Identifying putative modes-of-action for environmental chemicals using high-throughput phenotypic profiling

Johanna Nyffeler[1,2], Clinton Willis[1], Grace Patlewicz[1], Daniel Chang[1], Imran Shah[1], Joshua Harrill[1]

[1] Center for Computational Toxicology & Exposure, Office of Research & Development, US Environmental Protection Agency, Durham NC, United States.
[2] Oak Ridge Institute for Science and Education (ORISE) Postdoctoral Fellow, Oak Ridge, TN, United States.

Johanna Nyffeler | Nyffeler.johanna@epa.gov | ORCiD 0000-0002-6155-9743

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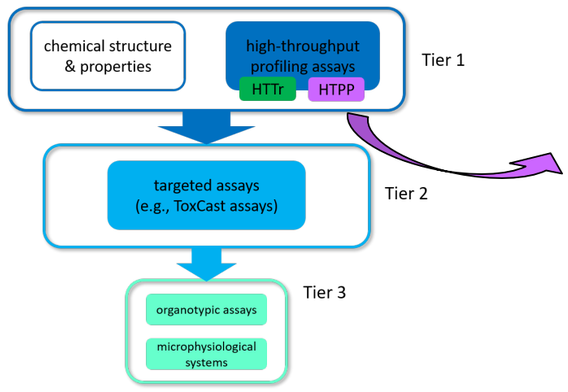
Introduction & Objective

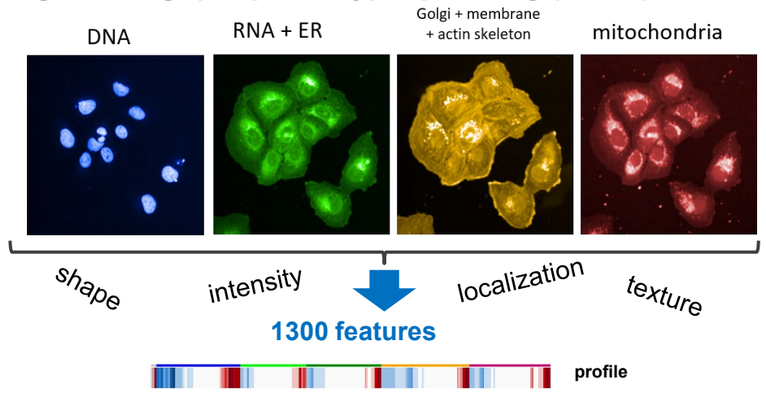
US EPA's tiered testing strategy



adapted from Thomas et al. 2019 [PMID: 30835285]

- The US EPA developed a tiered strategy for chemical hazard evaluation that is based on New Approach Methods (NAMs)
- Tier 1 includes 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 [PMID: 24312513], Bray et al. 2016 [PMID: 27560178]
- Amenable to many cell types
- Cost-effective

Aim: Determine if high-throughput phenotypic profiling provides information about putative modes-of-action as part of the tiered testing strategy for chemical hazard evaluation.

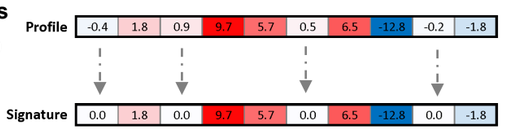
Method

Feature selection: 1300 reduced to 316 most informative features

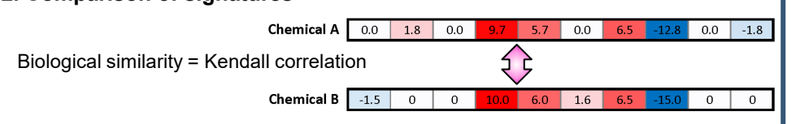
Calculation of biological similarity

1. Generation of signatures

replacing |values| < 1 with 0



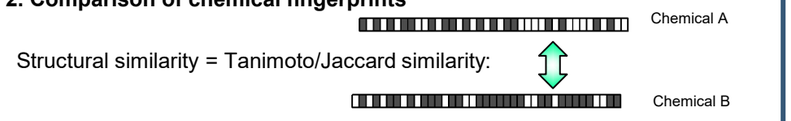
2. Comparison of signatures



Calculation of structural similarity

1. ToxPrint fingerprints

2. Comparison of chemical fingerprints



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

Study 1: Qualitative comparison

Experimental design	
Cell type	human U-2 OS osteosarcoma cells
Exposure time	24 h
# chemicals	120 reference + 441 environmental
# concentrations	8, ½ $\log_{10}$ dose spacing
Replicates	1 per plate | 4 independent experiments

120 reference chemicals with annotated modes-of-action

Can we identify putative modes-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 [PMID: 31899216])

Category	# of chems	Category	# of chems	Category	# of chems
1 Actin skeleton modulator		**6 DNA toxicants**		**10 Mitochondria complex inhibitor**	10
1.1 Destabilizer	4	6.1 Alkylators	10	**11 Autophagy**	
1.2 Stabilizer	2	6.2 Topoisomerase	8	11.1 Inhibitor	4
2 ER modulator	4	6.3 Antimetabolites	7	11.2 Activator	7
3 Golgi modulator	4	6.4 DNA damage	3	11.3 Rapamycin-like (activator)	4
4 Mitochondrial fission		6.5 DNA replication inhibitor	1	**12 Cell shape modulator**	1
4.1 Inhibitor	2	**7 Oxidative stress**	4	**13 Berberine chloride group**	2
4.2 Enhancer	1	**8 Protein homeostasis**		**14 Tetrandrine group**	2
5 Microtubuli modulator		8.1 Proteasome inhibitor	7	**15 - 20 Individual RefChem16 not contained in previous categories**	6
5.1 Colchicin-like	4	8.2 Protein synthesis inhibitor	2	**21 Negative controls**	5
5.2 Fenbendazole-like	9	8.3 RNA polymerase inhibitor	1		
5.3 Taxol-like (stabilizer)	3	**9 Uncoupler**	3		

Profiles of reference chemicals

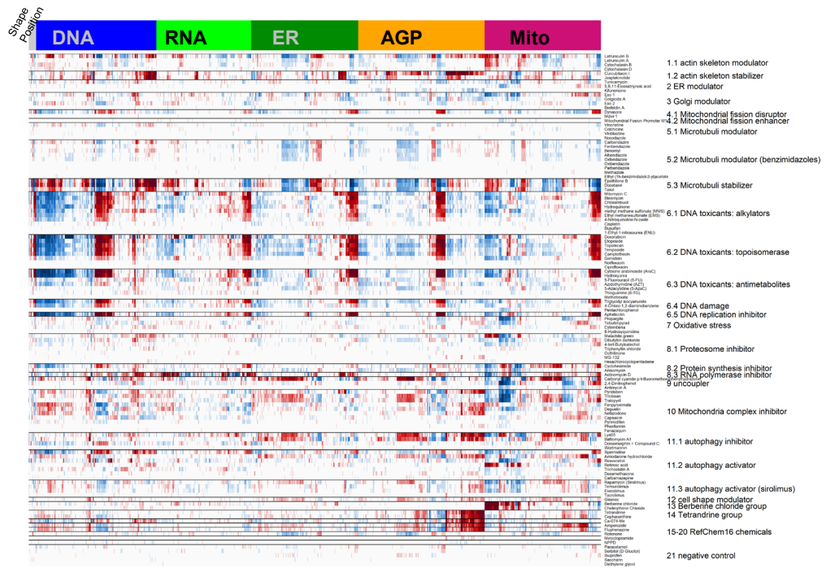


Fig 1: Signatures of 120 reference chemicals. Chemicals were manually grouped by their known mode-of-action. For each chemical, data from the highest non-cytotoxic concentration is displayed. Signatures were generated by flooring all absolute values < 1.5 to 0. Features (in columns) are ordered according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

⇨ **Different signatures are observed**

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

⇨ **Signatures of microtubule modulators (group 5) are different from DNA toxicants (group 6)**

Clustering of reference & environmental chemicals

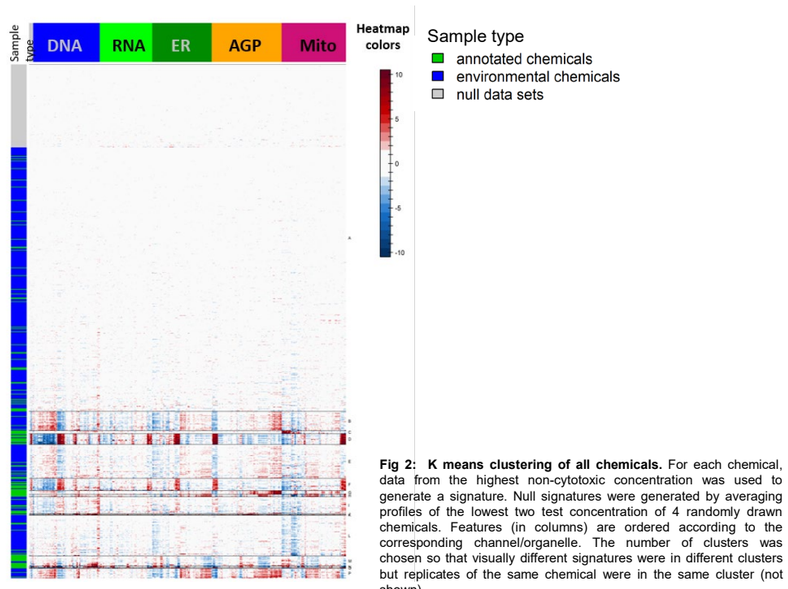


Fig 2: K means clustering of all chemicals. For each chemical, data from the highest non-cytotoxic concentration was used to generate a signature. Null signatures were generated by averaging profiles of the lowest two test concentration of 4 randomly drawn chemicals. Features (in columns) are ordered 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**

Study 2: Quantitative comparison

Experimental design	
Cell type	human U-2 OS osteosarcoma cells
Exposure time	24 h
# chemicals	1205
# concentrations	8, ½ $\log_{10}$ dose spacing
Replicates	1 per plate | 4 independent experiments

Biological similarity of nuclear receptor modulators

48 chemicals were annotated in ToxRefDB with targeting a nuclear receptor

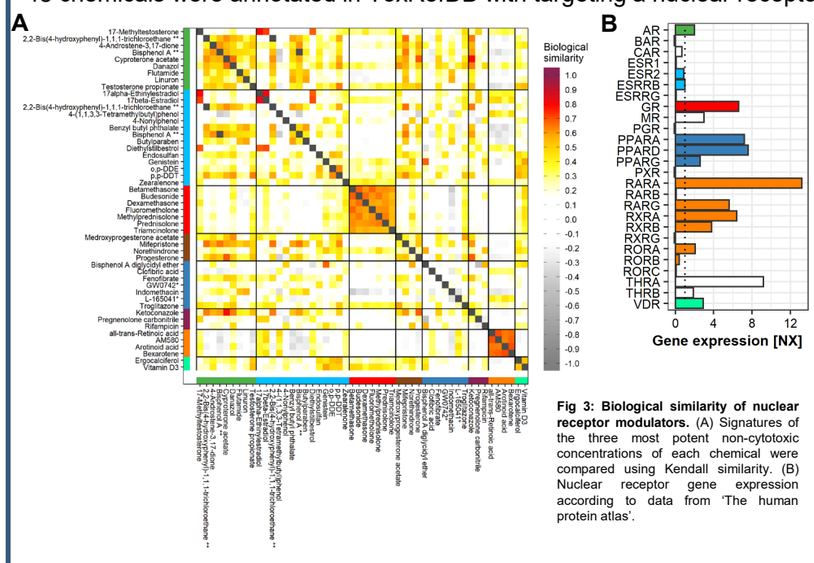


Fig 3: Biological similarity of nuclear receptor modulators. (A) Signatures of the three most potent non-cytotoxic concentrations of each chemical were compared using Kendall similarity. (B) Nuclear receptor gene expression according to data from 'The human protein atlas'.

⇨ **Glucocorticoids and retinoids each result in characteristic signatures**

⇨ **Glucocorticoid receptor (GR) and retinoic acid receptors (RAR) are expressed in U-2 OS cells.**

Biological similarity of all tested, active chemicals

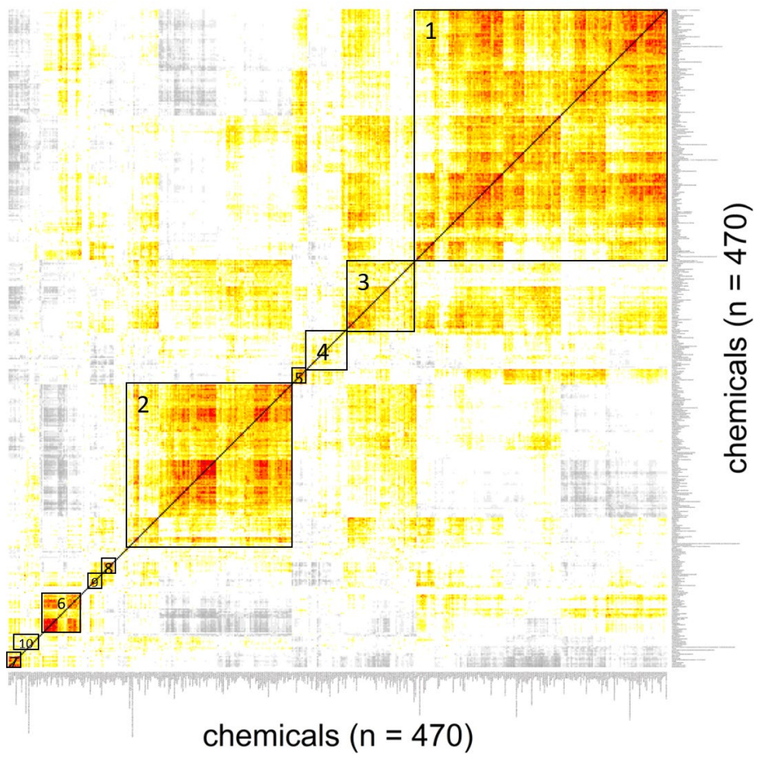


Fig 4: Biological similarity of nuclear receptor modulators. Signatures of the three most potent non-cytotoxic concentrations of each active chemical were compared using Kendall similarity. The clusters were manually drawn.

⇨ **The majority of chemicals cluster into two large groups that may represent non-specific biological effects such as cell stress.**

Biological similarity of structurally related chemicals

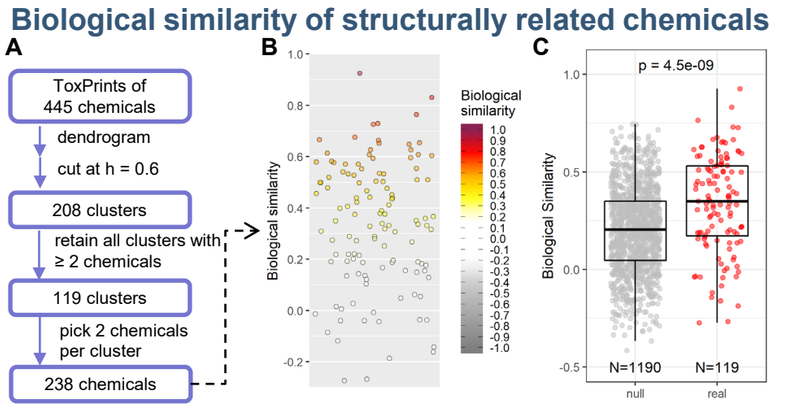


Fig 5: Biological similarity of structurally related chemicals. (A) Chemicals were grouped by their chemotype into clusters. (B) The biological similarity of a chemical pair was retained from each cluster. (C) The distribution of biological similarity values was compared to a 'null dataset' derived by assigning the same 445 chemicals to random clusters (with the same size distribution as the real data), repeated 10 times. The p-value was calculated using a one-sided Wilcoxon rank sum test (non-parametric).

⇨ **Chemicals that share structural similarity (i.e. are in the same cluster) are more phenotypically similar than expected by chance.**

Environmental chemicals

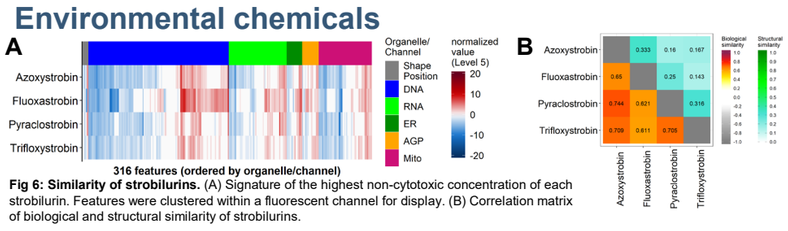


Fig 6: Similarity of strobilurins. (A) Signature of the highest non-cytotoxic concentration of each strobilurin. Features were clustered within a fluorescent channel for display. (B) Correlation matrix of biological and structural similarity of strobilurins.

⇨ **Strobilurins share high biological similarity but low structural similarity.**

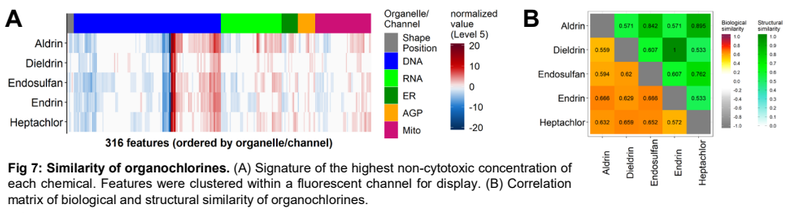


Fig 7: Similarity of organochlorines. (A) Signature of the highest non-cytotoxic concentration of each chemical. Features were clustered within a fluorescent channel for display. (B) Correlation matrix of biological and structural similarity of organochlorines.

⇨ **Several organochlorines share high structural and biological similarity.**

Conclusions: Different phenotypic profiles are observed, with some being characteristic for specific modes-of-action or chemical groups. Phenotypic profiles establish a basis for prioritizing chemicals for further hazard characterization using a tiered strategy.