Hazard assessment of diacetyl and structurally related diketones – a read-across approach

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Introduction

Primary human bronchial epithelial cells (PBECs) were isolated from tumor-free lung tissues from four donors and differentiated into mucociliary epithelial cells at air-liquid interface (ALI) conditions. The cells were exposed to the case study chemicals under (ALI) conditions using the P.R.I.T.® ExpoCube® device for 1h once or repeatedly on three consecutive days. Cellular viability was measured by LDH-leakage and barrier function by measuring the transepithelial electrical resistance (TEER) 24h after the final exposure. Exposure concentrations ranged from 100 to 1840 ppm (diacetyl) and from 50 to 5000 ppm (other diketone analogues). Chemical-induced transcriptomic responses were investigated utilizing targeted RNAseq with the Templated Oligo Detection Assay (TempO-Seq) based on a 3565 gene panel.

Experimental setup

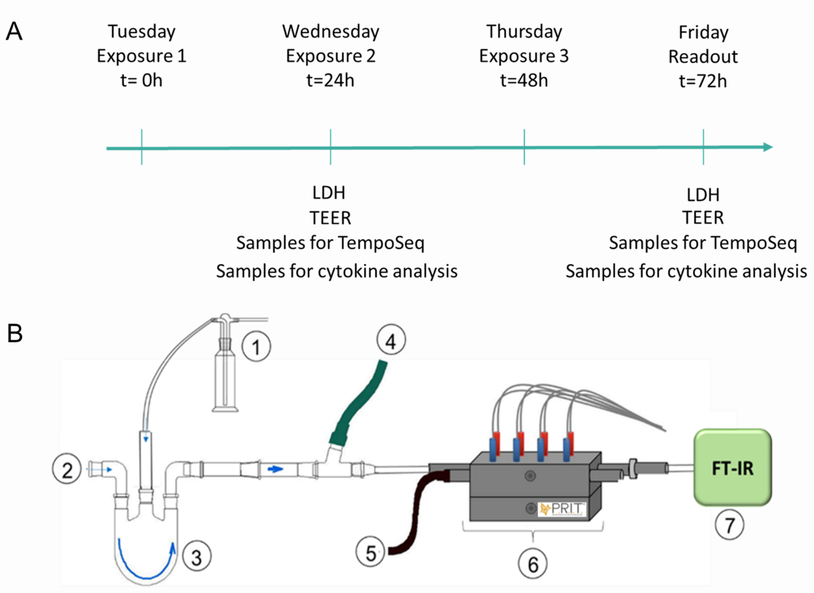

Figure 1: Experimental set-up
A) Exposure design: single exposure for 1h on exposure day 1, readout 24h after the start of exposure; repeated exposure for 1h on exposure days 1, 2 and 3, readout 72h after the start of exposure
B) The exposure P.R.I.T.® system: Generation and transport of the test substance atmosphere (1-3) or the clean air control (5) respectively. The exposure unit for the target cells, grown under air-liquid interphase conditions (P.R.I.T.® ExpoCube®; 6). Analyzing unit for test atmosphere (FT-IR Monitor; 7); Position 4 represents the exhaust of excess test atmosphere.

Cellular viability

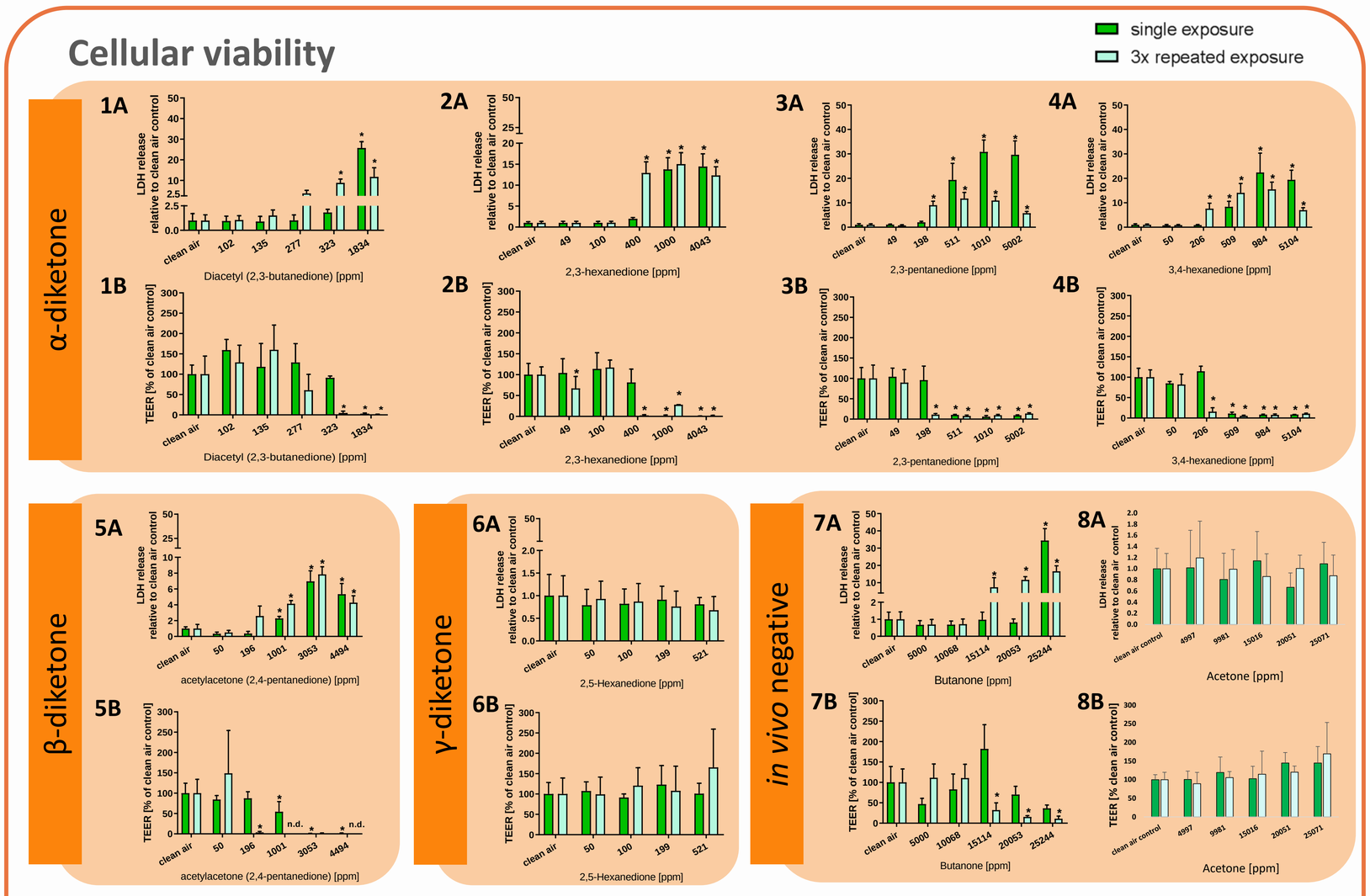

Figure 2: Primary human bronchial epithelial cells (PBECs) isolated from four different donors were exposed for 1h once (single exposure) or repeatedly on three consecutive days (3x repeated exposure) to increasing concentrations [ppm] of the tested compounds. LDH release (A) and TEER values (B) were measured 24h or 72h after the first exposure; *p<0.05 versus clean air control by one-way ANOVA followed by Tukey's test.

Chemical-induced transcriptomic response

The number of differentially expressed genes is shown (Figure 3, top). Cell loss led to insufficient number of reads at higher concentration. Samples with less than 500k reads were excluded.
Transcriptomic response for Diacetyl shows significantly (FDR<0.05) differentially expressed genes even at low concentrations. With increasing concentration the positive fold changes increase visibly (Figure 3, bottom).

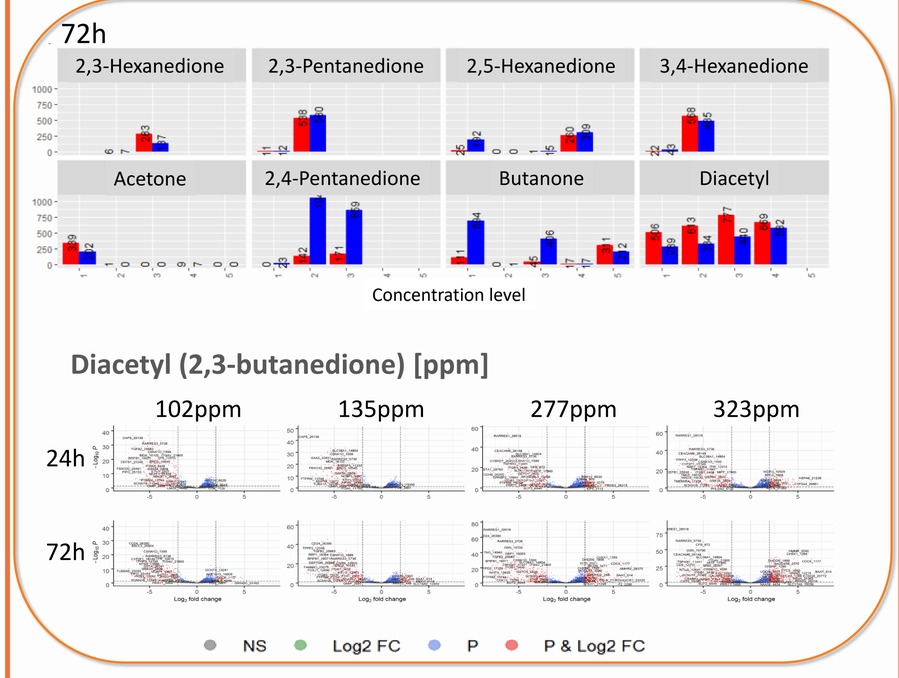

Figure 3: DEGs (top) and Volcano plot (bottom) - the adjusted p-values (FDR) is shown as negative $\log_{10}$-transformed and fold changes are represented in $\log_2$ form.

Transcriptome analyses to support similarity assessment

Unsupervised clustering of DEGs reveals similar expression patterns between negative compounds and the γ-diketone (Figure 4, right). Diacetyl on the other hand seems unrelated to its related compounds. However, certain subsets of genes creates clusters, which separates α-diketones clearly from other compounds (Figure 4, left).

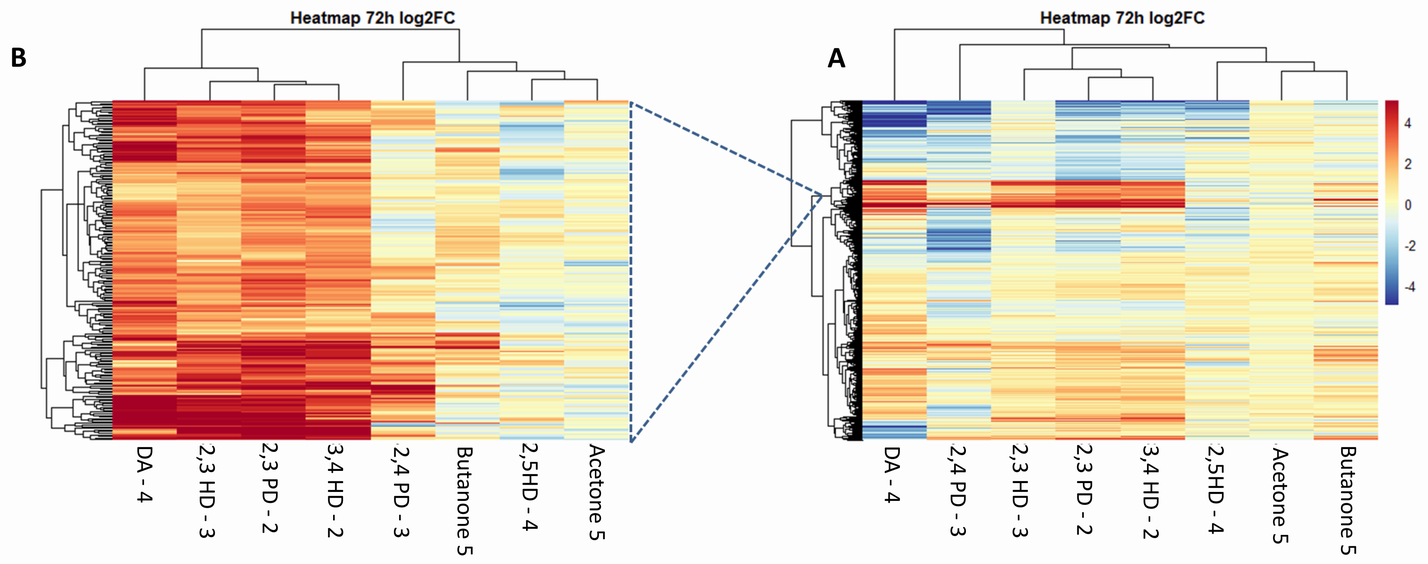

Figure 4: Heatmap - unsupervised clustering based on the fold changes in the highest non-toxic dose for each compound. A) Compounds are shown in columns, next to their neighbors based on complete linkage clustering. 2539 genes are selected by significance and clustered accordingly. B) Reclustering of the 209 upregulated genes showed highest differences between α-diketones and other analogues.

Conclusion

- in vitro testing of volatile gases requires special technical requirements like the P.R.I.T.® ExpoCube® to provide correct quantitative statements and to avoid false positive or negative results. Under these conditions, the various diketones revealed a dose-dependent toxic effect on primary human lung cells.
- TempO-Seq analysis revealed up or down regulated differentially expressed genes (DEGs) in a dose and exposure time dependent manner.
- Analysis of DEGs, show shared expression patterns, which might indicate a shared mode of action.
- Further upstream analyses will be carried out to link these in vitro observations to relevant adverse human outcomes like pulmonary fibrosis and inflammation.

The EU-ToxRisk project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002.

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