UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND TOXIC
SUBSTANCES

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MEMORANDUM

DATE: March 20, 2006

SUBJECT: **Human Studies Review Board:** Final Weight of Evidence Comparison of Human and Animal Toxicology Studies and Endpoints for DDVP Human Health Risk Assessment and Discussion of Interspecies Extrapolation in the Organophosphate Cumulative Risk Assessment.

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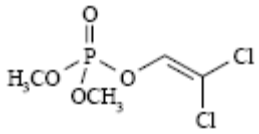
This weight of evidence (WOE) document for dichlorvos (DDVP) describes the Agency's rationale for selecting a repeated dose human toxicity study (MRID 44248801) to assess risks from short- and intermediate term residential and occupational exposure to DDVP. In addition, the WOE document also discusses why an acute human toxicity study (MRID 44248802) was not considered appropriate to assess risks from a single exposure to DDVP. The WOE document also discusses the Agency's conclusions regarding the usefulness of this study in the cumulative risk assessment for the OPs.

Background. Dichlorvos is an organophosphate insecticide that is toxic to mammals, including humans, through inhibition of the acetylcholinesterase(s) of the peripheral and/or central nervous system. The technical registrant for dichlorvos has submitted a number of toxicity studies involving direct dosing of humans to support their contention that humans are no more or less sensitive to the effects of dichlorvos than rats, dogs or other experimental animals. In this document, HED scientists compare the strengths and weaknesses of the human and animal toxicity studies and present how the human studies compare with the animal studies, i.e., are the human data consistent with animal data in terms of types of effects and effect levels or are there notable differences between animals and humans.

This document focuses on two human studies in which humans were intentionally dosed with dichlorvos; a single dose oral study (MRID 44248802) and a repeated dose oral study (MRID 44248801). The human data are compared with animal data from similar studies, and recommendations for endpoint selection are made based on the most appropriate studies and uncertainty factors. All of the studies are discussed in summary form and then the weight of evidence discussion of endpoint selection follows.

Chemical and Hazard Characterization of Dichlorvos

Dichlorvos is a phosphate triester with a molecular formula of $C_4H_7O_4PCl_2$ and a molecular weight of 221. It is a liquid at room temperature with a relatively high vapor pressure of 0.032 mm (30 C).



The high vapor pressure of dichlorvos is the basis for its use as a fumigant for processed food commodities, food warehouses and food-handling establishments. Because of its volatility, dichlorvos is also incorporated into resin strips for use at many sites including family homes.

Like most other cholinesterase-inhibiting phosphotriesters, dichlorvos is asymmetrical with respect to the ester substituents, with two relatively difficult to hydrolyze methyl groups and a dichlorovinyl- “leaving group” which is more readily hydrolyzed and is the group displaced when dichlorvos reacts at the active site of cholinesterases. Many sulfur-containing organophosphate cholinesterase inhibitors require metabolic activation to convert an unsubstituted phosphorous-sulfur (P=S) group to a phosphorus-oxygen oxon (P=O) group before inhibition of cholinesterase can occur; however, dichlorvos already exists in the oxon form and needs no activation to inhibit cholinesterases.

Dichlorvos is well absorbed by all routes of exposure and extensively metabolized with excretion of metabolic products occurring mostly in the urine and through exhalation as CO_2. Absorbed dichlorvos is initially inactivated by an esterase found in plasma and liver. The esterase catalyzes the hydrolysis of dichlorvos to dimethyl phosphate and dichlorovinyl alcohol which

spontaneously rearranges to 2,2,-dichloroacetaldehyde which is then metabolized further. Dichlorvos may also be inactivated by a glutathione-dependent reaction to form desmethyl dichlorvos. The half-life of dichlorvos in the blood is very short, 15 minutes or less.

Dichlorvos inhibits plasma, erythrocyte, and brain cholinesterase in a variety of species, but does not cause organophosphate-induced delayed neurotoxicity (OPIDN) in the hen (MRID 43433501). In acute and 90-day neurotoxicity studies in rats (MRIDs 42497901, 41004101, there was no neuropathology associated with changes in FOB and motor activity. Subchronic and chronic oral exposures in rats and dogs (MRIDs 41004701, 41593101) as well as chronic inhalation exposure in rats (MRIDs 00057695, 00532569) resulted in significant decreases in plasma, red blood cell and/or brain cholinesterase activity. Animal toxicity studies do not show evidence of gender susceptibility. Repeated, oral subchronic exposure in male humans was associated with statistically and biologically significant decreases in red blood cell cholinesterase activity (MRID 44248801).

There was no evidence of increased susceptibility following *in utero* exposure to rats and rabbits as well as pre/post natal exposure to rats in developmental and reproduction studies (MRIDs 41802401, 41951501, 42483901). However, increased sensitivity to dichlorvos-induced inhibition of brain cholinesterase activity was observed in repeated gavage studies in preweaning rats in comparison to young adult rats (MRID 46153304). These findings necessitated that a special Food Quality Protection Act (FQPA) 3x safety factor be retained for assessment of risks (other than risks from acute exposure) where the endpoint is based on RBC cholinesterase inhibition as a result of repeated exposure. A factor of 3x is considered appropriate since the differences in brain cholinesterase inhibition were minimal. The factor of 3x is not needed for assessing acute risks, since there was no increased sensitivity in brain cholinesterase activity in preweaning rats in comparison to young adults following a single gavage dose of dichlorvos.

Specific Toxicity Studies

Single Dose Oral Studies:

A. Human

In a single dose human study with DDVP (MRID 44248802), the NOAEL for RBC cholinesterase depression is 1.0 mg/kg bw based on the absence of statistically significant ChE depression in 6 fasted young healthy male volunteers administered a 70 mg oral dose of DDVP. In this study, the first cholinesterase measurement was recorded 24 hours after dosing. In another study (MRID 46153303) on the measurement of RBC and brain ChE activity in pre-weaning and adult female rats treated with a single dose of 15 mg/kg dichlorvos, time-course data demonstrate that the time of peak effect for both RBC and brain ChE measurements is 1-3 hours post-dosing and that by 24 hours post-dosing, RBC cholinesterase activity has recovered to levels similar to the controls (MRID 46153303). Therefore, the absence of biologically significant RBC ChE depression in the human study may be due to the absence of blood

sampling at the time of peak effect (1-3 hours), since in the human study, the first measurement did not occur until 24 hours after dosing.

B. Animals

Single dose comparative cholinesterase studies in preweaning and adult rats which measured both RBC and brain ChE depression at 1 hour following oral exposure were analyzed by a Benchmark Dose (BMD) procedure (MRIDs 45805703, 45842301). The BMDS (Benchmark Dose Software version 1.3.2) model was used to derive the BMD_{10}, the estimated dose that results in 10% inhibition of cholinesterase, and the $BMDL_{10}$, the lower 95% confidence interval on the BMD_{10}, for the cholinesterase data evaluated. For this analysis, the continuous polynomial model was used with the default option of relative deviation for the benchmark response (BMR) type. A BMR factor of 0.1 was the basis for BMD_{10} and $BMDL_{10}$ derivation.

A $BMDL_{10}$ of 0.8 mg/kg (BMD = 1.6 mg/kg) based on female brain ChE depression was selected as the lowest value of all the studies available which were analyzed. Consistent with EPA's Draft Technical Benchmark Dose Guidance (2000), the BMDL, not the BMD, is used to extrapolate risk. BMD analysis of studies with pup and adult ChE depression results did not demonstrate any substantial age-related numerical differences in BMDL values (all values were approximately 1 mg/kg) for either RBC or brain cholinesterase.

Repeated Dose Oral Studies

A. Human

In a single blind oral study (MRID 44248801), 6 fasted male volunteers were administered 7 mg of DDVP in corn oil (equivalent to approximately 0.1 mg/kg/d) via capsule daily for 21 days. Three control subjects received corn oil as a placebo. Baseline values for RBC cholinesterase activity for each study participant were determined. After dosing started, RBC cholinesterase activity was monitored on days 2, 4, 7, 9, 11, 14, 16, and 18, then on day 25 or 28 post dosing. No toxicity attributable to administration of DDVP was reported. Mean RBC cholinesterase activity was statistically significantly reduced in treated subjects on days 7, 11, 14, 16, and 18. These values were 8, 10, 14, 14, and 16 percent below the pre-dose mean. Although the percent mean depression was less than 20%, the blood samples were not taken until just before the next day's dose, at the point of maximum recovery. Under the conditions of the study, a LOAEL for RBC cholinesterase inhibition was established at 0.1 mg/kg/d based on the consistent, statistically significant ChE depression over time, although ChE depression during the study was less than 20%. A NOAEL was not established.

The repeated dose human study has been criticized for a number of reasons including:

1) Too few subjects. There were six treated adult males and three adult males served as placebo controls. All of the treated males responded to some extent to repeated dosing of dichlorvos with a mean response of 16%. If there had been no response, then the argument that there were

insufficient subjects might have more merit, however, the Agency has determined that the administered dose is a LOAEL.

2) Use of males only. All subjects were adult males. Animal toxicity studies do not show evidence of gender susceptibility.

3) Administration of only a single dose level. A single dose level of 7 mg per person per day was administered for 21 days. This dose resulted in sufficient RBC cholinesterase inhibition that we consider the response to be a LOAEL. If there had been no response, interpretation of the results would have been problematic.

4) Blood sampling did not occur until 24 hours after dosing. This is considered a critical deficiency for the single dose study; however, after 21-days there is a clear response which the Agency considers a LOAEL. If blood had been sampled at 1-3 hours after dosing, RBC cholinesterase inhibition may have been somewhat greater.

B. Animal

Comparative cholinesterase (7-day rat). In a comparative cholinesterase inhibition study (MRID 46153304), dichlorvos was administered by gavage in seven daily doses of 0, 0.1, 7.5, or 15 mg/kg/day to groups of 5 rats/sex beginning on either PND 12 (pre-weaning rats) or 42 (young adults) and animals were sacrificed one hour after the last dose. RBC and brain ChE activities were measured in all animals in each study. In pre-weaning rats, tremors were observed in 5/5 males and 5/5 females at 15 mg/kg/day on 3-5 days of the dosing interval. In young adult rats at 15 mg/kg/day, tremors were observed in 3/5 males and 5/5 females on one to four days of the dosing interval. In addition, tremors were seen in one adult male after the last dose of 7.5 mg/kg/day. No clinical signs of toxicity were observed in the remaining groups. Dose-related inhibition of RBC and brain ChE activities was apparent after repeated dosing in both adult and pre-weaning rats. Biologically significant inhibition of RBC enzyme activity (>50%) occurred at doses of 7.5 and 15 mg/kg/day in both sexes of adults and pre-weaning and at the low dose for adult animals (11-17%). Brain enzyme activity was statistically and biologically inhibited in both sexes at doses of 7.5 and 15 mg/kg/day for adults (>50%) and at all doses for pups (>20%). The LOAEL for inhibition of RBC cholinesterase was 0.1 mg/kg/day and a NOAEL was not identified based on findings in young adults. The LOAEL for inhibition of brain cholinesterase was 0.1 mg/kg/day and a NOAEL was not identified based on findings in pre-weaning pups.

Subchronic neurotoxicity (90-day rat). In a subchronic oral neurotoxicity study (MRID 42958101), dichlorvos was administered in deionized water to 15 Sprague-Dawley rats/sex/group at gavage doses of 0, 0.1, 7.5, or 15.0 mg/kg/day for 90 days. Within each dose group, 10 rats/sex were allocated for brain cholinesterase determination and 5 rats/sex were allocated for neuropathology evaluation. Additionally, blood samples were collected for cholinesterase measurements prestudy and on study weeks 3, 7, and 13 . Five rats/sex/dose from the cholinesterase group and 5/sex/dose from the neuropathology group were evaluated with the Functional Observational Battery (FOB) and motor activity tests pretest and on study weeks 3, 7, and 12. Body weight and food consumption were measured weekly.

There was no treatment-related mortality. Mean body weight in high-dose females was consistently lower than the control (11-21%) throughout the study. No body weight effects were observed in any other animals, and there was no treatment-related effect on food consumption. Tremors, salivation, exophthalmos, lacrimation, and clear material on the forelimbs were observed in high-dose males and females approximately 15 minutes post-dosing. Rales, chromodacryorrhea, and red/yellow/orange material around the nose and mouth were also seen in high-dose rats. Tremors were observed in three mid-dose males and nine mid-dose females. Generally, the clinical signs occurred during the third week of treatment in the mid-dose animals and as early as the first week of dosing and throughout the study in the high-dose rats. Cholinesterase activity was decreased in mid- and high-dose male and female rats as follows: plasma 30-58%; erythrocyte 8-35%; brainstem and brain cortex 10-16%. There were no treatment-related effects in the FOB or motor activity tests. No treatment-related neurohistopathological lesions and no apparent changes in brain weight or size were observed. Based on decreased cholinesterase activity and clinical cholinergic signs, the LOAEL for dichlorvos is 7.50 mg/kg and the NOAEL is 0.1 mg/kg.

Chronic (One-year dog). In a chronic feeding study (MRID 41593101), groups of 4/sex/dose beagle dogs were administered dichlorvos by capsule for 52 weeks at dose levels of 0, 0.1, 1.0 and 3.0 mg/kg/day. The 0.1 mg/kg/day dose was lowered to 0.05 mg/kg/day on day 22 due to the inhibition of plasma cholinesterase noted after 12 days (plasma cholinesterase was decreased in males (21.1%) and females (25.7%) at week 2 in the 0.1 mg/kg/day group). At time points after week 2, plasma cholinesterase activity was only significantly reduced in males (39.1 to 59.2%) and females (41.0 to 56.7%) in the mid-dose group and in males (65.1 to 74.3%) and females (61.1 to 74.2%)in the high dose group. Although RBC cholinesterase activity was reduced in males (23.6%) and females (50.1%) at week 6 in the low-dose group, this was believed to be residual effect on RBC cholinesterase of the higher dose of 0.1 mg/kg/day. RBC cholinesterase inhibition was not observed in this group after week 6. At time points after week 6, RBC cholinesterase activity was only significantly decreased in males (43.0 to 53.9) and females (38.0 to 51.9) in the mid-dose group and in males (81.2 to 86.9%) and females (79.2 to 82.5%) in the high-dose groups. Brain cholinesterase activity was significantly reduced in males (22%) in the mid-dose group and in males (47%) and females (29%) in the high dose group. The NOAEL was 0.05 mg/kg/day and the LOAEL was 0.1 mg/kg/day based on plasma and RBC cholinesterase inhibition in males and females.

Other animal studies. There are several other animal studies by the oral route in which RBC and plasma cholinesterase were measured: 1) 90-day rat subchronic in rats (MRID 41004701), LOAEL = 1.5 mg/kg/day based on plasma and RBC cholinesterase inhibition, NOAEL = 0.1 mg/kg/day; 2) Range-finding study for the rabbit developmental study (MRID 41802401), LOAEL = 1 mg/kg/day based on RBC cholinesterase inhibition, NOAEL = 0.1 mg/kg/day. Repeated dose (28-day) delayed neurotoxicity study in hens (MRID 43433501), LOAEL = 0.3 mg/kg/day based on inhibition of brain cholinesterase, NOAEL = 0.1 mg/kg/day.

Endpoint Selection.

Acute RfD. In the past, the acute dosing study in humans was considered suitable for use in establishing an acute RfD, but recently received time-course data in rats indicate that peak inhibition of RBC cholinesterase occurs 1-3 hours after oral dosing and that by 24-hours post-dosing, cholinesterase activity returns to near control levels. The risk assessment team concludes that the lack of cholinesterase measurements prior to 24 hours post-treatment in the acute human study is a deficiency so critical that it has opted not to rely on the acute human study for either establishing an acute RfD or to decrease the interspecies uncertainty factor.

The rat acute $BMDL_{10}$, 0.8 mg/kg, was selected for assessment of acute exposure scenarios. An uncertainty factor of 100 (10x for interspecies differences and 10x for intraspecies variation) was applied. It was concluded that an additional special FQPA factor is not needed, since BMD analysis of studies with pup and adult ChE depression results for either RBC or brain cholinesterase inhibition did not demonstrate any substantial numerical differences in the acute BMDL values for either RBC or brain cholinesterase inhibition (all values were approximately 1 mg/kg). Based on this assessment, an acute RfD of 0.008 mg/kg/day for the general population was derived for DDVP.

Short term residential & occupational exposure. There are a number of repeated dose studies that are under consideration, either individually or collectively, for providing appropriate endpoints for risk assessment for short- term durations.

- The 21-day repeated dose study in humans with a LOAEL for RBC cholinesterase inhibition of 0.1 mg/kg/day is of an adequate duration for selection of endpoints.
- In the 7-day repeated dose comparative cholinesterase study in rats, the LOAEL for adult rats for **RBC** cholinesterase inhibition was 0.1 mg/kg/day, whereas in pre-weaning rats, 0.1 mg/kg was a NOAEL for RBC cholinesterase inhibition.
- In the 7-day repeated dose comparative cholinesterase study in rats, the NOAEL for inhibition of **brain** cholinesterase inhibition in adult rats was 0.1 mg/kg/day, whereas 0.1 mg/kg/day was a LOAEL in preweaning rats.

The DDVP risk assessment team concludes that the 21-day human study is sufficiently robust and is more reflective of the short-term exposure duration of 30 days or less, and has therefore selected that study for assessment of short-term risks via all routes of exposure.

Intermediate term residential & occupational exposure. There are a number of repeated dose studies that are under consideration, either individually or collectively, for providing appropriate endpoints for risk assessment for intermediate-term durations.

- The 21-day repeated dose study in humans with a LOAEL for RBC cholinesterase inhibition of 0.1 mg/kg/day is of an adequate duration for selection of endpoints.
- The NOAEL in a 90-day rat subchronic neurotoxicity study was 0.1 mg/kg/day and the LOAEL was 7.5 mg/kg/day based on clinical signs of neurotoxicity and inhibition of plasma, RBC and brain cholinesterase.
- In a chronic feeding study in dogs, the LOAEL for plasma and RBC cholinesterase inhibition was 0.1 mg/kg/day and the NOAEL was 0.05 mg/kg/day measured at 3 and 6 weeks. The NOAEL for brain cholinesterase inhibition measured at the end of the study was 0.05 mg/kg/day.

The findings in the repeated dose studies are consistent across species and over study durations ranging from seven days to one year, with the LOAEL or NOAEL fluctuating around 0.1 mg/kg/day for RBC or brain cholinesterase inhibition. The HED dichlorvos risk assessment team is of the opinion that the endpoint of RBC cholinesterase inhibition in the human repeated dose study is well supported by several animal studies and should serve as the basis for comparison in assessing short- and intermediate-term risks. A MOE of 100 is recommended to account for intraspecies variability (10x), the lack of NOAEL in this study (3x) and a Special FQPA factor of 3x. The Special FQPA factor is based on residual concern for the sensitivity shown by young rats to brain cholinesterase inhibition in the repeated dose comparative cholinesterase study (MRID 46153304). A Special Factor of 3x is considered sufficient since the percent inhibition (25%) in brain cholinesterase in preweaning rats at 0.1 mg/kg/day is not substantial. The factor should be applied in any situation where the endpoint is based on RBC cholinesterase inhibition. A factor of 3x rather than 10x was used to account for a lack of a NOAEL since the RBC cholinesterase inhibition in humans during the exposure period of 21 days was minimal.

The dichlorvos risk assessment team acknowledges that there may be some uncertainty associated using the 21-day study for exposures up to 6 months in duration, particularly given that steady state has not yet been reached in the human study. However, the Team notes that for purposes of quantitative risk assessment, use of the steady state $BMDL_{10}$ of 0.4 mg/kg/day from the rat 90 day subchronic study (see below) with 10X factors for intra- and inter-species extrapolation yields a regulatory endpoint of 0.004 mg/kg/day. Use of the human LOAEL of 0.1 mg/kg/day with a 3X for LOAEL to NOAEL with a 10X factor for intra-species extrapolation yields a regulatory endpoint of 0.003 mg/kg/day. These two approaches provide very similar regulatory endpoints. Thus, the human LOAEL provides a reasonable endpoint for extrapolating human health intermediate-term risk.

Chronic RfD and long term residential & occupational. The same rationale described under short- and intermediate-term scenarios could be used for long-term scenarios since study duration didn't seem to make any difference in the NOAELs and LOAELs; however, the risk assessment team concluded that it would be difficult to justify using a 21-day human study for chronic or long-term scenarios. The risk assessment team recommends continuing to use the one-year dog study for establishing a chronic oral RfD and to assess long-term occupational or residential risk.

For the chronic dog study with a NOAEL of 0.05 mg/kg/day, an uncertainty factor of 300x was selected (10x for interspecies variation, 10x for intraspecies extrapolation, and 3x for a Special FQPA factor as described above. The risk assessment team considered reducing the interspecies factor to 3x based on the similarity of the human data to the dog data, but chose to continue with the standard 10x value based again on the difficulty in justifying use of a 21-day study (with a LOAEL) to decrease the interspecies factor for a chronic study. Based on this information, a chronic oral RfD of 0.00017 mg/kg/day was established for DDVP based on the dog study.

Note on Human Studies Cited in MacGregor et al (2005)

In its submissions to, and discussions with the Agency about the risk assessment of dichlorvos, the registrant has consistently made the argument that the body of experimental animal and human data indicate that humans are no more sensitive to the cholinesterase-inhibiting effects of dichlorvos than are laboratory animals, and therefore, the interspecies factor reflecting uncertainty about sensitivity across species should be 1x. In a publication appearing last year (MacGregor et al, 2005), the registrant and its consultants reiterated their argument for removing the interspecies factor and cited key human and experimental animal studies conducted over four decades in support of their thesis.

EPA has looked at these studies and remain of the opinion that a weight of evidence argument such as MacGregor et al. present is only as robust as the individual studies that are used to build the argument. The publication lists 29 studies "available for assessment of DDVP toxicity potential", but only 11 of these are used for the quantitative interspecies comparison (capsulized in Appendix A). There are four oral studies listed for quantitative comparison, two of which are discussed in this weight of evidence document (Gledhill, 1997a, 1997c). One of the remaining oral studies, (Slomka and Hine, 1981) utilizes a slow-release formulation of dichlorvos entrained in plastic beads. The availability of dichlorvos in this formulation is unknown. The remaining study (Gledhill, 1997b) is deficient as well (Appendix A).

There are 7 human inhalation studies, 3 of which use children as subjects and are therefore are excluded in accord with the Human Studies Rule. Two of the remaining 4 studies (Smith et al., 1972 and Witter et al., 1961) limit exposure to 2 hours or less which is of little use in assessing current use patterns, or for comparing with animal data. One study (Stein et al., 1966) monitors exposure to workers over a few weeks, but it isn't possible from the study to determine what the actual exposures to the subjects were. One study (Ueda and Nishimura, 1967) in which four subjects were exposed to relatively high levels for 2 days may have limited use for comparing with animal data.

The Agency has concluded that these studies are insufficient to support a weight of evidence argument that the interspecies factor should be reduced to 1X.

OP Cumulative Risk Assessment

The Food Quality Protection Act (FQPA) was passed by Congress in 1996. The FQPA made key changes to the approaches used by EPA to assess pesticide chemicals. One of these changes was the requirement to consider cumulative risk to those pesticides which act by a common mechanism of toxicity. Pesticides are determined to have a "common mechanism of toxicity" if they act the same way in the body--that is, the same toxic effect occurs in the same organ or tissue by essentially the same sequence of major biochemical events. OPP established the organophosphate pesticides (OPs) as a common mechanism group and in accordance with FQPA has developed a cumulative risk assessment for this group of pesticides (USEPA, 2002a). DDVP is a member of the OP common mechanism group.

OPP has developed a guidance document for developing cumulative risks assessments under FQPA (USEPA, 2002b). This guidance indicates that when developing multi-chemical hazard assessments, comparison of toxic potency should be made using a uniform basis of comparison, by using to the extent possible a common response derived from a comparable measurement methodology, species, and sex for all the exposure routes of interest. In the OP cumulative risk assessment, brain ChE data from rat toxicity studies in duration of 28 days and longer have been used by EPA to estimate relative potency and to develop the points of departure for extrapolating cumulative risk. ChE inhibition of OPs typically reaches steady state *in rats* at or near 28 days of exposure. Thus, potency estimates for exposures in duration from approximately one month or longer are consistent and show less variation than potency estimates for shorter exposure durations. Brain data have been selected over RBC data as brain ChE inhibition represents a direct measure of the target tissue (as opposed to blood data which is considered a surrogate measure) and brain ChE tend to have less variation and thus confer less uncertainty on cumulative risk estimates.

In the DDVP human multi-dosing study, the subjects exhibited 8, 10, 14, 14, and 16 percent below the pre-dose mean on days 7, 11, 14, 16, and 18 respectively. These data indicate that there is an increase in inhibition from days 7 to 18 of exposure. As such, steady state in humans may not have yet been reached during the DDVP study. The Agency notes given the relatively small increase (2%) in inhibition observed from days 14 to 18, it is unlikely that RBC ChE inhibition would increase substantially with prolonged exposure.

Incorporation of the results from the DDVP human multi-dosing study may create a mis-match with the relative potency factors and points of departure being used in the cumulative risk assessment which are based on ChE inhibition at steady state. The Agency has determined that for the cumulative risk assessment, the inter-species factor for oral exposure to DDVP will be **10X**. This determination is based, in part, on uncertainties associated with lack of steady state ChE inhibition in the human study and potential mis-matching with the data being used for relative potency and points of departure. The 10X inter-species extrapolation factor is further supported by a comparison of the results from the human study with RBC BMD_{10}s estimated by EPA previously (USEPA, 2001) from the DDVP rat subchronic study where steady state was reached (MRID no. 41004701; see table below). Dividing rat BMD_{10} of approximately 0.6 mg/kg/day by the human endpoint (0.1 mg/kg/day) yields a rat to human extrapolation factor of approximately 6X. It is notable that rat benchmark response is based on 10% RBC ChE

inhibition whereas the human endpoint is 16%. It is reasonable to expect that the dose to result in 10% RBC ChE inhibition in humans would be lower than that resulting in 16% RBC ChE inhibition. As such, the 6X ratio is likely to be higher (ie, closer to 10X).

Sex and Age	RBC	
Human (male)	16% at 0.1 mg/kg/day on Day 18	
Rat	BMD_{10}	$BMDL_{10}$
Male	0.57- 0.60	0.41 – 0.49
Female	0.65	0.45 – 0.54

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Table I. Guideline Toxicology Studies for Dichlorvos in Experimental Animals and Humans

Study Type/Guideline No.	**MRID No.**	**Results**
Acute Oral Cholinesterase Inhibition Study (1st) in Adult Rats/870.1100 (modified)	45805701 Acceptable	ChEI NOAEL (RBC and Brain) = not established ChEI LOAEL (RBC and Brain) = 2.1 mg/kg
Acute Oral Cholinesterase Inhibition Study (2nd) in Adult Rats/870.1100 (modified)	45805702 Acceptable	ChEI NOAEL (RBC and Brain) = 1 mg/kg ChEI LOAEL (RBC and Brain) = not established
Acute Oral Cholinesterase Inhibition Study (3rd) in Adult Rats/870.1100 (modified)	45805703 Acceptable	ChEI NOAEL (RBC and Brain) = 1 mg/kg ChEI LOAEL (RBC and Brain) = 5 mg/kg RBC ChE (F/M) BMD_{10} = 1.4/1.7 mg/kg; RBC ChE (F/M) $BMDL_{10}$ = 1.2/1.3 mg/kg; Brain ChE (F/M) BMD_{10} = 1.3/1.6 mg/kg Brain ChE (F/M) $BMDL_{10}$ = 0.8/1.0 mg/kg
Acute Oral Cholinesterase Inhibition Study in Preweaning Rat Pups/870.1100 (modified)	45842301 Acceptable	ChEI NOAEL (RBC) = not established ChEI LOAEL (RBC) = 1 mg/kg ChEI NOAEL (Brain) = 1 mg/kg ChEI LOAEL (Brain) = 5 mg/kg PND8 RBC ChE (F/M)BMD_{10} = 1.5/1.8 mg/kg; PND8 RBC ChE (F/M)$BMDL_{10}$ = 1.0/1.3 mg/kg; PND8 Brain ChE (F/M)BMD_{10} = 2.2/1.8 mg/kg PND8 Brain ChE (F/M)$BMDL_{10}$ = 1.6./1.5 mg/kg PND15 RBC ChE (F/M)BMD_{10} = 1.3/1.5 mg/kg; PND15 RBC ChE (F/M)$BMDL_{10}$ = 1.1/1.2 mg/kg; PND15 Brain ChE (F/M)BMD_{10} = 1.4/1.6 mg/kg PND15 Brain ChE (F/M)$BMDL_{10}$ = 1.0./1.3 mg/kg
Single Dose Cholinesterase Inhibition Study-Humans (Non-Guideline)	44248802 Un-Acceptable	NOAEL = 1.0 mg/kg/day LOAEL = not established 70 mg/person, single oral (capsule) dose to 6 male volunteers with no placebos - **missed time of peak effect**
Time Course of Cholinesterase Inhibition in Preweaning and Adult Rats/870.8223 (Non-Guideline)	46153303 Acceptable	Brain and RBC enzyme activities were maximally inhibited one hour after single dosing in both adult and preweaning female rats. Thereafter, ChE inhibition in both compartments decreased to approximately control levels by 8 hours post dosing.

7-Day, Repeat Dose Cholinesterase Inhibition Study in Preweaning and Adult Rats/(Non-Guideline)	46153304 Acceptable	PND 18 (M/F) ChEI NOAEL (Brain) = not established PND 18 (M/F) ChEI LOAEL (Brain) = 0.1 mg/kg/d PND 48 (M/F) ChEI NOAEL (Brain) = 0.1 mg/kg/d PND 48 (M/F) ChEI LOAEL (Brain) = 7.5 mg/kg/d PND 18 (M/F) ChEI NOAEL (RBC) = 0.1 mg/kg/d PND 18 (M/F) ChEI LOAEL (RBC) = 7.5 mg/kg/d PND 48 (M/F) ChEI NOAEL (RBC) = not established PND 48 (M/F) ChEI LOAEL (RBC) = 0.1 mg/kg/d
21-Day Oral (capsule) Cholinesterase Inhibition Study-Humans (Non-Guideline)	44248801 Acceptable	NOAEL = not established LOAEL = 0.1 mg/kg/day (RBC ChE) 7 mg/day for 21 Days in 6 male volunteers plus 3 male volunteers as placebos
Single Dose and Repeated Dose Cholinesterase Inhibition Studies-Humans (Non-Guideline)	44317901 Acceptable	Phase I (Single Dose of 35 mg) NOAEL = 0.5 mg/kg/day (RBC ChE) Phase II (Repeated Dose of 21 mg/day for 12 or 15 Days) NOAEL = not established LOAEL = 0.3 mg/kg/day (RBC ChE)
28-Day Delayed Neurotoxicity-Hen/870.6100	43433501 Acceptable	Cholinesterase inhibition (brain ChEI) NOAEL = 0.1 mg/kg/day LOAEL = 0.3 mg/kg/day No neuropathology.
90-Day Subchronic Oral Toxicity - Rat/870.3100	41004701 Acceptable	NOAEL = 0.1 mg/kg/day LOAEL = 1.5 mg/kg/day (plasma and RBC ChE)
90-Day Neurotoxicity - Rat/870.6200	42958101 Acceptable	NOAEL = 0.1 mg/day LOAEL = 7.5 mg/kg/day (plasma, red blood cell (RBC) and brain ChEI).
Chronic-Feeding-Dog/870.4100	41593101 Acceptable	NOAEL = 0.05 mg/kg/day LOAEL = 0.1 mg/kg/day (plasma and RBC ChEI in both sexes).
Chronic-Inhalation-Rats/ Guideline	00057695, 00632569 Acceptable	NOAEL = 0.00005 mg/L LOAEL = 0.0005 mg/L based on plasma, RBC and brain cholinesterase inhibition.

[a]Chronic-Inhalation-Human/870.4100	45812001, 00060486 Acceptable	NOAEL = 0.125 μg/L (0.000125 mg/L) LOAEL = 0.180 μg/L (0.000180 mg/L) (Headaches and RBC ChEI)
Developmental Toxicity-Rat/870.3700	41951501 Acceptable	Maternal toxicity NOAEL = 3 mg/kg/day LOAEL = 21 mg/kg/day (clinical signs, decreased body weight gain and reductions in food consumption and efficiency) Developmental toxicity NOAEL = $\geq$ 21 mg/kg/day (HDT)
Developmental Toxicity-Rabbit/870.3700	41802401 Acceptable	Maternal toxicity NOAEL = 0.1 mg/kg/day LOAEL = 2.5 mg/kg/day (mortality, decreased body weight gain at LOAEL) Developmental toxicity NOAEL= $\geq$ 7 mg/kg/day (HDT) ChEI was not measured.in main study Range-Finding: Doses were 0, 0.1, 1.0, 2.5, 5.0, 10 mg/kg/day Maternal toxicity ChE NOAEL = 0.1 mg/kg/day ChE LOAEL = 1.0 mg/kg/day
Reproductive Toxicity - Rat/870.3800	42483901 Acceptable	Parental/Systemic NOAEL = 2.3 mg/kg/day LOAEL = 8.3 mg/kg/day (decreased % of females with estrous cycle and increased % of females with abnormal cycling) Offspring NOAEL= 2.3 mg/kg/day LOAEL = 8.3 mg/kg/day (reduced # dams bearing litter, fertility index, pregnancy index and pup weight).

[a] Although this study has been used previously to assess inhalation risk, children are included in the study as subjects, and therefore the study can not be relied on for risk assessment purposes in compliance with the [HS Rule].

Preliminary Developmental Neurotoxicity - Rat/(Non-Guideline)	46153301 Acceptable	Systemic NOAEL = 7.5 mg/kg/day Maternal Systemic LOAEL = not identified Maternal RBC ChEI NOAEL = 0.1 mg/kg/day Maternal RBC ChEI LOAEL = 1.0 mg/kg/day Maternal Brain ChEI NOAEL = 1.0 mg/kg/day Maternal Brain ChEI LOAEL = 7.5 mg/kg/day Maternal Systemic NOAEL = 7.5 mg/kg/day Offspring Systemic LOAEL = not identified Offspring RBC ChEI NOAEL = 1.0 mg/kg/day Fetuses (GD 22) RBC ChEI LOAEL = 7.5 mg/kg/day Fetuses (GD 22) Brain ChEI NOAEL = 1.0 mg/kg/day Fetuses (GD 22) Brain ChEI LOAEL = 7.5 mg/kg/day Fetuses (GD22) Offspring (Pups) did not demonstrate ChEI during PND 2-22
Developmental Neurotoxicity - Rat/870.6300	46153302 Not-acceptable	Maternal LOAEL/NOAEL could not be identified due to low viability indices Offspring LOAEL/NOAEL could not be identified due to low viability indices Offspring Effects at 7.5 mg/kg/day (HDT) included increased startle response in males on PND 23, impaired memory in males on PND 27 and 62, and alterations in brain morphometry on PND 62. Study is unacceptable/not upgradable
Developmental Neurotoxicity - Rat/870.6300	46239801 Not-acceptable	LOAEL/NOAEL could not be identified due to low viability index in controls. No maternal or offspring effects in FOB, motor activity, auditory startle reflex habituation, learning and memory tests, brain weight, neuropathology and morphometry at 7.5 mg/kg/day (HDT). Study is unacceptable/not upgradable

Appendix A.

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Usable?	Citation
Oral studies used in the interspecies comparison	
No _ Missed peak effect	Gledhill, A. (1997a) Dichlorvos: A Study to Investigate the Effect of a Single Oral Dose on Erythrocyte Cholinesterase Inhibition in Healthy Male Volunteers: Lab Project Number: CTL/P/5393: XH6064. Unpublished study prepared by Zeneca Central Toxicology Lab. 44 p.
No _ Missed peak effect (acute) & Gledhill 1997b dose is lower (multidose)	Gledhill, A. (1997b) Dichlorvos: A Study to Investigate Erythrocyte Cholinesterase Inhibition Following Oral Administration to Healthy Male Volunteers: Lab Project Number: XH5170: CTL/P/5251. Unpublished study prepared by Central Toxicology Lab. 66 p.
Yes _ Multidose study used in the RA	Gledhill, A. (1997c) Dichlorvos: A Single Blind, Placebo Controlled, Randomised Study to Investigate the Effects of Multiple Oral Dosing on Erythrocyte Cholinesterase Inhibition in Healthy Male Volunteers: Lab Project Number: CTL/P/5392: XH6063. Unpublished study prepared by Zeneca Central Toxicology Lab. 52 p.
No _ Plastic bead formulation _ limited bioavailability	Slomka, M.B. and C.H. Hine. 1981. Clinical pharmacology of dichlorvos. Acta. Pharmacol. Toxicol. 49(Suppl. 5): 105_108.
Inhalation studies used in the interspecies comparison	
No _ children exposed	Funckes, A.J., Miller, S., Hayes, W., 1963. Initial field studies in upper volta with dichlorvos residual fumigant as a malaria eradication technique. Bull. World Health Org. 29, 243_246.
No _ children exposed _ **study had been used in RA**	Johnston, J.E., Barraj, L., Petersen, B., Youngren, S.H., 2002. A reanalysis of observations on occupants of Arizona homes containing 20% vapona insecticide resin strips, Arizona II home study, Exponent Inc., Project Identification Number DDVP_ 02_01, December 4, 2002.
No _ children exposed	Shell Chemical Com pany, 1970. The third Arizona home study: quantitation of DDVP residues in foods consumed by human volunteers exposed to No_Pest Strip insecticide. May 1970, unpublished report from Shell Chemical Company.
No _ 1 hr exposure _ not relevant to use	Smith, P.W., Mertens, H., Lewis, M.F., Funkhouser, G.E., Higgins, E.A., Crane, C.R., Sanders, D.C., Endecott, B.R., Flux, M., 1972. Toxicology of dichlorvos at operational aircraft cabin altitudes. Aerosp. Med. 43, 473_478.
No _ Exposure levels to individuals uncertain	Stein, W.J., Miller, S., Fetzer, L.E., 1966. Studies with dichlorvos residual fumigant as a malaria eradication technique in Haiti. III. Toxicological studies. Am. J. Trop. Med. Hyg. 15, 672_675.
Limited _ 4 subjects exposed 2 days to high conc.	Ueda, K.; Nishimura, M. (1967) Effect of Vapona/Strips to Human Beings. (Unpublished study prepared by Tokyo Dental College, Japan)
No _ 1-2 hr exposure _ not relevant to current use	Witter, R.F.; Gaines, T.B.; Short, J.G.; et al. (1961) Studies on the safety of DDVP for the disinsection of commercial aircraft. Bulletin of the World Health Organization 24(?): 635_642.