Introduction to Leveraging Non-Mammalian Models For Developmental Neurotoxicity Testing

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The developing nervous system is a sensitive target for chemical exposure in both humans and animal models, and early life-stage exposures can lead to long-term effects on motor activity, sensory function, and cognition. To adequately protect human health and the environment, there is a recognized need for cellular and alternative non-mammalian models to support rapid and cost-effective screening, hazard identification, and prioritization of chemicals for developmental neurotoxicity testing in rodents. The vast majority of high-throughput screening (HTS) and high-content screening (HCS) assays used for developmental neurotoxicity testing utilize cell-free and cell-based methods that model key biological events across a wide range of toxicologically-relevant pathways. However, these assays do not adequately reflect the complex physiology of an intact organism and uniformly lack the ability to test for specific neurodevelopmental events such as myelination or complex behaviors including activity, learning, and memory. Therefore, the use of smaller, alternative non-mammalian animal models (such as amphibians, nematodes, flies, and fish embryos) have been proposed as complementary models to cell-free and cell-based methods, as these model systems contain complex, intact nervous systems and are also suitable for both microplate-based HTS/HCS assays and mechanistic neurotoxicological research. Moreover, such models often have distinct advantages over mammalian models, such as fully mapped cell lineages and easily manipulatable genetics.

In order to highlight the use of non-mammalian models for developmental neurotoxicity testing, this Virtual Special Issue of *Neurotoxicology and Teratology* focused on the theme of "*Leveraging Non-Mammalian Models for Developmental Neurotoxicity Testing*". The primary goals of this Virtual Special Issue were to highlight the utility of non-mammalian models for 1) testing a wide variety of developmental neurotoxicants – including emerging, understudied contaminants such as flame retardants (brominated and phosphorous) and per- and polyfluoroalkyl substances (PFAS) and 2) uncovering mechanisms underlying environmental chemical-induced neurotoxicity. Non-mammalian models include, but were not limited to, fish

(e.g., zebrafish), nematodes (e.g., *Caenorhabditis elegans*), fruit flies (e.g., *Drosophila melanogaster*), echinoderms (e.g., sea urchins), and planarians.

Out of eight papers published within this Virtual Special Issue, three non-mammalian models were utilized – zebrafish (Cadena et al., 2020; Hawkey et al., 2021; Maharaj et al., 2020; Ogungbemi et al., 2020; Oliveri et al., 2020; Ahkin Chin Tai et al., 2021), frogs (Foguth et al., 2020), and nematodes (Ke et al., 2021) – and a diverse array of drugs and environmental contaminants that are suspected or known developmental neurotoxicants were tested. Moreover, seven out of eight papers investigated chemically-induced alterations on behavior, using automated tests. This demonstrates the growing use of complex, functional readouts of neurobehavioral development as indicators of developmental neurotoxicity events. While classical locomotor responses to light-to-dark and dark-to-light transitions were commonly used, one paper provided significant advances in automated analysis of spontaneous tail coiling in zebrafish embryos – an increasingly used read-out that is valuable for rapid, high-throughput screening and identification of neuroactive chemicals during early development (Ogungbemi et al., 2020).

Another theme explored in this collection is the use of alternative models to evaluate the developmental neurotoxicity of chemical mixtures. Mixture toxicity was assessed in two zebrafish studies (Cadena et al., 2020; Hawkey et al., 2021), and one paper focused on the effects of individual PFAS and their mixtures on brain neurochemistry the developing Northern leopard frog (Foguth et al., 2020). Using alternative models, these papers underscore the ability to evaluate neurotoxicological endpoints within assays that better recapitulate complex environmental exposures.

Finally, from a translational perspective, one paper showed conservation of major atrazine metabolites to those detected in mammals (Ahkin Chin Tai et al., 2021). While current alternative neurotoxicity assays are clearly useful for the prioritization of further neurotoxicological testing in

mammals, studies such as this are necessary to build confidence in hazard identification generated in alternative tests to ultimately avoid evaluations in mammalian tests.

Overall, this collection of papers highlights the versatility and power of non-mammalian models for developmental neurotoxicity testing, and further supports the need to continue promoting these models as complementary tools to cell-free and cell-based methods.

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