P18-03
DMSO-induced drastic changes in cellular processes and epigenetic landscape *in vitro*

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The use of 0.1% DMSO (dimethyl sulfoxide) as solvent for in vitro assays is widespread and effects are often assumed to be negligible. Initially, DMSO was researched for medical applications although clinical trials were halted in 1965 due to adverse effects whereupon DMSO was labeled extremely toxic. Years later, upon reviewing new results, the FDA classified DMSO in the safest category. Thereafter, DMSO was used in applications such as cryopreservation or cell differentiation inducer, but mainly as a solvent. This study uses complete transcriptome and epigenome sequencing (RNA-seq & MeDIP-seq) to chart the effects of 0.1% DMSO on 3D cardiac and hepatic microtissues for the purpose of assessing whether DMSO induces bias in analyzing findings from toxicant-treated in vitro assays. RNA-seq detected over two thousand differentially expressed genes (DEGs; FDR > 0.05) in both cardiac and hepatic tissue (compared to untreated microtissues). Pathway analysis of these DEGs identified hundreds significantly overrepresented pathways (FDR < 0.05). Although the amount of DEGs per biological process differs between the tissues, similar processes are affected, indicating a consistent mode of action of DMSO. Since affected pathways displayed a majority of downregulated genes, processes of transcriptional regulation were investigated in detail, focusing on DNA methylation. Differentially methylated regions (FDR < 0.05) found in both tissues indicate changes in the epigenetic landscape, suggesting that DMSO can interfere with regulatory systems in cardiac and hepatic tissues. While the field is evolving towards more sophisticated in vitro models, using 3D conformation and/or physiological-consistent low dose concentrations, omics technologies clearly demonstrate that the effect of DMSO on cell regulation has to be kept in mind when designing in vitro studies and interpreting the data. Consequently, the lowest possible dose of DMSO should be used as even low incubation concentrations may induce solvent-induced bias.

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P18-05
Omics-based analysis of the impact of cigarette smoke and cessation in mouse liver and kidney following a 6-month exposure

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Smoking cessation has been reported as one of the most effective approaches to reducing the effects of cigarette smoke (CS). We used integrated omics analyses to investigate the impact of smoking cessation in murine liver and kidney, the primary organs involved in xenobiotic response. Female C57BL/6J mice were exposed to filtered room air (sham) or K3R4F reference CS for 6 months. Moreover, two additional groups as smoking cessation groups, mice were exposed to CS for 5 or 13 week, followed by filtered air up to 6 months. Livers and kidneys were subjected to transcriptomic and proteomic analysis. The number of differentially expressed genes (DEGs) was higher in livers than in kidneys in all exposure groups, suggesting a greater impact on liver. Notably, the number of DEGs decreased as the duration of the cessation period increased, in particular DEGs related to cell stress, oxidative stress, and inflammatory responses. Cessation periods reduced the numbers of DEGs in both organs, approaching the numbers in the sham group. Canonical pathway analysis predicted that CS inhalation would activate the aryl hydrocarbon receptor signaling pathway in the liver, showing a good correlation between gene expression and the abundance of proteins related to aryl hydrocarbon receptor signaling. These findings suggest that CS-inducible cell stress, especially in the liver, is mediated by aryl hydrocarbon receptor signaling. Moreover, this impact can decrease with the duration of smoking cessation.

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P18-06
Data-driven identification of interspecies pathway perturbations *in vivo* and *in vitro*: the case of Nrf2

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The dynamic, time- and dose-dependent perturbation of intracellular pathways is an important mechanism underlying toxic responses in several species, *in vivo* and *in vitro*; the identification of such perturbations is essential to understand the mechanisms and to design effective predictive models. Despite the large amount of gene expression data generated for this purpose, certain limitations exist when using available analytical methods, including database annotation, arbitrary thresholds for statistical testing, as well as species- and model- specific responses. In this study, we sought to overcome these limitations by applying a data-driven approach to identify perturbations to pathways/gene sets applying ordinary differential equations (ODEs) to exposed time series data. Our hypothesis is that different stimuli will lead to different dose-dependent perturbations of component nodes. For this, we focused on perturbations to (orthologue) genes from the Nrf2 pathway, which were then modelled using TG-GATEs sets (rat *in vivo* repeated and single dose; rat *in vitro* and human *in vitro*) exposed to compounds with different pathological outcomes after chronic exposure *in vivo*: acetaminophen (APAP), carbon tetrachloride (CCl4) and diazepam (DIZ). A clear separation of the perturbation clusters following single and repeated exposure regimens