Dimethoate:

Issues Related to the Hazard and Dose Response Assessment

July 6, 2004

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

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ChE	Cholinesterase
DCI	Data call-in (refers to notice)
DER	Data evaluation record
DPF	Dermal penetration factor
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

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DIMETHOATE: 2nd Report of the Hazard Identification Assessment Review Committee. Paul Chin. March 26, 2002.

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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
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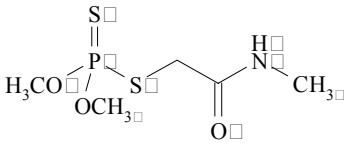
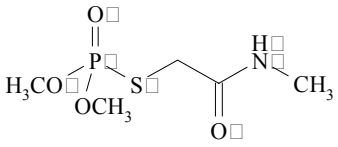
I. BACKGROUND

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 pesticides (OPs), 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 the cholinesterase (ChE) inhibition. In the case of dimethoate, the oxon metabolite is called omethoate. Although never 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).

Table 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* and *dermal absorption* 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 developmental neurotoxicity for dimethoate have become available. These include: development neurotoxicity (DNT; OPPTS 870.6300) study, a comparative ChE study on dimethoate, and a cross-fostering study. In addition, data evaluating dermal penetration have also been submitted which are important for characterizing dermal risk from occupational activities.

B. Issue 1: 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 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 have been 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 and the impact of maternal toxicity and pre-natal and post-natal dam exposures on pup mortality.

C. Issue 2: Dermal absorption of dimethoate

Dermal exposure to dimethoate and/or omethoate can occur during occupational activities. The toxic effects critical for risk assessment were identified from the dimethoate DNT study. Because this is an oral study and potential exposures to humans can occur by the dermal route, a dermal penetration factor (DPF) is needed for purposes of route-to-route extrapolation.

The registrant has submitted three dermal penetration studies (2 *in vivo* and 1 *in vitro*) in support of a DPF for dimethoate. The EPA and PMRA have evaluated these three studies. The results and uncertainties/deficiencies associated with each study are discussed in Section III; EPA and PMRA are soliciting comment on the utility of each.

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

A. Introduction

Several studies which characterize developmental neurotoxicity 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. 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 2-6.

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 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).

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. 1, 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.

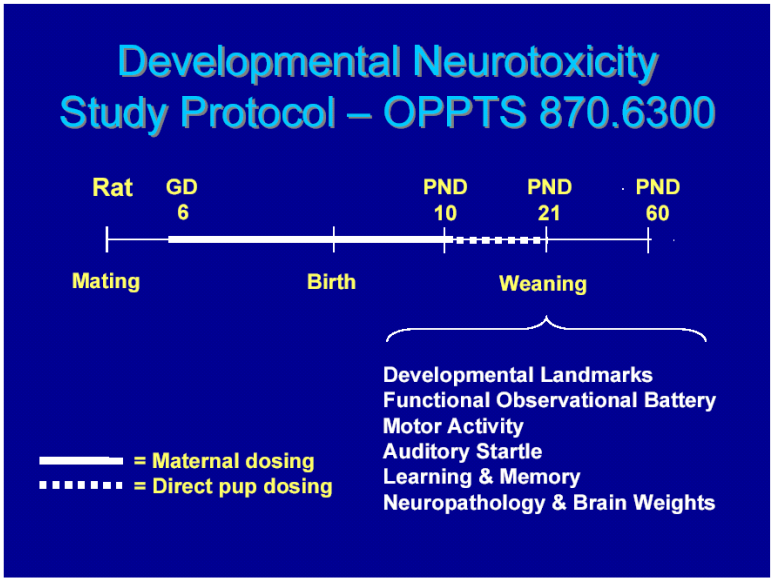


Figure 1.

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. 2, ChE). To enable comparison of pup versus adult sensitivity following a similar dosing regimen, cholinesterase 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 (pups and adults):	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 (pups):	39 days post-dosing
Day 1 (adults):	2 h post-dosing
Day 11 (adults):	2 h post-dosing

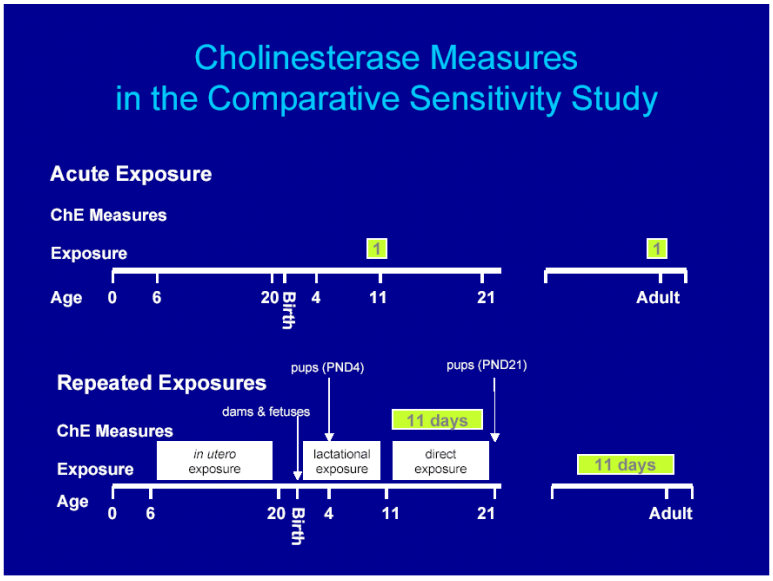


Figure 2.

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, Appendix 6) was conducted by the registrant. 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. Cross-fostering allows maternal animals to rear unrelated offspring. In this study 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 contaning 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-fostoring study).

C. Results: pup mortality, brain cholinesterase inhibition, maternal neglect

Findings from the main DNT study, comparative ChE and range-finding studies, and a cross-fostering study are briefly described below.

1. Main DNT study, comparative ChE study, and range-finding DNT study

Reproduction data from the main DNT study, comparative ChE study, and range-finding DNT study, including both pup and litter incidence, are presented in Tables 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]).

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 2). No increase in pup mortality was seen in the comparative ChE study (8-10 litters/group; see Table 3). 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 group (8-10 litters/group; see Table 4). Two litters at the 6 mg/kg dose in the range-finding study accounted for 26 of 41 deaths. One litter at 0.5 mg/kg dose and three at 3.0 mg/kg dose in the main DNT

study accounted for 15 of 43 and 38 of 89 deaths, respectively. It is notable that there was no excess mortality during the period of direct dosing to pups. Most of the pup mortality in the DNT and DNT range-finding studies was seen during early lactation (PNDs 1-11), and was accompanied by an increase in total litter loss.

Table 2. 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)	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)
p<0.01, Chi Square test.

Table 3. Post-natal Pup Mortality – Dimethoate Comparative ChE study

Dose (mg/kg/day)	live pups /litters born	Days of Lactation[a]					Total litter loss (day)	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
0.1 (LDT)	142/10	2	0	2	NA	NA	0	0.2
0.5 (MDT)	146/10	2	0	2	NA	NA	0	0.2
3.0 (HDT)	137/10	1	1	2	NA	NA	0	0.2

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

Table 4. 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 (day)	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)	2 (2,5)	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).
Statistical analysis not performed

2. Brain cholinesterase inhibition data.

ChE inhibition is the primary mode of action for an OP such as dimethoate; it is, therefore, important to consider the concordance between pup mortality and ChE inhibition in adult and juvenile animals. 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. ChE inhibition data are provided for plasma and RBC in Appendix 4. This document will focus on analysis and interpretation of brain ChE measurements from the comparative ChE and the DNT range-finding studies.

As seen in Table 6, statistically significant increases in brain ChE inhibition of 12-18% have been observed following acute exposures to 3.0 mg/kg dimethoate in pups and adults. Following acute exposures at the dose of 0.5 mg/kg, consistent, yet small, decreases of 2-4% brain ChE inhibition were observed in pups and adults. Statistically significant increases in brain ChE inhibition of 22-75% have been observed in GD 20, PND 21, and day 11 adults following multiple exposures to 3.0 mg/kg in the comparative ChE and range-finding studies (Table 6). Following multiple exposures, statistically significant decreases in brain ChE of 6-13% were observed at 0.5 mg/kg/day consistently across all groups of pups and adults. Results observed at the lowest dose of 0.1 mg/kg/day following acute and multiple exposures were less consistent, ranging from 4% higher than control values to inhibition levels of 12% with no clear pattern of effect across age or sex (Table 5).

Table 5. 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]			
	0.0	0.1	0.5	3.0
Acute Exposures (n=8/sex/group)				
Adult Males	13,794 ± 247	13,544 ± 802 (2)[g]	13,294* ± 241 (4)	12,131** ± 1096 (12)
Adult Females	14,150 ± 555	13,625 ± 445 (4)	13,850 ± 687 (2)	12,106** ± 827 (14)
PND 11 Males	6475 ± 244	6363 ± 236 (2)	6144* ± 360 (5)	5375** ± 290 (17)
PND 11 Females	6256 ± 195	6350 ± 338 (-2)	6125 ± 298 (2)	5144** ± 532 (18)
Repeated Exposures				
GD 20 Dams (n=8/group)[b]	12,838 ± 1373	13,044 ± 530 (-2)	11,563* ± 300 (10)	5094** ± 1081 (60)
GD 20 Fetuses (n=8/group)[b]	1781 ± 175	1569* ± 173 (12)	1600* ± 136 (10)	1188** ± 164 (33)
PND 4 Males (n=14-19/group)[c]	3137 ± 322	2817* ± 434 (10)	2889* ± 215 (8)	2744** ± 335 (13)
PND 4 Females (n=12-16/group)[c]	2823 ± 310	2941 ± 253 (-4)	2650 ± 287 (6)	2638 ± 269 (7)
PND 21 Males (n=8/group)[d]	10,375 ± 207	9944* ± 331 (4)	9044** ± 340 (13)	5675** ± 551 (45)
PND 21 Females (n=8/group)[d]	10,275 ± 376	9906 ± 313 (4)	9019** ± 248 (12)	5956** ± 965 (42)
Adult Males (n=8/group)[e]	14,100 ± 529	13.988 ± 662 (1)	12,700* ± 548 (10)	7469** ± 2484 (47)
Adult Females (n=8/group)[e]	14,869 ± 1400	13,913 ± 446 (7)	12,881**±845 (13)	6188** ± 1078 (58)
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)
PND 60 Females[f]	13,275 ± 277	12,950 ± 317 (2)	12,738* ± 243 (4)	12,744* ± 586 (4)

[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] Anmals 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# 0.05, **p # 0.01

Data from the range-finding study present similar findings to those from the comparative ChE study (Table 6). 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.

Table 6. Brain cholinesterase activities (µg/kg) from dimethoate range-finding study				
Dose (mg/kg/day)	**0**	**0.2**	**3**	**6**
Gestation day 20 (n=5/group)				
Dams	12710 ± 1333.9	12680 ± 640.9 (0)	3240 ± 411.4 (75)	1580 ± 195.6 (88)
Male fetuses	2150 ± 562.4	2320 ± 675.1 (+8)	1670 ± 564.1 (22)	1390 ± 780.5 (35)
Female fetuses	1970 ± 288.5	2100 ± 871.8 (+7)	1500 ± 500.0 (24)	1140 ± 638.7 (42)
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)
Female pups	9338 ± 2709.6	9886 ± 445.2 (+6)	5414 ± 756.7 (42)	3186 ± 827.3 (66)
Data taken from Tables 28-32, pp. 78-82, MRID 45529701. Number in parentheses is percent inhibition; data were not statistically analyzed				

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.

In the 3 mg/kg/day 'pre-natal only' and 'post-natal only' groups, there was a very slight increase in the total number of pup deaths. 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 (this effect was not as pronounced as that seen at the higher dose; see Table 8. Note, results at 3 mg/kg/day are difficult to interpret as 1) both pre-natal and post-natal exposure 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).

Table 7. 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)
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

Table 8. 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
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 strong indication of treatment-related maternal toxicity or clinical signs during gestation. 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 9a). 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 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 9b). 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 9b). 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.

Table 9a. 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						
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

Table 9b. 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
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, these maternal behaviors were not the sole cause of pup mortality. Rather, a combination of pre- and post-natal toxicity to pups and/or dams appear to have contributed to the observed pup mortality.

4. Standard developmental and reproductive toxicity studies in dimethoate and omethoate.

As animals are sacrificed prior to partition, typical developmental toxicity studies do not provide appropriate data for purposes of evaluating post-natal pup survival. However, to evaluate all the relevant information pertinent to the pup mortality, EPA and PMRA have considered the standard development toxicity studies with rat and rabbit for dimethoate and its oxon metabolite, omethoate. In the dimethoate developmental toxicity study with the rat, maternal toxicity was indicated by small pellet-like feces ($ 6 mg/kg/day) and body weight decrement ($ 18 mg/kg/day); no effects on resorptions or fetal body weight were observed. In the rabbit dimethoate developmental study, body weight decrement, tremors and unsteady gait were seen ($ 20 mg/kg/day) in maternal animals with decreased fetal weight at 40 mg/kg/day. In the omethoate rat study, maternal toxicity was noted by decreases in body weight or body weight gain, clinical signs including tremors and ataxia in rats ($ 3 mg/kg/day); no differences in pup weight or resorptions were noted in the rat study. In the rabbit omethoate study, ChE inhibition was noted in maternal animals at doses $ 1 mg/kg/day; increases in resorptions/dam and increased post-implantation loss compared to control at 1 and 5 mg/kg/day were also noted.

EPA and PMRA have also evaluated the available data related to pup survival from reproductive toxicity studies in dimethoate and omethoate. 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. Two omethoate reproductive toxicity studies are available; one administered in the feed and one administered in drinking water. The available dimethoate study was administered in the feed (Appendix 7). The following tables (10a & b, 11) provide viability and lactation indices and, when available, side by side comparison of brain ChE inhibition.

There is a poor dose-response relationship between pup morality and maternal dose in the dimethoate reproduction toxicity study even at doses resulting in approximately 60% brain ChE inhibition. A drinking water study found pup mortality at the highest dose tested (Table 10a & b, 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). In studies with omethoate and dimethoate, the dose-response for maternal ChE inhibition appears more steep than that of the pups–i.e., maternal animals exhibited higher levels of brain ChE inhibition.

Table 10 a. Comparison of results from omethoate reproductive toxicity studies. F_1 Generation

Omethoate Feeding (MRID 46195301)					Omethoate Drinking water (MRID 45806201)				
F1 Generation									
Dose	Index	Breeding $F1_A$	Breeding $F1_B$	Brain Cholinesterase Inhibition	Dose [b]	Index	Breeding $F1_A$	Brain Cholinesterase Inhibition (:mol-SH/mL)[a]	
								Parental	F1 Pups LD 21
Control	Viability	81.7 ± 2.6	91.2 ± 2.0	NA: Not measured in feeding study	**Control**	Viability	98.0	4.11 ± 0.32 F 4.61 ±0.25 M	6.33 ± 0.45 F 6.05 ±0.67 M
	Lactation	95.3 ± 1.7	97.1 ± 1.4			Lactation	100		
1 ppm (0.05 mg/kg/day)	Viability	93.2 ± 1.8**	93.7 ± 1.8		**0.5 ppm (0.04 mg/kg/day)**	Viability	99.0	4.00 ± 0.23 F 4.56 ±0.51 M	6.30 ± 0.57 F 5.92 ±0.74 M
	Lactation	95.7 ± 1.6	98.6 ± 1.0			Lactation	100		
3 ppm (0.15 mg/kg/day)	Viability	89.1 ± 2.1*	93.7 ± 1.8		**3 ppm (0.23 mg/kg/day)**	Viability	97.1	3.26** ± 0.13 (21)[a] F 3.80** ±0.24 (18) M	6.33 ± 0.53 F 6.25 ±0.52 M
	Lactation	93.4 ± 1.9	97.2 ± 1.4			Lactation	97.9		
10 ppm (0.5 mg/kg/day)	Viability	87.8 ± 2.4	96.5 ± 1.5		**18 ppm (1.2 mg/kg/day)**	Viability	95.9	2.70** ± 0.18 (34) F 2.83** ±0.16 (39) M	5.56**± 0.56 (12) F 5.80 ±0.45 M
	Lactation	91.3 ± 2.3	96.7 ± 1.6			Lactation	86.4		

* p<0.05, ** p<0.01, */** Dunnett test based on pooled variance.
[a] Number in parentheses is percent inhibition calculated by the reviewer.
[b] Dose expressed as minima of range for male animals
Viability index = No. live pups at birth/No. live pups at day 4 or 5 (pre-cull) × 100
Lactation index = No. live pups on day 4 or 5 (post-cull)/No. live pups day 21 or 28 × 100

Table 10b. Comparison of results from omethoate reproductive toxicity studies. F_2 and F_3 Generations

Omethoate Feeding (MRID 46195301)					Omethoate Drinking water (MRID 45806201)				
F2 Generation									
Dose	**Index**	**Breeding $F2_A$**	**Breeding $F2_B$**	**Brain Cholinesterase Inhibition**	**Dose [b]**	**Index**	**Breeding $F2_A$**	**Brain Cholinesterase Inhibition (:mol-SH/mL)[a]**	
								Adult	**Pups LD 21**
Control	Viability	93.1 ± 1.7	78.6 ± 3.7	NA: Not measured in feeding study	**Control**	Viability	90.3	6.75 ± 0.55 F 7.55 ±0.68 M	8.08 ± 0.56 F 7.77 ±0.41 M
	Lactation	92.8 ± 2.0	83.1 ± 4.1			Lactation	98.9		
1 ppm (0.05 mg/kg/day)	Viability	94.2 ± 1.5	85.3 ± 2.4		**0.5 ppm (0.03 mg/kg/day)**	Viability	96.8	5.68** ± 0.54 (16)[a] F 6.84* ±0.50 (9) M	7.67 ± 0.61 F 7.57 ±0.45 M
	Lactation	90.3 ± 2.2	88.4 ± 2.7			Lactation	98.9		
3 ppm (0.15 mg/kg/day)	Viability	80.0 ± 2.6**	64.2 ± 3.4**		**3 ppm (0.2 mg/kg/day)**	Viability	96.3	4.72** ± 0.48 (30) F 5.32** ±0.53 (29) M	6.86**± 0.95 (15) F 7.60 ±0.69 M
	Lactation	90.7 ± 2.4	76.3 ± 4.0			Lactation	100		
10 ppm (0.5 mg/kg/day)	Viability	85.6 ± 2.5*	62.5 ± 4.2**		**18 ppm (1.06 mg/kg/day)**	Viability	84.8	3.04** ± 0.16 (55) F 3.35** ±0.60 (56) M	6.45**± 0.77 (20) F 6.44** ±0.74 (17) M
	Lactation	88.2 ± 2.7	67.1 ± 5.4*			Lactation	85.8		
F3 Generation									
Dose	**Index**	**Breeding $F3_A$**	**Breeding $F3_B$**	**Brain Cholinesterase Inhibition**	**Dose [b]**	**Index**	**Breeding $F3_A$**	**Brain Cholinesterase Inhibition (:mol-SH/mL)[a]**	
								Adults	**Pups**
Control	Viability	95.7 ± 1.9	94.0 ± 1.9	NA: Not measured in feeding study	NA: Only 2 generations in drinking water study.				
	Lactation	97.9 ± 1.5	96.3 ± 1.6						
1 ppm (0.05 mg/kg/day)	Viability	94.1 ± 1.6	97.6 ± 1.1						
	Lactation	91.8 ± 2.1	98.2 ± 1.0						
3 ppm (0.15 mg/kg/day)	Viability	94.5 ± 1.6	97.2 ± 1.4						
	Lactation	91.3 ± 2.3	86.7 ± 3.1*						
10 ppm (0.5 mg/kg/day)	Viability	93.3 ± 1.9	92.5 ± 2.0						
	Lactation	82.7 ± 3.3**	92.6 ± 2.2						

* p<0.05, ** p<0.01,
[a] Number in parentheses is percent inhibition calculated by the reviewer.
[b]Dose expressed as minima of range for male animals
Viability index = No. live pups at birth/No. live pups at day 4 or 5 (pre-cull) × 100
Lactation index = No. live pups on day 4 or 5 (post-cull)/No. live pups day 21 or 28 × 100

Table 11a. Results from dimethoate reproductive toxicity study

Feeding (MRID 42251501)						
F1 Generation						
Dose	**Index**	**Breeding A**	**Breeding B**	**Brain Cholinesterase Inhibition (∶mol/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

Table 11b. Results from dimethoate developmental neurotoxicity (DNT) and comparative ChE study

DNT (MRID 45529703)				Comparative ChE (MRID 45529702)				
							Brain Cholinesterase Inhibition Acute Exposures (µ/kg)[a]	
Dose	**Index**	**Breeding‡ $F1_A$**	**Brain ChE Inhibition**	**Dose**	**Index**	**Breeding $F1_A$**	**Adults**	**Offspring PND 11**
Control	Viability	97.6	NA: Not measured in feeding study	**Control**	Viability	100	14,150 ± 555 F 13,794 ± 247 M	6256 ± 195 F 6475 ± 244 M
	Lactation	86.2			Lactation	100		
0.1 mg/kg/day	Viability	98.8		**0.1 mg/kg/day**	Viability	98.5	13,625 ± 445 (4)[a] F 13,544 ± 802 (2) M	6350 ± 338 (-2) F 6363 ± 236 (2) M
Lactation	Lactation	87.2			Lactation	100		
0.5 mg/kg/day	Viability	92.9		**0.5 mg/kg/day**	Viability	98.5	13,850 ± 687 (2) F 13,294* ± 241 (4) M	6125 ± 298 (2) F 6144* ± 360 (5) M
Lactation	Lactation	87.0			Lactation	100		
3.0 mg/kg/day	Viability	82.7		**3.0 mg/kg/day**	Viability	99.3	12,106** ± 827 (14) F 12,131** ± 1096 (12) M	5144** ± 532 (18) F 5375**± 290 (17) M
Lactation	Lactation	84.0			Lactation	98.8		

Brain Cholinesterase Inhibition Repeated Exposures (µ/kg)[a]					
Dose	**GD 20 Dams**	**GD 20 Fetuses**	**Day 11 Adults**	**PND 4 Offspring (grp 1-4)**	**PND 21 Offspring (grp 1-4)**
Control	12,838 ± 1373	1781 ± 175	14,869 ± 1400 F 14,100 ± 529 M	2823 ± 310 F 3137 ± 322 M	10,275 ± 376 F 10,375 ± 207 M
0.1 mg/kg/day	13,044 ± 530 (-2)	1569* ± 173 (2)	13,913 ± 446 (7) F 13,988 ± 662 (1) M	2941 ± 253 (-4) F 2817* ± 434 (10) M	9906 ± 313 (4) F 9944* ± 331 (4) M
0.5 mg/kg/day	11,563* ± 300 (10)	1600* ± 136 (10)	12,881* ± 845 (13) F 12,700 ± 548 (10) M	2650 ± 287 (6) F 2889* ± 215 (8) M	9019** ± 248 (12) F 9044** ± 340 (13) M
3.0 mg/kg/day	5094** ± 1081 (60)	1188** ± 164 (33)	6188** ± 1078 (58) F 7469** ± 2484 (47) M	2638 ± 269 (7) 2744** ± 335 (13)	5956** ± 965(42) F 5675** ± 551 (45) M

* p<0.05, ** p<0.01, [a] Number in parentheses is percent inhibtion calculated by reviewer,
‡ Lactation index for day 21 was calculated using day 11 (prior to the scheduled sacrifice) as the baseline, thus animals sacrificed for neuropathological evaluation are included as deaths in this calculation, but deaths prior to day 11 are not included.

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 is 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). This effect has not been replicated 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 is unknown. A potential reason may be related to the smaller number of litters/group used in those studies (8-10/dose, compared with 23-24 in the main study) and, therefore, less power to detect the effect. The effect seen at 6 mg/kg in the DNT range-finding study was replicated 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 are 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 dams 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.

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 (EPA and PMRA notes that 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 9a & 9b). 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 amounts or concentrations 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.

The link between ChE inhibition in pups or fetuses and the pup mortality observed in the dimethoate DNT study also remains unclear. With the exception of the main DNT study, pup mortality was observed at doses resulting in approximately 12% brain ChE inhibition following acute exposures and and approximately 40-60% following 11 exposures. In the main DNT, at 0.5 mg/kg/day, the LOAEL for pup mortality, only 2% and 13% brain ChE inhibition in pups (Table 5) were observed in acute and multidosing regimes, respectively. Although the level of brain inhibition was similar in pups, fetuses, and dams in the companion ChE inhibition study (see Table 5), cholinergic symptoms were not observed in treated dams and no treatment-related mortality was seen in dams. The cause of the pup mortality is unknown.

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.

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 (Table 2). 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, 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.

In the cross-fostering study, there is also an increase in the number of litters with multiple pups dying, as detailed in Table 7 (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 was 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 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 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 believes, uniformly applied, there is no reason to expect that it has biased the results.

D. Summary and Weight of the Evidence

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 the OPs is the inhibition of ChE (in the brain or blood compartment). However, in the case of dimethoate, both cholinesterase 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-generational 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 increase 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

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

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.

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 an additional three studies. Although *two of these five* recent studies show whole litter loss in untreated animals, overall the historical control provides evidence for low incidence of whole litter losses in untreated rats.

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 8- 10 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, the results at this level are difficult to interpret. Lastly, pup mortality was found at 6 mg/kg/day following post-natal only treatment and pre- and postnatal exposure.

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. The two-generation reproductive study on dimethoate is a dietary study conducted at approximately 0.1, 1, and 6 mg/kg/day. At the highest dose tested, no pup deaths are found. Reduced live births were, however, found at the 6 mg/kg/day dose level. Two multi- generation reproductive studies are available for omethoate. 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 cholinesterase 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 post-natal pup deaths were found the multi-generation reproductive study with dimethoate 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 only determining factor. In some studies where significant maternal brain ChE inhibition was observed, increases in pup mortality was 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.

In conclusion, several studies (i.e., the main DNT, range-finding study, and cross fostering studies) demonstrate increased pup mortality. In addition, there were reduced live births in the rat multi-generation reproductive study. 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 can not 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, EPA and PMRA consider this finding 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 increase 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 a treatment-related effect following dimethoate administration at 0.5 mg/kg in the main DNT study. Submission of additional data (the cross-fostering study) has also provided further information regarding maternal toxicity and pup death in addition to information regarding the influence of maternal toxicity or neglect on pup mortality. EPA is seeking input from the panel on appropriate analysis and interpretation of data regarding the increase in pup mortality following maternal dimethoate exposure.

III. DERMAL PENETRATION FACTOR

A. Introduction

"Data from dermal absorption studies allow the EPA to make risk determinations in cases where the toxic effect has been determined by the oral or inhalation route in the experimental animal, and the exposure to humans is by the dermal route." (OPPTS Guideline 870.7600) Dermal exposure to dimethoate and omethoate occur during occupational activities. Because toxic effects for dimethoate have been determined by the oral route in the experimental animal and exposure to humans can occur by the dermal route, a dermal penetration factor (DPF) is required for route-to-route extrapolation.

The registrant has submitted three (3) studies in support of a DPF for dimethoate. The studies submitted are:

- **^{14}C-Dimethoate: The biokinetics and metabolism in the rat**

  OPP first utilized a DPF of 11% derived from this study for the short- and intermediate- term dermal risk assessment. This *in vivo* dermal penetration study used dimethoate prepared in aqueous sodium carboxymethyl cellulose.

- **Study on the dermal penetration of ^{14}C-dimethoate in rats**

  Subsequent to the first study, OPP utilized a 28% DPF derived from this study for the short- and intermediate-term dermal risk assessment. This *in vivo* dermal penetration study used dimethoate prepared in formulation concentrate and in 10% and 0.5% aqueous dilutions thereof.

- **Dimethoate: In Vitro Absorption from a 400 g/L EC formulation through human and rat epidermis**

  This study showed that dermal penetration is at least 5-fold greater in rat skin than in human skin. Based on this *in vitro* study, the registrant recommends the use of a 5.6% DPF by dividing 28% from study II by 5.

The EPA and PMRA have evaluated these three studies; and the results, conclusions, the scientific merit, and uncertainties/deficiencies associated with each study will be discussed here. EPA and PMRA are seeking comment on the usefulness of each study for establishing a DPF for purposes of route-to-route extrapolation.

B. Study I - ^{14}C-Dimethoate: The Biokinetics and Metabolism in the Rat

1. Methods

In this study (MRID# 43964001, Appendix 8), five Wistar rats/sex/dose were exposed dermally to a suspension of radiolabeled technical dimethoate diluted in an aqueous sodium carboxymethyl cellulose solution (1% w/v) at doses of 10 and100 mg/kg dimethoate. Six hours after dosing, the application site was washed with cotton wool swabs soaked in soapy water. The animals were transferred to glass metabolism cages until sacrifice at 120 hours. Urine and feces were collected at 6, 12, and 24 hours and then at 24-hour intervals. Dermal absorption was calculated as the sum of the radioactivity in urine and cage wash, total tissues, and feces. This dermal dosing regimen in and of itself does not follow the minimal requirements for a dermal absorption study (see discussion below). Therefore, only a brief summary of this study will be presented below.

2. Results

Five days after treatment, dermal absorption based on the amount of radioactivity recovered in urine, feces and tissues was 9.9 % of the administered dose at 0.2 mg/cm^2 and 1-2% at 2 mg/cm^2. Radioactivity remaining in treated skin was 15.3% and 2.9% at the low and high dose respectively. Absorption values at 1 and 2 days are based on radioactivity in urine and feces only and are therefore not useful in the determination of absorption factors. However, comparison with the 5 day values at 0.2 mg/cm^2 suggests skin bound residues were released over time. Dermal absorption was approximately 1 mg/kg in terms of weight equivalent of dimethoate absorbed at each dose level suggesting that absorption was saturated at these treated doses.

Table 12

Dose Level	Mean Percentage of Dose Absorbed		
	1 day	2 day	5 day
10 mg/kg; 0.2 mg/cm^2 (2 mg/animal)	Urine, Feces: 7.9	Urine, Feces: 8.2	Urine, Feces: 9.2 Absorbed (Urine, Feces, Tissues): 9.9 Absorbable (Skin) : 15.3 Absorbed + Absorbable: 25.2
100 mg/kg; 2 mg/cm^2 (20 mg/animal)	Urine, Feces: 1-2	Urine, Feces: 1-2	Urine, Feces: 1-2 Absorbed (Urine, Feces, Tissues): 1.5 Absorbable (Skin): 2.9 Absorbed + Absorbable: 4.4

3. Uncertainties/Deficiencies

- The dose levels do not span the anticipated range of dermal residues.
- This study used a suspension of radiolabeled technical dimethoate diluted in aqueous sodium carboxymethyl cellulose (1% w/v). EPA guidelines recommend that the commercial formulation be the vehicle/solvent used in the dermal absorption studies; Use of a suspension of a technical dimethoate may produce less dermal penetration than use of a solution of the commercial formulation.
- The study was not adequate to characterize the fate of skin bound residues; if skin bound (potentially absorbable) residues were included in the absorption estimates, estimated DPFs may be higher.
- The exposure duration was limited to 6 hours.

C. Study II - Study on the Dermal Penetration of ^{14}C-Dimethoate in Rats

1. Methods

a. Structure of test material

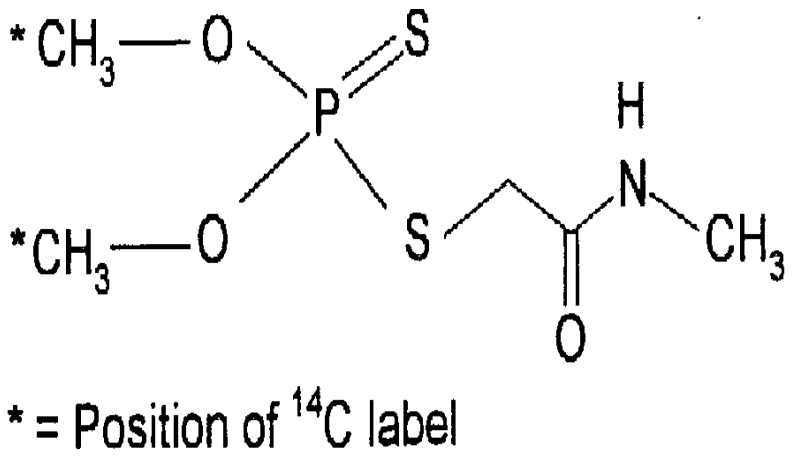


Figure 3.

This study (MRID# 45530501, Appendix 9) investigated dermal absorption in male rats following a single dermal administration of ^{14}C-Dimethoate (^{14}C was labeled in the O-methyl group) in a formulation concentrate (DIMETHOATE 400 g/l EC; 38 % active ingredient) and in 10% and 0.5% aqueous dilutions thereof.

b. Dose Selection

^{14}C-Dimethoate was applied at 3 concentrations. The nominal doses of 4.0 mg/cm^2 (formulation concentrate), 0.4 mg/cm^2 (1/10 aqueous dilution of formulation concentrate) and 0.02 mg/cm^2 (1/200 aqueous dilution of formulation concentrate) were selected to simulate user specific exposure scenarios and to cover a broad range of potential exposures. Calculation of the dermal dose was based on a 100 µl dose of the test item preparation applied to a total area of about 10 cm^2. The following table summarizes dose selection and grouping.

Table 13.

Dose Group	Dose (Nominal) (mg/cm^2)	Number of Animals	Exposure Duration (hrs)	Termination Time (hrs)*
I	0.02	16	1, 10 or 24	1, 10, 24, 72
II	0.4	16	1, 10 or 24	1, 10, 24, 72
III	4.0	16	1, 10 or 24	1, 10, 24, 72

* 72 hour group underwent skin wash at 24 hours and were carried to 72 hours

c. Dose Preparation

A stock solution of the radiolabeled test item was prepared in acetone. For the formulation concentrate dose, respective aliquots of the stock solution were taken and the acetone evaporated to dryness. The residue was dissolved and filled up to the final volume with the formulation concentrate. Dilutions of formulation concentrate were prepared in the same manner except that after the residue was dissolved in the respective volume of the formulation concentrate, this mixture was filled up to the final volume with the respective volume of doubly distilled water. The preparations were stirred to ensure a homogeneous preparation.

d. Animal Preparation and Dosing

An approximate area of about 10 cm^2 of the back skin of the rats was clipped free of hair and washed with acetone 24 h prior to dosing. A silicone rubber ring was glued onto the skin with tissue glue. The test material (about 10 µl/cm^2) was administered with a syringe. A nylon mesh was then glued to the surface of the silicone ring and a porous (semi-occlusive) bandage was wrapped around

the trunk of the animal. After dosing, animals were placed in metabolism cages for up to 72 hours.

e. Animal Observations and Sample Collection

After the respective exposure periods the protective cover was removed and the exposed skin was washed with a mild soap solution. For animals with a post-observation period, a new gauze and a new bandage was applied and a second skin wash was performed before sacrifice. Animals were sacrificed at the end of the various collection periods.

The following specimens/tissues were checked for remaining radioactivity: excreta (urine, feces), blood, carcass, skin, and skin wash(es). For mass balance estimates the cage wash as well as the protective cover (including the gauze with bandage and the silicone rubber ring) were also checked for radioactivity.

f. Dermal Absorption

The total amount of test compound absorbed by each animal is the sum of the quantity found in the excreta (urine, feces), organs/tissues, carcass and cage wash.

2. Results

Results from the high dose group are not presented because an excessive amount of the applied material was found on the application site cover and surrounding skin at all exposure periods for this dose group. Material on the cover is not available for absorption. Therefore data from the high dose group is unusable for determining the dermally absorbed portion of the applied dose.

A comparison of skin penetration at the low and intermediate dose showed that the percentage of radioactivity absorbed decreased with increasing dose indicating saturation of skin penetration after a 24 hour exposure period.

Mean recoveries of applied radioactivity from all dose groups ranged from 94 to 107%. The following table summarizes mean percent absorption at each time period for the low and intermediate dose groups.

Table 14				
Dose Level	Mean Percentage of Dose Absorbed			
	1 hour	10 hours	24 hours	72 hours [a]
0.02 mg/cm^2 (0.2 mg/animal)	Absorbed [b] : 5.68 Absorbable [c]: 13.69 Total: 19.37	Absorbed [b]: 28.15 Absorbable [c]: 14.19 Total: 42.34	Absorbed [b]: 38.06 Absorbable [c]: 12.97 Total:51.03	Absorbed [b]: 41.81 Absorbable [c]: 9.06 Total: 50.87
0.4 mg/cm^2 (4.0 mg/animal)	Absorbed [b]: 5.68 Absorbable [c]: 9.23 Total:14.91	Absorbed [b]: 24.98 Absorbable [c]: 8.29 Total: 33.27	Absorbed [b]: 25.33 Absorbable [c]: 6.58 Total: 31.91	Absorbed [b]: 31.69 Absorbable [c]: 4.40 Total: 36.09

[a] 72 hour group underwent skin wash at 24 hours and were carried to 72 hours
[b] Absorbed = urine, feces, cage wash, blood and carcass
[c] Absorbable = application site and surrounding skin

The study was not adequate to fully characterize the fate of skin bound residues; if skin bound (potentially absorbable) residues were included in the absorption estimates, estimated DPFs would be higher.

Results of the dermal penetration studies are described in detail in the DER (Appendix 9).

D. Study III - Dimethoate: *In Vitro* Absorption from a 400 g/L EC Formulation Through Human and Rat Epidermis

1. Methods

In an *in vitro* dermal penetration study (MRID# 45922602, Appendix 10), dimethoate (99.5% a.i., batch #291-Bse-75B) was administered to the isolated epidermal membrane from human and rat skin. The test samples were undiluted formulation (400 g/L EC formulation) and a 1:200 v/v water dilution of the formulation (2 g dimethoate/L). The undiluted formulation and the water dilution were applied to the epidermal membranes at a dose of 10 ul/cm^2; all applications were left unoccluded throughout the entire exposure period (8 or 24 hours).

For the human segment of the study, the skin samples were immersed in water at 60^{o}C for 40-45 seconds and the epidermis was teased away from the dermis. For the rat segment of the study the skins were soaked for approximately 20 hours in 1.5 M sodium bromide then rinsed with distilled water. The epidermis was carefully peeled from the dermis (Scott, R.C. et al, 1986). Exposure durations were 8 and 24 hours at the end of which time a sample of the receptor fluid was taken, the application site was washed, and the epidermal membrane was collected.

The samples analyzed were; skin wash, membrane, skin strippings (from human samples), epidermis and receptor fluid. The mean % of applied dose penetrated takes into account the radiolabeled material

found in the receptor fluid and the amount in the epidermis and tape strips (tape strips were used on human, but not rat skin).

2. Results

The mean total penetration was 0.6% in human skin and 25.67% in rat skin after 24-hour exposure to the 4 EC Concentrate (see Table 15 below). For the spray dilution, the total penetration was 5.1% in human skin and 62% in rat skin.

Based on the findings from the 8-hour experiment, dermal penetration is at least 5-fold greater in rat skin than in human skin to the spray dilution.

Table 15. Summary of results of *in vitro* dermal penetration study

	4 EC Concentrate Mean % of applied dose		1:200 spray dilution Mean % of applied dose	
	8 Hour Experiment	24 Hour Experiment	8 Hour Experiment	24 Hour Experiment
Human epidermis (receptor fluid + epidermis + tape strips)	1.05	0.6	14.46	5.1
Rat epidermis (receptor fluid + epidermis)	22.76	25.67	68.9	62.0
Ratio of % absorption of dimethoate by rat vs human skin	**22**	43	**5**	12

3. Uncertainties/Deficiencies

The EPA has received adequate comparative dermal absorption data on chemicals tested *in vivo* in the rat and *in vitro* with this preparation of rat epidermis to conclude that the preparation does not accurately model *in vivo* dermal absorption. The EPA's analysis of the data clearly shows that this particular *in vitro* procedure in the rat greatly overestimated dermal absorption for the majority of doses and exposure durations with the greatest error in the lower doses. They usually overestimate penetration but may underestimate and appear to be random. There is no common factor which would indicate a systematic error in the *in vitro* procedure. The overestimate ranged from 2 to 6.3 fold. The errors of the procedure do not appear to be correctable (Zendzian and Dellarco 2003).

There is an additional problem with the species comparison data. Skin preparation was different for human and rat. The human epidermis was obtained from human, female, whole skin samples. The skin samples were immersed in water at 60°C for 40-45 seconds and the epidermis was teased away from the dermis. For the rat, the skins were soaked for approximately 20 hours in 1.5 M sodium bromide then rinsed with distilled water. The epidermis was carefully peeled from the dermis. Scott, R.C. et al. (1986) presented the rationale for the use of 1.5 M sodium bromide for

effectively removing an epidermal membrane from both rat and human skin. However, in this study, and in all similar studies using human skin submitted to the EPA, heat separation has been used during preparation of human skin. Neither explanation nor reference has been given for using this method. Heat processing can be expected to denature the protein matrix of the epidermis (of the stratum corneum) resulting in an unpredictable decrease in permeability (Appendix 11).

E. Summary of dermal penetration factor issue

The results of Study I (^{14}C-Dimethoate: The Biokinetics and Metabolism in the Rat), suggest a DPF of 11%. However, this study had many limitations. For example, the dose levels do not span the anticipated range of dermal residues, doses were administered as a suspension of technical dimethoate diluted in aqueous sodium carboxymethyl cellulose (1% w/v) and exposure duration was limited to 6 hours.

A DPF of 28% was derived from Study II (Study on the dermal penetration of ^{14}C-dimethoate in rats). This dermal absorption study does satisfy the guideline requirement for a dermal absorption study (85-3) in rat. The EPA acknowledges that this dermal penetration study used the 4EC formulation which is not sold in the U.S.. EPA guidelines for a dermal absorption study recommend that the vehicle/solvent be the material used in the commercial formulation. Because both the 4EC and 4E formulations comprise dimethoate dissolved in similar organic solvents, little difference in dermal penetration is expected between these two formulations.

It is noted that neither of the *in vivo* studies is adequate to fully characterize the fate of skin bound residues. If these potentially absorbable residues were added to absorbed doses, estimated DPFs would increase substantially.

Study III (Dimethoate: *In Vitro* absorption from a 400 g/L EC formulation through human and rat epidermis) suggests dermal penetration is at least 5-fold greater in rat skin than in human skin. Based on this *in vitro* study, the registrant recommends the use of a 5.6% human DPF (by dividing 28% from Study II by 5). However, as described earlier, this *in vitro* methodology does not accurately model *in vivo* dermal absorption and can over- or underestimate dermal absorption (Zendzian and Dellarco 2003).

IV. REFERENCES

Hassan, A., S.M.A.D. Zayed, and M.R.E. Bahig. (1969). Metabolism of organophosphorus insecticides–XI: Metabolic fate of dimethoate in rat. Biochem. Pharmacol 18:2429-2438.

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Scott, R.C. ; M., Walker; Dugard P.H. 1986. In vitro percutaneous absorption experiments: A technique for the production of intact epidermal membranes from rat skin. J. Soc. Cosmet. Chem. 37, J/F 35-41

USEPA (2002). Revised Organophosphorus Pesticide Cumulative Risk Assessment. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, DC. June 10, 2002. http://www.epa.gov/pesticides/cumulative/rra-op/

Zendzian, R.P. and Dellarco, M. Validating in vitro Dermal Absorption Studies, An Introductory Case Study. in Alterative Toxicological Methods CRC Press (2003) pp 207-219.