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ScienceDirect
Neurotoxicology and Teratology 30 (2008) 349–381
NEUROTOXICOLOGY AND TERATOLOGY
www.elsevier.com/locate/neutera

Identification and interpretation of developmental neurotoxicity effects A report from the ILSI Research Foundation/Risk Science Institute expert working group on neurodevelopmental endpoints

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Received 5 January 2007; received in revised form 25 July 2007; accepted 29 July 2007
Available online 3 August 2007

Abstract

The reliable detection, measurement, and interpretation of treatment-related developmental neurotoxicity (DNT) effects depend on appropriate study design and execution, using scientifically established methodologies, with appropriate controls to minimize confounding factors. Appropriate statistical approaches should be optimized for the specific endpoints in advance, analyzing effects across time and functional domains as far as possible. If available, biomarkers of exposure are useful to assess the bioavailability of toxicants to the dam and offspring in utero and after birth. Finally, "weight of evidence" principles are used to aid assessment of the biological significance of differences from concurrent controls. These effects should be interpreted in light of available information from historical controls, positive controls, maternal and offspring systemic toxicity, and other relevant toxicological data. This review provides a framework for the integration of all these types of information in the interpretation of DNT studies.

Keywords: Developmental neurotoxicity testing; DNT; DNT treatment-related effects; Interpretation of treatment-related effects

1. Introduction

The development and maturation of the mammalian brain is an extremely complex process, spanning the period from conception through adolescence to young adult ages. The timing and rate of development differ by cell type, tissue layer, brain region, and species [96]. Brain development involves cell division, migration and differentiation, programmed cell death (apoptosis), cell-to-cell interactions (e.g., for migration and synaptic communication), and multiple other processes under different timetables for the various brain regions [97,98]. Genetic, epigenetic, and environmental factors (e.g., exposure to toxic chemicals, including certain heavy metals, industrial chemicals and pesticides), particularly during the susceptible periods of development and aging, can result in many possible adverse CNS consequences, ranging from mild to severe and involving various functions (e.g., cognition, motor or sensory dysfunction) [93].

Developmental neurotoxicity (DNT) refers to any adverse effect of perinatal exposure to a toxic substance on the normal development of nervous system structure and/or function [73,121]. For regulatory purposes, information from a variety of studies is used to assess the potential neurotoxic hazard and risk associated with exposure to a compound during the period

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doi:10.1016/j.ntt.2007.07.008

of nervous system development. To generate the appropriate types of information, one United States Federal (EPA) and one international (OECD TG 426, in draft) regulatory bodies have developed test guidelines for the conduct of DNT studies [79,121], which are discussed in Section 2 of this paper. In fact, the first formal DNT study, compliant with the early EPA draft guidelines, was published by Bates et al. in 1994 [6]. In parallel efforts, academic and other research centers have been developing methods/procedures for improving our understanding and evaluation of perturbations in mammalian neurodevelopment, which may be useful to the regulatory arena in the future [41]. Among the methodological developments that would be particularly useful for these purposes is an integrated assessment of functional (neurological, behavioral, and physiological) and neuropathologic changes using appropriate biomarkers of effect [10,21,44,120,125]. Neurochemical and genomic/proteomic/metabolomic changes are being considered as additional complementary biomarkers, not just of exposure but also of neurotoxicity that may signal underlying mechanistic events and enhance data interpretation and extrapolation to humans [3,4,27,105]. The EPA Office of the Inspector General recently commented on requiring DNT tests to protect children's health when evaluating pesticides [122]. There are advocates who believe that DNT tests provide valuable information on how pesticides affect development of the nervous system, information that might otherwise be overlooked [19,50,59,77,110]. In contrast, industry sources believe the test to be unnecessarily costly and difficult to interpret [58].

The goal of this manuscript is to provide guidance to researchers and reviewers of DNT studies on the interpretation of the data generated in these studies. Proper interpretation involves expert judgment that takes into account both the biological and statistical significance of the results. Data evaluation involves a series of considerations, including:

- adequacy of study design — to address the question(s) being asked;
- reliable conduct of study — use of appropriate controls and experimental procedures;
- reasonableness of baseline data — consistent with historical data and data profiles from other laboratories (e.g., published literature);
- analysis of findings for biological relevance; and
- evaluation of findings in the context of other available data.

Section 2 of this chapter addresses issues that need to be considered when planning and conducting a DNT study. Section 3 addresses the importance of understanding the benefits and limitations of the different tests used, given the biological processes involved, to evaluate the biological and statistical significance of the results. Information about the severity, duration, and reversibility of effects is an important component of these evaluations. The overall assessment of biological relevance of treatment-related effects must be carried out in the context of all available data; e.g., historical (untreated or vehicle) controls, positive controls, offspring systemic toxicity data, effects on offspring in relation to maternal toxicity, and any other toxicity data. Data interpretation ultimately requires the exercise of scientific judgment based on an appropriate background, including a knowledge-and experience-based expertise in developmental neurotoxicology. Case studies are presented to exemplify how developmental neurotoxicity data may be interpreted/used to assess dose-and time-effect relationships to establish no effect or threshold effect levels. In Section 4, recommendations are presented to highlight the principal issues to consider in the evaluation of DNT data.

2. Identification of treatment-related effects: impact of study design

Data should be collected using an appropriate study design and scientifically established methodologies, with appropriate controls to minimize confounding factors that may affect collection and interpretation of data [53]. The selected statistical approaches for the specific endpoints should be appropriate for the experimental design and be determined *a priori*.

2.1. Defining study objective(s) and design

All available information about the test chemical should be considered in defining clear study objective(s) and to help in formulation of the study design. Such information would include: (1) levels and routes of human exposure; (2) available toxicological profile; and (3) physicochemical and pharmaco/toxicokinetic properties of the test substance. For example, if human exposure occurs only via inhalation, the experimental data in animals should, if possible, also be collected via inhalation. Although, if good toxicokinetic data are available, it may be possible to use data from a study design that uses one route of exposure to extrapolate to risk from another route [33].

2.2. Selection of study design elements and conduct of study

Guidelines for developmental neurotoxicity studies have been generated to provide a general strategy for testing and to recommend certain specific criteria for various study design elements [79,121]. The occurrence, detection, and interpretation of adverse effects induced by any particular developmentally neurotoxic chemical may be influenced by a variety of basic study design elements in DNT studies as listed in Table 1. The following text will briefly discuss some of the more critical components.

2.2.1. Selection of animal model

Testing should routinely be performed in the rat. The EPA DNT testing guidelines recommend that the Fischer 344 strain not be used [121]. If the Fischer 344 rat or a mammalian species other than the rat is used, ample justification/reasoning should be provided. Among the multiple factors to be considered in the selection of the most appropriate experimental animal model are:

1. Pharmacokinetic profile — the availability of chemical-specific pharmacokinetic information in humans and laboratory animals could provide support for selection of appropriate animal species/strains.