Available online at www.sciencedirect.com
SCIENCE DIRECT®
Regulatory Toxicology and Pharmacology 43 (2005) 55–65
Regulatory Toxicology and Pharmacology
www.elsevier.com/locate/yrtph

Use of benchmark dose and meta-analysis to determine the most sensitive endpoint for risk assessment for dimethoate

Richard Reiss *, David Gaylor

Sciences International, Inc., 1800 Diagonal Road, Suite 500, Alexandria, VA 22304, USA

Received 5 April 2005
Available online 15 August 2005

Abstract

A meta-analysis of several rat toxicity studies for dimethoate was conducted to determine the most sensitive endpoint for use in risk assessment. The analysis was motivated by a recent developmental neurotoxicity (DNT) study, which identified the same no observed adverse effect level (NOAEL) for pup mortality and cholinesterase inhibition. The pup mortality NOAEL was lower than that determined in a range-finding study for the DNT and other reproduction studies, and was highly influenced by a single total litter loss in the middle dose group, which made interpretation difficult. First, a meta-analysis was conducted of four recent studies by gavage dosing with very similar designs, including the DNT. Benchmark dose (BMD) modeling was used to determine the appropriate point of departure for regulatory purposes, the lower limit of the BMD for a 5% incidence for pup mortality ($BMDL_5$) and the lower limit of a 10% inhibition of brain cholinesterase ($BMDL^*_{10}$), the asterisk denotes that the BMD is based on continuous response variable as opposed to an incidence level. For pup mortality, the $BMDL_5$ for post-natal days (PND) 1–4 was 0.64 mg/kg/day. For cholinesterase inhibition, the lowest $BMDL^*_{10}$ was 0.19 mg/kg/day for the dams at gestation day 20. These results show that the regulatory point-of-departure for cholinesterase inhibition is more than threefold lower than pup mortality. Thus, risk assessments protecting against cholinesterase inhibition are likely to also be protective of pup mortality. In addition, cholinesterase inhibition and pup mortality were evaluated in two 2-generation reproduction studies by dietary exposure. Also, cholinesterase inhibition was evaluated in a 28-day dietary study. Dietary exposure is more relevant than gavage exposures for many human risk assessment scenarios. There was no consistent pup mortality at the highest doses of the two 2-generation dietary studies (6.0 and 6.5 mg/kg/day). The average BMD_{10}s for brain cholinesterase inhibition for the 2-generation studies was 0.65 mg/kg/day, with a range of 0.49–0.96 mg/kg/day. This suggests that cholinesterase inhibition is at least a 10-fold more sensitive endpoint than pup mortality for dietary exposures. For the 28-day dietary study, the BMD_{10} for brain cholinesterase inhibition was 1.1 mg/kg/day for males and 0.70 mg/kg/day for females. The exposure duration in the 28-day dietary study is closest to the durations in the gavage studies. Compared to the dams in the gavage studies, which had a $BMDL_{10}$ of 0.19 mg/kg/day, the animals were more than threefold more sensitive to cholinesterase inhibition by gavage compared to dietary exposure.

Keywords: Dimethoate; Insecticide; Benchmark dose; Cholinesterase; Developmental effects; Meta-analysis

1. Introduction

Dimethoate (*O*,*O*-dimethyl *S*-[*N*-methylcarbamoylmethyl]phosphorodithioate) is an organophosphate insecticide with numerous uses on field and agricultural crops and ornamentals. Like most organophosphates, dimethoate has been traditionally regulated based on its potential to cause inhibition of the acetylcholinesterase enzyme (Mileson et al., 1998). However, a recent developmental neurotoxicity study (DNT) with gavage exposure identified the same no observed adverse effect level (NOAEL) for pup mortality and cholinesterase inhibition (Myers, 2001a). The result in this study was highly

* Corresponding author. Fax: +1 703 684 2223.
E-mail address: rreiss@sciences.com (R. Reiss).

0273-2300/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2005.06.012

influenced by a single total litter loss at the middle dose group. The mortality at the middle dose group was not statistically significant. Nonetheless, these results raised questions about whether cholinesterase inhibition or pup mortality is the most sensitive endpoint on which to base a risk assessment for dimethoate, and whether dosing by gavage was a factor in the DNT study results. To address these questions, the extensive database of cholinesterase inhibition and reproductive and developmental tests for dimethoate were analyzed in a meta-analysis to determine the most appropriate endpoint for dimethoate risk assessment. First, the DNT data and several other studies with gavage exposure were analyzed in a meta-analysis using benchmark dose (BMD) modeling to ascertain the most sensitive endpoint by gavage exposure. In addition, studies assessing reproduction and cholinesterase inhibition by dietary exposure were analyzed to ascertain the difference in the relative sensitivity of cholinesterase inhibition and pup mortality by the dietary exposure route.

2. Materials and methods

2.1. Toxicity Studies

The analysis in this article relies on studies conducted by dimethoate registrants in the US and Europe. There were four separate, but related, gavage studies. These studies were conducted according to international Good Laboratory Practice (GLP) standards, audited, and then submitted to the US EPA in support of the reregistration of dimethoate. The US EPA prepared summaries and evaluations of the studies which are available on the EPA website. The principal study was the DNT, and the other three studies followed a similar design. In the DNT, dimethoate was administered to 24 parent female Crl:CD BR rats by gavage dosing at dose levels of 0, 0.1, 0.5, and 3.0 mg/kg/day (Myers, 2001a). The exposure began on gestation day (GD) 6 and continued through postnatal day (PND) 10. The offspring were also exposed through gavage dosing at the same levels as the dams from PND 4 to 10. The study included functional observation battery (FOB) tests on the dams. For the offspring, the testing included FOB, automated motor activity, auditory startle response, and assessment of learning and memory (Morris Water Maze). For these tests, there were either no effects with dosage or possible effects at the highest dosage. Therefore, the focus of the analysis is on the pup mortality data. The DNT was conducted under US EPA Test Guideline OPPTS 870.6300 (US EPA, 1998a).

In addition to the DNT, there were three other studies conducted with virtually identical designs with the same types of rats, and were done in the same laboratory during similar time periods. First, there was a companion cholinesterase study, which included cholinesterase measurements and pup mortality data (Myers, 2001b). The dose groups in the companion cholinesterase study were identical to the main DNT study. Plasma, red blood cell (RBC), and brain cholinesterase measurements were made for dams on GD20, for pups on PND4, PND11, PND21, and PND60, and for adult animals on days 1 and 11 of treatment. The cholinesterase analysis for all of the studies (including the dietary studies discussed below), was conducted with a modified Ellman method (US EPA, 1996). Although, each study separately measured plasma, RBC, and brain cholinesterase, dimethoate is regulated with the brain cholinesterase inhibition endpoint. Therefore, only the brain cholinesterase data are considered herein.

There is also a range-finding study to the DNT, which included both cholinesterase and pup mortality measurements (Myers, 2001c). The range-finding study had the following dose groups: 0, 0.2, 3.0, and 6.0 mg/kg/day. Finally, a cross-fostering study was conducted to further investigate the cause of the pup mortality (i.e., from exposure to the dams during gestation or exposure to the dams or pups during lactation) (Myers, 2004). There were six dose groups in the cross-fostering study that included varying dosages to the birth dam and the dam that raised the pups. However, the meta-analysis only relied on the two groups in the study that were not cross-fostered, including the control group and the 6 mg/kg/day dose group, because these are the only groups that can be considered replicate data with the other studies.

Additionally, there are two dimethoate 2-generation reproduction studies with dietary exposure. The first study (Brooker and Stubbs, 1992) was conducted under US EPA Test Guidelines OPPTS 870.3800 (US EPA, 1998b, most recent version). Dimethoate was administered in the diet to Crl:CD BR rats at 0, 0.09, 1.3, and 6.0 mg/kg/day. The F0 animals (24/sex/group) were treated for 10 weeks prior to the first mating. The F1 generation was selected from the F1A litters (24/sex/group) and was first mated at week 16. There was also a second mating of the F1A generation, followed by a partial third mating involving animals which had not been successful at either of their first two pairings.

The second 2-generation reproduction study (Mellert et al., 2003) was conducted under OECD Guideline no. 416 (OECD, 1983) and US EPA Test Guidelines OPPTS 870.3800 (US EPA, 1998b). Dimethoate was administered via the diet to Wistar rats (CrlGlxBrlHan:WI) at 0, 0.2, 1.0, and 6.5 mg/kg/day. The F0 animals (25/sex/group) were treated for at least 10 weeks prior to the first mating. The F0 animals were mated to produce a first litter (F1A) and subsequently remated (after about 20 additional weeks of treatment) to produce a second litter (F1B). A group from the F1A generation (25/sex/group) was selected as the F1 parental generation and were