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

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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: RfD/Peer Review Report of Chlorpyrifos [O,O-Diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate]

CASRN. 2921-88-2
EPA Chem. Code: 059101
Caswell No. 219AA

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THRU: William Burnam
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The Health Effects Division-RfD/Peer Review Committee met on May 25, 1995 and subsequently on November 23, 1995 to reconsider its position on the Reference Dose (RfD) for chlorpyrifos in light of the registrant's petition of April 28, 1995.

In addressing this issue, the Committee reviewed the registrant's petition and the supporting documents, published reports relevant to the issue at hand, the RfD/Peer Review Report, data evaluation records for human studies and chronic toxicity studies in animals.

After thorough consideration of all aspects and based on all weight of the evidence, the Committee recommended that the repeated exposure RfD for chlorpyrifos remain unchanged.

1/8

A. Background:

The RfD for chlorpyrifos has been assessed by the Health Effects Division-RfD/Peer Review Committee on February 21, 1986 and verified by the Agency RfD Work Group on March 11, 1986. The Committee then reconvened on March 4, 1988 and reaffirmed its previous conclusion. The RfD was based on a cholinesterase inhibition study conducted on human volunteers with a NOEL of 0.03 mg/kg/day. Plasma cholinesterase inhibition was observed at the next higher dose level of 0.1 mg/kg/day. An uncertainty factor (UF) of 10 was applied to account for the intraspecies variability. On this basis the RfD was calculated to be 0.003 mg/kg/day.

Subsequently, the Health Effects Division-RfD/Peer Review Committee met on September 9, 1993 to discuss and evaluate the entire human safety/toxicology data base for chlorpyrifos and to reassess the RfD for this chemical for reregistration purposes. In the meeting of September 9, 1993 the Committee recommended that the existing RfD remain unchanged. In doing so, the Committee was aware that an Acceptable Daily Intake (ADI) of 0.01 mg/kg/day for chlorpyrifos has been established by the FAO/WHO joint meeting on pesticide residues (JMPR) in 1982.

B. Registrant's Rebuttal:

The registrant, DowElanco, has recently petitioned the Agency to revise the existing RfD for chlorpyrifos (a letter signed by Dr. W. Chin and Dr. R. Nolan, DowElanco, dated April 28, 1995). In their letter, the registrant questioned the validity of using plasma cholinesterase inhibition as a toxicological end-point for setting the Reference Dose for chlorpyrifos indicating that chlorpyrifos inhibits plasma cholinesterase at much lower doses than that which is required to inhibit acetylcholinesterase found in red blood cells and in brain. The registrant also questioned the NOEL established by the Agency for the human study. The registrant proposed to reestablish an RfD for chlorpyrifos based on inhibition of red blood cells (RBC) cholinesterase, rather than inhibition of plasma cholinesterase, observed in the human studies

The registrant's points of contention can be summarized as follows:

1) Chlorpyrifos, like other organic phosphorothioates, exerts its effect by inhibition of acetylcholinesterase in the neuroeffector junction, e.g. synapse and myoneural junctions. Human plasma as pseudo-cholinesterase is entirely made up of butyrylcholinesterase, which is a different enzyme than acetylcholinesterase. Plasma butyrylcholinesterase has no known biological function and it plays no role in cholinergic transmission.

2) Human and animal studies have shown that chlorpyrifos

inhibits plasma cholinesterase at much lower doses than that which is required to inhibit acetylcholinesterase found in red blood cells and in brain.

3) Plasma cholinesterase activity is a poor predictor of cholinesterase activity in the target tissues of brain and muscle.

4) Although acetylcholinesterase found in erythrocytes does not play a role in cholinergic transmission, it is a better reflection of the acetylcholinesterase activity in brain, since the two enzymes are considered biochemically identical. In recent studies, cholinesterase activity in the red blood cells correlated better with the activity in brain and muscle than did cholinesterase activity in plasma.

5) Brain acetylcholinesterase is the best measure of the cholinergic effects of chlorpyrifos anticholinesterase pesticide. However, in the absence of data on brain acetylcholinesterase, human red blood cell values should be used to assess the toxicity of the chemical.

The registrant believes that the human data indicate that 0.05 and 0.01 mg/kg body weight would be appropriate and conservative standards for evaluating single and repeated dietary and non-dietary (occupational) exposures to chlorpyrifos. The following calculations were provided by the registrant for establishing the reference dose(s):

a. Single exposure RfD: using a NOEL of 0.50 mg/kg/day for human RBC cholinesterase inhibition and an uncertainty factor of 10 to account for intraspecies variability (yielding an RfD of 0.05 mg/kg/day).

b. Repeated exposure RfD: using a NOEL of 0.1 mg/kg/day for human red blood cell cholinesterase inhibition and uncertainty factor of 10 to account for intraspecies variability (yielding an RfD of 0.01 mg/kg/day). Another approach for deriving the same RfD was suggested by using a NOEL of 1.00 mg/kg/day for brain cholinesterase inhibition in the two-year chronic rat study and an uncertainty factor 100 for interspecies extrapolation and intraspecies variability yielding an RfD of 0.01 mg/kg/day.

C. Committee's Conclusions and Recommendations:

In addressing the registrant's rebuttal, the Committee was aware that the issue at hand is a multicomponent issue involving scientific, regulatory and policy aspects.

In the registrant letter of rebuttal, several issues were raised. The registrant questioned the Agency's policy of using plasma cholinesterase inhibition as a toxicological end-point for setting the Reference Dose. The registrant pointed out that

chlorpyrifos inhibits plasma cholinesterase at much lower doses than that which is required to inhibit acetylcholinesterase found in red blood cells and in brain. The registrant proposed to reestablish an RfD for chlorpyrifos based on inhibition of red blood cells (RBC) cholinesterase, rather than inhibition of plasma cholinesterase, observed in the human studies. Furthermore, the registrant, indirectly, questioned the NOEL for plasma cholinesterase inhibition established by the Agency for the human study used in setting the RfD for chlorpyrifos.

After thorough consideration of all aspects and based on all weight of the evidence, the Committee recommended that the repeated exposure RfD for chlorpyrifos remain unchanged.

The following factors were cited by the Committee and were considered significant in shaping the Committee's position and conclusion on these issues:

The Committee indicated that its evaluation of cholinesterase inhibitors is usually performed on a case-by-case basis since anticholinesterase agents differ in their pharmacokinetics and pharmacodynamic aspects, and that the use of plasma cholinesterase inhibition in human studies in setting the RfD for chlorpyrifos is supported by the weight of evidence as discussed below.

The Committee disagreed with the registrant's comments regarding the difference between acetylcholinesterase found in the erythrocyte and in the neuroeffector junctions (synapse and myoneural junctions) and plasma pseudo-cholinesterase (butyrylcholinesterase). It is the Committee's position that although the two esterases may vary in their substrate specificity, they are similarly susceptible to inhibition by organophosphorus pesticides and other cholinesterase inhibitors and that the two enzymes bind to inhibitors in the same manner.

The Committee agreed with the registrant position in that both plasma pseudo-cholinesterase and erythrocyte cholinesterase have no known biological function and play no role in cholinergic transmission. However, the Committee disagreed with the registrant statement that plasma cholinesterase activity is a poor predictor of cholinesterase activity in the target tissues of brain and muscle and that acetylcholinesterase found in erythrocytes is a better reflection of the acetylcholinesterase activity in brain. Contrary to the registrant's claim in the rebuttal letter, open literature information reviewed by the Committee demonstrated that, in the rat, plasma cholinesterase inhibition has been observed to correlate very well with brain cholinesterase inhibition following the administration of single doses of certain organophosphorus pesticides. In some cases organophosphorus pesticides caused brain cholinesterase inhibition at dose levels comparable to those causing inhibition of plasma cholinesterase without causing similar depression of the red blood cell cholinesterase, i. e., with

certain organophosphorus pesticides there was a correlation between brain and plasma cholinesterase activities but not a good correlation between either brain and erythrocytes cholinesterase activities or plasma and erythrocytes cholinesterase activities [Pope, C. N. and Chakraborti, T. K. (1992); Pope, C. N., et al., (1992); Padilla, S. et al.(1994)]. It is clear from the following discussion of the three animal studies cited here that plasma cholinesterase inhibition has predictive value for brain cholinesterase inhibition in the case of chlorpyrifos (see Section D of this memo).

Considering all weight of the evidence, the magnitude of plasma cholinesterase inhibition observed in the human study used by the Agency in setting the RfD for chlorpyrifos was sufficient to define the NOEL and LOEL. In this study there were clinical signs, some of which are cholinergic in nature, accompanying plasma cholinesterase inhibition. Although, there was no evidence to suggest otherwise, other contributing factors such as common cold were not precluded and could have resulted in some, but not all, of these symptoms in some human subjects used in this study. For example, blurred vision observed in, at least, one subject is a typical cholinergic sign of cholinesterase inhibition and can not be attributed to common cold. Personal communication by Dr. Brian Dementi, HED, OPP. (January 29, 1996) with Dr. Jean Hollingsworth and Dr. Joe Bresee of the Center for Disease Control and Prevention indicated that blurred vision is not a sign of cold/influenza. The Committee also indicated that although, no pronounced effect was noted on red blood cell cholinesterase in the human study, given the limited number of subjects used in this study and the variability of the cholinesterase assay, this kind of results are expected.

With respect to the registrant proposal to use the rat study as an alternative for RfD setting, the Committee indicated that it is the Agency's position that the use of human data, when available, is preferred over animal data since it eliminates an unnecessary level of uncertainties, i.e. the need for interspecies extrapolation.

Finally, there were no new data submitted by the registrant to the agency to change its position.

D. Material Reviewed and Detailed Considerations:

In addition to the toxicology data base available to the Agency, the Committee searched the open literature in order to better address the issue of plasma and brain cholinesterase correlation. Open literature information reviewed by the Committee demonstrated that, in the rat, plasma cholinesterase inhibition has been observed to correlate very well with brain cholinesterase inhibition following the administration of single doses of certain organophosphorus pesticides. The following are excerpts taken from

a review by Dr. Brian Dementi, HED, OPP for three open literature studies used by the RfD Peer Review Committee in partial support of its current position on issues raised by the registrant.

In a study by Pope, C. N. and Chakraborti, T. K. (Toxicology, 73, 35-43, 1992) with three organophosphorus pesticides; parathion, methyl parathion and chlorpyrifos administered subcutaneously, as evaluated in both adults and neonates SD rats, good correlations were observed between ED_{50} values (dose inhibiting the enzyme 50%) and the Maximum Tolerated Dose (MTD), used as an indication of toxicity. In this publication the authors stated (P.41): "For example, when brain cholinesterase ED_{50} values were correlated with MTDs for both age groups, a correlation (r) value of 0.932 was obtained, indicating a good correlation between brain cholinesterase inhibitory potency and acute toxicity among the inhibitors. An even higher correlation (r=0.992) was noted, however, between plasma cholinesterase ED_{50} values and MTDs. In addition, there were no significant differences in the ED_{50} values of brain cholinesterase relative to plasma cholinesterase with either of the OP treatments in either age group."

The authors indicated that, while plasma cholinesterase levels, under defined experimental conditions, may provide a quantitative estimate of the extent of cholinesterase inhibition in the central nervous system following organophosphate exposure, factors such as route of exposure and time after treatment when cholinesterase is assayed could influence the degree of correlation.

In another publication by Pope, C. N., et al. (Pharm. Biochem. Behav., 42, 251-256, 1992), the authors investigated effects in the rat of chlorpyrifos, as administered subcutaneously at the MTD, on a number of parameters, including plasma and brain cholinesterase inhibition SD rats. Following administration of the test material, cholinesterase activity was assessed periodically over a 12-week period. The following is a quotation from the study report (P.253):

"Cholinesterase inhibition in plasma was not as extensive as in either cortex or striatum at any time point during the observation period, but roughly equivalent rates of recovery of enzyme activity were noted between plasma and the brain regions."

Inhibition in the striatum and cortex were essentially identical. Inhibition for these brain regions were 94-96%, 82-83%, 58-60% and approximately 20% at weeks 2, 4, 6 and 12, respectively. By comparison, plasma cholinesterase was inhibited at the same respective time points by about 90%, 55%, 30% and 0%. The authors advise that cholinesterase activities were not significantly different between treatment groups at the 12-week time point.

Although erythrocyte cholinesterase was not assayed in either of the above referenced publications, the data indicate that plasma cholinesterase inhibition correlated well with brain cholinesterase inhibition, and toxicity under certain conditions of each study. While in view of the author's discussion, this correlation may not hold to be true under all exposure scenarios, the correlation should be considered as substantive.

In a third publication by Padilla, S. et al. (Toxicology, 92, 11-25; 1994) on Long Evans rats, correlations between plasma, whole blood and erythrocyte cholinesterase inhibition and brain cholinesterase inhibition were determined over a 35 day period following a single subcutaneous dose of chlorpyrifos administration in the rat. The study revealed high correlation coefficients between inhibition of all three blood components enzymes and that of the frontal cortex during days 4-21 post-dosing. At the 35 day time point, plasma cholinesterase activity was less well correlated than was whole blood or erythrocyte cholinesterase activity with brain cholinesterase inhibition.

Collectively, the three published articles reveal a good correlation between plasma and brain cholinesterase inhibition. In rats, at least in the Padilla study, erythrocyte cholinesterase appears to be more remarkably inhibited by chlorpyrifos than either the plasma or brain enzyme activity.

It is clear from the above discussion of the three animal studies that plasma cholinesterase inhibition has predictive value for brain cholinesterase inhibition in the case of chlorpyrifos.

In the human study in question in the chlorpyrifos data base, and at issue in this review, the plasma enzyme activity was reportedly inhibited at a lower dose than the erythrocyte cholinesterase activity. We have no explanation for this reversal of effect with respect to plasma and erythrocyte cholinesterase responses except that it may have to do with inherent differences between human and rat, or the circumstance of exposure. The limited number of subjects and variability of the cholinesterase assay methodology were also cited by the Committee as possible factors.

In any case, there is no reason to conclude that brain cholinesterase inhibition in the human case study would not be a correlate of plasma cholinesterase inhibition.

E. Individuals in Attendance:

Peer Review Committee members and associates present, at least in one meeting, were William Burnam (Chief, SAB; chairman, RfD/Peer Review Committee), Karl Baetcke (Chief, TB I), Marcia Van Gemert (Chief, TB II), George Ghali (Manager, RfD/Peer Review Committee), Rick Whiting, William Sette, Henry Spencer, David Anderson, Stephen Dapson, Brian Dementi and Karen Hamernik.

Scientific reviewer (Committee or non-committee member(s) responsible for data presentation; signature (s) indicate technical accuracy of panel report)

Virginia Dobozy

Alan Levy

Mike Ioannou

Respective branch chief (Committee member; Signature indicates concurrence with the peer review unless otherwise stated)

Karl Baetcke

CC: Stephanie Irene
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