TXR NO. 0050229

DATE: October 26, 2001

MEMORANDUM

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

From:: Jess Rowland, Co-Chair
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To: Margaret Stasikowski,
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On July 16, 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, Elizabeth Mendez, David Nixon, Jess Rowland and Yung Yang
Member(s) in absentia:	Pam Hurley and Brenda Tarplee
Data was presented by:	John Doherty

Introduction

On July 16, 2001, the Hazard Identification Assessment Committee (HIARC) met to discuss the potential impact of consideration of a single oral 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: Single Dose Cholinesterase Inhibition in Human Subjects

Reference: P. McFarlane and S. Freestone, 1998. "A Randomized Double Blind Ascending Single Oral Dose Study with Azinphos-methyl to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity". Inveresk Research, Elphinstone Research Centre, Scotland, Study No.: ICR 0132319, December 21, 1998.
MRID No. 44786901.

Executive Summary: In this special study (MRID No.: 44786901) that assessed plasma and RBC cholinesterase inhibition, as well as signs of clinical toxicity in human subjects, five groups of males were given a single oral capsule dose as placebo, 0.25, 0.50, 0.75 or 1.0 mg/kg of Azinphos methyl. The placebo group consisted of 12 subjects and each group dosed with Azinphos methyl consisted of 7 subjects. Two groups of females were also dosed as placebo (3 subjects) and 0.75 mg/kg (7 subjects). Blood samples (4.5 mL) were taken at pretest (days -10, -8, -4, -2, -1 and -30 min) and post dosing (1, 2, 4, 8, 12, 24, 48 and 72 hours and days 7 and 14) for analysis of cholinesterase. All subjects were examined by medical staff for possible reactions to treatment in this double blind study.

There were no clinical reactions to treatment including effects on pulse, heart rate, body temperature or electrocardiogram (ECG) in repeated assessments made within 24 hours post treatment or obvious signs within 72 hours. Plasma and RBC cholinesterase activity, as assessed relative to each subjects pretreatment baseline mean, varied with decreases by as much as about 11% to increases of the about same magnitude.

No consistent pattern of decrease indicating inhibition by Azinphos methyl was noted and decreases in the 1 mg/kg male dose group were not significantly greater than the placebo or lower dose groups. The NOAEL and LOAEL for both clinical signs and inhibition of plasma and RBC cholinesterase is $\geq$ 1.0 mg/kg in males and $\geq$ 0.75 mg/kg in females for a single oral dose.

Compliance: Statements of Data Confidentiality (no claim of confidentiality under FIFRA), Good Clinical Research Laboratory Practice (for principles of Good Clinical Practice under CPMP/ICH/135/95 for studies with humans and OECD Good Laboratory Practice) and Quality Assurance were provided. The report stated that the study was conducted in accordance with guidelines set out in the Declaration of Helsinki, 1964 as amended by the 29th Medical World Assembly in Tokyo 1975, the 35th Medical World Assembly in Venice 1983, the 41st Medical World Assembly in Hong Kong 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996. Appendix B (41 pages) of the report provides additional information of the ethics committees and on information for subjects and consent form.

HIARC's Review

Study Design: Double-Blind, Ascending, Randomized Single Dose Oral Study

Test Material: 91.6% AI. As stated, however, HPLC analysis indicated 93.6%

Subject Selection: Healthy subjects; 33 males and 10 females. The mean age for males was 32.7 years and for females was 31.0 years. All subjects underwent an adequate pre-exposure screening within 21-days of commencement to assure that they were in good health, not taking medications, females were not pregnant, or had personal habits that would confound the purpose of the study. The study had an adequate set of exclusion and inclusion criteria.

Treatment Groups: Males: 0.25, 0.5, 0.75, 1.0 mg/kg
Females: 0.75 mg/kg

Control Group: Males: 12 subjects
Females: 3 subjects

Number of Test Subjects per Treated Group: Males: 7 subjects per group
Females: 7 subjects per group

Dose Selection: Based upon the results of a previously conducted repeated dose study in humans (NOAEL = 0.29 mg/kg/day) and an acute neurotoxicity study in rats [(NOAEL = 1.0 mg/kg (Registrant); LOAEL= 1 mg/kg (HED)].

Dose Preparation and Administration: Test material was administered in gelatin capsules. Doses were prepared based upon individual body weights (weights provided). Prior to dosing, subjects were given breakfast.

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

Sample Handling: Blood was centrifuged at 3000 rpm for 15 min to separate plasma and RBCs. This supernatant was assayed directly for plasma cholinesterase activity. An aliquot of 100 of the packed RBCs was transferred to an Eppendorf tube and 200 μl of a 1% Triton x 100 solution was vortexes to produce a homogeneous haemolysate. The haemolysate was then centrifuged at approximately 10,000 rpm for 2 min to remove debris. The supernatant containing the RBC acetylcholinesterase was then assayed for activity.

The samples up to four hours postdosing were shipped to laboratory at the Elphistone Research Centre on wet ice and assayed the same day. Samples later than 4 hours post dose were centrifuged, separated and stored frozen overnight before assay. Assay for cholinesterase was by the Inveresk SOP. This method used a Hitachi 717 Analyzer with the Boehringer-Mannheim Cat No.: 124117 test kit. In this assay, acetylthiocholine is hydrolyzed by cholinesterase to yield thiocholine which reacts with dithio-bis (nitrobenzoate) to yield 2-nitro-5- mercaptobenzoate. The latter compound is measured calorimetrically. The actual assay of the plasma and RBC samples was the responsibility of Mr. Jim Milner of the Inversek laboratory. The SOP for the assay procedure was provided.

There was no specific statement that described the number of replicates for each given sample time for each individual. Based on the data tables it appears that only a single sample was run for each time/individual

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. No treatment related effects were observed on plasma or red blood cell cholinesterase activity. For plasma and RBC cholinesterase no inhibition, was observed at the highest dose tested; the NOAEL was 1.0 mg/kg/day in males and 0.75 mg/kg/day in females.

Use of the Study in Risk Assessment: The current acute RfD for azinphos-methyl is based upon a LOAEL of 1.0 mg/kg/day from an acute neurotoxicity study in rats. This LOAEL of 1.0 mg/kg based on plasma, RBC and brain cholinesterase inhibition, in the acute neurotoxicity study is the same as the NOAEL of 1.0 mg/kg indicated in the single dose study in human subjects.

Comparison of the plasma and RBC cholinesterase dose response data for the human subjects with the data from the acute neurotoxicity stuy in rats, the HIARC noted that the curve for the rat was shifted to the left. This observation was interpreted by the HIARC to indicate that for a single oral bolus dose of azinphos methyl, rats were more sensitive for cholinesterase inhibition than humans.

Therefore, HIARC concluded that an UF of 1 would be appropriate to reflect the difference in species sensitivity to azinphosmethyl between rats and humans and thus support the reduction of the inter-species uncertainty factor from 10 to 1.

Six members and the reviewer supported the reduction of the inter-species uncertainty factor from 10 to 1x because there was no indication of greater sensitivity in humans than in rats. In fact, the data suggested that rats were more sensitive. However, two members were concerned that one dose (0.75 mg/kg) was tested in females whereas four doses were tested in males, and therefore, considered the reduction of the inter-species uncertainty factor to a 3x to be more prudent due to concern for gender differences in sensitivity.

The HIARC could not envision any likely use of this data beyond the acute time frame because the relationship between cholinesterase levels in rats and human test subjects following repeated doses could not be anticipated. Therefore, any change in hazard evaluation due to the results of this study would not extend beyond an acute endpoint.

On August 29, 2001, the HIARC evaluated the results of a repeated dose study in human subjects. The Committee determined that the 10x interspecies uncertainty factor can not be removed for chronic risk assessment because of the concern for offspring toxicity other than cholinesterase inhibition seen 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 HIARC concluded that the results of the repeated dose study had no bearing on the acute assessment since the effect of concern (i.e., offspring toxicity) is attributable to repeated dosing and does not result from a single exposure (dose).