UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF RESEARCH AND DEVELOPMENT

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: March 13, 2023

SUBJECT: Response to the Final Report of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) on the Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment

1.0 Introduction

Consistent with the National Research Council's report on *Toxicity Testing in 21st Century: A Vision and a Strategy* and the Environmental Protection Agency's New Approach Methods (NAMs) Workplan[1], the EPA's Office of Pesticide Programs has been actively engaged in numerous efforts over the past decade to reduce use of laboratory animals and implement NAMs that are more efficient and human relevant than traditional methods of hazard evaluation. In September 2020, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) was convened to review two proposed approaches for implementing NAMs to inform uncertainty and safety factors for human health risk assessment in lieu of reliance on default factors.

The first proposed approach presented work completed by the Office of Research and Development (ORD) thus far to develop a battery of NAMs to evaluate developmental neurotoxicity (DNT) potential as part of an international effort. The internationally developed battery is comprised of *in vitro* assays that evaluate critical processes of neurodevelopment, including proliferation of neuroprogenitor cells (NPC), differentiation of neuroprogenitors into glial and neuronal subtypes, apoptosis, migration of neurons and oligodendrocytes, neurite outgrowth, synaptogenesis, and neural network formation (Sachana et al., 2018). For the September 2020 SAP, organophosphate (OP) pesticides and their metabolites were presented as a case study. EPA solicited comment from the SAP on the use of the *in vitro* assays developed by ORD for evaluating neurodevelopmental endpoints using two technology platforms: microelectrode arrays with neuronal cell types to understand neuronal network formation (MEA NFA) and high-content imaging (HCI) assays of neural cells to understand key processes relevant to neurodevelopment. Additionally, EPA solicited comment on the ability of the current full battery of assays, which includes the ORD assays and assays developed by laboratories sponsored by the European Food Safety Authority (EFSA), to cover critical processes in neurodevelopment. Furthermore, EPA solicited comment on the proposed process taken to compare the relative sensitivity of concentrations eliciting activity in these DNT NAMs to doses that inhibit acetylcholinesterase (AChE) in laboratory animals, which is the basis of current OP human health risk assessments. This was accomplished by comparing administered equivalent doses (AEDs) for the DNT NAMs to benchmark dose (BMD) and associated lower confidence bound (BMDL) values estimated from AChE inhibition data in *in vivo* rat studies. AEDs were derived by *in vitro* to *in vivo* extrapolation (IVIVE), using generic high-throughput toxicokinetic (HTTK) models and the primary assumption that the nominal micromolar concentrations bioactive in the DNT NAMs is equivalent to average plasma concentrations in rats and humans.

The second proposed approach presented experiments performed by academia on behalf of pesticide registrants and their consultant (Exponent) for 16 OP compounds. The experiments evaluated *in vitro* AChE inhibition constants in rats and humans for the intended purpose of developing interspecies and intraspecies pharmacodynamic (PD) data-derived extrapolation

[1] https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical

factors (DDEFs). EPA solicited comment from the SAP on the study design and methods utilized to generate the *in vitro* data, statistical analyses employed to calculate proposed DDEFs, and analyses that were performed on a small subset of OP compounds to evaluate contributions of experimental and intrinsic variability.

In December 2020, the meeting minutes and final report with comments from the SAP were published.[2] OPP and ORD have reviewed the final report from the SAP panel and considered their comments and recommendations. This document is intended to address the salient comments from the SAP focusing on consensus statements and points of clarification with discussion and responses grouped by NAM approach (i.e., DNT NAM battery in Section 2.0 and DDEFs for OPs in Section 3.0).

2.0 DNT NAM Battery

Summary of SAP Comment: The panel was largely supportive of the DNT NAM battery and complimented the EPA on its efforts to advance the development and evaluation of NAMs for assessing DNT potential. The panel agreed with EPA that a battery of assays is needed at this time since no single *in vitro* assay can recapitulate all the critical processes of neurodevelopment. Overall, the panel agreed that the focused battery of assays reflects, if not directly models, critical processes for neurodevelopment and "agreed that if the Agency uses published data in their evaluation, then there is no reason to exclude peer-reviewed published *in vitro* assay data – whether screening or mechanistic – in that final weight of evidence."

> EPA Response: The Agency appreciates the support and encouragement from the panel on its efforts. EPA staff have been at the forefront of national and international efforts to reduce its reliance on animal testing and improve human health risk assessment through the development and implementation of NAMs. This has included over a decade of working with international collaborators to develop the current DNT NAM battery, as described in the Agency's issue paper[3], to provide high quality mechanistic data to evaluate DNT potential. OPP strongly advocates for the use of weight of evidence (WOE) approaches and has a long history of utilizing WOE evaluations in chemical risk assessments, including pesticides. As such, we appreciate the panel's agreement that data from the DNT NAM battery should be considered as part of an overall WOE when evaluating the DNT potential of a chemical.

[2] Transmittal of meeting minutes and final report of the Federal Insecticide, Fungicide and Rodenticide Act, Scientific Advisory Panel (FIFRA SAP) Virtual Meeting held on September 15-18, 2020. EPA-HQ-OPP-2020-0263-0054. https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0054

[3] Agency Issue Paper: Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment. July 2020. https://www.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006

Summary of SAP Comment: Several important processes and cell types, such as glial components, neurovascular units, chemotaxic cues, and cell-cell interactions, are missing in the battery and it underestimates the complexity of nervous system development.

EPA Response: It is well recognized that any given assay, or even collective endpoints in a battery of DNT NAM assays, will not recapitulate the full complexity nor evaluate all components of the nervous system. The latter is also true of the *in vivo* DNT guideline study, as it does not completely evaluate all aspects of nervous system structure and function/behavior. When NAM research for DNT was initiated, it was recognized that brain development was complex and took place with different timelines in different brain regions, involving many different cell types. The concept of evaluating "key neurodevelopmental processes" (Lein et al., 2005; Coecke et al., 2007) was designed to address this issue, as proliferation, migration, differentiation, etc., must take place across all brain regions and neurotransmitter types for proper nervous system development, and the mechanisms underlying these processes are well conserved. In addition to the strengths of the DNT NAM battery identified in the SAP report, the DNT NAM battery can provide mechanistically relevant information related to DNT potential and evaluate early perturbations in critical processes that are difficult to obtain or evaluate *in vivo*. As such, the strengths and uncertainties associated with the DNT NAM battery need to be considered and balanced with those associated with *in vivo* studies, including the quality and human relevance of the data obtained from those studies.

The current battery is not entirely lacking the processes and cell types the were pointed out by the panel. For instance, the panel encouraged the development and inclusion of glial-based (astrocyte, oligodendrocyte, and microglia) targeted NAMs in the battery. EPA recognizes that glia-derived cells are critical to nervous system development and agrees that more glial-specific endpoints, such as myelination, could be represented in the battery. However, it is important to note that several assays in the battery include glia and that changes in these assays may reflect chemical effects on glia or neurons. Specifically, the battery includes oligodendrocyte differentiation and radial glia migration assays (NPC2, NPC5), in which migration is measured at two time points. Further, the neurite outgrowth, synaptogenesis, and network formation assays (NFA) utilize mixed cortical cultures which contain primarily glutamatergic and gabaergic neurons, astrocytes, and a small percentage of microglia. While these three latter assays do not specifically measure glial endpoints, alterations in glial health may contribute to the effects observed in these assays allowing for potential detection of effects through a glial mediated mechanism (Mauch et al., 2001; Fester et al., 2009; Suzuki et al., 2007). Indeed, there are numerous studies that demonstrate the role of astrocytes in promoting synaptogenesis and network formation.

The panel also commented on limited representation of different neurotransmitter types, neuronal types, and brain regions in the DNT NAM battery. However, the current DNT NAM battery, comprised of the ORD assays and assays developed by international collaborators, does in fact cover a broad variety of different cell types. The current DNT

NAM battery utilizes neuronal models that include glutamatergic, gabaergic, cholinergic (assays with rat primary cortical cells or CDI human neurons), and human dopaminergic (UKN4) neurons as well as radial glial, oligodendrocyte (NPC assays), and neural crest (UKN2) cells and human peripheral neurons (UKN5). It also includes different types of two- and three-dimensional human NPCs (hNP1, neurospheres) as well as primary rodent mixed cell type cultures for both central and peripheral models. Additionally, further characterization of the expression of different neural substrates (e.g., receptors, cell types, proteins, etc) using transcriptomic approaches is in progress for the EPA assays.

The panel noted that the DNT NAM battery lacks chemotaxic/chemoattractant cues for neurite outgrowth and migration. While directional cues may not be represented in dispersed, two-dimensional cultures, these models (e.g., the primary cortical model) do release signaling molecules such as brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF) and other cues, as well as express the receptors and signaling pathways for those molecules. Modulation BNDF and FGF signaling pathways affects *in vitro* neurodevelopment processes such as neurite outgrowth and synapse formation in dispersed two-dimensional culture models. Thus, chemical impacts on these signaling pathways may still be observed in many cases. In addition, the neurosphere assays (IUF models) also have some chemotaxic cues for migration of neurons, as they migrate along the tracts laid down by radial glia, and signaling molecules are not completely lacking in the proliferation culture system given the presence of growth factors.

Additionally, the panel commented that cell-cell interactions are not accounted for in the DNT NAM battery. However, this is not entirely accurate. While some assays in the DNT NAM battery are limited in these interactions, in other assays these interactions are critical components. For example, the synaptogenesis and NFAs demonstrate the structural and functional formation of synapses in primary cortical cultures. These cultures contain excitatory and inhibitory neurons, as well as glia (astrocytes), which all contact each other in the development of synapses and functional networks. Additionally, the NFA has several assay endpoints (e.g., network spikes, mutual information, correlation coefficient) that evaluate the connectivity of the networks and thus provide metrics of how well groups of neurons are communicating with each other. Further, the suite of NPC assays from one of the laboratories sponsored by EFSA relies on neurospheres which require interaction of neuroprogenitors, neurons, oligodendrocytes and radial glia for several key processes evaluated in the assay(s).

As stated above, it is recognized that any given assay will not evaluate fully all components of the nervous system and therefore utilizing information from other available studies could help address some of the processes and cell types that may be underrepresented in the battery. For example, the panel encouraged the development and inclusion of neurovascular-based targeted NAMs in the battery. While the DNT NAM assays presented to the SAP did not include assays related to neurovascular unit development, assays, and computational models (Saili et al., 2017; Zurlinden et al., 2020) evaluating chemical effects