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Undertaking positive control studies as part of developmental neurotoxicity testing A report from the ILSI Research Foundation/Risk Science Institute expert working group on neurodevelopmental endpoints

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Abstract

Developmental neurotoxicity testing involves functional and neurohistological assessments in offspring during and following maternal and/or neonatal exposure. Data from positive control studies are an integral component in developmental neurotoxicity risk assessments. Positive control data are crucial for evaluating a laboratory's capability to detect chemical-induced changes in measured endpoints. Positive control data are also valuable in a weight-of-evidence approach to help determine the biological significance of results and provide confidence in negative results from developmental neurotoxicity (DNT) studies. This review is a practical guide for the selection and use of positive control agents in developmental neurotoxicology. The advantages and disadvantages of various positive control agents are discussed for the endpoints in developmental neurotoxicity studies. Design issues specific to positive control studies in developmental neurotoxicity are considered and recommendations on how to interpret and report positive control data are made. Positive control studies should be conducted as an integral component of the incorporation and use of developmental neurotoxicity testing methods in laboratories that generate data used in risk decisions.
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1. Introduction

Numerous groups have advocated the use of positive controls in neurotoxicity screening [3,34,65,76,91,97,108,129,138,199,245]. Positive control studies are one of many tools used to demonstrate the proficiency of a laboratory, and in this review, it is assumed that other tools necessary to calibrate and validate equipment and test methods have already been utilized [34,43,91,130,193,213,221,230]. The importance of positive control studies in neurotoxicology cannot be overstated. While most neurotoxicological tests used in toxicology and pharmacology have undergone extensive validation [167], this does not necessarily translate into proper implementation (i.e., installation and use) in all test laboratories. This review provides guidance for undertaking these positive control studies for developmental neurotoxicology.

The developmental neurotoxicity (DNT) guideline [215] requires that "positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used" are provided by the test laboratory. These recommendations have been clarified in discussions for the USEPA Office of Pesticide Program's data call-in for DNT testing on food-use organophosphate pesticides as follows: "Positive control data from the laboratories that performed the DNT studies should be provided to the Agency at the time of

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study submission. These positive control data should demonstrate the sensitivity of the procedures used, including, for some measures, the ability to detect both increases and decreases in measured parameters. While the positive control studies do not need to be performed using prenatal exposures, the laboratory must demonstrate competence in the evaluation of effects in neonatal animals perinatally exposed to chemicals and establish test norms for all critical endpoints, and for appropriate age groups. The positive control data should be derived from relatively recent studies, that is, studies that were performed in the same laboratory within the past few years, utilizing (to the greatest extent possible) the staff and equipment that will be used in conducting the current studies." [216].

Positive controls are classically used in a toxicological context as reference standards that can serve multiple purposes. Maurissen and Marable [138] state that complementary data (e.g., historical and positive controls, parametric proficiency data) can aid in the interpretation of the biological significance of an effect (see also [79,107]). Positive controls are a tool used to help determine the relative proficiency of a testing laboratory in detecting chemical-induced changes in the measured endpoint, and serve as reference standards for the measured endpoint [43]. They help demonstrate the dynamic range of a biological response beyond that observed in control animals, create a realistic environment to verify training proficiency of laboratory personnel, and characterize inter-observer, intra-laboratory, and inter-laboratory reliability [24,25,40,43,63,64,72,154,155,168,181,199,221]. Positive control data, along with historical control and other proficiency data, are often used to establish confidence in test results from new laboratories or help interpret data from previously untested chemicals [43,57,79,89,107,130,138,139,180]. Characterizing the effects of positive controls in animals provides valuable information on normal variability in animals [24,40,43,58,63,132,138,154,168,219,221,227,228]. In addition, such testing provides valuable knowledge and experience on how to correctly perform the test.

A recent survey of DNT studies submitted to the USEPA revealed a number of deficiencies in positive control studies [43]. These deficiencies included problems in study design, data reporting and report structure (Table 1). This review, therefore, provides a context for the use, interpretation, and reporting of positive control studies for regulatory developmental neurotoxicity testing. Guidance is given on how to select and use positive controls and other issues specific to the design of positive control studies. Data tables provide summaries of the advantages and disadvantages of different chemicals when used as positive controls for different endpoints in developmental neurotoxicity studies. Differences between acute age-relevant studies and full developmental studies are discussed. Recommendations on proper and efficient reporting of positive control data are made. It is important to note that there is no single positive control chemical that can demonstrate changes in every DNT endpoint, and positive control studies are just one tool that helps demonstrate proficiency. They also can insure proper use and biological calibration of test equipment. This will likely reduce the use of animals by insuring that testing methods are used in an accurate and effective fashion. The use of positive control studies requires significant use of animals and concerns for animal welfare are discussed.

Table 1
Reported deficiencies in positive control studies for developmental neurotoxicology

Experimental design
Use of only one gender
Dissimilar ages at time of testing
Small or inadequate group size
Lack of dose–response
Lack of positive control
Use of inappropriate positive controls
Results
Lack of effect of positive controls
Report structure
Lack of either summary or individual data
No statistical analyses
Inadequate methodological details
Age of data
Absence of quality assurance review

Adapted from [43].

2. Design issues

The DNT guideline [215] requires that "positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used" are provided by the test laboratory. Since the purpose of requiring positive control data is to demonstrate laboratory proficiency and estimate the sensitivity of the procedures being used to detect changes in the structure and function of the developing nervous system, the design (including equipment parameters and settings, time and order of testing, and data collection techniques) should track and be consistent with the DNT design (Table 2). Thus, age of testing, gender, and number of animals/group should be equivalent to those in the DNT guideline. Since DNT testing is performed using both sexes, and sex-related differences in responses to neuroactive substances [30,86,142,200], are often observed, positive control studies should be performed in both sexes. Also as recommended by the DNT guideline, when performing observational assessments, the observer should be unaware of the treatment of the animal [215]. Current guidance for DNT testing does not require the use of developmentally-exposed animals, but allows use of appropriately-aged, acutely-exposed animals, using the same procedures used in the DNT. Most data submitted to the USEPA have used this latter approach [43].

The design of studies using positive controls may involve one of three approaches: 1) a design similar to that used in DNT studies with developmental exposure to the positive control agent; 2) evaluation of the individual test parameters using animals similar in age to those recommended in the guideline (age-relevant); or, 3) evaluations of test parameters using adult age animals (Table 3). The advantages and disadvantages for these three approaches are discussed below.

Positive control studies designed similar to a formal DNT study have many advantages, including: use of animals with similar handling experiences, testing of animals following developmental exposure, and testing animals at the same age