Substantiating Chemical Categories with Omics-derived Mechanistic Evidence

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and Case Study team

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Disclaimer

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Overview

- Regulatory need and case study objective
- Study design
- Substance selection: azo dyes
- Conventional (Q)SAR profiling to group the azo dyes
  - Defining the ‘conventional grouping hypothesis’
- Omics-based grouping of the azo dyes
  - *Daphnia* toxicity testing
  - Grouping with transcriptomics and metabolomics data
  - Testing the ‘conventional grouping hypothesis’
- Conclusions

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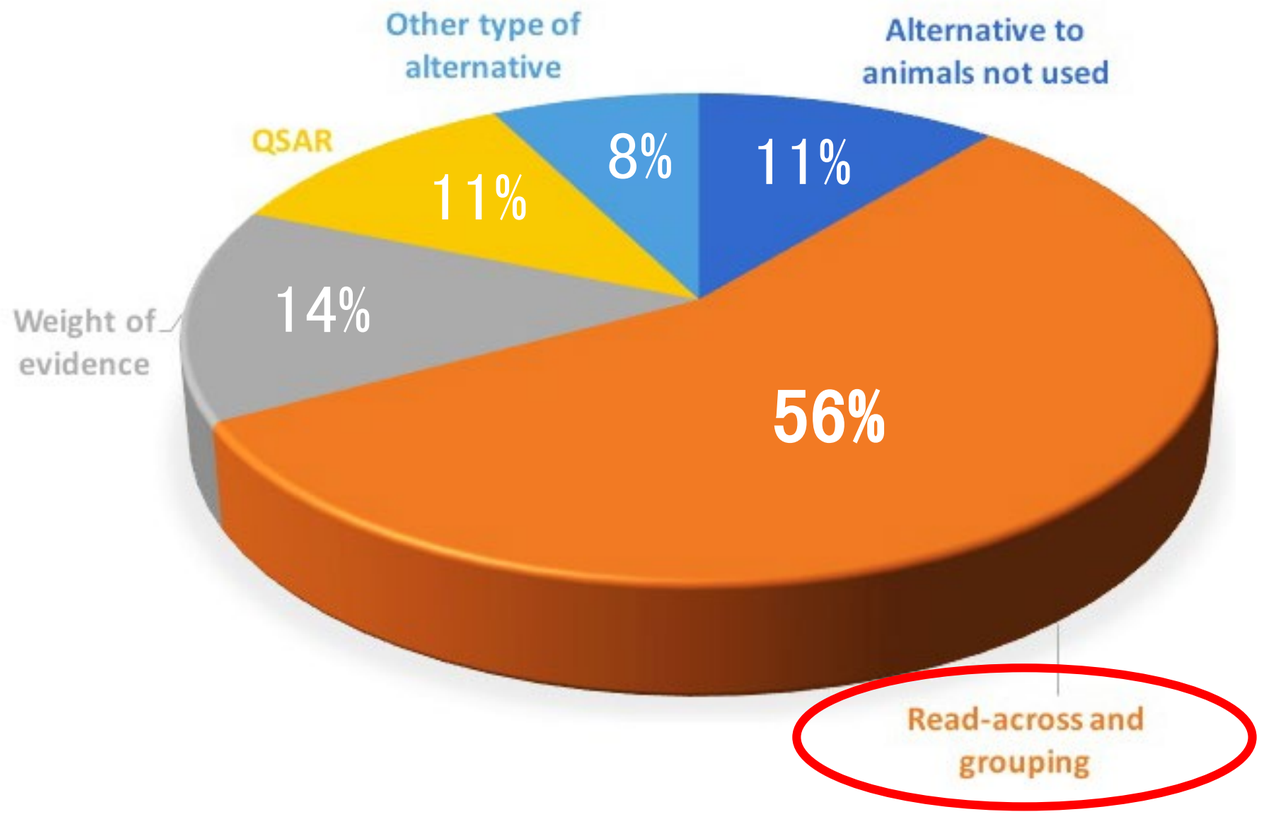
Regulatory need

Dossiers submitted to ECHA between 2008 and 2016 for 6290 substances



ECHA
EUROPEAN CHEMICALS AGENCY

Read-Across Assessment Framework (RAAF)

Can we increase the scientific evidence and therefore the acceptance rate of grouping/read-across dossiers by substantiating them with grouping based upon molecular mechanistic data?

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Case study objective

- To evaluate the capability of multi-omics and computational approaches to group substances based on molecular mechanistic data.
- To use these results to substantiate (or not) the formation of chemical groups derived from conventional QSAR approaches, ultimately to improve the reliably of the read-across.

Daphnia magna

BioSpyder™

TempO-Seq
transcriptomics

Mass spectrometry
metabolomics

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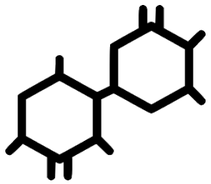
Study design



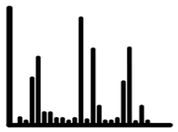
Conventional grouping of 7 substances
- Structure
- Phys-chem properties
- (Q)SAR profiling

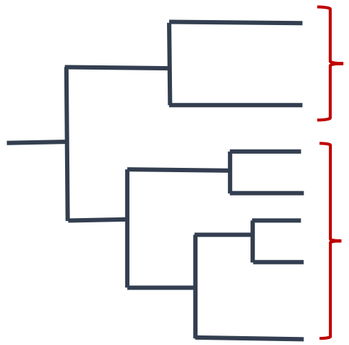
QSAR TOOLBOX — The OECD QSAR Toolbox for Grouping Chemicals into Categories

Group	Substance
A	??
B…	??

Data-poor substances (n=7)

Omics-based grouping of 7 substances
- Transcriptomics
- Metabolomics





Molecular pathway analysis to search for a mechanistic rationale for the grouping

IPA INGENUITY PATHWAY ANALYSIS

Group	Substance
A	??
B…	??

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Chemical selection criteria

- Structure: a specific core & different functional groups
- Log K_{ow}: ~4-7 (some solubility)
- *Daphnia* acute toxicity data (OECD 202): available or predicted
- *Daphnia* chronic toxicity (OECD 211): available
- Ideally REACH registered

- Known to induce toxicity in *Daphnia*
- (Partial) water solubility with low volatility
- Commercially available
- ≥ 95% purity

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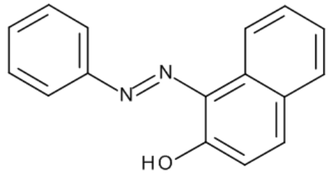
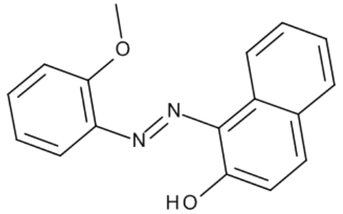
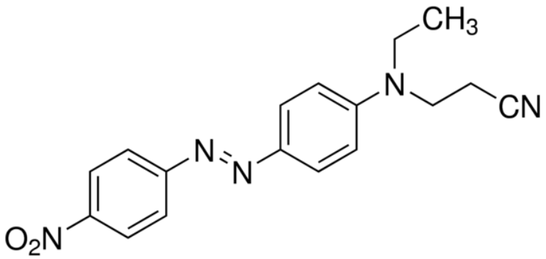
Substance selection: azo dyes



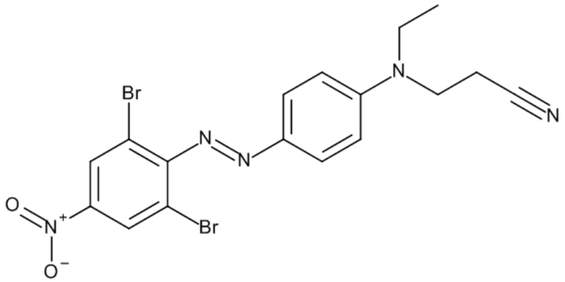
Sudan 1 (S1)



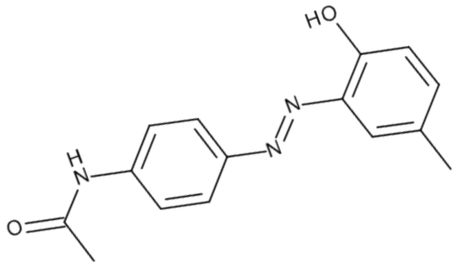
Sudan red G (SRG)



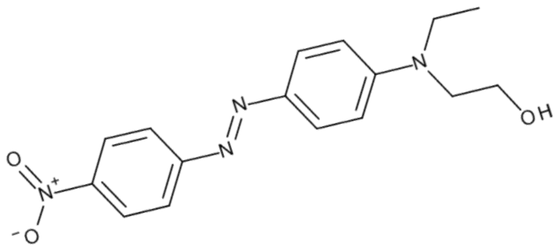
Disperse orange 25 (DO25)



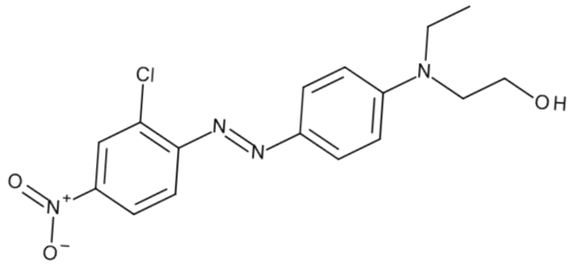
Disperse orange 61 (DO61)



Disperse yellow 3 (DY3)



Disperse red 1 (DR1)



Disperse red 13 (DR13)

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Conventional (Q)SAR profiling to group the 7 azo dyes

Approach	Grouping hypothesis
Structural similarity	**Group A** - S1, SRG **Group B** - DO25, DO61 **Group C** - DR1, DR13 **Group D** - DY3
(Q)SAR profiling	**Group A** - S1, SRG **Group B** - DO25, DO61, DR1, DR13 **Group C** - DY3

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Omics-based grouping of substances: the approach

Dose selection for omics via *Daphnia* OECD TG202

- 7 analytical grade azo dyes
- 48-hr study

BMD modelling of acute toxicity data

- PROAST
- Derived benchmark response = BMR (10% immobilisation)

Daphnia exposures for omics study

- 48-hr study
- 3 sampling times (2, 24, 48 hrs)
- DMSO control and 3 doses (anchored to BMR(10%), sub-lethal)

Omics data generation and analysis

- Transcriptomics using new custom-designed BioSpyder platform (1991 genes)
- Metabolomics using Thermo Scientific direct infusion mass spectrometry approach

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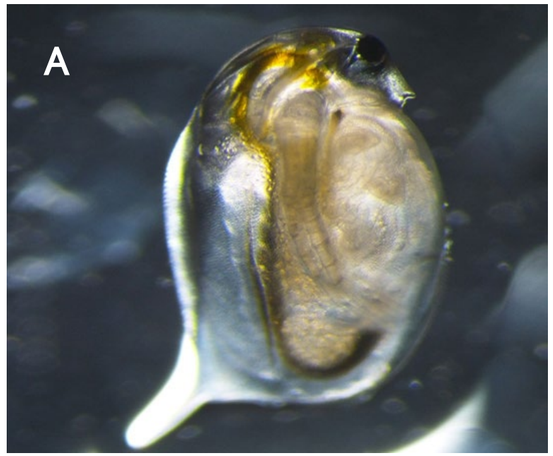
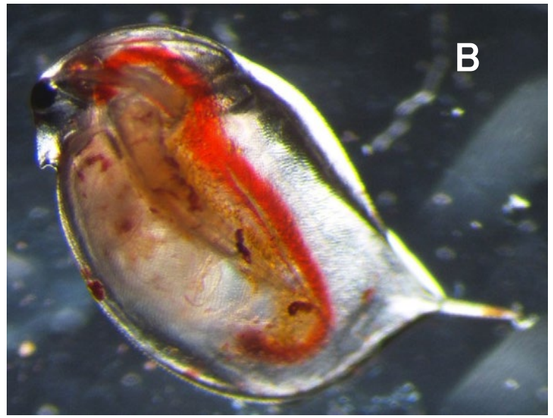
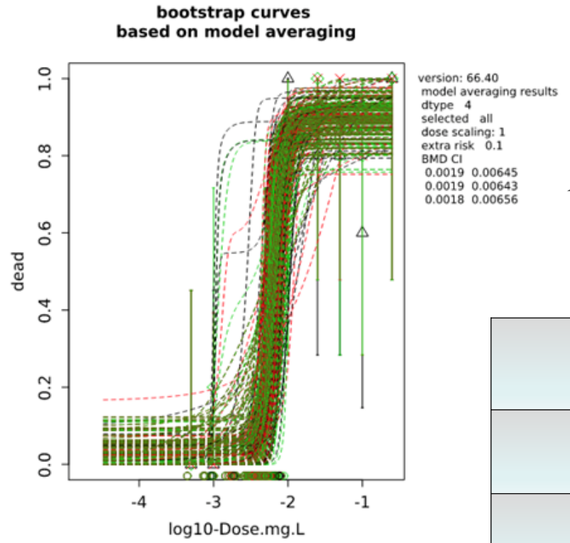
Daphnia toxicity testing results





Light microscopy: **A** – *Daphnia* control,
B – *Daphnia* exposed to Sudan red G
for 24 hrs



Substance	BMR(10%) mg/L
DO61	0.0042
DR13	0.018
Sudan 1	0.078
DR1	0.099
DY3	0.91
Sudan red G	3.4
DO25	No toxicity detected

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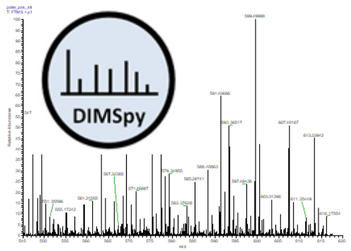
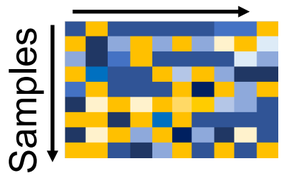
Defined multi-omics workflow

1a. Metabolomics data stream



2a. Metabolic response matrix

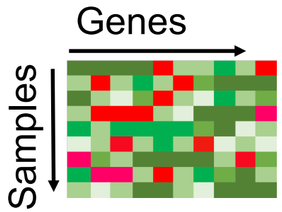
$\log_2$ [mean(treated) / mean(cont)]



1b. Transcriptomics data stream

BioSpyder TempOSeq

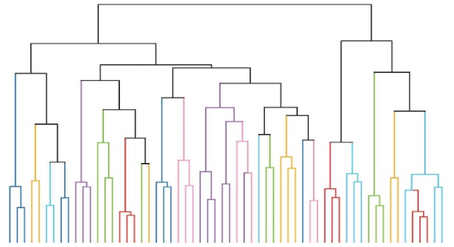
2b. Transcriptional response matrix



3. Fuse molecular data streams

- All 3 doses, 3 time points, 7 dyes
- Unit variance scaling

4. Measure similarities of molecular responses across 7 dyes using hierarchical cluster analysis



Group	Substance
A	??
B…	??

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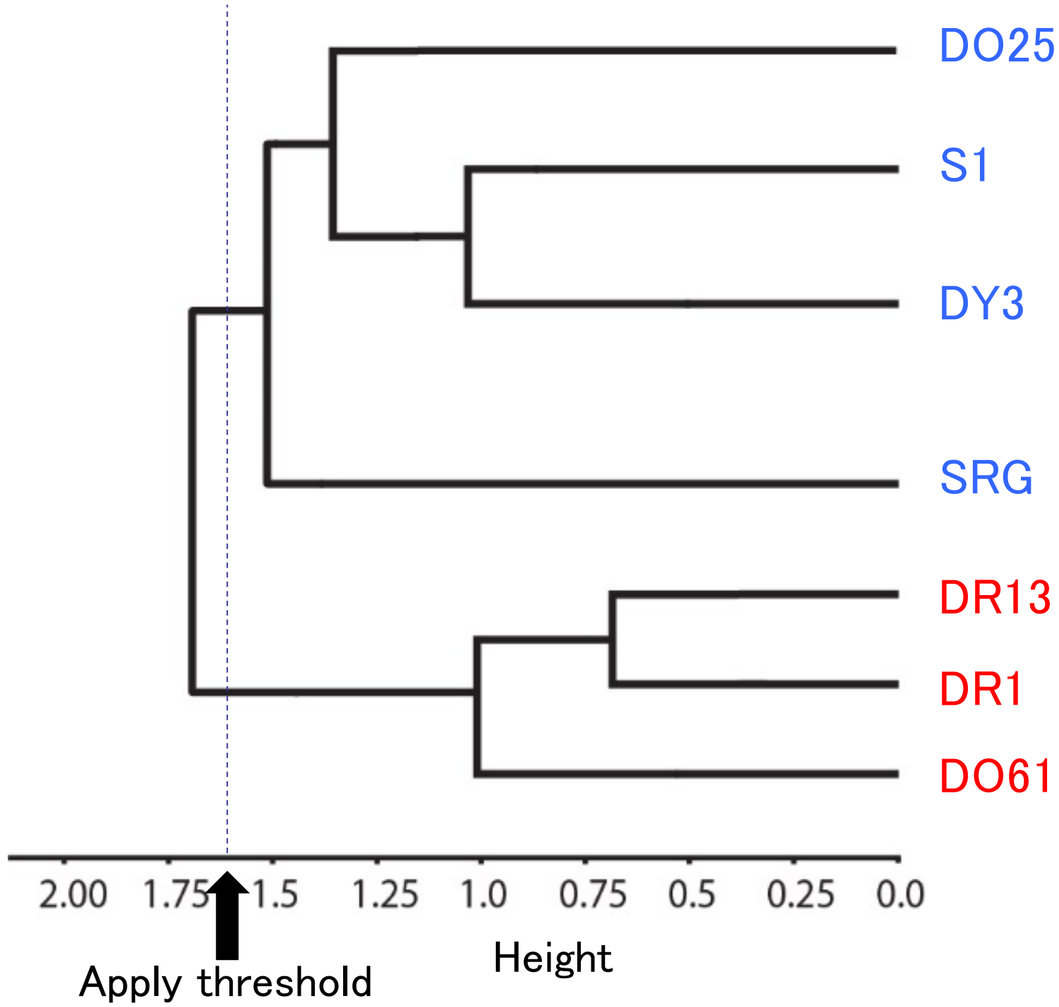
Omics-based grouping results

Focus on highest sub-lethal dose for each of 7 dyes, at 48 hrs only



Group	Substance
A	S1, SRG, DO25, DY3
B	DO61, DR1, DR13

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Testing the ‘conventional grouping hypothesis’

Conventional grouping hypothesis
from (Q)SAR profiling

Group	Substance
A	S1, SRG
B	DO25, DO61, DR1, DR13
C	DY3

Omics-based grouping

Group	Substance
A	S1, SRG, DO25, DY3
B	DO61, DR1, DR13

Which proposed grouping (for read-across) is most reliable?

Can a molecular mechanistic rationale be provided to add confidence to the omics-based grouping?

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Molecular pathway analysis to provide mechanistic support for the grouping

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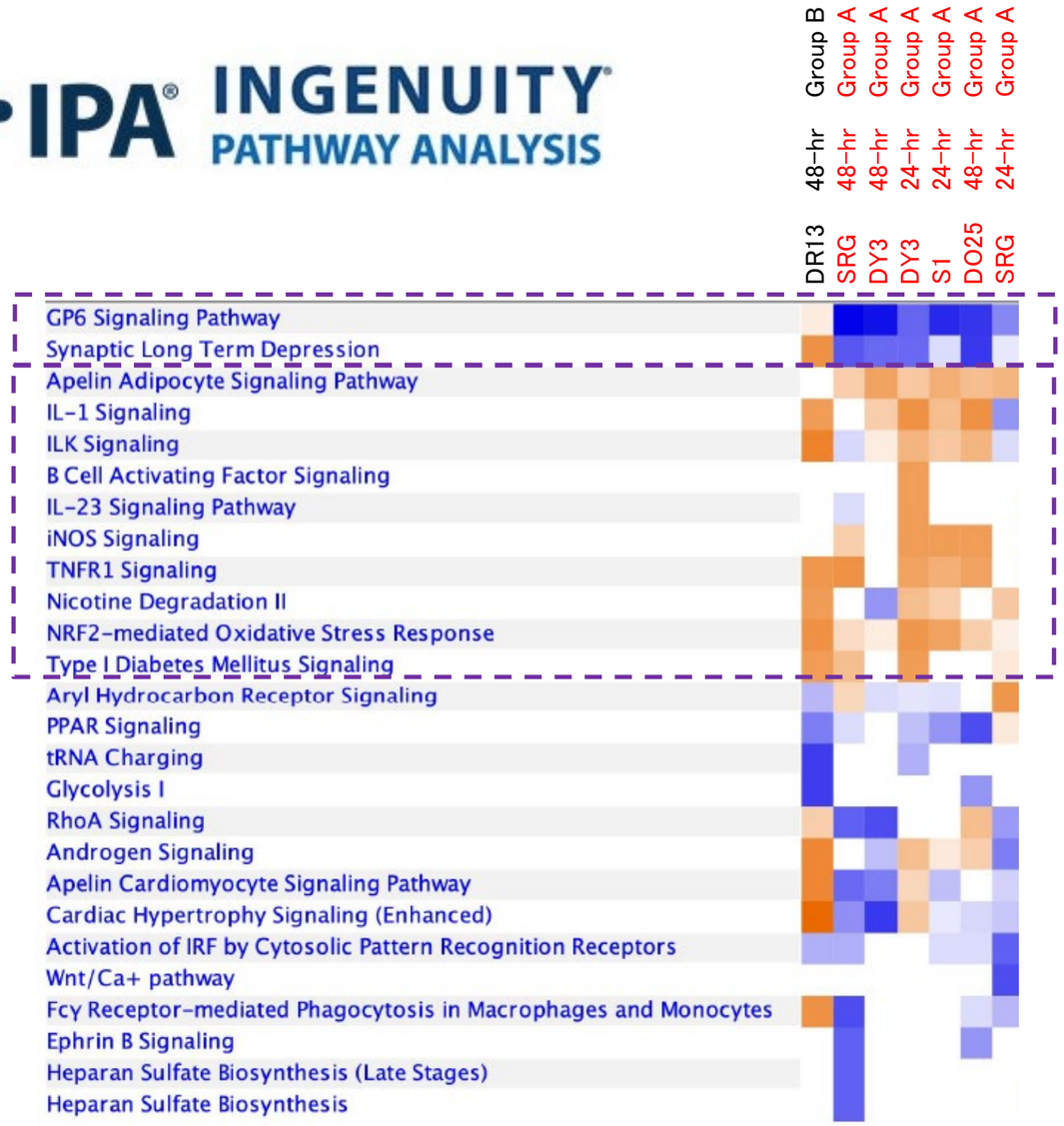

Cellular stress, injury

Inflammation, apoptosis, oxidative stress

Omics-based grouping

Group	Substance
A	S1, SRG, DO25, DY3
B	DO61, DR1, DR13

Multi-omics grouping of S1, SRG, DO25, DY3 – which differs from the ‘conventional QSAR grouping hypothesis’ – is supported by the consistency of the molecular pathway responses

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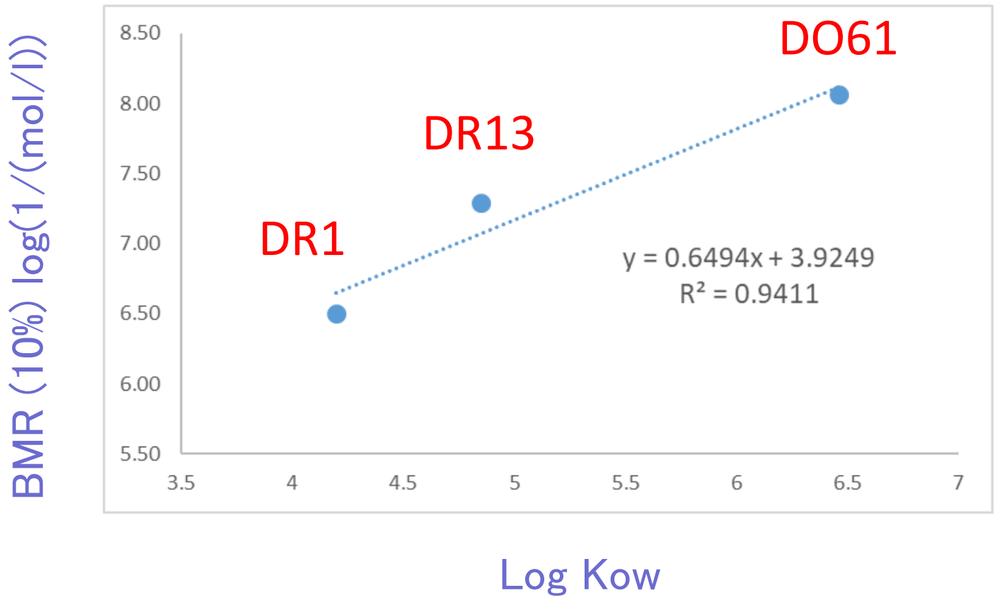
Further support for Group B membership?



Omics-based grouping

Group	Substance
A	S1, SRG, DO25, DY3
B	DO61, DR1, DR13

Group B toxicities correlate well with log Kow

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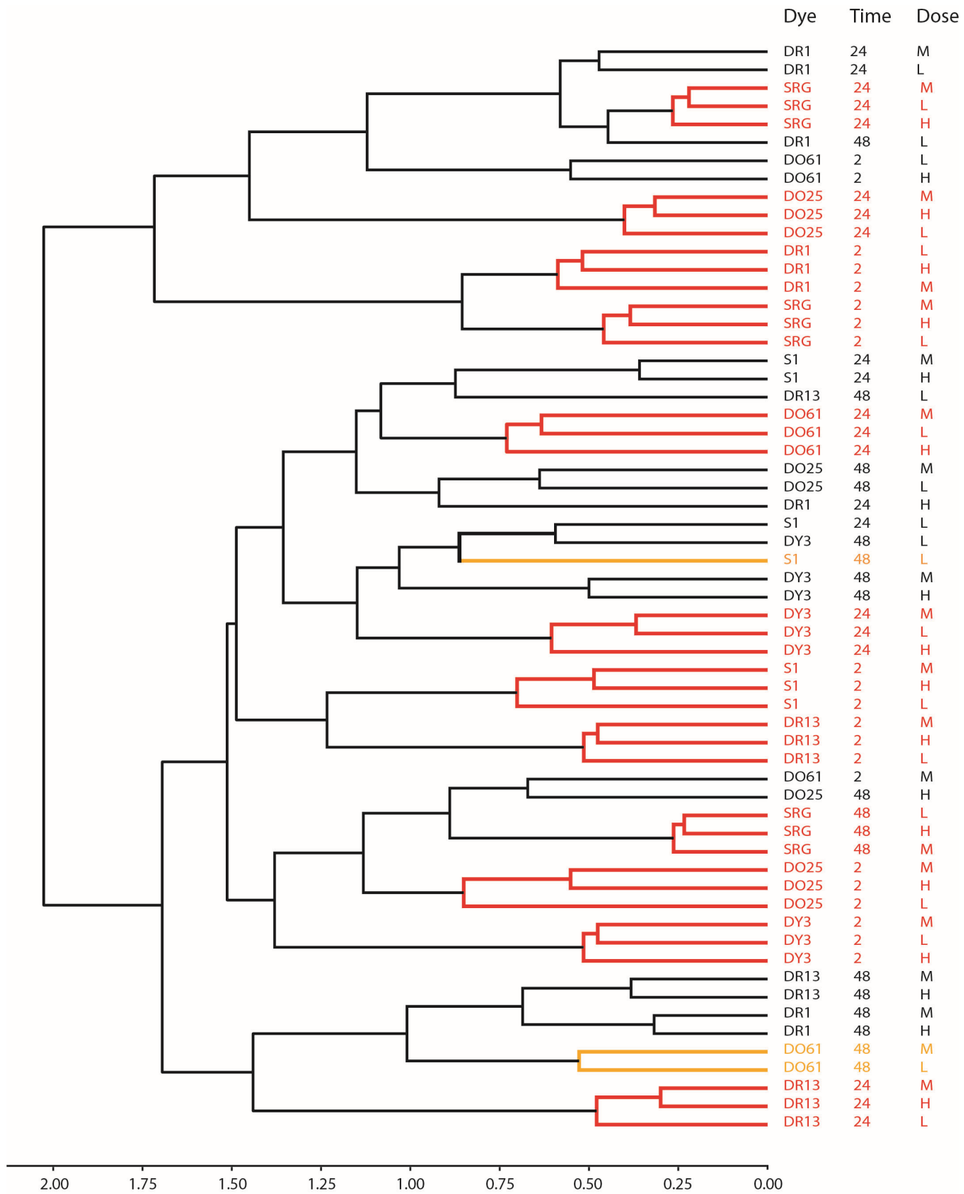
Omics-based grouping results: all doses and time points



3 doses cluster together

Subtle changes of dose levels induce relatively consistent molecular responses within any one dye, compared to differences in responses across times and different azo dyes

Dyes with missing doses (due to lethality; DO61 & S1), the remaining dose groups still cluster together

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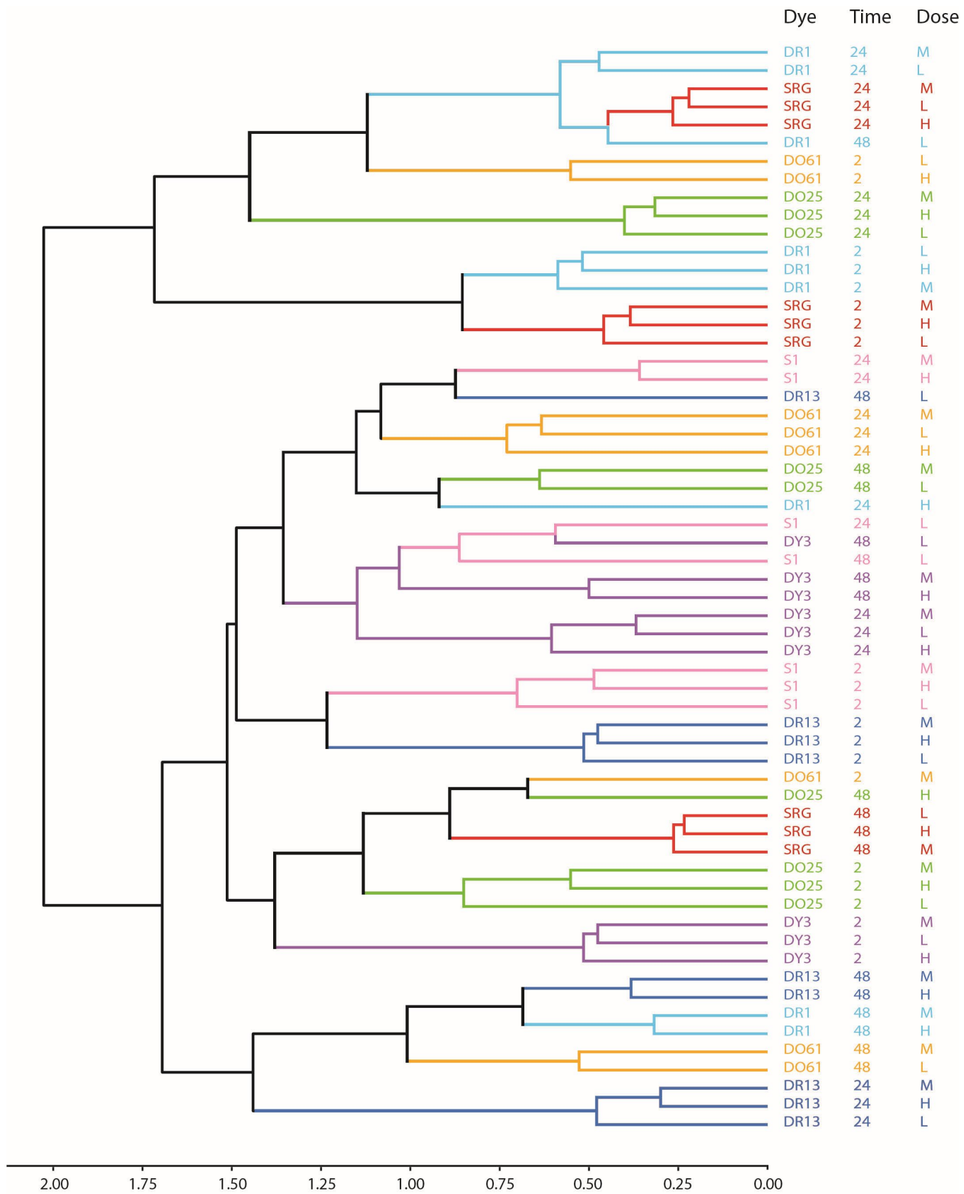
Omics-based grouping results: all doses and time points



Sudan Red G 24h

Sudan Red G 2h

Sudan Red G 48h

Time of observation of the dynamic molecular changes has a significant impact on grouping

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Conclusions

- Multi-omics responses can generate a grouping of the 7 azo dyes (for subsequent read-across).
- Omics-based grouping is similar to that from the conventional QSAR profiling, but not identical, most likely because the omics-based grouping is considering a broader range of MoAs.
- Molecular pathway analyses provide mechanistic insights into the omics-based grouping, thereby increasing confidence in group membership through shared mechanisms.
- On-going work includes setting thresholds for group membership, and assigning confidence to group membership.

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Thank You for Listening!!

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