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MEMORANDUM

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FROM: Anna M. Fan, Ph.D., Chief
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DATE: April 11, 2003

SUBJECT: COMMENTS AND RECOMMENDATIONS ON THE DRAFT AZINPHOS-METHYL RISK CHARACTERIZATION DOCUMENT PREPARED BY THE DEPARTMENT OF PESTICIDE REGULATION

Thank you for the opportunity to review the draft risk characterization document (RCD) for azinphos-methyl (AZM) prepared by the Department of Pesticide Regulation (DPR). The Office of Environmental Health Hazard Assessment (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 (FAC), Section 13129, in which OEHHA has the authority to provide advice, consultation, and recommendations to DPR concerning the risks to human health associated with exposure to pesticide active ingredients.

There is much history involving DPR and OEHHA in evaluating the health risks associated with exposure to the active ingredient, AZM. OEHHA has reviewed two previous versions of the draft RCD for AZM, one prepared in 1995 and the other in 1998. OEHHA has also reviewed DPR's draft toxic air contaminant document (TAC) for AZM (dated July 2000) and prepared findings under the authority of FAC, Sections 14022 and 14023. In addition, OEHHA has reviewed draft regulations developed by DPR to change the re-entry intervals for workers in fields where AZM has been applied.

We have found it difficult to follow the changes made in these documents and the implications of these changes for the protection of public and worker health. Therefore, some of the comments OEHHA has submitted to DPR on its previous attempts to evaluate the risks of

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AZM exposure might still apply to the current version. However, in reviewing the current (May 28, 2002) draft RCD, we have limited our comments and recommendations to only those issues we feel are the most important to address at this time. In the future, we might have an opportunity to revisit our previous comments and recommend further changes. Furthermore, under a separate cover, we will be submitting revised draft findings on the TAC document for AZM based on our most recent review of the available information.

1. We recommend that the data reported in the MacFarlane and Freestone (1998) volunteer study not be used in the RCD for quantifying human health risks from acute exposures to AZM. In this study, healthy men (seven per treatment group and three in the control group) were administered AZM orally in capsules at 0 (lactose), 0.25, 0.5, 0.75, or 1.0 mg/kg and followed for 14 days after dosing. Seven healthy women were also administered AZM at 0.75 mg/kg along with three control subjects and followed for the same time-course. The mean ages for these subjects were 32.7 for the men and 31 years for the women. Plasma and red blood cell (RBC) cholinesterase (ChE) activities were measured at several time-points over the course of the investigation. It should be noted that individual baseline ChE activities were also measured and reported for the study subjects. Other parameters such as vital signs, electrocardiograms, hematology, clinical chemistry, and urine chemistry were also measured at various time-points. No-observed-adverse-effect-levels (NOAELs) of 1.0 and 0.75 mg/kg were identified in the study for men and women, respectively, and were based on the absence of effects (no statistically significant RBC or plasma ChE inhibition at any dose when compared to the controls). We reviewed and analyzed the data reported by MacFarlane and Freestone (1998) and have the following observations:
 - (a) This study was not designed according to any standardized scientific or regulatory guidelines. The Federal Insecticide Fungicide and Rodenticide Act (FIFRA) does not require human exposure studies nor are there federal or state manuals guiding the design of such studies. Furthermore, to our knowledge this study is not published in the peer-reviewed scientific literature.
 - (b) Adverse symptoms were not systematically recorded or analyzed in the study. Although some adverse symptoms were reported by the study subjects and appeared to be dose-related, the effects were discounted because they occurred in the absence of ChE activity inhibition. Because not all adverse effects of organophosphorous pesticides are mediated by inhibition of ChE activity, markers of toxicity such as ChE inhibition and symptom reports should be interpreted independently of each other.

- (c) Normal variation of RBC or plasma ChE activity in the human population can be quite high (typically 10 to 20 percent but as high as 40 percent or more), depending on the analytical method used, the tissue and enzyme activity of interest, and the number of “control” subjects used. One way to help control for this variation in studying human exposures is to measure pre-exposure baseline levels of ChE activity. By doing this, the individual acts as his/her own control. In addition, using relatively large numbers of subjects would increase the statistical power of the study to allow for smaller changes in enzyme activity (relative to unexposed controls) to be detected. Although the MacFarlane and Freestone (1998) study did measure pre-exposure baseline ChE activities for each individual subject, only small numbers of control subjects (unexposed to AZM) were used.
- (d) The study was not designed to examine possible sequelae. Volunteers did not undergo neurobehavioral or neurophysiological testing to evaluate for more subtle neurological effects in cognition or nerve conduction. Furthermore, no follow-up examinations were offered to test for any longer-term effects. Without including the more sensitive neurological examinations or follow-up, an overall conclusion that there was no effect of AZM exposure in these volunteers might not be valid and at the very least such a statement should be qualified.
- (e) On August 31, 1999, OEHHA and DPR staff met to discuss the available toxicology data for AZM. During that meeting, OEHHA staff presented some evidence of laboratory or other systematic errors in the MacFarlane and Freestone (1998) study, which compromise the reliability of the results. In summary, there are two unexplained results:
 - i. Dosed and non-dosed (placebo) plasma and RBC ChE values are correlated, that is, an increase or decrease in the daily measurement of control plasma ChE activity is more likely than chance to be accompanied by a similar increase or decrease in ChE activity in the treatment groups. RBC values are particularly of concern, as RBC is more stable and not subject to daily variation. We believe this is evidence of laboratory or other systemic error.
 - ii. Thirty-two percent of the reported values for RBC ChE activity exceed the normal range of clinical values for RBC ChE activity. The authors do not comment on the frequency of these elevated values. These results also suggest a problem with the analytical protocol.

- (f) The NOAEL of 1 mg/kg identified from the MacFarlane and Freestone (1998) study is based on no observable effects at the highest dose tested and is approximately 10-fold higher than NOAELs identified from other available human AZM exposure studies. For example, Carrier and Brunet (1999) estimated a NOAEL of 0.1 mg/kg based on statistically significant reduction in RBC ChE activity. Similar human NOAELs of approximately 0.2 mg/kg based on inhibition of RBC ChE have been previously reported (Rider et al., 1967; 1968; 1970; 1971; 1972).
2. We recommend that the data from Sheets (1994) be used as the basis for calculating human health risks from acute AZM exposure. This neurotoxicity study in rats identifies a NOAEL of 1.0 mg/kg based on inhibition of brain ChE activity and effects on the functional observed battery (FOB) at the next higher dose. A NOAEL of 0.1 mg/kg was also estimated for inhibition of RBC ChE activity based on a lowest-observed-adverse-effect level (LOAEL) of 1.0 mg/kg. It is also noted that the NOAEL of 1.0 mg/kg from Sheets (1994) was used in the 1998 draft RCD as well as the 2000 draft TAC document for AZM as the critical endpoint for evaluating acute AZM exposures. This study was designed according to and meets FIFRA guidelines. The U.S. Environmental Protection Agency (U.S. EPA) also selected this neurotoxicity study for evaluating AZM acute dietary exposure.
 3. We recommend that the data reported in the MacFarlane and Freestone (1999) volunteer study not be used in the RCD for quantifying human health risks from subchronic exposures to AZM. The reasons to not use the results of this study relate to the flaws in study design, the inappropriate utilization of statistical methods, and the lack of detail in reporting the study results. In this study, 12 healthy men were administered either a placebo (four subjects) or AZM (eight subjects) at 0.25 mg/kg-day in a gelatin capsule for 28 days. The mean ages for the control group were 35.3 years and 29.3 for the exposed group. Plasma and RBC ChE activities were measured four-hours after each daily dose on selected days during the study. Individual baseline ChE activities were measured and reported for the study subjects. Other parameters such as vital signs, electrocardiograms, hematology, clinical chemistry, and urine chemistry were also measured at various time-points. A NOAEL of 0.25 mg/kg-day was identified based on the absence of effects. We reviewed and analyzed the data reported by MacFarlane and Freestone (1999) and have the following observations:

- (a) This study was not designed according to any standardized scientific or regulatory guidelines. The Federal Insecticide Fungicide and Rodenticide Act (FIFRA) does not require human exposure studies nor are there federal or state manuals guiding the design of such studies. Furthermore, to our knowledge this study is not published in the peer-reviewed scientific literature.
- (b) As with the acute human exposure study (MacFarlane and Freestone, 1998), adverse symptoms were not systematically recorded or analyzed in the study. Although the AZM exposed subjects recorded more symptoms than the unexposed (placebo) subjects, the effects were discounted because they occurred in the absence of ChE activity inhibition. Because not all adverse effects of organophosphorous pesticides are mediated by ChE activity inhibition, markers of toxicity such as ChE activity inhibition and symptom reports should be interpreted independently of each other.
- (c) As noted in comment 1(b) above, normal variation of RBC or plasma ChE activity in the human population can be quite high and to help control for this variation in studying human exposures, measuring pre-exposure baseline levels of ChE activity is important. By doing this, the individual acts as his/her own control. Although the MacFarlane and Freestone (1999) study did measure pre-exposure baseline ChE activities for each individual subject these values were apparently not used in the statistical analysis. Additionally, only small numbers of control subjects (unexposed to AZM) were used in the study. The report defined baseline as the mean of all available pre-exposure values. Accordingly, the baseline is calculated in the RCD as an average of 96 values (eight time points: days -14, -12, -10, -8, -6, -4, -2, and -1 averaged for 12 study subjects). In general, inter-individual ChE activity values vary significantly, and as seen in the study, differences between individuals can be as high as 20 to 40 percent. Therefore, in order to consider that the 15 percent difference of blood ChE is an acceptable criterion, each individual should act as his/her own control. Namely, the mean of the pre-exposure values from each individual should be used to compare to his/her post-exposure values. This does not appear to be the method used by the study investigators, based on what was presented in the study report on pages 39 and appendix M, pages 45 to 56. It is not appropriate to consider any inferences of no effect drawn from the analysis of ChE data if baselines were calculated in this manner.
- (d) Analysis of Variance could only determine the overall treatment effect. There are two study parameters, one is the treatment effect and the other is the effect of time. It is not appropriate to compare placebo and dosed groups at each day without adjusting

for multiple comparisons, that is, dividing the p value by the number of comparisons made and determining whether each comparison was significant at the new reduced p value. For example, the controls show greater ChE inhibition than the treated subjects in the plasma and RBC on certain days, and this may or may not be due to chance alone.

- (e) Although two blood samples were taken per time point for ChE measurements, only one value for ChE activity (one replicate per subject per time point) was provided in the results. Because only one sample per time point was used in the results, it is difficult to assess the variability of the experimental procedure.
 - (f) As with the acute human exposure study (MacFarlane and Freestone, 1998), the 1999 study was not designed to examine possible sequelae. Volunteers did not undergo neurobehavioral or neurophysiological testing to evaluate for more subtle neurological effects in cognition or nerve conduction. Furthermore, no follow-up examinations were offered to test for any longer-term effects.
4. We recommend that the data from the Sheets and Hamilton study (1995) be used as the basis for calculating human health risks from subchronic (seasonal) AZM exposures. This study was designed according to and meets FIFRA guidelines. In this 13 week feeding study in rats, a NOAEL of 0.09 mg/kg was estimated from a LOAEL of 0.9 mg/kg for inhibition of RBC and plasma ChE activity. The results from this study indicate effects of AZM at doses lower than those reported in the MacFarlane and Freestone (1999) human subjects study. The results of the Sheets and Hamilton (1995) study are consistent with other laboratory animal studies. For example, a NOAEL of 0.33 mg/kg was identified from a reproductive toxicity study in rats based on the decreased viability (percent of pups born live that survived to day four) and lactation indices at the next higher dose (Eiben and Janda, 1984). In addition, a NOAEL of 1 mg/kg based on reduced brain ChE activity at the next higher dose was identified from a developmental toxicity study in rats (Kowalski et al., 1987). In another developmental toxicity study, a NOAEL of less than 1 mg/kg in rabbits was reported based on reduced RBC ChE activity (Clemens et al., 1998).
 5. We agree with the draft RCD in selecting the NOAEL of 0.15 mg/kg-day from the one-year oral dog study (Allen, 1990) as the critical endpoint for calculating human health risks from chronic exposures to AZM. The use of this NOAEL is also in agreement with U.S. EPA (1999, 2001).

6. In most, if not all of the RCDs previously reviewed by OEHHA, only margins of exposure (MOEs) of 100 or greater were considered public health protective by DPR when using animal data for the risk assessment. However, the draft RCD on page 79 recommends that MOEs greater than 30, rather than 100, are sufficiently health protective for subchronic (seasonal) and chronic exposures to AZM. For AZM, the basis for the determination made in the draft RCD that an MOE of 30 is sufficient is that an uncertainty factor of ten is not needed for inter-species extrapolation for chronic exposures. Instead, an additional uncertainty factor of three was applied to account for a study design flaw (lack of female subjects) in the MacFarlane and Freestone (1999) study resulting in a total uncertainty factor of 30 for issues to account for intra-species (human) variability and data gaps. The following lines of evidence were used to support this determination made in the draft RCD:

- (a) The acute NOAELs for brain (1 mg/kg) and RBC (less than 1 mg/kg) inhibition of ChE activity in rats (Sheets, 1994) are greater than the NOAEL of 0.75 mg/kg identified in humans from MacFarlane and Freestone (1998).
- (b) For subchronic and chronic exposures, the subchronic NOAEL of 0.25 mg/kg-day for inhibition of RBC ChE activity in humans (MacFarlane and Freestone, 1999) is equal to the chronic NOAEL of 0.25 mg/kg-day in rats based on reduced RBC and brain ChE activity (Schmidt and Chevalier, 1984).
- (c) Both NOAELs are less than the subchronic NOAEL of 0.91 mg/kg-day based on reduced blood and brain ChE activity in rats (Sheet and Hamilton, 1995).

OEHHA disagrees with the recommendation on page 79 that an MOE less than 100 is sufficiently health protective for subchronic (seasonal) and chronic exposures to AZM because:

- (a) The data from MacFarlane and Freestone (1998, 1999) are not defensible and appropriate for quantitative risk assessment (see our discussion above). Furthermore, the validity of comparing a NOAEL identified from an acute or a chronic rat study for the inhibition of brain ChE activity with a NOAEL identified from human volunteer exposure studies based on inhibition of plasma or RBC ChE activity is not adequately justified in the draft RCD. In addition, no scientific rationale was provided in the draft RCD supporting the comparison of data from a subchronic human study to data from a chronic animal study.

- (b) Insufficient scientific evidence is presented in the draft RCD demonstrating that a value of less than ten for inter-species extrapolation should be applied. That is, there is insufficient evidence supporting the conclusion that compared to rats, humans are equally sensitive to the toxic effects of AZM. It should be noted that, in reference to argument (c) above, the NOAEL of 0.91 mg/kg-day (Sheet and Hamilton, 1995) used to justify that “the uncertainty factor for interspecies extrapolation can probably reduced even for chronic exposure” (page 79, draft RCD) is the incorrect value for the NOAEL from that study. The NOAEL established from the study is 0.09 mg/kg-day (see comment number four for a more detailed explanation).
 - (c) The chronic rat study quoted in DPR argument (b) above (Schmidt and Chevalier, 1984) is not the animal study (Allen, 1990) that was used in the draft RCD or by U.S. EPA to calculate human health risks from chronic exposure to AZM. The chronic NOAEL of 0.15 mg/kg-day based on diarrhea and RBC ChE activity inhibition used for calculating health risks from a one-year dog study (Allen, 1990) is lower than the NOAEL of 0.25 mg/kg-day identified from a two-year rat study (Schmidt and Chevalier, 1984).
8. From our previous comments, we recommend that the experimental data from animals as reported by Sheets (1994), Sheets and Hamilton (1995), and Allen (1990) be used in developing reference exposures levels for acute, subchronic, and chronic AZM exposure, respectively. OEHHA also recommends that uncertainty factors of 100 (ten for inter-species extrapolation and ten for intra-species variability) be applied to the NOAELs identified from the animal data for acute, subchronic, and chronic exposures. Therefore, we recommend MOEs of at least 100 to protect humans from acute, subchronic, and chronic exposures to AZM using the animal data.
9. We agree with the analysis of the developmental and reproductive toxicity studies presented in the draft RCD. There appears to be insufficient evidence of increased pre- and post-natal sensitivity to AZM. In addition, we agree with the analysis of the weight-of-evidence for oncogenicity presented in the draft RCD and concur with U.S. EPA’s classification of Group E carcinogen or “not likely” to be a human carcinogen.

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If you have any questions regarding our review, please call me or
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References

In addition to the major comments and recommendations listed above, we also refer you to some references cited in the text of the draft RCD that we did not find in the bibliography. These include: Ellenhorn and Barceloux (1997), Li et al. (2000), Lefkowitz et al. (1990), Pantuck (1993), DPR (1993a), Levine and Murphy (1976), Everett et al. (1977), Bianchi-Santamaria (1997), De Ferrari et al. (1991), Gomez-Arroyo et al. (1992), Lander and Ronne (1995), Bianchi et al. (1994), Shah et al. (1997), Machemer (1975), Grisaru et al. (1999), Sette (1998), JMPR (1999), Clemens (1998), EDSTAC (1998), and Lewis (1998).

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