Neurotoxicology and Teratology 52 (2015) 62–67

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Assessment of learning, memory and attention in developmental neurotoxicity regulatory studies: Introduction

Susan L. Makris [a], Charles V. Vorhees [b,*]

[a] U.S. Environmental Protection Agency, National Center for Environmental Assessment, 1200 Pennsylvania Ave., NW (8623P), Washington, DC 20460, United States
[b] Cincinnati Children's Research Foundation, University of Cincinnati College of Medicine, United States

ARTICLE INFO

Article history:
Received 19 December 2014
Received in revised form 28 May 2015
Accepted 29 May 2015
Available online 3 June 2015

Keywords:
Learning and memory testing
Developmental Neurotoxicity Testing
Regulatory studies
Environmental Protection Agency
Organization for Economic Cooperation and Development

ABSTRACT

There are a variety of chemicals, including pharmaceuticals, that alter neurobehavior following developmental exposure and guidelines for the conduct of studies to detect such effects by statute in the United States and Europe. Guidelines for Developmental Neurotoxicity Testing (DNT) studies issued by the U.S. Environmental Protection Agency (EPA) under prevailing law and European Organization for Economic Cooperation and Development (OECD) recommendations to member countries provide that such studies include a series of neurobehavioral and neuropathological assessments. Among these are assessment of cognitive function, specifically learning and memory. After reviewing 69 DNT studies submitted to the EPA, tests of learning and memory were noted to have detected the lowest observed adverse effect level (LOAELs) less frequently than behavioral tests of locomotor activity and acoustic/auditory startle, but slightly more than for the developmental Functional Observational Battery (devFOB; which is less extensive than the full FOB), but the reasons for the lower LOAEL detection rate for learning and memory assessment could not be determined. A major concern identified in the review, however, was the adequacy of the methods employed in these studies rather than on the importance of learning and memory to the proper assessment of brain function. Accordingly, a symposium was conducted to consider how the guidelines for tests of learning and memory might be improved. Four laboratories with established histories investigating the effects of chemical exposures during development on learning, memory, and attention, were invited to review the topic and offer recommendations, both theoretical and practical, on approaches to improve the assessment of these vital CNS functions. Reviewers were asked to recommend methods that are grounded in functional importance to CNS integrity, well-validated, reliable, and amenable to the context of regulatory studies as well as to basic research on the underlying processes they measure. This Introduction sets the stage for the reviews by providing the background and regulatory context for improved tests for learning and memory in DNT and other regulatory studies, such as single- or multi-generational studies where similar methods are incorporated.

1. Introduction

There are a variety of chemicals, including pharmaceuticals, that alter cognition following developmental exposure and may persist into adulthood. Effects of this nature have been identified in laboratory animals and humans. Perturbations in learning and memory can be sensitive biomarkers of alterations to the developing nervous system but only when the appropriate test paradigm is selected. Unfortunately, studies conducted in the past to detect the potential for cognitive alterations in developing laboratory animals for regulatory purposes have not been found to be as sensitive as other methods (Bushnell, 2014). First, we describe the current regulatory testing paradigm, discuss trends in developmental neurotoxicity screening and testing, and introduce four review papers that examine the question of how rodent cognitive assessments can be improved.

Developmental Neurotoxicity Testing (DNT) is conducted for chemical hazard assessment, including to support registration for some pesticides in accordance with 40 CFR Part 158 of the Federal Insecticide Fungicide Rodenticide Act (FIFRA) (U.S. Environmental Protection Agency, 2007) and guidance published by the Office of Pesticide Programs (OPP) (U.S. Environmental Protection Agency, 2013). Standard guideline protocols have been developed and refined for such studies (Makris et al., 2009). Both the U.S. Environmental Protection Agency (U.S. EPA) and the European International Organization for Economic Cooperation and Development (OECD) have published DNT guidelines, titled OPPTS 870.6300 (U.S. Environmental Protection Agency, 1998b) and OECD 426 (OECD, 2007), respectively. The EPA guideline was last revised in 1998; the OECD guideline incorporates some enhancements developed through discussion and international agreement prior to

* Corresponding author at: Cincinnati Children's Research Foundation, Division of Neurology, 3333 Burnet Avenue, Cincinnati, OH 45229, United States.
E-mail addresses: makris.susan@epa.gov (S.L. Makris), Charles.vorhees@cchmc.org (C.V. Vorhees).

http://dx.doi.org/10.1016/j.ntt.2015.05.010
0892-0362/

finalization in 2007 as OECD 426, i.e., extension of the offspring dosing period from PND (postnatal day) 11 to PND 21, consideration of the direct postnatal administration of the test substance to the offspring, required measures of behavioral ontogeny, and increased numbers of offspring assigned to neuropathology examination. Thus, OECD 426 represents the most updated version of the DNT study protocol. Studies conducted using harmonized test methods are mutually accepted by the U.S. EPA and OECD (OECD, 2008); therefore EPA considers OECD 426 acceptable to meet regulatory DNT testing requirements. It is described as follows.

A guideline DNT study (Fig. 1) is typically conducted in rats, although other species may be used if deemed more appropriate (e.g., due to toxicokinetics, species-related differential susceptibility or sensitivity, or known predictivity for human response). Pregnant rats are administered the test chemical from gestation day (GD) 6 through PND 21. Therefore, the offspring are exposed to the test substance via the maternal circulation and/or milk and subsequently through direct exposure, during in utero and preweaning development (a total of approximately 37 days). Detailed clinical observations are conducted (outside of the home cage) on approximately half of the dams in each group twice during gestation and twice during lactation, and maternal body weight is recorded at least weekly. The offspring are assessed for evidence of deficits in functional development. Litters are randomly standardized on PND 4 to yield four pups per sex per litter, and the pups are randomly assigned to testing subgroups for neurobehavioral and neuropathological assessment. End points that are evaluated between birth and approximately PND 60, when the offspring are young adults, include measures of physical development, reflex ontogeny, motor activity, motor function, sensory function, and learning and memory. Daily cage-side observations are conducted, and 10 pups/sex/treatment group are examined outside the cage in a modified Functional Observational Battery (devFOB) on PNDs 4, 11, 21, 35, 45, and 60. Pups are counted and weighed individually at birth, on PNDs 4, 11, 17, 21, and at regular intervals during the postweaning period. At a minimum, developmental landmarks recorded include the age of vaginal opening and balanopreputial separation. Motor activity is monitored at early time points and at termination; the EPA guideline specifies the use of an automated activity recording apparatus on postnatal days 13, 17, 21, and 60 (± 2). Tests of auditory startle habituation (preferably using prepulse inhibition) and associative learning and memory are performed on the offspring around the time of weaning (PND 21) and around PND 60. At an early time point (around PND 21) and at study termination, the offspring are subjected to neuropathological examination including morphometric analysis. One pup per sex per litter is killed on PND 21, and of these, 10 pups per sex per group are assigned to neuropathological evaluation. At study termination, all remaining offspring are killed; 10 rats per sex per group are prepared for neuropathological evaluation after perfusion fixation. Brain weight is recorded at both an early time point (around PND 21) and at study termination (around PND 60). Qualitative neuropathological examination is conducted for the control and high-dose groups, and if a treatment-related finding is evident, the low- and mid-dose groups are also examined. Guidance is provided regarding the regions of the brain to be examined and the types of alterations upon which to focus, particularly emphasizing structural changes. Simple morphometric analysis, performed on offspring killed at the early time point and at termination, is defined as consisting, at a minimum, of a reliable estimate of the thickness of major layers at representative locations within the neocortex, hippocampus, and cerebellum.

This study protocol provides some guidance regarding the scheduling and performance of offspring neurobehavioral and neuropathological testing. The least guidance is provided for cognitive testing performed around weaning (PND 21) and near study termination (PND 60). The guideline provides that learning and memory testing meet two criteria:

1. "Learning must be assessed either as a change across several repeated learning trials or sessions, or, in tests involving a single trial, with reference to a condition that controls for non-associative effects of the training experience."
2. "The tests should include some measure of memory (short-term or long-term) in addition to original learning (acquisition)." It is also noted that if a treatment-related effect is observed, it might be prudent to conduct additional tests to rule out alternative interpretations, which could be based on alterations in sensory, motivational, and/or motor capacities.

In addition, the guideline states that "the test of learning and memory be chosen on the basis of its demonstrated sensitivity to the class of compound under investigation, if such information is available in the

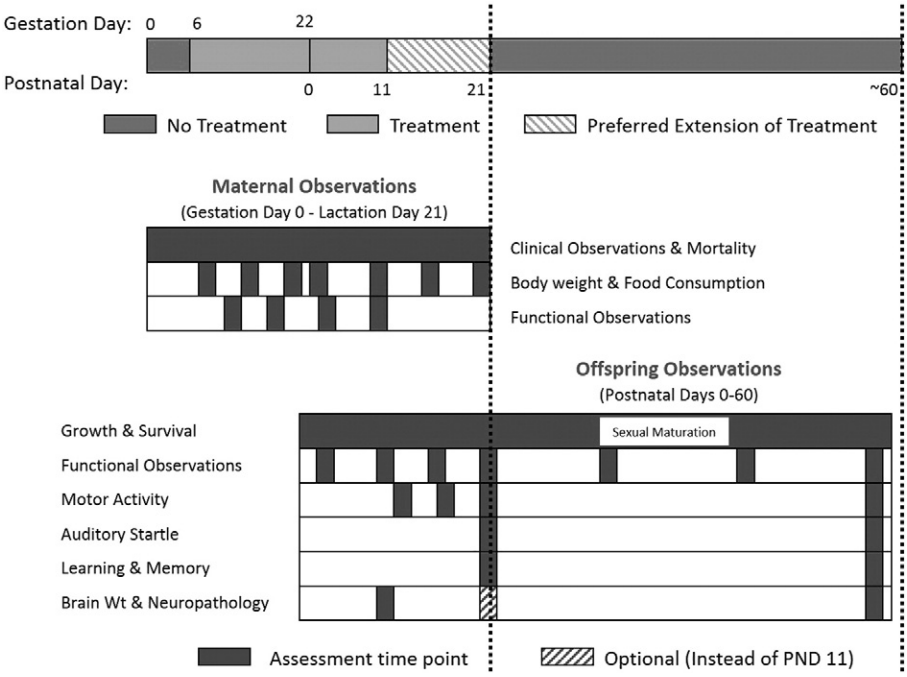

Fig. 1. Developmental Neurotoxicity (DNT) study — OPPTS 870.6300 (U.S. Environmental Protection Agency, 1998a,b) and OECD 426 (OECD, 2007).