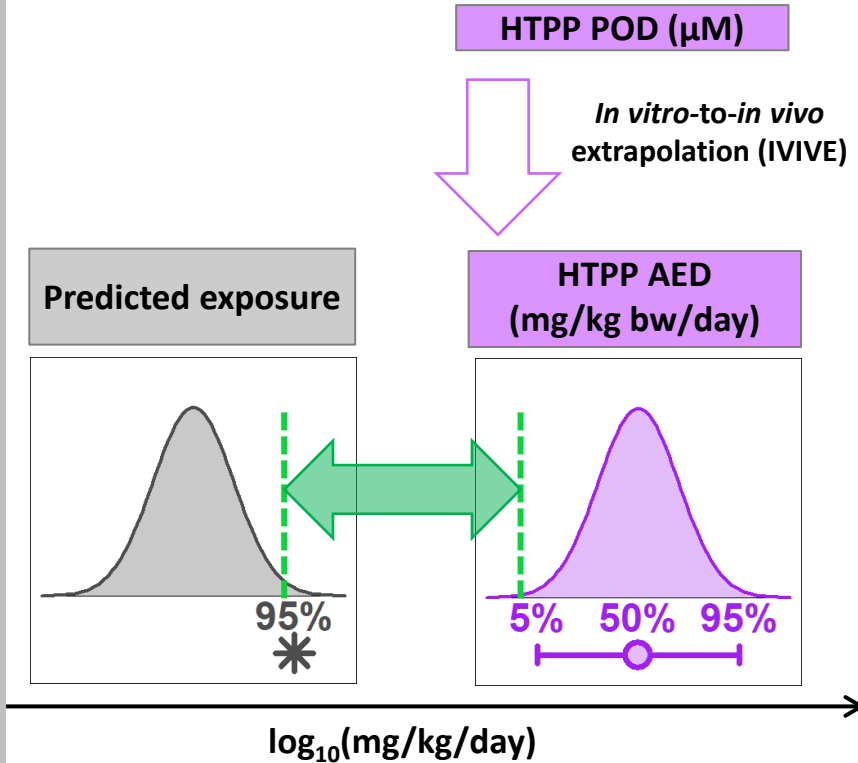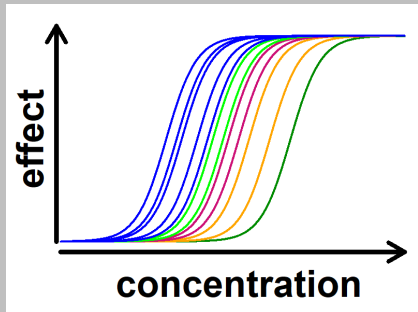
Bioactivity-Exposure Ratio
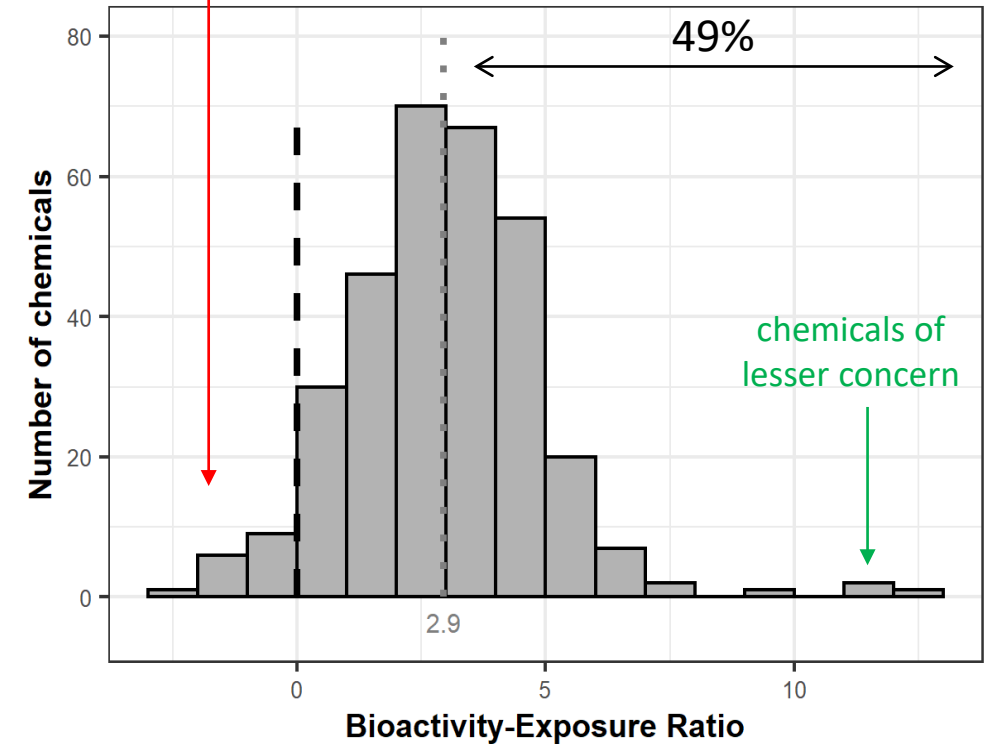
⇨ **for 49% of chemicals, predicted exposure is > 1000x lower than estimated bioactivity**

⇨ **for a small set of chemicals, the BER was negative, indicating a potential for humans to be exposed to bioactive concentrations of these chemicals**

Feature Selection & Profile Comparison

Application 2: Mechanistic prediction
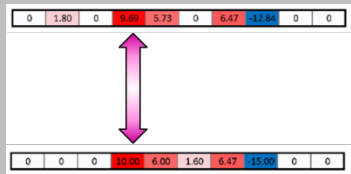
Feature Selection

1300 features

↓

317 features

Profile Comparison

	feature_1	feature_2	feature_3	feature_4	feature_5	feature_6	feature_7	feature_8	feature_9	feature_10
Profile	-1.1	1.8	0.9	9.7	5.7	0.5	6.5	-12.8	-0.2	-1.4

remove low-magnitude effects

Signature	-1.1	1.8	0.0	9.7	5.7	0.0	6.5	-12.8	0.0	-1.4

Kendall correlation

Chemical C	0	-7.0	0	2.0	0	5.0	2.0	-2.0	0	9.0

Chemical A	-1.5	1.5	0	5.0	3.0	0	5.0	-7.0	0	-1.8
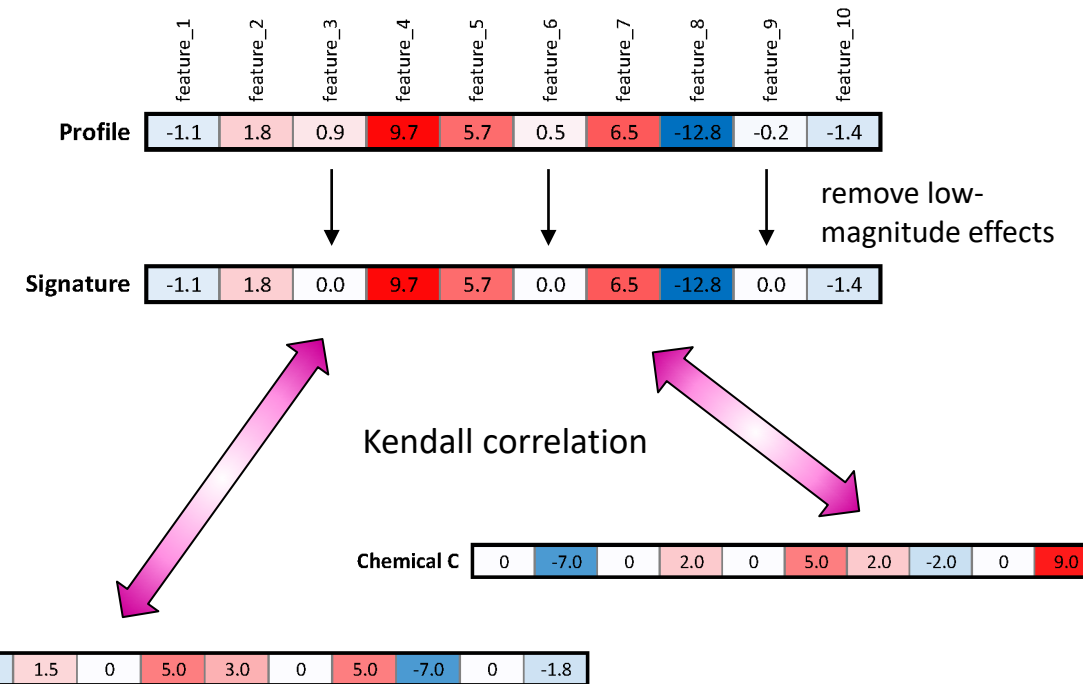
Application 2:

Mechanistic prediction
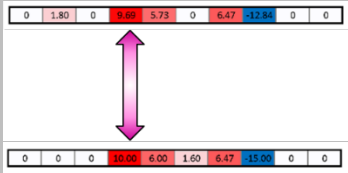
- 50 chemicals were annotated as targeting a nuclear receptor

Biological similarity in HTPP

50 chemicals

target

- **AR**
- **ESR**
- **GR**
- **PGR**
- **PPAR**
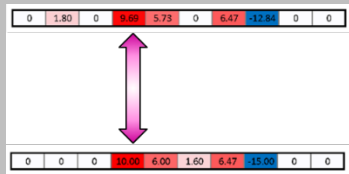- **PXR**
- **RAR**
- **VDR**
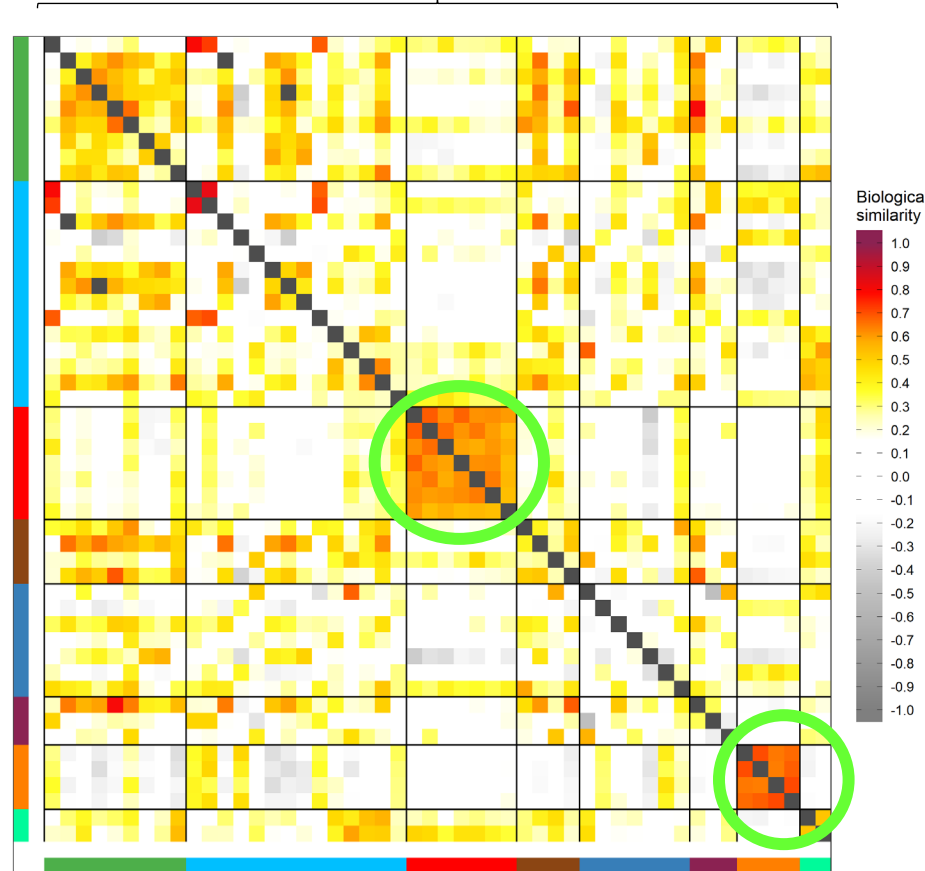
Gene expression in U-2 OS
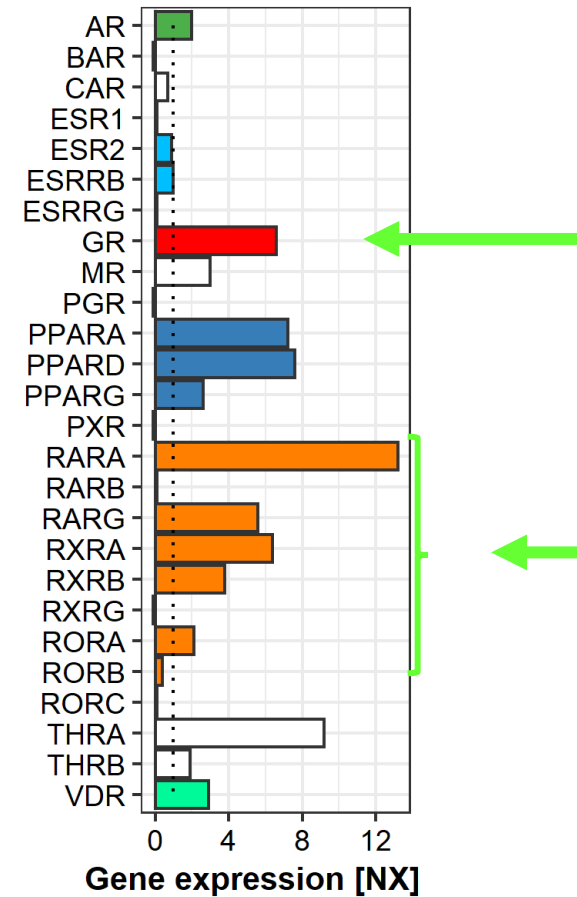
⇒ **Agonists of the GR and of RAR/RXR display characteristic profiles**
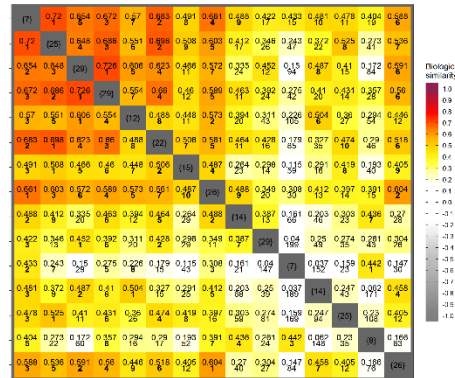
Preliminary results. Do not cite or quote.

Application 2:

Mechanistic prediction

1. Compare profiles to 5 known retinoids:

→ 10 candidate chemicals

2. Repeat HTPP experiments:
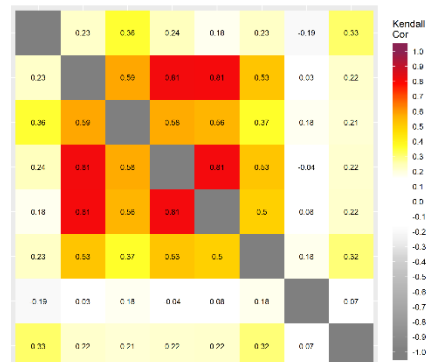
→ 4/10 chemicals confirmed

3. Compare to ToxCast results:

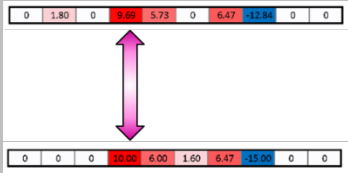RAR/RXR assays
other assays
HTPP potency
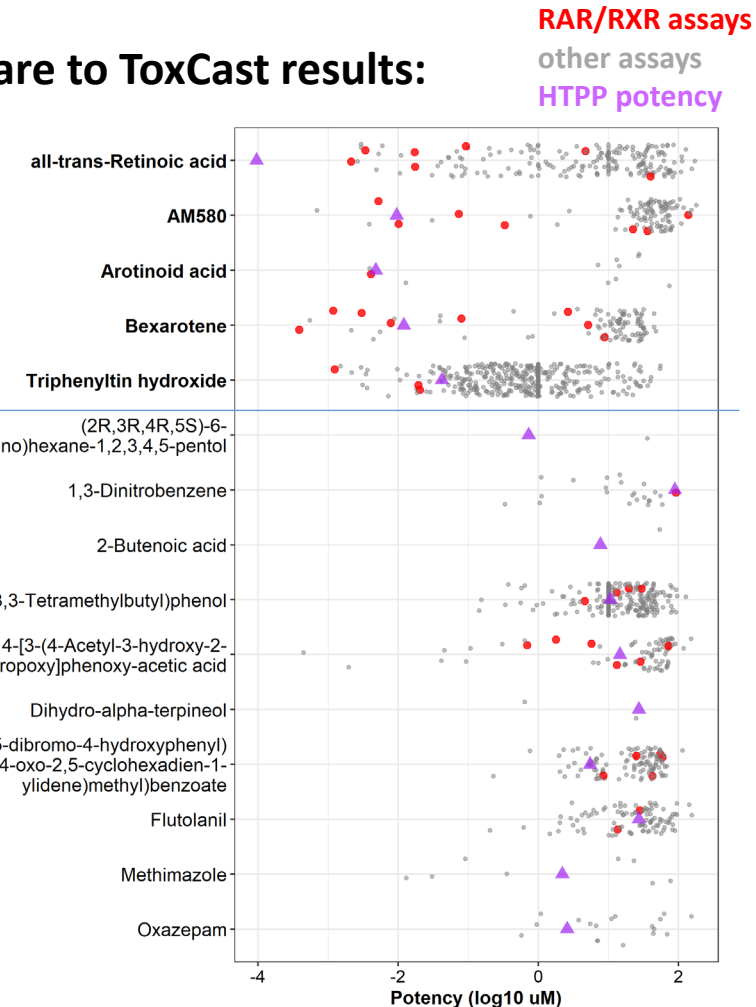
→ These 4 chemicals had activity in ToxCast assays targeting RAR/RXR

⇨ **HTPP has the potential to identify environmental chemicals with specific activities**

Preliminary results. Do not cite or quote.

Specific vs Non-specific Phenotypes

Application 2:

Mechanistic prediction

small groups of chemicals with a very **specific** phenotype

large groups of chemicals with similar phenotype → **non-specific** toxicity mechanisms?
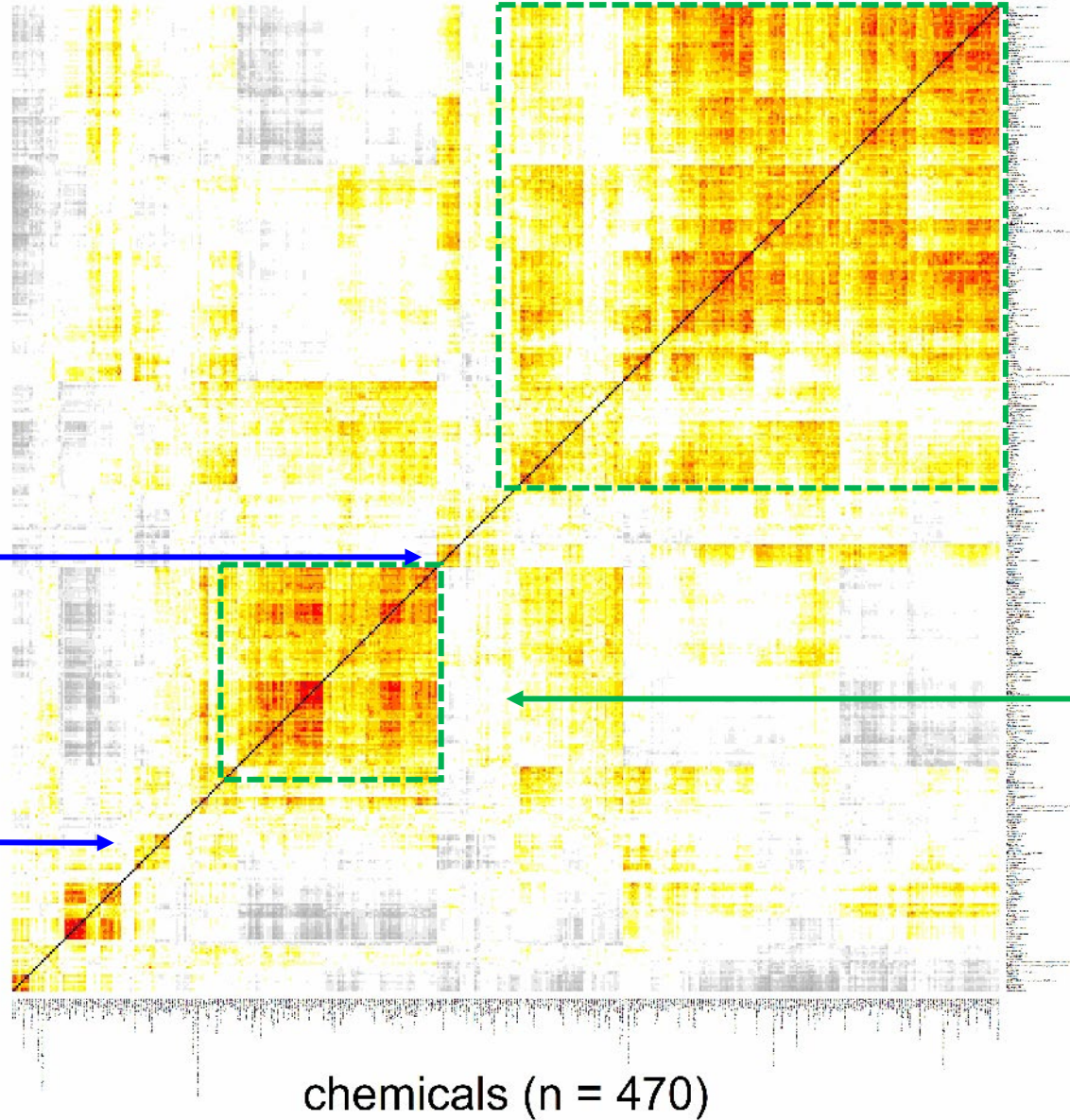
chemicals (n = 470)

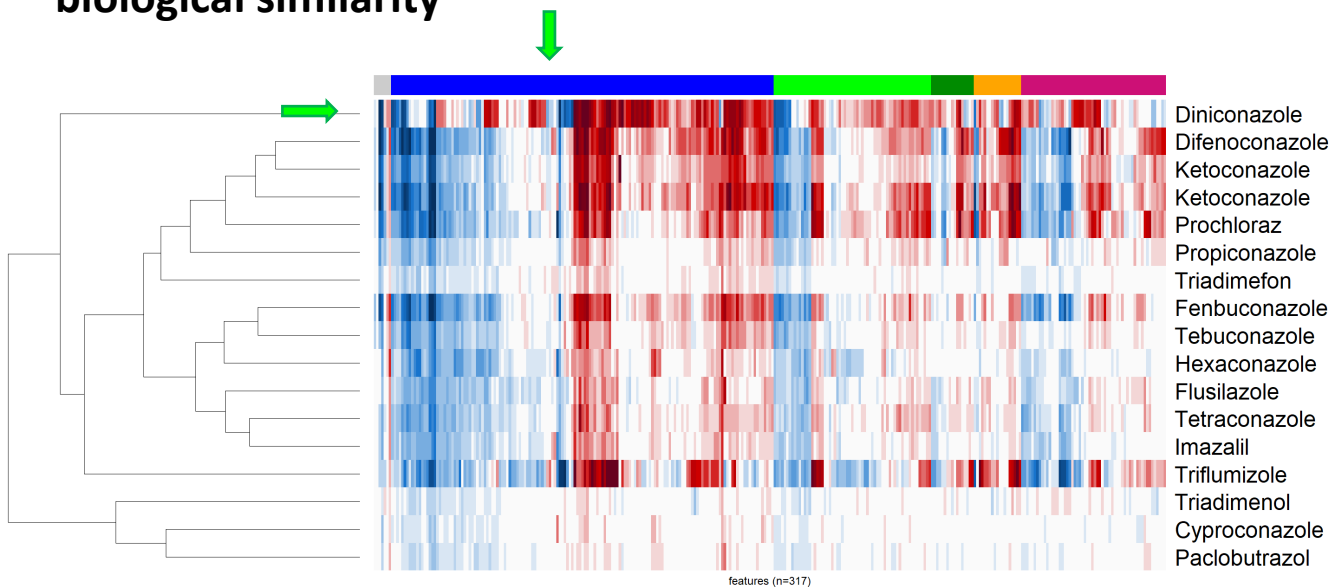chemicals (n = 470)

Color Key and Histogram
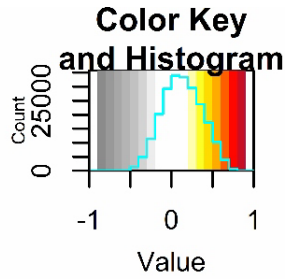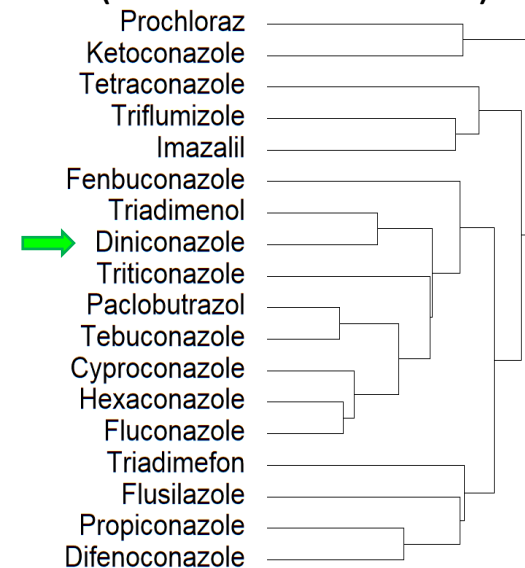
Application: Grouping of Conazoles

Application 2:

Mechanistic prediction

- group of fungicides
- disturb ergosterol synthesis via CYP51 and CYP61 (target absent in mammals)
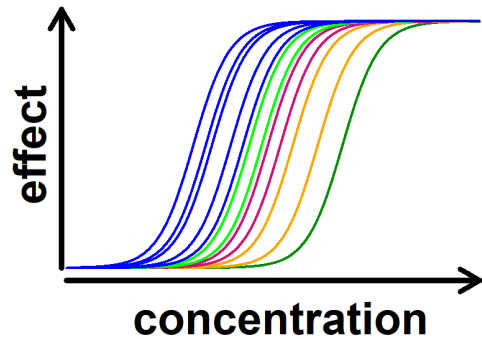

biological similarity

structural similarity
(based on ToxPrints)

⇨ **most conazoles are phenotypically similar**

⇨ **Diniconazole is phenotypically different from the other active conazoles**

*Preliminary results. Do not cite or quote.*

Conclusions

Application 1: Potency estimation

- HTPP can be used to derive *in vitro* potency estimates

- *in vitro* potency estimates often comparable or more conservative than *in vivo* PODs

- used for Bioactivity-Exposure-Ratio (BER) analysis

Application 2: Mechanistic prediction

- Identification of chemicals with specific mechanisms e.g., chemicals with retinoid-like activity

- Biological grouping of structurally related chemicals e.g., conazoles
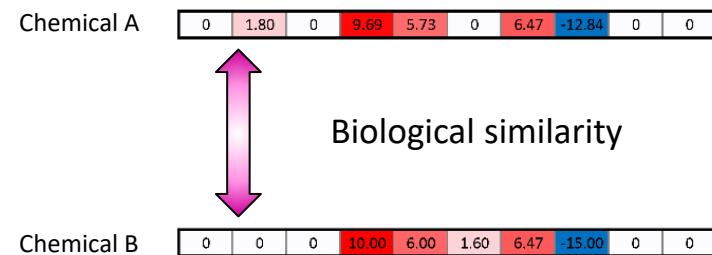
Acknowledgements

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