Can We Get The Full Picture? Comparison of a Series of Volatile Chemicals' Action on Gene Expression in A549 Cells as Part of a Read-Across Approach.

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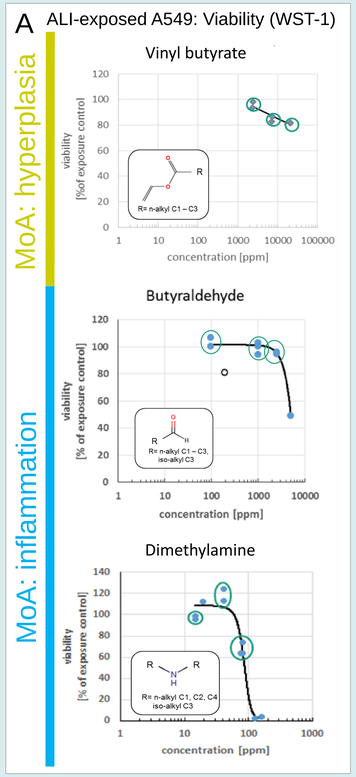
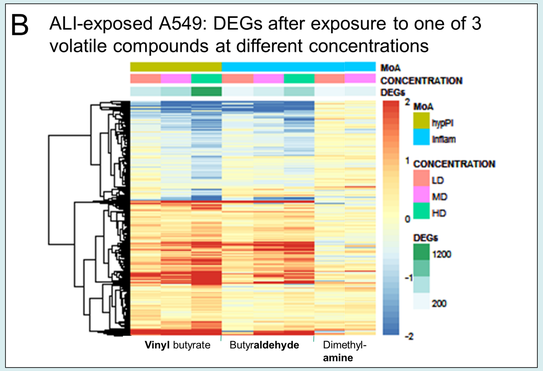
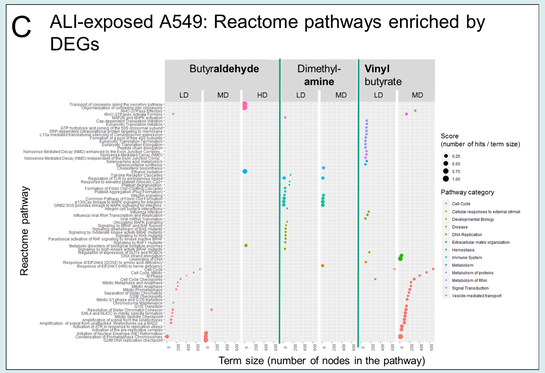
Results

- Three chemically heterogenous compounds showed a dose-response in terms of differentially expressed gene (DEG) counts and log2 fold changes at subcytotoxic concentrations (Fig. A, B)
- DEG profiles indicate high concordance between the vinylester and aldehyde, whereas the secondary amine shows a different pattern, indicating mechanistic dissimilarity or lacking equipotency. (Fig. B)
- Mechanistic similarity of the vinylester and aldehyde is confirmed in a Reactome pathway enrichment of the DEGs. Similiarities indicated relate to the cell cycle and the regulation of the cytoskeleton. (Fig. C)
- Genes with a consistent differential expression within a given group of structurally similar read-across compounds confirm some mechanistic similarity of tested vinylesters and aldehydes and dissimilarity of secondary amines (Fig. D: red and green box, respectively). However, concordance among chemically similar compounds is low with regard to DEG patterns, probably due to the limited number of concentrations tested.







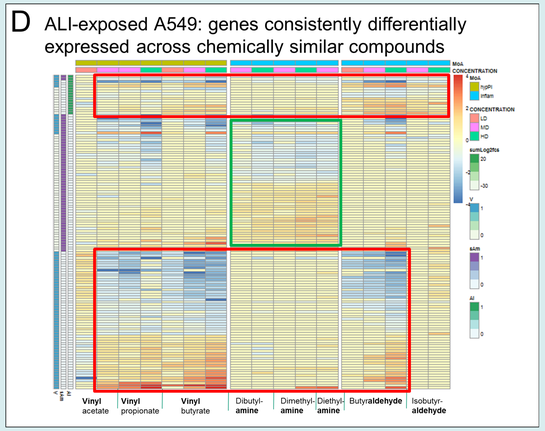


Figure A) Viability (WST-1) of A549 cells after three days with 1h ALI-exposure each. Green circles indicate the three doses chosen for the transcriptomics experiment.

Figure B) Heatmap of log2 fold-changes of ALI-exposed A549 cells' DEGs of selected compounds. Values outside the range of [-2,2] are are colour-coded -2 and 2, respectively. (N=1771; number of DEGs per condition: Vinlybutyrate: LD: 297, MD: 504, HD: 1362; Butyraldehyde: LD: 58, MD: 211, HD: 579, Dimethylamine: LD: 18, MD: 105.

Figure C) Reactome pathways enriched by gost (gprofiler2) in an GSEA-style analysis of the DEGs ordered according to log2 fold change.

Figure D) Heatmap of genes with a consistent differential expression within a given group of structurally similar read-across compounds (N=128). Values outside the range of [-4,4] are are colour-coded -4 and 4, respectively. (hypPl, hyperplasia; inflam, inflammation; V, vinyl esters; sAm, secondary amines; Al, aldehydes)

Introduction

- In practice, the read-across approach's **bottleneck** is often **proving similarity** of compounds. As far as REACH is concerned, chemical similarity is never enough. Therefore, we aimed to elucidate requirements for transcriptional data to serve as evidence indicating **mechanistic similarity**. Volatile model substances with different modes of action (MoA) were selected. The different MoA were selected based on common apical effects observed in repeated dose toxicity studies with inhalation exposure.
  - **MoA hyperplasia**: vinyl esters (model compounds: vinyl acetate, vinyl propionate, vinyl butyrate)
  - **MoA inflammation**: aldehydes (model compounds: dimethylamine, diethylamine, dibutylamine), Secondary Amines (model compounds: butyraldehyde, isobutyraldehyde)

Conclusions

- Transcriptome data and thereof derived pathways can be used to demonstrate biological similarity e.g. in read-across approaches.
- It is difficult to hit right **non-cytotoxic concentrations**, which still give an **pronounced (and dose-dependent) transcriptional response**, with less than 5 concentrations.
- Three concentrations may allow for confidently identifying genes consistently differentially expressed across concentrations of a compound and across compounds. However, they don't usually allow for dose-response analysis, which can increase confidence in gene sets claimed to constitute a characteristic response.

Materials and Methods

- **A549** cells were exposed to volatile test substances via **air-liquid interface (ALI)** using the **P.R.I.T.® ExpoCube®** for 1h per day on three consecutive days.
- Read-outs 72h after the first exposure included those of the WST-1 assay (viability) and the TempO-Seq™ Human Whole Transcriptome Assay.
- Assessment of concordance of chemically similar compounds: Genes were considered concordant if they were differentially expressed in at least 5 of 7, 4 of 5 and 4 of 5 conditions for vinylesters, secondary amines and aldehydes, respectively.
- Reactome pathway enrichment was done through the gprofiler2 R package.

Acknowledgement

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References

1. https://www.rdocumentation.org/packages/gprofiler2/versions/0.2.0/topics/gost