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

Title and Code Number:
Mining the developmental toxicity biomarker genome in the zebrafish embryo test. - **LRI C9**

Background

Developmental toxicity testing mainly relies on *in vivo* methods that are conducted in both rodents (rat) and non-rodents (rabbits) species. These very descriptive and time-consuming animal studies are mainly focusing on death, structural abnormalities, or altered growth in the foetus and pose some ethical issues**.**

- The zebrafish is a vertebrate which shares high physiological, morphological, and histological similarities with mammals including common characteristics in developmental biology. In compliance with international animal welfare regulations, the fish embryo model provides an ethically acceptable test system with the complexity of a complete vertebrate organism**.** This model is therefore often used as a developmental toxicity alternative, still in a descriptive manner, for screening purposes in low throughput assays. However, the zebrafish embryo test, usually based on morphological effect assessment, is not employed to its full potential. The zebrafish embryo test provides opportunities to study molecular effects of chemicals at the level of gene expression in an intact vertebrate embryo. Given the highly conserved genetic regulation throughout early vertebrate embryogenesis, molecular markers of embryogenesis may provide significant added value to developmental toxicity testing relevant for mammalian species including man. It is therefore proposed to identify sensitive key molecular biomarkers of vertebrate developmental toxicity and use them in a predictive manner.

Objectives
This project is looking to mine the zebrafish developmental genome for biomarkers of developmental toxicity, including but beyond those related to the retinoic acid pathway, to enhance the applicability domain of the zebrafish embryo test for chemical safety assessment.

The project objectives are to comprehensively identify, in a mechanistic approach, the group of principal molecular drivers of (dys)morphogenesis, as a basis for developmental toxicity assessment as follows:

1. Develop a quantitative spatiotemporal map of the major vertebrate morphogenetic and developmental gene families in the zebrafish embryo model,

based on mining existing literature and databases. Data selection and curation should be documented systematically.

2. Develop a quantitative spatiotemporal map of how chemical exposures perturb ZF gene expression based on existing literature and databases.
3. Perform some limited and dedicated experimentation in the zebrafish embryo model to fill knowledge gaps that have been identified through the literature and database reviews.
4. Update the gene expression pathway network that integrates the quantitative spatio-temporal maps from 1, 2 & 3 into a quantitative map of how chemical exposure perturbs the spatiotemporal map of ZF development as the overarching concept for monitoring vertebrate developmental toxicity after compound exposure in the zebrafish embryo model.
5. Identify a shortlist of candidate genes that when monitored adequately cover this embryotoxicity gene expression network in the zebrafish embryo model. Validate the effect biomarkers in zebrafish embryos, and recommend strategies to validate them in in vivo mammal models to evaluate translatability to mammals.
6. The gene expression pathway network should ultimately form the basis for a predictive in silico tool for developmental toxicity, that can be fed by data from the zebrafish embryo test and other dedicated in vitro assays.

Scope

The zebrafish embryo test is considered an acceptable alternative to mammalian testing under European legislation. For developmental toxicity testing it currently provides the only model available for studying the effects of compounds on intact vertebrate embryos that expresses the full morphogenetic spectrum of gene expression. Cell lines do not express all morphogenetic genes given their ex vivo conditions. Up to now classical approaches starting from individual alternative assays have failed to gain regulatory acceptance primarily because important mechanisms were not covered by these assays, resulting in incorrect predictions. Therefore, the strategy of defining a comprehensive gene set for embryotoxicity testing that is common to all vertebrates is the crucial starting point for predicting potential developmental toxicity in humans and to eventually gain regulatory acceptance of this assay.

Deliverables

The final report shall contain an executive summary (2 pages max), a main part (max. 50 pages) and a detailed bibliography. 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).

The spatiotemporal maps of ZF developmental gene expression must also be developed in a suitable machine-readable format to permit rapid re-use and integration. Where possible, the use of publicly available standard controlled vocabularies, ontologies and data standards & formats are preferred.

Cost and Timing
Start in Q1 2021
Duration 2 years
Budget in the order of 250.000 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

Using zebrafish in systems toxicology for developmental toxicity testing (2016)
Y. Nishimura, A. Inoue, S. Sasagawa , J. Koiwa , K. Kawaguchi , R. Kawase , T. Maruyama S. Kim, T. Tanaka
Congenit Anom (Kyoto) 56(1):18-27.

Transcriptomic analysis in the developing zebrafish embryo after compound exposure: individual gene expression and pathway regulation (2013).
S. Hermsen, T. Pronk, E. van den Brandhof, L. van der Ven, A. Piersma
Toxicol Appl Pharmacol 272:161–171.

Transcriptomic Changes in Zebrafish Embryos and Larvae Following Benzo[a]pyrene Exposure (2015). X. Fang, J. Corrales, C. Thornton, T. Clerk, B. Scheffler, K. Willett
Toxicol Sci 146, 395–411.

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.