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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: **FORTRESS® (CHLORETHOXYFOS). ID #000352-00553**. Review of Protocols for Acute and 28-day Hen Delayed Neurotoxicity Studies (81-7 and 82-6, Respectively) and Acute Rat Neurotoxicity Screening Study (81-8SS).

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I. CONCLUSIONS

TB-I has reviewed the submitted protocols for neurotoxicity studies on Fortress® and has the following comments:

Hen acute oral LD_{50} and delayed acute neurotoxicity studies: The hen LD_{50} study describes a standard assay using 5 animals/dose group plus a second test for evaluating atropine protection at the LD_{50} and 2X LD_{50}. The delayed neurotoxicity study also describes a standard assay and will expose hens to a single gavage dose of Fortress® at doses based on the results of the LD_{50} study. Twelve animals per control group (vehicle and TOCP) and 15 animals/test groups will be evaluated at a single dose level (3/test group for neurotoxic esterase and cholinesterase evaluations at 48 hr following dosing). The protocols describe appropriate studies to satisfy Guideline 81-7. However, TB-I notes the following:

1. The dose levels for the LD_{50} study were not specified; the earlier hen study (MRID 40898702) should provide guidance for dose selection.

2. The vehicle to be used in the LD_{50} and the acute delayed neurotoxicity studies was not specified in the protocol. Corn oil was used in the earlier hen study and has been used in other gavage studies using Fortress®. Other studies have used aqueous solutions of methyl cellulose. TB-I has no clear preference, but corn oil may be preferable since it was used in the other hen study.

3. The Guideline number for the hen LD_{50} and acute delayed neurotoxicity study should be 81-7 instead of 82-6 as noted on the cover page of the protocols.

4. Individual animal data should be included in the study report (not listed under the items to be included in the study report).

5. Care should be taken to ensure homogeneity and accurate concentration of dosing solutions of Fortress®.

Hen 28-day delayed neurotoxicity study: This study would only be conducted if neurotoxic esterase showed significant inhibition in the acute study (greater than 30% of controls) or if delayed neurotoxicity was observed. The protocol describes a standard guideline study using 3 dose levels, with assessment of neurotoxic esterase and acetylcholinesterase at 48 hrs following the last dose (4 animals/group) and neuropathology at 21 days after the last dose (10 animals/group). A TOCP positive control is included. TB-I has the following comments:

1. See Comments 2, 4 and 5, above.

2. Dose selection or a rationale for dose selection were not included in the protocol. Guidelines indicate that hens in a 28-day delayed neurotoxicity study should be tested at 3 doses, with a low dose that causes either minimum toxicity (eg. ED10) or at a NOEL, and the high dose should be a maximum tolerated dose or cause delayed neurotoxicity, but without causing significant fatalities that would compromise interpretation of the data.

Rat acute neurotoxicity screening study: The protocol describes an abbreviated acute neurotoxicity screening study designed to address evaluations missing in the original study: neuropathology and body temperature. A total of 7 rats/sex/dose would be treated with 0, 0.75, 1.3 or 1.75 mg/kg/day (males) or 0, 0.25, 0.50 or 0.75 mg/kg/day (females). Parameters that would be evaluated include daily clinical signs, weekly body weight, weekly body temperature and gross/microscopic neuropathology of 6 animals/sex/dose at 14 days post-dosing. Cholinesterase measurements also had been requested by the Agency but were not included because the Registrant considered the existing database adequate for assessment of

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cholinesterase inhibition by Fortress® and concern that the Agency has not finalized its decision on methods for assessment of cholinesterase inhibition. TB-I has the following comments:

1. TB-I has no objection to waiving functional observational battery and motor activity testing in this study since adequate data were already submitted (MRID 42559210).

2. TB-I has no objection to the proposed dose selections for males and females, based on the results of the previously submitted rat acute neurotoxicity study and its range-finding study.

3. The study should include animals for measurement of plasma, red blood cell and brain cholinesterase. Because of the variability in effects noted in different studies at similar doses, inclusion of cholinesterase assessment will assist in interpretation of the data and provide some indication of degree of inhibition associated with neuropathology, if present, and to neurobehavioral signs seen in the earlier acute neurotoxicity study. Although data are available for plasma and red blood cell cholinesterase following a one-day dietary exposure, there are currently no data available on inhibition of brain cholinesterase following an acute exposure to Fortress®. TB-I notes that assessment of regional brain cholinesterase inhibition is NOT required for this study.

4. Care should be taken to ensure homogeneity and accurate concentration of dosing solutions of Fortress®.

5. TB-I recommends inclusion of additional observations for clinical signs on the day of dosing around the time of peak effect since no neurobehavioral testing will be conducted.

II. ACTION REQUESTED

TB-I received for comment study protocols for acute and 28-day hen delayed neurotoxicity studies and for an acute rat neurotoxicity screening study (submitted November 9, 1995 by Linda Carter of DuPont de Nemours and Co., Inc.). These studies were required following the HED RfD/Peer Review Committee's evaluation of the toxicology database for Fortress®. Evaluation of neurotoxic esterase in hen was required, which was not conducted in the original hen acute delayed neurotoxicity study. A new rat acute neurotoxicity study that included neuropathology, body temperature measurements and plasma, red blood cell and brain cholinesterase measurements was also required since these parameters were not evaluated in the original study.