CEFIC Long-range Research Initiative
Request for Proposals (RfP)

Title and Code Number:
Expansion of a regulatory accepted *in vitro* testing battery for developmental neurotoxicity (DNT) evaluation **– LRI AIMT11**

Background
The increasing prevalence of neurodevelopmental disorders in infants and children (e.g. cognitive deficits, dysfunctional learning) and the rise in spectrum disorders like autism or attention deficit hyperactivity (ADHD), are suspected to be *inter alia* linked to chemical exposure. Despite this, developmental neurotoxicity can be considered the least studied toxicological endpoint which bears potential risks especially for foetuses, infants and small children as the developing brain is, in some cases, more susceptible to (chemical) insults than the adult nervous system. This higher sensitivity and vulnerability are likely related to the complex interactions required for brain development coupled with developmental differences in CNS transporters and the presence of intercellular junctions in the ventricle-lining neuroependymal cells. These complex interactions include specific developmental processes involving differentiation of neural progenitor cells, neuronal and glial cell proliferation, migration, synaptogenesis, myelination, networking and terminal functional neuronal and glial maturation.

Based on the apparent lack of DNT data for most chemicals, DNT testing and assessment is emerging as a basic regulatory requirement during substance evaluations, e.g. under REACH. Currently only higher-tier *in vivo* studies are officially recognized for DNT testing, i.e. OECD TG 426 or OECD TG 443. However, technical aspects and methodological deficiencies are limiting their appropriateness and relevance, leading to a lack of available data and mechanistic DNT understanding. Although at present a full replacement of such animal studies for DNT testing is not feasible, alternative non-animal approaches like AOP-informed *in vitro* methods may have the potential to close data gaps through more efficient screening and by mode-of-action based testing. A DNT screening program aimed at the development of respective *in vitro* assays has been initiated and an *in vitro* testing battery for DNT assessment is currently under development by EFSA, the JRC, the US EPA and the OECD. However, focus has been given so far on the neurodevelopmental processes NPC proliferation, migration, neuronal and oligodendrocyte differentiation, neuronal morphology, synaptogenesis and neuronal network formation, whereas *in vitro* assays on radial and astroglial differentiation processes as well as *in vitro* co-culture systems to study the interactions and interdependencies of neuronal and glia cells with microglia are still missing. In this respect it is important to note, that glia cells including microglia play key roles during brain development by regulating essential processes like neuronal differentiation, axon growth, synapse formation and pruning. Disturbances in glia cell differentiation thus may result in a wide range of detrimental effects. Likewise,

normal functioning astrocytes are essential for *inter alia* sustaining brain homeostasis by maintaining the blood-brain-barrier.

Considering the gaps in the current DNT testing battery especially regarding methods that assess the impact of chemical exposure on radial glia and astrocyte differentiation, maturation and functioning, as well as on the contribution of microglia to neurodevelopmental toxicity, there is a significant need for expanding the existing DNT *in vitro* testing battery with reliable and efficient *in vitro* tests necessary for a holistic DNT testing and assessment strategy.

Objectives

This project is expected to complement the current DNT *in vitro* testing battery by developing cell culture models regarding

i) differentiation of radial glia cells as important neuronal progenitor cells playing a central role in brain development,
ii) differentiation, maturation, and functioning of astrocytes as important cells *inter alia* for maintaining brain homeostasis and blood-brain-barrier and
iii) the impact of microglia on interactions and interdependencies of neurons and glia cells in co-culture for studying the effects of microglia activation for DNT chemical sensitivity.

By adding the above-mentioned test methods to the existing DNT *in vitro* testing battery, uncertainties of the battery will be reduced as the biological and toxicological applicability domains of the whole battery are enlarged. However, considering that full replacements of *in vivo* studies regarding DNT in the short-term seems unrealistic and that there is an absence of scientific guidance as to when DNT testing is necessary, the project should also help identifying mode-of-action-based triggers (e.g. set of molecular biomarkers) for subsequent targeted *in vivo* testing instead of performing full higher tier studies.

Scope

- For eventually relating data to rodent *in vivo* studies, set up of rodent *in vitro* cultures corresponding to the human tests is advantageous.
- Cells used for test method set up should reflect human and as far as possible also rodent physiology in cell type and cell function.
- Appropriate quality control needs to be applied to the cells used for test method set up.
- Test methods need to be developed according to Crofton et al. ALTEX 2011.
- Cell Systems as the basis for test methods need to undergo molecular characterization.
- An appropriate number of positive and negative controls need to be applied for identifying the assays' dynamic ranges.
- Technical, biological and toxicological application domains of the assays need to be defined.

- Carefully chosen test compounds with focus on man-made and natural stressors need to be tested in the assays.
- Developmental endpoints on the cellular/functional level need to be supported by molecular data applying 'omics.
- Standard operation procedures (SOPs) need to be produced for the developed test methods.

Deliverables

The final report shall contain an executive summary (2 pages max), a main part (max. 50 pages) and a detailed bibliography. The main report should include a brief section on cross-species neurodevelopment (rat and human) for the three specific mechanistic questions in scope and a discussion of the added value of the new models regarding the identification of potential developmental neurotoxicants. It is expected that the findings will be developed into at least one peer reviewed publication, following poster(s) and presentation(s) at suitable scientific conference(s). SOPs for test methods need to be attached to the report. The report shall contain aspects of scientific validation of test methods in a regulatory context, i.e. information on the scientific basis, the reproducibility and the relevance. Hypothetical AOPs (even if partial) should be developed for all three test methods, i.e. radial glia differentiation, astrocyte differentiation and function and microglia contribution to DNT.

Cost and Timing

Start in Q1 2021
Duration: 2 years
Budget in the order of 320.000 Euro

Partnering / Co-funding

Applicants should provide an indication of additional partners and funding opportunities that can be appropriately leveraged as part of their proposal. Partners can include, but are not limited to industry, government/regulatory organizations, research institutes, etc. Statements from potential partners should be included in the proposal package.

Fit with LRI objectives / Possible regulatory and policy impact involvements / Dissemination

Applicants should provide information on the fit of their proposal with LRI objectives and an indication on how and where they could play a role in the regulatory and policy areas. Dissemination plans should also be laid down.

References

For Further Reading:

Crofton et al. (2011) : Developmental Neurotoxicity Testing : Recommendations for Developing Alternative Methods for the Screening and Prioritization of Chemicals ; http://www.altex.ch/resources/altex_2011_1_9_15_Crofton.pdf

Fritsche et al. (2015): Literature Review on In Vitro and Alternative Developmental Neurotoxicity (DNT) Testing Methods;
https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2015.EN-778
Bal-Price et al. (2018): Strategies to improve the regulatory assessment of developmental neurotoxicity (DNT) using in vitro methods;
https://www.sciencedirect.com/science/article/pii/S0041008X18300541?via%3Dihub
Bal-Prive et al. (2018): Recommendation on Test Readiness Criteria for New Approach Methods in Toxicology: Exemplified for Developmental Neurotoxicity;
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6545888/
Fritsche et al. (2018): Development of the Concept for Stem Cell-Based Developmental Neurotoxicity Evaluation; https://academic.oup.com/toxsci/article/165/1/14/5046970
Fritsche et al. (2018): Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes;
https://www.sciencedirect.com/science/article/pii/S0041008X18300437?via%3Dihub
Kraft (2015): The use of glial data in human health assessments of environmental contaminants; https://www.sciencedirect.com/science/article/pii/S0300483X15000827
OECD (2017): Report of the OECD/EFSA workshop on developmental neurotoxicity (DNT): The use of non-animal test methods for regulatory purposes;
http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2017)4/ANN1&docLanguage=En
Siracusa et al. (2019): Astrocytes: Role and Functions in Brain Pathologies;
https://www.ncbi.nlm.nih.gov/pubmed/31611796

DEADLINE FOR SUBMISSIONS: August 31, 2020

Please see www.cefic-lri.org/funding-opportunities/apply-for-a-grant/ for general LRI objectives information, project proposal form and further guidance for grant applications.