# Utilizing In vitro cytotoxicity data of advanced material in life cycle assessment and human risk assessment

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## 1. Introduction

Over the past three decades, the field of advanced materials (AdMa) has evolved significantly, positioning itself as a key enabling technology in the 21st century. Despite acknowledging their benefits, concerns have arisen about potential adverse environmental and health effects, primarily due to the distinctive risks of involuntary exposure and unique toxicity profiles associated with AdMa compared to bulk materials [1]. To address these challenges, the integration of toxicological data with general risk methodologies, such as Risk Assessment (RA) and Life Cycle Assessment (LCA), is crucial for early risk identification [2].

Traditionally, RA and LCA have relied on epidemiological or in vivo toxicological data assessing dose-response relationships. However, the transition from phenomenological to more cell based mechanistic toxicological studies demands a proactive adaptation of risk methodologies to accommodate the newly evolving data sources and types. [3]. The *in vitro* technologies are starting to anticipate this complexity by moving from cancer cell lines to primary human cells, from monocultures to co-cultures, from 2D to 3D systems, and from static to dynamic conditions, creating novel systems such as organs-on-a-chip mimicking the physiological environment of the cells. Thus, this shift requires an adaptation of risk methodologies to accommodate evolving data sources and types, minimizing reliance on animal testing [4].

Despite significant strides, establishing a comprehensive strategy for a quantitative assessment of the hazard impacts of advanced materials, using available non-animal data and in silico models, remains a challenge [5;6]. Our objective is to explore pathways towards quantitative In Vitro In Vivo Extrapolation (QIVIVE) of AdMa, emphasizing the integration of in vitro data into hazard assessment for a next-generation toxicity evaluation.

Building upon our previous work, which identified the combination of in vitro dosimetry and lung dosimetry as a mature approach for QIVIVE, particularly for inhaled particles affecting the lungs (Romeo, et al., 2022), we have developed a simplified model for inhaled spherical nanomaterials and their impact on the lungs to extract toxicity effect factors (Romeo, et al., 2022). Additionally, our research extends to Machine Learning and

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Quantitative Structure-Activity Relationship (QSAR) models, exploring their potential to predict in vitro activity of Graphene-related Materials (GRMs)(Romeo, et al., 2022).

Given the development of in vitro techniques, our studies aim to bridge the gap between currently increasing in vitro data pool, RA and LCA.

## 2. Screening of in vitro to in vivo extrapolation methods

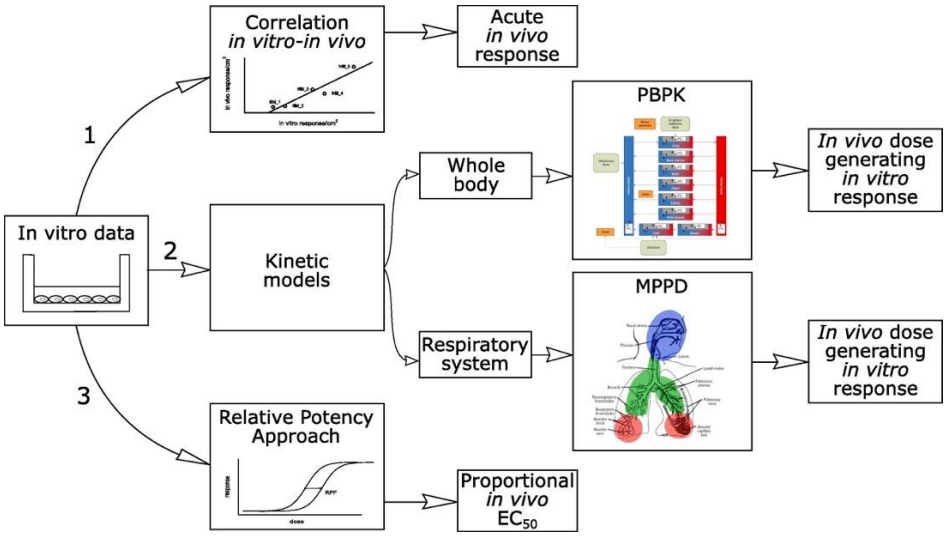


*Figure 1: Pathways to extract in vivo information from in vitro data.*

Utilizing mechanistic in vitro studies and in silico modelling, our objective is to bridge the gap between cellular responses and whole organism effects, as highlighted in [7]. The challenge lies in linking these two sets of information, for which there is currently no standard method. Our proposed solution involves three options, illustrated in the Fig.1.

We introduce the CoDo model [8](Romeo, et al., 2022), a novel approach that combines in vitro dosimetry and lung dosimetry to establish a link between in vitro doses and human intakes. Illustrated through a case study on titanium dioxide, the CoDo model effectively demonstrates realistic in vitro doses corresponding to exposure levels below 10 mg/m$^3$, highlighting its efficacy. A step-by-step procedure to calculate in vitro-to-in vivo extrapolation factors for estimating human Benchmark Doses and subsequent in vitro-based EFs for various inhaled non-soluble nanomaterials was developed [8] (Romeo, et al., 2022).

Except for in vivo benchmark dose extrapolation, we also step into the toxicity prediction using machine learning (ML) and QSAR. We have crafted a classification model to assess the viability effects of graphene-related materials (GRMs) [9] (Romeo, et al., 2022). The significance lies in comprehending potential adverse impacts on human health, particularly on the lungs, a sensitive exposure route. Employing ML approaches, we analysed in vitro cytotoxicity data derived from lung cell studies. Multiple regression models were employed to predict this endpoint based on material properties and experimental conditions.

## 3. Uncertainty space evaluation for hazard extrapolation

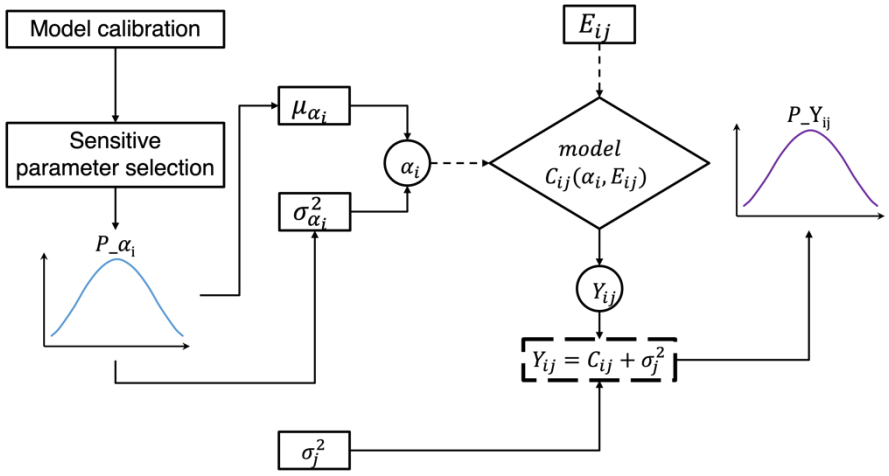


*Figure 2: Bayesian Model Structure to evaluate the parameter uncertainties.*

Bayesian statistical analysis serves as a crucial tool when dealing with the diversity of in vitro nanotoxicity datasets. Its utility extends to characterizing uncertainties originating from variations in experimental conditions, providing a resilient framework for comprehending the intricate dynamics of result variability. Through this analytical approach, we gain insights into the influence of these uncertainties on the extrapolation of safe AdMa concentration levels, a pivotal aspect of risk assessment. It enables the estimation and evaluation of these uncertainties, allowing for a nuanced examination of their impact on the estimation of in vivo effective doses.

This not only enhances the robustness of risk assessments but also contributes to a more informed and reliable understanding of the potential risks posed by nanomaterials to human health. In essence, Bayesian statistical analysis acts as a guiding compass in navigating the complexities of nanotoxicity assessment, providing a pathway towards more accurate and comprehensive risk evaluations.

## 4. Advanced in vitro methods as new source

As advancements in in-vitro technologies continue to progress, we anticipate a future where these cutting-edge methods could potentially streamline the existing procedures. The ultimate goal is to leverage advanced in vitro technologies directly for organ-to-human extrapolation, reducing intermediate steps and facilitating a more direct translation of in vitro data into in vivo effects. This paradigm shift envisions a time when in vitro data will be harnessed in a manner analogous to the current utilization of animal data, employing extrapolation methods to directly derive in vivo effects.

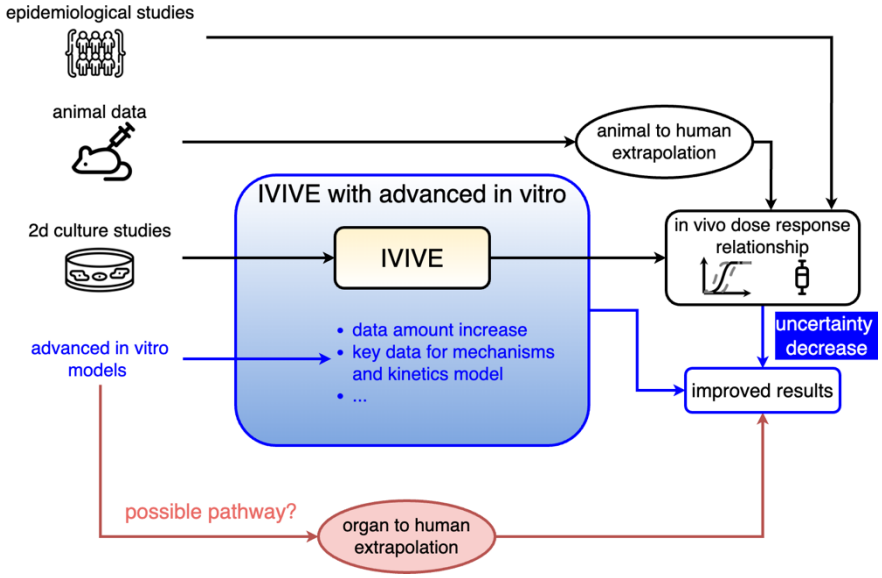


*Figure 3: In vivo dose response relationship generation pathways from different data source*

## 5. Conclusions

In conclusion, we are not yet there for a consistent and systematic calculation of in vitro-based EFs. However, we showed promising methods to calculate these factors and identified further steps are needed to reduce the uncertainty, improve and expand the results. Continued studies are needed to address remaining questions, with the future envisioning in vitro data usage comparable to current in vivo data through specific extrapolation steps.

## 6. Acknowledgment

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