UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 22, 2001

MEMORANDUM

SUBJECT: 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

TO: Patrick Dobak
Chemical Review Manager
Reregistration Branch 1
Special Review and Reregistration Division (7508C)

FROM: Kathleen C. Raffaele
Registration Action Branch 3
Health Effects Division (MC 7509C)
and
William F. Sette
Toxicology Branch
Health Effects Division (MC 7509C)

THRU: Stephen Dapson, Senior Scientist
Registration Action Branch 3
Health Effects Division (7509C)

CC: Paul Chin (MC 7509C)
Diana Locke (MC 7509C)

ACTION REQUESTED: Conduct a review of the 6(a) 2 submission, and review preliminary data from these 3 studies of Dimethoate.

In January, 2001, the Health Effects Division (HED) received a 6(a)(2) submission from Cheminova (12/15/00) transmitting information regarding adverse effects (early pup deaths) seen in a recently conducted developmental neurotoxicity study of Dimethoate.

Submissions on 2/13/01 and 3/09/01 provided additional data. A data audit of the developmental neurotoxicity study, a cholinesterase study, and a range finding study was conducted on 2/20/01-2/23/01 at Huntingdon Life Sciences Research Laboratories (Eye, Suffolk, England). A trip report (submitted on 3/7/01) describes the data gathered during the data audit. Only those elements of the data described below were evaluated for this memo.

The purpose of this memo is to provide a detailed review based on available preliminary data and to reach some preliminary conclusions about this data. The current memo builds on the trip report submitted on 3/7/01 and earlier reviews of this data and attempts to address 3 questions in considering the potential impact of this data on our current risk assessment of Dimethoate.

1. Is the pup mortality related to dimethoate exposure? Can it be attributed to maternal toxicity as proposed by the registrant? Or is it better seen as a direct effect of dimethoate on the pups? Do we know the basis of the mortality?
2. What is the lowest effect level for brain cholinesterase inhibition that is biologically significant in adults and in pups after acute and repeated exposure?
3. Does this study provide evidence of increased sensitivity or susceptibility in the pups?

Conclusions

These data and analyses are preliminary, and formal review of the final study report may warrant different conclusions from those given here.

1. **Pup Deaths.** Based on current evidence and analyses, we believe that the pup mortality is an effect of treatment. It is dose dependent, and appears significant at both 0.5 and 3.0 mg/kg. The cause of the deaths is unclear. They occur between the day of birth and day 11. A clear case has not been made by the registrant, nor appears evident to us from the data, that the deaths are primarily due either to maternal toxicity or to direct effects on the pups. Both dams (who are dosed directly during gestation) and their fetuses (who share a common blood supply) are exposed to the test material. The data on GD 20 show effects on ChEI in both dams and pups, confirming that biologically meaningful exposures to both are occurring. It is also not possible to separate the contribution of effects on the dams or on the offspring to pup deaths after birth.

2. **ChE LOAELs**. This study measures ChEI in pregnant dams and in fetuses after subacute exposure and in pups and in young adults after both acute and subacute exposures.

For acute exposures, we conclude that 0.5 mg/kg is an LOAEL in adult rats and in 11 day old pups, based on small (3-5%) but statistically significant decreases in brain ChE. Others may contest this view and a consensus conclusion may require further review.

For repeated exposures, we conclude that 0.1 mg/kg is an LOAEL for fetuses and pups, based on small but statistically significant inhibition of brain ChE in fetuses (12%), male pups on day 4 (10%), and in pups on day 21 (4%). Others may contest this view as well, so a

consensus conclusion may require further analyses and discussion. Significant inhibition of brain ChE in adults was seen at 0.5 mg/kg (10-13%).

3. **Susceptibility/Sensitivity.** We conclude that the data on pup mortality define increased susceptibility. In addition, brain cholinesterase data may provide evidence of increased sensitivity in the pups after repeated exposure.

4. **Implications for Risk Assessment.** Based on these conclusions, the endpoints previously selected for risk assessment, as well as the FQPA factor, should be re-examined.

The remainder of this memo provides more detailed analyses that provide the bases of these conclusions.

1. Pup Deaths

Main study. In our data audit, we spoke with study personnel to clarify several issues regarding interpretation of the litter and parturition records (provided by the registrant following the 6(a)(2) submission). They provided satisfactory answers to our questions, and using that information we have completed a tabulation of the pup deaths based on the litter and parturition records (see Table 1).

Table 1. Summary of reproduction data, including pup deaths occurring during PNDs 1-21.

Dose Group	Total litters	Mean pups/litter	Number of litters with pup loss	Total pup deaths (PNDs 1-21)	Mean number of dead pups/litter
Control	24	14.9±2.6	10	15	0.6
0.1 mg/kg/day	23	14.9±1.6	6	11	0.5
0.5 mg/kg/day	24	15.0±2.0	9 [1 TLL]	41	1.7
3.0 mg/kg/day	24	15.3±2.5	14 [3 TLL]	90	6.4

Data were extracted from submitted raw litter data record sheets. TLL = total litter loss

Total pup deaths and dead pups/litter were increased at 0.5 mg/kg/day and 3.0 mg/kg/day. The numbers of litters with pup loss were only increased at 3 mg/kg/day (14 vs. 10 controls). There were no changes in the total number of litters born or the number of pups/litter at birth. The number of pups dying at specified intervals during lactation are summarized below in Table 2.

Table 2. Postnatal Pup Mortality [a]

Dose (mg/kg/day)	Days of Lactation					Total litter loss (day)
	1-4	5-11	12-16	17-21	1-21	
0 (Control)	10 (7)	3(3)	2(2)	0(0)	15(10)	0
0.1 (LDT)	8 (5)	3(2)	0(0)	0(0)	11(6)	0
0.5 (MDT)	31* (8)	10* (3)	0(0)	0(0)	41*(9)	1 (2)
3 (HDT)	72 * (13)	13*(6)	1(1)	4(2)	90*(14)	3(2,3,4)

Number of pups (number of litters) [a] Includes pups that were found dead, missing and presumed dead, or sacrificed due to poor condition. * $p<0.05$, Chi Square test.

To evaluate the significance of the pup deaths we compared the total number of pup deaths on days 1-21, 1-4 and 5-11 with the number of live pups in a 2x2 table and using a Chi square analysis. At both 0.5 mg/kg and 3.0 mg/kg, pup deaths on days 1-21 and days 1-4 were significantly increased.

The number of deaths on days 5-11, after the period of total litter loss and prior to the beginning of direct dosing of the pups, was significantly increased at 3 mg/kg. There was also an increase at 0.5 mg/kg, but it was marginally significant statistically, with a Chi square p value of 0.047, and a non-significant yates corrected Chi square p value of 0.089.

Range-finding and Cholinesterase studies. We also examined litter records and death records for pups in the range-finding (CHV/068) and cholinesterase (CHV/070) studies on dimethoate. Each study had 10 litters/dose. The cholinesterase study used the same doses as the main study, while the range finding study used doses of 0, 0.2, 3, and 6 mg/kg/day, There was an increase in pup deaths at 6 mg/kg in the range-finding study, but no excess pup deaths were seen at 3 mg/kg in either study. The tabulated litter data from the range-finding and cholinesterase studies are shown in Table 3.

TABLE 3. PUP DEATHS IN RANGE FINDING AND CHE STUDIES

Dose (mg/kg)	Days 1- 4		Days 4_{ac} -11	
	Range-finding	ChE	Range-finding	ChE
0	5	0	1	0
0.1		2		0
0.2	1		0	
0.5		2		0
3.0	5	1	0	1
6.0	36; 1 TLL		1; 1 TLL	

N= 9-10 litters/group. from litter size tables. ac = after cull

2. Maternal Toxicity as a Contributor to Pup Deaths

One of the major questions we tried to address in our audit concerned the presence or absence of maternal toxicity during the dosing period, to evaluate the potential contribution of significant maternal toxicity to the pup deaths.

A. Total Litter Loss Evaluation

We tabulated a variety of available data on the dams that experienced total litter loss on postnatal days 1-4 and their offspring.

Table 4 . Data on Dams and Litters Sacrificed Between Days 1-4
[ ]: Numbers in brackets are the range for that dose group

	Day of Sacrifice	Body wt gain days 6-20	Litter Size	Mean litter wt (g)	Observations
0.5 mg/kg					
Dam 96	PND 2	137g [102-160]	17 [9-17]	5.7 % [5.0-7.6] 5.4 & [4.3-7.2]	All pups cold to touch; little food in stomach; underactive; 2 found dead
3.0 mg/kg					
Dam 54	PND 4	116g [87-156]	14 [8-19]	5.4 % [5.4-7.9] 4.9 & [4.9-7.4]	pups appear small and unfed; all but 3 found dead.
Dam 64	PND 3	141g	15	7.5% 6.9 &	cold to touch; little food in stomach; cold and underactive. 1 pup found dead
Dam 66	PND 2	107g	13	7.8% 7.1 &	cold to touch; little food in stomach; cold and underactive.

The observations in dams with total litter loss indicate that some pups were not nursing, cold to touch, i.e. quite ill. In a letter (16 Nov 2000) to Cheminova, the study director noted

that pups from 3 litters: 62, 64, and 66, "appear to be fed, but are cold to touch and scattered in the cages, perhaps suggestive of poor maternal care". Available copies of litter parturition records and other records do not show the observation of scattering or suggestion of poor maternal care. Nonetheless, it may have contributed to the litter loss. However, no observations related to poor nurturing were made of the other 2 dams that suffered total litter loss, nor in our record review was this noted in other observations of the dams.

Other measures for these dams and their offspring were generally within the range of that dose group. This was true for dam body weight gain during gestation. The litter size for Dam 96 was at the high end of the range for that 0.5 mg/kg group. For Dam 54, pup weights were at the low end of the range, but pups from dams 64 and 66 at the 3 mg/kg dose were nearer to the top of the range.

In summary, this data provides limited evidence of lack of maternal care, and scant evidence that large litter size or low birth weight pups played a critical role in the pup deaths. In view of the pup loss from 11 other litters, the incidence of scattered pups from 3 dams is much less than the scope of the effect noted.

B. Total Litter Loss Historical Control Data

It was also suggested by the registrant that total litter loss, noted earlier by the testing laboratory as a problem in reproduction studies in the CRL IGS rat, may have contributed to the total litter loss seen in this study, and therefore be an effect unrelated to treatment. So we attempted to evaluate any available data on this topic.

There are 2 important differences between the problem seen in the reproduction studies and the current studies. First, those dams were older at the time of breeding than the rats in this study, i.e., 16 weeks old in the reproduction studies, and 10-12 weeks old in these studies. Second, the testing laboratory provided 2 articles (Willoughby et. al., 1998, 2000) that demonstrate that animals bred at that later age showed an increased level of litter death in the first 4 days, and that reproductive performance, including offspring survival, is optimized when the VRF diet is used. The final protocol indicated that the diet in this study was the UAR VRF1 certified pelleted diet.

Data were provided on pre and postnatal studies in the IGS rat, where rats are most similar in age (11 weeks old) to those in the current studies (10-12 weeks old), although these did not use the VRF1 diet more recently found optimal. For 11 studies, conducted between 9/96 and 8/99, one study showed total litter loss in 2/25 litters. In the other 236 litters, there were no instances of total litter loss. Thus, the loss of 3 litters at the high dose in this study is outside the control range for this species and strain, prior to the revision in the diet used. The single litter loss exceeds that seen in 10/11 studies. Further, the pup loss seen in the main study goes well beyond the total litter losses seen in that study.

C. Maternal Observations

We spoke with study personnel regarding the types of observations conducted for dams and pups during lactation (for example, whether litters were gathered by dams, whether milk was detected in pup stomachs), and determined that such observations were conducted and that any findings would have been recorded on the litter and parturition data sheets. Other than as noted above, no observations related to poor maternal care were recorded in those records.

Evaluation of available data to determine whether any of the symptoms typically caused by agents interfering with cholinergic function (such as dimethoate) had been noted in dams during the main study revealed that there was no indication of cholinergic toxicity in the litter observation records, clinical observation records, or post-dosing observation records for pups or dams. We examined the list of symptoms evaluated for each animal, and found that cholinergic-type signs were included (PHM-OBS-001; File note #7).

There was also no indication of dose-related cholinergic toxicity found in the FOB results for dams or pups. Since the data were not tabulated, a comprehensive evaluation of all recorded signs could not be completed, however *ad hoc* tabulations were generated by us from the raw data sheets for several types of possible cholinergic signs, and no relationship to treatment was found. These tabulations included head shaking and gait changes for pups at several time points, and tremors, salivation, and urination for dams.

We tabulated tremors, salivation and urination as signs seen on survey of the FOB data frequently enough to be potential treatment effects, and because these were cholinergic signs (FOBs for dams were done prior to daily dosing on GD 12, 18, LD 4, 10). The following incidences were noted:

TABLE 5. Selected Tabulated Maternal FOB data:

	Tremors	Salivation	Urination (Scale of 1-3)			
			1	2	3	Total
Controls	6	6	7	1	2	10
0.1 mg/kg	5	6	4	6	4	14
0.5 mg/kg	4	7	5	6	6	17
3.0 mg/kg	6	9	4	6	1	11

# of rats affected; summed across 4 measurement days; # of litters 22-24.

Thus, there was no clear increase in the incidence of these cholinergic signs. These findings on cholinergic signs are consistent with the lack of cholinergic signs seen at similar doses in prior studies. It should also be noted that FOB observations were conducted prior to dosing, and thus might not reflect acute cholinergic effects that may have occurred (although any marked acute cholinergic symptoms should have been noted in post-dosing observations, as described above, and these were not evident).

D. Maternal and Pup Body Weights

Since changes in body weight can also be indicative of systemic toxicity, we examined available body weight data from all three studies for dams and pups during the relevant time period (GD20 to LD11). The data are presented in tables 6-8, below. None of these data have been statistically analyzed, so statistical significance of any changes cannot be determined at this time.

In the range-finding study, maternal body weight was slightly lower at 3.0 mg/kg (5-7%), and at 6.0 mg/kg (9-10%) than in controls. In that study there also was a lower pup body weight at 6.0 mg/kg (15-21%), which was apparent at birth and sustained throughout the day 1-11 time frame (see Table 6).

Table 6. Maternal and pup weight (g) - range-finding study.*

Group	LD 1	LD 4 (pre-cull)	LD 7	LD 11*
Dams				
Control	329±21	343±24	345±23	365±25
0.2 mg/kg	318±18	329±20	342±19	359±17
3.0 mg/kg	311±22	321±17	327±17	342±15
6.0 mg/kg	299±16	308±19	314±18	331±22
Male Pups				
Control	6.9±0.6	9.8±0.8	15.5±1.1	25.1±1.7
0.2 mg/kg	6.4±0.5	8.9±1.0	14.4±1.4	23.7±3.0
3.0 mg/kg	6.6±0.4	9.4±0.6	15.5±1.1	25.5±2.4
6.0 mg/kg	5.7±0.8	8.0±1.5	12.3±2.0	20.6±3.9
Female Pups				
Control	6.6±0.9	9.2±0.9	14.6±1.2	23.9±1.5
0.2 mg/kg	6.0±0.4	8.4±0.8	13.8±1.0	22.1±2.9
3.0 mg/kg	6.2±0.3	9.0±0.6	14.9±1.0	24.3±2.1
6.0 mg/kg	5.5±0.7	7.6±1.7	11.7±2.4	20.4±3.5

*Pup weight was calculated by litter, except on day 11 where the mean includes selected offspring only, by individual. N=8-10/group for LD 1-7 (dams and pups); 15-20/group on LD 11 (pups).

As can be seen from Table 7, dimethoate administration had no effect (<5%) on maternal body weight at any dose or time point in the main study. This study also showed no effect on pup weight at birth (Day 1) for any dose, however there was a slight decrease in mean

body weight at later time points (6-10%), at the 3.0 mg/kg dose only.

Table 7. Maternal and pup weight (g) - Main study.

Group	GD 20	LD 1	LD 4 (pre-cull)	LD 7	LD 11
Dams					
Control	427.5±34.8	332.5±26.5	340.8±24.6	349.8±24.4	361.4±24.9
0.1 mg/kg	414.1±29.6	321.0±26.0	333.9±26.4	338.8±27.4	348.9±29.2
0.5 mg/kg	421.1±32.2	328.5±29.3	336.6±26.7	342.7±26.6	356.0±25.4
3.0 mg/kg	420.7±42.9	325.8±36.2	331.7±32.8	338.8±32.1	352.2±34.0
Male Pups					
Control		6.5±0.6	9.0±1.2	14.0±2.6	24.2±3.3
0.1 mg/kg		6.4±0.5	8.5±0.7	14.3±1.4	23.7±2.2
0.5 mg/kg		6.5±0.6	9.2±1.4	15.1±2.8	24.7±4.2
3.0 mg/kg		6.4±0.6	8.3±1.5	13.1±3.1	21.9±5.6
Female Pups					
Control		6.2±0.5	8.5±1.2	13.4±2.6	23.3±3.4
0.1 mg/kg		6.0±0.5	8.1±0.7	13.5±1.7	22.8±2.1
0.5 mg/kg		6.1±0.7	8.8±1.2	14.1±3.0	23.4±4.3
3.0 mg/kg		6.1±0.6	7.9±1.5	12.6±3.2	21.3±5.6

Pup weight was calculated by litter. N=21-24/group.

In the cholinesterase study, maternal data were not available and pup weights, shown in Table 8, showed no effect at any dose.

Table 8. Pup weight (g), by litter - Cholinesterase study.

Group	LD 1	LD 4 (pre-cull)	LD 7	LD 11*
Male Pups				
Control	6.6±0.6	9.7±1.1	15.4±1.9	25.6±2.2
0.1 mg/kg	6.4±0.5	9.6±0.8	15.5±1.1	25.7±1.8
0.5 mg/kg	6.6±1.0	10.0±1.1	15.7±1.5	25.6±1.5
3.0 mg/kg	6.7±1.0	9.9±1.3	15.5±2.0	25.2±3.0
Female Pups				
Control	6.4±0.6	9.2±1.0	14.5±1.6	24.4±2.4
0.1 mg/kg	6.1±0.3	9.2±0.8	14.9±0.8	24.7±1.2
0.5 mg/kg	6.2±1.0	9.4±1.2	14.8±1.6	24.5±1.8
3.0 mg/kg	6.2±1.0	9.6±1.5	14.9±2.0	24.4±3.1

Maternal body weight was not available for the cholinesterase study. N=10/group.

Overall, for the three studies, maternal body weight was not seriously affected at the 3.0 mg/kg dose. Pup weight at birth was also not affected at 3.0 mg/kg, but did show some decrease at later time points in the main study. There was no indication of an effect on maternal body weight or pup body weight at the 0.5 mg/kg dose, where excess pup deaths also occurred.

It should be noted again that these conclusions are preliminary, pending receipt of tabulated data and more detailed and definitive analyses of these (and other) study elements in the final report. Among other things, histopathological examination of day 21 offspring of all major organs, in addition to the central nervous system, will be made.

Discussion

While one can speculate about the extent to which these deaths reflect lack of maternal care, it is not clear that evidence of a lack of maternal care is itself a cause or effect of the pup morbidity, i.e., are the pups sick and uncared for because the dam is neglecting them, or did she abandon them because they are sick? This is not an issue that can be resolved within this study design. Second, these pups were exposed *in utero* throughout gestation, and the ChE data shows that both dams and pups had ChE inhibition on GD20, so how can one separate the potential pup effects related to such exposure from maternal effects? Third, if prenatal exposure to this material causes mothers to neglect their young to the point of their death, would not one prudently regard this as adverse?

Fourth, given that none of the dams in question were noted to be *in extremis*, or even to display cholinergic signs, the effects on the offspring, direct or indirect, were far more serious to those offspring.

In summary, at this time, there is limited evidence of maternal neglect (at the 3.0 mg/kg dose only), and no evidence of overt maternal toxicity in terms of clinical signs or body weight, or effects on reproductive indices, i.e., litter size, and pup body weight at birth (at either dose), that correlates with the pup deaths seen at the mid and high dose levels. Thus, it should be concluded that in the search for the cause of the pup deaths, neither maternal nor direct fetal/pup toxicity can be ruled out.

Cholinesterase Inhibition

This study measured ChEI in pregnant dams and fetuses at the end of gestation, and in pups and in young adults after both acute and subacute exposures. Tables 9-11 show the percent decrease in brain, plasma, and red blood cell cholinesterase activity, respectively. We reviewed copies of the individual data and examined the descriptions of the methods used. We found the methods to be acceptable, and nothing in the individual data raised questions concerning the conclusions suggested by the group data. For example, there were no outliers found that would impact the significance of a finding in one group or on one day.

Brain cholinesterase. At the end of gestation, statistically significant inhibition of brain cholinesterase was seen at all doses for fetuses (12-33%), while for dams significant inhibition was seen only at the mid and high dose (10-60%).

Statistically significant decreases were also seen at all doses for male pups at PND 4 and PND 21 (4-10%).

Statistically significant inhibition was not found following a single dose to pups or adults at 0.1 mg/kg/day, but was found in males at both ages following a single dose at 0.5 mg/kg/day. The magnitude of inhibition was small, but statistically significant (3-5%).

Table 9. Percent decrease of brain cholinesterase activity, by treatment levels, when compared to control levels.

Time point	Group	Dose (mg/kg/day)		
		0.1	0.5	3.0
GD20 (dams treated from GD6-GD20)	Dams Fetuses	----- 11.9%*	9.9%* 10.2%*	60.3%** 33.3%**
PND4 pups (pups from treated dams; dams treated GD6 to PND 10)	Males Females	10.2%* -----	7.9%* 6.1%	12.5%** 6.6%
PND 21 pups (11 doses to pups from treated dams; dams treated GD6 to PND 10, pups treated PND11-21)	Males Females	4.1%* 3.6%	12.8%** 12.2%**	45.3%** 42.0%**
Adult (11 Days treatment)	Males Females	----- 6.4%	9.9%* 13.4%**	47.0%** 58.4%**
PND 11 pups (single dose to pups from untreated dams)	Males Females	1.7% -----	5.1%* [2.1%]	16.9%** [17.8%]
Adult (Single treatment)	Males Females	1.8% 3.7%	3.6%* 2.2%	12.1%** 14.4%**

*Statistically significant difference from controls ($p<0.01$ [**] or 0.05 [*]). Numbers in brackets were calculated from raw data and have not been statistically analyzed.

Blood Cholinesterase. Dams and fetuses consistently significant inhibition in blood ChEs only after 3 mg/kg of exposure. In pups tested on PND 4 or PND 21, only male pups on PND 4 at 0.5 mg/kg showed significant inhibition in plasma ChE, but the magnitude of the effect (7.7%) was less than is usually considered meaningful for this compartment. Female pups on PND 21 given 0.5 mg/kg showed significant RBC inhibition (22.8%). At 3 mg/kg, both plasma and RBCs were affected in PND 21 pups, with a lesser response in PND 4 pups (note that exposure for PND4 pups is unknown, since direct pup dosing started only on PND11).

Acute exposures to pups on day 11 and to young adults showed significant effects on plasma in males and female pups, and lesser response in adult females after 3 mg/kg. RBCs were affected in females after an acute dose of 3 mg/kg, with lesser response in males.

After 11 days of exposure in adults, there were no significant effects on blood ChEs at 0.5 mg/kg, but significant effects at 3 mg/kg were noted.

Table 10. Percent decrease of plasma cholinesterase activity, by treatment levels, when compared to control levels.

Time point	Group	Dose (mg/kg/day)		
		0.1	0.5	3.0
GD20 (dams treated from GD6-GD20)	Dams Fetuses	11.9% -----	14.3% 7.4%	43.8%** 43.0%**
PND4 pups (pups from treated dams; dams treated GD6 to PND 10)	Males Females	----- 5.5%	3.9% 7.7%*	7.5% 10.0%**
PND 21 pups (11 doses to pups from treated dams; dams treated GD6 to PND 10, pups treated PND11-21)	Males Females	----- -----	5.5% 4.9%	39.3%** 37.6%**
Adult (11 Days treatment)	Males Females	4.7% -----	11.9% 2.5%	37.3%** 21.0%
PND 11 pups (single dose to pups from untreated dams)	Males Females	1.1% [5.6%]	9.0% [3.0%]	18.8%** [17.9%]
Adult (Single treatment)	Males Females	----- 4.5%	2.9% -----	18.7%* 12.5%

*Statistically significant difference from controls ($p<0.01$ [**] or 0.05 [*]). Numbers in brackets were calculated from raw data and have not been statistically analyzed.

Table 11. Percent decrease of red blood cell cholinesterase activity, by treatment levels, when compared to control levels.

Time point	Group	Dose (mg/kg/day)		
		0.1	0.5	3.0
GD20 (dams treated from GD6-GD20)	Dams Fetuses	6.4% -----	12.6% 2.6%	57.5%** 31.2%**
PND4 pups (pups from treated dams; dams treated GD6 to PND 10)	Males Females	----- -----	2.9% -----	17.0%** 13.7%
PND 21 pups (11 doses to pups from treated dams; dams treated GD6 to PND 10, pups treated PND11-21)	Males Females	----- 14.8%	8.8% 22.8%*	59.1%** 65.1%**
Adult (11 Days treatment)	Males Females	----- 2.7%	17.5% 6.8%	58.3%** 63.2%**
PND 11 pups (single dose to pups from untreated dams)	Males Females	1.7% [17.5%]	4.0% [5.2%]	7.2% [26.1%]
Adult (Single treatment)	Males Females	----- 6.7%	----- 8.5%	17.3%* 27.1%**

*Statistically significant difference from controls ($p<0.01$ [**] or 0.05 [*]). Numbers in brackets were calculated from raw data and have not been statistically analyzed.

For acute exposures, we conclude that 0.5 mg/kg is an LOAEL in adult rats and in 11 day old pups, based on small (3-5%) but statistically significant decreases in brain ChE. Others may contest this view and a consensus conclusion may require further review.

For repeated exposures, we conclude that 0.1 mg/kg is an LOAEL in fetuses and pups, based on small but statistically significant inhibition of brain cholinesterase in fetuses (12%), male pups on day 4 (10%), and in pups on day 21 (4%). It is likely that others would contest this view as well, so a consensus conclusion must await further analyses and discussion. No significant effects on adults were seen at 0.1 mg/kg; the LOAEL for adults following repeated exposures is 0.5 mg/kg/day, based on statistically significant inhibition of brain cholinesterase (10-13%).

Implications for risk assessment

1. Data submitted on pup deaths and on ChE inhibition suggest that both may impact the current risk assessment in terms of the selection of endpoints and of an FQPA factor.

2. The current acute dietary and short term inhalation risk assessments are based on an NOAEL of 2 mg/kg for ChEI. In this study, statistically significant inhibition of brain ChE was seen in pups and adults after a single dose of 0.5 mg/kg [3-5%].

3. Similarly, current intermediate endpoints are based on LOAELs of 3.2 mg/kg for ChEI. In this study, statistically significant inhibition of brain ChEI was seen at 0.1 mg/kg [4-12%] in fetuses and pups (PNDs 4 and 21). Statistically significant inhibition of brain ChEI was seen in adults following 11 doses at 0.5 mg/kg (10-13 %).

4. The chronic dietary endpoint is based on an LOAEL of 0.25 mg/kg, and an NOAEL of 0.05 mg/kg. If one considers 0.1 mg/kg as an effect level based on brain ChEI in pups, then the NOAEL might be regarded as 0.033 mg/kg (LOAEL/3), which is lower than the current NOAEL.

5. Currently, the FQPA factor for dimethoate has been removed. The excess pup deaths occurring at 0.5 and 3.0 mg/kg, doses without significant overt maternal toxicity, warrant reconsideration of an FQPA factor based on this differential susceptibility. In addition, there may be increased sensitivity in the brain cholinesterase data if one considers 0.1 mg/kg an effect level in pups.

References

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