Dichlorvos (DDVP)

Risk Characterization Document

Third Addendum

Medical Toxicology Branch

Department of Pesticide Regulation

California Environmental Protection Agency

September 2008

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Acknowledgment

A draft of this document was reviewed by the Office of Environmental Health and Hazard Assessment, California Environmental Protection Agency.

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I. Introduction

The Department of Pesticide Regulation (DPR) completed a Risk Characterization Document (RCD), First Addendum, and Second Addendum for the use of DDVP in California (DPR, 1996, 1997 and 1998; Appendices A, B, and C, respectively). The conclusion was that mitigation measures were needed for some occupational and residential exposure scenarios. Dietary exposure to residues in food posed no health concerns.

This Third Addendum was conducted to reevaluate the risks associated with DDVP use in California. It was prompted by label changes, updated assessments from the U.S. Environmental Protection Agency (USEPA), as well as new toxicity information submitted by the registrant. In 2004, Amvac Chemical Corporation submitted several oral acute toxicity studies and developmental neurotoxicity studies. The results provided relevant information regarding time-course, as well as age and gender in acetylcholinesterase (AChE) inhibition by DDVP. As part of the comments to a draft of this Addendum, Amvac Chemical Corporation also submitted an acute inhalation LC50 study with rats (Debets, 1986) for consideration as the acute critical study and raised concerns regarding the use of NOEL versus benchmark dose and the oncogenic potential of DDVP (Chart, 2007; Chart, *et al*., 2007 and 2008; Starr, 2007a and b). An updated Toxicology Summary is included in Appendix D.

I.A. U.S. Environmental Protection Agency

In 2006, the USEPA completed the Interim Reregistration Eligibility Document (IRED) for DDVP, which resulted in cancellation of some uses and amendment to the labels (USEPA, 2006a-c; 2007a-d). The cancelled uses included: handheld fogger for mushroom house, greenhouse, and warehouse; total release fogger; and use on lawn, turf, ornamentals or crack and crevice. Amendments required coveralls for mushroom house hose end spray, and post-application reentry interval requirements for mushroom houses (18 hours) and greenhouses (12 hours). The pest strips size and use conditions were modified. The 21-gram strip was replaced by a 16-gram strip. The available strip products were 65 and 80-gram strips for unoccupied areas (including garages), non-home areas with less than 4-hours of occupation, as well as in warehouses in the fumigation of packaged/bagged/bulk stored processed and raw agricultural commodities. Only the smaller size strips (5.25, 10.5, and 16 gram) were allowed in homes with no time restriction, but are limited to closets, wardrobes, and cupboards. However, the IRED recommendation not to use the strips where infants, children, and the sick or aged would be present for an extended period of time (USEPA, 2006a and 2007e) was not included on the label (National Pesticide Information Retrieval System, 2008).

I.B. California

After the completion of the 1996 RCD, DPR and all relevant parties, including the registrants, signed the Stipulated Final Order regarding conditions for the continued registration of the DDVP pest strip products (DPR, 2001). A mitigating proposal was completed to require an increase of worker reentry interval to 24 hours for warehouse and other enclosed structural application, and to require protective clothing and respirators (DPR, 2002). In addition, the 80-gram pest strips could not be used in any living areas. Strips of 5.25-gram and 21-gram would be allowed in kitchen cupboards and closet, respectively. However, none could be used in closets of rooms occupied by infants, children, elderly, and ill people. Use-specific monitoring studies

would be needed to support any attenuation of the restrictions. To date, such a study has not been conducted. Amvac Chemical Corporation considered available data sufficient for exposure assessment (Chart, 2007).

While the large strips are available in California, DPR recently denied the registration of the small strips for home use (DPR, 2008a). The registrant failed to amend the federally approved label to include statements to prevent the use in closets, as well as wardrobe and cupboard where infants, children, or the sick or aged are or will be present for any extended period of confinement, and to prohibit the use of strips in more than one closet or wardrobe located in the same room (DPR, 2008b).

As of July, 2008, there are 26 active products registered in California (DPR, 2008c). They are in forms of spray, fly bait, pest strip, and liquid. The Pesticide Use Report showed 3,800 to 8,477 pounds used in the last year. For 2006, the total was 6,500 pounds, primarily in structural fumigation, landscape maintenance, and animal premise (DPR, 2006). The Use Report, however, does not include amount used in home use products such as pest strips. There are no longer home fogger or flea collar registered products.

II. Toxicology Profile and Hazard Identification

This section contains only recently submitted studies and those relevant for critical study selection.

II.A. Acute Toxicity

In the DPR First Addendum (DPR, 1997), two human oral toxicity studies were selected to derive the acute critical NOEL to assess oral and dermal exposures. They were conducted with Caucasian male healthy volunteers (20-32 years old) (Gledhill, 1997a and b). The volunteers were given DDVP (purity 97.7%) in corn oil in capsules, and then returned 24 hours later for blood samples and interview for symptoms. Only RBC AChE activity was reported. In the first study at 0.5 mg/kg/day (n=4), there was a statistically significant ($p<0.05$) decrease (90%-95% of pre-dose level) in the group mean RBC AChE activity. In the second study, the volunteers (n=6) were given a higher dose, 1.0 mg/kg of DDVP (purity 98%). Compared to the pre-dose levels, the group mean RBC AChE activity was significantly ($p<0.01$) reduced (about 10%) on days 5, 7, and 14. No treatment-related clinical signs were observed. DPR established 0.5 mg/kg as the NOEL for acute oral and dermal exposures. The ethical conduct of these human studies was reviewed by the USEPA Human Studies Review Board in 2006 (USEPA, 2006d and e). While the final review was not included in the IRED, an initial review concluded that there was no clear evidence of unethical conduct, while noting that there were gaps in the documentation. USEPA, therefore, included these human studies in the consideration of hazard identification and toxicity endpoint selection. The acute study (Gledhill, 1997a and b) eventually was not selected to assess acute exposure in the IRED because the blood sampling was done at 24 hours after dosing. This late sampling might have missed the peak effect occurring at 1 to 3 hours, as shown in the time course study with rats (to be described in this document). However, the result from the repeated dose human study (Gledhill, 1997c) was selected to address dermal exposure (short, intermediate, and long-term exposures).

For acute inhalation exposure, the DPR critical NOEL was 1.25 µg/L (0.65 mg/kg/day) based on mortality from two rabbit developmental toxicity studies exposed to DDVP (0.25 to 6.25 µg/L) during gestation for 28 days and 23 hours per day in two experiments (Thorpe *et al*., 1971) (Table 1). There was one dead pregnant rabbit at 2 µg/L after "2-3" days of exposure, with additional deaths at 4 and 6.25 µg/L after additional days of exposure. Cholinesterase activity was measured at the end of the experiment with a NOEL of 0.25 µg/L (0.13 mg/kg/day). There were uncertainties in the use of this study in risk assessment because problems with controlling the air concentration, which resulted in higher concentrations and deaths. There was also a question as to when the 1 death at 2 µg/L occurred. In the published report of this study (Thorpe *et al*., 1972), the time of death was noted as after 23 days, not 2-3 days as in the original report. Since this discrepancy could not be resolved, DPR had relied on the original report for the time of death. Since the completion of the risk assessment, additional studies submitted to DPR prompted re-evaluation of this critical NOEL for inhalation.

Table 1. Effects on pregnant rabbits from inhalation exposure to DDVP during gestation.[a]

Effects	**Concentration in µg/L (mg/kg/day,** not corrected for absorption**)**					
	0	**0.25 (0.13)**	**1.25 (0.65)**	**2 (1.04)**	**4 (2.08)**	**6.25 (3.25)**
Mortality	0/38	0/20	0/20	1/20	6/20	16/20
				1 died after 2-3 days of exposure	Six does died or were killed because of clinical signs[b]	Severe toxicity and mortality on day 6[c]
Cholinesterase inhibition – % control (measured after 28 days of exposure)						
Plasma		100	65*			27*
RBC		100	32*			7*
Brain		100	44*			15*

a/ Data from Thorpe *et al*., 1971. Pregnant rabbits were exposed to DDVP from gestation day 1 to 28.
b/ Five of the six does in the 4 µg/L group were affected in the week when the DDVP concentration reached as high as 6.6 µg/L due to a blockage of the chamber filter.
c/ Nine of the losses occurring within 7 days of the chamber concentration reaching 8 µg/L.

II.A.1. Additional Oral Toxicity Studies

Potential developmental neurotoxicity (DNT) and age-dependent cholinesterase inhibition by DDVP were examined in several oral toxicity studies. The studies conducted in rats included *in utero* and via the milk exposure using the standard DNT protocol (Milburn, 2003a and b; 2004), single direct dosing (preweaning and young adult rats) (Milburn, 2003c; Twomey, 2002a, b, and c; Moxon, 2002), and repeated dosing (Moxon 2003 and 2004).

II.A.1.a. Developmental Neurotoxicity Studies

In the preliminary developmental neurotoxicity study, Wistar-derived timed-mated dams (15/group) were given DDVP (purity 99%; 0, 0.1, 1, or 7.5 mg/kg/day) by gavage from gestation day 7 until lactation day 22 (Milburn, 2003a). Dams were evaluated for body weight, food consumption (during gestation), and clinical signs. Offspring were evaluated for litter size, survival, clinical signs, and body weight. Brain and RBC AChE activities were determined in dams at gestation day 22 and lactation day 22 (sacrifice 2 to 3 hours after the last dosing). They

were measured in the fetuses (five litters/group) at gestation day 22, with pooling of brain tissues from 4 male or 4 female fetuses per sample, and blood was likewise pooled from multiple fetuses per sex per litter for analysis. They were also measured in pups on lactation days 2, 8, 15, and 22.

For dams, irregular breathing was noted in one high dose dam on three consecutive days. AChE measurements for gestation and lactation day 22 demonstrated statistically significant inhibition of brain AChE at 7.5 mg/kg/day (59% and 67% inhibition) and RBC AChE at 1 mg/kg/day (25% and 18% inhibition) (Table 2).

Mean gestation length, initial litter sizes, pup survival, and body weight were unaffected by treatment. There was a higher incidence of "cold" pups in the 7.5 mg/kg/day group during the first two days of lactation than in other groups (litter incidences were 1 or 2 for all groups) (Table 3). This large incidence was due to a single dam, which had 14 "cold" pups on lactation day 1, and 11 cold pups on lactation day 2, without subsequent recurrences. These pups maintained lower than average weights throughout the study. "Cold" pup was not a treatment effect in the definitive study (Milburn, 2003b; to be discussed), and thus may not be a treatment effect. Brain and RBC AChE activities of the 7.5 mg/kg/day fetuses were inhibited (16-21%) on gestation day 22, but not those of the pups during lactation day 2 to 22 (Table 4).

The maternal AChE inhibition NOEL was 1 mg/kg/day for brain, and 0.1 mg/kg/day for RBC. The maternal NOEL (other than AChE inhibition) was 7.5 mg/kg/day (highest dose tested, HDT). The fetal AChE inhibition NOEL was 1 mg/kg/day for brain and RBC. The pup AChE inhibition NOEL was 7.5 mg/kg/day (HDT). The fetal/pup NOEL (other than AChE inhibition) was 7.5 mg/kg/day (HDT). DPR considered this a supplemental study. These NOELs were the same as those (NOAEL) established by the USEPA for this study (MRID 46153301; USEPA, 2006a).

Table 2. Acetylcholinesterase activity in the dams given DDVP during gestation and lactation.[a]

Sacrifice Time	**Dose (mg/kg/day)**							
	Brain (IU/g)				**RBC (U/l)**			
	0	**0.1**	**1**	**7.5**	**0**	**0.1**	**1**	**7.5**
Gestation Day 22	4.74 ±0.61	5.26 ±0.35	5.27 ±1.05	1.96 ±0.34** (59%)	2750 ±262	2724 ±233	2073 ±155** (25%)	1432 ±75** (52%)
Lactation Day 22	4.51 ±0.79	5.19 ±0.85	3.98 ±0.20 (12%)	1.49 ±0.22** (67%)	2372 ±322	2393 ±271	1948 ±131* (18%)	1283 ±128** (46%)

a/ Data from Milburn, 2003a. Statistical analysis by investigators with *, ** significant, $p < 0.05$ and $p < 0.01$, respectively. n=5. Cholinesterase inhibition as percent inhibition in parenthesis.

Table 3. Effects of DDVP in pups exposed to DDVP during gestation and lactation.[a]

Effects	**Dose (mg/kg/day)**			
	0	**0.1**	**1.0**	**7.5**
"Cold" No. pup (litters) Days from – to	 2 (2) 1-1	 3 (1) 1-1	 13 (1) 1-2	 27 (2) 1-2
Mean body weight (g, mean of mean pup weight in each litter), n=9-10				
Day 1 male female	6.06±0.77 5.70±0.64	6.34±0.80 5.88±0.61	6.08±0.49 5.77±0.52	6.56±0.69 6.07±0.75
Day 22 male female	49.21±10.76 49.67±6.75	51.33±5.19 50.21±6.64	50.64±4.80 40.54±4.50	48.40±5.46 47.07±5.34

a/ Data from Milburn, 2003a.

Table 4. Effects of DDVP on acetylcholinesterase activity of offspring.[a]

Effects **Sacrifice Time**	**Dose (mg/kg/day)**							
	Males				**Females**			
	0	**0.1**	**1**	**7.5**	**0**	**0.1**	**1**	**7.5**
Brain AChE (IU/g), mean±sd, % inhibition in parenthesis								
Gestation Day 22	1.39 ±0.07	1.26 ±0.16	1.39 ±0.23	1.17 ±0.11*(16%)[b]	1.35 ±0.12	1.30 ±0.07	1.37 ±0.17	1.07 ±0.11**(21%)
Lactation Day 2	1.96 ±0.15	1.83 ±0.12	1.85 ±0.18	1.89 ±0.12	1.97 ±0.25	1.98 ±0.35	1.98 ±0.28	1.91 ±0.25
Lactation Day 8	3.27 ±0.44	3.01 ±0.24	3.18 ±0.55	3.00 ±0.71	3.06 ±0.35	2.99 ±0.40	3.10 ±0.61	2.94 ±0.29
Lactation Day 15	4.57 ±0.59	5.03 ±0.57	4.84 ±0.27	4.37 ±0.62	4.62 ±0.49	5.29 ±0.49	4.79 ±0.46	5.24 ±0.57
Lactation Day 22	5.28 ±0.24	4.99 ±0.54	5.02 ±0.42	4.92 ±0.42	4.98 ±0.36	4.92 ±0.41	5.49 ±0.78	5.72 ±1.35
RBC AChE (U/L), mean±sd, % inhibition in parenthesis								
Gestation Day 22	2584 ±429	2231 ±601	2671 ±278	1863 ±223*(18%)	2864 ±614	2571 ±733	2451 ±538	2254 ±286(21%)
Lactation Day 2	2353 ±579	2110 ±273	2043 ±499	1970 ±427	2073 ±302	2191 ±417	2221 ±325	1830 ±280
Lactation Day 8	3074 ±757	2681 ±757	2862 ±361	2591 ±629	3376 ±269	3045 ±841	3264 ±1000	3061 ±732
Lactation Day 15	3460 ±780	3171 ±420	3371 ±416	3279 ±205	3462 ±924	3426 ±480	3167 ±674	3588 ±447
Lactation Day 22	3179 ±213	2752 ±541	2612 ±519	2762 ±193	3073 ±381	2837 ±291	2954 ±874	2777 ±308

a/ Data from Milburn, 2003a. Statistical analysis by investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively. n=2.

The definitive developmental neurotoxicity study consisted of two studies (Milburn, 2003b). A supplementary study (Milburn, 2004) was conducted to increase the number of litters for the high dose group (7.5 mg/kg/day). In the primary study, there were only 14 litters for this group. Timed-mated Wistar-derived females (30/group) were dosed with DDVP from gestation day 7 through lactation day 7, after which the pups were dosed (lactation days 8 through 22). Both the dams and pups were dosed with 0, 0.1, 1, or 7.5 mg/kg/day DDVP (purity 99%) by gavage. Functional observational battery (FOB) evaluations of dams were conducted on gestation days 10 and 17, and on lactation days 2 and 9. FOB evaluations on F1 rats were made on postnatal day (PND) 5, 12, 22, 36, 46, and 61. One male or one female F1 pup or rat per litter was used, except that in the 7.5 mg/kg/day group in the primary study, both a male and a female F1 animal per litter was sometimes employed in an effort to provide at least 10/sex/group. Other parameters assessed included automated motor activity evaluations, assessment of developmental landmarks in pups (preputial separation or vaginal opening), auditory startle, learning and memory (Y-shaped water maze), and neurohistopathology of selected F1 rats at termination (PNDs 12 and 63). Neurological tissues were examined only for the control and 7.5 mg/kg group. Brains and peripheral nerves were examined by histopathology and the brains were subjected to morphometric measurements.

In the dams, there were no treatment-related clinical signs or body weight changes observed. The FOB showed a few randomly distributed effects such as pale and thin in appearance, and piloerection. However, various parameters (such as general activity level), which might have revealed subtle changes in levels of arousal or amount of normal movement, were not recorded.

Other maternal measures without evidence of treatment effects included gestation length, percent live born pups, mean litter size, or whole litter losses. The numbers of available litters at 7.5 mg/kg/day were low (14 compared to 23+ in other groups) (Table 5); thus the supplementary study was needed. The high dose group had statistically significantly reduced pup survival (excluding total litter losses) (Table 5). This finding might not be treatment-related for several reasons. There was a small difference between survival at 7.5 mg/kg/day and 0.1 mg/kg/day. In the supplementary study, survival at 7.5 mg/kg/day was significantly ($p < 0.01$) higher than its concurrent control (85.9% in control *versus*. 94.1% at 7.5 mg/kg/day).

There were no treatment effects on sex distribution of pups, pup body weights at day 1 or at culling, or of total mean litter weights. General clinical signs data for PND 1-5 were comparable between groups. For pups selected to continue after culling, there were general increases in non-surviving male pups/young adults during the evaluation period. At the same time, high dose female F1 animals had the best survival of any female groups.

Body weights of F1 animals were not affected by treatment. High dose pups were slightly heavier than controls, significantly elevated for some time points. Consistent with slightly larger body weights in high dose males, preputial separation occurred about 1 day earlier in this group compared to corresponding controls. Day to vaginal opening did not vary significantly between groups. In the supplementary study, both developmental landmarks were achieved about 1 day earlier in 7.5 mg/kg/day F1 pups than in concurrent controls, consistent with slightly higher body weights in treated pups in that study.

FOB evaluation at PND 5, 12, 22, 36, 46, and 61 showed no treatment-related effects. “Normal” was reported for virtually all categories, including those (*i.e*., decreased activity, increased

activity) for which graded responses would be expected. Motor activity patterns did not indicate treatment effects at either of the four evaluation times in either sex. Startle amplitude was significantly elevated in high dose males on PND 23 from both studies (Table 5). In general, amplitudes decreased over the 5 blocks of 10 repetitions, regardless of group. Values in all treated groups were similar over a 75-fold range of exposures, suggesting that an atypical control sampling in the males was more likely a cause of differences than a treatment effect. There were no treatment effects in PND 23 females, or in either gender at PND 61.

There were no systematic changes in swimming times in the straight channel or in the Y-maze learning or memory phases in PND 24-27 rats. PND 59-62 rats displayed no systematic differences in swimming times in the learning phase. High dose males had a significantly increased mean time in the first trial of the memory phase, but thereafter showed a pattern comparable to other groups. This was not repeated in the supplementary study (Milburn, 2004), which had a much larger sample size for 7.5 mg/kg/day rats than this original study. Treated females were unaffected in the memory phase.

Whole brain and cerebellum weights at PND 12 and PND 63 were unaffected by treatment. Microscopic examination of the brain showed increased incidences of peripheral nerve demyelination of the distal and proximal tibial nerve when data for both genders are combined (Table 6).

Morphometric measurements of the brain were made only for controls and high dose groups at PND 12 and 63. Those with statistically significant changes from this or the supplementary study are presented in Tables 7 and 8. The changes might not be treatment related. Several significant values appeared to derive from comparison with atypical concurrent control values. Rarely was a statistically significant measurement for a particular parameter in one study associated with a remarkable change in the same direction in the second study.

The Medical Toxicology Branch reviewer established a NOEL of 7.5 mg/kg/day (HDT) for maternal toxicity and developmental toxicity (including developmental neurotoxicity). This study was upgraded to acceptable after the submission of validation study.

USEPA established the maternal NOAEL at 7.5 mg/kg/day for no treatment-related effects. The developmental NOAEL was 1.0 mg/kg/day for increased auditory startle reflex (Vmax) in PND 23 of the 7.5 mg/kg males in both studies (MRID 46153302 and 46239801; USEPA, 2006a).

Table 5. Reproductive and developmental effects in rats exposed to DDVP.[a]

Effects	**Dose (mg/kg/day)**			
	0	**0.1**	**1**	**7.5**
Dams on study	30	30	30	30
Failed to Litter	0	0	1	3
Total litter loss	6	3	5	5
Died to Dystocia	0	0	1	0
Sacrificed *in extremis* on PND 3	0	0	0	1
Insufficient pups (min. 3/sex)	1	6	2	7
Litters available for study[b]	23	21	21	14
Pup Survival (determined from Day 1 until Day 5)				
Excluding total litter losses:				
Percentage of pups surviving	96.3	93.3	98.9	91.3**
Proportion of litters with all pups surviving	19/24	20/27	21/23	14/21
Including total litter losses:				
Percentage of pups surviving	77.0	84.0	84.2	73.8
Proportion of litters with all pups surviving	19/30	20/30	21/27	14/26
Repetition Series, Startle Amplitude (Vmax) in PND 23 Males[c]				
1-10	362±151 (913±187)	464±216	452±108	459±124 (942±429)
11-20	237±57 (735±173)	292±68	259±55	332±90** (921±338)
21-30	221±64 (592±347)	273±51	271±54	327±128** (779±220)
31-40	200±99 (547±190)	243±37	224±45	285±73** (803±262*)
41-50	180±73 (633±283)	227±44	219±64	249±45* (642±223)

a/ Data from Milburn, 2003b. Dams were exposed to DDVP from gestation day 7 to lactation day 7. Pups were exposed to DDVP *in utero*, via the milk, and directly dosed on lactation days 8 through 22. Analysis by investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively.

b/ These values are also consistent with numbers of litters carried beyond PND 6.

c/ Data from a repeated experiment with higher number of litters (Milburn, 2004) are presented in parenthesis.

Table 6. Peripheral nerve demyelination in PND 63 rats.

Sites		Dose (mg/kg/day)			
		Milburn, 2003b		Milburn, 2004	
		0	7.5	0	7.5
Distal Tibia	minimal	4/23	6/21	3/22	10/24
Proximal Sciatic	minimal	15/23	11/21	10/22	10/24
	slight	0/23	2/21	2/22	4/24
Proximal Tibia	minimal	11/23	14/21	10/22	15/24
	slight	0/23	0/21	1/22	4/24

Table 7. Morphometric measurements in PND 12 rats.[a]

Location	Dose (mg/kg/day)							
	Milburn, 2003b				Milburn, 2004			
	Males		Females		Males		Females	
	0	7.5	0	7.5	0	7.5	0	7.5
PND 12 N[b]	12	6	11	7	8	12	8	10
Section Level and Structure Measured								
Level 3, Dorsal cortex 1, thickness	1.46± 0.07	1.46± 0.13	1.42± 0.08	1.42± 0.10	1.18± 0.13	1.25± 0.09	1.10± 0.12	1.23± 0.06**
Level 3, Dorsal cortex 2, thickness	1.60± 0.08	1.57± 0.08	1.58± 0.08	1.58± 0.09	1.24± 0.09	1.33± 0.16	1.20± 0.10	1.35± 0.12*
Level 4, Dorsal cortex, thickness	1.39± 0.09	1.40± 0.14	1.37± 0.08	1.38± 0.12	1.11± 0.12	1.14± 0.12	1.06± 0.06	1.14± 0.09*
Level 4, Thalamus width	8.16± 0.47	8.41± 0.26	8.24± 0.34	8.17± 0.48	8.35± 0.57	8.14± 0.52	8.30± 0.20	7.86± 0.43*
Level 4, Hippocampus, length from midline	4.07± 0.24	4.14± 0.28	4.13± 0.21	4.16± 0.13	4.32± 0.27	3.88± 0.42*	4.23± 0.15	3.88± 0.34*
Level 5, Thalamus width	7.39± 0.45	7.74± 0.27	7.58± 0.33	7.43± 0.51	7.53± 0.55	6.90± 0.52*	6.93± 0.23	7.05± 0.39
Level 5, Hippocampus, width dentate gyrus	0.80± 0.08	0.80± 0.05	0.77± 0.06	0.81± 0.06	0.69± 0.07	0.62± 0.12	0.59± 0.06	0.69± 0.09*
Level 5, Hippocampus, overall width	1.47± 0.12	1.48± 0.06	1.48± 0.07	1.56± 0.11	1.31± 0.08	1.18± 0.16*	1.21± 0.14	1.35± 0.07*
Cerebellum, height	3.84± 0.20	3.91± 0.32	3.81± 0.29	3.87± 0.22	3.74± 0.16	3.56± 0.17*	3.38± 0.37	3.72± 0.19*
Cerebellum, Prepyramidal Fissure								
Thickness of inner granular layer	145± 29	132± 24	134± 11	146± 21	137± 22	142± 22	123± 9	138± 16*
Thickness of molecular layer	62.0± 6.2	62.2± 10.2	58.2± NA	70.1± 10.2*	65.0± 10.2	57.7± 6.3	56.4± 8.8	57.2± 8.2
Thickness of outer granular layer	44.2± 6.8	48.8± 4.5	49± 10.1	48.7± 4.2	44.6± 3.5	49.3± 9.8	44.8± 7.0	48.5± 9.5

a/ Statistical analysis performed by the investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively.
b/ N=number of animals examined (in some cases, fewer animals were examined). Units were not given in the report's tables.

Table 8. Morphometric measurements in PND 63 rats.[a]

Location	Dose (mg/kg/day)							
	Milburn, 2003b				Milburn, 2004			
	Males		Females		Males		Females	
	0	7.5	0	7.5	0	7.5	0	7.5
PND 63 N[b] =	11	10	12	11	11	12	11	12
Section Level and Structure Measured								
Level 4, Piriform cortex, thickness	1.05± 0.12	1.15± 0.10	1.08± 0.13	1.21± 0.11*	1.06± 0.06	1.15± 0.09**	1.20± 0.07	1.13± 0.07*
Level 4, Corpus callosum, thickness	0.36± 0.06	0.32± 0.05	0.37± 0.07	0.31± 0.04*	0.36± 0.05	0.38± 0.05	0.42± 0.05	0.39± 0.07
Level 4, Thalamus, height	5.39± 0.27	4.96± 0.46*	5.32± 0.29	5.43± 0.27	5.37± 0.23	5.20± 0.28	5.42± 0.22	5.32± 0.29
Level 4, Hippocampus Width of dentate gyrus	0.54± 0.05	0.65± 0.04**	0.58± 0.08	0.68± 0.05**	0.57± 0.04	0.58± 0.06	0.57± 0.02	0.58± 0.05
Level 5, Piriform cortex, thickness	1.06± 0.11	1.17± 0.08*	1.09± 0.10	1.16± 0.08	1.08± 0.04	1.08± 0.10	1.09± 0.07	1.09± 0.10
Level 5, Hippocampus Width of dentate gyrus	0.65± 0.04	0.78± 0.05**	0.66± 0.06	0.76± 0.04**	0.64± 0.15	0.68± 0.18	0.59± 0.05	0.61± 0.08
Level 5, Hippocampus, overall width	1.44± 0.08	1.57± 0.09**	1.43± 0.10	1.53± 0.06**	1.31± 0.11	1.31± 0.15	1.34± 0.06	1.36± 0.07
Cerebellum: Prepyramidal Fissure[c]								
Thickness of inner granular layer	157± 6	134± 20*	153± 19	139± 24	73± 10	81± 7*	77± 10	77± 10
Thickness of molecular layer	208± 15	210± 18	198± 16	203± 15	119± 20	117± 19	114± 11	109± 13

a/ Data from Milburn, 2003b and 2004. Statistical analysis performed by the investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively.

b/ N=number of animals examined (in some cases, fewer animals were examined). Units were not given in the report's tables, and units are obviously not the same for all measured widths and thicknesses.

c/ It appears that a sum of two measurements was used in the original study (Milburn, 2003b), and a single measurement or average of two measurements was used in the second study (Milburn, 2004) for the cerebellar measurements.

II.A.1.b. Single and Repeated Dose Studies

In a time course study of AChE inhibition, female Wistar-derived rats (PND 15 preweaning and PND 42 young adult rats, 5/age/dose interval) were given a single oral dose of DDVP (purity 99%; 0 or 15 mg/kg) and sacrificed at 1, 3, 8, 24, or 72 hour post-dosing (Milburn, 2003c). There were no clinical signs observed. At 1 hour post-dosing, both the brain and RBC AChE were inhibited by about 50% for both preweaning and young adult rats (Table 9). Apparent recovery of AChE activity started at 8 hours post dosing. Substantial recovery occurred by 24 hours after treatment. Note that the 8-hour control brain AChE for young adults was unusually low. In another study from the same facility, the typical brain AChE activities in young adult males and females range around 5 to 6 IU/g (Moxon, 2003). The NOEL for brain and RBC AChE inhibition was <15 mg/kg/day. DPR considered this a supplementary study.

USEPA noted that AChE was maximally inhibited at one hour. By 8 hours after dosing, AChE activity level was restored to a level comparable to that of the control group for the same time period (MRID 46153303; USEPA, 2006a).

Table 9. Time course of acetylcholinesterase inhibition in preweaning and young adult rats given a single oral dose of DDVP.[a]

Hours After Dosing	PND 15 Treatment Preweaning		PND 42 Treatment Young Adults	
	Control	15 mg/kg	Control	15 mg/kg
Brain AChE (IU/g)				
1	6.46±0.97	2.67±0.52** (59%)	7.48±1.82	3.52±0.45** (47%)
3	6.34±1.03	3.08±0.60** (51%)	7.40±1.44	4.59±0.78* (38%)
8	5.58±0.45	4.65±0.88 (17%)	3.05±0.42[b]	3.14±1.15
24	5.19±0.05	4.55±0.29* (12%)	5.42±0.23	4.73±0.29* (13%)
72	5.39±0.98	5.59±0.95	5.06±0.41	4.69±0.38 (7%)
RBC AChE (U/L)				
1	3708±451	1745±140** (53%)	3064±200	1649±176** (46%)
3	4538±1466	2190±264* (52%)	2949±102	1934±45** (34%)
8	3712±772	2699±555* (27%)	2441±587	2017±208 (17%)
24	4339±433	3677±365 (15%)	3065±200	2712±272* (12%)
72	3344±218	3338±541 (2%)	2974±108	2541±149** (15%)

a/ Data from Milburn, 2003c. Statistical analysis performed by investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively. Cholinesterase activity as percent inhibition in parenthesis.

b/ Value lower than all controls.

Three acute toxicity studies were conducted on the effects of DDVP on young adult rats. In the first study, Wistar-derived rats (5/sex/group) were given a single oral dose of DDVP (purity 99%; 0, 2, 5, or 39 mg/kg) and sacrificed at either 1 hour[1], 8 days, or 15 days post dosing for AChE measurements (Twomey, 2002a). Clinical signs were reported only for the 39 mg/kg groups on the first day (Table 10).

One hour after dosing, cerebellar AChE activity was significantly reduced in all male treated groups, and only 39 mg/kg females (Table 10). The inhibition was 17% for 5 mg/kg females; it was not statistically significant. By day 8 and 15, no significant inhibition of the cerebellar AChE was detected. The RBC AChE activity at 1 hour was significantly inhibited for all doses and both genders (Table 10). The inhibition of the 39 mg/kg groups on day 8 was at a lower level but was statistically significant. The acute NOEL for AChE inhibition and clinical signs were <2 mg/kg and 5 mg/kg, respectively. DPR considered this a supplementary study. USEPA considered the 2 mg/kg/day dose as the LOAEL (MRID 45805701; USEPA, 2006a).

In the second study with a lower dose, Wistar-derived young adult rats (5/sex/group) were given a single oral dose of DDVP (purity 99%; 0 or 1 mg/kg) and sacrificed one hour after dosing (Twomey, 2002b). No treatment-related clinical signs were observed. RBC and cerebellar AChE activities were unaffected by treatment (Table 11). The NOEL for RBC and cerebellum AChE inhibition was 1 mg/kg/day. USEPA also established the 1 mg/kg/day as the NOAEL (MRID 45805702; USEPA, 2006a).

In the third study with different brain regions, Wistar-derived rats (at least PND 42 days old, 15/sex/group) were given a single oral dose of DDVP (purity 99%; 0, 1, 5, or 35 mg/kg) (Twomey, 2002c). RBC and brain region AChE activities were measured either one hour[1] or 8 days after dosing. Limited assessments were performed at 15 days after dosing when indicated by results of Day 1 and Day 8. Excessive toxicity including mortalities in 35 mg/kg males prompted a discontinuation of that group. Females, which had not yet been dosed at that level, were re-allocated to provide an extra group of 15 controls and a revised high dose of fifteen females at 15 mg/kg. Body weights and brain weights were unaffected by treatment.

Clinical signs were observed primarily in the 35 mg/kg males (Table 12). They included decreased activity, fasciculation, gasping, prostrate, reduced righting reflex, salivation, reduced splay reflex, stained around nose, and curved up spine. There was a dose-related increase in the incidence of mydriasis (pupil dilation) for the males, but not the females (Table 12). Note that mydriasis is not generally associated with AChE inhibition. At 15 mg/kg, one female displayed fasciculations and miosis only during the first hour after dosing.

When measured 1 hour after dosing, there was statistically significant AChE inhibition in all regions for the 35 mg/kg males, as well as the 5 and 15 mg/kg females (Table 13). For the 5 mg/kg males, cortex was the only region significantly inhibited (35%). The Medical Toxicology data reviewer noted that the measurements were highly variable with % CV's on the order of 50%, and thus it was not possible to establish a NOEL at 1 mg/kg for brain AChE inhibition. For risk assessment purpose, the 1 mg/kg could be considered the NOEL for brain AChE inhibition (35%) based on statistical significance for the 5 mg/kg males. With RBC AChE, statistically

[1] While the protocol of these studies stated that the AChE activities were measured at 1 hour post dosing, the report data tables indicated the time as "Day 1".

significant AChE inhibition in the first hour was determined for 5, 15, and 35 mg/kg groups (Table 13). At 1 mg/kg, the only significant inhibition was 7% on day 8. Since no inhibition was noted for day 1 and the magnitude is relatively low, this finding on day 8 was likely not treatment-related. Thus, the 1 mg/kg was the NOEL for RBC AChE inhibition. BMD analysis showed $BMDL_{10}$ of 0.8 to 1.01 mg/kg for brain AChE inhibition, and 1.17 to 1.35 mg/kg for RBC AChE inhibition (**II.A.1.c., Table 16**).

The USEPA determined 1 mg/kg as the NOAEL for both RBC and brain AChE inhibition (MRID 45805703; USEPA, 2006a).

Table 10. Effect in young adult rats given a single dose (2 to 39 mg/kg) oral of DDVP.[a]

Effects	**Dose (mg/kg)**							
	Males				**Females**			
	0	**2**	**5**	**39**	**0**	**2**	**5**	**39**
Clinical signs (all observed on the first day) incidence=animals affected/total animals								
Reduced Activity	0/15	0/15	0/15	15/15	0/15	0/15	0/15	14/15
Fasciculations	0/15	0/15	0/15	15/15	0/15	0/15	0/15	14/15
Lachrimation	0/15	0/15	0/15	7/15	0/15	0/15	0/15	6/15
Miosis	0/15	0/15	0/15	11/15	0/15	0/15	0/15	10/15
Reduced Splay reflex	0/15	0/15	0/15	0/15	0/15	0/15	0/15	2/15
Salivation	0/15	0/15	0/15	10/15	0/15	0/15	0/15	7/15
Tremors	0/15	0/15	0/15	2/15	0/15	0/15	0/15	0/15
Brain (cerebellum) AChE (IU/g), % inhibition in parenthesis, n=5.								
Day 1	2.17± 0.15	1.92± 0.17* (12%)	1.43± 0.15** (34%)	0.79± 0.10** (64%)	1.96± 0.53	2.03± 0.35	1.62± 0.42 (17%)	0.83± 0.18** (58%)
Day 8	2.46± 0.36	2.57± 0.35	2.31± 0.47 (6%)	2.13± 0.15 (13%)	2.43± 0.23	2.37± 0.41 (2%)	2.37± 0.26 (2%)	2.32± 0.09 (5%)
Day 15	2.57± 0.59	2.30± 0.27 (11%)	2.35± 0.26 (9%)	2.25± 0.24 (12%)	2.35± 0.32	2.19± 0.39 (7%)	2.22± 0.22 (6%)	2.11± 0.24 (10%)
RBC AChE (U/L), % inhibition in parenthesis, n=5.								
Day 1	2458± 169	1950± 118** (21%)	1555± 113** (37%)	1307± 114** (47%)	2485± 147	2047± 196** (18%)	1683± 89** (32%)	1327± 66** (47%)
Day 8	2480± 256	2143± 165* (14%)	2250± 179 (9%)	2111± 152** (15%)	2385± 181	2508± 167	2284± 228 (4%)	2113± 154* (11%)
Day 15	2193± 129	2249± 221	2197± 107	2241± 173	2342± 209	2395± 108	2247± 333(4%)	2187± 450(7%)

a/ Data from Twomey, 2002a. Statistical analysis performed by investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively.

Table 11. Cholinesterase activity in young adult rats given a single 1 mg/kg oral dose of DDVP.[a]

Effects	Dose (mg/kg)			
	Males		Females	
	0	1	0	1
Cerebellum AChE (IU/g)	2.88±0.36	2.46±0.34 (15%)	2.21±0.20	2.38±0.35
RBC AChE (U/L)	2234±86	2198±110 (2%)	2262±121	2146±192 (5%)

a/ Data from Twomey, 2002b. Cholinesterase activity as percent inhibition in parenthesis.

Table 12. Clinical signs in young adult rats given a single oral dose (1 to 35 mg/kg) of DDVP.[a]

Clinical signs	Dose (mg/kg)							
	Males				Females			
	0	1	5	35 [b]	0 [c]	1	5	15
Decreased activity	0/15	0/15	0/15	5/9	0/15	0/15	0/15	0/15
Fasciculations	0/15	0/15	0/15	8/9	0/15	0/15	0/15	1/15
Gasping	0/15	0/15	0/15	4/9	0/15	0/15	0/15	0/15
Mydriasis	0/15	1/15	6/15	4/9	0/15	0/15	0/15	0/15
Prostrate	0/15	0/15	0/15	5/9	0/15	0/15	0/15	0/15
Reduced righting reflex	0/15	0/15	0/15	2/9	0/15	0/15	0/15	0/15
Salivation	0/15	0/15	0/15	4/9	0/15	0/15	0/15	0/15
Reduced splay reflex	0/15	0/15	0/15	2/9	0/15	0/15	0/15	0/15
Stained around nose	0/15	0/15	0/15	2/9	0/15	0/15	0/15	0/15
Spine curved upward	0/15	0/15	0/15	2/9	0/15	0/15	0/15	0/15
Miosis	0/15	0/15	0/15	0/9	0/15	0/15	0/15	1/15

a/ Data from Twomey, 2002c, n=15.
b/ Treatment proved excessive, and treatment ceased for this group after 9 males were dosed.
c/ There were two control groups, one in the initial protocol, then one for the 15 mg/kg females.

Table 13. Cholinesterase activity in young adult rats given a single oral dose (1 to 35 mg/kg) of DDVP.[a]

AChE[b]	**Dose (mg/kg)**							
	Males				**Females**[b]			
	0	**1**	**5**	**35**	**0** **0'**	**1**	**5**	**15**
Brain (cerebellum) AChE (IU/g)								
Day 1	2.26± 0.57	2.31± 0.32	1.88± 0.46 (17%)	0.99± 0.32** (56%)	2.92±0.34 2.93±0.24	2.51±0.25 (14%)	2.39±0.34* (18%)	1.41±0.30**(52%)
Day 8	2.31± 0.43	2.60± 0.46	2.61± 0.43	2.47± 0.48	3.53±1.79 2.24±0.39	4.26±2.13	2.95±0.34 (16%)	2.53±0.20
Brain (cortex) AChE (IU/g)								
Day 1	7.71± 0.80	7.66± 2.59 (6%)	5.04± 1.16* (35%)	1.82± 0.56** (76%)	10.39±0.95 7.21±2.19	7.70±1.82 (26%)	6.57±1.72** (37%)	4.05±1.42*(44%)
Day 8	5.24± 2.82	7.11± 3.46	6.27± 0.45	5.65± 0.71	8.04±3.21 6.95±1.69	6.43±2.26 (20%)	7.74±1.69 (4%)	6.65±1.62(17%)
Brain (hippocampus) AChE (IU/g)								
Day 1	4.80± 2.14	4.55± 1.13	3.57± 0.97 (26%)	2.34± 1.39* (51%)	5.51±0.79 7.29±2.06	5.53±1.48	3.94±0.46* (29%)	3.32±1.05**(55%)
Day 8	6.04± 1.04	7.25± 3.21	7.82± 3.75	6.27± 0.88	18.83±9.59 6.63±1.29	15.98±9.41 (15%)	17.12±5.12 (10%)	5.99±0.71(10%)
Brain (remainder) AChE (IU/g)								
Day 1	6.96± 1.54	7.52± 2.17	5.57± 2.27 (20%)	2.19± 1.00** (69%)	9.10±0.85 11.12±3.32	8.16±1.81 (10%)	6.07±0.64** (33%)	4.55±1.11**(59%)
Day 8	6.87± 0.82	8.37± 0.78**	6.42± 0.47	7.02± 1.36	8.64±1.52 8.29±1.10	8.34±2.93	7.92±0.99 (8%)	6.97±1.20(19%)
Brain (half) AChE (IU/g)								
Day 1	7.14± 2.05	7.42± 1.81	5.22± 1.01 (27%)	1.77± 0.74** (75%)	9.17±2.19 7.73±3.24	7.48±2.19 (18%)	6.03±2.25* (34%)	3.36±1.00*(57%)
Day 8	6.14± 0.60	7.20± 2.54	5.53± 0.39 (10%)	4.65± 0.64 (24%)	7.66±0.86 5.71±0.77	7.81±1.97	8.40±1.64	5.31±0.38 (31%)
RBC AChE (U/L)								
Day 1	2705± 343	2719± 170	2015± 94** (26%)	1616± 245** (40%)	2597±179 2555±87	2373±264 (9%)	1835±101** (29%)	1589±108**(38%)
Day 8	2654± 79	2473± 133* (7%)	2418± 69** (9%)	2256± 92 (15%)	2540±49 2454±183	2556±124	2499±199 (2%)	2221±152(9%)
Day 15	2574± 312	NA	2414±113 (6%)	NA	2527±191	NA	NA	NA

a/ Data from Twomey, 2002c, n=5, except n=4 day 1-35 mg/kg/day males, n=2 for day 8- 35 mg/kg/day males. NA=no data. Statistical analysis performed by investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively. Cholinesterase activity as percent inhibition in parenthesis.

b/ There were two control groups, one in the initial protocol, then one for the 15 mg/kg females.

In an acute study with preweaning rats of various ages, Wistar-derived pups (PND 8, 15, or 22; 5/sex/group) were given a single oral dose of DDVP (purity 99%; 0, 1, 5 or 15 mg/kg) and sacrificed one hour later for AChE measurements (Moxon, 2002). Tremor was the only clinical sign observed, and was reported for the 15 mg/kg group (PND 8 and 22, Table 14). Cholinesterase inhibition was statistically significant in all 5 mg/kg pups (at least $p < 0.05$) and all 15 mg/kg pups ($p < 0.01$) for both brain and RBC AChE (Table 14). There was no obvious difference in response between genders or age at the higher two dose levels. Brain cholinesterase was unaffected at 1 mg/kg in either sex. RBC cholinesterase at 1 mg/kg was inhibited 22% and 27% in PND 8 females and PND 15 females, respectively, and 9% in PND 15 males. The NOELs for brain and RBC AChE inhibition were 1 mg/kg and <1 mg/kg, respectively. DPR considered this a supplementary study. BMD analysis showed $BMDL_{10}$ of 1.01 to 1.59 mg/kg for brain AChE inhibition, and 0.80 to 1.30 mg/kg for RBC AChE inhibition (**II.A.1.c., Table 16**).

USEPA established the brain AChE NOAEL of 1 mg/kg, and RBC AChE LOAEL of 1 mg/kg (MRID 45842301; USEPA, 2006a).

Table 14. Effects in pups exposed to DDVP as a single oral dose.[a]

Effects/ days postpartum	Dose (mg/kg)							
	Males				Females			
	0	**1**	**5**	**15**	**0**	**1**	**5**	**15**
Clinical signs								
Tremor, slight Day 8 Day 22	 0/5 0/5	 0/5 0/5	 0/5 0/5	 1/5 1/5	 0/5 0/5	 0/5 0/5	 0/5 0/5	 1/5 1/5
Whole brain AChE (IU/g)								
Day 8	3.14± 0.20	3.04± 0.10	2.33± 0.24** (26%)	1.11± 0.09** (65%)	3.08± 0.21	2.98± 0.23	2.41± 0.23** (22%)	1.42± 0.24** (54%)
Day 15	4.64± 0.42	4.38± 0.50	3.35± 0.38** (28%)	1.83± 0.10** (61%)	4.80± 0.59	5.00± 1.45	3.52± 0.32* (27%)	1.86± 0.32** (61%)
Day 22	5.03± 0.65	4.68± 0.30	3.49± 0.25** (31%)	2.09± 0.23** (58%)	5.12± 1.02	4.46± 0.53	3.86± 0.13** (25%)	2.04± 0.33** (60%)
RBC AChE (U/L)								
Day 8	3620± 276	3473± 411	2693± 442** (26%)	1384± 359** (62%)	3830± 733	2991± 662* (22%)	2636± 315** (31%)	1689± 349** (56%)
Day 15	3420± 263	3107± 208* (9%)	2412± 116** (29%)	1593± 211** (53%)	3615± 301	2626± 691** (27%)	2190± 317** (39%)	1563± 163** (57%)
Day 22	3507± 575	3095± 159	2281± 348** (35%)	1787± 168** (49%)	3121± 309	3111± 230	2233± 145** (28%)	1708± 144** (45%)

a/ Data from Moxon, 2002. Statistical analysis performed by investigators with *, ** significant for $p < 0.05$ and $p < 0.01$, respectively, n=5. Cholinesterase activity as percent inhibition in parenthesis.

In a repeated dose study, Wistar-derived rats (5/sex/dose; PND 12 preweaning or PND 42 young adult) were given a daily oral dose of DDVP (purity 99%; 0, 0.1, 7.5 or 15 mg/kg) for 7 days and sacrificed 1 hour after the last dose (PND 18 or PND 48) (Moxon, 2003). Additional statistical analysis was submitted in Moxon (2004).

For preweaning rats, one litter was allocated to each treatment group for the evaluation of cholinesterase activity. This design was necessary, as the pups needed to stay with their female parent. Mixing of pups from different litters, or allocating different parents to the pups could confound the results. Therefore, the data were compared on an animal basis and on a litter basis. In addition, the cholinesterase activity data were compared with the concurrent control data and pooled control data from this study and two contemporary studies conducted (one month before and 6 months after the current study) with the same study design and time frame.

The only clinical sign was slight tremors seen on multiple occasions during post-dosing in preweaning and young adult rats given 15 mg/kg/day, and on day 48 for one 7.5 mg/kg/day young adult male (Table 15). No clinical signs were reported for the other groups.

There was no apparent difference in the gender or age in the effect of DDVP on AChE activity. Brain AChE was significantly inhibited at 7.5 and 15 mg/kg/day, when compared on per animal basis using concurrent control or pooled controls for preweaning and young adult rats, and on per litter basis for preweaning rats (Table 15). Statistically significant inhibition (26% and 24% for males and females) was observed at the 0.1 mg/kg/day dose only for preweaning rats compared with the concurrent control and on per animal basis. The investigator attributed this difference to the high concurrent control value (7.09 IU/g for males, 6.59 IU/g for females) compared to the pooled control (5.76 IU/g for males, 5.54 IU/g for males) for both genders. However, the Medical Toxicology reviewer noted that the control values obtained in this experiment were consistent with other studies using Wistar rats.

With RBC AChE, the inhibition was statistically significant for the 7.5 and 15 mg/kg/day groups regardless of how the values were compared (Table 15). In contrast with brain AChE, the 0.1 mg/kg/day young rats showed significant RBC AChE inhibition. The Medical Toxicology Branch reviewer considered the 0.1 mg/kg/day dose as the LOEL for brain AChE inhibition in preweaning and RBC AChE inhibition in young adult rats. DPR considered this a supplemental study. BMD analysis showed $BMDL_{10}$ of 0.77 to 0.95 mg/kg/day for brain AChE inhibition, and 0.79 to 0.89 mg/kg/day for RBC AChE inhibition (**II.A.1.c., Table 16**).

USEPA determined $BMDL_{10}$ of 0.8 mg/kg/day brain AChE inhibition in males (PND 48) and RBC AChE inhibition in females (PND 18) (USEPA, 2004). In a separate analysis for aggregate and cumulative risk assessment, most of the groups showed $BMDL_{10}$ between 0.5 and 1.2 mg/kg/day (USEPA, 2006f).

Table 15. Effects in preweaning and young adult rats given DDVP oral doses for 7 days.[a]

Effects	Dose (mg/kg/day)							
	Males				Females			
	0	**0.1**	**7.5**	**15**	**0**	**0.1**	**7.5**	**15**
Clinical sign- slight tremors observed post-dosing (d=Postnatal day)								
Preweaning	none	none	none	5/5 (d13) 5/5 (d14) 2/5 (d16) 1/5 (d17) 3/5 (d18)	none	none	none	5/5 (d13) 5/5 (d14) 3/5 (d16) 2/5 (d17) 4/5 (d18)
Young adult	none	none	1/5 (d48)	1/5 (d44) 1/5 (d45) 2/5 (d46) 1/5 (d48)	none	none	none	2/5 (d44) 2/5 (d45) 2/5 (d46) 3/5 (d47) 1/5 (d48)
Brain AChE (IU/g) mean±sd								
PND 18 preweaning per animal basis	7.09 ± 0.64	5.27± 0.44** (26%)	2.53± 0.41** (64%)	1.58± 0.21** (78%)	6.59 ± 0.59	5.02± 0.46** (24%)	2.54± 0.54** (61%)	1.71± 0.22** (74%)
	5.76[b] ± 1.05(n=24)	(9%)	**(56%)	**(73%)	5.54[b] ± 1.18(n=21)	(9%)	**(54%)	**(69%)
PND 18 per litter basis	5.77± 0.84(n=4)	5.27	2.53*	1.58*	5.50± 0.86(n=4)	5.02	2.54*	1.71*
PND 48 adult	5.84 ± 0.40	5.52± 0.42 (5%)	2.32± 0.68** (60%)	1.51± 0.26** (74%)	5.51 ± 0.34	5.36± 0.25 (3%)	2.51± 0.56** (54%)	1.57± 0.15** (72%)
	5.41[b] ± 0.61(n=25)	(0%)	(57%)**	(72%)**	5.32[b] ± 0.60(n=25)	(0%)	(53%)**	(70%)**
RBC AChE (U/L) mean±sd								
PND 18 preweaning per animal basis	3299 ± 353	3441± 484 (0%)	1423± 186** (57%)	1280± 146** (61%)	3418[b] ± 470	3254± 257 (0%)	1424± 201** (58%)	1208± 145** (65%)
	3162[b] ± 385(n=20)	(0%)	(55%)**	(60%)**	3185[b] ± 377(n=18)	(0%)	(55%)**	(62%)**
PND 18 per litter basis	3162 ± 252(n=4)	3441	1423**	1280**	3200 ± 209(n=4)	3254	1424**	1208**
PND 48 adult	2908 ± 610	2413± 111* (17%)	1346± 236** (54%)	1159± 155** (60%)	2727 ± 150	2414± 158** (11%)	1247± 109** (54%)	1270± 70** (53%)
	2650[b] ± 411(n=20)	(9%)	(49%)**	(56%)**	2458[b] ± 399(n=20)	(2%)	(49%)**	(48%)**

a/ Data from Moxon, 2003 and 2004. Rats were given DDVP starting on PND 12 (preweaning) or PND 42 (young adult) for 7 days. Statistical analysis performed by investigators with significance based on two-sided Student's t-test *($p<0.05$) **($p<0.01$). Unless specified, n=5. Cholinesterase activity as percent inhibition in parenthesis.

b/ Values for the treatment groups were compared with pooled control values from two similar studies.

II.A.1.c. Benchmark Dose Analysis

The use of benchmark dose analysis, instead of No-Observed-Effect Level (NOEL) or extrapolation from Lowest-Effect Level (LOEL), allowed comparison of the threshold doses for a given response level (*e.g.*, 10% for AChE inhibition) under various experimental conditions (*e.g.*, age and gender of animals, single versus repeated doses).[2] Data for the 1-hour post-dosing measurement from the three studies (Moxon, 2002; Twomey, 2002c; Moxon, 2003) were subjected to BMD analysis to calculate the threshold doses (BMD_{10} and $BMDL_{10}$). These values are considered in the identification of the critical NOEL, in the comparison of responses between parameters (Table 16). The lowest $BMDL_{10}$ was 0.8 mg/kg/day for brain AChE inhibition in PND 42+ rats (Twomey, 2002c) and for RBC AChE inhibition in PND 15 rats (Moxon, 2002). This value should be used as the revised critical acute oral NOEL.

There was no indication of increased sensitivity by age, gender, or tissue source of the animals to DDVP exposure. The brain AChE inhibition $BMDL_{10}$ values for PND 8 animals were higher than those for the older animals (PND 15, 22, and 42+). This difference was not evident for RBC AChE data. Therefore, it could be concluded that there was no increased sensitivity of the young to DDVP induced AChE inhibition. The ranges of BMD values between genders (male versus female) and tissue source (brain versus RBC) were similar. These conclusions are consistent with those determined by the USEPA (2006f). Most BMD values for the 7-day repeated exposure study (Moxon, 2003) were lower than those after a single oral dose (Moxon, 2002; Twomey, 2002c).

Table 16. Benchmark dose analysis of acetylcholinesterase activity data.[a]

Age	AChE Tissue	Males		Females		References
		BMD_{10} mg/kg/day	$BMDL_{10}$ mg/kg/day	BMD_{10} mg/kg/day	$BMDL_{10}$ mg/kg/day	
Single Oral Dose						
PND 8	whole brain	1.81	1.48	2.16	1.59	Moxon, 2002
PND 15	whole brain	1.64	1.26	1.63	1.01	
PND 22	whole brain	1.45	1.15	2.05	1.30	
PND 42+	half-brain	1.62	1.01	1.34	0.80	Twomey, 2002c
PND 8	RBC	1.82	1.28	1.52	0.97	Moxon, 2002
PND 15	RBC	1.51	1.24	1.11	0.80	
PND 22	RBC	2.05	1.30	1.46	1.17	
PND 42+	RBC	1.72	1.35	1.43	1.17	Twomey, 2002c
7 Repeated Doses						
PND 18	whole brain	0.95	0.77	0.99	0.80	Moxon, 2003
PND 48	whole brain	0.95	0.83	1.07	0.95	
PND 18	RBC	0.92	0.79	0.94	0.81	
PND 48	RBC	1.10	0.85	1.00	0.89	

a/ BMD analysis for AChE measured 1 day after dosing using USEPA BMD polynomial model revision 2.2 (dated 9/12/2002) with rho set to 0, relative risk, BMDR=10%. For the Moxon study (2003), PND 18 data for per animal basis, instead of per litter basis (see Table 15), were used in the analysis.

[2] DPR has a guidance for the use of BMD analysis (DPR MT-2, 2004). USEPA used the ED_{10} of brain AChE as the response level in cumulative risk assessment of organophosphates (USEPA, 2002). The 10% inhibition level is generally at or near the sensitivity limit for statistical significance and is close to the background response level.

II.A.2. Additional Acute Inhalation Study

The Denka study (Debets, 1986) was submitted for consideration as the critical study for acute inhalation exposure. In this LC50 study, Wistar rats (5/sex/group) were exposed to DDVP (97% purity) at reported analytical concentrations of 0.17, 0.20, or 0.24 mg/L by nose-only inhalation in a 4-hour experiment. Mortality was reported in all dose groups (Table 17). Clinical observations included lethargy, ataxia, tremors, hyponea, and bloody eye or nose encrustation. The surviving rats appeared normal after 4 days. One week after exposure, the mid- and high dose groups showed normal weight gain. Necropsy showed lung hemorrhages, bloody mouth or nose, and bloody trachea. The reported LC50s were 0.23 mg/L (both gender), 0.29 mg/L (male), and 0.22 mg/L (female). DPR considered this study unacceptable, but possibly upgradeable with the submission of standard curves, chromatographical values and calculations used to establish the analytical concentration. In addition to the deficiencies, this study was inappropriate for the critical NOEL because it was essentially a single dose experiment as there was only a 1.4-fold between the high dose and low dose. While the data for mortality showed an apparent dose-response relationship, it was not the case for clinical signs as similar incidences were reported for all doses. When a default uncertainty factor of 10 is applied to extrapolate the NOEL, the estimated NOEL was 0.017 mg/L (16 mg/kg/day). This level was higher than the existing critical NOEL of 1.25 µg/L or 0.65 mg/kg/day for death calculated from the rabbit developmental toxicity study (Thorpe *et al*., 1971).

Table 17. Effects on rats from inhalation exposure to DDVP.[a].

Effects	**Concentration (µg/L)**			
	0	**170**	**200**	**240**
Deaths	0/10	1/10 1 male	2/10 1 male, 1 female	6/10 2 males, 4 females
Time to death (from start of exposure)	na	2.5 hours	15 minutes and 1.75 hours	between 1 and 2.5 hours
Clinical Observations after 4 hours of exposure				
Lethargy	0/10	8/9	8/8	4/4
Bloody nose encrustration	0/10	4/9	5/8	2/4

a/ Data from Debets, 1986. na=not applicable.

II.A.3. Acute Critical NOEL Selection

Review of the additional toxicity studies (Table 18) showed that the acute critical NOEL should be 0.8 mg/kg/day, the $BMDL_{10}$ for brain AChE inhibition in young adult rats (Twomey, 2002c) and RBC AChE inhibition in preweaning rats (Moxon, 2002). This value is applicable for oral and dermal exposures. Considering that the 0.8 mg/kg/day value is derived from well-conducted studies and is similar in magnitude to the previously determined inhalation critical NOEL of 0.65 mg/kg/day (1.25 µg/L, with 100% inhalation absorption[3]) from the rabbit inhalation study (Thorpe *et al*., 1971), it is appropriate to apply this value for the inhalation route. The USEPA critical NOEL is 0.8 mg/kg/day for all routes of exposure (USEPA, 2006a).

[3] The current DPR default inhalation absorption is 100%, a change from the previous policy of 50%.

Table 18. Acute toxicity studies in rats.

Species/route/ duration	When AChE measured	NOEL mg/kg/day	LOEL mg/kg/day	Effects	Ref.[a]
Acute LC50 study					
Rats/inhalation/4 hours	not measured	16	160	Death and clinical signs	1
Exposure during gestation and lactation					
Pregnant rabbits/inhalation/ GD 1-28	Dam: GD 28	0.13 0.65	0.65 1.04	Maternal brain, RBC AChEI (28 days) Maternal death (2-3 days)	2
Pregnant rats/oral/ GD 7 to LD 22	Dam: GD 22, LD22	1 0.1	7.5 1	Maternal-brain AChEI Maternal-RBC AChEI	3
	Fetus: GD 22	1	7.5	Fetal- brain and RBC AChEI	
	Pup: LD 2, 8, 15, 22	7.5 (HDT)	>7.5	Pup- no brain or RBC AChEI	
Pregnant rats/oral GD 7 to LD 7 Pups/oral/LD 8 to 22	not measured (FOB on GD, LD, PND)	7.5 (HDT)	>7.5	Maternal and pups- no effects on clinical observation, functional observation battery tests, or brain morphometry	4
Single dose in preweaning and young adult rats					
Female PW (PND 15) or YA (PND 22)/oral/1 dose	1 to 72 hours post dose	<15	15 (only dose tested)	Brain and RBC AChEI	5
YA/oral/1 dose	1 hour, 8 days, or 15 days post dose	<2 5	2 39	Brain and RBC AChEI on day 1 Clinical signs	6
YA/oral/1 dose	1 hour post dose	1	>1	No brain or RBC AChEI, or clinical signs at 1 mg/kg/day	7
YA/oral/1dose PND 42+	**1 or 8 hours post dose**	15 **($BMDL_{10}$=0.8)** ($BMDL_{10}$=1.17)	35	Clinical signs **Brain AChEI** RBC AChEI	**8**
PW (PND 8, 15, 22)/oral /1dose	**1 hour post dose**	($BMDL_{10}$=1.01) **($BMDL_{10}$=0.8)**		Brain AChEI **RBC AChEI**	**9**
Repeated doses in preweaning and young adult rats					
PW (PND 18) or YA (PND 48)/oral/ 7 doses	1 hour post last dose	($BMDL_{10}$=0.77) ($BMDL_{10}$=0.79) ($BMDL_{10}$=0.83) ($BMDL_{10}$=0.85) 0.1	 7.5	PW: Brain AChEI PW: RBC AChEI YA: Brain AChEI YA: RBC AChEI Tremors in PW and YA (after 7 days)	10

a/ References: 1. Debets, 1986; 2. Thorpe *et al*., 1971; 3. Milburn, 2003a; 4. Milburn, 2003b and 2004; 5. Milburn, 2003c; 6. Twomey, 2002a; 7. Twomey, 2002b; 8. Twomey, 2002c; 9. Moxon, 2002; 10. Moxon, 2003 and 2004. Abbreviations: AChEI=acetylcholinesterase inhibition, FOB=functional observation battery, GD=gestation day, LD=lactation day, PND=postnatal day, PW=preweaning, YA=young adult.

II.B. Subchronic Toxicity

Subchronic critical NOELs were established in the 1996 RCD, but were not applied because there were no subchronic exposure scenarios. They are presented in this Addendum because the USEPA worker exposure assessment included intermediate exposure scenarios, and for comparison with the critical study selected by the USEPA.

II.B.1. Subchronic Studies

Rabbit - Inhalation

The study of Thorpe et al., 1971 with pregnant rabbits exposed to DDVP by inhalation was already presented in **II.A. Acute Toxicity**. The endpoint of concern was brain and RBC AChE inhibition after 28 days of exposure with a NOEL of 0. 25 µg/L (0.13 mg/kg/day). The inhibition of brain AChE activity was dose-related and statistically significant ($p \leq 0.05$); and was 44% and 15% of control values for the 1.25 µg/L (0.65 mg/kg-day) and 6.25 µg/L (3.25 mg/kg/day) groups, respectively (Table 1).

Rat – Gavage

In a subchronic neurotoxicity study, Sprague-Dawley rats (Crl:CD.BR, 15/sex/group) were given DDVP (97.87% purity; 0. 0.1, 7.5, or 15 mg/kg/day) by gavage 7 days per week for 13 weeks (Lamb, 1993). Tremors, salivation, exophthalmus, lacrimation, clear material on forelimbs, rales, chromodacryorrhea and material around the mouth were observed in the 15 mg/kg/day group with most of the clinical signs observed shortly after dosing (about 15 minutes). Tremors, salivation, and exophthalmus were observed in the 7.5 mg/kg/day group starting at week 3. Body weights of the 15 mg/kg/day females were significantly lower (91% of control value) by week 13. Plasma ChE activity of the 7.5 and 15 mg/kg/day groups was significantly ($p \leq 0.05$) inhibited at weeks 3, 7, and 13, and the activity ranged from 42% to 66% of control values. RBC AChE activity was not significantly inhibited, except for the 35% decrease (65% of control) in the 15 mg/kg/day group after 3 weeks of treatment.

At week 13, brain stem AChE activity was reduced for the 7.5 mg/kg (88% of control, both genders) and 15 mg/kg (84% for males and 90% for females, of controls) groups. These reductions were not statistically significant. However, similar inhibition levels were statistically significant for the cerebral cortex AChE activity. They were 88-87% of control, and 85-90% of control for 7.5 and 15 mg/kg/day, respectively. No adverse effects were observed in the functional observational battery, locomotor, brain weight, brain dimension or neuropathological parameters. The NOEL was 0.1 mg/kg/day (0.1 mg/m^3)[4] based on cholinergic signs and brain cholinesterase inhibition.

Human – Oral

In a single blind, randomized, placebo controlled multiple dose study in 9 volunteers (age 19-34 years, weight 61-90 kg), six volunteers were given DDVP (98% pure; 7 mg/day or 0.1

[4] Calculated based on default rat breathing rate of 0.96 m^3/kg/day or human adult breathing rate of 0.28 m^3/kg/day For example: 0.1 mg/kg/day ÷ 0.96 m^3/kg/day=0.1 mg/m^3

mg/kg/day for a 70 kg male) and three volunteers received corn oil in capsules for 21 days (Gledhill, 1997c). Compared with the placebo group, the treated group mean RBC AChE activity (% of predose level) was significantly ($p<0.01$) decreased on days 7 (91%), 11 (90%), 14 (86%), 16 (86%), and 18 (84%). No treatment-related clinical signs were observed. The LOEL was 0.1 mg/kg/day for RBC AChE inhibition at this dose. This study was reviewed by the USEPA Human Studies Review Board (HSRB) and found to be appropriate to use for risk characterization (USEPA, 2006d). There were strengths (repeated dose, RBC AChE activity measured before and after treatment) and weaknesses (single dose, male only, small sample size) to the study.

Table 19. Selected no-observed-effect levels (NOELs) and lowest-observed-effect levels (LOELs) of DDVP from subchronic studies.[a]

Species/route/ duration	NOEL	LOEL	Effects	Ref.
	mg/kg/day			
Rat, gavage 5d/w x 13 w	**0.1** 7.5	**7.5** 15	**Brain AChE inhibition; tremors, salivation (onset on week 3)** RBC AChE inhibition	**1***
Rabbit, inhale 23hr/d, gd 1-28	0.13	0.65	Plasma, RBC, and brain AChE inhibition	2
Human, capsule 21 days	<0.1	0.1	RBC AChE inhibition	3

a/ * after the reference number indicates the study was acceptable to DPR according to FIFRA guidelines. References: 1. Lamb, 1993; 2. Thorpe *et al*., 1971; 3. Gledhill, 1997c. Abbreviations: d= days, gd=gestation day, hr=hour, w=week.

II.B.2. Subchronic Critical NOEL Selection

In the 1996 RCD, the critical NOEL for subchronic inhalation toxicity was established at 0.25 µg/L (0.13 mg/kg/day) based on brain AChE inhibition in pregnant rabbits exposed to DDVP from day 1 to 28 of gestation (Thorpe *et al.*, 1971). This NOEL is similar in magnitude as the critical NOEL for subchronic oral toxicity. The NOEL was 0.1 mg/kg/day from a rat gavage study for tremors and brain AChE inhibition at 7.5 mg/kg/day (Lamb, 1993). The 21-day repeated dose human study (Gledhill, 1997c) was not available at that time.

In the IRED, the USEPA selected the human study as the critical study (USEPA, 2006a). The LOAEL was 0.1 mg/kg/day and the estimated NOEL was 0.03 mg/kg/day using a 3-fold LOEL to NOEL extrapolation factor. However, the DPR estimated NOEL for this study would be lower since the DPR default factor is 10-fold. Since this is a single dose study, the use of a 10-fold factor seems more prudent than a 3-fold factor, resulting in an estimated NOEL of 0.01 mg/kg/day. While this study yielded a lower NOEL than the animal studies, both NOELs would yield the same reference concentration using the DPR methodology. The higher NOEL of 0.1 mg/kg/day from the animal studies would be reduced by a factor for 10-fold interspecies extrapolation factor. Since the Lamb study (1993) is designed as a subchronic neurotoxicity, its NOEL is thus considered the critical NOEL, with supporting from the other studies.

II.C. Chronic Toxicity

In the 1996 RCD, DPR had considered only brain AChE inhibition in the selection of the critical inhalation NOEL from the 2-year rat study. The use of the NOEL as the point of departure is being reevaluated using the benchmark dose methodology. The critical study for oral exposure is included in this section to show the basis for the endpoint and NOEL of 0.05 mg/kg/day.

II.C.1. Chronic Studies

Dog – Oral

DDVP (97.3-99.5% purity) was administered orally in gelatin capsules at 0 (gelatin capsules), 0.05 (0.1 for the first 3 weeks of study), 1.0, or 3.0 mg/kg-day to purebred beagle dogs (4/sex/group) daily for 52 weeks (Markiewicz, 1990). The dosage of the 0.1 mg/kg-day group was lowered to 0.05 mg/kg-day because of plasma ChE inhibition (21-26%) observed after 2 weeks of dosing. There were no treatment-related changes in the mean food consumption, ophthalmology, necropsy, or histopathology. Cholinergic signs (soft stools, lacrimation, emesis, salivation, ataxia, and dyspnea) were intermittently observed at low incidences with no increase in the frequency of the observations with continued exposure. The occurrences (at least one observation noted per week) of emesis of food, soft stools, and salivation were dose-related. DDVP capsules were found in the emesis of 1 male and 3 females of the 3 mg/kg-day groups. However, only 7 occurrences were reported throughout the 1 year study. There was no apparent dose-related effect on lacrimation as it was observed more frequently in the lower doses. The earliest observation, reported on week 1, was soft stools in the 1.0 and 3.0 mg/kg-day males and emesis in the 3.0 mg/kg-day males. The NOEL for cholinergic signs was 1.0 mg/kg-day.

Plasma and RBC AChE activities were determined on weeks 2, 6, 13, 26, 39, and 52. Brain AChE activity was measured only at the end of the experiment. From week 13 to week 52, the plasma and RBC AChE activities were significantly ($p \leq 0.05$) depressed in the 1.0 and 3.0 mg/kg-day groups. At 52 weeks, RBC and brain AChE inhibitions for these two dose groups were significantly different from control ($p \leq 0.05$). At 1.0 mg/kg/day, RBC AChE activity was 40% (male) and 53% (female) of control, and brain AChE activity was 78% (male) and 93% (female, not significant) of control. At 3.0 mg/kg/day, RBC AChE activity was 12% (male) and 20% (female) of control, and brain AChE activity was 53% (male) and 71% (female) of control. The NOEL for both plasma and RBC AChE inhibition was 0.05 mg/kg-day. The brain AChE activities were inhibited in the 1.0 and 3.0 mg/kg-day males (78 and 53% of control values respectively), and in the 3.0 mg/kg-day females (71% of control value). The NOELs for brain AChE inhibition were 0.05 and 1.0 for males and females, respectively. This study was considered acceptable to DPR according to FIFRA guidelines.

Rat - Inhalation

In this study, rats (Carworth Farm E strain, 50/sex/group) were exposed to DDVP (purity not specified; at nominal concentrations of 0, 0.05, 0.5 or 5.0 mg/m^3) by whole-body inhalation for 23 hours per day, 7 days per week for 2 years (Blair *et al*., 1974 and 1976). The equivalent dosages were 0, 0.05, 0.5, and 4.6 mg/kg/day. The survival of the control groups was lower than

that for the treated groups. Daily observations were made and cholinesterase activity was measured only for survivors at the end of the experiment.

At 104 weeks, the survival was 64 and 72% for males and females, respectively, of the high dose groups, compared to 22 and 47% for males and females, respectively, for the control groups. The body weights of the 5.0 mg/m^3 group were consistently lower than those of the control groups. As an example, the decrease in the mean body weights for weeks 28, 56, 80, and 100 ranged from 13-19% for the males and 9-11% for the females, and were statistically significant ($p \leq 0.05$). The NOEL for decreased body weight in the males was 0.5 mg/m^3 (0.5 mg/kg-day). The food consumption rates of the treated groups were either the same or slightly higher than those for the controls.

No cholinergic signs were observed in any groups. Cholinesterase activities of plasma, erythrocyte, and brain showed dose-related depression (Table 20). The NOEL was 0.05 mg/m^3 based on statistically significant brain AChE inhibition at 0.5 mg/m^3 (Table 20). At a given dose, RBC AChE inhibition was greater than that for plasma or brain AChE inhibition.

Table 20. Cholinesterase inhibition in rats exposed to DDVP by inhalation for 2 years.[a]

Cholinesterase activity	**Dose (mg/m^3)**			
	0	**0.05**	**0.5**	**5.0**
Males				
n=	8	18	12	29
Plasma ChE (ml/min)	1.02±0.22	0.98±0.23 (94%)	0.78±0.14* (76%)	0.38±0.09** (37%)
RBC AChE (ml/min)	0.99±0.28	0.99±0.25 (100%)	0.71±0.46 (72%)	0.04±0.08** (4%)
Brain AChE (g/min)	12.13±0.50	11.64±0.86 (96%)	10.93±1.10** (90%)	2.51±0.48** (n=28) (21%)
Females				
n=	18	24	23	31
Plasma ChE (ml/min)	1.82±0.48	1.68±0.43 (92%)	1.52±0.50* (84%)	0.40±0.11** (22%)
RBC AChE (ml/min)	1.28±0.23	1.13±0.21* (n=23) (88%)	0.88±0.26** (78%)	0.07±0.10** (5%)
Brain AChE (g/min)	11.28±1.21 (n=19)	10.90±0.89 (n=23) (97%)	10.10±1.12** (90%)	2.13±0.47** (19%)

a/ Data from the publication (Blair *et al*., 1976) of the original report (Blair *et al*., 1974). This publication provided standard deviation values, whereas the original report has standard errors. n=number of animals per group, except where indicated. Percent of control activity indicated in parentheses.

II.C.2. Chronic Critical NOEL Selection

The critical oral NOEL for chronic toxicity remained at 0.05 mg/kg/day based on brain AChE inhibition from the dog study (Markiewicz, 1990). The data were not reanalyzed using the BMD analysis.

For the chronic inhalation study, the BMD analysis was performed by the USEPA and showed $BMDL_{10}$ and $BMDL_{20}$ of 0.078 and 0.196 mg/m^3, respectively, for RBC AChE inhibition, and $BMDL_{10}$ of 0.41 mg/m^3 for brain AChE inhibition (USEPA, 2007f). The $BMDL_{20}$ for RBC AChE inhibition was considered as the appropriate point of departure since there was a large margin between that and the $BMDL_{10}$ for brain AChE inhibition, and no cholinergic signs were observed in this study. In the USEPA risk assessment of the strips, all three BMDL values were used to calculate the MOEs (USEPA, 2007e and f).

For this Addendum, the RBC AChE inhibition is considered the most sensitive endpoint and is a surrogate for peripheral AChE, which was not measured in any of the studies. Both $BMDL_{10}$ and $BMDL_{20}$ for RBC AChE inhibition will be considered in the calculation of margins of exposure for the following reasons:

1. The current DPR default policy for RBC AChE inhibition threshold is 20%. And the USEPA considers 20% appropriate for chronic toxicity of DDVP (USEPA, 2007f).

2. However, USEPA selected RBC AChE inhibition at 10% response to address the acute toxicity of DDVP (USEPA, 2007f). After a single dose or 7-repeated doses, the $BMDL_{10}$ for RBC AChE is the same as that for brain AChE inhibition (Table 16).

3. The use of 20% AChE inhibition would result in a threshold that is higher than shown by the data. The 12% inhibition of RBC AChE for the 0.05 mg/m^3 female group, was statistically significantly different from the control (Table 20).

4. Furthermore, the lack of reported cholinergic sign at 5 mg/kg/day (highest dose tested) with chronic exposure in this study raised questions about the adequacy of the observation when the study was conducted in 1974. More recent studies suggested that clinical signs would be expected at that dose with long-term exposures. In Twomey (2002c), fasciculations and miosis were reported in young adult female given an oral dose of 15 mg/kg (Table 12; Twomey, 2002c). At a lower dose of 7.5 mg/kg/day, slight tremors were reported in rats after 7 repeated doses (Table 15; Moxon, 2003). In the subchronic neurotoxicity study, tremors, salivation, and exophthalmus were observed in the 7.5 mg/kg-day group starting at week 3 (Lamb, 1993). The only chronic gavage study reported mild diarrhea but neither the frequency nor the treatment group (2.9 and 5.7 mg/kg/day) was specified (Chan, 1989).

5. The availability of AChE activity measurements only for the survivors, and only at the end of the Blair *et al*. 2 year study created uncertainty about whether the animals which died were affected. A lower level of 10% inhibition as the threshold would provide more health protection.

II.D. Mutagenicity and Oncogenicity

In DPR 1996 RCD, DDVP was considered to be potentially oncogenic in humans based on positive results, mononuclear cell leukemia in rats (Table 21) and forestomach adenoma and carcinoma in mice, from the NTP study (Chan, 1989) and positive results from *in vitro* genotoxicity studies.

For this Addendum, the available literature and USEPA detailed discussion of the change in cancer classification (USEPA, 2000 and 2007e) were considered. For genotoxicity, DDVP showed positive responses in some *in vitro* studies, but not in *in vivo* studies. Oncogenicity studies with experimental animals showed no tumors after dietary (Witherup *et al.*, 1967; NCI, 1977a and b), inhalation (Blair *et al*., 1974 and 1976), drinking water (Enomoto, 1978), or gavage (Horn *et al*., 1987 and 1988) exposures. The only positive findings were those in the NTP study (Chan, 1989). In mice, the forestomach tumors were likely associated with chronic irritation on the tissue associated with gavage dosing, a route not expected in human exposure. However, the finding of significantly increased incidences of mononuclear cell leukemia (MCL) in male rats with DDVP remains a concern, as an indication of oncogenic potential. There is no evidence to dismiss the finding as irrelevant to humans, even though MCL is a common tumor in rats with high variability, and strain specificity (USEPA, 2007e).

Table 21.The incidences of tumors in rats treated with DDVP by gavage for 2 years[a].

Tumor types	Dose (mg/kg-day)[b]		
	0	**2.9**	**5.7**
Mononuclear leukemia, males	11/50^{+} (22%)	20/50* (40%)	21/50* (42%)
Mononuclear leukemia, females	17/50 (34%)	21/48 (44%)	23/50 (46%)

a/ Data were from Chan (1989). Incidences were expressed as the number of animals bearing tumors per animals at risk. All animals with at least 52 weeks of exposure or alive when the first tumor was detected, whichever occurs first, were considered at risk. Level of statistical significance, $p \leq 0.05$ (* or $^{+}$), is indicated after each incidence. Significance at the control value is based on a dose-weighted chi-square trend test, and significance at the dosed groups is for the Fisher's Exact Test.

b/ Doses adjusted for 5 days per week dosing.

The epidemiologic study by Koutros *et al.* (2008) is the largest prospective study of adults (1101 applicators in North Carolina and Iowa) enrolled from 1993 to 1997, and followed for cancer through 2004. It is a continued study of the Agricultural Health Study workers, and was prompted by the several reports suggesting increased cancer risks associated with a history of DDVP exposures (Reeves *et al*., 1981; Brown *et al*., 1990; Cantor *et al*., 1992; De Roos *et al*., 2003; Mills and Yang, 2003; Alavanja *et al.*, 2003; Flower *et al*., 2004). The investigators reported no increased incidence of lymphohematopoietic cancers as a group. However, they considered the number of cases for leukemia and non-Hodgkins lymphoma too few for adequate analysis. There was a slight increase (not statistically significant) in prostate cancer incidences for workers with a family history for this cancer. Previous study of the same cohort, but with smaller number of cases, had shown a statistically significant excess risk for prostate cancer and DDVP exposure for men with a family history of this cancer (Alavanja *et al*., 2003). The

investigators plan to follow-up with this group to further study the association between DDVP and these cancers.

Thus, DPR concluded that DDVP remains a potential oncogen for humans because of the significantly increased incidences of mononuclear cell leukemia in male rats and concerns for increased cancer risk for workers with a family history of prostate cancer. The potential for DDVP to cause cancer in children has not been adequately studied. Given the high background and the narrow dose range in the results in the male rat data for mononuclear leukemia (Chan, 1989), MT concurs with USEPA that quantitative risk assessment should not be conducted.

III. Risk Assessment

There was sufficient new information on toxicology and exposure, and amended uses to reexamine the previous DPR risk assessments. The acute critical NOEL for all routes is a $BMDL_{10}$ of 0.8 mg/kg/day for brain AChE inhibition in young adult rats and RBC AChE inhibition in preweaning rats (Table 22). This is a change from the previous value of 0.65 mg/kg/day for death in pregnant rabbits after inhalation exposure. The chronic inhalation critical NOEL is revised based on BMD analysis to derive BMDL levels of 0.072 mg/kg/day (10% inhibition) and 0.18 mg/kg/day (20% inhibition), for RBC AChE inhibition in rats. The previous value was a NOEL of 0.05 mg/kg/day for brain AChE inhibition. The subchronic critical NOEL (oral and inhalation) of 0.1 mg/kg/day, and chronic critical oral NOEL of 0.05 mg/kg/day remained the same. DDVP should continue to be considered a potential oncogen for humans, but quantitative risks would not be calculated.

When compared with the USEPA, many of the critical NOELs are the same. However, conclusions regarding the MOEs and the reference concentration are different for the inhalation route because differences in reference concentration methodology resulting in MOE benchmarks of 100 and 30, respectively, for DPR an USEPA (Table 22). The difference is how pharmacokinetic differences between species are addressed. For extrapolation of results from laboratory animals to humans, DPR uses breathing rate adjustment to account for intake differences between species. Since this adjustment does not address potential pharmacokinetic (absorption, distribution, metabolism, and excretion) differences, DPR applied an uncertainty factor of $\sqrt{10}$. In comparison, the USEPA uncertainty factor is 1 in the absence of pharmacokinetic data (USEPA, 1994). Both USEPA and DPR applied uncertainty factors of $\sqrt{10}$-fold to address the species pharmacodynamic differences, and a 10-fold factor to address intraspecies variations. As for subchronic exposure, there were additional differences in the magnitude of the LOEL to NOEL extrapolation factor (3 versus 10) and the selection of the critical study (human versus rat study), as already discussed. For oral and dermal exposures, the benchmarks for acceptable risks are MOEs of at least 100 for both DPR and USEPA.

Table 22. Revised critical no-observed-effects levels (NOELs) for risk characterization.

Scenarios	NOEL or BMDL	RfD or RfC		Effects	Ref[a]
Acute Oral/Dermal	0.5 mg/kg/day	0.05 mg/kg/day (UF=10)		RBC AChEI in humans	1
Inhalation	1.25 mg/m^3 (0.65 mg/kg/day)	0.0065 mg/kg/day (UF=100)		Death in pregnant rabbit	2
Subchronic Oral/Dermal	0.1 mg/kg/day	0.001 mg/kg/day (UF=100)		Tremors in rats	3
Inhalation	0.25 mg/m^3 (0.13 mg/kg/day)	0.0013 mg/kg/day (UF=100)		Brain AChEI in rabbits	2
Chronic Oral	0.05 mg/kg/day	0.0005 mg/kg/day (UF=100)		Brain AChEI in dogs	4
Inhalation	0.05 mg/m^3 (0.05 mg/kg/day)	0.0005 mg/kg/day (UF=100)		Brain AChEI in rats	5
DPR Current Assessment[b]					
Acute All routes	$BMDL_{10}$=0.8 mg/kg/day	0.008 mg/kg/day (UF=100)		Brain AChEI in young adult and RBC AChEI in preweaning rats	6
Subchronic All routes	0.1 mg/kg/day	0.001 mg/kg/day (UF=100)		Brain AChEI and clinical signs in rats	3
Chronic Oral	0.05 mg/kg/day	0.0005 mg/kg/day (UF=100)		Brain AChEI in dogs	4
Inhalation	$BMDL_{10}$= 0.078 mg/m^3 (0.072 mg/kg/day)	Adult 4 hours: 0.0154 mg/m^3 24 hours: 0.0026 mg/m^3 (BR, UF=100)	Child 4 hours: 0.0074 mg/m^3 24 hours: 0.0012 mg/m^3 (BR, UF=100)	RBC AChEI in rats	5
	$BMDL_{20}$= 0.196 mg/m^3 (0.18 mg/kg/day)	Adult 4 hours: 0.0386 mg/m^3 24 hours: 0.0064 mg/m^3 (BR, UF=100)	Child 4 hours: 0.0183 mg/m^3 24 hours: 0.0031 mg/m^3 (BR, UF=100)		
USEPA, 2006a and 2007f					
Acute Oral	$BMDL_{10}$=0.8 mg/kg/day	0.008 mg/kg/day (UF=100)		Brain AChEI in young adult and RBC AChEI in preweaning rats	6
Inhalation		0.03 mg/kg/day (UF=30)			
Subchronic All routes	0.1 mg/kg/day LOEL	0.003 mg/kg/day (UF=30)		RBC AChEI in humans	7
Chronic Oral	0.05 mg/kg/day	0.0005 mg/kg/day (UF=100)		Plasma and RBC AChEI in dogs	4
Inhalation	$BMDL_{10}$= 0.078 mg/m^3	0.003 mg/m^3 (UF=30)		RBC AChEI in rats	5
	$BMDL_{20}$= 0.196 mg/m^3	0.007 mg/m^3 (UF=30)			
	$BMDL_{10}$=0.41 mg/m^3	0.014 mg/m^3 (UF=30)		Brain AChEI in rats	

a/ References: 1. Gledhill, 1997a and b; 2. Thorpe *et al*., 1971; 3. Lamb, 1993; 4. Markiewicz, 1990; 5. Blair *et al.*, 1974; 6. Twomey, 2002c; and Moxon, 2002; 7. Gledhill, 1997c. Abbreviations: AChEI=acetylcholinesterase inhibition, BR=breathing rate adjustment, ENEL=estimated NOEL using a factor of 3, UF=uncertainty factors applied. 1 µg/L=1 mg/m^3

b/ Calculations are shown in Appendix E.

III.A. Occupational Exposure

For workers, exposure from DDVP use alone was evaluated because it is unlikely that they would be handling or exposed to DDVP, naled or trichlorfon at the same time since each pesticide has different application sites.

In the absence of new monitoring data, the exposure estimates were obtained from USEPA documents because they reflected current products and use restrictions on the federal labels (USEPA, 2006a and 2007e). They were determined for application and post-application in mushroom houses, greenhouses, barns, rail cars, and trucks. The values were mainly from surrogate databases such as the Outdoor Residential Exposure Task Force (ORETF) database, a chlorpyrifos/dichlorvos study, and PHED V1.1, with assumptions described in the IRED (USEPA, 2006a). The exposures and margins of exposure are presented in Table 23.

The MOEs for all the workers, with few exceptions, were greater than 100 (Table 23). The exceptions are mainly concerned with workers in mushroom house and green house. The MOEs for the dermal exposure of mushroom house and green house applicators using ORETF hose-end sprayer without wearing overalls was 47. With the addition of overalls, the MOE increased to 91, this could be considered acceptable. For post application, the inhalation MOEs for workers in mushroom house were 24 and 67 for restricted entry intervals (REIs) of 12 and 24 hours, respectively. Since the USEPA MOE benchmark is 30, the current label REI of 18 hours is sufficient. However, based on the DPR benchmark of 100, the REI needs to be extended to more than 24 hours.

Table 23. Exposures and margins of exposure for workers exposed to DDVP.[a].

Scenarios	Exposure (mg/kg/day)		Margin of Exposure[b]	
	Dermal	**Inhalation**	**Dermal**	**Inhalation**
Application - intermediate term exposures, 8 hours/day				
Mushroom house and green house				
ORETF hose end sprayer	0.00212	0.00004	47	2500
ORETF hose end sprayer+overall	0.0011	0.00004	91	2500
Direct animal treatment				
hand held sprayer	0.000025	0.000023	4000	4348
backpack sprayer (471)	0.000376	0.000023	266	4348
backpack sprayer (416)	0.000039	0.000023	2564	4348
portable sprayer on cart	0.0001	0.000068	1000	1471
Dairy barns-space spray				
hand held sprayer	0.000009	0.000008	11111	12500
backpack sprayer (471)	0.000135	0.000008	741	12500
backpack sprayer (416)	0.000014	0.000008	7143	12500
portable sprayer on cart	0.000036	0.000025	2778	4000
Dairy barns-surface spray				
hand held sprayer	0.000014	0.000013	7143	7692
backpack sprayer (471)	0.000217	0.000013	461	7692
backpack sprayer (416)	0.000022	0.000013	4545	7692
portable sprayer on cart	0.000057	0.000039	1754	2564
Rail cars and trucks surface spray	0.0003	0.000013	333	7692
Post Application - intermediate term exposures, 8 hours/day except noted				
Mushroom house reentry REI=12 hr	0.0002	0.004	500	24
reentry REI=24 hr	0.0002	0.001	500	67
Greenhouse reentry REI=2 hr	0.0012	0.0003	83	3061
reentry REI=12 hr	0.00012	0.0003	833	3061
reentry REI=24 hr	0.00012	0.0003	833	3061
Rail cars and trucks (4 hours/day)				
reentry REI=8 hr	NA	0.001	NA	115
Post Application - acute exposures, 8 hours/day				
Food manufacturing reentry REI=24 hr	0.00022	0.0049	3636	162
Warehouse treatment reentry REI=24 hr	0.00022	0.0069	3636	116

a/ Exposure values were determined by the USEPA (USEPA, 2006a). When exposures were given as air concentration, it was converted to dosage by using a default human breathing rate of 0.28 m^3/kg/day and adjusted for number of hours exposed/day. Abbreviations: hr=hours, NA=no exposure, ORETF= Outdoor Residential Exposure Task Force, REI=reentry interval.

b/ For all routes of exposure, DPR acute NOEL was 0.8 mg/kg/day ($BMDL_{10}$) for brain AChE inhibition in young adult rats (Twomey, 2002c) and RBC AChE inhibition in preweaning rats (Moxon, 2002). For all routes of exposure, DPR subchronic (intermediate term) NOEL was 0.1 mg/kg/day for cholinergic signs and brain AChE inhibition (Lamb, 1993). The benchmark MOE for acceptable risk was 100.

III.B. Residential Exposure

Residential exposure estimates for pest strips were also obtained from air concentrations determined by the USEPA, which were based on the values from Collins and DeVries (1973) study (USEPA, 2006a; 2007f) (Table 24-27). In this study, DDVP strips (80 or 100 grams) were placed at various locations in each house with one of them near the dining area, for 91 days. The air detector was placed near the dining room for each house.

For the 65-gram strips, the acute air concentration was the highest measured air concentration on day 1 for each house, while the chronic air concentration was the average concentration over the 91-day study period. These concentrations should also represent those for the 80-gram strips since the original study used both 80 and 100-gram strips. For the 16, 10.5, and 5.25-gram strips, the acute air concentrations were extrapolated from the 65-gram strips by reducing factors of 4, 6, and 12.4, respectively, with the assumption that air concentration is proportional to the mass of the strips. For the 16-gram strips, the chronic air concentration was based on the 91-day average detected measurement and the assumption that each house used 3-4 closet strips (USEPA, 2007f). From this value, the chronic air concentrations for 10.5 gram and 5.25 gram strips were extrapolated using reducing factors of 1.5 and 3, respectively. In Tables 24-27, the air concentrations were grouped as ranges instead of individual values, to simplify presentation.

For the 65-gram strip, the exposure duration was assumed to be the maximum of 4 hours, as indicated on the label. The affected residents include adults and children, since they can be present where the strips are allowed to be used in certain areas of the home (garages, attics, crawl spaces and sheds). For the 16, 10.5, and 5.25-gram strips, the federal label limits the use to closets, wardrobes, and cupboards, and the exposure duration was considered 24 hours since there is no time restriction. To convert to exposure, air concentrations were multiplied by DPR default adults and children breathing rates of 0.28 m^3/kg/24-hour day and 0.59 m^3/kg/24-hour day, respectively, and adjusted for exposure duration (4 or 24 hours).

For the 65-gram strip, acute air concentrations ranged from 0.01 to 0.11 mg/m^3 (Table 24). The acute MOEs for adults and children were 156-1714 and 74-814, respectively. For chronic exposure, the air concentrations ranged from 0.001 to 0.0061 mg/m^3. When based either on $BMDL_{10}$ or $BMDL_{20}$, all MOEs were greater than 100 for both groups (ranged 121 to 3896).

For the 16-gram strips used inside the home, acute air concentrations ranged from 0.003 to 0.028 mg/m^3 (Table 25). The acute MOEs were 104-1143 (adults) and 49-542 (children). For chronic exposure, the air concentrations ranged from 0.0015 to 0.0091 mg/m^3. When based on the $BMDL_{10}$ for toxicity, the MOEs were 28-174 (adults) and 13-82 (children). When based on the $BMDL_{20}$, the MOEs were 71-434 (adults) and 34-206 (children).

For the 10.5-gram strips used inside the home, the acute air concentrations ranged from 0.002 to 0.018 mg/m^3 (Table 26). The acute MOEs were 156-1714 (adults) and 74-814 (children). For chronic exposure, the air concentrations ranged from 0.001 to 0.0061 mg/m^3. When based on the $BMDL_{10}$ for toxicity, the MOEs were 42-260 (adults) and 20-123 (children). When based on the $BMDL_{20}$, the MOEs were 106-649 (adults) and 50-308 (children).

For the 5.25-gram strips used inside the home, the acute air concentrations ranged from 0.0008 to 0.0089 mg/m^3 (Table 27). The acute MOEs were 322-3543 (adults) and 153-1681 (children). For chronic exposure, the air concentrations ranged from 0.0005 to 0.003 mg/m^3. When based on the $BMDL_{10}$ for toxicity, the MOEs were 85-525 (adults) and 40-249 (children). When based on the $BMDL_{20}$, the MOEs were 212-1312 (adults) and 101-623 (children).

For acute exposure, the air concentration was acceptable only when it was at or below 0.08 mg/m^3 and 0.013 mg/m^3, for the 65-gram strip and the smaller strips, respectively. For chronic exposure, the air concentration should not be higher than 0.006 mg/m^3 for the 65-gram strips. For the smaller strips, the chronic air concentration has to be at or lower than 0.003, or below 0.0015 mg/m^3 when based on the $BMDL_{20}$ or $BMDL_{10}$, respectively. Since there are houses with air concentrations higher than these levels, all current strip sizes and exposure durations posed unacceptable risk, in particular for children.

Table 24. Exposures and margins of exposure to 65-gram pest strips for 4 hours.

Air concentration in houses[a] (mg/m^3)	**Adult**			**Child**		
	Exposure[b] mg/kg/day	**Margin of Exposure[c]**		**Exposure[b] mg/kg/day**	**Margin of Exposure[d]**	
Acute Exposure						
0.11 (2 houses)	0.005	156		0.011	74	
0.08 (2 houses)	0.004	214		0.008	102	
0.07 (2 houses)	0.003	245		0.007	116	
0.05 (3 houses)	0.002	343		0.005	163	
0.04 (2 houses)	0.002	429		0.004	203	
0.02 (3 houses)	0.001	857		0.002	407	
0.01 (1 house)	0.0005	1714		0.001	814	
Chronic Exposure		**$BMDL_{10}$**	**$BMDL_{20}$**		**$BMDL_{10}$**	**$BMDL_{20}$**
0.0061-0.0048 (3 houses)	0.00028-0.00023	254-319	635-799	0.0006-0.00047	121-152	302-379
0.0034-0.0033 (3 houses)	0.00016-0.00015	458-468	1145-1169	0.00033-0.00032	217-222	543-555
0.0021-0.0019 (4 houses)	0.00010-0.00009	728-804	1819-2009	0.00021-0.00019	345-381	863-953
0.0016-0.0010 (5 houses)	0.00008-0.00005	958-1558	2396-3896	0.00016-0.00010	455-740	1137-1849

a/ Air concentrations were determined by the USEPA (2007e and f) from the study of Collins and DeVries (1973) in 15 houses. The acute air concentrations were the highest estimated concentration on day 1 for each house. The chronic air concentrations were the average of 91 days of measurements for each house. In this table, houses with the same or similar air concentration reported are shown as a range, and are not listed individually.

b/ To convert to exposure, air concentrations were multiplied by DPR default adults and children breathing rates of 0.28m^3/kg/24-hour day and 0.59m^3/kg/24-hour day, respectively, and adjusted for 4 hours of exposure.

c/ The acute NOEL was 0.8 mg/kg/day ($BMDL_{10}$) for brain AChE inhibition in young adult rats (Twomey, 2002c) and RBC ChE inhibition in preweaning rats (Moxon, 2002).

d/ The chronic NOELs were 0.072 mg/kg/day ($BMDL_{10}$) and 0.18 mg/kg/day ($BMDL_{20}$) for RBC AChE inhibition in rats (Blair *et al*., 1974).

Table 25. Exposures and margins of exposure to 16-gram pest strips for 24 hours.

Air concentration in houses[a] (mg/m^3)	Adult			Child		
	Exposure[b] mg/kg/day	Margin of Exposure[c]		Exposure[b] mg/kg/day	Margin of Exposure[d]	
Acute Exposure						
0.028 (2 houses)	0.008	104		0.016	49	
0.020 (2 houses)	0.006	143		0.012	68	
0.018 (2 houses)	0.005	163		0.010	77	
0.013 (3 houses)	0.004	229		0.007	108	
0.010 (2 houses)	0.003	286		0.006	136	
0.005-0.003 (4 houses)	0.001	571-1143		0.003-0.001	271-542	
Chronic Exposure		**$BMDL_{10}$**	**$BMDL_{20}$**		**$BMDL_{10}$**	**$BMDL_{20}$**
0.0091-0.0073 (3 houses)	0.0025-0.0020	28-35	71-89	0.0054-0.0043	13-17	34-42
0.0051-0.0050 (3 houses)	0.0014	51-52	127-130	0.0030-0.0029	24-25	60-62
0.0032-0.0029 (4 houses)	0.0009-0.0008	81-89	202-223	0.0019-0.0017	38-42	96-106
0.0024-0.0019 (3 houses)	0.0007-0.0005	106-135	266-337	0.0014-0.0011	50-64	126-160
0.0016-0.0015 (2 houses)	0.0005-0.0004	157-174	392-434	0.0010-0.0009	74-82	186-206

a/ Air concentrations were determined by the USEPA (2007e and f) from the study of Collins and DeVries (1973) in 15 houses. The acute air concentrations were extrapolated from the data for the 65-gram strips and divided by a factor of 4. The chronic air concentrations were the average of 91 days of measurements for each house and the assumption that each house has 3-4 closet strips. In this table, houses with the same or similar air concentration reported are shown as a range, and are not listed individually.

b/ To convert to exposure, air concentrations were multiplied by DPR default adults and children breathing rates of $0.28 m^3$/kg/24-hour day and $0.59 m^3$/kg/24-hour day, respectively, and adjusted for 24 hours of exposure.

c/ The acute NOEL was 0.8 mg/kg/day ($BMDL_{10}$) for brain AChE inhibition in young adult rats (Twomey, 2002c) and RBC ChE inhibition in preweaning rats (Moxon, 2002).

d/ The chronic NOELs were 0.072 mg/kg/day ($BMDL_{10}$) and 0.18 mg/kg/day ($BMDL_{20}$) for RBC AChE inhibition in rats (Blair *et al*., 1974).

Table 26. Exposures and margins of exposure to 10.5-gram pest strips for 24 hours.

Air concentration in houses[a] (mg/m^3)	Adult			Child		
	Exposure[b] mg/kg/day	Margin of Exposure[c]		Exposure[b] mg/kg/day	Margin of Exposure[d]	
Acute Exposure						
0.018 (2 houses)	0.0051	156		0.011	74	
0.013-0.012 (4 houses)	0.0037-0.0033	214-245		0.008-0.007	102-116	
0.008-0.007 (5 houses)	0.0023-0.0019	343-429		0.005-0.004	163-203	
0.003-0.002 (4 houses)	0.0009-0.0005	857-1714		0.002-0.001	407-814	
Chronic Exposure		**$BMDL_{10}$**	**$BMDL_{20}$**		**$BMDL_{10}$**	**$BMDL_{20}$**
0.0061-0.0048 (3 houses)	0.0017-0.0014	42-53	106-133	0.0036-0.0028	20-25	50-63
0.0034-0.0033 (3 houses)	0.0009	76-78	191-195	0.0020-0.0019	36-37	91-92
0.0021-0.0014 (6 houses)	0.0006-0.0004	121-188	303-469	0.0013-0.0008	58-89	144-223
0.0013-0.0010 (3 houses)	0.0004-0.0003	202-260	506-649	0.0007-0.0006	96-123	240-308

a/ Air concentrations were determined by the USEPA (2007e and f) from the study of Collins and DeVries (1973) in 15 houses. The acute air concentrations were extrapolated from the acute data for the 65-gram strips and divided by a factor of 6. The chronic air concentrations were extrapolated from the chronic data for the 16-gram strips and divided by a factor of 1.5. In this table, houses with the same or similar air concentration reported are shown as a range, and are not listed individually.

b/ To convert to exposure, air concentrations were multiplied by DPR default adults and children breathing rates of $0.28 m^3$/kg/24-hour day and $0.59 m^3$/kg/24-hour day, respectively, and adjusted for 24 hours of exposure.

c/ The acute NOEL was 0.8 mg/kg/day ($BMDL_{10}$) for brain AChE inhibition in young adult rats (Twomey, 2002c) and RBC ChE inhibition in preweaning rats (Moxon, 2002).

d/ The chronic NOELs were 0.072 mg/kg/day ($BMDL_{10}$) and 0.18 mg/kg/day ($BMDL_{20}$) for RBC AChE inhibition in rats (Blair *et al*., 1974).

Table 27. Exposures and margins of exposure to 5.25-gram pest strips for 24 hours.

Air concentration in houses[a] (mg/m^3)	Adult			Child		
	Exposure[b] mg/kg/day	Margin of Exposure[c]		Exposure[b] mg/kg/day	Margin of Exposure[d]	
Acute Exposure						
0.0089 (2 houses)	0.0025	322		0.005	153	
0.0065-0.0056 (4 houses)	0.0018-0.0016	443-506		0.004-0.003	210-240	
0.0040-0.0032 (5 houses)	0.0011-0.0009	709-886		0.002	336-420	
0.0016-0.0008 (4 houses)	0.0005-0.0002	1771-3543		0.001-0.0005	841-1681	
Chronic Exposure		**$BMDL_{10}$**	**$BMDL_{20}$**		**$BMDL_{10}$**	**$BMDL_{20}$**
0.0030-0.0029 (2 houses)	0.0008	85-90	212-224	0.0018-0.0017	40-43	101-106
0.0024-0.0017 (4 houses)	0.0007-0.0005	106-156	266-390	0.0014-0.0010	50-74	126-185
0.0011-0.0010 (4 houses)	0.0003	243-268	606-670	0.0006	115-127	288-318
0.0008-0.0005 (4 houses)	0.0002-0.0001	317-525	794-1312	0.0005-0.0003	151-249	377-623

a/ Air concentrations were determined by the USEPA (2007e and f) from the study of Collins and DeVries (1973) in 15 houses. The acute air concentrations were extrapolated from the acute data for the 65-gram strips and divided by a factor of 12.4. The chronic air concentrations were extrapolated from the chronic data for the 16-gram strips and divided by a factor of 3. In this table, houses with the same or similar air concentration reported are shown as a range, and are not listed individually.

b/ To convert to exposure, air concentrations were multiplied by DPR default adults and children breathing rates of $0.28 m^3$/kg/24-hour day and $0.59 m^3$/kg/24-hour day, respectively, and adjusted for 24 hours of exposure.

c/ The acute NOEL was 0.8 mg/kg/day ($BMDL_{10}$) for brain AChE inhibition in young adult rats (Twomey, 2002c) and RBC ChE inhibition in preweaning rats (Moxon, 2002).

d/ The chronic NOELs were 0.072 mg/kg/day ($BMDL_{10}$) and 0.18 mg/kg/day ($BMDL_{20}$) for RBC AChE inhibition in rats (Blair *et al*., 1974).

III.C. Dietary Exposure

For the general population, there is a potential for exposure to DDVP residues from DDVP, naled and trichlorfon uses. The dietary exposure estimates were obtained from USEPA, which used residue data from monitoring studies, in particular the USDA Pesticide Data Program Data, and the exposure software DEEM-FCID^{TM} (USEPA, 2006a; 2007e and g). The DDVP residues in the diet were the sum of direct DDVP uses and as a metabolite of naled and trichlorfon use sites.

Monitoring studies showed that DDVP residues are generally below the detection limit in raw agricultural, processed commodities, and meats. Residue values were selected from the USDA Pesticide Data Program (PDP) for milk, poultry (fat, liver, muscle), beef (fat, liver, muscle), pork (muscle and fat), commodities with a preharvest use (including strawberries), cereal grain crops, and in crops from the use of naled. DDVP residues for other berries came from the Food and Drug Administration monitoring data. For processed commodities in warehouses, the residue levels were based on residue field trials, which used the maximum treatment rates and sampled the commodities 6 hours after treatment. DDVP residues in the drinking water were derived from modeling (Tier 2 PRZM-EXAMS analysis), and included consideration of naled and trichlorfon degradation from uses on agricultural commodities and turf. For all commodities, samples with non-detects were assigned ½ of the limit of detection. The food consumption rate relied on the CSFII surveys (1994-1996 and 1998). The USEPA estimated exposures for both 100% crop treatment as well as adjusted for percentage of crop treatment. For this Addendum, as a conservative measure, exposure values from 100% crop treatment were selected for comparison with previous values. As shown in Table 28, the current USEPA determined acute exposures were generally higher than those established by DPR in 1996 RCD, which included fewer commodities and did not include drinking water as a source for residues. Nevertheless, all the MOEs were >100 and thus the risk from dietary exposures remained acceptable.

Table 28. Exposures and margins of exposure for DDVP residues in the diet.

Population (age, years)	Exposures (mg/kg/day)			MOE[c] for 100% PCT USEPA exposures
	DPR 1996 RCD[a]	USEPA 100% PCT[b]	USEPA PCT adjusted[b]	
Acute Dietary Exposure		**99.9th percentile**		
General	0.00091	0.002274	0.001313	352
All infants	0.00144	0.004152	0.003735	193
1-2	0.00165	0.004663	0.001523	172
3-5		0.003533	0.001312	226
6-12	0.00115	0.002677	0.000911	299
13-19	0.00069	0.001660	0.000967	482
20-49	0.00069	0.001850	0.001475	432
50+	0.00057	0.001437	0.000929	557
Female 13-49	0.00064	0.001603	0.001	499
Chronic Dietary Exposure				
General	0.00022	0.000112	0.00006	446
All infants	0.00024	0.000154	0.000116	325
1-2	0.00053	0.000252	0.000111	198
3-5		0.000214	0.000103	234
6-12	0.00035	0.000138	0.000069	362
13-19	0.00024	0.000092	0.000048	543
20-49	0.00020	0.000102	0.000057	490
50+	NA	0.000088	0.000051	568
Female 13-49	0.00020	0.000097	0.00005	515

a/ When there are more than one subgroups for the age range, the highest value is selected from the RCD.
b/ Details on factors used for percent of crop (PCT) adjustment are in USEPA, 2007e and g.
c/ The acute NOEL was 0.8 mg/kg/day ($BMDL_{10}$) for brain AChE inhibition in young adult rats (Twomey, 2002c) and RBC AChE inhibition in preweaning rats (Moxon, 2002). The chronic NOEL was 0.05 mg/kg/day for brain AChE inhibition in dogs (Markiewicz, 1990).

IV. Risk Appraisal

The uncertainty of the margins of exposure values involved both the toxicology and exposure. While there are sufficient data to evaluate the acute and subchronic exposures, the chronic inhalation exposure to DDVP relied on a single study, which might not have adequate clinical observation, and cholinesterase activity was measured only at the end of the study. It is, therefore, more health protective to consider the MOEs based on the $BMDL_{10}$ instead of $BMDL_{20}$, for acceptability of exposure.

As for occupational exposure, the data were mostly from PHED and surrogate studies. It is not known if the values were overestimates or underestimates of actual exposure. For residential exposure, the air concentrations were based on a study in 1973, which may not reflect the current house construction with respect to air flow and gas retention inside the house. Actual monitoring data of current building construction are needed. Data are also needed to establish the relationship between strip size, air concentration, environmental condition (e.g., room temperature and air flow), and number of strips. The USEPA assumption of 3-4 strips per house may overestimate or underestimate actual exposures.

The dietary exposure assessment showed MOEs of >100. Since it was based on 100% crop treatment, the risk was likely overestimated.

No additional uncertainty factor for age-related differences in sensitivity was needed. USEPA made a determination of no increased sensitivity of juvenile rats to DDVP effect on AChE in the IRED. DPR had made such a conclusion in the first Addendum (DPR, 1997) but it was based on fewer studies. After a detailed analysis of the more recent studies, DPR also found no evidence of increased sensitivity of the young animals to the inhibition of AChE by DDVP. The developmental neurotoxicity studies showed the NOEL for fetal or pup AChE inhibition were the same or higher than those for the dams (Milburn, 2003a). A comparison of BMD values between preweaning and adult rats showed similar findings (Moxon, 2002 and Twomey, 2002c).

V. Conclusion

In this Addendum, reevaluation of the critical NOEL resulted in lowering of the reference dose from 0.05 mg/kg/day (based on the human study with a NOEL of 0.5 mg/kg/day and an uncertainty factor of 10) to 0.008 mg/kg/day (based on animal studies with a $BMDL_{10}$ of 0.8 mg/kg/day and an uncertainty factor of 100) for AChE inhibition by DDVP. This same value is applicable for inhalation exposure. The chronic critical NOEL for inhalation was increased from 0.05 mg/kg/day to 0.075 mg/kg/day and 0.188 mg/kg/day, respectively, for RBC AChE inhibition, because of the use of benchmark dose analysis. The subchronic critical NOEL and the chronic oral critical NOEL remained the same as in the previous assessments.

Using USEPA calculated exposures, most of the worker exposure scenarios were considered acceptable by DPR. The only exceptions are dermal exposure of applicator of mushroom house and green house without wearing overalls, and post application exposure of workers in a mushroom house with reentry of less than 24 hours. If overalls were added, the exposure of applicators would be acceptable. The post-application reentry to mushroom house needed to be longer than 24 hours.

Exposure of residents to DDVP in pest strips remained a health concern, as determined previously. In this Addendum, some air concentrations estimated for all strips >5.25 grams posed unacceptable risks for both children and adults. With the 5.25-gram strips, the exposures were acceptable for both adults and children when the toxicity was based on 20% RBC AChE inhibition, but not for children when based on 10% RBC AChE inhibition. However, there was sufficient uncertainty in the toxicology and exposure to be of concern with exposure to the 5.25-gram strips as evaluated using the 10% inhibition as the threshold. These conclusions for workers and residents are different than that from the USEPA because DPR required a higher benchmark of 100 for acceptable risk. The dietary exposure to DDVP residues, assuming 100% crop treatment) from DDVP, naled and trichlorfon uses, was acceptable for all population subgroups.

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Appendix A. Summary of Risk Characterization Document (1996)

INTRODUCTION

This Risk Characterization Document for dichlorvos (DDVP) addresses potential human exposures from its use in California. Oncogenic, genotoxic, and neurotoxic effects have been identified in animal studies. DDVP is listed under California Proposition 65, the Safe Drinking Water and Toxic Enforcement Act of 1986, as a chemical known to the State of California to cause cancer.

DDVP is an insecticide used for space spray treatment of food processing, handling, and storage plants; feedlots; stockyards; corrals; holding pens; animal buildings; poultry houses; as well as residential, commercial and institutional buildings. It is also used in flea collars for pets. The direct food uses are on vegetables grown in greenhouses, on livestock, and processed food items to control pests. Humans may be exposed to DDVP through inhalation, direct contact on the skin, and the diet.

THE RISK ASSESSMENT PROCESS

The risk assessment process consists of four aspects: hazard identification, dose response assessment, exposure evaluation, and risk characterization. Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and estimates the amount which could potentially cause an adverse effect. The amount which will not result in an observable or measurable effect is the no-observed-effect level, NOEL. A basic premise of toxicology is that at a high enough dose, virtually all substances will cause some toxic manifestation. Chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes. In reality, these terms describe chemicals which require low or high dosages, respectively, to cause toxic effects.

Toxicological activity is determined in a battery of experimental studies which define the types of toxic effects which can be caused, and the exposure levels (doses) at which effects may be seen. State and federal testing requirements mandate that substances be tested in laboratory animals at doses high enough to produce toxic effects, even if such levels are many times higher than those to which people might be exposed.

The exposure assessment includes an estimation of the potential exposure through the occupational, residential, and dietary routes on an acute (one time) and chronic (long-term and lifetime) basis. The levels of exposure are determined by the amount of pesticide residue in the air or on specific commodities and processed foods, and the exposure rates by inhalation or ingestion.

The risk characterization integrates the toxic effects observed in the laboratory studies, conducted with high dosages of pesticide, to potential human exposures to low dosages of pesticide residues in the air and diet. The potential for possible non-cancer adverse health effects in human population after acute and chronic exposures (long-term and lifetime) is generally expressed as the margin of safety, which is the ratio of the dosage which produced no effects in laboratory studies to the estimated exposure dosage. For cancer effects after potential lifetime

exposures, the probability of risk is calculated as the product of the cancer potency of the pesticide and the estimated exposure dosage.

BACKGROUND INFORMATION

DDVP is currently in Special Review, a process by the U.S. Environmental Protection Agency (USEPA) to determine the risk and benefit for the use of the pesticide. The major concerns are the inhibition of cholinesterase (ChE) activity, cancer, and nerve damage effects observed in animal studies. Cholinesterase is an essential enzyme in the body for the degradation of acetylcholine, a transmitter of signals from the nerve to another nerve or muscles. The inhibition of ChE results in cholinergic signs such as salivation, diarrhea, tremors, respiratory failure, and death due to the accumulation of acetylcholine and over-stimulation of nerves or muscles. USEPA has proposed the revocation of DDVP tolerances for processed commodities because of concerns on DDVP-induced cancer in experimental animals.

TOXICOLOGY

Cholinergic signs (tremors and diarrhea) and death in experimental animals were the most sensitive endpoints for the acute toxicity of DDVP after inhalation and oral exposures. The dose (the no-observed-effect level or NOEL) at which death and cholinergic signs did not occur was used to quantify the hazard for potential one-time exposure to humans. The long-term (chronic) health hazard to humans from repeated exposures to DDVP was evaluated based on the inhibition of cholinesterase activity in the brain observed in both inhalation and oral studies. The cancer risk from lifetime exposure was evaluated based on the finding of leukemia in rats after chronic oral exposure.

EXPOSURE ANALYSIS

The potential exposure scenarios of humans to DDVP include the work place, home, and the food. Workers are exposed to DDVP in the work place due to warehouse fumigation, livestock applications, and structural applications. The general population is exposed to DDVP in the home from the uses as directed spray, fogger, flea collars, and no-pest strips; as well as in the diet from the use of DDVP on vegetables, livestock, and processed foods. The worker exposure was also assessed in combination with exposure at home (from home use and in the diet).

RISK EVALUATION

A margin of safety (MOS) of at least 100 is generally considered sufficient to be protective of human health. The following exposure groups have MOSs greater than 100: chronic exposure of residents after structural fumigation; acute and chronic exposures of pet owners to flea collars; acute dietary exposure of all population subgroups; and chronic dietary exposure of all subgroups, except children 1 to 6 years old.

The following exposure groups have MOSs less than 100 for non-oncogenic effects: acute, chronic, and lifetime exposures for all workers exposed to DDVP only at work and in combination with exposure at home; acute exposure of residents after structural fumigation;

acute, chronic, and lifetime exposures of residents to home-use foggers; acute and chronic exposures of children to no-pest strips; and chronic dietary exposure of children 1 to 6 years old. For oncogenic effects, the excess lifetime oncogenic risks of the workers, residents, and the general population exposed to DDVP at work, at home, or in the diet and in combinations were greater than the benchmark oncogenic risk level of 1 x 10-6 which is generally considered protective of human health. The MOSs for the acute exposure to DDVP on vegetables and livestock products at tolerance levels are greater than 100.

CONCLUSIONS

The toxicological risk of potential exposure to DDVP was evaluated for occupational, residential, dietary and combined uses based on the inhibition of brain ChE activity, clinical signs, and the finding of mononuclear leukemia in animal studies. Using the conventional benchmark levels, a margin of safety of at least 100 for non-oncogenic effects and a risk level of 1 x 10-6 or less for oncogenic effects are generally considered sufficiently protective of human health. The exposure levels of only a few groups meet those benchmark levels. Groups which have exposure levels which do not meet the benchmark levels are: all people occupationally exposed to DDVP alone and in combination with home exposure on an acute, chronic, and lifetime basis; people exposed through residential use on an acute, chronic, and lifetime basis; and the general population exposed through the diet on a potentially lifetime basis.

Appendix B. Summary of First Addendum (1997)

INTRODUCTION

This addendum reevaluated the risk assessment of dichlorvos (DDVP) because of submitted human oral toxicity studies. In the 1996 Risk Characterization Document (RCD), the margins of exposure (MOE) were based on experimental animals. Most of the MOEs for workers and residents were below the conventional benchmark for human health protection.

TOXICOLOGY PROFILE

In the single dose studies, DDVP (1.0 mg/kg, highest dose tested) given in capsules to volunteers resulted in statistically significant inhibition (10%) of erythrocyte cholinesterase (ChE). Erythrocyte ChE inhibition was also inhibited (5-10%) at 0.5 mg/kg DDVP. In another study with volunteers given 0.3 mg/kg/day DDVP for 15 days, the erythrocyte ChE activity was significantly inhibited (15-30%) during and after exposure. At a lower dose of 0.1 mg/kg/day for 21 days, the erythrocyte ChE inhibition was 10-15% during the exposure.

RISK ASSESSMENT

The critical NOEL for acute oral exposure was revised to 0.5 mg/kg based on the human study. Because of uncertainties in route-to-route and time extrapolations, the critical NOELs for chronic dietary exposures and occupational and residential exposures remained the same as in the 1996 RCD. The margins of exposure for acute and chronic dietary exposures remained higher than the benchmark considered protective of human health. However, most of the occupational and residential exposures as well as the lifetime dietary exposures remained below the benchmarks.

RISK APPRAISAL

Depending on the endpoint and exposure duration, there were differences between humans and rats in the sensitivity to the toxicity of DDVP. Therefore, using an interspecies extrapolation factor was appropriate when the risk assessment was based on experimental animal studies. An additional uncertainty factor was not needed to account for the potential increased sensitivities of infants and children.

TOLERANCE ASSESSMENT

The MOEs for exposure to residue levels at tolerances remained the same.

CONCLUSIONS

This addendum did not change the conclusions in the 1996 RCD. The MOEs for acute and chronic dietary exposure remained above the benchmark considered sufficient for the protection of human health. For most of the non-dietary exposure scenarios, the MOEs or oncogenic risks did not meet the benchmarks considered protective of human health.

Appendix C. Summary of Second Addendum (1998)

INTRODUCTION

This second addendum reevaluated the risk assessment of dichlorvos (DDVP) because of new information on the toxicology and exposure of DDVP. The exposure scenarios assessed were: acute occupational and residential exposures, as well as chronic and lifetime dietary exposures.

RISK ASSESSMENT

Hazard identification

For acute occupational and residential exposures, a route-specific approach was considered. The critical no-observed-effect level (NOEL) used to assess dermal exposure was 0.5 mg/kg/day based on red blood cell cholinesterase inhibition in humans after oral dosing. The critical adjusted NOEL for inhalation exposure was 0.325 mg/kg/day for cholinergic signs and mortality in rabbits. In the 1996 Risk Characterization Document (RCD), the acute inhalation NOEL was used for the total exposure by both routes.

For lifetime exposures of all routes, the additional data submitted did not change the conclusion that there is sufficient evidence for DDVP oncogenicity.

Exposure

The occupational and residential exposures were determined for each route of exposure (inhalation and dermal) based on information given in the 1996 Risk Characterization Document.
The chronic dietary exposure was recalculated based on U.S. EPA analyses of the current monitoring data and field trials. Since the current data showed DDVP residues were essentially at the detection limit, the dietary exposure was substantially reduced.

CONCLUSIONS

This Addendum reassessed several acute, chronic, and lifetime exposure scenarios to DDVP.
For warehouse workers and livestock applicators, the inhalation route of exposure was of concern as the margins of exposures (MOEs) were below the benchmark. For residents, both the inhalation and dermal exposures were of concern as the MOEs were below the benchmark for all uses (structural, fogger, and resin-strip), except pet collar. The chronic and lifetime dietary exposures to DDVP were no longer of concern. The exposure estimates were reduced because current data showed residue levels at the detection limit for almost all foods.

Appendix D: Toxicology Summary

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH
SUMMARY OF TOXICOLOGY DATA

DDVP

Chemical Code # 187, Document Processing Number (DPN) # 235
SB 950 # 16

January 21, 1987
Revised 10/27/87, 6/2/89, 11/07/89,4/26/90, 7/5/90, 11/15/90, 07/11/91, 7/1/92, 12/11/92, 2/9/93, 9/2/93, 2/1/94, 2/10/95, 7/7/95, 12/12/95, 6/12/98, 6/17/99, 11/9/99, 2/28/05 and 6/3/08

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity:	No data gap, possible adverse effect

Toxicology one-liners are attached.
All record numbers applicable to SB-950 through 234880 (in Document No. 235-0250) were examined.
All relevant older records (Record Nos. > 900000) were examined. This includes all reports indexed as of 5/13/08. Note: Revision of 6/12/98 contains a publication from the open literature with a possible adverse effect (Gee, 6/12/98).
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: t20080603.wpd

Revised by: Stanton Morris, 7/11/91; J. Gee, 7/1/92; C. Aldous, 12/11/92; J. Gee, 2/9/93; T. Moore, 9/2/93; T. Kellner, 2/1/94; M. Silva, 2/10/95 & 7/7/95; J. Gee, 6/12/98, 6/17/99, 11/9/99; C. Aldous, 2/28/05 and June 3, 2008.

NOTE: Document No. 235-0186, Record No. 162850, contains a spreadsheet, indicating that worker health and safety branch has examined all volumes from 235-0186 through 235-209. Several of these records are also identified in this Summary of Toxicology Data.

Note: these pages contain summaries only. Individual worksheets may identify additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC TOXICITY, RAT

NOTE: Although no single rodent study independently fills the "rodent chronic" study data gap, the collective data on rodent chronic effects from three major rodent studies (095:074933, 071:035425, and 050:088033) adequately address general chronic toxicity in the rat. All three of the above studies exposed rats to levels near to an MTD, yet none found non-neoplasia effects of concern in any target tissue. A recent dog study (106:088784) also tested a dosage range at or near practical limits of exposure. In all cases, upper limits on acceptable dose levels elicited symptoms consistent with cholinesterase enzyme toxicity. The rodent chronic study data gap is considered "filled", with no adverse effects" indicated for non-neoplasia effects. Aldous, 11/14/90.

235-070 035423 "The Effects Exerted upon Rats during a Period of Two Years by the Introduction of Vapona Insecticide into their Daily Diets" (Kettering Lab, 2/14/67). DDVP (dichlorvos, Vapona), 93% initially by weight. 40/sex/group were fed at 0, 0.1, 1, 10, 100 or 500 ppm nominal; 22-80% loss of test article due to volatilization and hydrolysis in feed, which was mixed weekly. Interim sacrifices at 26, 52 and 78 weeks of 5/sex/group with 25/sex/group scheduled to be fed to term. ChE NOEL = 10 ppm nominal (blood cholinesterase inhibition), systemic NOEL = 100 ppm nominal (liver cell vacuolation). Unacceptable (inadequate numbers of animals at risk, protocol (surgical removal of tumors, use of animals in reproduction study), weekly preparation of diet when test article known to be volatile, inadequate histopathology and blood chemistry); text refers to vacuolated cytoplasm at high dose but no individual histology available. Not upgradeable. Aldous, 11/6/85.

NOTE: Memo of EPA to CDFA reconciling data gap differences (2/3/89) indicates that EPA classifies this study as "supplementary data".

235-011 911217 "Safety Evaluation of Vapona Strips" Summary of 035423.

SUBCHRONIC, RAT
(RELEVANT TO DOSE-SETTING FOR CHRONIC RAT STUDIES)

235-094 074932 "13-Week gavage toxicity study with DDVP in rats". Hazleton Laboratories America, Inc., Madison, WI, 12/28/88. Ten rats (Crl:CD®(SD)BR) per sex per group, dosed by gavage in deionized water vehicle for 5 days/wk, 13 weeks. Dose levels of 0, 0.1, 1.5, and 15 mg/kg/dose. Test article = Dichlorvos, Lot No. 902097, purity 98.3%. No adverse effects indicated. Findings associated with cholinergic activity included frequent salivation and urine staining during the period shortly after dosing in 15 mg/kg males and females. NOEL's for cholinesterase (ChE) enzyme inhibition were 0.1 mg/kg in males, and < 0.1 mg/kg in females (slight, but statistically significant decrease of RBC ChE in 0.1 mg/kg females at 14 weeks). Also, at termination, a significant decrease in brain ChE was noted at 15 mg/kg in females (nearly 50% reduction), and a non-statistically significant reduction of lesser magnitude was

noted in males. In addition, significant decreases in RBC parameters (RBC count, Hb, and HCT) were noted in both sexes at 15 mg/kg, and in males at 1.5 mg/kg. There was very equivocal evidence of ocular effects (phthisis bulbi) and also of slight degree of kidney tubular mineralization; both in 15 mg/kg females. If this study is to be used to set dose levels for a long-term aqueous vehicle gavage study, it would appear that a defensible MTD might be at or near to 15 mg/kg. Acceptable as a subchronic study. Aldous, 11/6/89.

CHRONIC TOXICITY, DOG

**235-106 088784, "A 52-Week Chronic Toxicity Study on DDVP in Dogs", (Victoria F. Markiewicz, M.P.H., Hazleton Laboratories America, Inc., Vienna, VA., Study # 2534-102, 8/6/90). DDVP Technical, 97.3% to 99.5% purity relative to an analytical standard, administered orally in gelatin capsules for 52 weeks at 0 (gelatin capsules), 0.05 (0.1 for the first 3 weeks of study), 1.0, and 3.0 mg/kg/day to 4 purebred beagle dogs/sex/group. Chronic NOEL = 1.0 mg/kg/day (increased frequency of emesis in 3 mg/kg/day males and females). Cholinesterase (ChE) NOEL = 0.05 mg/kg/day (dose-related inhibition of plasma and erythrocyte ChE in both sexes at 1.0 and 3.0 mg/kg/day). Also, brain ChE was inhibited significantly in dose-related fashion in 1 and 3 mg/kg/day males, and was also significantly inhibited in 3 mg/kg/day females. Acceptable. No adverse effects. H. Green and C. Aldous, 11/14/90.

235-070 035422 "The Effects Exerted upon Beagle Dogs during a Period of Two Years by the Introduction of Vapona Insecticide into Their Daily Diets" (Kettering Lab, 1/19/67). DDVP (dichlorvos, Vapona), 93%; fed in the diet to 3/sex/group at 0, 0.1, 1, 10, 100 or 500 ppm (nominal). Loss of about 64% of test article due to volatility. RBC and plasma cholinesterases were inhibited at 10 and 100 ppm (nominal) respectively to more than 30%, no effect on brain cholinesterase at term. ChE NOEL = 1 ppm; Systemic NOEL = 10 ppm (nominal) based on liver histological changes in females of rarefaction of cytoplasmic substance, enlargement of cells and prominence of cell membrane. Unacceptable (limited hematology and clinical chemistry, dose levels not adequately defined for actual exposure, inadequate tissues for histopathology, inadequate number of animals per group.) Mild liver effects without signs in blood chemistry of increase in SGOT, SGPT and alkaline phosphatase. Blood cholinesterases were inhibited but no behavioral signs were reported. Aldous 11/4/85.

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/3/89) notes EPA classification as "minimum".

235-011 035323 "Safety Evaluation of Vapona Strips". Summary of 035422.

CHRONIC TOXICITY, PIG

NOTE: At least 2 chronic pig studies have been performed. One was apparently completed in 1959 by Harris Laboratories. The volume containing the latter study (029:911216) has been lost, and no CDFA [now DPR] review has been performed. The second study was by Bio/Toxicological Research Associates, 1966. DPR has no report for this study, but it is referenced in 088:065499, p. 23. This brief summary does not indicate any adverse effects. There is no present indication that more information on chronic pig studies is necessary, particularly considering that a replacement dog study is completed. C. Aldous, 11/14/90, 12/07/92.

ONCOGENICITY, RAT

**235-095 074933 "NTP technical report on the toxicology and carcinogenesis studies of dichlorvos (CAS No. 62-73-7) in F344/N rats and B6C3F1 mice". Southern Research Institute, April, 1989. Tech. Vapona® [DDVP, dichlorvos], lot SDC 092179, Shell Development Co, Houston, 99% purity. 0, 4, or 8

mg/kg/day, 5 days/wk, to F344/N rats by corn oil gavage for 24 months. No NOEL was identified, however the dosage range was justified. Possible adverse effects: Principal indications of treatment-caused neoplasia were increased multiplicity of pancreatic exocrine cell adenomas in males (no increase in numbers of rats affected per group, but an increase in rats with multiple exocrine cell tumors in pancreas) and increased numbers of mononuclear cell leukemias in males. Both are comparatively common tumor types in males and both are considered to be equivocal evidence of oncogenicity. Also there was a minor increase in incidence of mammary gland fibroadenomas in females (not dose-related). No marked systemic toxicity. Acceptable for rat oncogenicity data requirement. C. Aldous, 10/17/89, 4/18/90 (rebuttal response).

235-050 (no record number). Rebuttal letter of 2/20/90 addressing study 235-095 074933, above. See CDFA response of 4/18/90.

235-0164 141583 (Supplementary to 235-095 074933, above): "Staging of Mononuclear Cell Leukemia in Male Rats From Toxicology and Carcinogenesis study of Dichlorvos in F344/N Rats (Pathology Working Group Review; PWGR)," (Brown, T. T., Jr., North Carolina State University; 1/31/95). The PWGR stated that the MCL staging results in this study were equivocal, and therefore, there is no evidence for any treatment-related progression of MCL in DDVP-treated rats. The status is unchanged, since DPR notes an overall tumor increase in DDVP-treated rats. M. Silva, 4/13/99.

235-081 069616 "A review of the interpretation of the NTP toxicology and carcinogenesis studies of dichlorvos (NTP Technical Report No. 342)". Date of submission of this review by John M. Mennear, Ph.D.: 6/30/88. [Date of galley draft of the referenced NTP report: April, 1989]. Dr. Mennear presented reasons why data on mononuclear cell tumors in male (M) rats, mammary gland tumors in female (F) rats, and pancreatic acinar tumors in M and F rats should collectively indicate only "equivocal evidence" of oncogenicity to rats, in contrast to the NTP Peer Review Panel conclusion of "some" evidence for M and "equivocal" evidence for F. Mouse forestomach tumors were also discussed, however the main focus of this discussion was lack of comparable effects on rats. The CDFA worksheet discusses major issues. No change in study status. C. Aldous, 10/24/89.

235-081 069617 "Mononuclear cell leukemia and pancreatic acinar-cell neoplasia in male F 344/N rats: A review of NTP study interpretations". Date of submission of this review by John M. Mennear, Ph.D.: this review of NTP interpretations was presumably prepared after 6/30/88. [Date of galley draft of the referenced NTP report: April, 1989]. Dr. Mennear presented reasons why the male rat incidence data for mononuclear cell leukemia and for pancreatic acinar-cell tumors should not be considered to represent "some evidence" of carcinogenicity. Salient points of Dr. Mennear's arguments and comments by this CDFA reviewer were noted for CDFA Health Assessment Group consideration. No change in study status. C. Aldous, 10/25/89.

235-094 074930 "Is dichlorvos a carcinogenic risk for humans?", Bremmer, J. N., et al., Mutation Research 209:39-44 (1988). This is a brief discussion of the overall data base for oncogenicity of DDVP. The conclusion of the authors was that DDVP "does not present a carcinogenic or mutagenic risk for man". Since the principal indications of oncogenic potential arise in the recent gavage NTP studies, which have been discussed in detail by Dr. Mennear, above, there is no need for a CDFA "review" of this record. Aldous, 10/26/89.

235-088 065499 "Dichlorvos: A review of carcinogenicity and mutagenicity studies". Several studies were discussed. Primary attention was given to the NTP studies in rats and mice (1989). Arguments for not assigning concern for oncogenic potential are comparable to those of Mennear, which have been reviewed by CDFA (Records 069616 and 069617). There is no apparent need for a separate CDFA review of this review paper. C. Aldous, 10/26/89.

235-089 067208 "Brief opinion on 'Board Draft' version of NTP Technical Report on toxicology and carcinogenesis studies on dichlorvos", 7/6/87, Dr. F. J. C. Roe. Major points are summarized in a review by C. Aldous, 10/27/89.

235-089 067209 "Comments on the Board Draft NTP Technical Report on the toxicological and carcinogenesis studies on dichlorvos [Report NTP TR 342 Draft 7/87]. Prof. P. Grasso, 7/9/87. There is comparatively little new in this opinion statement which has not been addressed in other opinions. No Data Review Group review is needed. C. Aldous, 10/27/89.

235-0211 164783 "An Evaluation of the Potential Carcinogenicity of Dichlorvos: Final Report of the Expert Panel," 7/27/98. A panel organized by the staff of SRA International, Inc. evaluated the evidence of oncogenicity in the 1989 NTP oncogenicity study in F344/N rats and B6C3F1 mice (Southern Research Institute, DPR Document # 235-095, Record # 074933). This record provides reasons for not considering dichlorvos as indicative of human oncogenic risk. This record offers useful interpretative perspectives, but does not provide new data. No DPR worksheet. Aldous, 2/8/05.

235-050 088033 Blair, D., et al. "Two year inhalation exposure of rats to Dichlorvos vapour". Tunstall Laboratory, June, 1974. Inhalation exposure was nearly continuous (rats were removed from chambers only once daily for inspection) over two years at dosages of 0, 0.05, 0.5 or 5.0 mg/m^3 of DDVP vapor) for groups of 50/sex rats (Carworth Farm E strain). No adverse effects were indicated. A cholinesterase (ChE) NOEL of 0.05 mg/m3 was observed in males. There was no ChE NOEL in females (slight inhibition of RBC ChE in low dose females). The NOAEL for other effects was 0.5 mg/m3 in both sexes (based primarily on decreased body weights in high dose males and females). The study is not acceptable, and not upgradeable, but provides useful data: A major deficiency was that the high dose appeared to have been slightly above the MTD [based on the high dose level "altering the normal life span" (lengthening) in both sexes]; yet the next lower dose was 10-fold lower, hence apparently well below the MTD. Thus there was no optimal high dose exposure. Aldous, 4/26/90.

NOTE: This study was recently re-examined by EPA and classified as "Minimum" for chronic and oncogenicity data gaps (EPA review dated 8/9/89 in Document No. 235-050, preceding text of the study report).

235-071 035425 "Bioassay of Dichlorvos for Possible Carcinogenicity" (Gulf South Res. Inst. for NCI, 1977) Dichlorvos technical, minimum purity 94%; fed in the diet for 80 weeks, followed by 30-31 weeks observation. Group sizes of 10/sex for concurrent controls (60 per sex for "pooled" controls) and 50/sex/test group at 150 or 300 ppm (the latter dosage began at 1000 ppm, but was lowered after 3 weeks because of excessive toxicity). Strain: Osborne-Mendel. Systemic NOEL = 150 ppm (body weight decrements). No evidence for oncogenicity effect reported. Unacceptable (no analysis of diet, no individual data, concurrent controls inadequate in number, staggered start for low and high doses with 4-week interval.) Gee, 1/20/87, C. Aldous, 6/1/89 (see note below).

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/3/89) notes EPA classification as "supplementary".

NOTE: study 071:035425 is referenced in the 2/17/88 review prepared for Amvac Chemical Corp. entitled "Dichlorvos: A review of carcinogenicity and mutagenicity studies", CDFA record No. 088:065499, pp. 18-22. The Amvac review was prepared in response to EPA reviews, and addressed 3 aspects of study design: (1) the use of only two dosage groups, (2) administration for 80 weeks instead of the full duration of the study, and (3) use of matched control groups of small size in conjunction with pooled control groups for comparison with treatment groups. This study and the 1/20/87 CDFA review were examined by C. Aldous. A concern previously stated in the 1/20/87 review about animals being in "poor condition" at term does not appear to reflect a husbandry problem, since geriatric animals would be

expected to reflect signs of aging, and since survival was very good in both treatment groups to termination. This study employed animals which were purchased from Charles River, Wilmington, MA. These were third generation offspring from Battelle Memorial Institute Osborne-Mendel stock. The pooled controls were purchased from Battelle Memorial (p. 7). Investigators felt that "there was probably no significant genetic drift influencing the incidence of tumors" (p. 11). Since EPA has recently examined the long-term rat study data base for DDVP, the Registrant is encouraged to submit EPA reviews of all these studies to CDFA. C. Aldous, 5/31/89, 10/27/89, 4/18/90.

235-057 911218 Partial duplicate of 071:035425-035426.

235-081 069614 "Studies on carcinogenicity of DDVP (2,2-dichlorovinyl dimethyl phosphate) mixed in drinking water in rats". Biosafety Research Center, Shizuoka, Japan. 12/2/78. DDVP, grade and purity not provided, at 0, 14, or 28 mg/kg/day nominal dosages, administered in aq solution, to F344 rats for 104 weeks. There were no definitive treatment effects, except that male body weights were reduced at 28 and possibly at 14 mg/kg/day. Apparent NOEL's are 14 mg/kg/day for M and 28 mg/kg/day for F. Study did not demonstrate adverse effects, however there was a slight increase in mononuclear cell leukemias in males. Incidence was 2, 6, and 6 for controls, 14, and 28 mg/kg/day groups: this was not statistically significant by standard two-group comparisons such as Fisher's exact test, but was noteworthy because an increase in male mononuclear cell leukemias was noted in a recent NTP study employing F344 rats. Not acceptable. C. Aldous, 5/24/89.

235-081 069615 "Stability of DDVP in drinking water". Brief document by T. Leafe and W. Feiler, indicating that DDVP is stable for some days in acidic or neutral water, and that hydrolysis of DDVP lowers water pH, limiting the further breakdown of DDVP in all but highly buffered, alkaline water. Also, the vapor pressure of DDVP was noted to be much lower than that of water. This document was submitted to show that dosages used in the above study (081:069614) were stable under conditions of the study. Aldous, June 1989.

ONCOGENICITY, MOUSE

**235-095 074933 "NTP technical report on the toxicology and carcinogenesis studies of dichlorvos (CAS No. 62-73-7) in F344/N rats and B6C3F1 mice". Southern Research Institute, April, 1989. Tech. Vapona® [DDVP, dichlorvos], lot SDC 092179, Shell Development Co, Houston, 99% purity. 0, 10, or 20 mg/kg/day (M) or 0, 20, 40 mg/kg/day (F), 5 days/wk, to B6C3F1 mice by corn oil gavage for 24 months. Cholinesterase inhibition was seen at all doses of DDVP. The high doses in males (20 mg/kg/day) and in females (40 mg/kg/day) were apparent LEL's for forestomach papillomas (possible adverse effect). There was no definitive other toxicity, hence the apparent NOEL for lesions outside of the forestomach is 20 mg/kg/day (male) or 40 mg/kg/day (female). Acceptable for the mouse oncogenicity data requirement. C. Aldous, 10/18/89.

NOTE: See records 141585, 141587 - 89 and 141592 below, under "DNA DAMAGE" for studies related to the forestomach effects in mice.

235-071 035426 "Bioassay of Dichlorvos for Possible Carcinogenicity" (Gulf South Res. Inst. for NCI, 1977). Dichlorvos technical, 94%, fed in the diet for 80 weeks plus 13-14 weeks of observation; 10/sex for concurrent controls (plus pooled control groups of 100 males and 80 females) and 50/sex/treatment group at 300 or 600 ppm, changed from 1000 and 2000 ppm, respectively, after 2 weeks because of toxicity. Systemic NOEL = 300 ppm (body weight), oncogenicity effect equivocal due to lack of control data for esophageal tumors. Unacceptable (no individual data, inadequate number of animals in concurrent control, no analysis of diets, two doses only.) Gee, 1/20/87.

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/3/89) notes EPA classification of the 1977 NCI study as Supplementary.

REPRODUCTION, RAT

**235-118 117629 "Two-Generation Reproductive Toxicity Study of DDVP Administered in the Drinking Water to CD® (Sprague-Dawley) Rats", (R.W. Tyl et al., Reproductive and Developmental Toxicology Laboratory, Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, NC., Report # 60C-4629-170, 31 August 1992). Production batch dichlorvos, 96.86% pure, Lot #802907. 30 albino CD® (Sprague-Dawley) Crl:CD®(SD)BR rats/sex/group were exposed to the test article in drinking water ad libitum continuously throughout the study (except for F1 females during mating for the F2b litters) at concentrations of 0, 5, 20, and 80 ppm. Rats were exposed for 10 and 11 wk in the pre-mating growth phases, respectively, for the F0 and F1 animals. A separate group of untreated males was used to sire the F2b litters in response to generally poor reproductive performance during production of the F2a litters. Parental NOEL (excluding cholinesterase) = 20 ppm (reduced water consumption in both sexes in both generations during most of the study, reduced body weights in F1 parents). Reproductive NOEL = 20 ppm [reduction in numbers of F1 dams pregnant, estrous cycling was not evident or was irregular in F1 females (not assessed in F0 females)]. Strictly speaking, there was no cholinesterase NOEL, due to small but statistically significant reductions at 5 ppm (RBC and plasma cholinesterase in F1 males, RBC cholinesterase in F0 and F1 females). Nevertheless, a practical NOAEL of 5 ppm is supportable for meaningful cholinesterase effects. At 20 and 80 ppm there were consistent, dose-related decrements in plasma, RBC and brain cholinesterase. No specific cholinergic signs were observed at any dose in this study. Acceptable. No adverse effects. (H. Green and C. Aldous, 12/11/92).

235-105 088543 Draft results of range-finding two-generation study, undertaken prior to the definitive study in Document No. 235-118, above.

Pages only, no record number, ID # SBC-131759-E, 11/22/91 Letter from D. Allemang, Jellinek, Schwartz, Connolly and Freshman, Inc., referring to the ongoing two-generation reproduction study, being conducted at Research Triangle Institute with DDVP. Data at this point suggested possible effect on testicular degeneration (but not on testicular atrophy). No worksheet. Gee, 6/30/92. (See DPR review for Document No. 235-118, above).

235-117 114476 Follow-up letter on the two-generation reproduction study in progress from D. Allemang, dated 2/20/92, (data not subjected to QA as of this date. Vaginal cytology data were presented (see DPR review for Document No. 235-118, above).

235-073 035434 "The Effects Exerted upon the Fertility of Rats and upon the Viability of Their Offspring by the Introduction of Vapona Insecticide into Their Diets" (Kettering Lab, 4/12/65). DDVP (dichlorvos, Vapona), 93% by weight, fed in the diets of CD rats, 15/sex/group, at 0, 0.1, 1, 10, 100 or 500 ppm for the first generation. There were 10 males 20 females per group for next two matings. Rats were dosed for six weeks before first mating for F1a litter; then mated with 3 males and 3 females per cage for F1 litters (cannot determine mates - group mating unacceptable). There were single litters in F2 and F3 generations. NOEL $\geq$ 500 ppm; no adverse reproductive effect reported. Unacceptable (dose selection unjustified: no evidence of toxicity, no analysis of diet (test article is known to be volatile and unstable in feed, yet the feed was prepared at weekly intervals), no individual data, no histopathology of adults of F1 or subsequent generations.) C. Aldous, 11/7/85, 5/26/89 (see concurrent rebuttal document).

TERATOLOGY, RAT

235-072 035428 "Toxicity Studies with Dichlorvos: Teratogenic Studies in Rats and Rabbits given Dichlorvos by Inhalation" (Tunstall Lab, 7/71, TLGR.0035.71). DDVP (dichlorvos), 97%. 16 rats in control group and 9 - 10 in each of treated groups. Treatment by inhalation, 23 hours/day, 7 days /week, at 0, 0.25, 1.25 or 6.25 µg/l nominal. Dose range appeared to be valid, judging by maternal cholinesterase (ChE) inhibition in RBC's, plasma and brain at 1.25 mg/l and above. No developmental toxicity reported. Unacceptable (too few litters per group, conditions of exposure not defined adequately for particle size, air flow, stability of test article during exposure, no individual data). Aldous, 11/8/85.

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/3/89) notes EPA classification as "supplementary".

**235-110 096391, R.W. Tyl, M.C. Marr, and C.B. Myers, "Developmental Toxicity Evaluation of DDVP Administered by Gavage to CD® (Sprague Dawley) Rats", RTI-60C-4629-10/20, Research Triangle Institute, Research Triangle Park, NC, 02/22/91. Twenty-five, mated (sperm positive = gestation day 0), female CD® (Sprague-Dawley) rats / dose were exposed on gestation days 6 through 15 by single daily oral gavages to DDVP (lot # 802097, 97% purity, water vehicle) at 0.0, 0.1, 3.0, or 21 mg/kg/day and sacrificed on gestation day 20. Treatment-related maternal effects were tremors and decreased food consumption and weight gain at 21 mg/kg/day. Maternal lethality (2/8) at 30 mg/kg/day and a NOEL for RBC and plasma ChE inhibition (0.1 mg/kg/day) were demonstrated in a pilot study. There were no treatment-related fetal effects. No adverse effect was indicated (maternal NOEL = 3 mg/kg/day $\leq$ fetal NOEL $\geq$ 21 mg/kg/day). The study was acceptable (S. Morris, 07/03/91).

TERATOLOGY, RABBIT

235-072 035432 "Teratogenic Potential of Dichlorvos given by Inhalation and Gavage to Mice and Rabbits" (National Institute of Environmental Health Sciences, 1979, publication in Teratology 20: 383-388 (1979) by B. A. Schwetz et al.) Dichlorvos, 96%, Batch no. 12-MMV-10. A gavage study involved dosages of 0 or 5 mg/kg/day to New Zealand White rabbits. There were 8 control litters and 12 test litters. The inhalation study involved exposure to 0 or 4 mg/l 7 hr/day (measured). There were 14 control litters and 19 exposed litters. No adverse effects were indicated. Unacceptable (no individual data, single dose per route at stated MTD but no clinical signs were observed.) The rabbit teratology data requirement may be subsequently filled, considering supportive information from the Tunstall Labs studies (072:035427), on receipt of individual data from the study published by Schwetz et al. C. Aldous, 10/23/85, 5/26/89.

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/3/89) notes EPA classification as "supplementary".

235-072 035430 "Teratological Studies with Dichlorvos in Rabbits" (Food and Drug Res. Labs, 6/30/69). Dichlorvos, no purity stated; given orally incorporated into polyvinyl chloride resin in capsules at 0, 3, 12, 36 and 60/24 (reduced for 7 ½ days) mg/kg/day, days 6 - 16 of gestation, 15 rabbits/group; 3 mg/kg dose added later. Litters/group: 11/15 in control, 8/15 in 12 mg/kg group and 12/15 at 3 mg/kg dose; no litters at 36 or 60/24 mg/kg. No NOEL can be established from study. Unacceptable (too few pregnant does, inadequate data for maternal toxicity, test article not described adequately, possible confounding effect of PVC resin, maturity of does not clear.) No teratogenic effect reported but inadequate for evaluation. Aldous, 11/12/85.

NOTE: This study was not available to EPA for review.

235-072 035429 "Teratology Studies in Rabbits" (Food and Drug Res. Labs, 6/30/69). DDVP (dichlorvos) given in capsules with polyvinyl chloride resin; 26 in control and 15, 15 and 20 in low (12 mg/kg), mid (36 mg/kg) and high (62 mg/kg) groups, days 6 - 18. NOEL cannot be determined. Unacceptable (test article not characterized and actual levels administered not clear, too few pregnant

animals per group with many stated as "immature", top dose subdivided into two groups and exposed for only part of organogenesis, too many deaths.) Aldous 11/8/85.

NOTE: This study was not available to EPA for review.

235-072 035431 Interpretative commentary on 035429 and 035430, above.

235-072 035427 "Toxicity Studies with Dichlorvos: Teratogenic Studies in Rats and Rabbits given Dichlorvos by Inhalation" (Tunstall Labs, 7/71, TLGR.0035.71). DDVP (dichlorvos), 97%, by inhalation 23 hours/day, 7 days/week, days 1 - 28 of gestation. Dutch rabbits, 19-20 per group at 0, 0.25, 1.25 or 6.25 μg/l nominal. 16/20 died in high dose group. In a second trial, doses of 0, 2 and 4 μg/l were used with 13-16 per group. ChE NOEL = 0.25 μg/l. Developmental toxicity NOEL not determined from study. Unacceptable (inadequate numbers of fetuses for evaluation - fetuses were examined for either skeletal or visceral findings - not both, conditions of exposure not thoroughly described, no corpora lutea counts, early and late fetal deaths not distinguished.) There was an apparent increase in late gestational fetal deaths at 4 μg/l, but this toxicity may have been due to technical problems: actual exposure in this group reached 6.6 mg/l for a time, and 6/20 dams died or were killed in extremis in this group. Aldous, 11/8/85.

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/3/89) notes EPA classification as "minimum".

235-090 068132 An omnibus collection of interpretative summaries of studies. This collection is listed here because several rabbit teratology studies were reviewed. Some of these comments were considered in the 5/31/89 CDFA Rebuttal Response (C. Aldous, 6/1/89).

**235-111 096392, "Developmental Toxicity Evaluation of DDVP Administered by Gavage to New Zealand White Rabbits", RTI-60C-4629-30/40, Research Triangle Institute, Research Triangle Park, NC, 02/22/91. Sixteen, artificially-inseminated (gestation day 0), female New Zealand White Rabbits/dose were exposed on gestation days 7 through 19 by single daily oral gavages to DDVP (lot # 802097, 97% purity, water vehicle) at 0.0, 0.1, 2.5, or 7.0 mg/kg/day and sacrificed on gestation day 30. Treatment-related maternal effects were clinical signs of cholinesterase inhibition and decreased food consumption at 7.0 mg/kg/day and lethality at 2.5 (2/16) and 7.0 mg/kg/day (4/16). A pilot study demonstrated lethality (5/8) and NOEL for RBC and plasma ChE inhibition (0.1 mg/kg/day). There were no treatment-related fetal effects. No adverse effect was indicated (maternal NOAEL = 0.1 mg/kg/day $\leq$ fetal NOAEL $\geq$ 7.0 mg/kg/day). The study was acceptable (S. Morris, 07/10/91).

TERATOLOGY, MOUSE

235-072 035433 "Teratogenic Potential of Dichlorvos given by Inhalation and Gavage to Mice and Rabbits" (National Institute of Env. Health Sciences, publication in Teratology 20: 383 - 388 (1979) by B. A. Schwetz et al.) Dichlorvos, 98%, Batch No. 12-MMV-10; mice were given 60 mg/kg/day by gavage with 28 control litters and 25 exposed litters; in a second series, mice were exposed to 4 μg/l 7 hr/day, by inhalation with 20 litters in the controls and 15 litters in the exposed group; no developmental effect was reported. Unacceptable, does not appear to be upgradeable (single dose, no individual data, although stated to be the maximum tolerated doses, no toxicity was reported.) Additional information will be examined (if submitted) on the mouse segment of this study, since the Registrant will probably be sending information on at least the rabbit segment of this study. Aldous, 11/12/85.

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/3/89) notes EPA classification as "supplementary".

TERATOLOGY, GUINEA PIG

No document number, no record number. "The Effect of Trichlorfon and other Organophosphates on Prenatal Brain Development in the Guinea Pig" (Mehl, A., Schanke, T. M., Johnsen, B. A., and Fonnum, F., *Neurochemical Research* 19: 569-574 (1994). Dichlorvos (99%) was given to guinea pigs i.p. [?] as follows: 15 mg/kg/day, single dose on days 42, 43 and 44 of gestation; 15 mg/kg, twice daily at 12h intervals, days 42, 43 and 44; 15 mg/kg, twice daily at 12h intervals, days 44, 45 and 46. There was a single pregnant dam per dosing regimen and one litter of pups (4 in each litter) was analyzed for the effect on the brain weight. Other organophosphates also tested were trichlorfon (125 mg/kg, days 42,43 and 44), ethyl-trichlorfon (125 mg/kg/day, days 44, 45 and 46), ethyl-trichlorfon (138 and 121 mg/kg/day, days 42 and 44), soman and TOCP. The pups were weighed and the brain recovered within 24 hours of natural birth. The brains were weighed and dissected into: medulla, cerebellum, quadrigemina, hippocampus, cerebral cortex and diencephalon. Each region was weighed. Selected regions (cerebellum, medulla and cerebral cortex) were homogenized and assayed for activity of glutamate decarboxylase, choline acetyltransferase and acetyl cholinesterase. The results showed that treatment with trichlorfon and dichlorvos (at 15 x 2 doses per day) during gestation days of the brain growth spurt caused significantly lower total brain weight and lower weight for selected regions of the brain. There was no effect on enzyme activity. Possible adverse effect. The study and report contain deficiencies including a single dam per dosing regimen and missing details of methodology. The study is supplemental. (Gee, 6/12/98)

GENE MUTATION

Microbial Systems

235-075 035438 "The Mutagenic Effect of Organophosphate Insecticides on E. coli" (Tunstall Lab for SDS Biotech, 8/71) Dichlorvos was one of nine insecticides tested with *E. Coli* B/r WP2, plated in triplicate with no adverse effect reported. Unacceptable (no data, no dose level stated.) Gee, 11/13/85.

235-075 035443 "Mutagenicity of Some Organophosphorus Compounds at the ade6 Locus of Schizosaccharomyces pombe" (Laboratoire de Genetique, Belgium, publication in Mutation Res. 117: 139 - 148 (1983)) DDVP (dichlorvos), >99%; strain SP-198 ade 6-60/rad 10-198/h^-, exposed for 1 hour to approximately 1.5, 4 or 14 mM (from graph); with and without mouse liver activation, replicates not stated; table indicates LD50 as 5.5 but no data; concentration-dependent increase in mutants; unacceptable (number of plates not specified, no individual colony counts, inadequate description of test.) Gee, 11/14/88.

235-075 035444 "Activity of OP Insecticides in Bacterial Tests for Mutagenicity and DNA Repair - Direct Alkylation versus Metabolic Activation," Zentralinstitut fur Genetik and Kulturpflanzenforschung, 8/7/81, publication in: Chem.-Biol. Interactions 39: 339 - 0350 (1982). DDVP (dichlorvos), 99%; tested with Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100, with and without activation with NMRI mouse liver; tested at 5, 10, 20 or 40 μM/plate, duplicate plates; cytotoxicity with TA100; replicate trials; increase in reversion reported in TA100 only without activation; unacceptable but possibly upgradeable (individual plate counts and replicates, clarification of "μM/plate" is needed.) Gee, 11/14/85.

Mammalian Systems

**235-083 050037 "L5178Y TK +/- Mouse Lymphoma Forward Mutation Assay with Dichlorvos" (Microbiological Assoc., 10/14/86, T-5211.702003, Doc. No. 132\12-86-0036-TX-002) Mouse lymphoma; Dichlorvos, 97.5%, lot 11381-23-5, liquid; tested with and without rat liver activation, two

acceptable trials each; without activation, at 0 to 0.33 in trial 1 and 0 to 0.12 µl/ml in trial 2; with activation, at 0 to 0.24 µl/ml in trial 1 and 0 to 0.12 µl/ml in trial 2; increase in mutation frequency to greater than twice control in ± activation, especially without activation; acceptable with a possible adverse effect for gene mutation. Gee, 1/20/87.

235-080 037219 "A Synopsis of a Mouse Lymphoma Forward Mutation Assay with Dichlorvos" (NIEHS/NTP, Litton Bionetics, 8/27/85, 5TX-85-0065) Dichlorvos, no purity stated; mouse lymphoma L5178Y TK +/-, clone 3.7.2C, tested without activation at 0, 6.25, 12.5, 25, 50, 100 or 200 nl/ml for 4 hours with cytotoxicity at > 50 nl/ml; concentration-related increase in mutation frequency at the three lowest exposures; unacceptable (no metabolic activation included, no purity of test article.) Gee, 2/3/86.

SUMMARY: The two mutagenicity studies in mammalian cells confirm each other for positive activity. The evidence in microbial systems is less certain with only one (TA100) of five strains of Salmonella responding positively. Gee, 11/88.

NOTE: The potential for mutagenicity in maximally dosed mammals is acknowledged, and must be noted as a "possible adverse effect". A relevant submission entitled "Discussion of the mutagenic potential of dichlorvos" [which begins on p. 29 of record #s 088:065499 and 090:068132 (this section of either record is identical)] should be examined by Health Assessment Section when the relevance of mammalian mutagenicity studies is assessed. The major point of this discussion is that dichlorvos metabolism generally proceeds by an esterase-catalyzed pathway, but when that pathway is saturated by heavy dosing, an alternative demethylation pathway becomes significant. Dr. Bernard (author of this discussion) suggests that the esterase cleavage products are likely to be less mutagenic than the demethylation products, and that the esterase products represent the relevant pathway for chronic human health risk assessment. This CDFA reviewer (C. Aldous) does not agree with the subsequent implication (see p. 29) that the esterase product, dichloroacetaldehyde, is a lesser concern because of its "extremely transient existence". On the contrary, this transience may attest to a high level of biological activity, with mutagenic potential. Unless the reactive molecule or molecules which are responsible for mutagenicity are identified, and demonstrated to be not significant intermediates in human metabolism, it would appear that the mutagenicity demonstrated in the above studies may not be discounted. Incidentally, Aquilina et al. (1984, CDFA [DPR] volume/record No. 075:035440) referenced two studies showing dichloroacetaldehyde to cause gene mutations in microorganisms and one study which found dichloroacetaldehyde to cause dominant lethal mutations in mice. C. Aldous, 6/2/89.

CHROMOSOME EFFECTS

235-080 037224, 037225 "A Dominant Lethal Assay in Mice with T-169-1" (Microbiological Assoc., 10/2/85, Study 695-5TX-85-0004) Dichlorvos, 98.4%; injected intraperitoneally for 5 consecutive days to 10 males per group at 0, 1, 3 or 10 mg/kg/day; mated each to 2 females/week for 8 weeks; TEM as positive control; no evidence of a dominant lethal effect; unacceptable (inadequate number of pregnant females, no explanation for 0% fertility in positive control group, no justification of dose selection.) Gee, 2/3/86.

235-075 035437 "Toxicity Studies with dichlorvos: Investigation of the Dominant Lethal Mutation Potential in the Mouse after an Inhalation Exposure" (Tunstall Lab, 5/71). DDVP (dichlorvos), technical, purity > 97%, given by inhalation at 30 or 55 µg/l (nominal) for 16 hours to 16 control males, and 8 males per treatment group. Each male mated to 3 females weekly for 8 weeks. Increase in early fetal deaths in week 6 of 8 weeks. Unacceptable (actual concentration measured but data not included, inadequate number of treated males, no justification for dose selection, no description of exposure conditions.) Gee, 11/13/85.

235-075 035439 "Toxicity Studies with Dichlorvos: Investigation of the Dominant Lethal Mutation in the Mouse after Multiple Inhalation Exposures" (Tunstall Lab, 8/71) DDVP (dichlorvos) technical, > 97%, given by inhalation 23 hours/day, 4 weeks, to 16 males in control and 8 males in each treatment group, at 2.1 or 5.8 μg/l (nominal); CF1 mice; treated males mated to 3 females weekly for 8 weeks; no deaths, no adverse effect reported; unacceptable (inadequate number of treated males, no justification of dose, no description of inhalation conditions, actual concentration measured but not reported, no individual data, no rational for route of exposure.) Gee, 11/13/85.

235-075 035441 "Cytogenetic Effects induced by Organophosphorus Pesticides in Mouse Spermatocytes" Laboratoire de Genetique, Belgium, publication in Toxicology Lett. 21: 315-319 (1984). DDVP (dichlorvos), given in a single i.p. injection at 10 mg/kg, 14 organophosphorous compounds tested; cytogenetic effects analyzed after 10-15 days in primary spermatocytes; 500 spermatocytes per animal; no increase in aberrations reported at any of three time intervals. Unacceptable (incomplete). Gee, 11/14/85.

235-075 035442 "Cytogenetic and Genetic Effects of Subchronic Treatments with Organophosphorus Insecticides" [Lab de Chimie Medicale, Belgium, publication in: Arch. Toxicol. 56: 66-67 (1984)]. DDVP (99%) given in the drinking water at 2 ppm (maximum residue allowed in Belgium) for 7 weeks to male mice; analyzed bone marrow and sperm for aberrations; no adverse effect reported; also included a dominant lethal assay with 20 males at same exposure mated to 4 females each for 1 week - no adverse effect reported. Unacceptable (incomplete report.) Not upgradeable. Gee, 11/14/85.

235-075 035446 "Test of Dichlorvos using the Sensitive-indicator Method for Dominant Skeletal Mutations in Mice" (Oak Ridge Nat. Lab, 3/82, publication in: Environmental Mutagenesis : 115 (1982). Abstract. Male and female mice were exposed to a resin strip impregnated with dichlorvos for 80 days before mating; scored fetal skeletons for effects - none reported. Unacceptable (protocol). Gee, 11/14/85.

**235-080 037220, 037221 "A Micronucleus Test in the Mouse using Dichlorvos" (Microbiological Assoc., 9/27/85, Study 695-5TX-83-0095-000) Dichlorvos, 98.4%; 5/sex/group/sacrifice interval injected i.p. with 0, 4, 13 or 40 mg/kg body weight twice at 24 hour interval, sacrificed at 30, 48 or 72 hours; LD50 approximately 56 mg/kg; 2 males and three females died at 40 mg/kg; no evidence of micronucleus formation. Acceptable. Gee, 2/3/86.

**235-084 055463 "A Dominant Lethal Assay in Mice with Dichlorvos" (Microbiological Associates, Inc., 3/9/87). Dichlorvos, 97.5%, administered intraperitoneally to male CD-1 mice at dosages of 0 (corn oil), 8, 16 or 32 mg/kg/day on each of 5 consecutive days, 30/group, 35/high group. Mated 1:2 weekly for 8 weeks. TEM was positive control. No adverse effect reported. Acceptable. Shimer & Luthra 10/87.

CHROMOSOME SUMMARY: No adverse effect was reported in an acceptable micronucleus test in mice and no dominant lethal effect was noted in several incomplete reports nor in an accepted replacement study. In addition, several publications reported no adverse cytogenetic effect in mouse spermatocytes or bone marrow. Thus there are no adverse chromosomal effects indicated. Gee, 10/27/87, 6/2/89.

DNA DAMAGE

**159 - 161 141585, 141587 - 88 "Investigation of the Genotoxic and/or Irritant Effects of Dichlorvos on Mouse Forestomach," (Benford, D.J.; Robens Institute of Health and Safety, Guildford, Surrey, UK; Report #: R190/0405; 9/25/91). Dichlorvos (99.8% pure) was administered in a single gavage dose to

B6C3F1 mice (5/sex/dose/time point) at 0 (corn oil), 10, 20, 40 and 100 mg/kg. Positive controls (MMNG - 200 mg/kg & BHA - 300 mg/kg) were also used. Food was withdrawn overnight before treatment of mice in the 2, 4 and 48 hour studies (unscheduled DNA synthesis, UDS) and 5 hours before treatment for the 12 hour study (proportions of S-phase cells were scored). Food was returned to the 48 hour exposure groups after dosing. Separate groups of 5 mice were left for 2, 4, 12 and 48 hour periods before autopsy. Sections from all time points were examined histologically. NOEL = 40 mg/kg (At 100 mg/kg a male (moribund at 1.5 hours) was terminated and 3 males died within 2 hours after dosing. The effect observed was dilation of the blood vessels in the stomach on 1 male mouse in the 12 hour group at 100 mg/kg.) No adverse effect. Acceptable. M. Silva, 8/4/99.

163 141592 "Investigation of the Irritant Effects of Dichlorvos on Mouse Forestomach," (Benford, D.J., Robens Institute of Health and Safety, Guildford, Surrey, UK; Report #: R191/0405; 11/16/92). Dichlorvos (99.8% pure) was administered in a single gavage dose to B6C3F1 mice (5/sex/dose/time point) at 0 (corn oil), 10, 20, 40 and 100 mg/kg. Positive controls (MMNG - 200 mg/kg & BHA - 300 mg/kg) were also used. At 8 and 10 hours after treatment, mice were sacrificed and forestomachs incubated with [^{3}H]-thymidine in order to assess replicative DNA synthesis (RDS). Forty-eight hours after exposure, mice were examined for histopathological changes in the forestomach. It was not possible to determine an adequate NOEL, as there were too many deaths and too many test samples were lost. Possible adverse effect could not be determined. Not acceptable and not upgradeable. M. Silva, 8/4/99.

162 141589 "Detection of Hyperplasia in Forestomach of B6C3F1 Mice Following Treatment with Butylated Hydroxyanisole," (Benford, D.J., Robens Institute of Health and Safety, University of Surrey, Surrey UK; Study #: 5/91/TX; Final Report #: R191/0403; 10/1/91). B6C3F1 mice (5/sex/dose/time period) were administered a single oral dose of butylated hydroxyanisole (BHA) at 0 (corn oil) or 300 mg/kg. After 6, 8, 10 or 12 hours, the mice were terminated and the forestomachs were removed for assessment of replicative DNA synthesis (RDS) by incorporation of ^{3}H-thymidine into DNA. The RDS was measured by autoradiography and liquid scintillation counting (LSC). The maximum RDS in forestomach occurred 10 hours post-treatment with BHA in both sexes. Histological examination revealed cellular damage induced by BHA but there was no evidence of hyperplasia at these times. No worksheet. M. Silva, 8/9/99.

235-075 035444 "Activity of OP Insecticides in Bacterial Tests for Mutagenicity and DNA Repair - Direct Alkylation versus Metabolic Activation and Breakdown" (Zentralinstitut fur Genetik und Kulturpflanzenforschung, 8/7/81, publication in Chem.-Biol. Interactions 39: 339-350 (1982). DDVP (dichlorvos), no purity stated; Proteus mirabilis, PG 713 and PG 273 with no activation, tested at 10 or 40 μM/plate, with Proteus in top agar and test article added in a well in 0.1 ml ethanol; results reported as "+" for dichlorvos - no data. Unacceptable (inadequate data.) J. Remsen (Gee), 11/14/85.

235-075 035440 "Genotoxic Activity of Dichlorvos, Trichlorfon and Dichloroacetaldehyde" Instituto Superiore di Sanita, Italy, publication in: Pest. Sci. 15: 439 (1984). Unscheduled DNA synthesis in human epithelial cells, DDVP (dichlorvos), no purity stated; cells (not a well-identified line/strain), exposed for 1 hour without activation at 0, 1.25, 2.5 or 5.0 mM as confluent monolayers, radioactive thymidine for 4 hours and autoradiography for analysis; also tested CHO V79 for ouabain resistant mutations; unacceptable (no activation or justification for not including it, inadequate details of methods, cytotoxicity data, justification for concentration selection, number of cells scored, others.) Positive, concentration-dependent effect for UDS reported for the two pesticides, but not for the dichloroacetaldehyde. Gee, 11/13/85.

**235-080 037222 "An In vivo Sister Chromatid Exchange Assay in Mice with Dichlorvos" (Microbiological Assoc., 9/27/85, Study 695-5TX-85-0003) Dichlorvos, 98.4%; injected once i.p. at 0, 3, 10 or 30 mg/kg into B6C3F1 mice, 5/sex/group; dose selection from preliminary study at doses to 100

mg/kg; sacrifice at 24 hours after injection; scored 50 metaphases per animal; no evidence for increase in sister chromatid exchanges; acceptable. Gee, 2/3/86.

235-123 120415 "In vivo Cytogenetics Assay: Analysis of Chromosomal Aberrations in Bone Marrow and Spermatogonial Cells Following Repeated Dose Administration." (Putman, D. L. and E. H. Shadly, Microbiological Associates, MD, Study No. TA458.109001, 12/18/92) Dichlorvos (DDVP), lot 802097, 98.09% purity, was given by oral gavage to 10 male ICR mice per dose at 0 (water), 12.5, 25 or 50 mg/kg body weight/day for 5 consecutive days. Bone marrow cells and spermatogonial cells were collected 24 hours after the last dosing. Dose selection was based on a range-finding study. Fifty metaphases of each cell type were scored per animal when possible. Cyclophosphamide was the positive control and functioned as expected. There was no increase in aberrations in either the bone marrow or the spermatogonial cells with dosing. Study is unacceptable and does not appear to be upgradeable (use of one sex only). No adverse effect. Gee, 2/8/93.

SUMMARY: Two in vitro tests give evidence for DNA damage (35444 and 035440) while the in vivo tests are negative. These tests, however, measure different endpoints and are, therefore, not directly comparable. In view of the results under "Gene Mutation", the overall assessment is that DDVP is genotoxic when measured in some systems including mammalian cells. Gee, 10/27/87, 6/2/89, 2/9/93.

MUTAGENICITY, GENERAL

235-066 027075 Summaries of studies in bacteria and fungi demonstrating genotoxic effects. EPA data call-in notice, 1983.

235-0210 164781 "An Evaluation of the Potential Genotoxicity of Dichlorvos: Final Report of the Expert Panel," 7/1/1998. A panel organized by the staff of SRA International, Inc. evaluated the evidence of mutagenicity in the body of available mutagenicity studies on dichlorvos. This record provides reasons for not considering dichlorvos as indicative of human mutagenic risk, while acknowledging an intrinsic potential for dichlorvos to elicit mutagenicity in *in vitro* systems. The panel concluded that genetic risks in humans appear low because *in vivo* metabolism in mammals does not favor formation of toxic products, and in particular, does not appear to lead to detectable alkylation of DNA. This record offers useful interpretative perspectives, but does not provide new data. No DPR worksheet. Aldous, 2/8/05.

158 141584 "Investigation of the Genotoxic and/or Irritant effects of Dichlorvos on Mouse Forestomach," a supplement to: "NTP technical report on the toxicology and carcinogenesis studies of dichlorvos (CAS No. 62-73-7) in F344/N rats and B6C3F1 mice (NTP, 1989)," (Bremmer, J. N.; Shell Internationale Petroleum Maatschappij B.V., The Hague Health, Safety and Environment Division; Occupational Health and Toxicology Division; 4/93). This volume contains an overview of several studies. The project was sponsored by the European Dichlorvos Working Group for the Robens Institute in England to investigate possible mechanisms by which DDVP may cause forestomach tumors in mice in a chronic corn oil gavage oncogenicity assay (NTP, 1989). In this project, a method was developed to distinguish between genotoxic forestomach carcinogens and substances causing hyperplasia via a non-genotoxic mechanism, which after chronic exposure may have tumor-promoting effects. Mice were exposed *in vivo* to DDVP and 3 parameters were measured: Unscheduled DNA synthesis (UDS); replicative DNA synthesis (RDS) and histopathological effects, including hyperplasia. BHA (antioxidant) was used as a non-genotoxic agent (cell proliferating/tumor promotor) for the forestomach. Measurement of UDS and RDS in the forestomach was done by autoradiography. Optimum time points to identify the maximum increase in RDS in forestomach cells were shown to vary with strain of mouse. Results showed that DDVP induced RDS and hyperplasia in forestomach epithelial cells (dose-related). The effects were comparable to the positive control BHA. UDS did not occur. These data are supplemental. M. Silva, 6/23/99.

ACUTE DELAYED NEUROTOXICITY, HEN

**235-094 074931 "DDVP: An acute delayed neurotoxic study in chickens". Wildlife International Ltd., 12/29/88. DDVP Technical, Lot #802097, purity 96.5%. White leghorn hens, 42 weeks old, treated by oral intubation with 16.5 mg/kg DDVP in distilled water. Negative control was distilled water; 600 mg/kg TOCP diluted in corn oil was positive control. Dosage volume 8 ml/kg b.w. in all cases. DDVP and negative controls were dosed day 1 and again day 22, and maintained for an additional 21 days. Positive controls were dosed on day 1 only. Sacrifice on day 22 (TOCP positive controls) or 43 (all others). A possible adverse effect was noted in histopathological examinations of sciatic nerve: one of 10 DDVP hens had nerve fiber degeneration in the proximal right sciatic nerve, and axonal swelling in proximal and distal parts of that nerve. No negative controls were affected, however 5 of 10 positive controls had nerve fiber degeneration and associated axonal swelling. All TOCP hens had some loss of coordination, and some had apparent lower limb weakness by day 21. Six out of 10 TOCP hens had some motor activity functional deficits consistent with organophosphorous compound - associated delayed distal neuropathy, compared with none of the control or DDVP groups. Study is acceptable. C. Aldous, 11/7/89.

NOTE: It is expected that reports of any subsequent neurotoxicity testing performed as a follow-up to this study will be submitted to CDFA for review.

235-074 035435 "Neurological Effects (Demyelination) of Vapona Insecticide on Chickens" (Shell Chemical, Agricultural Chem. Div., 2/18/85.) Hens were treated with 2.5 mg/kg for 5 days/week, 3 weeks. No TOCP-type neurotoxicity was observed. LD50 in hens was estimated to be 22.8 mg/kg. Unacceptable (protocol). No adverse effect reported. Aldous, 11/7/85.

NOTE: This study was not available to EPA for review as of 2/3/89.

235-074 035436 "Oral Neurotoxic Potential of Technical Dichlorvos (SD 1750) in the Chicken" (Shell, 5/3/71) Atropinized hens were given 22.9 mg/kg (stated as twice the LD50 - see 035435); 8/10 survived for 30 days. Unacceptable (protocol). No adverse effect reported. Aldous, 11/7/85.

NOTE: This study was not available to EPA for review as of 2/3/89.

ACUTE NEUROTOXICITY, RAT

124 120984; Acute Neurotoxicity Study; 818; Rat; WIL Research Laboratories, Inc., Ashland, OH. Dichlorvos (purity 97.87%); 12 animals/sex/group; Doses: 0, 0.5, 35, 70 mg/kg, by gavage; Mortality: 0 (M/F: 0/12), 0.5 (M/F:0/12), 35 (M/F: 0/12), 70 (M:1/11, F:5/12); Observations (signs observed in both sexes at 35 and 70 mg/kg unless noted): Functional Observational Battery-(Home Cage Observations) altered posture, clonic convulsions, whole body tremors, (Handling Observations) constricted pupils, (males only), salivation, increased eye prominence, decreased muscle tone, altered respiration, pale skin, poor grooming, (Open Field Observations) increased mean time to first step, impaired mobility and gait, decreased arousal, decreased rearing, (Sensory Observations) absence of approach response (except for 35 mg/kg females), absence of touch response, absence of tail pinch response, lack of response to olfactory stimuli (70 mg/kg only), absence of pupil response, impaired air righting reflex, (Neuromuscular Observations) reduced hindlimb resistance, reduced grip strength (70 mg/kg only), impaired rotarod performance, increased hindlimb footsplay (70 mg/kg only), (Physiological Observations) increased duration of catalepsy, decrease in body temperature, Locomotor Activity- reduced motor activity, recovery evident for all parameters by day 7 in all animals; Necropsy, Histopathology (animals which died): reddened cortico-medullary junction in the kidney (M:1/1, F:1/5), no treatment-related lesions in

the surviving animals; adverse effect: convulsions, tremors; NOEL-0.5 mg/kg (based on treatment-related signs in 35 mg/kg group); Study Supplemental. (Moore, 7/16/93)

Note: Study was performed according to the 818 guidelines for the evaluation of acute neurotoxicity. Although these results indicate the adverse effects of tremors and convulsions, they were readily reversible and distinguishable from the effects identifiable as those resulting from acute delayed neuropathy.

235-120 117929 Lamb, I. C., "An acute neurotoxicity study of dichlorvos in rats", WIL Research Laboratories, Inc., Study No. WIL-188003, 3/3/92. This study had been submitted in response to U.S. EPA requirements. The report will be reviewed at a later time by another working group in this Branch (the data do not apply at present to "SB-950" requirements). Aldous, 12/8/92.

235-119 117928 Lamb, I. C. (pilot study to 235-120 117929, above).

SUBCHRONIC NEUROTOXICITY

**133 126465 Lamb, I. "A Subchronic (13 Week) Neurotoxicity Study of Dichlorvos in Rats" (WIL Research Laboratories, Inc., Ashland, Ohio; WIL Study 188004, 9/30/93). Dichlorvos (DDVP) technical (lot# 802097, 97.87% purity) was administered orally (gavage) for a minimum of 91 consecutive days (7 days/week) to 15 Sprague-Dawley Crl:CD®BR rats/sex/dose at levels of 0, 0.1, 7.5 or 15 mg/kg/day. High-dose rats had tremors, salivation, exophthalmos, lacrimation, clear material on forelimbs, rales, chromodacryorrhea and material around the mouth. Body weights in the high-dose females were significantly lower than controls by week 13. Inhibition of plasma and RBC cholinesterase (ChE) was noted in the mid-dose and high-dose groups at weeks 3, 7 and 13; brain stem and/or cerebral cortex ChE inhibition ranged from 11-12% in the mid-dose and 10-16% in the high-dose rats at week 13. No Adverse Effects were noted in the FOB, locomotor, brain weight/dimension or other neuropathological parameters. NOEL (for systemic effects and ChE inhibition) = 0.1 mg/kg/day. ACCEPTABLE. Kellner and Gee, 1/28/94.

A letter was submitted by AMVAC Chemical Corporation to contest the adverse health effects observed by DPR in 235-143, 144/133037. No worksheet. M. Silva, 11/30/95

149 137355 "Response to California EPA Department of Pesticide Regulation Medical Toxicology Branch Review of: Dichlorvos (DDVP): 28-Day Neurotoxicity Study in the Domestic Hen," (W. F. Millar, AMVAC Chemical Corporation, City of Commerce, CA, 4/21/95). The volume contained a discussion of the DPR review of this study, specifically the adverse effects flag for DPR volume/record #: 235-143, 144/133037, 1332245 (reviewed above).

235-122 119717 "Dichlorvos (DDVP) 28 Day Neurotoxicity Study in Hens" Preliminary submission as an adverse effects disclosure of data not submitted to Quality Assurance inspection. Twenty-one hens per group were given 0, 0.3, 1.0 or 3.0 mg/kg/day. Birds were sacrificed after 49 or 77 days and sections of brain, spinal cord and peripheral nerves were examined. TOCP was the positive control. At 49 days, 2/6 examined showed minimal axonal degeneration of the spinal cord at 3.0 mg/kg. One bird in each of the 1.0 and 0.3 mg/kg showed minimal degeneration in one level of the spinal cord. At 77 days, 1/5 at 3.0 mg/kg showed minimal axonal degeneration at 2 levels in the spinal cord and 1/6 at 1.0 mg/kg showed an effect. No effect was reported at 0.3 mg/kg. No worksheet. Gee, 2/8/93.

** 143, 144, 184 133037, 133245, 161344 "DDVP: 28-Day Neurotoxicity in the Domestic Hen," (Manley, A., Huntingdon Research Centre Ltd., Cambridgeshire, UK; 10/21/94; AVC 1/921405). Pathology Working Group Peer review of DDVP 28-Day Neurotoxicity Study in the Domestic Hen,"

(Hardisty, J. F., Experimental Pathology Laboratories, Inc., Research Triangle Park, NC; EPL project #: 578-001). DDVP technical (97.87% pure; Batch #: 802097) was administered by gavage for 28 days to adult female domestic hens (Ross Hi-Sex Brown, Gallus gallus domesticus) at 0 (distilled water), 0.3, 1.0, 3.0 mg/kg (21/dose) and 0.1 mg/kg DDVP (3 hens--added later to determine brain ChE activities at day 30). TOCP (7.5 mg/kg) served as a positive control (21 hens) and corn oil served as vehicle control (4 hens--also used for brain ChE activities at day 30). The birds were observed for a total of 49 or 77 days after onset of dosing. Systemic NOEL = 0.3 mg/kg (Transitional weight loss occurred at 3.0 mg/kg. Unsteady gait and an inability to stand was observed in 2/21 hens at 1.0 mg/kg. At 3.0 mg/kg there were clinical signs of wings outstretched, birds being pecked, limping, inability to stand, quiet/subdued, unsteadiness and death which occurred in 1/21 at 1.0 mg/kg and 4/21 at 3.0 mg/kg.) Neurotoxicity NOEL = 3.0 mg/kg (The pathology working group re-evaluated all the histopathology slides and found no increases in neurotoxic effects, when compared to control.) ChE NOEL = 0.3 mg/kg (On day 4, brain ChE (BrChE) was inhibited 44% at 1.0 mg/kg and 63% at 3.0 mg/kg. By day 30, BrChE was inhibited 26% at 0.3 mg/kg, 34% at 1.0 mg/kg and 54% at 3.0 mg/kg. Brain neurotoxic esterase (B/NTE) and spinal cord NTE (SC/NTE) were not affected by DDVP treatment. The study was initially evaluated as having an adverse neurotoxic effect (Silva, 2/6/95). Upon re-evaluation of histopathology by a Pathology Working Group Peer Review, no increases in neurotoxic effects were observed. Acceptable. No adverse effect. M. Silva, 5/5/99.

METABOLISM/DISPOSITION STUDIES

235-101 086302 Jeffcoat, A.R., "Dermal absorption of Dichlorvos in rats". Research Triangle Institute, 3/23/90. This study was reviewed by Robert Zendzian (EPA HED) in a review signed off on 5/7/90. Study was classified as "Acceptable". Conclusions: "Dermal doses of 0.3, 3.0 or 30 μg/cm2, 10 hour wash and total exposures of 10, 24, and 120 hours per dose. 9.4 to 11.44% of the dose was absorbed, 12.1 to 20% of dose remained on skin after washing and 37.7 to 51.5% of the dose evaporated during the 10 hour exposure before washing. There was no dose or time relationship shown for any of these parameters." EPA review conclusions were recorded by Aldous, 7/5/90. Subsequently, CDFA WHS Branch reviewed the study, and classified it as "acceptable", recommending that 13% dermal absorption be used for human exposure.

CHOLINESTERASE STUDIES, RAT (supporting developmental neurotoxicity studies

235-0235 210700 Twomey, K., "Dichlorvos (DDVP): acute cholinesterase inhibition study in rats," (1st study), Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, 5/30/02. Laboratory Study # AR7079. RBC and cerebellar cholinesterase activities were determined in Alpk:AP_fSD (Wistar-derived) young adult rats (b.w. at Day 1 averaged 223 g for M and 169 g for F) following single oral doses of dichlorvos (99% purity) in 10 ml distilled water per kg b.w. Achieved doses were 0, 2, 5, and 39 mg/kg (nominal doses were 0, 1, 5, or 35 mg/kg). Five rats per sex per group were killed either one hour after dosing (near maximal tissue concentrations) or on days 8 or 15. Due to technical problems in dissecting several brain regions, only cerebellum samples were suitable for analysis. Treatment-related clinical signs were limited to 39 mg/kg rats, and were observed only on day 1. Common findings (with minimal sex differences) were decreased activity (29 rats), fasciculations (29 rats), miosis (21 rats), lacrimation (13 rats), and salivation (17 rats). Two 39 mg/kg males displaying tremors, and two 39 mg/kg females with "reduced splay reflex" were the only other signs observed in more than one rat/sex/group. Mydriasis, seemingly elevated in treated rats in Record No. 210702 (Laboratory Study # AR7138) was not observed in this study. RBC cholinesterase activity was reduced meaningfully at day 1 and was dose-related in all treated groups, without apparent sex difference (inhibition of 21, 37, and 47% in low to high dose males, and 18, 32, and 47% in corresponding females). Slight but statistically significant RBC cholinesterase reduction at Day 8 in 39 mg/kg males and females (15% and 11%, respectively) was also plausibly treatment-related. Cerebellar cholinesterase inhibition was statistically significant in all treated

male groups (inhibition of 12, 34, and 64% in increasing dose groups), and in 39 mg/kg females (with a plausibly treatment-related non-significant decrement in 5 mg/kg females). Inhibition was 17% and 58% in mid to high dose females. Noted technical errors necessitated a repeat of this study, but some useful supplementary information is provided. Aldous, 2/25/05.

235-0236 210701 Twomey, K., "Dichlorvos (DDVP): second acute cholinesterase inhibition study in rats," Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, 6/19/02. Laboratory Study # AR7126. RBC and cerebellar cholinesterase activities were determined in Alpk:AP_fSD (Wistar-derived) young adult rats (at least 42 days of age at dosing) following single oral doses of dichlorvos (99% purity) in 10 ml distilled water per kg b.w. Achieved doses were 0, and 1 mg/kg. Five rats per sex per group were killed one hour after dosing (near maximal tissue concentrations). Additional groups of 5/sex were dosed for sacrifice at days 8 or 15, however due to lack of effects at day 1, these rats were removed from the study. Due to technical problems in dissecting several brain regions, only cerebellum samples were suitable for analysis. No treatment-related clinical signs were observed. RBC and cerebellar cholinesterase activities were unaffected by treatment. This study provides cholinesterase NOEL for RBC and cerebellum of 1 mg/kg/day [some inhibition had been indicated in Record No. 210700 (Laboratory Study # AR7079) at 2 mg/kg/day in M and F for RBC, and for cerebellum (M only)]. Useful supplementary information. Aldous, 1/24/05.

235-0237 210702 Twomey, K., "Dichlorvos (DDVP): third acute cholinesterase inhibition study in rats," Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, 6/26/02. Laboratory Study #: AR7138. RBC and brain cholinesterase activities (brain was evaluated as "brain half", and as brain regions including cerebellum, cortex, hippocampus, and "remainder") were determined in Alpk:AP_fSD (Wistar-derived) rats (at least 42 days old at treatment) either one hour following single oral doses of dichlorvos (approximating peak tissue concentrations) or 8 days after dosing. Limited assessments were performed at 15 days after dosing where indicated by results of Day 1 and Day 8. Specifically, fifteen rats/sex/group were to be dosed with 0, 1, 5 or 35 mg/kg dichlorvos (99% purity) in 10 ml distilled water per kg b.w. Excessive toxicity including mortalities in 35 mg/kg males prompted a discontinuation of that group. Females, which had not yet been dosed at that level, were re-allocated to provide an extra group of 15 controls and a revised high dose of fifteen females at 15 mg/kg dichlorvos to assess the original study parameters. Clinical signs were almost entirely limited to 35 mg/kg rats (only males having been dosed at this level). The most common findings (each observed in at least four 35 mg/kg males) were decreased activity, fasciculations, gasping, mydriasis, prostration, and salivation). Four of the nine 35 mg/kg males were killed moribund. In a puzzling pattern, mydriasis (pupil dilatation) was observed in males at incidences of 0/15 controls, 1/15 at 1 mg/kg, 6/15 at 5 mg/kg, and 4/9 at 35 mg/kg. Mydriasis was not observed in any females. This pattern could not be entirely dismissed by investigators as incidental, although mydriasis is not a characteristic cholinesterase effect, and does not appear as an effect in any of the several related studies in the present submission. Investigators considered mydriasis findings not to be toxicologically significant, which is probably a valid assessment. Cholinesterase measurements in brain regions were highly variable (%CV's on the order of 50%), therefore the results are of very limited value in creating dose-response curves or assessing NOEL's. Nevertheless, it appears that 15 to 35 mg/kg elicited at least 50% cholinesterase inhibition, and that 5 mg/kg inhibited cholinesterase on the order of 30%. The present data cannot resolve whether measurable inhibition would occur at 1 mg/kg. The primary function that this study serves is to indicate that the high dose for a definitive repeat-dose study should not be higher than 15 mg/kg/day. This supplementary study is unacceptable for most other purposes. Aldous, 1/20/05.

NOTE: The above three studies together (Document Nos. 235-0235 through 235-0237) do not provide sufficient information to determine NOEL's for cholinesterase inhibition in major brain regions. It appears that the NOEL would be on the order of 1 mg/kg for a single acute oral dose in adult rats.

235-0232 210697 Milburn, G. M., "Dichlorvos: time course of cholinesterase inhibition in pre-weaning and adult rats," Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, 9/26/03. Laboratory Study #: AR7310. RBC and whole-brain cholinesterase activities were determined in Alpk:AP_fSD (Wistar-derived) rats following single oral doses of dichlorvos (99% purity) in 10 ml distilled water per kg b.w. Pups and young adult rats (aged PND 15 and PND 42), twenty-five females of each age per group, were dosed with 0 or 15 mg/kg a.i. as sets of 5/dose/interval, then sacrificed at 1, 3, 8, 24, or 72 hr post-dosing. There were no observed clinical signs. Cholinesterase inhibition at 1 hr after dosing was 59% and 53% in PND 15 pup brain and RBC's, and 53% and 46% in PND 42 rat brain and RBC's. Inhibition was only slightly lower at 3 hr. It is difficult to assess possible changes in cholinesterase activity at 8 hr after treatment and beyond. In general, there was appreciable variability within groups, and an unusually low value for young adult control female brain cholinesterase activity at 8 hr after treatment. These deficiencies limit the usefulness of this study to describe the dose-response after the first few hours of treatment. It appears, however, that substantial recovery had occurred by 24 hr after dosing. This study shows that 15 mg/kg would not be excessively high for acute dosing of rats with this compound, and possibly even for repeated daily dosing. Useful supplementary data with noted deficiencies. Aldous, 1/21/05.

235-0238 210703 Moxon, M. E., "Dichlorvos: acute cholinesterase inhibition study in pre-weaning rats," Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, 11/22/02. Laboratory Study #: AR7147. RBC and whole-brain cholinesterase activities were determined in Alpk:AP_fSD (Wistar-derived) pups one hour following single oral doses (approximating peak tissue concentrations). Five pups/sex (aged PND 8, 15, or 22), were dosed with 0, 1, 5 or 15 mg/kg dichlorvos (99% purity) in 10 ml distilled water per kg b.w. At 15 mg/kg dichlorvos, there were two PND 8 pups (1/sex) with slight tremors, and two PND 22 pups (1/sex) also with slight tremors. These were the only observed clinical signs. Cholinesterase inhibition was statistically significant in all 5 mg/kg pups (at least $p < 0.05$) and all 15 mg/kg pups ($p < 0.01$) for both brain and RBC. There was no obvious difference in response between sexes or over pup ages at the higher two dose levels. Ranges of cholinesterase activity inhibition were 22% to 31% for brain at 5 mg/kg, 54% to 65% for brain at 15 mg/kg, 26% to 39% for RBC at 5 mg/kg, and 45% to 62% for RBC at 15 mg/kg. Brain cholinesterase was unaffected at 1 mg/kg in either sex. RBC cholinesterase at 1 mg/kg was inhibited 22% and 27% in PND 8 females and PND 15 females, respectively, and 9% in PND 15 males, thus this study did not determine a NOEL for RBC cholinesterase inhibition. Useful supplementary data. Aldous, 2/25/05.

235-0233 210698 Moxon, M. E., "Dichlorvos: repeat dose cholinesterase inhibition study in pre-weaning and young adult rats," Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, 10/24/03. Laboratory Study #: KR1490. RBC and whole-brain cholinesterase activities were determined in Alpk:AP_fSD (Wistar-derived) rats following seven consecutive daily oral doses of dichlorvos (99% purity, in 10 ml distilled water per kg b.w.). Pups and young adult rats (aged PND 12 and PND 42 at dosing onset), five per sex of each age per group, were dosed by gavage at 0, 0.1, 7.5, or 15 mg/kg/day, then sacrificed at 1 hr post-dosing on day 7. All 15 mg/kg/day pre-weaning pups displayed slight tremors shortly after dosing on 2 or more treatment days, and the majority of 15 mg/kg/day PND 48 rats showed slight tremors on at least one occasion. One 7.5 mg/kg/day PND 48 male had tremors on one occasion: there were otherwise no clinical signs at 7.5 mg/kg/day or below. The NOEL for clinical signs, considering this study in isolation, is 0.1 mg/kg/day. At 7.5 to 15 mg/kg/day, there were no apparent sex or age differences in cholinesterase inhibition responses. In brain, inhibition ranged from 54% to 64% at 7.5 mg/kg/day and from 72-78% at 15 mg/kg/day. In RBC's, inhibition ranged from 54% to 58% at 7.5 mg/kg/day and from 53-65% at 15 mg/kg/day. Only PND 18 pups appeared to show inhibition in brain at 0.1 mg/kg/day (24-26%), and only PND 48 rats appeared to show inhibition in RBC's at 0.1 mg/kg/day (11-17%). Thus no NOEL for cholinesterase inhibition was determined in this study. This is a valid supplementary study, showing useful dose-response curves. Aldous, 2/25/05. Below is a DPR review of a slightly later version of this study.

235-0239 215893; Supplemental ChE Inhibition Study-Rats; Central Toxicology Laboratory, Alderley Park, Maccelesfield, Cheshire, UK; Study No.: KR1490; 8/27/04; Seven daily oral doses of 0 (control), 0.1, 7.5 or 15 mg dichlorvos/kg/day were given to five Alpk:APfSD (Wistar-derived) rats/sex/dose at 12 days (pre-weaning) or 42 days of age (young adult); rats were killed at the estimated time of peak effect, approximately 1 hour after the 7th dose (i.e., 18 or 48 days old); blood and brain were analyzed for cholinesterase (ChE) activity; slight tremors were seen in pre-weaning and young adult rats given 15 mg kg/day (multiple occasions) and in one young adult male rat at 7.5 mg/kg/day (day 48 only); no change in clinical condition was seen at 0.l mg/kg/day in either pre-weaning or young adult rats; brain ChE was significantly inhibited in pre-weaning rats at all dose levels (RBC ChE sig. inhibited at 7.5 and 15 mg/kg/day only); RBC ChE showed significant inhibition in young adult rats at all dose levels (Brain ChE sig. inhibited at 7.5 and 15 mg/kg/day); study author attributed the ChE inhibition seen at 0.1 mg/kg/day to unusually high control values (*i.e.*, NOEL for behavioral effects and ChE inhibition at 0.1 mg/kg/day); Med. Tox. reviewer considers this value a LOEL for ChE inhibition in pre-weaning and young adult Wistar rats. Supplemental data. (Kellner, 8/24/05).

DEVELOPMENTAL NEUROTOXICITY, RAT

**235-0231 210696 Milburn, G. M., "Dichlorvos: developmental neurotoxicity study in rats," Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, Nov. 10, 2003. Laboratory Study # RR0886. A supplementary study was undertaken because there were only 14 high dose litters available in this primary study, compared to Guideline recommendation of at least 20 per dose. The supplementary study is Document No. 235-0234, Record No. 210699, CTL Study No. RR0988, Milburn, G. M., "Dichlorvos: supplemental developmental neurotoxicity study in rats." Data from this supplementary study are included in this review of the primary study (similar design, but only 0 and 7.5 mg/kg/day dichlorvos, N = 30). In the primary study, thirty timed-mated Alpk:AP$_f$SD females/group were dosed from gestation day 7 through lactation day 7, after which the pups were dosed (lactation days 8 through 22): dosing to dams or pups being 0, 0.1, 1, or 7.5 mg/kg/day dichlorvos (99% purity). FOB evaluations of dams were at gestation days 10 and 17, and on lactation days 2 and 9. FOB evaluations on F1 rats were made on PND 5, 12, 22, 36, 46, and 61. Other parameters included automated motor activity evaluations, assessment of developmental landmarks in pups (preputial separation or vaginal opening), auditory startle, learning and memory (Y-shaped water maze), and neurohistopathology of selected F1 rats at termination (PND's 12 and 63). Histopathology at Day 12 included evaluations of immersion-fixed brains of 1 pup/litter, cut at 7 levels for examination after paraffin embedding, and stained with H&E. Histopathology at Day 63 involved perfusion fixation of at least one male or female per litter. Brains were prepared and examined as above. Peripheral structures in PND 63 rats were embedded in resin and stained with toluidine blue. Only controls and 7.5 mg/kg/day tissues were examined microscopically. A series of morphometric measurements was made in brains at both PND 12 and PND 63, for controls and 7.5 mg/kg/day groups. NOEL = 7.5 mg/kg/day (HDT) for maternal toxicity and developmental toxicity (including developmental neurotoxicity). This study is acceptable, with no adverse effects. The study was initially classified as unacceptable, with concerns about the methodology of the FOB, for which a key cited validation study was requested for support. A search by the DPR reviewer found that the desired validation study had been submitted in association with another product, and was considered acceptable. The present study (considered with the supplementary study) is now upgraded on that basis. Aldous, 2/25/05 and June 3, 2008.

NOTE: The U.S. EPA found this study (considering also DPR Document No. 235-0234, Record No. 210699, which is CTL Study No. RR0988) to be acceptable. U.S. EPA considered there to be a developmental NOAEL of 1 mg/kg/day, based on increased startle reflex habituation Vmax in PND 23 high dose males. DPR reviewer Aldous had presented the startle reflex data from the primary study in the 2005 review worksheet, and the data for the supplementary study in the May 2008 rebuttal response worksheet. DPR maintains that data do not demonstrate a treatment effect, considering the marginal high

dose increase and lack of credible dose-response in the primary study, and lack of remarkable treatment differences in the supplementary study. Thus DPR respectfully maintains a higher developmental NOAEL than U.S. EPA.

235-0230 210695 Milburn, G. M., "Dichlorvos: preliminary developmental neurotoxicity study in rats," Central Toxicology Laboratory, Alderley Park, Macclesfield, UK, 10/13/03. Laboratory Study # RR0885. Groups of 15 Alpk:AP_fSD (Wistar-derived) timed-mated dams were dosed from gestation day 7 until lactation day 22 with dichlorvos (99% purity) at 0, 0.1, 1, or 7.5 mg/kg/day by gavage in 10 ml/kg de-ionized water. Dams were evaluated for b.w., food consumption (during gestation), and clinical signs. Offspring were evaluated for number, survival, clinical signs, and body weight. Brain and RBC acetylcholinesterase (AChE) were evaluated in 5 dams/group at gestation day 22 and lactation day 22 (sacrifice 2-3 hr after the last dosing). Five litters/group were evaluated for these AChE activities at gestation day 22, with pooling of brain tissues from 4 male or 4 female fetuses per sample, and blood was likewise pooled from multiple fetuses per sex per litter for analysis. Five pups/sex/group were evaluated on lactation days 2, 8, 15, and 22 for brain and blood AChE (one male or one female pup per litter). This study was to assess dose levels for the definitive study. For parameters evaluated in this pilot study, maternal AChE NOEL = 1 mg/kg/day for brain (59% inhibition on gestation day 22, and 67% inhibition on lactation day 22 at 7.5 mg/kg/day), and 0.1 mg/kg/day for RBC (inhibition of 25% and 48% on gestation day 22, and of 24% and 50% on lactation day 22 for doses of 1 and 7.5 mg/kg/day). Maternal NOEL (other than AChE inhibition) = 7.5 mg/kg/day (HDT). Fetal AChE NOEL = 1 mg/kg/day (brain AChE inhibition of 16% and 21% for M and F, respectively, and RBC AChE inhibition of 28% in M: no significant difference in F). Pup AChE NOEL = 7.5 mg/kg/day (HDT). Fetal/pup NOEL (other than AChE inhibition) = 7.5 mg/kg/day (HDT). Useful supplementary data, with noted deficiencies in report preparation. The HDT of this study appears appropriate for the definitive study. Aldous, 2/25/05.

235-0241 219694 and 235-0242 219693 These are two brief records showing black-and-white photocopies of representative stained sections of tibial nerves, proximal and distal, transverse and longitudinal, of control and 7.5 mg/kg groups of above Record Nos. 210696 and 210695, respectively. Frequencies or appearances of lesions did not appear to reflect treatment responses in either record. These data had not been requested by DPR, and may have been solicited by U.S. EPA. These data do not impact study acceptability. No DPR worksheets. Aldous, 5/13/08.

235-0250 234880 "Benchmark Dose Estimates of Mortality from Dichlorvos Acute Inhalation Exposure for Use by the Cal-EPA's Dept. Of Pesticide Registration as a "Point of Departure" for Acute Inhalation Risk Characterization," Aug. 24, 2007. Statements from pp. 40-41 of that record were addressed in worksheet w210696 836 supp.wpd on June 3, 2008.

MISCELLANEOUS DOCUMENTS IN ALPHABETICAL ORDER BY AUTHORS
(with or without record numbers assigned)

[No record number] Anonymous, 1967. Safe use of pesticides in public health. WHO Technical Report Series 356, WHO, Geneva, p. 46-54. "8. Safety of dichlorvos (OMS-14) for disinfection of aircraft." The use of impregnated filters with compressed air to maintain a concentration of 0.2 - 0.25 µg/liter of air for ½ hour in airplanes for insecticidal use was discussed. The pharmacological activity is through inhibition of cholinesterase without causing permanent neurotoxicity. Results of a number of publications in which human populations were exposed to dichlorvos under several conditions were reviewed in brief. Cholinesterase activity was the primary parameter measured during exposure. A number of these publications have been examined by Medical Toxicology and are described in this "Summary of Toxicology Data." The conclusion of the report authors was that dichlorvos exposure to concentrations effective against insects (0.15 - 0.25 µg/liter of air for 30 minutes) would not produce adverse effects. (Gee, 5/25/99)

235-191 162857 Arts, J. H. E., "Inhalation toxicity of slow-release strips containing dichlorvos," June, 1995. This is a brief compilation of animal and human data associated with the use of slow-release strips. This report does not contain data sufficiently detailed for SB-950 analysis. The report provides some exposure ranges and associated human responses, such as cholinesterase inhibition data, of possible relevance to risk assessment. Aldous, 2/15/05.

[No record number] Boyer, A. C., 1975. Inhibition of human plasma cholinesterase by steady-state concentrations of dichlorvos. (Shell, Consumer Products, Technical Progress Report No. M-44-75, 7/75) Carbon-14-methoxy-labeled dichlorvos was incubated *in vitro* with human blood plasma (obtained from a blood bank) under several conditions. Degradation of dichlorvos over time was determined using concentrations of 1.14×10^{-5} M, 1.09^{-6} M, 1.08×10^{-8} M and 1.13×10^{-9} M. The curvature of the plots indicated that the disappearance from plasma was not a first-order kinetics reaction. There was a very rapid disappearance within one minute with all four concentrations. Other tests indicated that the degrading enzyme was stable at 37°C and was not inhibited by dichlorvos or its metabolites. Inhibition of plasma cholinesterase under steady state conditions was also measured over several hours with nonlinear results with less inhibition as a function of time. The author considered the cause as perhaps due to the dephosphorylation of the dimethyl phosphorylated enzyme. When infusion of dichlorvos was stopped, a rapid reversal of activity was measured but the reversal was not complete, stopping at about 70% inhibition, representing the amount of monomethyl phosphorylated state of the enzyme, or "aged" enzyme. From the results, the author concluded that it would take 7 to 8 hours to inhibit 5% of the enzyme at steady-state concentration of 1×10^{-9} M dichlorvos. Supplemental study. (Gee, 5/25/99)

[No record number] Boyer, A. C., L. J. Brown, M. B. Slomka and C. H. Hine, 1977. Inhibition of human plasma cholinesterase by ingested dichlorvos: Effect of formulation vehicle. (Shell and University of California, SF, in: Toxicol. Appl. Pharmacol. 41: 389 - 394.) Twenty-four male volunteers, ages 21 - 45 years, were divided into four groups: 1) 0.9 mg dichlorvos 3X daily in a pre-meal capsule in cottonseed oil; 2) in gelatin salad during meal or 3) placebo group receiving cottonseed oil capsule or 4) gelatin. Average weight was 81 kg with a range of 64 - 106 kg (dose equivalent to 0.01 mg/kg 3X daily). Dosing was carried out over 21 days. Plasma and RBC cholinesterase activities were measured pretest, twice a week during dosing, and for 7 weeks following dosing period. Blood for cholinesterase determinations was collected prior to breakfast. Plasma and RBC's were separated with plasma cholinesterase determined immediately and RBC the following day using a pH stat apparatus. Results: No signs or symptoms were noted. Only plasma cholinesterase was inhibited "to any significant extent". Dichlorvos in cottonseed oil was more effective than in gelatin at inhibiting plasma cholinesterase with gelatin being about 64% as effective. At 21 days, the percent inhibition with gelatin was about 30% and with cottonseed oil, 40%. The half-life of regeneration of plasma cholinesterase was estimated to be 13.7 days. Supplemental study. (Gee, 5/25/99)

[No record number] Cavagna, G., G. Locati and E. C. Vigliani, 1969. Clinical effects of exposure to DDVP (Vapona) insecticide in hospital wards. (University of Milan, Institute of Occupational Health, in: Arch. Environ. Health 19: 112 - 123 (1969)). Healthy adults, adult patients, sick children and women in labor were exposed to DDVP. Blood for cholinesterase determinations was obtained and activity determined by pH titration. The activity of cholinesterase in all patients/subjects was stated to be normal. Vapona strips were installed at 1 strip/30 m^3 (about 1/1000 ft^3). Air samples were taken at 1 meter above the floor in several ward locations for the first 15 days after the strips were installed. In addition, 12 babies aged 4 - 12 months of age wore clothes that had been exposed to DDVP. The air concentration in winter reached levels above 0.2 mg/m^3 with a maximum of 0.28 mg/m^3 fifteen days after installation, then decreasing. In 5 patients exposed for 24 hr/day to 0.1 to 0.28 mg/m^3, plasma cholinesterase was decreased to an average of 54% of pre-exposure activity with a range of 35 to 75%. No reduction was seen in RBC activity. In 17 patients exposed for 16 hrs/day at the same levels, neither plasma nor RBC cholinesterase was reduced. At 0.02 to 0.1 mg/m^3 for 16 and 24 hours/day, there was no inhibition of

cholinesterase. Patients with liver disease showed inhibition of plasma cholinesterase even below 0.1 mg/m^3 for 16 hours. No clinical symptoms were reported by these patients. Children showed a similar pattern. The authors concluded that exposure to concentrations of DDVP below 0.1 mg/m^3 even for 24 hours/day did not decrease plasma or RBC cholinesterase activity except for plasma in liver diseases. Although the report gives the hours per day for exposure, there are no data on the length of days of exposure for determination of acute effects on plasma cholinesterase, the only parameter showing an effect under the conditions of the study. No individual data. Supplemental study. (Gee, 6/3/99).

[No record number] Cavagna, G., G. Locati and E. C. Vigliani, 1970. Exposure of newborn babies to "Vapona" insecticide. (University of Milan, Institute of Occupational Health, in: Europ. J. Toxicol. 1: 49 - 57 (1970)) Healthy babies born to women exposed during labor and delivery or under 2 different conditions were compared with unexposed babies for blood cholinesterase activity at birth (umbilical cord) and 5 days later. Plasma and erythrocyte activities were measured by pH titration of Jensen-Holm. The activity in babies born to mothers exposed to DDVP (concentrations ranging from 0.095 to 0.25 mg/m^3), about 20 minutes 6 times daily, showed no alteration in cholinesterase activity compared with controls. Babies in a well ventilated nursery were exposed to DDVP from 1 strip/40 m^3 or in a less well ventilated nursery at 1 strip/30m^3 for 5 days. Concentrations under good ventilation ranged from 0.05 to a maximum of 0.125 mg/m^3 as estimated from a graph. With less ventilation, the concentration ranged from 0.05 to a maximum of 0.275 mg/m^3 (time-weighted concentrations were 0.152 and 0.159 mg/m^3 in the two poorly ventilated rooms). No significant effect on plasma or red cell cholinesterase was measured in any group. Limited individual data. The authors concluded that exposure of newborns to Vapona strips at 1/30 m^3 did not have an adverse effect under the conditions of the study. Supplemental study. (Gee, 6/3/99)

[No record number] Coulston, F. and T. Griffin, 1977. Cholinesterase activity and neuromuscular function of Rhesus monkeys exposed to DDVP vapors. (Albany Medical College, Draft document, 5/31/77) Male and female rhesus monkeys were exposed to a constant concentration of dichlorvos at 0.051 µg/l for 23 hours per day for three months [estimated at 20 µg/kg/day]. Analytical concentrations of DDVP in the chamber were determined at frequent intervals. Parameters measured included plasma and erythrocyte cholinesterase activity using the method of Ellman et al. Cholinesterase determinations were made pretest and at monthly intervals. Hematology and clinical chemistry parameters included hemoglobin concentration, packed cell volume, total erythrocyte and leucocyte counts, sodium, potassium, creatinine, bilirubin, glucose, total protein, SGPT, SGOT, LDH and inorganic phosphate. Electromyographic studies were performed on lightly anesthetized animals with stimulation of the ulnar nerve and electrodes placed on the tendon and the "belly" of the flexor carpi ulnaris muscle. The intensity of the stimulus was varied. RESULTS: Analytical concentrations ranged from 0.038 to 0.068 µg/l with an average of 0.051 µg/l. No changes in behavior, pharmacologic or toxicologic conditions were noted, including pupillary constriction, muscular fasciculation, tremors, hyper- or hypo-activity, anorexia, vomiting or stool consistency. No changes in clinical chemistry or hematology parameters were noted. No effect was seen on the electromyographic studies due to exposure to dichlorvos. CHOLINESTERASE: Plasma cholinesterase was reduced to 72% of baseline at 3 months ($p < 0.05$) and erythrocyte activity was reduced to 64% of baseline ($p < 0.01$). Individual data were included. Supplemental study. (Gee, 5/26/99)

[No record number] Durham, W. F., T. B. Gaines, R. H. McCauley, Jr., V. A. Sedlak, A. M. Mattson, and W. J. Hayes, Jr., 1957. Studies on the toxicity of O,O-dimethyl-2,2-dichlorovinyl phosphate (DDVP). (Public Health Service, Savannah, GA, in: Am. Med. Assoc. Archives of Indication. Health 15: 340 - 349) Effects of technical DDVP of rats, monkeys, hens and workers were studied by the oral, dermal or inhalation route. RAT: Acute oral $_{LD50}$ in males was 80 mg/kg and in females, 56 mg/kg. Dermal $_{LD50}$ was 107 mg/kg in males and 75 mg/kg in females. Subchronic : Groups of 10 female rats were given doses of 0, 5, 20, 50, 200, 500 or 1000 ppm in the diet over a period of 90 days. Plasma and

erythrocyte cholinesterase levels were determined periodically (days 3, 11, 30, 60 and 90) using the electrometric method of Michel. At 5 and 20 ppm (0.4 and 1.5 mg/kg), there was transitory depression in plasma but not erythrocyte cholinesterase with recovery by day 30. At 50 (3.5 mg/kg), depression of plasma activity continued through day 60 but recovered by day 90 and RBC activity recovered by day 30. At the higher doses, recovery was not complete by day 90. Inhalation: Walls and ceiling were sprayed with 2.5% DDVP in xylene and rats were exposed for two weeks total with plasma and RBC cholinesterase being determined after one and two weeks. The initial concentration peak value was 6 µg/L with a decrease by the third day to < 1 µg/L. Cholinesterase depression was marginal (5 - 17%). MONKEYS: Dermal applications at 50, 75 and 100 mg/kg/day were made for 5 days/week until the animal died. Cholinergic signs were seen after 10 to 20 min. Animals died after 8, 10 and 4 doses. Inhalation: Exposure the same as for rats. Both plasma and erythrocyte cholinesterase were inhibited after 1 week of exposure with a tendency for plasma to recover but little recovery with RBC cholinesterase. HENS: Results were reported elsewhere but no signs of paralysis or muscle weakness were noted after subcutaneous doses of 15 mg/kg. WORKERS: Five laboratory personnel exposed to "high" but undetermined levels of DDVP for 7 weeks via inhalation and dermal routes were studied for plasma and erythrocyte cholinesterase activity. Plasma levels were not affected but RBC activity was reduced to 68 - 71% of pre-exposure levels in 4 of 5 men. Supplemental study. (Gee, 5/27/99)

235-189 162853 Feiler, W. A., "Review of human incident data for DDVP," 12/20/05. This 3-page text may be suitable for qualitative background perspective in risk assessment documents, but provides no data relevant for SB-950 data review. Aldous, 2/15/05.

[No record number] Foll, C. V., C. P. Pant and P. E. Lietaert, 1965. A large-scale field trial with dichlorvos as a residual fumigant insecticide in Northern Nigeria. (WHO, in: Bull. World Health Org. 32: 531 - 550) The purpose of the field trial was to determine if either of two types of dispenser would provide sufficient concentration of DDVP to interrupt the malaria cycle. The two types consisted of 1) a solid, impregnated montan-wax strip with not less than 20 g DDVP or 2) a liquid dispenser with 14 g DDVP in 16 ml fluid. The dosage used was one dispenser per 15 m^3 of living space. Subjects were surveyed during the dry season and immediately after rains for parasites in blood samples. Mosquito densities in huts were also surveyed. The failure of dichlorvos to interrupt transmission was believed due primarily to the to the ventilation of the huts and lack of adequate concentration of dichlorvos. There was no discussion of toxic effects to the population. Supplemental study. (Gee, 5/27/99)

[No record number] Funckes, A. J., S. Miller and W. J. Hayes, Jr., 1963. Initial field studies in Upper Volta with dichlorvos residual fumigant as a malaria eradication technique. (CDC, Savannah, GA, in: Bull. World Health Org. 29: 243 - 246) The effect of dichlorvos in a solid dispenser (no details) on the exposed population was determined. Rates of one dispenser per 11 - 28 m^3 and one per 1.7 - 2.5 m^3 were used. The population consisted of 29 individuals with 17 males and 12 females and an age range of under 6 years to 64 years. Air samples were taken once a week primarily at 6 feet and in the morning and evening. The mean concentration varied with the time of year, being higher in August than in early summer. At one dispenser per 1.7 - 2.5 m^3, the range was 0.170 - 0.84 µg/L. The authors found no significant effect of exposure to dichlorvos on cholinesterase (plasma or RBC), hematocrit or hemoglobin levels. Supplemental study. (Gee, 5/27/99)

235-173, -175, -176 153926, 153928, 153929 Gledhill, A. J., "Dichlorvos: A Study to Investigate Erythrocyte Cholinesterase Inhibition Following Oral Administration to Healthy Male Volunteers," (Central Toxicology Laboratory, Alderley Park, Cheshire, UK, Report# CTUP/5251, 2/3/97; CTUP/5393, 3/25/97 and CTUP/5392, 3/24/97). Dichlorvos (DDVP, purity of 97.7%, dissolved in corn oil) was administered in gelatin capsules to fasted male human volunteers (weighing 67 to 80 kg) in three separate studies. The first study (CTUP/5251) was conducted in two phases, with the first involving a single oral dose (35 mg; 0.5 mg/kg for 70 kg male) followed by a placebo (corn oil) to four males; a week later, the

same four were given a second 35 mg dose (plus two more males that were given the second 35 mg dose only). The second phase included up to 15 consecutive daily doses of 21 mg to same 6 males. RBC ChE was measured before and after each phase; although no inhibition was seen after single oral dose (phase 1), inhibition of up to 31% was seen at day 22 (following cessation of multiple doses); return to baseline activity took about 40 days. No cholinergic symptoms were attributed to the test compound, although 2 volunteers had headaches and another was tired after phase I; after phase II, one felt tired on days 5-9 and on day 6 had headache and nausea. Another had abdominal colic on day 12. Note: documentation of clinical signs was minimal in all of reports, so little information on possible cholinergic effects was obtainable. The second study (CTL/P/5392) involved six males receiving 21 daily doses (7 mg) and a control group of 3 males getting the 21 doses of placebo. RBC ChE (measured daily) maximal group mean inhibition of 16% at day 18 (post dose inhibition was 17%). Clinical signs (again, not attributed to the test compound by the author) consisted of tiredness (2 volunteers on multiple occasions) and intermittent nausea (1 of the previously mentioned subjects) and mild headache between days 10 and 11 (a third subject). The final study (CTUP/5393) involved a single oral dose of 70 mg (approx. 1 mg/kg) to six males followed by RBC ChE monitoring at scheduled intervals after dosing. Group mean ChE activity on days 1, 3, 5, 7 and 14 after dosing was 94, 96, 90, 88 and 89% of the mean pre-dose activity (day 5, 7 and 14 were significantly different (1% level) from the pre-dose values using the paired t-test). There were no symptoms reported. Supplemental Data. Kellner, 9/10/97.

235-0194 162860 (Duplicate of 235-0175 153928, above, with additional appendices)

[No record number] Gratz, N. G., P. Bracha and A. Carmichael, 1963. A village-scale with dichlorvos as a residual fumigant insecticide in Southern Nigeria. (WHO Insecticide Testing Unit and NIH, in: Bull. World Health Org. 29: 251 - 270) Several types of dispensers were used: 1) solid mortan wax impregnated with dichlorvos containing 40 g of technical DDVP; 2) liquid type in a plastic container with 14 g technical DDVP and 3) a solid plastic dispenser with 30% DDVP or flat strips with 20% DDVP. Temperature and humidity were recorded over some months' time. The effectiveness of location within the hut (height) on mosquito control was determined using bioassays with several genus of mosquitos in cages. Placement of the dispensers gave best control when suspended at about 12 feet mid-way between the ridge pole and top of inner partition walls of the hut. Testing in various types of huts was also conducted. The concentration of dichlorvos in the air of huts was measured and found to be influenced by temperature, humidity and ventilation with higher temperature yielding higher concentration and higher humidity a decreased concentration. Also, concentrations at the 2-foot level were 10 - 20% that at the 12-foot level in the hut. Although dichlorvos is heavier than air, ventilation factors influence the concentrations. Plasma and red blood cell cholinesterase was estimated using Michel's micro-method. Activity was compared after 5 and 7 weeks exposure with that in a control village. The authors concluded that there was no significant change in plasma or RBC cholinesterase activity as a result of continual exposure to dichlorvos. Supplemental study. (Gee, 5/26/99)

[No record number] Hass, D. K., J. A. Collins and J. K. Kodama, 1972. Effects of orally administered dichlorvos in rhesus monkeys. (Shell Chemical, in: J. Am. Vet. Med. Assoc. 161: 714 - 719) Rhesus monkeys, either normal or infected with *Schistosoma mansoni*, were given one of several regimens of dichlorvos for 10 to 21 consecutive days. Dichlorvos, 20%, was given orally in a polyvinyl chloride resin pellet in a capsule once or twice daily. Uninfected monkeys, 2 per group, were given 20, 40 or 80 mg/kg b. wt. for 21 days. No deaths occurred but clinical signs included reduced appetite, diarrhea, emesis and salivation, increasing in number of days with dose. No tremors, ataxia, severe salivation or convulsions were noted. Although no data were reported, the text states that cholinesterase inhibition "was virtually complete" after 7 days and remained so up to the 21st day. Cholinesterase data for the infected monkeys, treated at one of several doses for 10 days (once or twice daily) indicated considerable inhibition of both plasma and erythrocyte cholinesterase with recovery toward normal activity being more rapid with plasma when dosing ceased. Erythrocyte activity required about 60 days

to recover to pretreatment levels. With twice daily treatment, the pattern was similar to once daily dosing. Other hematological parameters measured in infected animals (including SGOT, SGPT, Alkaline phosphatase, hemoglobin, white blood cell count) were similar to control values. Supplemental study. (Gee, 5/26/99)

[No record number] Hayes, W. J. Jr., 1961. Safety of DDVP for the disinfection of aircraft(Toxicology Section, Public Health Service, Atlanta, GA. In: Bull. World Health Org. 24: 629 - 633). This paper was presented to the WHO Expert Committee on Insecticides and contains no original data. The figure and table are based on Durham et al., 1959 and 1957 respectively. Supplemental reference. (Gee, 5/26/99)

235 - 215 164807 Hunter, C. G., 1969. "Report on initial studies of deliberate exposures to high concentrations of dichlorvos by human subjects." (Shell Research Ltd., Tunstall Laboratory, 1969). Six adult males were exposed to dichlorvos vapor (>98% purity), head and neck. Parameters measured included plasma and erythrocyte cholinesterases, creatinine, phosphate, others. Concentrations ranged from 6.3 to 52 mg/m^3 from 20 to 240 minutes. At 18.7 mg/m^3, 120 minutes, RBC was inhibited approximately 20% and required over 14 days to recover. At that same exposure, plasma cholinesterase was inhibited 66% with 11 days to recover. The percent depression for plasma cholinesterase was not strictly related to dose or time. No inhibition of red cell cholinesterase was found at 41.5 mg/m^3 for 24 min. No visual disturbances were noted. No other parameters were changed. Supplemental study. (Gee, 6/1/99)

235 - 215 164806 Hunter, C. G., 1970. "Dichlorvos: inhalation exposures with human subjects. Part I. " (Shell Research Ltd., Tunstall Laboratory, TLGR.0061.70, 1970) Adult male and female volunteers were exposed continuously by total body exposure wearing clothing from 2 to 7 ½ hours. Technical dichlorvos, > 94.6%, was used to generate atmospheres. Target level was 1 mg/m^3, the threshold limit value adopted by the Am. Conf. Gov, Indication. Hygienists. Clinical and physiological observations were made including plasma and erythrocyte cholinesterase activity, respiratory activity, EEG, urinalysis and hematology. Cholinesterase was measured pretest, immediately following exposure and after 16 - 18 hours. No effects were noted on parameters other than plasma cholinesterase. Exposures of 400 mg/min/m^3 caused decreased plasma cholinesterase compared with pretest levels, being reduced in the range of 20 to 30%. Continuous exposure for 6 - 7 hours were needed to decrease the plasma cholinesterase. Supplemental study. (Gee, 6/1/99)

235 - 215 164806 (part 2) Hunter, C. G., 1970. "Dichlorvos: inhalation exposures with human subjects. Part II. " (Shell Research Ltd., Tunstall Laboratory, TLGR.0067.70, 1970) Seven adult males were exposed by the head and neck using a "bell jar' to dichlorvos in concentrations ranging from 1 - 53 mg/m^3, 1 to 4 hours. Symptoms were confined to irritation of the throat, some rhinorrhea at the highest concentration, and slightly reduced erythrocyte cholinesterase in one subject only at 1,450 mg/min/m^3. No visual effects were reported. Plasma cholinesterase was affected by exposure related to the concentration and time of exposure both immediately after exposure and 16 - 20 hours later. One subject had approximately 90% inhibition of plasma cholinesterase immediately after exposure to 5,100 mg/min/m^3 with some inhibition still present at day 20 (30% inhibition compared with pretest value). Erythrocyte activity was not affected. Supplemental study. (Gee, 6/1/99).

[No record number] The Kettering Laboratory, 1965. Evaluation of safety in the use of Vapona® insecticide resin vaporizers. (University of Cincinnati, OH, 6/65) In part I, 10 volunteers were exposed to Vapona Resin Vaporizers (20% dichlorvos in resin) by either 30 minutes of handling the Vaporizers or by having a portion taped to the skin of the forearm for 30 minutes on 5 consecutive days. Plasma and erythrocyte cholinesterase activities were determined days -1, 3 and 5. There was no inhibition of cholinesterase activity as measured by the method of Michel. In part II, Vaporizers were installed in

homes at 1 per 1000 ft^3, the recommended rate. For two families, the Vaporizers were changed periodically over 6 months and for 6 families, for two months. Air samples taken from the homes of the first two families indicated a concentration of 0.087 - 0.097 μg/L. The concentration in the other 6 homes was not determined. Plasma and erythrocyte cholinesterase activities were measured over the course of the study. No inhibition of either cholinesterase was found. Supplemental study. (Gee, 5/27/99)

235-192 162858 Kirkland "Some aspects of acute inhalation pharmacology of dichlorvos in swine," Oct. 4, 1971. This is a paper describing some classic pharmacological parameters assessed for dichlorvos. No SB-950 review nor worksheet. Aldous, 2/15/05.

235 - 213 164803 Leary, J. S., W. R. Keane, C. Fontenot, E. F. Feichtmeir, D. Schultz, B. A. Koos, L. Hirsch, E. M. Lavor, C. C. Roan and C. H. Hine, 1974. Safety evaluation in the home of polyvinyl chloride resin strip containing dichlorvos (DDVP). (Shell Chemical Co., in: Arch. Environ. Health 29: 308 - 314 (1974)) Three studies were conducted in Arizona. I. Three families with 5 adults and 12 children, ages 1 - 20 years, were exposed to Vapona strips at 1/1000 ft^3 changed every three months for 1 year. The number of strips per home ranged from 7.5 to 18. Blood cholinesterases were determined using the micro-Michel method daily for 3 days prior to installation of the strips, once weekly for first month, every two weeks for two months, then monthly for 9 months. II. Twelve families with 22 adults and 32 children, ages 2 - 19 years, were exposed to Vapona strips at the above rate, 4 to 18 per home. The strips were changed monthly. Plasma and RBC cholinesterases were measured periodically. III. Vapona strips were installed at the same rate as in I and II except there were 1 strip per 500 ft^3 in the kitchen and dining areas. The test was conducted in winter with low ventilation. Air and food samples were taken. In addition, all volunteers were given physical exams and clinical profiles were taken. Records were kept of the time spent in each home. Results: In I, there was no difference between the plasma and red cell cholinesterase activities between exposed and control groups. In II, the plasma cholinesterase was slightly depressed (15 - 30%) in the exposed group during the winter. Results with RBC's were "erratic" and therefore, difficult to relate to exposure. In III, plasma activity did not differ significantly between exposed and control groups. The RBC activity, however, was slightly lower in the exposed group. The concentration in the air peaked at 0.12 - 0.13 mg/m^3 within several days of installation of the strips and declined to a plateau at 0.08 - 0.09 mg/m^3. Doubling the number of strips increased the air concentration to 0.16 mg/m^3 several days after installation. The air concentration declined to 0 within 17 days after removal of the strips. In food, the maximum of 0.11 to 0.12 ppm was found day 2 - 16. No effects on health were reported. Supplemental study. (Gee, 6/1/99).

235-0186 162850 Manley, A., "New evidence regarding dichlorvos carcinogenicity classification," 12/19/95. This record includes correspondence with U.S. EPA on dichlorvos issues, and includes within this record the previously reviewed record (235-0164 141583: Brown, T. T "Staging of Mononuclear Cell Leukemia in Male Rats From Toxicology and Carcinogenesis study of Dichlorvos in F344/N Rats"), previously examined by M. Silva and included in this Summary of Toxicology Data. This record was reviewed by T. Formoli of DPR Worker Health and Safety Branch. IN THE FRONT OF THIS VOLUME IS A SPREADSHEET, INDICATING THAT WORKER HEALTH AND SAFETY BRANCH HAS EXAMINED ALL VOLUMES FROM 235-0186 THROUGH 235-209.

00235-177 154698 Manley, A., "Metrifonate (MTF)/Dichlorvos (DDVP): Position Document on Long Term Administration in Humans," (Amvac Chemical Corp., Los Angeles, CA. Report# AM/001, 5/19/97). Metrifonate (MTF, transformed non-enzymatically to Dichlorvos or DDVP) was administered in multiple studies (longest duration was six years) to patients with Alzheimer's disease (AD). For example, acute doses of 7.5 mg/kg (Study I) or 2.5, 5.0 and 7.5 mg/kg (Study II) were administered to patients in a pharmacokinetic study. The half-life of MTF in plasma was 2.1 hours in Study I and 2.3 hours in Study II. Plasma ChE inhibition peaked at 78.5% at 15 min., while the maximum RBC ChE

inhibition seen at 1 hour was 61.0% in Study I. Other studies were longer in duration; a six-month study consisted of patients dosed initially with MTF to induce 50-70% RBC ChE inhibition within one week (2 mg/kg/day for five days, 0.95 mg/kg on the sixth day followed by 2.9 mg/kg/week for the remainder of the study). In the six-month double-blind treatment phase 23 patients received MTF and 24 received placebo. Mean RBC ChE inhibition was 62.2 ± 9.1% with a range of 44-77% (plasma ChE inhibition was similar). Although a total of 14 "adverse events" were reported for MTF treated patients (e.g., diarrhea, dizziness, vomiting and headache), all were rated as mild and transient and did not require adjustment of dose. In dose extrapolation experiments, it was shown that at MTF doses of about 0.5 mg/kg/day and below and DDVP doses of 0.25 mg/kg/day and below do not result in clinically significant levels of RBC ChE inhibition in humans. A pharmacodynamic model was used to predict MTF dosing in humans in order to achieve the desired steady-state levels of RBC ChE inhibition. This model indicated that a minimum daily dose of 0.6 mg/kg MTF will be necessary to maintain a level of 30%RBC ChE inhibition in humans. Supplemental Data. Kellner, 9/10/97.

[No record number] Mathis, W., A. St. Cloud, M. Eyraud, S. Miller and J. Hamon, 1963. Initial field studies in Upper Volta with dichlorvos residual fumigant as a malaria eradication technique. (Public Health Service, Savannah, GA, in: Bull. World Health Org. 29: 237 - 241) This was an efficacy study on the mortality of *Aedes aegypti* as function of exposure to dichlorvos during weeks 5 - 12 after installation of dispensers. No mammalian toxicological data although presumably the houses were occupied. Supplemental study. (Gee, 5/26/99).

[No record number] Quarterman, K. D., M. Lotte and H. F. Schoof, 1963. Initial field studies in Upper Volta with dichlorvos residual fumigant as a malaria eradication technique. (Public Health Service, Savannah, GA, in: Bull. World Health Org. 29: 231 - 235) This was an efficacy study on the mortality of mosquitos as function of exposure to dichlorvos for 3 to 5 months after installation of dispensers. No mammalian toxicological data. Supplemental study. (Gee, 5/26/99).

[No record number] Rasmussen, W. A., J. A. Jensen, W. J. Stein and W. J. Hayes, Jr., 1963. Toxicological studies of DDVP for disinfection of aircraft. (Public Health Service, Atlanta, GA, in: Aerospace Medicine 34: 593 - 600) Male volunteers, 15 per group, were exposed to dichlorvos in two phases. Phase I: 0 to 6 doses per night of 30-minute duration over 14 days with a total of 39 exposures ranging from 0.14 to 0.33 μg/L. Phase II: 8 30-minute exposures per night, 4 consecutive nights per week for 3 weeks increased to 10 30-minute exposures per night. For the first 10 weeks, the doses were 0.15 to 0.25 μg/L and increased to 0.40 to 0.55 μg/L, 10 doses per night, 4 days per week for 2 weeks. Parameters measured included plasma and erythrocyte cholinesterase activity, reaction time, a number of visual properties, and physical exams. RESULTS: Phase I: No difference in cholinesterase activity between control and exposed groups. Phase II: No effect was found on erythrocyte cholinesterase but plasma cholinesterase was depressed in the exposed group, occasionally reaching statistical significance. When the dose was increased, the plasma cholinesterase was further depressed but once exposure was discontinued, the activity returned to control level in 2.5 weeks. No significant changes in other parameters measured were reported as due to exposure to dichlorvos. The conclusion was that exposure to 0.15 to 0.25 μg/L caused no measurable changes in plasma or RBC cholinesterase activity, vision, airway resistance or reaction time. Supplemental study. (Gee, 5/27/99)

235-0212 164802 Richardson, R. J., Chair of Expert Panel organized by SRA International, Inc., "An Evaluation of the Significance of Dichlorvos Induced Alterations of Cholinesterase Levels in Biological Systems: Final Report of the Expert Panel," 11/13/98. The expert panel presented arguments that dichlorvos hazard assessment should consider human data, that metabolic disposition of dichlorvos should be considered with respect to reversibility of acetylcholinesterase (AChE) inhibition, and that observable symptoms rather than cholinesterase inhibition should be the primary considerations in assessment. Dichlorvos has a short plasma half-life, and its spontaneous reactivation time from bound

AChE is about 4.5 times more rapid than the aging of dichlorvos bound to AChE. As a result, occasional acute exposures, even if sufficient to elicit measurable AChE inhibition, would not be expected to cause cumulative effects. For this and other reasons, the Panel suggested that the U.S. EPA endpoints based on ChE inhibition in animal studies were unnecessarily conservative. Aldous, 2/10/05. No DPR worksheet.

[No record number] Rider, J. A., 1967. Determination of the minimal incipient toxicity of dichlorvos in humans. (Shell Chemical Company, by Gastrointestinal Research Laboratory, Franklin Hospital, San Francisco, 10/67) Male volunteers were given total doses of 1.0, 1.5, 2.0 or 2.5 mg/day in capsules, with one capsule given at 8 a.m. and one at 3 p.m. for 28 days. Further testing was done at 1.5 mg/day for 60 days followed by cholinesterase measurement for 74 days. Both plasma and erythrocyte cholinesterase activity were determined using the method of Michel. There were no significant effects on cholinesterase at 1.0 mg/day. At 1.5 mg/day [estimated as 0.02 mg/kg/day assuming 70 kg body weight], there was a maximum decrease of 15% in plasma cholinesterase the second day after cessation of dosing with no effect on RBC cholinesterase. At 2.0 mg/day, plasma cholinesterase was depressed beginning the second week of dosing with a maximum of 29% the second day after dosing ceased with no effect on RBC cholinesterase. At 2.5 mg/day [0.04 mg/kg/day], plasma cholinesterase was depressed during the second week with dosing stopped when it reached 30% depression after 20 days. The activity recovered to 99% of control in 15 days. With prolonged dosing at 1.5 mg/day for 60 days, the dose being selected as just below the level of minimal incipient toxicity, plasma cholinesterase was depressed during the second week of dosing and continued during the 60-day period reaching a maximum of 41%. Controls also showed some depression in plasma cholinesterase during the second half of the dosing period so that when the activity of the dichlorvos- treated group was adjusted, the maximum decrease was 27%. Following cessation of dosing, the plasma cholinesterase activity returned to within the range of pre-dosing levels within 2 weeks. Erythrocyte activity showed little effect with any of the exposure regimens. Using a criterion of 20 to 25% depression in plasma or erythrocyte cholinesterase activity as "minimal incipient toxicity", 1.5 mg/day [0.02 mg/kg/day] would be just below the dose of dichlorvos giving that effect. Supplemental study. (Gee, 5/27/99)

[No record number.] " Effects of dichlorvos (DDVP) inhalation on the activity of acetylcholinesterase in the bronchial tissue of rats" (Schmidt, G., M. Schmidt, M. Nenner and F. Vetterlein, Institut fur Pharmakologie und Toxikologie der Universität Göttingen, FRG), published in: *Arch. Toxicol.* 42: 191 - 198 (1979), Male Sprague-Dawley rats (180 - 220 g) were exposed to DDVP from Vapona strips for 3, 7 or 14 days. The DDVP atmosphere was generated by cutting commercial strips into equal portions of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32 or 1:64. Measured concentrations ranged from 0.83 to 56.64 µg/l in air as determined by the degree of inhibition of acetylcholinesterase from bovine erythrocytes compared with known concentrations of DDVP. At the end of the exposure period, rats were sacrificed and bronchial tissue isolated. The acetylcholinesterase activity of homogenates of bronchial tissue and of erythrocytes was determined with acetylcholine iodide. Histochemical detection of acetylcholinesterase was based on the thiolacetic acid method with the liberation of hydrogen sulfide and PbS precipitate formation in tissue. The reported NOEL for ACHE inhibition in bronchial homogenate was 0.2 µg/l, the reported concentration of DDVP from Vapona strip in a normally ventilated room. At 0.82 and 1.8 µg/l, the ACHE was inhibited to 63 and 52% of control. The NOEL for erythrocyte inhibition was 1.8 µg/l. No animals exposed to DDVP showed histochemical reaction indicating enzyme activity. Therefore, the NOEL for that assay was less than 0.2 µg/l. No clinical signs or pulmonary function data were included in the report. The toxicological significance of the findings could not be evaluated. The authors discussed the results in terms of a possible role in pathological conditions. Supplemental study. (Gee, 11/8/99)

[No record number] Slomka, M. B. and C. H. Hine, 1981. Clinical pharmacology of dichlorvos. (Shell, published in: Acta pharmacol. et toxicol. 4 (suppl. V): 105 - 108) Purified dichlorvos (97%) was incorporated into polyvinyl chloride resin pellets which were encapsulated into gelatin capsules for

dose administration. Male volunteers were given either a single dose, dosing for 7 days or dosing with an increasing dosage regimen each week with a maximum of two increases. Plasma and RBC cholinesterase activities were measured by the potentiometric titration method of Michel with the maximum depression at 24 hours or 48 hours after treatment reported. Single dose: 0, 0.1-1, 2-3, 4-6, 7-9, 10-12, 13-16, 17-20, 21-26 and 32 mg/kg dichlorvos. There were varying numbers of subjects per group. Both plasma and RBC cholinesterase activities were depressed in a dose-related manner. Plasma cholinesterase was more affected than RBC with a maximum depression of approximately 80% at 6 mg/kg, expressed as percent of pretreatment values. Less than 50% depression of RBC cholinesterase activity was found at 4 times that dose. Multiple doses: 0, 1, 2, 4, 8, 16 and 32 mg/kg for 7 days oral dosing except at 8 and 32 mg/kg which were of shorter duration due to cholinesterase depression. At 1 mg/kg, plasma was depressed 65 - 80%, RBC depressed 5 - 30%. At 2 mg/kg, plasma was depressed 75 - 85% and RBC, 25 - 45%. Side effects were reported in all groups including controls and involved the gastrointestinal tract or central nervous system. No data but there was a statement that no changes were noted in clinical or other laboratory examinations. Supplemental study. (Gee, 5/27/99)

235-0190 162855 Smith, C. A., J. H. Driver, and M. E. Ginevan, "Dichlorvos (DDVP): dietary cancer and non-cancer risk assessments for supported uses," 12/20/95. This record focuses on human dietary risk assessment. No SB-950 review is relevant. Aldous, 2/15/05.

[No record number] Stein, W. J., S. Miller and L. E. Fetzer, Jr., 1966. Studies with dichlorvos residual fumigant as a malaria eradication technique in Haiti. III. Toxicological studies. (Public Health Service, in: Am. J. Tropical Med. Hyg. 15: 672 - 675) Three groups of workers were studied: 1) twelve applicators who removed and installed new dispensers in houses, 2) two supervisors and 3) four laborers who changed the old for new dispensers. A medical history, physical examination and plasma and erythrocyte cholinesterase activities were obtained. The workers were observed for as long as three weeks with cholinesterase determined twice a week for applicators and three times per week for laborers. Air samples were taken in the shed used by the laborers at several locations during the study. There was no affect on RBC cholinesterase in any group. Plasma cholinesterase was depressed in both the applicators (still within normal limits after 2.5 weeks of exposure) and laborers (greater depression than applicators with the maximum depression reached in about a week with no further change in the final two weeks of observation. Data were presented graphically as the mean cholinesterase activity (ΔpH/hr) over the test period. The concentration of dichlorvos in the air of the shed varied with location and activity, being as high as 2.13 μg/L in the center of the work area at the end of a workday. On the following day, concentrations ranged from 0.29 to 1.18 μg/L, depending on location of the sampling. No illnesses were attributed to dichlorvos exposure. Because of the variation in the air samples, an accurate determination of worker exposure was not feasible. Supplemental study. (Gee, 5/27/99)

235-217 165876 Stevenson, D. E. And D. Blair, 1969. "A preliminary report on the inhalation toxicity of high concentrations of dichlorvos." (Shell Research Limited, Tunstall Laboratory, London, TLGR.0024.69, Project 507521, 5/27/69) Rats (CFE), mice (CF No. 1) and guinea pigs were exposed to DDVP and dichloroacetaldehyde (DCA) by inhalation. DCA is a hydrolysis product of DDVP. The concentration of DDVP in the chamber air was analyzed by gas chromatography. DCA was determined by a colorimetric method. A series of 10 experiments were conducted with exposure lasting 6 - 7 hours and the number of exposures ranging from 1 to 5. In the first four experiments with mice and rats, the authors state that the results were inconsistent in terms of mortality, the only specific endpoint given in Table 1. Doses were 40 to 80 μg/l DDVP with DCA content undetermined in experiments 1 - 3. For example, in Experiment. 1, 0/8 mice died, but had signs of toxicity(not described), at 80 μg/l, 5 exposures of 6 - 7 hours. In experiment. 2, 1/8 mice died after 4 exposures at 40 μg/l. In experiment. 3, 16/16 mice died at 50 - 80 μg/l, 4 exposures. The remark column contains the statement "1-2 days" without explanation as to the meaning. In experiment 4, 8/8 mice died at 80 μg/l after a single exposure. The authors concluded that the length of time the apparatus was in use and possibly the relative humidity,

which could influence the hydrolysis to DCA, caused the variability in results. Relative humidity was given as 20 - 30% in experiments1 - 4. Death in animals was stated to be preceded by clinical signs (including tremors, salivation/lachrymation, muscle paralysis, prostration). Rats under the same conditions were less susceptible to mortality. Guinea pigs were even less affected in experiment. 1, the only one in which they were included. In experiments 5 - 10, the DCA content was monitored and the relative humidity controlled. In mice at 80 - 134 μg/l DDVP (experiments. 5 and 6), there was mortality, although not 100%, being 4/12 females and 31/128, both sexes. At 41 to 80 μg/l (length of exposure to any given concentration not stated), no mortality occurred in mice receiving 4 or 5 exposures. The text states there were no clinical symptoms. The reason for the seemingly sharp difference in mortality with mice around 80 μg/l was not addressed. No mortality occurred in male or female mice exposed to 70, 135 or 130 μg/l DCA in a single exposure of 7 hours. Guinea pigs suffered no reported effects at 90 - 122 μg/l in 4 exposures of 7 hours. Rats showed an intermediate response. The authors concluded that the "no-visual-effect" levels were 130 μg/l for guinea pigs and 50 μg/l for rats and mice. Cholinesterase activity was not determined. The authors speculated that there would be inhibition of brain and blood cholinesterase activity but gave no data or citation. Inhaled doses were calculated by the authors to be 0.22, 0.61 and 0.72 l/kg min for guinea pig, rat and mouse, respectively, at the same atmospheric concentration of DDVP [not stated]. If the retention fraction were the same for all three species, then the mouse was stated as receiving over 3 times the dose of the guinea pig. Deficiencies in the report include a range of concentrations over time rather than a single concentration so that actual exposure cannot be determined, no individual data for animal responses/mortality (the only endpoint quantitated), no clinical chemistry for cholinesterase inhibition. Because of these deficiencies, this report cannot be used to determine a NOEL for acute effects. The report does suggest that the order of increasing sensitivity was guinea pig, rat, mouse under the conditions of the study. Supplemental study. (Gee, 6/16/99)

[No record number] Stevenson, D. E. and D. Blair, 1977. The uptake of dichlorvos during long-term inhalation studies. (Shell Research Ltd., Tunstall Laboratory, in: Proc. Eur. Soc. Toxicol. 18: 215 - 217) A general discussion of methods which could be used to determine uptake of dichlorvos by routes other than inhalation during an inhalation study. No data. (Gee, 5/27/99)

235-174 153927 Stonard, M. D., "Dichlorvos (DDVP): Position Document on Cholinesterase Inhibition" (Zeneca, Inc. Central Toxicology Laboratory, Alderley Park, Cheshire, UK, Report# CTL/P/5440, 2/13/97). The purpose of the position document was to propose the following NOEL's for DDVP exposure: 1 mg/kg (acute exposure), 0.3 mg/kg/day (subchronic) and 0.1 mg/kg/day (chronic). Data from recent human studies performed at the Central Toxicology Laboratory (Alderley Park, UK), from animal studies using DDVP and from ongoing human studies using Metrifonate (a pro-drug of DDVP) were used to establish these levels; the author placed the most emphasis on symptomology during the human studies (especially the long-term metrifonate exposures). The human data, while compelling, did not address subtle neurological changes that could accompany acute or long-term ChE inhibition; these changes are revealed during acute and subchronic neurotoxicity screens; for example in an acute neurotoxicity study in rats, a NOEL of 0.5 mg/kg was established based on cholinergic clinical signs. Neurotoxicity and ChE inhibition data from studies in humans and laboratory animals were used by the reviewer to establish the following NOEL's for DDVP exposure: 0.5 mg/kg (acute), 0.1 mg/kg/day (subchronic) and 0.05 to 0.1 mg/kg/day (chronic). Supplemental. Kellner, 9/10/97.
235-0193 162859 Exact duplicate of 235-174 153927, above.

235 - 214 164804 Thorpe, E., A. B. Wilson, K. M. Dix and D. Blair, 1972 "Teratological studies with dichlorvos vapour in rabbits and rats" (Shell Research Ltd., Tunstall Laboratory, in: Arch. Toxikol. 30: 29 - 38 (1972)) This is a publication of the studies reviewed in 235-072, Record numbers 035427 (rabbit) and 035428 (rat) in 1985. Neither study was found acceptable. In brief: Rats and rabbits were exposed to Dichlorvos technical (>97%) by inhalation at nominal doses of 0, 0.25, 1.25 and 0.625

µg/L of air. Carworth Farm E strain of rats were exposed from day 1 to day 20 of pregnancy, 15 / dose group, for 23 hours daily, 7 days per week. Dutch rabbits, 20/group, were exposed as above from day 1 to 28 of gestation. Fetuses were examined externally and approximately half were given visceral exams and half, skeletal exams. Plasma, erythrocyte and brain cholinesterase was determined from "a selection of the adult females". A second study with rabbits was performed at 0, 2 and 4 µg/L due to significant losses at 6.25 µg/L. RESULTS: Rats: at the lower two doses, no observations were made but at the high dose, 6.25 µg/L, they appeared "less active". At 0.25 µg/L, no significant cholinesterase inhibition was found (plasma = 97% and RBC = 105% with brain 98% compared with controls). At 1.25 µg/L, plasma was 67% of control, RBC was 71% and brain, 72%; at 6.25 µg/L, plasma was 27%, RBC was 12% and brain was 17% of control. NOEL = 0.25 µg/L. There was no effect on fetal resorption, late fetal death, litter size or fetal weight. Rabbits: 16 or 20 at 6.25 µg/L died or were terminated because of toxicity with 9 of the losses occurring within 7 days of the chamber concentration reaching 8 µg/L [presumably in error]. In the second experiment, 6 rabbits at 4 µg/L died or were killed because of toxicity, again due to a spike in the concentration of dichlorvos above the nominal level. At 0.25 µg/L, plasma cholinesterase activity was 85%, RBC was 86% and brain was 90% of control values. At 1.25 µg/L, plasma activity was 65% of control, RBC was 32% and brain, 44%. At 6.25 µg/L, no data were recorded due to toxicity. NOEL = 0.25 µg/L. In rabbits, there were no effects at 0.25 or 1.25 µg/L but there was a slight depression in fetal weight at 4 µg/L (20.2 g versus 23.1 g in control). CONCLUSION: There was no evidence of a teratogenic effect with exposure to dichlorvos under the conditions of the study in either rat or rabbit. NOTE: The authors calculated that the low concentration of 0.25 µg/L, based on respiratory properties, would be 110 µg/kg in the rabbit and 300 µg/kg in the rat over 24 hours. This was compared with 6 µg/kg for humans (no details). Supplemental study. (Gee, 5/28/99)

235-0217 165877 (Duplicate of 164804, above).

235 - 042 911129 Tracy, R. L., J. G. Woodcock and S. Chodroff, 1960. "Toxicological aspects of 2,2-dichlorovinyl dimethyl phosphate (DDVP) in cows, horses, and white rats." (Norda Essential Oil and Chemical Co., in: J. Econ. Entomol. 53: 593 - 601 (1960)) Horses: Five horses were exposed in a stable to dichlorvos at concentrations varying from 0.24 to 1.48 µg/L of air for 22 days. The dichlorvos was sprinkled daily onto a concrete walkway between the rows of stall and the 4 ventilators and 2 windows were open. Erythrocyte cholinesterase activity was depressed early in the exposure but appeared to recover by day 11. Plasma cholinesterase was unaffected by exposure compared with control level in unexposed horses. Cows: Two cows with suckling calves were fed DDVP in increasing doses beginning at 0 ppm and increasing to 3000 ppm (27 mg/kg). No significant depression of RBC cholinesterase activity was found up to 200 ppm (1.8 mg/kg) in the cows or the suckling calves. At 500 ppm (4.5 mg/kg)m RBC activity was significantly depressed in the cows but not the calves. Further increase in doses did not further depress the RBC activity in the cows. Assay of plasma activity varied markedly and was thought unreliable. At the highest dose tested for 1 day, 3000 ppm (27 mg/kg), the single cow collapsed but recovered. Plasma assay of the milk, urine and feces of the cows using *M. domestica* (house flies) did not produce kills. Rats: Seven rats with litters 1 - 12 days of age were given DDVP by gavage at 0, 10, 20, 30 or 40 mg/kg with increasing doses per female. Doses up to 20 mg/kg in dam did not cause symptoms although RBC cholinesterase was depressed nearly 50%. At 30 mg/kg, animals exhibited shock symptoms with recovery. At 30 and 40 mg/kg, erythrocyte cholinesterase was severely inhibited but plasma activity were not. The weight gain of the litters was near controls. The plasma and RBC cholinesterase activities in the litters were within normal range. Tissue studies: Several tissues (liver, brain, muscle and small intestine) were assayed with *M. domestica* for the ability to inactivate DDVP. The liver appeared to be the organ for detoxification. Supplemental study. (Gee, 5/28/99)

[No record number] Uchiyama, M., T. Kawakami and H. Hiuga, 1967. Effect of Vapona/strips to human beings and the method of determination of DDVP concentration in the air. (Tohoku Univ.,

Japan, 11/67) Pediatric patients were exposed in a ward at 3 strips Vapona per 93 m^3. Serum cholinesterase and liver function were determined over approximately 90 days. There was no effect on cholinesterase. Twenty adult patients were exposed to Vapona strips at 4/120 m^3 for 72 days. No effect due to the DDVP was noted. Cholinesterase was determined using a colorimetric assay based on Hestrin's procedure with the results based on index figures from 42 healthy persons as 100. Although an analytical method for determining DDVP in air was described, the concentrations in the air of the wards over time was not presented. Mice: Groups of ten mice were exposed to DDVP at 10 and 100 times the "standard" dosage in inhalation chambers. Standard was defined as 1 strip/28 m^3. Plasma, erythrocyte and brain cholinesterase activities were determined over a period of 3 weeks. The control group = 100%. At the standard exposure rate (0.46 to 0.2 μg/L), no cholinesterase inhibition occurred. At 10X (DDVP at 1.54 to 1.7 μg/L), no inhibition of plasma activity occurred but RBC was 94.5% of control after 2 weeks and brain was approximately 93% of control after 2 or 3 weeks. At 100X (DDVP at 12.5 - 14.5 μg/L), plasma was 75% of control at week 3, RBC was 83.8% at 2 weeks and brain was 28.3, 21.4 and 37.0 at weeks 1, 2 and 3 respectively. Supplemental study. (Gee, 5/28/99)

[No record number] Ueda, K. and M. Nishimura, 1967. Effect of Vapona/strips to human beings. (Tokyo Dental College, unpublished data, 1967) In part one, 47 hospital patients were exposed to DDVP at 1 strip/22 m^3, stated to be the standard dose. There was no evidence of inhibition of plasma or erythrocyte cholinesterase measured by Michel method. In part 2, two male subjects were exposed either to doses at 5 or 10 times the usual rate, or 10 strips/51.5 m^3 and 17 strips/46.8 m^3. At 5 times, the air concentrations varied from 2.2 μg/L at 3 hours to 0.8 by 48 hours. At 17 times, the air concentration varied from 8.5 μg/L in the first 12 hours to 2.4 μg/L by 48 hours. At 8.5 μg/L after 12 hours of exposure, [the dose was estimated to be 533 μg/kg/day] the plasma cholinesterase was inhibited by 15 and 24%. In these same individuals, at 4.4 μg/L, 24 hours, [dose estimated at 477 μg/kg/day], plasma cholinesterase was inhibited 33 and 28%. Changes in erythrocyte activity was less than in plasma. The plasma activity recovered by 7 days after exposure discontinued. At the 5 times exposure, no significant inhibition occurred. Supplemental study. (Gee, 5/28/99)

[No record number] Vigliani, E. C., 1971. Exposure of newborn babies to Vapona® insecticide. (Institute of Occupational Health, Milan, Italy, in: Toxicol. Appl. Pharmacol. 19: 379-380 (1971), abstract.) Healthy babies, 22 per group, were exposed for the first 5 days of life in rooms with 1) 1 Vapona® strip/40 m^3, 18 hours/day or 2) 1 strip/30 m^3 but poorly ventilated. The time-weighted average in 1) was 0.05 mg/m^3 and in 2)0.152 and 0.159 mg/m^3. Plasma and red blood cell cholinesterase was determined at birth and at the end of the exposure. The abstract states that there was no effect on either. No data. Supplemental data. (Gee, 5/28/99)

[No record number] Walker, A. I. T., D. Blair, E. D. Stevenson and P. L. Chambers, 1972. An inhalation toxicity study with dichlorvos. (Shell Research Ltd. Tunstall Laboratory, in: Arch. Toxikol. 30: 1-7) The effect of dichlorvos, 20% in polyvinyl chloride strips with either of two plasticizers, was measured in dogs, rabbits and cats with emphasis on electroencephalographic (EEG) patterns as an indication of central nervous system activity. Formulation A plasticizer was expected to give twice the concentration of formulation B; however, both were similar under test conditions. With group 1, strip A, one strip was located per 1200 ft^3 for 8 weeks with no change. With group 2, strip B, one strip was located per 1200 ft^3 for 6 weeks and replaced weekly thereafter with strip A. The air concentration with strip A ranged from a high of 0.2 μg/L after installation, reached 0.1 μg/L in a few days and decreased to 0.05 μg/L over 50 days. With B strips, the high was close to 0.3 μg/L and took 30 to 35 days to reach 0.05 μg/L from 0.1 μg/L with an increase each time the strip was replaced. A limited number of animals per species and group were used due to implantation of the electrodes. Two male dogs and rabbits served as controls with 4 or 5 of both sexes exposed to group A strips and 2 or 3 to strip B. The number of cats was even more limited. The relationship of pre- and post-exposure activities for plasma and erythrocyte cholinesterases was determined, using a modified method from Michel. The

conclusion was that neither exposure produced any treatment effect. No changes were seen in the EEG recordings. The authors concluded that at concentrations higher than those achieved with normal DDVP strip use, no effects on general health, behavior, cholinesterase activity of EEG patterns were found in dogs, rabbits and cats. Supplemental study. (Gee, 5/28/99)

235-188 162852 Wilkinson, C. F., "Dichlorvos (DDVP): An analysis of the human data on dichlorvos in relation to occupational and residential risk assessment," 12/20/05. These risk assessment data are not relevant for SB-950 review. Aldous, 2/15/05.

[No record number] Witter, R. F., T. B. Gaines, J. G. Short, V. A. Sedlak and D. R. Maddock, 1961. Studies on the safety of DDVP for the disinfection of commercial aircraft. (Public Health Service, Savannah, GA, in: Bull. World Health Org. 24: 635 - 642) Seven men and 8 Rhesus monkeys were used in the study. Exposure regimens were designed to simulate disinsection of aircraft. Measurements included plasma and erythrocyte cholinesterase activities by the method of Michel and examination of the eyes for miosis. In the first exposure, the men were exposed for one or two hours on four consecutive days to a range of 0.26 - 0.88 μg/L (mean of 0.48 μg/L). In the second, they were exposed to a range of 0.09 to 3.5 μg/L (mean of 2.1 μg/L) for a total of 4 or 8 hours. Post-exposure blood samples were taken up to 7 days. At the lower range of exposure, there was no effect on cholinesterase. At the higher range, there was a "slight" decrease in plasma activity in 2 of 3 men exposed a total of 8 hours (data presented graphically only). No change was noted in RBC activity or in those exposed for 4 hours total. No miosis was found. The monkeys were divided into 4 groups and exposed for 2 hours on 4 consecutive days. Four ranges were used: 0.32 - 0.66 (mean of 0.48 μg/L), 1.2 - 3.2 (mean of 2.3 μg/L), 1.9 - 3.3 (mean of 2.6 μg/L) and 7.5 - 17.9 (mean of 12.9 μg/L). In the first three groups, results were stated to be negative or questionable for plasma cholinesterase. At the highest range, both monkeys had miosis which disappeared, a significant drop in red blood cell and plasma cholinesterase which lasted several weeks after testing was discontinued. The authors concluded that the threshold range for man was 0.09 to 3.5 μg/L compared to an effective concentration for insects of 0.15 to 0.25 μg/L DDVP. Supplemental study. (Gee, 5/28/99)

Appendix E. Calculations

1. Conversion of DDVP air concentration to mg/kg/day for laboratory animals adjusting for breathing rates[5] and hours exposed:

$$\text{DDVP in mg/m}^3 \text{ x animal breathing rate x hours exposed/24 hours x days exposed/7 days week} = \text{mg/kg/day}$$

Example: What is the $BMDL_{10}$ for RBC AChE inhibition from Blair *et al.*, 1974 expressed as mg/kg/day:

$$0.078 \text{ mg/m}^3 \text{ x } 0.96 \text{ m}^3\text{/kg/day x } 23 \text{ hours exposed/24 hours x } 7 \text{ days/7 days} = 0.072 \text{ mg/kg/day}$$

2. Calculation of human equivalent NOEL and reference concentration:

$$\text{DDVP in mg/m}^3 \text{ x human BR x hrs exposed/24 hrs} = \text{animal dose in mg/kg/day}$$

Example: What is the $[DDVP]_{air}$ for human adult exposure of 4 hours to receive an equivalent of 0.072 mg/kg/day as in the Blair rat study?

$$? \text{ mg/m}^3 \text{ x } 0.28 \text{ m}^3\text{/kg/day x } 4 \text{ hrs exposed/24 hrs} = 0.072 \text{ mg/kg/day}$$

$$\text{Human equivalent NOEL} = 1.54 \text{ mg/m}^3$$

Example: what is the $[DDVP]_{air}$ for human children exposure of 24 hours to receive an equivalent of 0.072 mg/kg/day as in the Blair rat study?

$$? \text{ mg/m}^3 \text{ x } 0.59 \text{ m}^3\text{/kg/day x } 24 \text{ hrs exposed/24 hrs} = 0.072 \text{ mg/kg/day}$$

$$\text{Human equivalent NOEL} = 0.12 \text{ mg/m}^3$$

3. Calculation of reference concentration:

$$\text{Human equivalent NOEL in mg/m}^3 \text{ x } 1/10_{\text{interspecies UF}} \text{ x } 1/10_{\text{intraspecies UF}} = \text{RfC}$$

Example: What is the reference concentration for human adult 4-hour chronic exposure?

$$1.54 \text{ mg/m}^3 \text{ x } 1/10_{\text{interspecies UF}} \text{ x } 1/10_{\text{intraspecies UF}} = 0.0154 \text{ mg/m}^3$$

4. Calculation of margin of exposure:

MOE= NOEL or BMDL / human exposure

[5] The DPR default breathing rates (m^3/kg/day) are: 0.96 (rat), 1.8 (mouse), 0.54 (rabbit), 0.39 (dog), 0.28 (human adult), and 0.59 (human child).

Appendix F. Comments and Response to Comments from the Office of Environmental Health Hazard Assessment

M E M O R A N D U M

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FROM: Anna M. Fan, Ph.D. Chief
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DATE: July 31, 2007

SUBJECT: COMMENTS ON DPR'S DRAFT RISK CHARACTERIZATION, THIRD ADDENDUM DOCUMENT FOR THE ACTIVE INGREDIENT DICHLORVOS (DDVP)

We have received for our review the Department of Pesticide Regulation's (DPR) draft risk characterization document, third addendum, for the pesticide active ingredient dichlorvos (also known as 2, 2-dichlorovinyl dimethyl phosphate or DDVP), dated June 20, 2007. The Office of Environmental Health Hazard Assessment (OEHHA) reviewed and commented on DPR's draft risk characterization document (RCD) for dichlorvos in 1994, but did not review the first and second addenda of the final RCD for dichlorvos.

OEHHA reviews risk assessments prepared by DPR under the general authority of the Health and Safety Code, section 59004, and also under the Food and Agricultural Code, section 13129, which gives OEHHA the authority to provide advice, consultation, and recommendations to DPR concerning the risks to human health associated with exposure to pesticide active ingredients.

Dichlorvos is used to control a wide variety of insects in agricultural, commercial, and industrial settings. It is also used in and around homes (i.e., as resin strips) and on pets. Dichlorvos is an organophosphate, its neurotoxicity related to cholinesterase activity inhibition is the main concern in human exposure. Our comments on the draft third addendum are presented below, divided into general and specific comments.

GENERAL COMMENTS

1. We agree with DPR's decision in using the cholinesterase activity inhibition data derived from animal toxicity studies (as indicated in the draft third addendum) instead of the acute human toxicity studies reported by Gledhil (1997a and b) (as used in the first addendum issued in 1997) for the evaluation of acute oral and dermal exposures. As pointed out in the draft addendum, the inappropriate sampling time of the human studies might have missed the peak effects and under-estimated the cholinesterase activity inhibition potential of dichlorvos. We also agree with DPR in the use of benchmark dose modeling for evaluating the dose-response relationship of the cholinesterase activity inhibition data. In general, benchmark dose modeling is superior to the LOEL/NOEL approach as it uses all the data points in the study and the result is less affected by the spacing of the doses. The ability of benchmark dose modeling to include the variability of the data in its estimation is another advantage.

2. At present, if one needs to understand the risk assessment of dichlorvos by DPR, one has to read four documents: the dichlorvos RCD (DPR, 1996), the first (DPR, 1997) and second addenda to the dichlorvos RCD (DPR, 1998) and the subject of this review – the draft third addendum to the dichlorvos RCD. All three addenda were used by DPR, when new toxicological and/or exposure data became available, to modify and update the original dichlorvos RCD of 1996. However, this method of revision has increased the complexity of the risk assessment and made it difficult to understand. For instance, the main focus of the draft third addendum is to nullify some of the changes made in the first addendum. We suggest DPR consider either updating the RCD document itself or consolidating all the changes into one single addendum.

3. OEHHA notes that using the new critical equivalent NOEL of 0.8 mg/kg-day, some of the margins of exposure (MOE) calculated for home fogger exposure scenarios are below the benchmark of 100. For the dermal route, the MOEs calculated for the adult and the child are 14 and 10, respectively. For the oral route, the MOE calculated for the child is 48.
4. The current draft third addendum focuses only on dichlorvos. We suggest DPR includes exposures to naled and trichlorfon in the evaluation since these two pesticides are metabolized or degraded to dichlorvos in food, water, or the environment.

SPECIFIC COMMENTS

1. Table 1. Some exposure scenarios (e.g., 1-30 days oral and short to intermediate term inhalation) were evaluated by the U.S. EPA (2006) but not by the DPR. The draft third addendum should explain the differences.

2. Table 7. The title of Table 7 does not match the footnote of the table.

3. Table 11. Based on the acute toxicity data reported by Milburn (2003), DPR found that at 1 hour post-dosing, both the brain and red blood cell (RBC) acetylcholinesterase (AChE) were inhibited by about 50% for both the pre-weaning and the young adult rats. However, we noted that at 3 hours post-dosing, the AChE inhibitions in the brain (51%) and RBC (52%) of preweaning rats were much higher than the inhibitions in the brain (38%) and RBC (34%) of young adult rats. This information suggests that the pre-weaning rats were less capable of recovering from the exposure to dichlorvos than the young adult rats.

4. Page 27, the first paragraph. A 10% inhibition of AChE activity was chosen as the reference point in the benchmark dose analyses. The draft third addendum should discuss the reason(s) for this decision.

5. Table 18. The region (i.e., cerebellum, cortex, hippocampus, or the remainder) of brain should be specified for the PND42+ rat data (Twomey, 2002) reported in the table.

6. Page 30, the third paragraph. There is a typo. It should be “Table 21" instead of “Table 2.”

Thank you for the opportunity to review this document. We hope that you find our comments useful. Should you have any questions regarding OEHHA’s review, please contact Dr. David Ting, Dr. Jolanta Bankowska, or me at 510-622-3170.

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DATE: September 8, 2008

SUBJECT: RESPONSE TO OEHHA'S COMMMENTS ON DRAFT RISK CHARACTERIZATION THIRD ADDENDUM DOCUMENT FOR THE ACTIVE INGREDIENT DICHLORVOS (DDVP)

I have reviewed the comments from the Office of Environmental Health Hazard Assessment (OEHHA) on my draft third addendum (June 20, 2007) to the Risk Characterization Document (RCD) on dichlorvos (DDVP). The main purpose of this addendum was to revise the critical No-Observed-Effect Level (NOEL) for acute oral and dermal exposure. In the comments, OEHHA agreed with the proposed change in the NOEL. The following are responses to other comments:

General Comments #4: OEHHA suggested including naled and trichlorfon exposures in the addendum.

Response: It is unlikely that workers would be working with and be exposed to DDVP, naled or trichlorfon at the same time. DDVP is used in structures, animal facilities, and warehouses. Naled is applied on agricultural commodities and for mosquito control. Trichlorfon is used on turf. For the general population, there is a potential for exposure to DDVP from these three compounds in the diet. Therefore, the Addendum will include a discussion and risk assessment using the USEPA recently conducted dietary exposure assessment

Specific Comment #1: OEHHA suggested an explanation on why some exposure scenarios were evaluated by the USEPA but not by DPR.

Response: The Addendum will adopt the scenarios and exposures in the USEPA IRED, and evaluate the exposures based on DPR approaches.

Specific Comment #2: The title of Table 7 does not match the footnote of the table.

Response: The title (Table 5 in final document) will be clarified.

Specific Comment #3: Based the data in Table 11, OEHHA generalized the inhibition as 50% for both pre-weaning and adult rats at 1 hour after exposure, and compared this value with those obtained at 3 hours. They concluded that pre-weaning rats were less capable of recovering from the exposure to dichlorvos than the young adult rats.

Response: This table (Table 9 in final document), the 1 and 3 hour data showed a slower

initial recovery, rather than "less capable of recovering" for preweaning rats compared to young adult rats. This slower recovery was probably due to the greater 1 hour AChE inhibition of the preweaning rats (59% and 53%) compared to the young adult rats (47% and 46%) after DDVP exposure. Subsequent time points showed little difference between these two groups at 24 hours, and complete recovery by 72 hours in the preweaning rats.

Table 9. Time course of acetylcholinesterase inhibition in preweaning and young adult rats given a single oral dose of DDVP.[a]

Hours After Dosing	PND 15 Treatment-Preweaning		PND 42 Treatment -Young Adults	
	Control	15 mg/kg	Control	15 mg/kg
Brain Cholinesterase (IU/g)				
1	6.46±0.97	2.67±0.52** (59%)	7.48±1.82	3.52±0.45** (47%)
3	6.34±1.03	3.08±0.60** (51%)	7.40±1.44	4.59±0.78* (38%)
8	5.58±0.45	4.65±0.88 (17%)	3.05±0.42[b]	3.14±1.15
24	5.19±0.05	4.55±0.29* (12%)	5.42±0.23	4.73±0.29* (13%)
72	5.39±0.98	5.59±0.95	5.06±0.41	4.69±0.38 (7%)
RBC Cholinesterase (U/l)				
1	3708±451	1745±140** (53%)	3064±200	1649±176** (46%)
3	4538±1466	2190±264* (52%)	2949±102	1934±45** (34%)
8	3712±772	2699±555* (27%)	2441±587	2017±208 (17%)
24	4339±433	3677±365 (15%)	3065±200	2712±272* (12%)
72	3344±218	3338±541 (2%)	2974±108	2541±149** (15%)

a/ Data from Milburn, 2003c. Statistical analysis performed by investigators with *, ** significant at $p < 0.05$ and $p < 0.01$, respectively. Cholinesterase activity as percent inhibition is in parenthesis.

b/ Value lower than all controls.

Specific Comment #4: OEHHA suggested a discussion on why a 10% benchmark response was used.

Response: It will be added in the discussion.

Specific Comment #5: OEHHA suggested adding brain regions to the Table 18.

Response: It will be added (Table 16 in final document).

Specific Comment #6: OEHHA suggested that Table 21 should be cited instead of Table 2.

Response: This section has been revised.