Comparative Case Studies on NAMs: A Step Towards Enhancing Specific Target Organ Toxicity Analysis

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Introduction Risk assessment has traditionally relied on animal testing, but concerns exist about interspecies consistency, reproducibility, time, costs, and ethics. New Approach Methodologies (NAMs), like cell-based omics analysis, offer promise by understanding underlying mechanisms, rather than assessing organ pathology. However, NAMs face limitations, such as the absence of a whole organism system and limited toxicokinetic interactions. The question remains whether omics data can sufficiently predict target organ toxicity. Here, comparative studies between *in vitro* and *in vivo* results can help improve the acceptance of NAM-generated data.

Methods Six pesticide active substances with known *in vivo* hepato- and nephrotoxicity were tested on two cell lines, the liver cell line HepaRG and the kidney cell line RPTEC. Two non-cytotoxic concentrations were selected for targeted protein and transcriptomics analysis using multiplexed microsphere-based sandwich immunoassays and quantitative real-time PCR arrays, respectively.

Results Protein and mRNA from cultivated cells was successfully isolated and analyzed. A Weight of Evidence approach was established to identify relevant pathways from the data. Where possible, *in vitro* endpoints were connected to *in vivo* observations. Analyses of this data set from substances with a good *in vivo* database revealed various affected pathways, some of which could not be connected to *in vivo* observations.

Conclusion The challenges associated with generating risk assessment conclusions from *in vitro* data and extrapolate to the *in vivo* situation can be addressed by comprehensive assessment frameworks incorporating kinetic considerations.