CS8: In vitro testing of diacetyl and analogues at ALI conditions
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Methods
Primary bronchial epithelial cells were exposed to the alpha-diketones diacetyl, 2,3-pentanedione and 2,3-hexanediol or the beta-diketone 2,4-pentanedione under air-liquid interface (ALI) conditions using the P.R.I.T.® ExpoCube® device. This unique exposure device provides a highly efficient exposure situation by preventing contact between the test compound and the culture medium. Primary human bronchial epithelial cells (PBECs) from tumor-free lung tissues from four donors were differentiated into airway epithelium at ALI conditions. Test atmospheres were generated by evaporation of the volatile test compounds and diluted in clean air. FT-IR spectroscopy enabled online analysis of the exposure concentration. PBECs were exposed for 1h once or repeatedly on three consecutive days. Cellular viability was measured by LDH-leakage and monolayer integrity 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). Lowest observed adverse effect levels (LOAELs) were lower after repeated exposure compared to the single exposure protocol.

Experimental setup
A) exposure design and time points of the conducted readouts: 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, readouts 72h after start of exposure B) The system consists of three parts: A: Generation and transport of the test substance atmosphere (1-4) or the clean air (5) control respectively. B: The exposure unit for the target cells, grown under air-liquid interphase conditions (P.R.I.T.® ExpoCube®). C: Analysing unit for testatmosphere (FT-IR Monitor). Position 4 represents the exhaust of excess testatmosphere.

Results
Measurement of transepithelial electrical resistance (TEER) values and LDH release after a single or 3x repeated exposure to case study compounds: Primary human bronchial epithelial cells isolated from four different donors (PBEC) were exposed for 1h to increasing concentrations of the chemicals. 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 and Tukey’s test.

Conclusions
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.

Next steps
• Analysis of TempO-Seq data
• ALI exposure of cryopreserved precision cut lung slices (PCLs)