Meeting Report

International STakeholder NETwork (ISTNET) Workshop for Creating a Developmental and Reproductive Toxicity (DART) Testing Roadmap for Regulatory Purposes

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Currently, there is a paradigm shift in regulatory chemical hazard and risk assessment moving from the evaluation of apical endpoints in animals towards the use of human-relevant new approach methodologies (NAMs) (NRC, 2007; Berggren et al., 2017; Thomas et al., 2019; Krewski et al., 2020; Pistollato et al., 2021; Magurany et al., 2023). These methods include *in vitro* and *in silico* techniques that capture key biological processes and mechanisms of toxicity. This paradigm shift is based on the need to acquire safety information on the tens of thousands of untested chemicals in a cost- and time-effective way (Berggren and Worth, 2023; Schmeisser et al., 2023). Ethical considerations have also been driving the establishment of NAMs as the utilization of thousands of animals in chemical safety testing is being challenged by animal rights organizations and the general public[1,2]. Most importantly, scientific considerations strongly support this notion, as NAMs can provide more accurate and relevant data for human health risk assessments. For instance, the high attrition rate in the drug development process has been educating us on the value of using human-relevant models to better predict safety and efficacy for human pharmaceuticals (Weaver and Valentin, 2019; Pognan et al., 2023).

An area of high animal utilization and known species-specificity is developmental and reproductive toxicity (DART) (Rovida et al., 2023). Exposure to certain chemicals and drugs has been linked to reproductive and developmental disorders in humans. Preconception exposure of men and women can potentially lead to infertility, miscarriage, and birth defects. During pregnancy, fetal exposure is particularly concerning as certain chemicals and drugs can cross the placental barrier, potentially resulting in neurodevelopmental disorders, congenital abnormalities, and fetal death. Prenatal and perinatal exposure has also been linked to immune disorders, childhood cancer, and learning disabilities (Frazier and Fromer, 2017; Wager and Thompson, 2024). These concerns, coupled with advances in developmental and reproductive biology, have prompted calls for increased testing of industrial chemicals, pesticides, cosmetic products, food additives, and pharmaceuticals for DART by multiple stakeholders. New methods, including human stem cell-derived *in vitro* models, hold great promise for more efficient and protective models for DART and can be used in an integrated manner to solve regulatory challenges. In order to develop a roadmap to define a NAM-based integrated testing strategy (ITS) to meet DART regulatory requirements, there needs to be truly effective communication and discussion among the different stakeholders (regulators, industry, and academia). To establish this, the first meeting of the International STakeholder NETwork (ISTNET) for DART was held in Zurich, Switzerland on September 12-13, 2024, with the aim of building consensus on the development and use of *in silico* and *in vitro* methods to deliver useful data for regulatory decisions.

The meeting included 61 participants from 12 countries representing regulatory, academic and industry scientists. Experts in the regulation and management of risks attended from the European Commission, EC; European Food Safety Authority, EFSA; European Chemicals Agency, ECHA; United States Environmental Protection Agency, US EPA; Danish EPA; US Food and Drug Administration, US FDA; German Federal Institute for Risk Assessment, BfR; Japanese National Institute of Public Health, NIPH; Swissmedic and Swiss Federal Office of Public Health. Academic and industry scientists spanned 15 international universities/academic research institutions as well as 15 different private companies. Non-government organizations were represented by Doctors Against Animal Experiments.

Discussions at the meeting started by presenting the successful pathway followed for NAM development in the context of developmental neurotoxicity (DNT). The ISTNET-DNT meeting, which took place in Zurich in 2014 (Crofton et al., 2014; Bal-Price et al., 2015), produced a roadmap that paved the way for developing DNT-NAMs towards regulatory application (Masjosthusmann et al., 2020; Crofton and Mundy, 2021) that summited in OECD (Organisation for Economic Co-operation and Development) initial recommendations in 2023 (OECD, 2023). The success of the DNT *in vitro* battery (DNT-IVB) was based on the early involvement of all stakeholders, i.e., regulatory, academic and industry scientists, in creating the roadmap and on basing the DNT-IVB assays on the biology underlying human neurodevelopment.

[1] EIC (European Citizens' Initiative) Save Cruelty Free Cosmetics – Commit to a Europe Without Animal Testing. https://citizens-initiative.europa.eu/initiatives/details/2021/000006_en (accessed 09.10.2024)

[2] EIC (European Citizens' Initiative) Stop Vivisection. https://citizens-initiative.europa.eu/initiatives/details/2012/000007/stop-vivisection_en (accessed 09.10.2024)

For the ISTNET-DART workshop, likewise, the biology of the reproductive and developmental cycles was laid out in 18 short talks accompanied by insights into the current status of *in vitro* methods available for DART testing and related physiology-based kinetic (PBK) modelling. These scientific talks were framed by insights from regulatory agencies into present strategies for DART testing and their perspectives on the use of NAMs in this complex area, views on readiness and validation of test methods, DART implementation into next generation risk assessment (NGRA) frameworks, and the overall strategy of the European roadmap for phasing out animal experiments, a response to a European Citizens' Initiative in 2023[3].

The meeting was followed by two half days of breakout groups for female and male reproductive toxicity, developmental toxicity, and PBK modelling. In these breakout groups the experts mapped the respective biology to the currently available NAMs in the fields and identified areas of further research needs. In general, barriers and drivers for the use of DART NAMs were discussed. Here, especially the timing of development of reproductive organs as well as the whole embryo/fetus, including the postnatal and juvenile period up to sexual maturity, were seen as a particular challenge because covering these different life stages will potentially require a high number of NAMs. Smart integrating solutions need to be found. While for some areas, like DNT and early embryogenesis, NAMs are already advanced, other areas are less well covered or largely lack appropriate strategies. Small numbers of adverse outcome pathways (AOPs) in the different areas were identified, highlighting the need for advancing AOPs for DART. To relate DART-relevant hazard with exposure during the reproductive cycle, prediction of internal concentrations during the different life stages, including PBK models for pregnancy and fetal exposure, were discussed.

There was consensus at the meeting that NAMs for DART testing still bear biological uncertainties. Once the most important life stages and key events of the reproductive and developmental cycles are covered by NAMs, they need to be placed into *in vitro* batteries (DART-IVBs), as – like for DNT – a one-to-one replacement for such complex endpoints is not possible. The implementation of such batteries would also benefit from tiered testing strategies as suggested in the breakout groups. For advancing DART testing using NAMs, there is a large body of methodologies and strategies in the stem cell and human reproduction fields that can be applied to toxicological questions. Besides biological and toxicological coverage, it was also discussed that novel DART methods should be time- and cost-effective to promote their uptake for safety assessments in industrial and regulatory contexts.

Utilization of *in silico* approaches such as computational networks of gene expression, computational tools for assembly and assessment of AOP networks, QSARs (quantitative structure activity relationships), and most highlighted, the "virtual embryo" (Marx, 2023) were deemed extremely useful tools on several levels. In addition to the toxicodynamic means, estimation of toxicokinetics in the DART context by using computational models was extensively discussed, and also here a tiered approach was suggested. In analogy to DNT, also DART-IVBs can be used in the future to determine the most sensitive endpoints across multiple assays that can serve as points of departure. In combination with exposure estimation, such tests can hence be used in NGRA frameworks.

Overall, the meeting was very successful as a high number of stakeholder representatives intensively discussed their different perspectives and views with the goal of finding consensus. While regulators learned about some cutting-edge science, scientists were educated on the sector-specific regulatory needs in various countries. These discussions are critical for the development of methods and strategies that provide useful data for regulatory purposes.

The next steps include dissemination of the meeting outcomes as a DART roadmap to engage more scientists and regulators in this endeavor. With this, we also aim to raise political attention to the topic to support the development of efficient tiered testing strategies for DART that can provide not only an initial screening but a comprehensive human health safety assessment that is useful for regulatory purposes.

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