

***In Vitro* Toxicology Lecture and Luncheon for Students Host Guide**

Dedication to the Use of *In Vitro* Alternative Techniques to Study Toxicologic Mechanisms: Case Study of Developmental Neurotoxicity

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The lecture will be opened by introducing a general understanding on the use of *in vitro* alternative test systems by providing the example of neurospheres for developmental neurotoxicity (DNT) evaluation. The 3Rs concept and the nature of “alternative techniques” will be explained. Benefits and limitations of using alternative approaches while planning experiments will be addressed. Finally, the neurosphere assays as parts of the DNT *in vitro* battery will be introduced and their use explained. A special focus will lie on the mechanistic applications of neurospheres and in their suitability for species-overarching studies. Especially the latter make alternative methods attractive tools for translational studies, which are extremely valuable in drug safety and environmental risk assessment.

Table Discussion

1. Concerning the aim of replacing animal data with *in vitro* alternatives, what are the general uncertainties?

Discussion points:

- *In vitro* does not resemble a whole organism hence, indirect effects are not assessed.
- Lack of, or unknown metabolic competence of the cells.
- Limitations of testing methods for volatiles or DMSO insoluble chemicals.
- Limited exposure durations.
- Potency estimates based on nominal media concentrations.

2. Concerning the aim of replacing animal data with *in vitro* alternatives, what are the specific uncertainties for the DNT *in vitro* battery?

Discussion points:

- The lack of assays for some cellular processes and systemic processes known to be critical for normal neurological development.
- Need for development of additional AOPs to increase mapping of the KEs covered in the DNT IVB.
- A relatively limited number of tested chemicals as compared to current accepted batteries (e.g., ER activation).
- Uncertainty in the overall specificity and sensitivity of the DNT IVB due to limited testing of DNT reference chemicals and comparison of results to a curated *in vivo* developmental neurotoxicity study database.
- A need for a consensus-based and regulatory driven tiered testing strategy to be used in IATAs.

3. Which organs or organ functions do you consider most challenging for modelling *in vitro*? Discuss a few examples.

Discussion points:

- Brain -> consciousness, learning, memory, experiences.
- Kidney -> integrated organ function with the different parts of the nephron.
- Reproduction and Development -> assessing phases of reproduction and embryonic as well as fetal development comprehensively.
- Heart -> e.g., mechanical issues with heart valves or specific actions on pace maker cells
- Blood vessels -> in general as part of organ toxicity.
- Immune system -> modelling immune system development and also function with the plethora of immune cells in the human body.