OPFR Prioritization using the Developmental Neurotoxicity In Vitro Test Battery: An Update to OECD IATA Case Study

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Background and Purpose

Organophosphorus flame retardants (OPFRs) are abundant and persistent in the environment due to their extensive use in industrial processes and products. In vivo and in vitro studies suggest that OPFRs may cause adverse health effects. Additionally, their similarity in structure to organophosphate pesticides presents a great concern for potential acute neurotoxicity and developmental neurotoxicity (DNT) commonly associated with organophosphates. However, current in vivo DNT guideline tests (OECD TG 426 and 443) have challenges and limitations such as high costs, extensive timelines, and questionable human relevance. A DNT in vitro battery (DNT-IVB) was developed to help resolve these in vivo testing challenges. In September 2022, an OECD Integrated Approach for Testing and Assessment (IATA) case study was developed and published for DNT, with OPFRs as the case example, to demonstrate how applying DNT-IVB can prioritize a class of compounds. In the IATA case study, eight compounds were used, including aromatic OPFRs such as triphenyl phosphate (TPHP), isopropylated phenyl phosphate (IPP), 2-ethylhexyl diphenyl phosphate (EHDP), tricresyl phosphate (TMPP), isodecyl diphenyl phosphate (IDDP), tert-butylphenyl diphenyl phosphate (BPDP); halogenated (tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(2-chloroethyl) phosphate (TCEP); as well as two classical brominated flame retardants (BFRs), 2,2'4,4'-tetrabromodiphenyl ether (BDE-47) and 3,3',5,5'-tetrabromobisphenol A (TBBPA). Here, we enhance the OECD IATA case study using the same chemicals with additional in vitro assay data, and contextualize the data with human exposure information and toxicokinetic parameters for relevant endpoints and mechanisms not covered in the initial case study.

Methods

The DNT-IVB consists of in vitro assays that evaluate processes in neurodevelopment and function (e.g., neural proliferation, neurite outgrowth, oligodendrocyte differentiation, cell migration, and neuronal firing/network formation). As an addition, behavioral data from complementary non-mammalian animal models, zebrafish and planaria, were included. We also added exposure data from new sources that sampled breast milk, urine, house dust, etc., to provide more thorough evaluation of human exposure to OPFRs. In vitro assay data were re-evaluated using the benchmark concentration (BMC) approach. Physiologically based pharmacokinetic modeling was performed to predict the maximum plasma concentration from exposure data to be compared to the in vitro BMCs. Furthermore, we collated additional information for endpoints and processes not covered in the current DNT-IVB, such as glia differentiation and function, ontogeny of neurotransmitters and receptors, and endocrine disruption from the literature. We also used curated high-throughput screening data from the

Integrated Chemical Environment provided by U.S. federal ToxCast and Tox21 initiative that measured diverse endpoints.

Results

Considering compound class for the extended DNT-IVB endpoints, the classic BFRs and aromatic OPFRs were generally more active than halogenated OPFRs. Out of a total of 20 assays, BFRs and aromatic OPFRs was active in 10 assays on average, while halogenated OPFRs was active in 2-3 assays. The aromatic OPFR BPDP affected most of the endpoints with activity in 16 assays, while the classic BFR BDE-47 was most potent with lowest BMC, followed by other novel OPFRs such as EHDP and TPHP. Of all the assays, behavioral and neurite outgrowth were most sensitive, with zebrafish showing more sensitivity in the behavior assays than the planaria species. Additionally, further characterizing the results of the DNT-IVB revealed that the in vitro BMC for many assays were within 10-fold of one another, with only BDE-47 and TPHP observed as the more potent in the assays. The lowest in vitro activity concentration of the most sensitive endpoint falls in the range of maximum plasma concentration estimated from human exposure through breast milk for BDE-47, TPHP, TDCIPP, and EHDP, suggesting a human risk concern. Data from the literature and ICE included endpoints that showed some effects at lower concentrations than the DNT-IVB for, e.g., thyroid inhibition, glial differentiation, and ontogeny of neurotransmitters. However, these studies used different analysis pipelines and cannot be directly compared.

Conclusions

Our updated data reveal that human exposure to some OPFRs could lead to a plasma concentration similar as those exerting in vitro activities, indicating potential concern for human health. Moreover, there were some endpoints that were more sensitive to OPFRs than the DNT-IVB and need further investigation. Overall, our study can refine the DNT-IVB and improve confidence in integrating new approach methodologies in DNT assessment thereby helping to prioritize the ranking of OPFRs that are being used as replacements for some BFRs. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

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