TXR NO. 0050230

DATE: October 26, 2001

MEMORANDUM

SUBJECT: **AZINPHOS-METHYL - Repeated Dose** Oral Study - Report of the Hazard Identification Assessment Review Committee.

From: Jess Rowland, Co-Chair
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

To: Margaret Stasikowski,
Director
Health Effects Division

On August 29, 2001, the Hazard Identification Assessment Committee (HIARC) met to discuss the potential impact of consideration of a toxicity study for azinphos-methyl conducted in human test subjects in the hazard assessment process. The Committee's recommendations are presented in this report.

Committee Members in Attendance

Members present were: Ayaad Assaad, William Burnam, Johnathan Chen, Elizabeth Doyle, Pam Hurley, Elizabeth Mendez, David Nixon, Jess Rowland, Brenda Tarplee and Yung Yang

Data was presented by: John Doherty

Introduction

On August 29, 2001, the Hazard Identification Assessment Committee (HIARC) met to discuss the potential impact of consideration of a repeated dose study with azinphos-methyl conducted in human test subjects in the hazard assessment process. The HIARC examination of this study focused on the content and results of the study considered as part of the weight of all available data for evaluating and quantifying the hazard potential of azinphos-methyl. The results of HIARC's work represents how this study could affect the OPP's conclusions about hazard assessment for azinphos-methyl.

Study Review

Study Type: Repeated Dose Cholinesterase Inhibition in Human Subjects

Reference: P. McFarlane and S. Freestone, "A Randomized Double Blind Placebo Controlled Study with Azinphos-Methyl to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity After Repeated Doses." Inveresk Research, Study No.: ICR 013580, April 15, 1999. MRID No.: 45476101

Executive Summary: In this 28-day dosing study (MRID No.: 45476101) with human subjects (males only), a group of 4 volunteers were dosed with placebo (lactose) and a group of 8 volunteers were dosed with 0.25 mg/kg/day of azinphos methyl (89.1% to 91.6% purity) for 28 consecutive days. Dosing was orally by capsule and the subjects were housed in a clinic throughout the dosing period. The subjects were monitored for reactions and samples of their blood were taken at predose (8 times) and daily throughout the dosing period. On some days, a blood sample was taken both prior to administering the dose and 4 hours after. The blood was separated and assessed for both plasma cholinesterase (ChE) and red blood cell acetylcholinesterase (RBC AChE). In addition, hematology, clinical chemistry and urinalysis were evaluated as well as blood pressure and ECG.

There were no reactions to treatment and the blood pressure and ECG parameters were not affected. Eight of the 12 volunteers were noted to have symptoms of a viral infection and some were treated with paracetamol. This was not considered to affect the interpretation of the study.

The plasma ChE in IU/mL for the baseline (mean ± the standard deviation) was 6965± 1327 (19%) for the placebo group (4 subjects) and 5307±1048 (20%) for the azinphos methyl treated group (8 subjects) meaning that the azinphos methyl group was already 24% decreased prior to test material administration.

The RBC AChE in IU/mL for the baseline was 10933±255 (±2.3%) for the placebo group and 11049±478 (4.3%) for the azinphos methyl treated group prior to dosing. The mean

values and variance are as would be expected for assessment of plasma ChE and RBC AChE from human subjects. The postdosing group mean data were compared three ways in an effort to see if azinphos methyl caused inhibition of either plasma ChE or RBC AChE. Neither comparison of the group mean for the dosed group with 1) the placebo group at each time interval or 2) the dosed groups baseline mean (the most appropriate method) indicated inhibition of either plasma ChE or RBC AChE. In particular, the maximum decrease in plasma ChE was only 6% and the maximum decrease for RBC AChE was 9% (which occurred only on one day) when compared to the baseline mean. Also, samples taken 4 hours after dosing on several occasions did not indicate inhibition.

In conclusion, a dose of 0.25 mg/kg/day of azinphos methyl for 28 days did not result in either clinical symptomology or inhibition of either plasma ChE or RBC AChE in human males. The NOAEL is 0.25 mg/kg/day.

Compliance: A Combined Good Clinical Practice (CPMP/ICH/135/95) for treatment of human subjects and a OECD Good Laboratory Practice statement was provided (dated August 3, 1999). A Statement of Data Confidentiality (no claim) was also provided (dated August 13, 1999). The report provided Information on the volunteer consent form and other aspects of the ethical treatment of the subjects such as a statement that the study being conducted in accordance with the Declaration of Helsinki, as amended in 1975, 1983, 1989 and 1996.

HIARC's Review

Study Design: Double-Blind, Randomized Repeated Dose Oral Study

Test Material: 89-92% AI

Subject Selection: Each subject was given a screening examination to assure that there were no medical reasons for exclusion. In general, only individuals in good health and not on drugs (prescription or otherwise), non-smokers and capable of communicating with the medical staff were selected. Objection from the subjects personal physician also was cause to preclude a persons participation in the study. Agricultural workers and pesticide applicators or persons exposed to an anti-cholinesterase agents within a month of the dosing were not eligible. During the study, no alcohol or caffeine or other medications were allowed. The study had an adequate set of exclusion and inclusion criteria.

Treatment Group: 0.25 mg/kg/kg

Control Group: Males: 4 subjects

Number of Test Subjects: Males: 8

Dose Selection: The dose level of 0.25 mg/kg/day was selected based on the results of animal studies and an earlier single dose oral study with human subjects that established a NOAEL of 0.03 mg/kg for plasma and RBC cholinesterase.

Dose Preparation and Administration: Each subject was dosed by capsule containing either the placebo (lactose) or azinphos methyl approximately 5 minutes *after* breakfast for 28 consecutive days.

Endpoint Measurements: Cholinesterase measurements were made on plasma and red blood cells using the Ellman (modified) colorimetric method.

Sample Handling: Blood samples (4.5 mL) were taken by venipuncture each day at screening, on days -14, -12, -10, -8, -6, -4, -2, and -1 (before dosing) and on days 1, 2, 3, 4, 5, 7, 10, 14, 17, 21, 24 and 28 postdosing. The samples were collected in tubes containing EDTA and centrifuged to separate the plasma from the RBCs. These were shipped to IR laboratory at Elphinstone Research Centre on wet ice and assayed the same day. The actual time between sample collection and assay time was not stated and may have been different for each day. Plasma ChE and RBC AChE was assessed following the SOP/CLC/043 using an Hitachi Analyzer. This analyzer utilizes acetylthiocholine as the substrate and is a modification of Ellman's original method.

Clinical Observations/Signs: Adequate monitoring for signs was conducted.

Clinical Pathology: Adequate hematological, clinical chemistry and urinalysis measures were conducted.

Statistical Analysis: Appropriate/adequate statistical analyses were performed.

Evaluation of Data: Clinical observations and signs reported were non-specific and did not correspond to treatment. In addition, clinical chemistries, hematology and urinalysis indicated no effect due to treatment with azinphos-methyl. Twenty eight consecutive days of dosing with 0.25 mg/kg/day did not result in biologically significant changes in plasma or RBC cholinesterase activity. For plasma and RBC, the NOAEL was 0.25 mg/kg/day.

Use of the Study in Risk Assessment: The current chronic RfD for azinphos-methyl is based upon a NOAEL of 0.15 mg/kg from a 1-year dog study; the LOAEL was 0.7 mg/kg/day based on RBC cholinesterase inhibition.

The 10x interspecies UF can NOT be removed when assessing chronic dietary or dermal risk because of the concern for offspring toxicity other than cholinesterase inhibition seen at the same dose (0.7 mg/kg/day) in the one and two generation reproduction studies. The repeated dose study in humans could not provide any information on the relative sensitivity with respect to offspring toxicity.

The dose response data from the human study did demonstrate that humans are no more sensitive than animals with respect to plasma and RBC cholinesterase inhibition based on observations that are comparable between humans and animals as shown below:

The lack of cholinesterase inhibition at 0.25 mg/kg/day is similar/comparable to the NOAELs established in : the 28-day study in rats (0.25 mg/kg/day); the 13-week subchronic neurotoxicity study in rats (0.25 mg/kg/day); the 1-year chronic toxicity study in rats (0.28 mg/kg/day); the 1-generation reproduction study in rats (0.25 mg/kg/day); the 2-generation reproduction study in rats (0.25 mg/kg/day); and the 1-year study in dogs (0.17 mg/kg/day).

IF cholinesterase inhibition were the only known effects of concern, then, this demonstration of comparable sensitivity with respect to cholinesterase inhibition in animals and humans, could be an element in the decision to reduce the 10x inter-species uncertainty factor.

However, the repeated dose study in humans could not demonstrate that humans are no more sensitive than animals with respect to offspring toxicity. The following concerns/uncertainties remain:

- In both the 1-and the 2 generation studies, pup death occurred during early lactation (Days 0 to 5) and decreased pup weight during late lactation (Days 5-28). These findings raise concern for short, intermediate and chronic exposures for females during pregnancy, while nursing, and to nursing infants.

- Pup deaths are most likely due to postnatal exposure and not to *in utero* exposure since there was no fetal deaths were seen in the developmental studies in rats..

- Although the pup death occurred at the same dose (LOAEL=0.75 mg/kg/day) that also caused cholinesterase inhibition in rats and dogs, this may not be the case in humans (i.e., offspring effects may occur at lower doses in humans).

- The current available data do not substantiate that the decreased pup viability seen is directly related to maternal toxicity from cholinesterase inhibition. In the one-generation study, no clinical signs indicative of cholinesterase inhibition (e.g., lethargy, hypoactivity, decreased motor activity, convulsions etc) were seen in maternal animals at the dose (0.75 mg/kg/day) that caused plasma, red blood cell and brain cholinesterase inhibition and at higher doses. An association between maternal toxicity and pup death can not made in the two generation study, since cholinesterase inhibition was not measured either in the maternal animals or the pups in that study. There are no data to indicate that the pup deaths are due to maternal neglect or reduced body temperature in young pups.

- The human study did not include female subjects, and therefore gender differences are also not addressed.

Based on these considerations, the HIARC determined that the inter-species uncertainty factor can not be reduced or removed for chronic dietary and or occupational/residential exposure risk assessments.

On August 16, 2001, the HIARC evaluated the results of a single dose study in human subjects. The results of the single dose study supported the reduction of the inter-species uncertainty factor from 10 to 1 for acute dietary risk assessment. The results of the repeated dose study have no bearing on the acute assessment since the offspring toxicity was seen after repeated exposure in the multi generation studies.