High-throughput phenotypic profiling to inform putative mode of action for environmental chemicals

Abstract #320

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INTRODUCTION & OBJECTIVE

- US EPA has a tiered testing strategy for chemical hazard evaluation
- High-throughput phenotypic profiling (HTPP) is an imaging based, untargeted screening approach
- **Can HTPP provide information about putative modes of action (MoA) as part of US EPA's tiered testing strategy?**

APPROACH

1. Test 120 chemicals with annotated MoA and 441 environmental chemicals
2. Compute biological similarities of the phenotypic profiles and compare among chemicals

MAIN RESULTS

- Different phenotypic profiles are observed
- Structurally similar chemicals often induce similar profiles
- Structurally diverse chemicals with shared MoA often induce similar profiles

IMPACT

HTPP can be used to derive putative MoA information – both for structurally similar and structurally diverse chemicals - that could be used in the context of a tiered testing strategy for chemical hazard evaluation

This work does not reflect US EPA policy.
Mention of tradenames or products does not represent endorsement for use.

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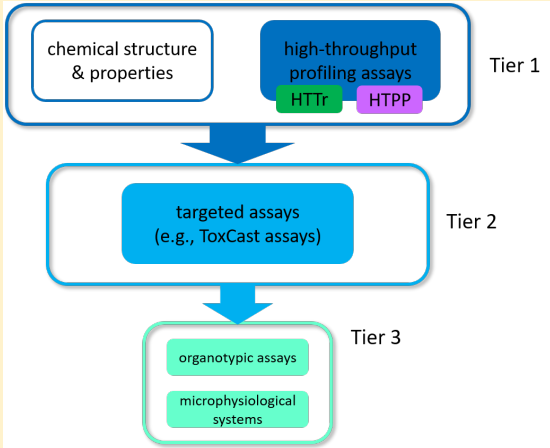
INTRODUCTION & OBJECTIVE

US EPA's tiered testing strategy

- The US EPA developed a tiered strategy for chemical hazard evaluation that is based on New Approach Methods (NAMs)



adapted from Thomas et al. 2019

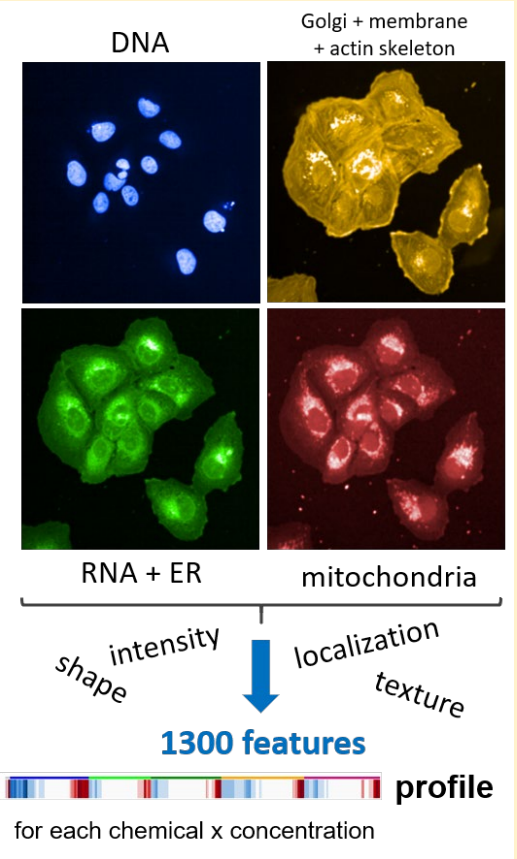
- Tier 1 consists of two high-throughput profiling assays:
  - high-throughput transcriptomics (HTTr)
  - high-throughput phenotypic profiling (HTPP)
- Goals:
  - potency estimation
  - prediction of putative modes of action (MoA)

High-throughput phenotypic profiling (HTPP)

- Labeling of various cell organelles with fluorescent probes in *in vitro* cultures
- Assessing a large variety of morphological features
- 'Cell Painting' assay:
  Gustafsdottir et al. 2013, Bray et al. 2016

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

- Amenable to many cell types
- Cost-effective

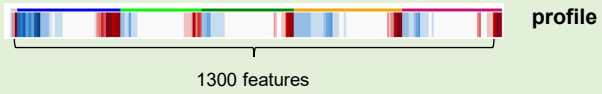


Can high-throughput phenotypic profiling provide information about putative modes of action as part of the tiered testing strategy for chemical hazard evaluation?

High-throughput phenotypic profiling to inform putative mode of action for environmental chemicals

APPROACH

1. Test reference chemicals and environmental chemicals in the 'Cell Painting' assay
   - human U-2 OS osteosarcoma cells
   - 24 h exposure
   - 8 concentrations
2. Derive profiles for the highest non-cytotoxic concentration of each chemical



120 reference chemicals
with annotated mode-of-actions

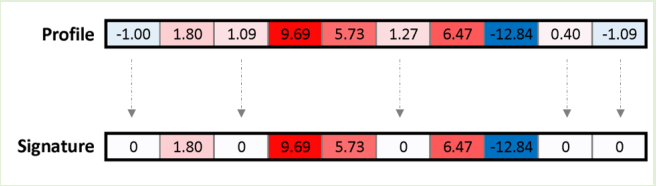
Can we identify putative mode of action for environmental chemicals based on similarities in phenotypic response?

441 environmental chemicals
75% pesticides, drug-like, food additives, industrial chemicals, …
(Nyffeler *et al.* 2020)
https://comptox.epa.gov/dashboard/chemical_lists/HTPP2019_SCREEN

Category	# of chems
1 actin skeleton modulator	
1.1 destabilizer	4
1.2 stabilizer	2
2 ER modulator	4
3 Golgi modulator	4
4 Mitochondrial fission	
4.1 inhibitor	2
4.2 enhancer	1
5 Microtubuli modulator	
5.1 Colchicin-like	4
5.2 Fenbendazole-like	9
5.3 Taxol-like (stabilizer)	3
6 DNA toxicants	
6.1 alkylators	10
6.2 topoisomerase	8
6.3 antimetabolites	7
6.4 DNA damage	3
6.5 DNA replication inhibitor	1

Category	# of chems
7 Oxidative stress	4
8 Protein homeostasis	
8.1 Proteasome inhibitor	7
8.2 Protein synthesis inhibitor	2
8.3 RNA polymerase inhibitor	1
9 uncoupler	3
10 mitochondria complex inhibitor	10
11 autophagy	
11.1 inhibitor	4
11.2 activator	7
11.3 Rapamycin-like (activator)	4
12 cell shape modulator	1
13 Berberine chloride group	2
14 Tetrandrine group	2
15 - 20 individual RefChem16 not contained in previous categories	6
21 negative controls	5

3. Calculate biological similarity:

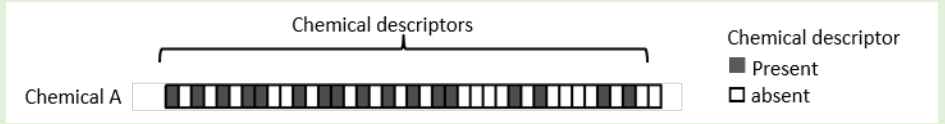


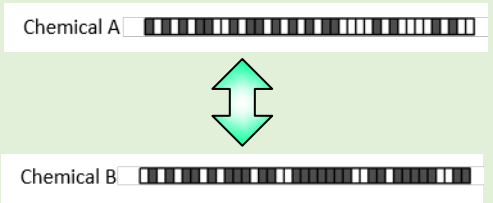
1. Generate signatures
by replacing |values| < 1.5 with 0

2. Compare signatures
biological similarity = Pearson correlation

4. Calculate structural similarity:

1. Morgan fingerprints are used to represent chemical structures





2. Compare fingerprints
Structural similarity = Tanimoto/Jaccard similarity:

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} = \frac{\#\text{ shared structural features}}{\text{total number of measured features}}$$

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MAIN RESULTS: Profiles of reference chemicals

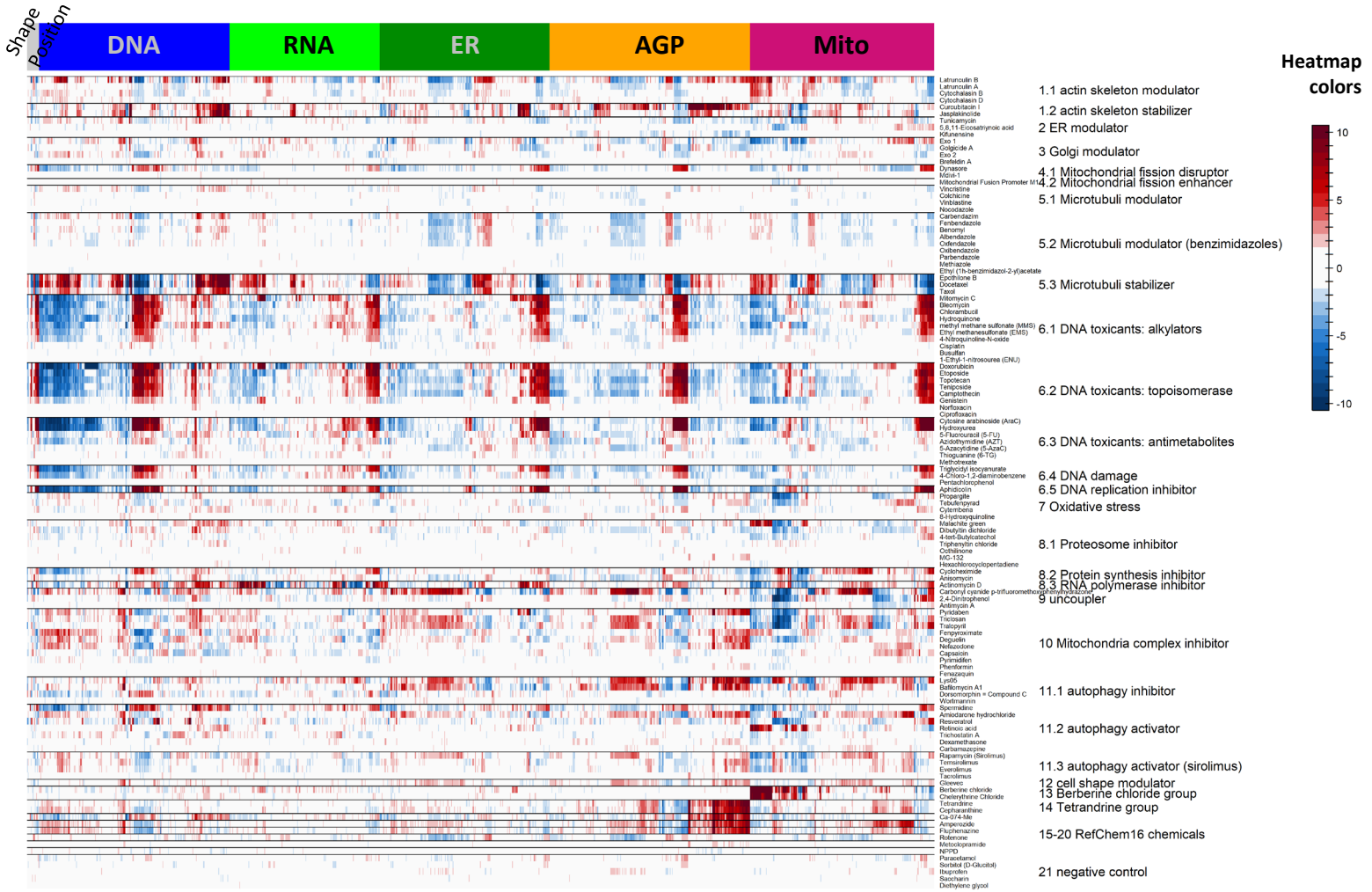

Fig 1: Signatures of 120 reference chemicals. Chemicals were manually grouped by their known mechanism-of-action. For each chemical, data from the highest non-cytotoxic concentration is displayed. Signatures were generated by flooring all absolute values < 1.5 to 0. Features (in columns) are ordered according to the corresponding channel/organelle.

⇨ Different signatures are observed

⇨ Different classes of DNA toxicants (group 6) share similar signatures

Example: Microtubule stabilizers

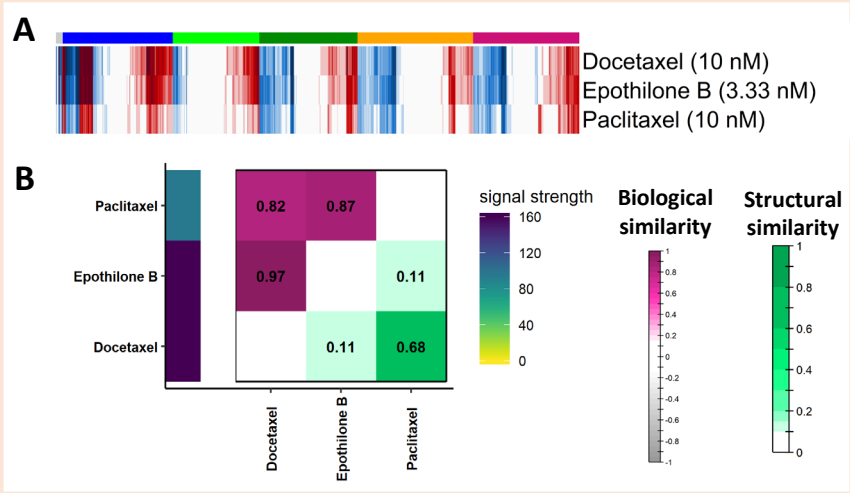

Fig 2: Structural and biological similarities of microtubule stabilizers. (A) Signature of the highest non-cytotoxic concentration of each chemical. Features were clustered within a fluorescent channel for display. (B) Correlation matrix of biological and structural similarity.

⇨ Epothilone B is structurally different from paclitaxel and docetaxel but phenotypically similar

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MAIN RESULTS: Clustering of reference & environmental chemicals

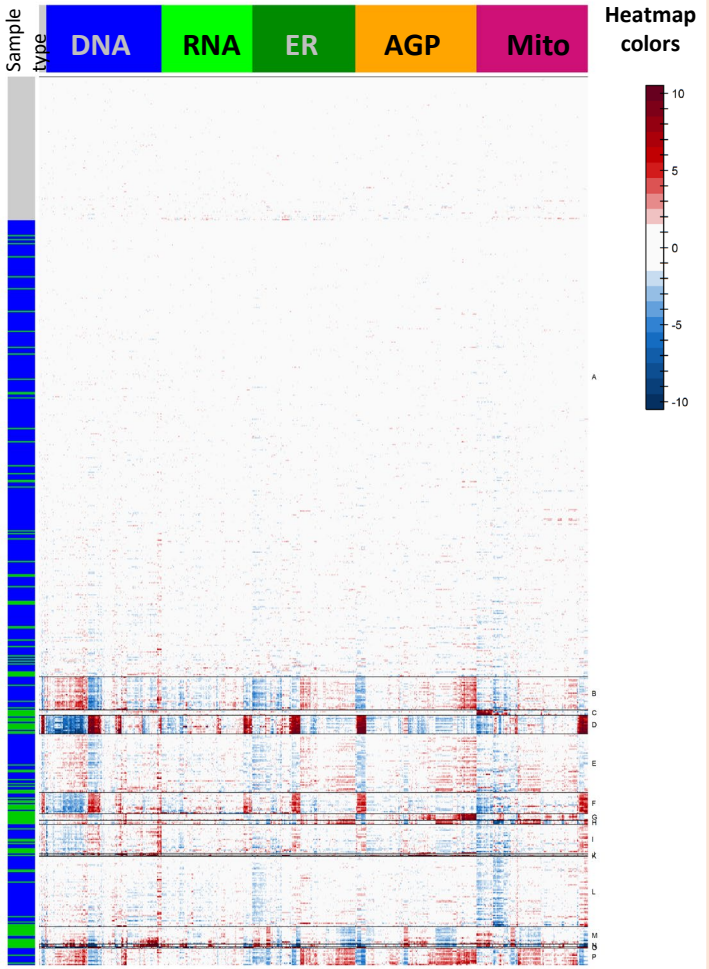
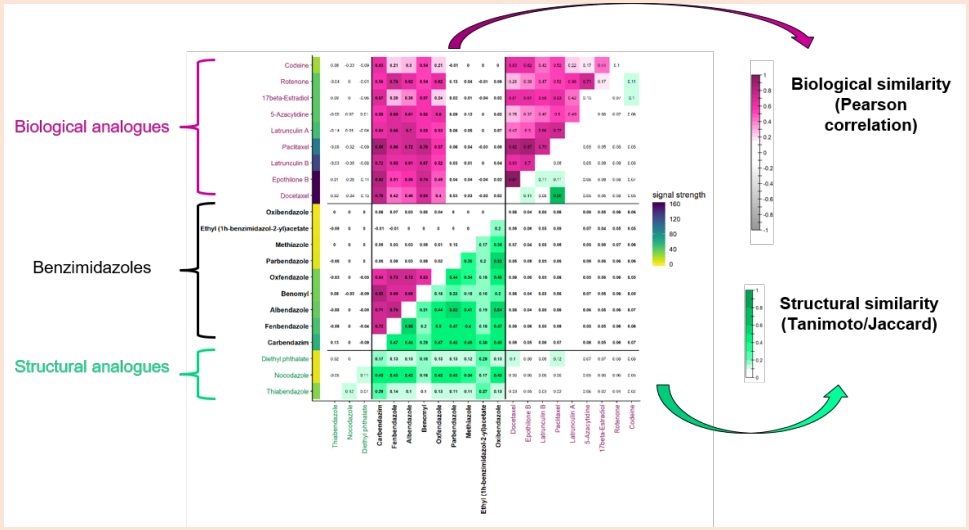


Fig 3: K means clustering of all chemicals. For each chemical, data from the highest non-cytotoxic concentration was used to generate a signature. Features (in columns) are ordered according to the corresponding channel/organelle. The number of clusters was chosen so that visually different signatures were in different clusters but replicates of the same chemical were in the same cluster (not shown).

- ⇨ Approximately 16 signature clusters are observed
- ⇨ 300/441 environmental chemicals clustered with the null data sets (i.e. have no distinctive signature at the highest non-cytotoxic concentration)
- ⇨ The remaining environmental chemicals mostly shared signatures with reference chemicals



Example: Benzimidazoles

- ⇨ All benzimidazoles are structurally similar, but only 5 had biological similarity (the other 4 have low signal strength)
- ⇨ Among the biological analogues are microtubule stabilizer (group 5.3) as well as actin cytoskeleton modulators (group 1.1).

Fig 4: Correlation matrix of biological and structural analogues of benzimidazoles All tested chemicals were searched for structural and biological similarity to any of the benzimidazoles. Chemicals with a structural similarity > 0.25 or biological similarity > 0.6 to any benzimidazole are displayed. The upper left half of the correlation matrix displays biological similarity (as pearson correlation), while the lower right half of the matrix displays structural similarity (measured as Tanimoto similarity).

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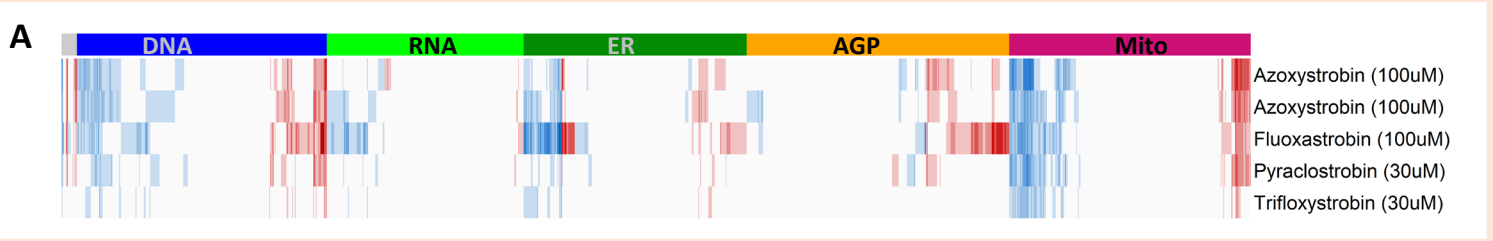
MAIN RESULTS: Environmental chemicals

Example: Strobilurins



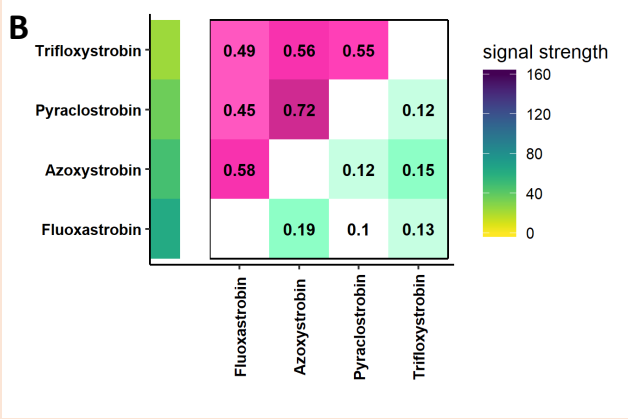
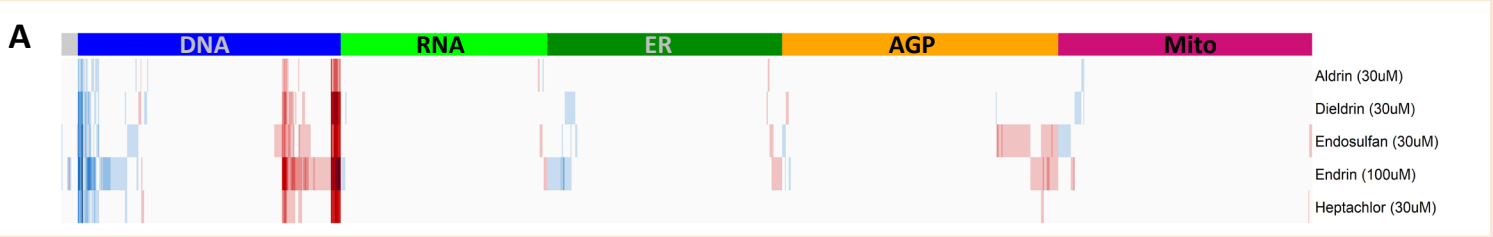


Fig 5: Similarity of strobilurins. (A) Signature of the highest non-cytotoxic concentration of each strobilurin (Azoxystrobin was tested in duplicate). Features were clustered within a fluorescent channel for display. (B) Correlation matrix of biological and structural similarity of strobilurins.

⇨ Strobilurins have similar signatures with many mitochondrial features affected.

Example: Dieldrin



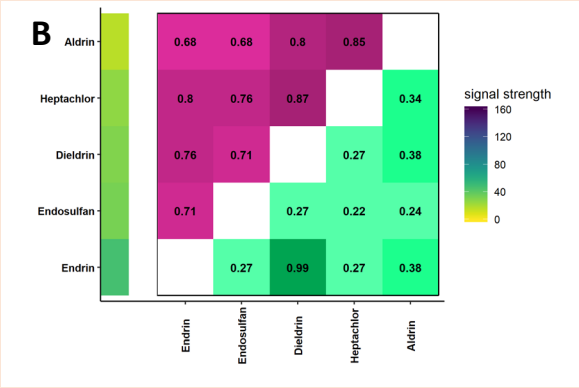
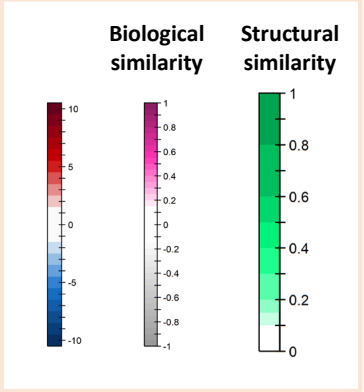


Fig 6: Structural and biological analogues of dieldrin. All tested chemicals with a structural similarity of > 0.2 are displayed. (A) Signature of the highest non-cytotoxic concentration of each chemical. Features were clustered within a fluorescent channel for display. (B) Correlation matrix of biological and structural similarity.

⇨ Four structural analogues to dieldrin displayed high biological similarity with dieldrin, with changes in the DNA channel.



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IMPACT/SIGNIFICANCE

- HTPP revealed different phenotypic profiles for different MoAs
- Chemicals with similar structure often induce similar phenotypes

HTPP can be used to derive putative MoA information – both for structurally similar and structurally diverse chemicals - that could be used in the context of the tiered testing strategy for chemical hazard evaluation

OUTLOOK

- Refine biological similarity calculation:
  - Redundant features might distort biological similarity measurements
    → evaluate feature reduction and feature selection approaches prior to similarity calculations
  - How to incorporate information from multiple concentrations
- Apply similarity calculation to a larger screen of 1200 environmental chemicals

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