Bioavailability of organic micropollutants in cell-based bioassays

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Introduction

Humanity is facing complex challenges like overpopulation, climate change, and the rapid global technologization. A rather inconspicuous but serious risk for human and environmental health represent anthropogenic organic chemicals that had been and are continuously emitted into the environment. The application of *in vitro* cell-based bioassays is increasing in chemical risk and hazard assessment and their implementation in high-throughput screening (HTS) format can contribute significantly to meet the high demands on effect data for the increasing number and variety of anthropogenic chemicals. The suitability of *in vitro* cell-based bioassays to replace whole-organism tests in human health risk assessment depends on the ability to quantitatively predict effects in humans, referred to as quantitative *in vitro-in vivo* extrapolation (QIVIVE) (Figure 1).

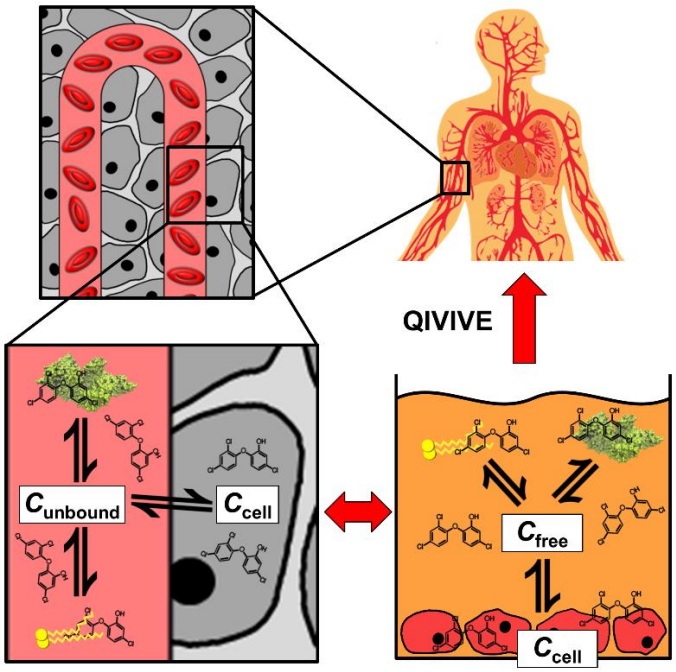

Figure 1 Framework of *in vitro* bioassay exposure modeling and link to QIVIVE: Quantifying exposure on the *in vitro* level to anchor *in vitro* effects for reliable extrapolation to expose concentrations in the human body [1].

For better comparability and interpretability of *in vitro* effect data, the freely dissolved concentration (C_{free}) was proposed as quantitative metric because it is considered the chemical concentration that is available for passive uptake by diffusion through membranes [2]. In QIVIVE models, nominal concentrations (C_{nom}) in the *in vitro* exposure medium are typically directly related to the steady-state plasma concentration *in vivo* but these QIVIVE models can be improved by comparing C_{free} *in vitro* with $C_{unbound}$ in human plasma. However, because of the miniaturization of the test systems, experimental quantification and adjustment of C_{free} are difficult to integrate into the routine application of *in vitro* cell assays in HTS.

This study aimed to progress the assessment of chemical exposure in *in vitro* cell-based bioassays as basis for improved dosing of chemicals, analysis and interpretation of *in vitro* effect data, and extrapolation to *in vivo* exposure scenarios. Experimentally parameterized exposure models were developed that enable the prediction of C_{free} of neutral and ionizable chemicals in various cell-based bioassays. In a first step, a steady-state mass balance model was parameterized and applied to the effect data of 100 neutral, anionic, cationic and multiprotic chemicals from the reporter gene assays of the HTS platform “Toxicology in the 21st Century” (Tox21) [3]. The influence of cellular uptake kinetics on cell exposure during the typical 24-hour bioassay was evaluated while emphasizing the role of the medium composition on the kinetics and extent of uptake. Chemical sorption to the plastic of multi-well plates (polystyrene) that are used for HTS was measured and predicted by a kinetic model for various multi-well plate formats and medium compositions.

Results

The results of the Tox21 modeling study demonstrated that the fetal bovine serum (FBS) that is commonly supplemented to mammalian cell-based bioassays represents a dominant sorptive phase for a wide range of chemicals. The extent of chemical partitioning to the medium increased with increasing medium volume and FBS content, with molar fractions of any chemical in the medium of >84%. The extent of chemical partitioning to the medium increased with increasing medium volume and FBS content, which ranged between 0.5% and 10% FBS in the Tox21 reporter gene assays [3]. Despite reducing exposure and the apparent assay sensitivity, the proteins and lipids in the FBS represent a large reservoir of reversibly bound chemicals that can compensate for the chemical depletion of the exposure medium by cellular uptake, growth, sorption to well plate materials, and volatilization, keeping the exposure fairly constant during the bioassay duration (Figure 2). Due to the analogy to conventional passive dosing [4], in which chemical exposure in the medium is kept constant by desorption from a chemical-saturated polymer, the idea was termed serum-mediated passive dosing (SMPD).

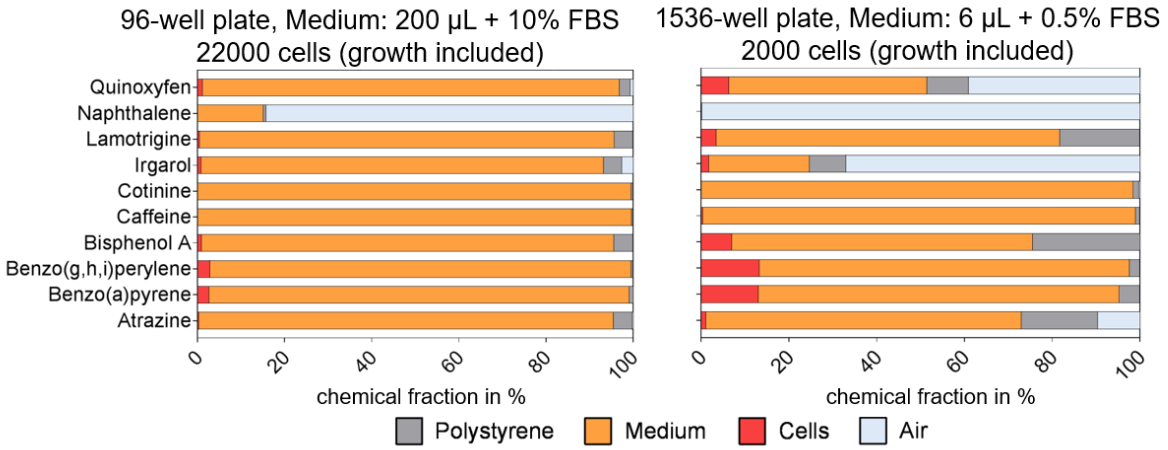

Figure 2 Chemical partitioning of ten neutral organic chemicals between the assay compartments multi-well plate plastic (polystyrene), medium, air and cells after the common assay duration of 24 hours given two widely applied experimental setups.

Kinetics of cellular uptake do not need to be considered in a first approximation because uptake kinetics in cells were measured to be relatively fast for a set of ten neutral and ionizable chemicals using a fluorescence microscope and automated image analysis. The application of higher medium FBS contents maximized the capacity of the chemical reservoir and decreased the relevance of kinetic processes, leading to an increased predictive power of the developed equilibrium mass balance model. An area under the curve analysis showed that the delayed cellular uptake kinetics reduced the cell exposure by <20% over the typical assay duration of 24 h. More importantly, increasing the medium FBS content from 0.5% to 10% accelerated the cell-medium equilibration substantially, favoring the use of higher FBS contents to decrease the impact of cellular uptake kinetics on the attainment of the chemical equilibrium in the cells.

The results of the kinetic model on multi-well plate sorption showed that even though the polystyrene walls in standardized multi-well plates are thick (up to 1 mm), the effective diffusion depth of the chemicals during the 24-h assay was in the range of 20 µm because of the low chemical diffusion coefficients in polystyrene that were measured in a water-polystyrene system (1.25 - 8.0 · 10^{-16} (m^2/s)). Experiments demonstrated that for hydrophobic chemicals exhibiting high sorptive affinity to polystyrene, the chemical concentrations in the FBS-free exposure medium of *in vivo* assays were significantly reduced. Contrarily, the depletion in *in vitro* assays was minor because of the high sorptive capacity of the FBS proteins and lipids. The depletion of the exposure medium was lower when increasing the medium FBS content from 0.5% to 10% because of the increased sorptive capacity of the SMPD reservoir in the medium and the decreased polystyrene-medium partition constant, suggesting again to use higher FBS contents in the medium to stabilize exposure.

Integrating all evaluated processes that can influence the bioavailability of chemicals in *in vitro* cell-based bioassays, it was demonstrated that the medium FBS is the major determinant for *in vitro* chemical exposure. Despite pointing out that the variable chemical bioavailability can lead to a reduced assay sensitivity and lower comparability between different *in vitro* assays, the results emphasize that the medium FBS can be utilized to adjust defined and stable C_{free} in the medium by simple equations that apply predicted parameters [5]. The ability of the medium FBS to compensate for continuous losses depends on the applied FBS content, suggesting the application of higher FBS contents in cell-based bioassays that apply low medium volumes in HTS. Because it can be expected that similar C_{free} in different exposure scenarios result in similar effects [6], the predicted C_{free} can be compared between different chemicals, between *in vitro* assays and to *in vivo* assays to assess differences in the chemicals' potency. The application of the predicted C_{free} increases the suitability of *in vitro* effect data for QIVIVE approaches as differences in the partitioning between different chemicals and test systems cancel out, as well as differences between C_{free} in the assay and plasma. The methods and results significantly improve the suitability of high-throughput cell-based bioassays to predict adverse effects in human, contributing to alternative animal-free test strategies that meet the high demands on effect characterization for the increasing number and variety of anthropogenic chemicals.

References

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