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Review article

Expanding the test set: Chemicals with potential to disrupt mammalian brain development

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ABSTRACT

High-throughput test methods including molecular, cellular, and alternative species-based assays that examine critical events of normal brain development are being developed for detection of developmental neurotoxicants. As new assays are developed, a "training set" of chemicals is used to evaluate the relevance of individual assays for specific endpoints. Different training sets are necessary for each assay that would comprise a developmental neurotoxicity test battery. In contrast, evaluation of the predictive ability of a comprehensive test battery requires a set of chemicals that have been shown to alter brain development after in vivo exposure ("test set"). Because only a small number of substances have been well documented to alter human neurodevelopment, we have proposed an expanded test set that includes chemicals demonstrated to adversely affect neurodevelopment in animals. To compile a list of potential developmental neurotoxicants, a literature review of compounds that have been examined for effects on the developing nervous system was conducted. The search was limited to mammalian studies published in the peer-reviewed literature and regulatory studies submitted to the U.S. EPA. The definition of developmental neurotoxicity encompassed changes in behavior, brain morphology, and neurochemistry after gestational or lactational exposure. Reports that indicated developmental neurotoxicity was observed only at doses that resulted in significant maternal toxicity or were lethal to the fetus or offspring were not considered. As a basic indication of reproducibility, we only included a chemical if data on its developmental neurotoxicity were available from more than one laboratory (defined as studies originating from laboratories with a different senior investigator). Evidence from human studies was included when available. Approximately 100 developmental neurotoxicity test set chemicals were identified, with 22% having evidence in humans.

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1. Introduction

Traditional toxicity testing requires collecting data on one chemical at a time using common laboratory species (e.g., rats, rabbits, mice). With tens of thousands of chemicals now in commerce with limited toxicology data, higher throughput methods need to be employed to enable the rapid collection of data on these chemicals (NRC, 2007). These high-throughput designs include in silico modeling, in vitro assays, and the use of small model organisms as alternative species for toxicity testing. A number of efforts, including the United States Environmental Protection Agency's (U.S. EPA) ToxCast™ program (www.epa.gov/ncct/toxcast/) and the joint U.S. EPA/NIH/FDA Tox21 initiative (ncats.nih.gov/tox21.html), are employing hundreds of high-throughput assays that investigate molecular targets and key events related to pathways that can potentially lead to adverse health effects, including reproductive and developmental toxicity (Tice et al., 2013). To date, there is limited use of high-throughput assays for endpoints relevant to developmental neurotoxicity (Bal-Price et al., 2015a).

Traditional animal testing to determine if a chemical has the potential to cause adverse effects in the developing nervous system is time and resource intensive. Studies based on U.S. EPA or the Organization for Economic Co-operation and Development (OECD) guidelines can take months to years to complete, cost hundreds of thousands of dollars, and use hundreds of laboratory animals. In light of the concern regarding the potential of environmental chemicals to contribute to neurodevelopmental disorders in children (Grandjean and Landrigan, 2006, 2014; Braun et al., 2006; Hertz-Picciotto et al., 2006; Karr, 2012), there are ongoing efforts to develop medium- and high-throughput assays to facilitate the detection of chemicals that are likely to affect brain development (Coecke et al., 2007; Bal-Price et al., 2012). The methods being developed probe multiple levels of biological organization including molecular, cellular, and alternative species (Lein et al., 2007). Due to the complexity of the events regulating brain development, along with the known and unknown modes of action of neurotoxic chemicals (Bal-Price et al., 2015b), it is unlikely that any individual assay will be sufficient to detect all chemicals with the potential to disrupt neurodevelopment. Thus, a battery of assays covering multiple molecular targets, intracellular signaling pathways, critical cellular events, and integrated neural functions is needed (Lein et al., 2007; Radio and Mundy, 2008; de Groot et al., 2013). Several references have provided general principles for developing and evaluating models and assays to screen and prioritize chemicals that affect neurodevelopment (Crofton et al., 2011, 2012; Kadereit et al., 2012). An important step in the development and evaluation of alternative methods is the use of "training set" and "test set" chemicals (as defined by consensus at the international TestSmart DNT II meeting (http://caat.jhsph.edu/programs/workshops/dnt2.html; Crofton et al., 2011). Note that this terminology should not be confused with training set data and test set data used to build and validate QSAR models. In the context of alternative methods development, the "training set" refers to chemicals that have been previously shown to reliably and consistently alter a specific endpoint that the assay is designed to assess. Typically, these are chemicals with well-documented modes of action or that have been repeatedly tested in multiple in vitro model systems. Use of these chemicals can demonstrate the relevance and performance of the assay, as well as its practical ability to test moderate numbers of chemicals in a screening mode (Judson et al., 2013). As an example, training sets for in vitro assays of the critical neurodevelopmental event of neurite outgrowth can be found in Radio et al. (2008) and Krug et al. (2013). An important consideration is that training set chemicals must be specific to the endpoint being measured, and different training sets may be necessary for each of the multiple assays that would comprise a developmental neurotoxicity test battery. A discussion of training sets for evaluation of endpoint-specific assays can be found in Crofton et al. (2011) and Kadereit et al. (2012).

In contrast to individual assay development, evaluation of the ability of a battery of in vitro assays or non-mammalian test species to predict whether chemicals are likely to affect neurodevelopment in vivo in mammals requires a different set of chemicals, described as a "test set" (Crofton et al., 2011). Test set chemicals are those that have been shown to alter brain development after in vivo exposure. Ideally, because the goal of these assays is to protect human populations, the test set should be comprised of chemicals known to produce developmental neurotoxicity in humans. However, only a small number of chemicals (e.g., methylmercury, lead, ethanol, valproic acid, PCBs, arsenic, toluene) have been well documented to alter human neurodevelopment (Giordano and Costa, 2012), and in some cases, the evidence is based on small increases in the relative risk determined in a limited set of epidemiologic studies (Grandjean and Landrigan, 2006, 2014; Kadereit et al., 2012). This small number of "known developmental neurotoxicants" is unlikely to be representative of all the potential mechanisms by which chemicals may produce developmental neurotoxicity, and thus does not comprise a sufficient test set to validate the predictive ability of a developmental neurotoxicity test battery. For example, to evaluate the predictive ability of a battery of three in vitro genotoxicity tests, Kirkland et al. (2006) tested over 700 chemicals classified as rodent carcinogens based on in vivo cancer bioassays. Similarly, a set of 60 chemicals was generated for use as a test set for evaluating alternatives to whole fish toxicity tests (Schirmer et al., 2008).

In order to develop a test set of developmental neurotoxicants, we propose that chemicals that have been demonstrated to adversely affect neurodevelopment in experimental animals should be included. This would expand the chemical space covered in the test set and presumably increase confidence in the relevance of the in vitro test battery to the in vivo outcome. It is acknowledged that animal studies must interpreted with caution when extrapolating to humans based on differences in timing of neurodevelopmental processes, size and complexity of the nervous system and pharmacokinetics between species (Rodier, 1994; Rice and Barone, 2000). Still, there is a wealth of animal data concerning developmental neurotoxicity that should be considered, and animal studies in rodents and primates are currently the basis of many regulatory decisions. The present effort was undertaken to identify chemicals with data in the peer-reviewed literature demonstrating effects on neurodevelopment in vivo. This list of chemicals provides a starting point for selecting an expanded test set that would include both potential human and animal developmental neurotoxicants.

2. Definition and criteria for developmental neurotoxicity

Neurotoxicity is defined as an adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biologic agent (US EPA, 1998). For the purposes of this review, developmental neurotoxicity was defined as a change in the structure or function of the nervous system after exposure to a substance during the period of gestation and/or lactation. For rodent species, this would include the period of the brain growth spurt (Bayer et al., 1993; Rodier, 1994). Our definition of neurotoxicity was broad by intention, in order to encompass the range of endpoints reported in the literature. Evidence of neurotoxicity from literature and other reports was classified into three categories: behavior, morphology, or neurochemistry. Behavioral endpoints included neurobehavioral impairments (e.g., motor impairments, sensory changes, learning and memory, including I.Q. in humans) as well as changes in developmental landmarks (e.g., negative geotaxis, startle response, righting response). Morphological endpoints included gross structural changes (e.g., reduced brain weight, spina bifida, and exencephaly), brain pathology and morphometry (e.g., cell death, changes in neuron or glial numbers, loss of myelin, reduced cortical thickness). Neurochemical indices included changes in neurotransmitters and/or