ToxStrategies

Rapid Risk Assessment of Potential Neurotoxicants Using Non-Animal Test Methods

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Abstract:

Background and Purpose: Across the Department of Defense, concerns for warfighter safety span complex potential exposures that include mixed stressors (chemical, particulate, physical), as well as diverse exposure durations (acute, short-term deployment, long-term on base occupation). Over the last two decades, the focus of chemical risk research has been moving toward development of methods and strategies to support rapid chemical risk assessment using new approach methods (NAMs), including human cell in vitro assays and computational models. Several case studies with these tools have demonstrated utility for prioritizing potential public health concerns for environmental chemicals with expected long-term low dose exposures. However, it is likely that many current models will need to be expanded to account for deployment situational concerns to apply NAM strategies to military relevant exposures. As a case study, we evaluated the utility of current high throughput NAMs for rapid risk assessment of drinking water exposures to 220 potentially neurotoxic chemicals during a 1-year deployment. In vitro bioactivity data were used together with the US Environmental Protection Agency (EPA) high-throughput toxicokinetic modeling platform "httk" to estimate human equivalent doses (HEDs) in the active-duty Air Force population and to compare these HEDs to in vivo points of departure (PODs) and provisional 1-year drinking water Military Exposure Guidelines (MEGs).

Methods: Acute neurotoxicity data (oxidative stress, cytotoxicity, neurite outgrowth) were collected in human induced pluripotent stem cell (iPSC)-derived neurons for 220 potentially neurotoxic compounds after a 4-hour treatment (Pre et al. 2022). The USEPA ToxCast Pipeline was used to calculate AC_{50} (concentration with 50% maximum activity) values for these data. USEPA's InVitroDB (v.3.5) was accessed to download all bioactivity concentrations for the chemical set. Combined, these datasets were used to derive NAM-based chemical-specific PODs (POD-NAMs) for all in vitro endpoints regardless of association with neurotoxicity as a health outcome, as described by Paul-Friedman et al. (2020). Briefly, the AC_{50} was derived for each chemical/assay combination and an overall chemical-specific POD-NAM was calculated from the 5^{th} percentile of AC_{50} values. Neuro-specific POD-NAM also were derived from assays that were manually identified to be directly related to neurotoxicity. Reverse dosimetry was performed using the USEPA httk package v.2.2.2 to estimate the HED from a given POD-NAM (all or neuro-specific NAMs). To model the US Air Force (USAF) population specifically, the httk model was altered to reflect demographic parameters for active-duty personnel (Zehner and Mullenger, 2020). In vivo PODs were calculated from the 5^{th} percentile of the PODs listed in USEPA's ToxValDB for each of the case study chemicals. Provisional MEGs were calculated from existing toxicity data using the 2013 US Army technical guidance TG230.

Results: In vitro-derived POD-NAMs for the neurotoxicity-associated endpoints generally less conservative compared to those calculated from the combined in vitro endpoints (99/137 chemicals). For subsequent

comparisons with in vivo derived values, HEDs were calculated using POD-NAMs derived from all in vitro endpoints. In vitro-based HEDs were compared to traditional animal study-derived PODs to evaluate the utility of in vitro assays as surrogates for traditional toxicology studies. In vitro derived HEDs were lower (more conservative) than traditional in vivo PODs for 86% and 87% of the chemicals when evaluated for the US general population and the USAF population, respectively. To evaluate the feasibility of using in vitro-derived HEDs to recommend operational exposure limits, HEDs calculated for the USAF population were compared to provisional MEGs for each chemical. In vitro derived HEDs were lower than the provisional 1-year drinking water MEGs for 66% of the tested chemicals. Adjusting the derived HEDs by an empirical uncertainty factor of 1,000 to ensure a conservative estimated screening exposure limit for 95% of the test chemicals.

Conclusions: In vitro derived HEDs were generally more conservative than PODs derived from traditional in vivo toxicity studies in laboratory animals. POD-NAMs derived from neuro-specific assays were less conservative than values derived from all available in vitro data. Using high throughput in vitro data together with the httk model tailored to the USAF population, we were able to derive drinking water screening exposure limits for all 220 potentially neurotoxic compounds. Use of an uncertainty factor of 1,000 to calculate a conservative operational exposure screening limit is in line with traditional risk assessment methods that assign default 10-fold uncertainty factors each for intraspecies, interspecies, and sub-chronic to chronic exposure extrapolation (10 x 10 x 10 = 1000) to calculate in vivo derived exposure limits. This work supports the use of NAM-based tools to derive conservative (health protective) PODs for screening level assessments.

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