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Dimethoate:

Issues Related to the Hazard and Dose Response Assessment

November 2, 2004

Office of Prevention, Pesticides & Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C. 20460

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ChE	Cholinesterase
DCI	Data call-in (refers to notice)
DER	Data evaluation record
DNT	Developmental neurotoxicity
FOB	Functional observational battery
FQPA	Food Quality Protection Act
FRN	Federal register notice
GD	Gestation day
LOAEL	Lowest-Observed-Adverse-Effect Level
NOAEL	No-Observed-Adverse-Effect Level
OP	Organophosphate pesticide
OPP	Office of Pesticide Programs
PMRA	Pest Management Regulatory Agency (refers to Canada)
PND	Post-natal day
RBC	Red blood cells
SAP	Scientific Advisory Panel

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

LIST OF APPENDICES

Appendix 1
E-file name: 035001ha.003.wpd
DIMETHOATE: 2nd Report of the Hazard Identification Assessment Review Committee. Paul Chin. March 26, 2002.

Appendix 2
E-file name: 45529703.der.wpd
DATA EVALUATION RECORD. DIMETHOATE/035001. STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300. MRID 45529703. EPA Reviewer: K. Raffaele. January 14, 2002.

Appendix 3
E-file name: 45529701.der.wpd
DATA EVALUATION RECORD. DIMETHOATE. Study Type: DOSE-FINDING DEVELOPMENTAL NEUROTOXICITY [NON-GUIDELINE] MRID 45529701. EPA Reviewer: K. Raffaele. January 14, 2002.

Appendix 4
E-file name: 45529702.der.wpd
DATA EVALUATION RECORD. DIMETHOATE. Study Type: SPECIAL STUDY, CHOLINESTERASE INHIBITION [NON-GUIDELINE]. MRID 45529702. EPA Reviewer: K. Raffaele. January 18, 2002.

Appendix 5
E-file name: D273221.me2.wpd
D273221: Dimethoate (035001). Review of Data on Developmental Neurotoxicity Based on: a 6(a) 2 Report; Preliminary Data Submissions from a Range Finding Study (CHV/068), a Developmental Neurotoxicity Study (CHV/069), and a Cholinesterase Study (CHV/070); and a Data Audit of these 3 Studies. Kathleen Raffaele and William F. Sette. March 22, 2001.

Appendix 6
E-file name: 46214501.der.wpd
Cross Fostering Study (Non Guideline) - Rat (MRID 46214501). Elissa Reaves and Susan Makris. June 24, 2004.

Appendix 7
E-file name: 035001_0013000_030393_TX010065_R014928.tif
EPA ID# 035001: Dimethoate - Review of Reproductive Toxicity in Rats. Paul Chin. March 3, 1993.

Appendix 8
E-file name: dimethoate_appendix8_final.pdf
BMD Analysis of Pup Death Mortality Data

Appendix 9
E-file name: dimethoate_appendix9_final.pdf
BMD Analysis of Brain Cholinesterase Data

Appendix 10
E-file name: 46181001.der.2-gen repro.wpd
DATA EVALUATION RECORD. MRID 46181001. STUDY TYPE: §83-4; Multigeneration Reproduction Study in Rats.

Appendix 11
E-file name: 46348201.der.1-gen repro.wpd
DATA EVALUATION RECORD. MRID 46348201. STUDY TYPE: Non-guideline; Range-finding One-generation Reproduction Study in Rats

Appendix 12
E-file name: omethoate_reproductivetox.wpd
Results of Reproductive Toxicity Studies with Omethoate

Appendix 13
E-file name: Meta analysis report-v1 of 2.pdf AND Meta analysis report-v2 of 2.pdf
A Meta Analysis of Pup Death and Cholinesterase Inhibition Data for Dimethoate. September 1, 2004.

Appendix 14
E-file name: 46288001.der.28-day oral tox in rats.wpd
DATA EVALUATION RECORD. MRID 46288001. STUDY TYPE: Repeated Dose (28-day) Oral Toxicity Study in Rats

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

PREFACE

A Scientific Advisory Panel (SAP) meeting to discuss issues related to the hazard assessment for dimethoate was originally scheduled for July 29-30, 2004. Shortly before the meeting, EPA became aware of additional information that could impact the issues scheduled for discussion. This information included three additional toxicity studies with dimethoate (two reproductive toxicity studies and a 28-day dietary toxicity study) and additional data analyses conducted by the registrant [Cheminova's Position Concerning the Appropriate Toxicological Endpoints for the Regulation of Dimethoate (MRID 46245901) and A Meta Analysis of Pup Death and Cholinesterase Inhibition Data for Dimethoate, September 1, 2004 (MRID 46386001, Appendix 13)]. Following receipt of this information, EPA conducted additional data analyses, including benchmark dose (BMD) modeling, to further evaluate the dose-response relationships for cholinesterase (ChE) inhibition and pup mortality following exposure to dimethoate. These analyses resulted in different interpretations from the July 6, 2004 EPA document, in particular the new BMD analyses supported the selection of brain ChE inhibition as an appropriate endpoint for all exposure scenarios in the dimethoate risk assessment. Given this new analysis, the availability of a 28-day dermal toxicity study on dimethoate, which included brain ChE measurements, obviates the need for route-to-route extrapolation. Therefore, the dermal absorption discussion, originally in the July 6, 2004 EPA document, is not included. The results of the analyses and the additional data have been incorporated into the revised background documents and materials made available for the current SAP meeting.

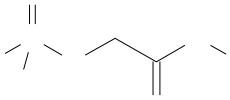
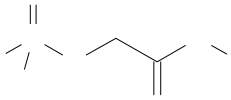
US EPA ARCHIVE DOCUMENT

I. BACKGROUND

A. Introduction

The Food Quality Protection Act of 1996 requires EPA to reassess all previously approved pesticide tolerances by August 2006. As part of the reassessment process, EPA's Office of Pesticide Programs (OPP) is developing risk assessments for each of the individual organophosphate (OP) pesticides, including dimethoate, in addition to a cumulative risk assessment for the OP common mechanism group (EPA, 2002). The primary mode of toxic action for OPs is inhibition of acetylcholinesterase through phosphorylation of the enzyme active site. This inhibition leads to accumulation of acetylcholine and results in cholinergic toxicity due to continuous stimulation of cholinergic receptors throughout the central and peripheral nervous systems. Some OPs require activation *in vivo* to the oxon metabolite prior to the ChE inhibition. In the case of dimethoate, the oxon metabolite is called omethoate. Although not registered for use in the US or Canada, omethoate is registered as a pesticide in some other countries. Dimethoate is used to control a wide variety of insect pests on a range of foods, feeds, ornamentals, and non-crop areas. Potential sources of exposure to dimethoate are food, drinking water, and occupational exposures (dermal and inhalation).

Figure 1: Chemical structures of Dimethoate and Omethoate

Dimethoate	**Omethoate**
	

The present document was developed jointly between the U.S. EPA and Canada's Pest Management Regulatory Agency (PMRA). The purpose of this document is to discuss specific scientific issues related to the dimethoate hazard and dose-response assessment. These issues relate to data evaluating *developmental neurotoxicity* (DNT) which have become available to EPA and PMRA since the 1999 release of EPA's Preliminary Human Health Risk Assessment for Dimethoate. Several key studies characterizing DNT for dimethoate have become available. These include: a development neurotoxicity (DNT; OPPTS 870.6300) study, a comparative ChE study on dimethoate, and a cross-fostering study.

B. Interpretation of the Dimethoate Developmental Neurotoxicity and Related Studies

In 1999, EPA issued a data call in (DCI) notice [Federal Register Notice (FRN) October 5, 1999 OPP-34192 FRL 6097-9] requiring DNT studies to be submitted for all registered OP pesticides. In addition to the information collected in the standard guideline study (which includes behavioral and neuropathological assessments of offspring following exposure of dams during gestation and early lactation), the DCI included several additional requirements: (1) that exposure be continued throughout lactation (through post-natal day 21); (2) that adequacy of exposure to pups be considered; and (3) that studies specifically evaluate the relative, or comparative, sensitivity of pups and adults to ChE inhibition.

Following discussions with the EPA regarding the protocol, the DNT study for dimethoate was initiated in October, 2000. On January 3, 2001, Cheminova (registrant for dimethoate), notified the EPA of unanticipated adverse effects in the DNT study for dimethoate (L000608). Specifically, an apparent increase in the number of deaths in young pups when the dams were exposed to dimethoate during gestation and early lactation was observed. Following a complete review of data from the DNT, range-finding, and comparative ChE studies, including an on-site audit of the performing laboratory, the no-observed-adverse-effect-level (NOAEL) for offspring in the main DNT study was determined by EPA and PMRA to be 0.1 mg/kg/day, with a lowest-observed-adverse-effect-level (LOAEL) of 0.5 mg/kg/day, based on increased pup death and increases in motor activity. In the companion ChE study, the NOAEL for ChE inhibition was 0.5 mg/kg following acute administration and 0.1 mg/kg/day following repeated administration; similar inhibition levels were seen in adults and young animals and there were no differences in the NOAELs among age groups. Because these effects occurred at doses lower than effects seen in previous studies, the endpoints from the DNT study were used in the revised dimethoate hazard assessment (Appendix 1).

Section II of this document discusses the results of the DNT, comparative ChE inhibition, and cross-fostering studies. EPA and PMRA are seeking comment from the SAP on key aspects of these studies, namely the interpretation of available pup mortality data, the possible impact of maternal toxicity and pre-natal and post-natal dam exposures on pup mortality, and the interpretation of a benchmark dose analysis for brain ChE inhibition and pup mortality data.

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II. DIMETHOATE DNT STUDY AND RELATED CHOLINESTERASE AND CROSS-FOSTERING STUDIES

A. Introduction

Several studies that characterize DNT and/or potential maternal neglect are available for dimethoate. Along with the main DNT study, a range-finding study, and a companion, comparative ChE inhibition study were submitted. Several reproductive toxicity studies are also available for dimethoate (2 full studies and one range-finding study), and the registrant has recently performed a cross-fostering study to provide further information regarding the relative contribution of pre- and post-natal maternal exposure to post-natal pup mortality. The effects observed in these studies are key to the hazard characterization of dimethoate. The design of the DNT, the range-finding, and comparative ChE inhibition studies, in addition to the cross-fostering study are described in Section B below. Section C contains discussion of results related to pup mortality along with the potential cause. Full data evaluation reviews (DERs) for each study are provided in Appendices 2-7, 14, 15, and 18.

B. Summary of Study Design for Relevant Data Sources

1. Developmental Neurotoxicity Study, Companion Cholinesterase Study, and Range-finding study

The DNT study guideline (OPPTS 870.6300) is designed to evaluate potential neurotoxic effects in offspring following developmental exposure to pesticides or other toxic substances. Briefly, dams are exposed to a test substance starting 6 days after mating and continuing until 11 or 21 days after birth of the young (post-natal day [PND] 11 or 21). Birth and development of the offspring are monitored (including litter size, survival, body weight, clinical signs, etc.). Offspring are also evaluated behaviorally, using specific tests of neurological function (including detailed observations, motor activity, auditory startle habituation, and learning and memory testing), at several time points during and after weaning. Neuropathological evaluations (including brain weights and measurements as well as histopathology) are conducted at two time points (PND 11 or 21, and as adults).

a. Range-finding Study

Prior to the start of the main DNT study, a range-finding study was conducted to determine appropriate dose levels for use in the main study (MRID 45529701, Appendix 3). The range-finding study used the same exposure regimen used in the main study. Dams (8-10/dose) were dosed from gestation day (GD) 6 through PND 10 at 0, 0.2, 3.0, and 6.0 mg/kg/day, via gavage. Pups were potentially exposed *in utero* starting on GD 6 and via milk through lactation day 10. Pups were dosed directly, using the same doses (on a mg/kg basis) administered to their dams, from PND 11 until weaning on PND 21. Reproductive parameters were collected as in the main study. ChE inhibition was evaluated at GD 20 in dams and fetuses, and at PND 21 in pups only. Post-dose sampling times for purposes of ChE inhibition measurements were the same as those used in the comparative ChE study (see below).

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b. Main DNT Study

In the main DNT study (MRID 45529703, Appendix 2), dams (23-24/dose) were dosed from gestation day (GD) 6 through post-natal day (PND) 10 at 0, 0.1, 0.5, or 3.0 mg/kg/day, via gavage. Pups were potentially exposed *in utero* starting on GD 6 and via milk through lactation day 10. Pups were dosed directly, using the same doses (on a mg/kg basis) administered to their dams, from PND 11 until weaning on PND 21 (Fig. 2, DNT). All parameters detailed in the guideline were evaluated in the main DNT study, including reproductive data, pup development and survival, and behavioral and neuropathological evaluations.

Figure 2

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c. Comparative Cholinesterase Study

ChE activity levels (in whole brain, plasma, and red blood cells [RBC]) were measured in a companion study (MRID 45529702) to the main DNT study, using the same dosing and exposure regimen as the main study (DER Appendix 4) with 8-10 dams/dose. ChE activity was evaluated at several time points: in fetuses and dams on GD 20, and in pups on PNDs 4, 21, and 60. In addition, to evaluate ChE inhibition following a single exposure, pups from a satellite group of unexposed dams received a single oral dose of dimethoate on PND 11, at the same doses used in the DNT study (Fig. 3, ChE). To enable comparison of pup versus adult sensitivity following a similar dosing regimen, ChE inhibition was also assessed in a group of adult males and females following a single dose or 10 repeated doses of dimethoate, at the same doses received by pups in the DNT study.

The study report stated that times for assessment were chosen based on previously available data for time to peak effect for adult rat behavioral observations. Sampling times were:

GD 20 (fetuses and dams):	3 h post-dosing
PND 4 (pups):	4 h after dosing to dams
PND 11 (pups):	2 h post-dosing
PND 21 (pups):	2 h post-dosing
PND 60 (adult offspring):	39 days post-dosing
Day 1 (adults):	2 h post-dosing
Day 11 (adults):	2 h post-dosing

Figure 3

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2. Cross-fostering Study

To obtain further information regarding the relative contribution of pre- and post-natal maternal exposure to post-natal pup mortality, a cross-fostering study (MRID 46214501, DER Appendix 6) was conducted by the registrant. Cross-fostering allows maternal animals to rear unrelated offspring, allowing separate assessment of the effects of pre-natal or post-natal exposure to pups. In this study, dimethoate was administered to dams at doses of 0, 3, or 6 mg/kg, from GD 6 through PND 11, in a regimen similar to that used in the main DNT study. On PND 1, pups from dams treated with dimethoate at 3 or 6 mg/kg were cross-fostered to control dams, and control pups were cross-fostered to dimethoate-treated dams; pups from two additional groups (control or 6 mg/kg dams) were not cross-fostered. Thus, half of the litters containing 12 or more pups from dams receiving 0 and 6 mg/kg/day were cross-fostered to different dose groups whereas all of the litters containing 12 or more pups from dams receiving 3 mg/kg/day were cross-fostered. Detailed behavioral observations of pups and dams were conducted at several time points during gestation and lactation. At PND 11, surviving pups and dams were sacrificed and a number of clinical chemistry and hematological parameters were measured. ChE inhibition was not evaluated in the cross-fostering study.

3. Reproductive Toxicity Studies

In a reproductive toxicity study, parental (F0 generation) animals are continuously exposed for 6-8 weeks before mating, and during mating, gestation, and lactation, typically in feed or drinking water. First generation offspring (F1 generation) are exposed *in utero* followed by exposure before mating, during mating, gestation, and lactation typically in feed or drinking water. Second generation offspring (F2 pups) are exposed *in utero* and during lactation. Reproductive parameters, including litter size and pup survival, are evaluated. Some ChE inhibition data are also available from the reproductive toxicity studies for dimethoate.

4. Benchmark Dose Analyses

Benchmark dose modeling offers an alternative approach to the use of NOAELs and LOAELs as points of departure for purposes of developing risk assessments (USEPA, 2000). In benchmark dose (BMD) modeling, mathematical and statistical techniques are used to estimate the dose at which a defined level of response (benchmark response, or BMR) is expected to occur. Typically, the lower limit on the BMD (BMDL) is also calculated and reported. As discussed in EPA's Draft Benchmark Dose Technical Guidance (2000), a lower confidence limit is placed on the BMD to obtain a dose (BMDL) that assures with high confidence (*e.g.*,

95%) that the BMR is not exceeded. In cases where data are sufficiently robust to support an analysis, BMD modeling is preferred over the use of NOAELs and LOAELs since NOAELs and LOAELs do not necessarily reflect the relationship between dose and response for a given chemical but instead reflect the dose levels selected for testing. In a recent submission, the registrant for dimethoate has performed several BMD analyses of ChE inhibition and pup mortality in available studies for dimethoate (see Appendix 17). EPA and PMRA have also conducted several BMD analyses for these data; the results of these analyses are discussed below.

US EPA ARCHIVE DOCUMENT

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C. Results

Findings from the main DNT study, comparative ChE and range-finding studies, and a cross-fostering study, as well as relevant reproductive toxicity studies, are briefly described below. Results of the BMD analyses of brain ChE inhibition and pup mortality are also provided.

1. Brain Cholinesterase Inhibition Data

Following exposure to dimethoate, inhibition of brain ChE most often occurs at doses similar to or below those causing ChE inhibition in the blood compartments (see Appendix 1, DIMETHOATE: 2nd Report of the Hazard Identification Assessment Review Committee). Results from the DNT companion ChE study are consistent with this pattern: for all ages, brain ChE inhibition was seen at or below doses causing inhibition in other compartments (see Appendix 4, DER for companion ChE study). This document will focus on analysis and interpretation of brain ChE measurements from the comparative ChE and the DNT range-finding studies. ChE inhibition data for plasma and red blood cells (RBC) are provided in Appendix 4.

As seen in Table 1, statistically significant decreases in brain ChE activity of 12-18% have been observed following acute (single dose) exposures to 3.0 mg/kg dimethoate in pups and adults. Following acute exposures at the dose of 0.5 mg/kg, consistent, but small, decreases in brain ChE activity of 2-4% were observed in pups and adults. Although the decreases at 0.5 mg/kg following single exposures were statistically significant for two treatment groups (adult and PND11 males), they were not of sufficient magnitude to be considered toxicologically significant.

Following multiple exposures to 3.0 mg/kg in the comparative ChE and range-finding studies, statistically significant decreases in brain ChE activity of 22-75% were observed in GD 20 dams, PND 21 offspring, and day 11 adults (Tables 1 and 2). Statistically significant decreases in brain ChE of 6-13% were consistently observed at 0.5 mg/kg/day across all groups of pups and adults. Results observed at the lowest dose of 0.1 mg/kg/day following repeated exposures were less consistent, ranging from increases of 4% to decreases of 12%, when compared to control values, with no clear pattern of effect across age or sex (Table 1).

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Table 1. Brain Cholinesterase Activity in Adults, Fetuses, and Offspring of Rats Treated with Dimethoate in Comparative ChE Inhibition Study.

Dose (mg/kg/day)	Brain Cholinesterase Activity (μ/kg)[a]				Benchmark Doses[g] (mg/kg/day)	
	0.0	0.1	0.5	3.0	BMD_{10}	$BMDL_{10}$
Acute Exposures (n=8/sex/group)						
Adult Males	13,794 ± 247	13,544 ± 802 (2)[g]	13,294* ± 241 (4)	12,131** ± 1096 (12)	2.6	2.0
Adult Females	14,150 ± 555	13,625 ± 445 (4)	13,850 ± 687 (2)	12,106** ± 827 (14)	2.2	1.8
PND 11 Males	6475 ± 244	6363 ± 236 (2)	6144* ± 360 (5)	5375** ± 290 (17)	1.8	1.5
PND 11 Females	6256 ± 195	6350 ± 338 (-2)	6125 ± 298 (2)	5144** ± 532 (18)	1.5	1.3
Repeated Exposures						
GD 20 Dams (n=8/group)[b]	12,838 ± 1373	13,044 ± 530 (-2)	11,563* ± 300 (10)	5094** ± 1081 (60)	0.3	0.3
GD 20 Fetuses (n=8/group)[b]	1781 ± 175	1569* ± 173 (12)	1600* ± 136 (10)	1188** ± 164 (33)	0.9	0.7
PND 4 Males (n=14-19/group)[c]	3137 ± 322	2817* ± 434 (10)	2889* ± 215 (8)	2744** ± 335 (13)	4.3@	2.3@
PND 4 Females (n=12-16/group)[c]	2823 ± 310	2941 ± 253 (-4)	2650 ± 287 (6)	2638 ± 269 (7)	4.5@	2.3@
PND 21 Males (n=8/group)[d]	10,375 ± 207	9944* ± 331 (4)	9044** ± 340 (13)	5675** ± 551 (45)	0.4	0.3
PND 21 Females (n=8/group)[d]	10,275 ± 376	9906 ± 313 (4)	9019** ± 248 (12)	5956** ± 965 (42)	0.4	0.3
Adult Males (n=8/group)[e]	14,100 ± 529	13.988 ± 662 (1)	12,700* ± 548 (10)	7469** ± 2484 (47)	0.5	0.2
Adult Females (n=8/group)[e]	14,869 ± 1400	13,913 ± 446 (7)	12,881**±845 (13)	6188** ± 1078 (58)	0.4	0.3
Post Exposure (n=8/sex/group)						
PND 60 Males[f]	13,000 ± 450	13,100 ± 411 (-1)	12,988 ± 422 (0)	13,044 ± 756 (0)	NE	NE
PND 60 Females[f]	13,275 ± 277	12,950 ± 317 (2)	12,738* ± 243 (4)	12,744* ± 586 (4)	NE	NE

[a] Results in parenthesis ( ) are percent inhibition relative to control
[b] Animals exposed from gestation day 6 to 20
[c] Animals exposed from gestation day 6 to post-natal day 4
[d] Animals exposed from gestation day 6 to post-natal day 21
[e] Animals exposed for 11 days
[f] Animals exposed from gestation day 6 to post-natal day 21
* = $p \leq 0.05$, ** $p \leq 0.01$

NE=not evaluated
@=poor model fit or values outside dose range
[g]See Appendix 8 for details of analysis doses in mg/kg/day

Data from the range-finding study present similar findings to those from the comparative ChE study (Table 2). Although the data were not statistically analyzed, there was a dose-related decrease in brain ChE activity at the 3 and 6 mg/kg/day doses. It is notable that the degree of inhibition observed at 3 mg/kg/day is consistent with that observed in the comparative ChE study at the same dose.

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Table 2. Brain cholinesterase activities (μg/kg) from dimethoate range-finding study

Dose (mg/kg/day)	0	0.2	3	6	Benchmark Dose (mg/kg/day)[a]	
					BMD_{10}	$BMDL_{10}$
Gestation day 20 (n=5/group)						
Dams	12710 ± 1333.9	12680 ± 640.9 (0)	3240 ± 411.4 (75)	1580 ± 195.6 (88)	0.2	0.2
Male fetuses	2150 ± 562.4	2320 ± 675.1 (+8)	1670 ± 564.1 (22)	1390 ± 780.5 (35)	1.0	0.3
Female fetuses	1970 ± 288.5	2100 ± 871.8 (+7)	1500 ± 500.0 (24)	1140 ± 638.7 (42)	1.0	0.4
Post-natal day 21 (n=14-20/group; up to 2/litter)						
Male pups	10555 ± 623.8	9942 ± 614.1 (6)	5839 ± 749.6 (45)	4720 ± 1654.5 (55)	0.4	0.3
Female pups	9338 ± 2709.6	9886 ± 445.2 (+6)	5414 ± 756.7 (42)	3186 ± 827.3 (66)	0.5	0.4

Data taken from Tables 28-32, pp. 78-82, MRID 45529701. Number in parentheses is percent inhibition; data were not statistically analyzed	[a]See Appendix 9 for details of analysis

As discussed earlier, BMD analyses provide a way to model the dose-response relationship for a given endpoint based on available data. Additional analyses of dimethoate data were recently submitted by the registrant for dimethoate (Appendix 13), using BMD modeling to refine the hazard assessment for dimethoate. EPA has also conducted additional analyses of the dimethoate data, using BMD modeling.

BMD models are used to interpolate a dose at which a specific response level of effect occurs (the benchmark response, or BMR). Thus, use of these models in hazard assessment requires selection of a BMR, which can then be compared across different treatment groups. A BMD_{10} (using the 'extra risk' model) is the dose resulting in a 10% change from the estimated background level for the endpoint being evaluated. For the endpoint of brain ChE inhibition, BMD_{10} is defined as the estimated dose which is expected to result in a 10% reduction in enzyme activity, when compared with control levels. A $BMDL_{10}$ is the lower 95% confidence limit on the BMD_{10} estimate. As described in detail in EPA's Revised Cumulative Risk Assessment for the OPs (USEPA, 2002), EPA has previously performed a power analysis of brain ChE data extracted from rat toxicity studies submitted to OPP for purposes of pesticide registration. This analysis included data from more than 30 OPs and over 100 studies. The power for each study to detect a difference of 1%, 5%, 7.5%, 10%, 15%, and 20% in mean brain ChE activity between control and a single treatment group was calculated. This analysis showed that a 10% change in mean activity was at the low end of detectability of assays for brain ChE activity as they were conducted in those studies. In the dimethoate comparative ChE study, the method used to measure brain ChE and also the number of animals in each dose group are similar to those used in the studies considered in the power analysis. Since a 10% change in mean brain ChE activity has been previously determined to be at the lower limit of detectability, it is considered appropriate for use as a benchmark response in the dimethoate evaluation.

To better compare relative sensitivity across groups, BMD_{10} and $BMDL_{10}$ values for brain ChE inhibition were computed for each exposure group in the comparative ChE and range-finding DNT studies using the same dose-response model (i.e., exponential model) as utilized in the OP Cumulative Risk Assessment. This method has been previously evaluated by the FIFRA SAP (2001, 2002). To provide an indication of the consistency of these values across different studies, BMD_{10} and $BMDL_{10}$ values were also determined for several additional studies in the

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

dimethoate database (a range-finding one generation reproductive toxicity study in rats, a 28-day dietary study in rats, and two two-generation reproductive toxicity studies in rats). Complete results of these analyses are provided in Appendix 9. BMD_{10} and $BMDL_{10}$ values for the companion ChE and DNT range-finding studies are included with the results for ChE inhibition in Tables 1 and 2, above. BMD_{10} and $BMDL_{10}$ values for additional studies are provided in Table 3, below.

For repeated exposures in the DNT companion ChE and range-finding studies, results of the BMD analysis demonstrate very similar BMD_{10} and $BMDL_{10}$ values for all groups (range 0.20-1.0 mg/kg/day for BMD_{10} and 0.2-0.7 mg/kg/day for $BMDL_{10}$). No age or sex-related differences were seen. Similar BMD values were estimated from other studies and thus support the findings from the comparative ChE and range-finding DNT studies. Computed values from other studies ranged from 0.3-1.0 mg/kg/day for BMD_{10}, and 0.2-0.8 mg/kg/day for $BMDL_{10}$, a very similar range to those from the DNT-related studies. Generally, the various brain ChE BMD values (both BMDs and BMDLs) provided in the analysis submitted by the pesticide registrant for dimethoate, Cheminova, are comparable to those computed by OPP.

Clear dose-response relationships were exhibited by a majority of the brain ChE data from repeated dosing and adequate model fits were attained for most of the data sets. Although the dose-response curve appeared to adequately model the data (by visual inspection), the goodness-of-fit statistic for the PND 42 males from the one generation reproductive toxicity study and the day 28 adult females from the 28-day dietary study resulted in highly significant p-values (i.e. p-value < 0.01) indicating the model's lack of fit. Additionally, the goodness-of-fit statistic for the day 218 adult females from the "new" two-generation reproductive toxicity study has a borderline significant p-value of 0.057 indicating the fit of that model is also questionable.

Table 3. BMD Values for brain cholinesterase inhibition for related dimethoate studies.

Study Type	MRID No.	Subpopulation	Sex	BMD_{10}	$BMDL_{10}$
Two-generation dietary Reproductive Toxicity	4225501	Day 224, F_0 adults	M	0.7	0.7
		Day 224, F_0 adults	F	0.3	0.3
		Day 308 F_1 adults	M	0.4	0.3
		Day 308 F_1 adults	F	0.4	0.3
Two-generation dietary Reproductive Toxicity	46181001	Day 205, F_0 adults	M	0.3	0.2
		Day 205, F_0 adults	F	0.5	0.3
		Day 218 F_1 adults	M	0.8	0.4
		Day 218 F_1 adults	F	0.6@	0.5@

One-generation dietary range-finding reproductive toxicity	46348201	Day 91 adults	M	0.4	0.3
		Day 91 adults	F	0.5	0.4
		PND42 offspring	M	0.3@	0.2@
		PND42 offspring	F	0.4	0.3
28-day dietary toxicity	46288001	Day 28 adults	M	1.0	0.8
		Day 28 adults	F	0.8@	0.7@
@=poor model fit; details of analysis provided in Appendix 9					

For acute exposures, one data set for adults and one for PND11 offspring (both from the comparative ChE study) were available. The BMD analyses of these data also support similar BMD_{10} and $BMDL_{10}$ values across age and sex (1.5-2.6 mg/kg/day for BMD_{10}, 1.3-2.0 mg/kg/day for $BMDL_{10}$). As expected, values are higher for acute than for repeated exposure scenarios, because a larger dose is needed to produce a similar amount of inhibition following single exposures at a given dose, compared to repeated dosing.

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2. Pup Mortality: Main DNT Study, Comparative ChE Study, & Range-finding DNT Study

a. Study Results

Complete reproduction data (i.e. litter size, pup body weight, mortality, etc.) from the main DNT study, comparative ChE study, and range-finding DNT study, are available in attached DERs (Appendices 2-4). There was no difference in litter size or pup weight at birth for any groups, with the possible exception of the 6 mg/kg dose in the range-finding study (there was a slight decrease in live litter size on day 1 in that study [12.4 pups per litter compared with 14.4 for controls], with no difference in total litter size at birth [14.1 at the high dose, 14.4 for controls]).

Pup mortality data, including both pup and litter incidence, are presented in Tables 4-6. A dose-related increase in pup mortality was seen in the 0.5 and 3 mg/kg/day groups of the main dimethoate DNT study (23-24 litters/group; see Table 4). No increase in pup mortality was seen in the comparative ChE study (8-10 litters/group; see Table 5). There was an increase in pup mortality at the 6 mg/kg/day dose level in the range-finding study but not at the 3 mg/kg/day level (8-10 litters/group; see Table 6). Total litter loss was seen in two litters at the 6 mg/kg dose in the range-finding study, and in one litter at the 0.5 mg/kg dose and three litters at the 3.0 mg/kg dose in the main DNT study. Most of the pup mortality in the DNT and DNT range-finding studies was seen during early lactation (PNDs 1-11). It is notable that there was no excess mortality during the period of direct dosing to pups.

Table 4. Post-natal Pup Mortality – Dimethoate DNT study [a]

Dose (mg/kg/day)	live pups /litters born	Days of Lactation[b]					Total litter loss (pups/ litters)[c]	Mean # dead pups/ litter
		1-4	5-11	12-16	17-21	1-21		
0 (Control)	371/24	10 (7)	3 (3)	2 (2)	0 (0)	15 (10)	0	0.6

0.1 (LDT)	343/23	8 (5)	3 (2)	0 (0)	0 (0)	11 (6)	0	0.5
0.5 (MDT)	360/24	32* (9)	10 (3)	1 (1)	0 (0)	43* (10)	15/1	1.8
3.0 (HDT)	366/24	71* (13)	15 (7)	1 (1)	2 (2)	89* (14)	38/3	3.7

[a] Includes pups that were found dead, missing and presumed dead, or sacrificed due to poor condition; slightly revised numbers recently submitted by Cheminova vary from these totals by no more than 1 death/group;
[b] Number of pups affected (number of litters affected)
[c] Number of pups humanely sacrificed; additional pups from the affected litters died prior to sacrifice
* $p<0.01$, Chi Square test.

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Table 5. Post-natal Pup Mortality – Dimethoate Comparative ChE study

Dose (mg/kg/day)	live pups /litters born	Days of Lactation[a]					Total litter loss (pups/ litters)	Mean # dead pups/ litter
		1-4	5-11	1-11	11-21	1-21		
0 (Control)	133/10	0	0	0	NA	NA	0/0	0
0.1 (LDT)	142/10	2	0	2	NA	NA	0/0	0.2
0.5 (MDT)	146/10	2	0	2	NA	NA	0/0	0.2
3.0 (HDT)	137/10	1	1	2	NA	NA	0/0	0.2

[a] Number of pups affected
NA=data not available.
Statistical analysis not performed

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Table 6. Post-natal Pup Mortality – Dimethoate range-finding DNT study [a]

Dose (mg/kg/day)	live pups /litters born	Days of Lactation[b]					Total litter loss (pups/ litters)[c]	Mean # dead pups/ litter
		1-4	5-11	1-11	11-21	1-21		
0 (Control)	144/10	5(4)	0(0)	5(4)	0	5(4)	0	0.5
0.2 (LDT)	128/9	1 (1)	0(0)	1(1)	0	1(1)	0	0.1
3.0 (MDT)	129/9	5 (4)	1(1)	6(4)	0	6(4)	0	0.7
6.0 (HDT)	113/8	38 (7)	1(1)	39(7)	2(2)	41(7)	3/2	5.1

[a] Includes pups that were found dead, missing and presumed dead, or sacrificed due to poor condition.
[b] Number of pups affected (number of litters affected)
[c] Number of pups humanely sacrificed; additional pups from the affected litters died prior to sacrifice
Statistical analysis not performed

b. Benchmark Dose Analysis

As noted previously, recently submitted analyses from the registrant included BMD analyses of available pup mortality data for dimethoate (Appendix 13). To confirm these analyses, and to further explore the dose/response relationship for the increase in pup mortality in the main DNT study, a separate Benchmark Dose (BMD) analysis was performed by EPA. Although analyses submitted by the registrant included data from several related studies, EPA analyses were conducted using data from the main DNT study only (see Appendix 8 for full details of the analysis).

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The EPA analysis was conducted using EPA's Benchmark Dose Software (BMDS; www.epa.gov/ncea/bmds.htm). The response of pup death due to dosing was modeled using the available BMDS nested models: NLogistic, NCTR, and RaiVR (Technical details provided in Appendix 8). A culling event on PND 4 artificially reduced the sizes of the litters making the periods, PND 1-4 and PND 5-11 incomparable. Consequently pup death data for the two periods were modeled separately. For both study periods, NCTR and RaiVR produced very similar BMD values. The minimal Akaike Information Criterion (AIC) was used to identify the most parsimonious model within and among model families. Although the NCTR model resulted in the smallest AIC for both study periods, NLogistic had an only slightly larger AIC, but resulted in smaller BMD values. Therefore the NLogistic BMD values were chosen for purposes of comparison with ChE BMD estimates. BMDs and their corresponding lower limits (BMDLs) were computed for benchmark responses of 5% and 10% based on the pup death data from the PND 1-4 period. Computed values for the BMDs and BMDLs are provided below (Table 7); complete results of the analysis are provided in Appendix 8.

Table 7. Benchmark Dose Values for Increased Pup Mortality during PNDs 1-4 in the main DNT study

BMD Level	BMD (mg/kg/day)	
	BMD	BMDL
BMD_5	0.47	0.27
BMD_{10}	0.99	0.57

BMD=Benchmark dose
BMDL=statistical lower limit on BMD

The goodness of fit statistic supports an adequate fit of the NLogistic model for the PND1-4 pup death data. The BMD values ranged from 0.47 to 0.99 mg/kg/day, depending on the specified benchmark response. As expected, BMDL values were somewhat lower than BMD values, ranging from 0.27 to 0.57 mg/kg/day. $BMD/BMDL_5$ levels for PND1-4 pup mortality are consistent with study findings for that endpoint, falling between the study LOAEL of 0.5 mg/kg/day and the study NOAEL of 0.1 mg/kg/day. The BMD_{10} estimates for the PND5-11 pup death data are higher than those for PND1-4 and outside the dose range for the main DNT study (see Appendix 8 for details). No analysis of the sensitivity of the benchmark dose was performed for the PND 5-11 data.

Pup mortality in control groups from the main DNT, companion ChE, and range finding study ranged from 0-3.5% of live born for PNDs1-4. In the cross-fostering study, conducted

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using a similar protocol in the same laboratory (see below), pup mortality in the control group was very similar: 2.7% of liveborn for PND1-4. Insufficient information was available to compute pup mortality as a percent of liveborn for available historical control data. Based on these background levels for pup mortality, an increase of 5% above background (i.e. a BMD_5) was considered to be the smallest detectable change from background and therefore an appropriate BMR for this effect. Additional support for this selection is provided by several analyses in the literature (Faustman et al, 1994; Allen et al., 1994a; Allen et al. 1994b; Kavlock et al., 1995), which report that the use of a $BMDL_5$ for developmental endpoints results in values similar to available NOAELs within the same studies. Available EPA guidance also indicates that a BMR of 5% has typically been used for developmental studies (EPA, 2000).

3. Dimethoate Cross-fostering Study

In an attempt to determine whether the increased pup mortality seen in the dimethoate DNT study could be attributed specifically to pre- or post-natal exposure to dams, a cross-fostering study was conducted by the dimethoate registrant (MRID 46214501, Appendix 6). Dimethoate was administered by gavage to dams from GD 6 through PND 10, at doses of 0, 3, or 6 mg/kg/day. Pups were cross-fostered on PND 1, to create groups with no exposure (control), maternal pre-natal exposure only (3 and 6 mg/kg), maternal post-natal exposure only (3 and 6 mg/kg), or maternal pre and post-natal exposure (6 mg/kg group only). In addition to pup survival and reproductive outcome data, detailed observations were conducted on both pups and dams to evaluate possible treatment-related toxicity that might contribute to increased mortality. Clinical chemistry and hematological parameters were also evaluated in pups on PND11, but ChE activity was not evaluated.

At the 6 mg/kg dose, the timing of deaths appeared related to the timing of exposure: 24/25 deaths in the 'pre-natal only' exposure group occurred during PND 1-4, 22/31 deaths in the 'post-natal' only group occurred during PND 4-11. In the 'pre- and post-natal' group, 28/38 deaths occurred during PND1-4 and 10/38 deaths occurred during PND 4-11 (see Table 8).

In the 3 mg/kg/day 'pre-natal only' and 'post-natal only' groups, there was a slight increase in the total number of pup deaths (12 deaths in controls, 16 deaths in each of the 3 mg/kg groups; see Table 8). There was no increase in the total number of litters with pup death, but there was a small increase in the number of litters with multiple deaths (0 litters with multiple deaths in controls, 5 in 'pre-natal only' and 3 in 'postnatal only'

groups treated at 3 mg/kg/day). The increase in litters with multiple deaths at 3 mg/kg was not as pronounced as that seen at the 6 mg/kg (5 litters in the 'prenatal only', 9 litters in 'postnatal only', and 10 litters in 'pre- and post-natal' groups; see Table 9). Note, interpretation of results at 3 mg/kg/day is complicated by two features of the study design: 1) a group exposed both pre- and post-natally at that dose was not included in the study; and 2) a control for cross-fostering was not included in the study.

Results of the hematological evaluation are described in detail in the DER (Appendix 6).

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Table 8 Pup Mortality in the Cross-fostering study [dead/missing pups (litters)] [a]

Post-natal Day	Group					
	1C Dam: Control Pup: Own litter	**1A Dam: Control Pup: 3 mg/kg/day[e]**	**1B Dam: Control Pup: 6 mg/kg/day[e]**	**2 Dam: 3 mg/kg/day Pup: Control[e]**	**3A Dam: 6 mg/kg/day Pup: Control[e]**	**3B Dam: 6 mg/kg/day Pup: Own litter**
No. pups/ litters born	**375/25**	**347/23**	**352/23**	**352/23**	**352/23**	**341/22**
Day 1 [b]	7 (7)	7 (5)	12 (6)	7 (4)	3 (2)	7 (7)
PND 1-4 [c]	3 (3)	2 (1)	12 (5)	2 (2)	6 (6)	21 (11)
PND 1-4 [d]	10 (10)	9 (6)	24 (9)	9 (5)	9 (7)	28 (14)
PND 4-7	1 (1)	3 (3)	1 (1)	3 (3)	6 (6)	6 (4)
PND 7-11	1 (1)	4 (4)	0 (0)	4 (4)	16 (10)	4 (4)
PND 4-11	2 (2)	7 (7)	1 (1)	7 (6)	22 (12)	10 (6)

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PND 1-11[c]	5 (5)	9 (8)	13 (6)	9 (8)	28 (14)	31 (13)
PND 1-11 [d]	12 (12)	16 (10)	25 (10)	16 (11)	31 (15)	38 (16)
total pup death as % live birth, PND1-11	3.2	4.6	7.1	4.5	8.8	11.1

[a] Data extracted from Appendix 22, pp. 281-286, MRID 46214501
[b] Includes stillborn and other nonviable pups
[c] Without Day 1 stillborn and other nonviable pups
[d] Includes Day 1 stillborn and other nonviable pups
[e] Pups from mothers treated with listed dose.
1A - dams in control group fostering pups from dams treated at 3 mg/kg/day
1B - dams in control group fostering pups from dams treated at 6 mg/kg/day
1C - dams in control group rearing own litter
2 - dams treated at 3 mg/kg/day fostering pups from a dam in the control group
3A - dams treated at 6 mg/kg/day fostering pups from a dam in the control group
3B - dams at the 6 mg/kg/day rearing own litter

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Table 9. Distribution of Pup Deaths in cross-fostering study [no. of dams with dead/missing pups] [a]

No. of dead/ missing pups [a]	Group					
	1C Dam: Control Pup: Own litter	1A Dam: Control Pup: 3 mg/kg/day[c]	1B Dam: Control Pup: 6 mg/kg/day[c]	2 Dam: 3 mg/kg/day Pup: Control[c]	3A Dam: 6 mg/kg/day Pup: Control[c]	3B Dam: 6 mg/kg/day Pup: Own litter
No. litters	**25**	**23**	**23**	**23**	**23**	**22**
0	13	13	13	12	8	6
1	12	5	5	8	6	6
2	0	4	2	1	3	4
3	0	1	1	2	5	3
4	0	0	0	0	1	2
5	0	0	1	0	0	0
6	0	0	0	0	0	0

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7	0	0	0	0	0	1
8	0	0	1	0	0	0

[a] Data obtained from Appendix 22, pages 281-286, MRID 46214501.
[b] Includes stillborn and other nonviable pups
[c] Pups from mothers treated with listed dose.
1A - dams in control group fostering pups from dams treated at 3 mg/kg/day
1B - dams in control group fostering pups from dams treated at 6 mg/kg/day
1C - dams in control group rearing own litter
2 - dams treated at 3 mg/kg/day fostering pups from a dam in the control group
3A - dams treated at 6 mg/kg/day fostering pups from a dam in the control group
3B - dams at the 6 mg/kg/day rearing own litter

There was no indication of treatment-related maternal toxicity or clinical signs during gestation; there were no treatment-related clinical signs and no difference in body weight/body weight gain among treatment groups (see DER, appendix 6). However, during the lactation period, there was a higher proportion of dams exposed at 3 and 6 mg/kg/day showing restlessness on 2 days or more, regardless of whether they were rearing their own litters (group 3B) or control offspring (groups 2 and 3A) (Table 10). Scattering of offspring in the cage on two or more days of lactation was also increased in dams exposed during the study (2, 3A & 3B) at 3 and 6 mg/kg/day.

The number of pups with the umbilicus still attached during the early perinatal period (PND 1) was increased in groups 1B (15), 3A (10), and 3B (13) compared to controls (4) (Table 11). However, the number of pups with umbilicus attached after PND 1 was similar between control and treated groups and therefore did not suggest reduced maternal care of pups, even after cross-fostering.

During lactation, the incidence of pups with “no milk in the stomach” was increased in groups 2, 3A and 3B (15, 28, and 11, respectively) compared to control (group 1C: 4) (Table 11). It is noted that this finding is supported by pup necropsy results, specifically that the incidence of pups found dead after cross-fostering with no milk in the stomach was increased in groups 2, 3A, and 3B (14, 24, and 28 pups, respectively), as well as in group 1B (12 pups), compared to control (group 1C: 7 pups) (see Table 19 in the DER). It cannot be determined whether this finding is due to adverse effects on the dams or on the pups.

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Table 10. Incidence of maternal restlessness and offspring scattering[a]

Observation	Number of dams or litters affected [# observations/# days]					
	1C (n=25) Dam: Control Pup: Own litter	**1A (n=23) Dam: Control Pup: 3 mg/kg/day[b]**	**1B (n=23) Dam: Control Pup: 6 mg/kg/day[b]**	**2 (n=23) Dam: 3 mg/kg/day Pup: Control[b]**	**3A (n=23) Dam: 6 mg/kg/day Pup: Control[b]**	**3B (n=22) Dam: 6 mg/kg/day Pup: Own litter**
Maternal restlessness						
0 Days	15	13	15	8	3	1
1 Day	7 [7 obs/7 days]	10 [11 obs/10 days]	7 [8 obs/6 days]	6 [8 obs/6 days]	5 [6 obs/5 days]	6 [8 obs/5 days]
2-3 Days	3 [7 obs/6 days]	0	1 [4 obs/2 days]	8 [20 obs/19 days]	13 [40 obs/33 days]	12 [41 obs/32 days]
4 or more days	0	0	0	1 [6 obs/4 days]	3 [19 obs/12 days]	3 [24 obs/14 days]
Scattering of offspring						

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0 Days	13	13	9	6	1	5
1 Day	6 [6 obs/6 days]	3 [3 obs/3 days]	6 [9 obs/7 days]	4 [6 obs/5 days]	3 [4 obs/3 days]	4 [6 obs/5 days]
2-3 Days	6 [17 obs/14 days]	4 [10 obs/10 days]	6 [14 obs/12 days]	8 [29 obs/19 days]	12 [53 obs/33 days]	7 [23 obs/17 days]
4 or more days	0	3 [19 obs/13 days]	1 [4 obs/4 days]	5 [36 obs/28 days]	7 [37 obs/30 days]	6 [37 obs/29 days]

[a] Data obtained from Table 19, page 84, MRID 46214501.
[b] Pups from mothers treated with listed dose
1A - dams in control group fostering pups from dams treated at 3 mg/kg/day
1B - dams in control group fostering pups from dams treated at 6 mg/kg/day
1C - dams in control group rearing own litter
2 - dams treated at 3 mg/kg/day fostering pups from a dam in the control group
3A - dams treated at 6 mg/kg/day fostering pups from a dam in the control group
3B - dams at the 6 mg/kg/day rearing own litter

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Table 11. Observations of umbilicus attached and no milk in the stomach of pups (PND 1-11) [a]

Clinical Sign	Group					
	1C Dam: Control Pup: Own litter	**1A Dam: Control Pup: 3 mg/kg/day[b]**	**1B Dam: Control Pup: 6 mg/kg/day[b]**	**2 Dam: 3 mg/kg/day Pup: Control[b]**	**3A Dam: 6 mg/kg/day Pup: Control[b]**	**3B Dam: 6 mg/kg/day Pup: Own litter**
Umbilicus Attached [c]						
PND 1	4	5	15	1	10	13
PNDs 2-4	4	0	4	0	4	2
after PND 4	3	0	0	0	3	0
No Milk in Stomach						
Incidences of no milk in stomach[d]	4	3	4	15	28	11
Number of litters	4	3	4	9	13	9

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Specific days of observation	2, 4, 5, 7	4, 5, 8	1, 5, 7	2,3,4,5,6,7	1,3,4,5,6,7, 8,9	3,4,6,7

[a] Data extracted from Appendix 20, page 245-265,and Appendix 21, pp 270-279, MRID 46214501
[b] Pups from mothers treated with listed dose
[c] Number of observations of umbilicus still attached after cross-fostering of litters. Observations of offspring not allocated to cross-fostering include umbilicus attached for 15 control pups on PND 1, none for 3 mg/kg/day litters, and 5 pups for 6 mg/kg/day litters pre-PND 1.
[d] Observation of one or more pups in a litter with the finding at a scheduled observation time.
1A - dams in control group fostering pups from dams treated at 3 mg/kg/day
1B - dams in control group fostering pups from dams treated at 6 mg/kg/day
1C - dams in control group rearing own litter
2 - dams treated at 3 mg/kg/day fostering pups from a dam in the control group
3A - dams treated at 6 mg/kg/day fostering pups from a dam in the control group
3B - dams at the 6 mg/kg/day rearing own litter

In summary, pup mortality in the cross-fostering study was increased after cross-fostering, days 1-4, and again on post-natal days 4-11, suggesting that pup mortality increased regardless of pre- or post-natal maternal exposure to dimethoate at 3 or 6 mg/kg/day. Post-natal deaths appeared to be correlated to some extent with the incidences of maternal restlessness and litter scattering for groups 2, 3A, and 3B. However, the cause of maternal behaviors and their contribution to pup mortality cannot be determined. A combination of pre- and post-natal exposure to pups and/or dams contributed to the observed pup mortality.

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4. Standard Reproductive Toxicity Studies in Dimethoate and Omethoate

EPA and PMRA have evaluated the available data related to pup survival from three reproductive toxicity studies in dimethoate (2 full studies and one range-finding study) and two reproductive toxicity studies in omethoate. Results from dimethoate studies are presented below; results from omethoate studies are presented in Appendix 12. The purpose of this evaluation is to further characterize available pup mortality/survival data and related ChE inhibition in pup/offspring and/or maternal animals. In available dimethoate reproductive toxicity studies, dimethoate was administered in the feed (Appendices 7, 10, 11). Results of the BMD analyses of the ChE data from these studies have been previously discussed (see above). Results related to pup mortality are presented in Tables 12, 13, and 14, below.

Table 12. Pup Death Data from Dimethoate first dietary reproductive toxicity study (MRID 42251501).				
	live pups/ litters born	deaths* PND 0-4	deaths PND 4-21	total deaths PND 0-21
F0/first mating				
control	421/26	23/11	2/2	25/12
1 ppm (0.09 mg/kg)	409/27	14/8	2/2	16/9
15 ppm (1.3 mg/kg)	367/24	7/6	3/3	10/7
65 ppm (6.04 mg/kg)	355/24(23)	8/4	2/1	10/4
F0/second mating				
control	369/25	7/7	1/1	8/7
1 ppm (0.09 mg/kg)	379/26	6/4	1/1	7/4
15 ppm (1.3 mg/kg)	350/25	7/6	2/2	9/7
65 ppm (6.04 mg/kg)	282/20	7/6	7/4	14/9
F1/first mating				
control	279/23	10/7	2/2	12/8
1 ppm (0.09 mg/kg)	199/17	5/4	0/0	5/4
15 ppm (1.3 mg/kg) TLL=2	247/17	27/6	11/4	38/7
65 ppm (6.04 mg/kg) TLL=1	180/15	19/5	8/4	27/6
F1/second mating				
control	222/16	1/1	1/1	2/1
1 ppm (0.09 mg/kg)	213/16	3/3	1/1	4/2
15 ppm (1.3 mg/kg) TLL=1	178/14	14/3	5/3	19/5
65 ppm (6.04 mg/kg)	120/12	2/1	2/1	4/2
*values represent total pup deaths/total litters with pup deaths				

No clear increase in pup mortality was seen in the two full reproductive toxicity studies with dimethoate, at maternal doses up to 6 mg/kg/day (see tables 12 and 14). There was some indication of decreased fertility (fewer pups/litters born) at 6.04 mg/kg/day in the first study, complicating the interpretation of pup mortality in that study (see Table 12). Similar findings were not seen in the second study (Table 13), although there was some indication in the histopathology of effects on the male reproductive system (it should be noted that the studies were performed in different laboratories, using different strains of rat). In both studies, there was increased pup mortality for some matings at the high dose. However, this increase was not consistent across matings and generations, and its toxicological significance is difficult to assess.

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Table 13. Pup Death Data from Dimethoate second dietary reproductive toxicity study (MRID 46181001).

	live pups/ litters born	deaths* PND 0-4	deaths PND5-11	deaths PND 5-21	total deaths PND 0-21
F0/first mating					
control	206/21	0/0	1/1	1/1	1/1
0.2 mg/kg	244/24	1/1	0/0	0/0	1/1
1.0 mg/kg	259/25	2/2	0/0	0/0	2/2
6.5 mg/kg	234**/23	3/3	0/0	0/0**	3/3
F0/second mating					
control	262/24	2/2	0/0	1/1	3/3
0.2 mg/kg	232/22	1/1	0/0	0/0	1/1
1.0 mg/kg	244/21	1/1	0/0	0/0	1/1
6.5 mg/kg	251/25	10/4	0/0	0/0	10/4
F1/first mating					
control	247/22	8/3	1/1	1/1	9/4
0.2 mg/kg	251/24	8/6	0/0	0/0	8/6
1.0 mg/kg	281/24	5/4	1/1	1/1	6/5
6.5 mg/kg	261/25	1/1	0/0	0/0	1/1
F1/second mating					
control	256/22	9/7	0/0	0/0	9/7
0.2 mg/kg	285/25	4/4	2/2	3/3	7/6
1.0 mg/kg	248/22	3/2	1/1	1/1	4/2
6.5 mg/kg	265/25	6/5	11/3	12/4	18/6

a Data were obtained from pages 627 - 634 and 1005 - 1012 of the study report.
*values represent total pup deaths/total litters with pup deaths. Pup deaths included cannibalized pups.
** Pup which suffered from accidental death on Day 17 is omitted.

In a one-generation range-finding reproductive toxicity study, treatment-related effects were seen on several reproductive parameters, including decreased number of implantation sites, increased post-implantation loss, and decreased litter size at birth. These dose-related effects were seen in all treatment groups, at doses ranging from 3.9-7.5 mg/kg/day (50, 75, or 100 ppm in the diet). Although there was no increase in pup mortality between PND1-4, there was an increase in mortality from PND4-21 at doses of 75 and 100 ppm (5.8 or 7.5 mg/kg/day, respectively); this finding was also reflected in a lower lactation index at these doses, as well as a continued decrease in mean litter size.

Pup mortality findings from this study are summarized in Table 14; the complete study review (DER) is provided in Appendix 11.

Table 14. Pup Death Data from Dimethoate dietary one-generation range-finding reproductive toxicity study.(MRID 46348201)

	live pups/ litters born	deaths* PND 0-4	deaths PND 5-11	deaths PND 5-21	total deaths PND 0-21
F0					
control	153/10	1	0	0	1
50 ppm (3.9 mg/kg)	137/9	3	0	1	4
75 ppm (5.8 mg/kg)	140/10	1	13	13	14
100 ppm (7.5 mg/kg)	132/10	1	4	9	10

* values represent total pup deaths/total litters with pup deaths

Dose-related decreases in brain ChE activity were seen in both of the dimethoate two-generation reproductive toxicity studies (see Tables 15 and 16) and in the one-generation range-finding reproductive toxicity study (see Table 17). BMD analyses conducted using these data have been presented above, and result in BMD values similar to those seen in the DNT companion ChE study. Due to differences in exposure (lack of direct exposure to pups during lactation due to dietary route of administration), data suitable for comparing young and adult dose/response curves are not

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available from these studies. However, based on the absence of a clear increase in pup mortality at doses up to 6 mg/kg/day (doses resulting in approximately 60% brain ChE inhibition), it is clear that the increase in pup mortality seen in the DNT study is not solely due to maternal or fetal ChE inhibition.

Relevant data from available reproductive toxicity studies for omethoate (the active metabolite of dimethoate) were also examined (see Appendix 12). Two multi-generation reproductive studies are available: the drinking water study found pup mortality at the highest dose tested (1 mg/kg/day), most notably in the second generation. In a feeding study conducted at doses up to 0.5 mg/kg/day of omethoate, small increases in pup mortality were noted in the second generation (note: significant deficiencies were noted in the study protocol of the feeding study). The increases in pup mortality seen in these studies provide support for the findings in the main DNT study, the range-finding DNT study, and the cross-fostering study, but differences in study design preclude any direct comparison of dose/response relationships.

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In summary, there is some indication in available dimethoate reproductive toxicity studies of offspring toxicity, manifested as decreases in pups and/or litters born or as increased pup mortality. However, effects seen in these studies generally occurred at higher doses than similar effects in the DNT study. Increased pup mortality was also seen in available reproductive toxicity studies for omethoate (see Appendix 12). Taken together, these results support the determination that treatment-related increases in offspring mortality can occur following exposure to dimethoate, but provide conflicting information regarding the doses at which that effect occurs.

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Table 15 . Results from first dimethoate reproductive toxicity study

Feeding (MRID 42251501)						
F1 Generation						
Dose	**Index**	**Breeding A**	**Breeding B**	**Brain Cholinesterase Inhibition (µmol/g/min) Males/Females [a]**		
				Parental Generation	**Pups (PND 4)**	**Adult F1 (44 weeks)**
Control	Viability	93	97	5.94 ± 0.663 F 5.89 ± 0.730 M	2.47 ± 0.285 F 2.78 ± 0.397 M	6.73 ± 0.988 F 7.87 ± 1.527 M
	Lactation	99	99			
1 ppm (0.09 mg/kg/day)	Viability	96	96	6.02 ± 0.743 (1%) F 5.63 ± 0.783 (4%)[a] M	2.61 ± 0.276 (6%) F 2.62 ± 0.360 (6%) M	6.61 ± 0.848 (2%) F 8.13 ± 1.630 (3%) M
	Lactation	99	99			
15 ppm (1.30 mg/kg/day)	Viability	98	97	4.03** ± 0.656 (32%) F 4.83** ± 0.854 (18%) M	2.70 ± 0.323 (9%) F 2.57 ± 0.421 (8%) M	4.43** ± 0.801 (30%) F 5.64** ± 1.365 (28%) M
	Lactation	98	99			
65 ppm (6.04 mg/kg/day)	Viability	98	97	2.24** ± 0.455 (62%) F 2.38** ± 0.755 (60%) M	2.57 ± 0.321 (4%) F 2.42 ± 0.289 (13%)[b] M	1.97** ± 0.609 (71%) F 3.07** ± 1.061 (61%) M
	Lactation	99	96			
F2 Generation						
Control	Viability	95	98	NA: Not measured		
	Lactation	99	99			
1 ppm (0.09 mg/kg/day)	Viability	96	99			
	Lactation	100	99			
15 ppm (1.30 mg/kg/day)	Viability	89	86			
	Lactation	98	95			
65 ppm (6.04 mg/kg/day)	Viability	89	98			
	Lactation	93	98			

* p<0.05, ** p<0.01, Analysis of variance followed by intergroup comparison with the control (Williams Ttest)
Viability index = No. live pups at birth/No. live pups at day 5 (pre-cull) × 100
Lactation index = No. live pups on day 5 (post-cull)/No. live pups day 28 × 100
[a] Number in parentheses is percent inhibition calculated by the reviewer.
[b] statistics not provided in study

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Table 16. Results from second dimethoate reproductive toxicity study

Feeding (MRID 46181001)						
F1 Generation						
Dose	**Index**	**Breeding A**	**Breeding B**	**Brain Cholinesterase Inhibition (µmol/g/min) Males/Females [a]**		
				Parental Generation	**Pups (PND 4)**	**Adult F1 (44 weeks)**
Control	Viability	100	99	2.46 ± 0.79 F 2.26 ± 0.92 M	1.12 ± 0.08 F 1.07 ± 0.08 M	1.62 ± 0.33 F 1.68 ± 0.45 M
	Lactation	99	99			
0.2 mg/kg/day	Viability	100	100	2.66 ± 1.16 F 2.26 ± 0.93 M	NA	1.46 ± 0.26 F 1.66 ± 0.63 M
	Lactation	100	100			
1.0 mg/kg/day	Viability	99	100	2.03* ± 0.82 (17%) F 1.80** ± 0.74 (20%) M	NA	1.30** ± 0.23 (20%) F 1.47** ± 0.48 (13%) M
	Lactation	100	100			
6.5 mg/kg/day	Viability	99	96*	0.76** ± 0.25 (69%) F 1.09** ± 0.57 (52%) M	1.02* ± 0.10 (10%) F 1.03 ± 0.09 M	0.52** ± 0.11 (68%) F 0.70** ± 0.21 (58%) M
	Lactation	99	100			
F2 Generation						
Control	Viability	97	96		1.24 ± 0.16 F 1.23 ± 0.12 M	
	Lactation	99	100			
0.2 mg/kg/day	Viability	97	99		NA	
	Lactation	100	98			
1.0 mg/kg/day	Viability	98	99		NA	
	Lactation	99	99			
6.5 mg/kg/day	Viability	100	98		1.15 ± 0.22 (7%) F 1.16 ± 0.21 (6%) M	
	Lactation	100	94**			

* p<0.05, ** p<0.01, Analysis of variance followed by intergroup comparison with the control (Williams Ttest)
Viability index = No. live pups at birth/No. live pups at day 5 (pre-cull) × 100
Lactation index = No. live pups on day 5 (post-cull)/No. live pups day 28 × 100
[a] Number in parentheses is percent inhibition calculated by the reviewer.
[b] statistics not provided in study

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Table 17. Results from dimethoate one generation range-finding reproductive toxicity study

Feeding (MRID 46348201)[b]				
F1 Generation				
Dose	**Index**	**Breeding A**	**Brain Cholinesterase Inhibition (µmol/g/min) Males/Females[a]**	
			Parental Generation	**Offspring (Week 6)**
Control	Viability	99	7.89 F 7.45 M	7.58 F 7.19 M
	Lactation	100		
50 ppm (2.9/3.9 mg/kg/day	Viability	98	3.70 (53%) F	3.13 (59%) F

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Table 17. Results from dimethoate one generation range-finding reproductive toxicity study

Feeding (MRID 46348201)[b]				
[M/F])	Lactation	99	4.20 (44%) M	2.75 (62%) M
75 ppm (4.4/5.8 mg/kg/day [M/F])	Viability	99	3.17 (60%) F 3.61 (52%) M	2.30 (70%) F 2.62 (64%) M
	Lactation	91		
100 ppm (6.1/7.5 mg/kg/day [M/F])	Viability	99	2.55 (68%) F 3.31 (56%) M	2.14 (72%) F 2.00 (72%) M
	Lactation	93		

Viability index = No. live pups at birth/No. live pups at day 5 (pre-cull) × 100
Lactation index = No. live pups on day 5 (post-cull)/No. live pups day 28 × 100
[a] Number in parentheses is percent inhibition calculated by the reviewer, s.d. not provided.
[b] statistics not provided in study

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5. Discussion

Increased pup mortality was seen in several recently conducted studies following maternal exposure to dimethoate during gestation and early lactation. However, the dose at which the pup mortality was observed varies among the studies. The doses at which increased mortality is seen in the main DNT study (0.5 and 3 mg/kg/day) are lower than that at which the effect is seen in other dimethoate studies conducted using similar exposure regimes (6 mg/kg/day). No increase in pup mortality was seen at the 0.5 mg/kg/day in the comparative ChE study and at 3.0 mg/kg/day in the comparative ChE or range-finding studies. The reason for this difference in results is unknown, but may be related to the smaller number of litters/group used in the comparative ChE and range-finding studies (8-10/dose, compared with 23-24 in the main study) and, therefore, less statistical power to detect the effect. The effect seen at 6 mg/kg in the DNT range-finding study was found at that same dose in the cross-fostering study. In the cross-fostering study, although a 3 mg/kg dose was administered, no pups were exposed at that dose both *in utero* and during lactation, as were the pups in the main DNT study, comparative ChE, and range-finding studies.

a. Relationship Between Maternal Toxicity and Pup Mortality

It has been suggested that the increase in pup death seen during early lactation following maternal exposure to dimethoate is attributable to maternal toxicity (see discussion in trip audit memo, 3/22/2001, Appendix 5). Although there was no indication of overt maternal toxicity in the main dimethoate DNT study, there is significant ChE inhibition at both the 0.5 and 3.0 mg/kg/day doses in both dams and fetuses in the comparative ChE inhibition study. Since both pups and dams have been exposed in the DNT study design, it is not possible to separate effects on pups from effects on dams. Consequently EPA and PMRA conclude that data from the main DNT, range-finding, and comparative ChE inhibition studies do not support a determination regarding the dams as the exclusive cause of the pup mortality seen in the main DNT study.

To obtain further information regarding a possible link between maternal toxicity and pup mortality, the registrant conducted the cross-fostering study described above. In that study, detailed behavioral observations were conducted to look for symptoms of maternal toxicity that may not have been apparent in the cageside evaluations conducted in the main DNT study. In addition, separate groups of pups were exposed exclusively pre-natally (*in utero* only) or post-natally (via maternal dosing), in order

to characterize what factor(s) may lead to pup mortality. Although ChE inhibition was not measured in the cross-fostering study, the exposure and dosing regime were similar to that used in the DNT range-finding study.

Results from the cross-fostering study showed that increases in pup mortality could result from either pre- or post-natal exposure to the dams. The number and timing of the pup deaths appeared to vary depending on the timing of the dam's exposure. At the 6 mg/kg level: in the 'pre-natal' group, there were 25 deaths, 24 of 25 deaths during PND1-4; in the 'post-natal' group; 31 deaths, 22 of 31 during PND 4-11; and in the 'pre- and post-natal' group, 38 deaths, spread throughout PND1-11. The increased incidence of death during the period immediately following birth (i.e. on day 1, prior to cross-fostering) is consistent with the pattern seen in prior studies. No clear-cut increase in pup mortality was seen at the 3.0 mg/kg/day dose in the cross-fostering study, possibly because of the lack of combined pre- and post-natal exposure to pups.

Although cholinergic signs were not observed in the dams during the main DNT study at any dose, based on the results of the comparative ChE inhibition study significant cholinergic inhibition in the maternal brains is expected and could have impacted maternal behavior. In order to address possible maternal toxicity not detected by routine clinical observations, detailed observations of maternal care were conducted for dams in the cross-fostering study (note: behavioral observations were performed by individuals aware of the treatment received by animals they were observing, raising the possibility of observer bias). There was an increase in incidence of several observations in treated dams and pups (maternal restlessness and scattering of offspring, no milk in stomach of pups, and umbilicus attached in pups).

Careful examination of the data indicated no clear relationship between these observations and increased pup mortality within the affected litters. First, there was an increase in pup death in the 6 mg/kg 'pre-natal only' group, but no increase in scattering when compared to controls. In addition, the increase in restlessness/scattering in dams treated with 3.0 mg/kg was similar in magnitude to the increase in symptoms in dams treated with 6.0 mg/kg, with a much smaller increase in pup death in the 3 mg/kg 'post-natal only' group. Evaluation of in-life observations of 'no milk in the stomach of pups' leads to similar conclusions; the incidence is increased in pups of all dams receiving post-natal treatment (on a pup or litter basis), regardless of dose, and thus cannot be specifically related to the increase in pup death (Tables 10 & 11).

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An increase in the number of pups with umbilicus attached on PND 1 was seen in pups treated at 6 mg/kg, either pre- or post-natally, but in no other group. However, the number of pups with umbilicus attached after PND 1 was similar between control and treated groups and therefore did not suggest reduced maternal care of pups, even after cross-fostering.

Decreases in maternal care may originate from effects in the pups or the dams (i.e. ill mothers neglect their pups, or mothers may neglect their ill pups). Actual exposure of pups to dimethoate or its metabolites via lactation, following maternal exposure to dimethoate, has not been quantified. Available data from the companion ChE inhibition study indicate similar levels of ChE inhibition in fetuses and dams following *in utero* exposure; lower levels of inhibition found in PND 4 pups could suggest less exposure via lactation, but the actual exposure levels are unknown. Detailed pup observations and clinical chemistry/ hematology evaluations conducted in the cross-fostering study provide some indication of pup toxicity in addition to the increase in mortality. There was an apparent delay in development of the surface righting reflex (the righting reflex took more than 3 seconds) in male and female pups reared by 6 mg/kg treated dams (3A) and in pups of dams dosed at 6 mg/kg (3B). Increases in several hematological parameters (most consistently hematocrit and mean red cell volume) were seen in pups reared by 6 mg/kg treated dams, and increases in urea were seen in pups from dams treated post-natally at 3 or 6 mg/kg (Appendix 6). These results reinforce the finding of pup toxicity in dams treated with dimethoate, but do not provide information regarding the cause of that toxicity. At this time, available data regarding the role of maternal toxicity in the pup mortality seen following dimethoate exposure do not allow for a definitive conclusion on this issue, but support a contribution of both pre- and post-natal exposure.

b. Litters vs. Pups as the Unit of Analysis

Most sources regard the litter as the most appropriate unit of statistical analysis for developmental toxicity studies and related studies, e.g. dominant lethal studies, where exposures are purely pre-natal and the dose to all fetuses in the litter are largely determined by the maternal variables, and also where the fetus depends on the mother for life. Pups from a single dam are considered to be closely related in terms of their exposure *in utero*, and thus lack statistical independence.

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Following weaning in a reproduction study, when the pups are directly exposed after lactation, it seems clear that the individual pup again becomes the appropriate unit of analysis. Between birth and lactation, independent living and exposure depend not only on the dose to the lactating dam and maternal behavior and health, but also on the behavior/health of each pup in successfully suckling and on the amount the pup consumes. Thus, determination of the most appropriate statistical evaluation during that time period is more complex.

In the dimethoate DNT study, there was an increase in the number of litters with pup death at 3.0 mg/kg (14 litters with at least one pup death, as opposed to 10 control litters); at 0.5 mg/kg, the number of affected litters (10 with at least one death) is the same as for controls. When the total number of pup deaths is compared across groups, there is an increase at 0.5 mg/kg (43 deaths), when compared with control (15 deaths) or low dose (11 deaths) groups. The increase in number of deaths is seen during both the PND 1-4 and PND 5-11 intervals, at both 0.5 and 3.0 mg/kg doses. Evaluation of pup death as mean number of dead pups per litter or as percent of liveborn also shows a dose-related increase. The increase in pup death is statistically significant at the mid-dose (0.5 mg/kg/day) when evaluated as the total number of deaths, but not when evaluated as a litter effect. A BMD analysis of pup mortality from the main DNT study resulted in a $BMDL_5$ of 0.27 mg/kg/day, falling between the mid and low doses, and consistent with the NOAEL/LOAELs determined for the study. All of the candidate BMDS models considered for estimating BMD values for pup death provide options for modeling pup death data at the individual level or the litter level. In the model selected for use in this analysis, no litter specific covariates were specified, however interlitter correlation (i.e. a litter effect) was modeled.

In the cross-fostering study, there is also an increase in the number of litters with multiple pups dying, as detailed in Table 8 (distribution of number of deaths by litters):

- ❏ No control litters have more than one death
- ❏ There are 5 litters with multiple deaths in pups exposed to 3.0 mg/kg only pre-natally
- ❏ 3 litters with multiple deaths in pups exposed to 3.0 mg/kg only post-natally

- ❑ 5 litters with multiple deaths in pups exposed to 6.0 mg/kg only pre-natally

- ❑ 9 litters with multiple deaths in pups exposed to 6.0 mg/kg only post-natally

- ❑ 16 litters with multiple deaths in pups exposed to 6.0 mg/kg both pre- and post-natally.

Thus, although the number of litters with at least one pup death was increased only in the two groups of pups exposed post-natally at 6 mg/kg (the 'post-natal only' and 'pre-and post-natal' 6 mg/kg groups had at least one death in 15-16 litters, compared to 12 litters for controls and 10-11 litters in other treatment groups), the number of litters with multiple deaths was increased in all treatment groups.

Another issue raised with respect to the analysis of the pup mortality seen in the dimethoate DNT study was the inclusion in the analysis of pups from litters that were humanely sacrificed. In the main dimethoate DNT study, a substantial portion of the total pup deaths at the mid dose can be attributed to a single incident of total litter loss occurring at that dose (15 of 43 pup deaths at 0.5 mg/kg occurred due to litter sacrifice; two additional pups from that litter died prior to the litter sacrifice). Three dams with total litter loss were also observed at the high dose (3.0 mg/kg), with 38 of 89 pup deaths at that dose attributed to humane sacrifice. There were no instances of total litter loss in the control or low dose groups. Although total litter loss is sometimes excluded from evaluation of pup survival, the EPA and PMRA included them in this instance.

Total litter loss is uncommon in the relevant historical control data base for the performing laboratory: for 11 studies, conducted between September, 1996 and August, 1999, one study showed total litter loss in 2/25 litters; in the other 236 litters, there were no instances of total litter loss. Historical control data recently submitted from 5 additional studies conducted from October, 2000 to September, 2002 show total litter loss in 1/24 litters for each of two studies (both conducted in 2002), with no litter loss in the other 3 studies. Thus, the loss of 3 litters at the high dose in the DNT main study (conducted in 2000/2001) is outside the control range for this species and strain, and exceeds that seen in 16 available historical control studies.

The sacrificed pups were moribund and were from litters where other pups had already died (two pups in the mid-dose litter had died prior to sacrifice; at the high dose 11/14 pups from one

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litter, and 2/16 pups from a second litter had died prior to sacrifice). Observations conducted during the study indicate that all pups from the sacrificed litters (both mid-dose and high dose) were cold to touch, underactive, and had little food in their stomachs (Appendix 5). EPA and PMRA must rely on the judgement of the investigator that the pups were moribund and that the sacrifice of these pups was a humane procedure to prevent additional suffering. It is, therefore, appropriate to consider these pups as having been rendered fatally ill, assume they would have died nevertheless, and include them in the total count of pup deaths. While use of such a procedure does introduce a measure of uncertainty about what otherwise might have been the fate of these pups, if such procedure was, as EPA and PMRA believe, uniformly applied, there is no reason to expect that it has biased the results.

c. Pup Mortality

Since developmental effects, including increased mortality, have been shown to occur as a result of single exposures during development (USEPA, 1991), this endpoint (increased pup mortality) is considered to be appropriate for use in risk assessment for single dose exposures. Results from the cross-fostering study at the 6 mg/kg dose, which demonstrated an increase in pup death following pre-natal only, post-natal only, or combined pre- and post-natal exposure, provide no data to contradict the assumption that a single exposure to dimethoate at 6 mg/kg could result in increased pup mortality. However, a clear effect on pup mortality was not seen in the cross-fostering study following pre- or post-natal exposure to 10 or 15 doses of 3.0 mg/kg. These results suggest that the increases in pup death seen at the 0.5 or 3.0 mg/kg/day doses in the main DNT study were not due to a single exposure during a critical window, but were more likely a result of continuing exposure throughout gestation and early lactation. Thus, the assumption that the increase in pup mortality seen at the 0.5 mg/kg/day dose could have been the result of a single maternal exposure is not supported by the results of the cross-fostering study.

d. Brain Cholinesterase Inhibition: Protective for Pup Death Endpoint?

In the main DNT study, increases in pup mortality were seen at doses that also caused inhibition of brain ChE following repeated dosing. BMD analyses for pup mortality and brain ChE were conducted to provide more information regarding the relationship between brain ChE inhibition and pup mortality, as well as to

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evaluate the relative sensitivity of young and adult animals to brain ChE inhibition following dimethoate exposure.

Evaluation of results from the BMD analysis for the repeated-dose studies reveals no age-related difference in susceptibility to dimethoate-induced brain ChE inhibition. BMD_{10} and $BMDL_{10}$ values for brain ChE inhibition following repeated dosing are very similar across age groups (range 0.20-1.0 mg/kg/day for BMD_{10} and 0.2-0.7 mg/kg/day for $BMDL_{10}$). Low end $BMDL_{10}$ values for brain ChE inhibition (0.2 mg/kg/day) are lower than the $BMDL_{10}$ values for pup mortality (0.6 mg/kg/day) and are comparable to $BMDL_5$ values for pup mortality (0.3 mg/kg/day).

$BMDL_{10}$ estimates for brain ChE inhibition following single doses of dimethoate are also comparable for pups and adults (1.3-2.0 mg/kg). Although these doses are higher than the $BMDL_5$ and $BMDL_{10}$ for pup mortality in the main DNT study, they are lower than the dose producing minimal effects in relation to pup death in the cross-fostering study (3.0 mg/kg/day following isolated pre-natal or post-natal exposure).

Although these results provide no information to support brain ChE inhibition as a cause of pup mortality, comparison of the results of the BMD analyses for pup mortality and brain ChE inhibition provide a reasonable basis for the conclusion that brain ChE inhibition occurs at doses similar to or lower than those causing increases in pup mortality, given similar exposure scenarios.

These analyses lead to the following conclusions:

1) The BMD analyses indicate that dose/response relationships for brain ChE inhibition are similar across age groups;

2) Brain ChE inhibition occurs at doses similar to or slightly lower than those causing increases in pup mortality;

3) The $BMDL_{10}$ for brain ChE inhibition is similar to the $BMDL_5$ for increased pup mortality following repeated dosing.

4) The $BMDL_{10}$ for brain ChE inhibition is lower than the dose assumed to be associated with pup mortality following acute dosing

Based on this analysis: (1) dimethoate risk assessments based on a lack of inhibition of brain ChE will be protective against pup

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mortality for acute and repeated-dose scenarios; and (2) for dimethoate, use of adult ChE inhibition endpoints will be protective of effects in the young, for exposure routes where age-specific data are not available.

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D. Weight of the Evidence and Summary

Dimethoate is an OP that exerts its neurotoxicity by binding to, and phosphorylation of, the enzyme acetylcholinesterase in the central (brain) and peripheral nervous systems. Dimethoate undergoes oxidative desulfuration to its more potent ChE inhibiting oxon (omethoate). In the rat, approximately 5% of dimethoate is converted to omethoate.

The critical effect[1] for many OPs is the inhibition of ChE (in the brain or blood compartment). However, in the case of dimethoate, both ChE inhibition and pup mortality are its critical effects. This is a unique finding for this OP because pup mortality has not been found to be the critical effect or the lowest observed adverse effect, for other OPs based on two-generation reproductive rat studies and on the rat DNT studies submitted and reviewed to date.

Pup mortality as a critical effect for dimethoate was first observed in a rat DNT gavage study. In the main DNT study, there is a statistically significant and dose-related increase in total pup mortality at the 0.5 and 3.0 mg/kg/day dose groups when pups are evaluated as individuals. Similarly, although not statistically evaluated, a dose-related increase in mean pup mortality/litter was observed. No effects on pup mortality are found at 0.1 mg/kg/day. Most of the deaths occur on PND 1-4.

The litter is typically considered the appropriate unit of analysis for developmental and reproductive toxicity studies. For study designs such as the DNT, however, where post-natal pup survival is dependant on the behavior and health of both the dam and the pup, it is important to consider both the litter and the individual pup separately as units of analysis. As seen in the 0.5 and 3.0 mg/kg/day dose groups of the main DNT study, when the whole litter(s) from one or two dams die or are humanely sacrificed, it is important to evaluate the impact of whole litter loss on the total pup mortality and to consider the degree to which this entire litter loss influences the total count. In the 0.5 and 3.0 mg/kg/day groups, the whole litter from one and three dams, respectively, died or were humanely sacrificed. These losses accounted for 15 of 43 pups and for 38 of 89 pups in the 0.5 and 3.0 mg/kg/day dose groups, respectively. This represents mean pup mortality/litter of 1.8 for the 0.5 mg/kg/day dose group and 3.7 for the 3.0 mg/kg/day dose level, whereas the control group only shows that 0.6 pups/litter died. Removal of whole litter losses from the mean/litter calculations result in 1.2 and 2.4 for the 0.5 and 3 mg/kg/day dose groups, respectively. Thus, on recalculation of the mean pup mortality/litter without the pups from whole litter losses, a dose-related increase in pup mortality is still observed.

[1] A *critical* effect is one considered the most sensitive endpoint from the most appropriate species.

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It is important to point out that total litter loss appears to be an infrequent occurrence in the DNT study. In the historical control data, which included 11 studies conducted between September, 1996 and August, 1999, only one study showed total litter loss in 2/25 litters; in the other 236 litters from these 11 studies, there were no instances of total litter loss. In more recent studies conducted from October, 2000 to September, 2002, however, total litter loss was observed in 1/24 litters for each of two studies (both conducted in 2002), no litter loss was observed in the additional three studies. Although two of these five recent studies show whole litter loss in untreated animals, overall the historical control data provide evidence for low incidence of whole litter losses in untreated rats. The BMD analysis includes both the individual pups and the litter as part of the model, taking into account both these variables.

Although the pup mortality observed at both the 0.5 and 3.0 mg/kg/day dose levels in the DNT study appears to be dose- related, and thus treatment-related, this finding is not supported by other studies that had similar exposure regimes (repeated gavage dosing at similar dose levels). In the comparative ChE inhibition study, no pup mortality was observed at any dose (i.e., 0.1, 0.5 and 3 mg/kg/day). In addition, the range-finding study showed no increased pup mortality at 0.2 or 3.0 mg/kg/day. An increase in pup mortality was observed (total litter loss = 2 of litters) at the highest dose tested (6.0 mg/kg/day) in this study. In the cross-fostering gavage study of dimethoate, although a slight increase in total number of pup deaths was observed at 3.0 mg/kg/day following either pre-natal only or post-natal only exposure, the results at this level are difficult to interpret. Lastly, pup mortality was found at 6 mg/kg/day following pre-natal only, post-natal only, and combined pre- and postnatal exposure in the cross-fostering study.

The rat multi-generation reproductive studies on dimethoate and omethoate are important to evaluate given that exposure extends over the entire period of development up to sexual maturation, and viability is evaluated. Although doses are not comparable for dimethoate and omethoate, similar effects on pup survival are seen for both chemicals. Both two-generation reproductive toxicity studies on dimethoate are dietary studies; similar high doses were used, of approximately 6 mg/kg/day. No clear increase in pup death was seen in either study; however a reduction in live births was seen in one study at the 6 mg/kg/day dose level. In a one-generation range-finding reproductive toxicity study with dimethoate, dose-related changes in reproductive parameters were seen starting at 3.9 mg/kg/day (decreases in implantation rate and litter size at birth, increases in post-implantation loss), and increases in pup mortality were seen at doses of 5.8 and 7.5 mg/kg/day. Two multi-generation reproductive toxicity studies are also available for omethoate (see Appendix 12). A drinking water study found pup mortality at the highest dose tested (1 mg/kg/day), most notably in the second generation. In a feeding study conducted at doses up to 0.5 mg/kg/day of omethoate, small increases in pup mortality were noted in the

second generation (note: significant deficiencies were noted in the study protocol of the feeding study).

The association between pup mortality observed in the DNT study and brain ChE inhibition is unclear. Following treatment with the lowest dose producing pup mortality (0.5 mg/kg/day) in the DNT study, only minimal brain ChE inhibition was found in the GD 20 dams (10%), fetus (10%) and the PND 4 pups (8%) for males. At the next highest dose (3 mg/kg/day), there was more pronounced brain ChE inhibition (dams 60%, fetus 33%). The small amount of brain ChE inhibition (7-13%) in PND 4 pups does not support a link between pup mortality nor a "burst" of exposure to dimethoate via lactation. The association between brain ChE inhibition as a causative factor in the pup deaths is also called into question by the results of the comparative ChE and range finding studies. In the comparative ChE study no pup mortality was observed, but the highest dose tested (3 mg/kg/day) produced pronounced brain ChE inhibition in the dams (60%) and fetuses (33%) (albeit, minimal inhibition (13%) was found in the PND 4 pups). In the range-finding study, no pup mortality was found at 3 mg/kg/day of dimethoate although greater than 70% brain ChE inhibition was found in the dams and 22-24% inhibition in the fetus. In addition, no increase in post-natal pup deaths was found in the multi-generation reproductive study with dimethoate, where greater than 60% brain ChE inhibition was found in dams (albeit, little brain ChE inhibition found in PND 4 pups) at the highest dose tested (6 mg/kg/day).

Although the underlying basis of the pup mortality is unclear, maternal toxicity does not appear to be the only determining factor. In some studies where significant maternal brain ChE inhibition was observed, increases in pup mortality were not observed. With the exception of the special observations made in the cross-fostering study, no clinical signs of overt toxicity were observed in dams at any dose even where pup death occurred. Lastly, in the cross fostering study, which was designed to address this issue, no clear correlation could be drawn between maternal behavior and pup death.

Although no clear association can be made between a specific level of brain ChE inhibition and an increase in pup death, results of the BMD analyses would appear to support the conclusion that protection against brain ChE inhibition will also result in protection against increased pup mortality following repeated dosing. No consistent age-related differences were seen in calculated BMD_{10} or $BMDL_{10}$ values for brain ChE inhibition; these values were similar to or lower than those calculated for increases in pup mortality from the main DNT study (the most sensitive study for that effect). Although the BMD_{10}/$BMDL_{10}$ is higher for ChE inhibition following a single dose, results of the recent cross-fostering study may support a conclusion that increased mortality is not seen following a single exposure at doses up to 3.0 mg/kg. Thus, use of the acute $BMDL_{10}$ values for brain ChE inhibition (1.3-2.0 mg/kg) could also be protective for increased pup mortality which might be seen after a single exposure at doses

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greater than 3.0 mg/kg (for example, at 6.0 mg/kg/day in the cross-fostering study).

In conclusion, several studies (i.e., the main DNT, range-finding study, and cross fostering studies, the one-generation range-finding reproductive toxicity study, and the omethoate reproductive toxicity studies) demonstrate increased pup mortality following maternal exposure to dimethoate. In addition, there were reduced live births in a rat multi-generation reproductive toxicity study and in the one-generation range-finding reproductive toxicity study with dimethoate. The important issues to address in this hazard assessment are the characterization of the dose response and also the underlying basis for the pup deaths. Although the comparative ChE inhibition, range-finding, and cross-fostering studies are not consistent with the findings of pup mortality in the main DNT study, it is concluded that the pup mortality observed at both the 0.5 and 3.0 mg/kg/day dose levels cannot be discounted as treatment related. This conclusion is based on the statistically significant response at both the 0.5 and 3 mg/kg/day doses and the dose-related nature of the response. The underlying basis of pup mortality is not understood. The available data do not support maternal toxicity as being the only determinant of pup mortality.

Based on the overall weight of the evidence which shows a dose-related increase in pup death in the mid- and high-dose pups, this finding is considered to be treatment-related and adverse at both the mid- and high doses. The key evidence includes: pups were reported to be cold to the touch and unresponsive; low incidence of total litter loss in performing laboratory; similar effect observed in other studies–although dose levels differed; qualitative increased pup death/litter (although does not reach statistical significance until 3 mg/kg/day); and quantitative increase in pup death when evaluated as individuals. It is appropriate to consider the increase in pup mortality as a treatment-related effect following dimethoate administration at 0.5 mg/kg in the main DNT study.

In conclusion, the current analysis supports the use of brain ChE inhibition as an appropriate endpoint for acute or repeated-dose risk assessment scenarios, based on the following:

- ❏ Brain ChE inhibition occurs at doses similar to or lower than those causing ChE inhibition in other compartments

- ❏ BMD analyses results indicate a very robust dose-response curve for brain ChE inhibition, with similar BMD_{10} values from studies with varying modes of administration (dietary or gavage) and durations (short term for DNT studies and longer term for reproduction studies);

- ❏ BMD analyses results indicate similar dose-response curves at all ages, with no difference in BMD_{10} values for different age groups following similar exposure durations;

- ❑ Comparison of BMR dose levels for brain ChE inhibition and pup mortality following repeated dosing indicates that ChE inhibition occurs at doses similar to those associated with increases in pup mortality;

- ❑ Evaluation of pup mortality data from the cross-fostering study reveals clear increases in mortality only at the highest dose following short-term exposure, indicating that increased mortality at lower doses occurs only with repeated dosing;

- ❑ Comparison of the NOAEL for increased pup mortality from limited dosing with the BMD_{10} for brain ChE inhibition following a single dose indicates that brain ChE inhibition occurs at doses below those causing a clear increase in pup mortality.

Therefore, regulation of dimethoate exposure at levels below those causing brain ChE inhibition in adults will also protect against brain ChE inhibition and increased mortality in pups.

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