28-Day Rat Inhalation Study – How Systems Toxicology Complements OECD Inhalation Studies

Kogel U., Schlage W.K., Martin F., Ansari S., Xiang Y., Meurrens K., Vanscheeuwijck P., Hoeng J., and Peitsch M.C.
Philip Morris International R&D, Biological Systems Research Department, CH-2000, Neuchâtel, Switzerland

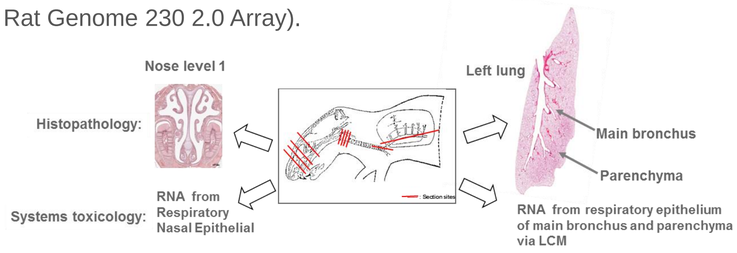
Introduction

OECD 28-day rat inhalation studies are designed to characterize test article toxicity. In this study, we augmented the classical OECD study with transcriptomics analysis with the view to developing a systems toxicology-based risk assessment approach for Modified Risk Tobacco Products (MRTPs). This new risk assessment approach uses state-of-the-art technologies for sample preparation and analysis of gene expression arrays. Here, we assessed the feasibility of investigating disease-relevant molecular perturbations occurring at the major sites impacted by cigarette smoke in the respiratory tract.

In order to place the molecular profiling data into the context of known biology, a novel computational-modeling approach[1] based on our recently built tissue-specific causal biological networks[2-5] was applied. The computable biological network models are specific to non-diseased pulmonary and cardiovascular cells/tissues and capture the molecular events that can be activated following exposure to environmental toxicants. To date, we have built six causal biological network models using the Biological Expression Language (BEL) and Selventa's (http://www.selventa.com) computable framework for biological network representation. The BEL framework is available for public use (http://belframework.org/). The biological mechanisms covered by our networks encompass cell proliferation[2], cellular stress[3], lung inflammation[4], DNA damage, autophagy, cell death and senescence (DACS)[5], cardiovascular inflammation, tissue repair, angiogenesis, and wound healing[6]. Each network is built in a modular way where each module (sub-network) describes a specific biological aspect of the entire network. The networks are provided in XGMML (eXtensible Graph Markup and Modeling Language) format and can be viewed using freely available software such as Cytoscape (http://www.cytoscape.org). To enable a quantitative comparison of perturbation in the biological networks impacted by an exposure, we have developed a computational approach that translates transcriptomics analysis into Network Perturbation Amplitude (NPA) scores[7]. In addition, the mechanisms leading to the network perturbations can be investigated by analyzing the regulation of nodes in the network backbones.

Materials and Methods

In a 28-day rat inhalation study performed according to the Organization for Economic Cooperation and Development (OECD) Test Guideline 412, Sprague-Dawley rats were exposed to filtered fresh air (sham), or to a low, medium, or high concentration of mainstream smoke (MS) from the Kentucky Reference Cigarette 3R4F (i.e., 0, 8, 15 and 23 µg nicotine/l). Care and use of the animals was in accordance with the American Association for Laboratory Animal Science Policy (1996). All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC). The histopathological evaluation was performed at defined anatomical sites of the nose and of the left lung according to a defined grading system. Free lung cells were determined in bronchoalveolar lavage fluid by flow cytometry, and inflammatory mediators were measured by multi-analytes profiling (MAP). For the Systems Toxicology approach, RNA samples from specific sites in the respiratory tract were obtained, i.e., respiratory nasal epithelium (RNE) and lung. For lung RNA isolation, respiratory epithelium of main bronchus and lung parenchyma was separated by Laser Capture Microdissection (LCM) and further processed, and analyzed on whole genome Affymetrix microarrays (GeneChip® Rat Genome 230 2.0 Array).



Gene expression fold-changes were translated into differential values for each node of the network. The node differential values are in turn summarized into a quantitative measure of network perturbation amplitude[7].

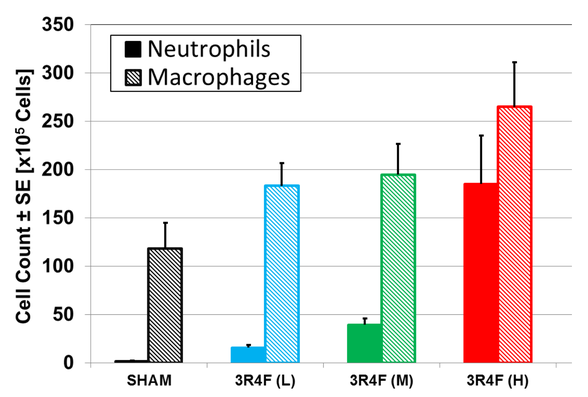
References

1) Hoeng, J. ,et al., 2012. *A network-based approach to quantifying the impact of biologically active substances*. Drug Discov Today 17, 413-418.
2) Westra, J.W., et al., 2011. Construction of a computable cell proliferation network focused on non-diseased lung cells. BMC Syst Biol,. **5**: 105.
3) Schlage, W.K., et al., 2011. A computable cellular stress network model for non-diseased pulmonary and cardiovascular tissue. BMC Syst Biol, 2011. **5**: 168.
4) Westra, J.W., et al., A modular cell-type focused inflammatory process network model for non-diseased pulmonary tissue. *Bioinformatics and biology insights. 2013;7:1-26.*
5) Gebel et al., 2013. Construction of a computable network model for DNA damage, cell death, autophagy, and senescence. Bioinformatics and Biology Insights 7, 97-117.
6) Park, J.S. et al., 2013; *Construction of a Computable Network Model of Tissue Repair and Angiogenesis in the Lung*. J Clinic Toxicol 2013, S12-002.
7) Martin et al., 2012. *Assessment of network perturbation amplitudes by applying high-throughput data to causal biological networks*. BMC Syst Biol 6, 54.

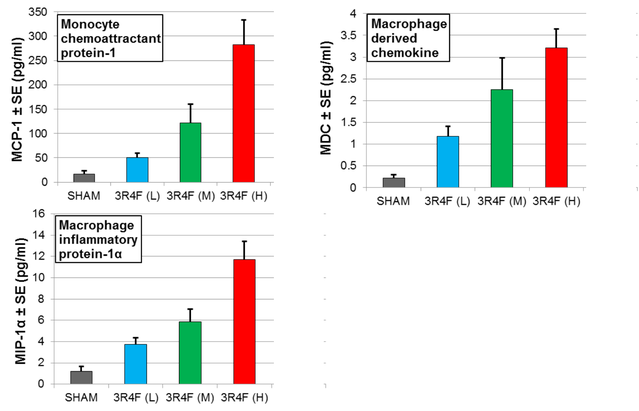
Results

Dose-dependent Inflammatory Response in Bronchoalveolar Lavage Fluid after 3R4F exposure

Differential Cell Count of Free Lung Cells in BALF of male Rats



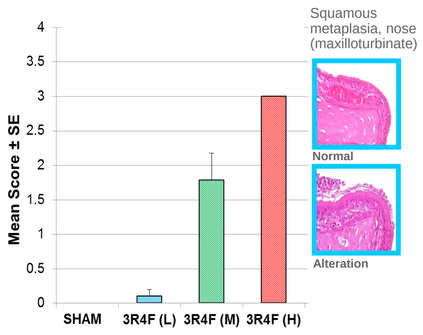
Measurement of BALF Chemokines from male Rats



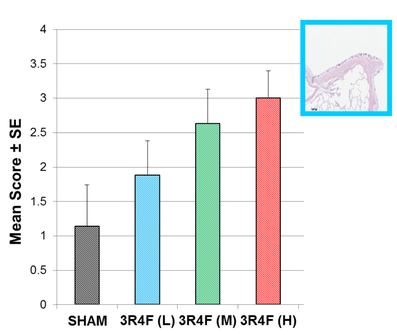
An increase in neutrophil and macrophage counts concomitant with an increase in chemokines indicates an inflammatory response.

Dose-dependent Adaptive Changes and Inflammatory Response in Respiratory Tract after 3R4F exposure

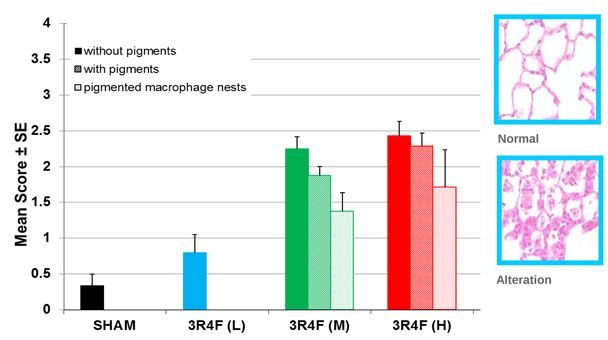
Histopathological Findings, Nose, Respiratory Nasal Epithelium (RNE), Amount of Cornification



Histopathological Findings, Left lung, Main Bronchus, Goblet Cell Hyperplasia



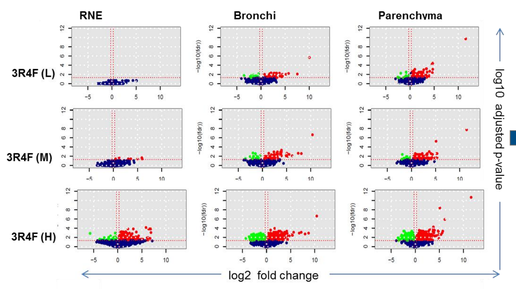
Histopathological Findings, Left lung, Parenchyma, Alveolar macrophages



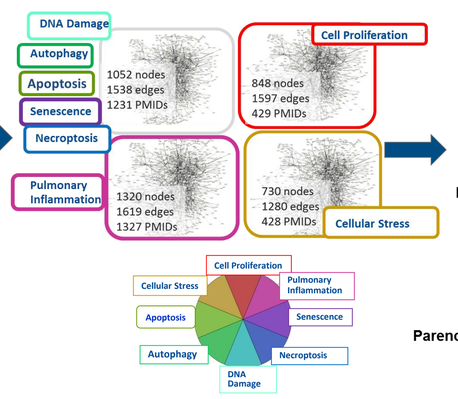
The typical smoke-related as well as dose-dependent adaptive and inflammatory response after exposure to 3R4F was observed in nose and in lung, e.g., squamous metaplasia with cornification of the RNE, goblet cell hyperplasia in the bronchus, and increased number of macrophages and macrophage nests in the lung alveolar parenchyma.

Translation of Gene Expression into Network Perturbations

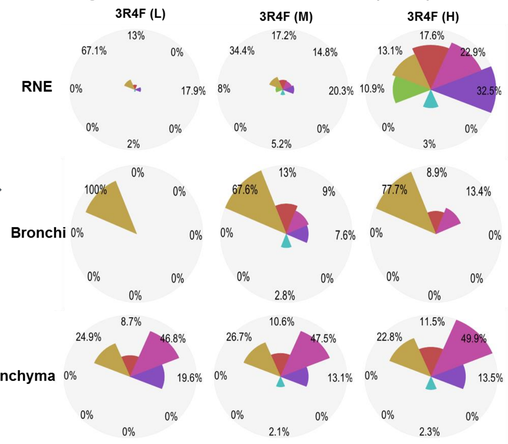
Systems Response Profiles (SRP)



Non-diseased Biological Processes



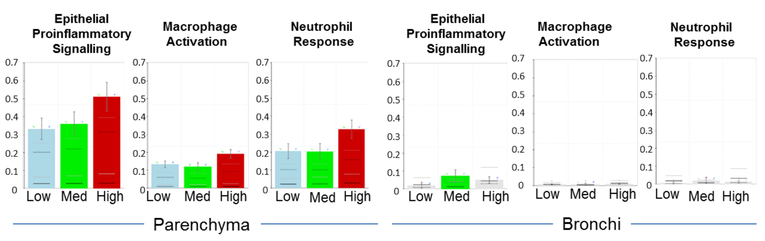
Biological Network Perturbation (NPA)



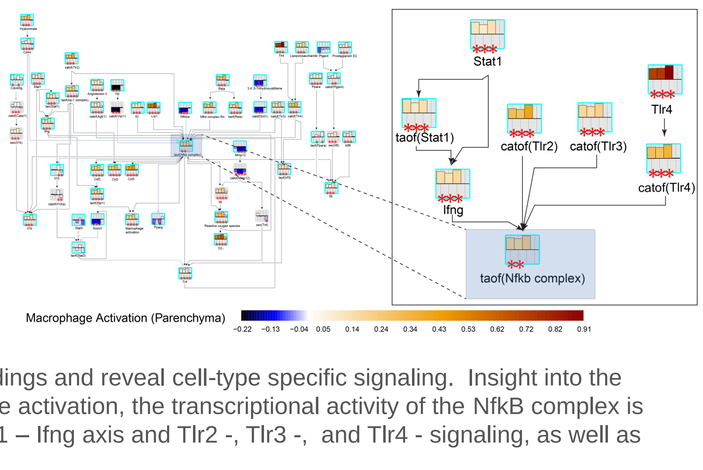
SRP demonstrate a dose-dependent up-regulation of differentially expressed genes after 3R4F exposure. Using a computational approach that is based on causal models of tissue-specific biological networks, gene fold-changes were translated into NPA scores, a quantitative measure for the perturbation of a network[7]. Major perturbations were found in networks related to inflammation, cell stress, cell proliferation, and senescence. Corresponding to the histopathological changes, the stress-related response was more pronounced in the RNE and bronchi, while the inflammatory response was more pronounced in lung parenchyma.

NPA of Exemplary Sub-networks from the Inflammatory Processes Network (IPN)

Quantification of the Biological Perturbations observed in the IPN using NPA Scoring Method[7]



Backbone Macrophage Activation sub-network



Three exemplary sub-networks show agreement with histopathological findings and reveal cell-type specific signaling. Insight into the molecular mechanisms is gained from the backbone of IPN: for macrophage activation, the transcriptional activity of the NfkB complex is the central node integrating input from activating pathways such as the Stat1 – Ifng axis and Tlr2 -, Tlr3 -, and Tlr4 - signaling, as well as inhibitory input from Cd44, Vipr1, and Sirt1.

Conclusion

In conclusion, observed histopathological endpoints correlate well with the perturbation of their associated biological networks. This indicates the applicability of this approach as a powerful tool to investigate disease mechanisms *in vivo* and to develop a systems toxicology-based risk assessment for product testing of environmental toxicants, including tobacco products .

PMI RESEARCH & DEVELOPMENT

ICT Seoul The XIII International Congress of Toxicology 2013

Philip Morris International Research & Development, Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland
T: +41 58 242 21 13, **F**: +41 58 242 28 11, **W**: www.pmi.com