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

JUN 18 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 8340-17: Review of protocols for a 90 day dog study, 13 week rat study, 2-generation rat reproduction study, and chromosome aberration study with triphenyltin hydroxide.

TOX CHEM 896E

FROM: J.D. Doherty 6/18/85
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TO: H.A. Jacoby, Product Manager #21
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THRU: E.R. Budd, Section Head
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Background:

The American Hoechst Corporation has requested Toxicology Branch (TB) review four protocols related to the toxicity testing of the fungicide triphenyltin hydroxide (TPTH). These studies are apparently being conducted to satisfy data requirements indicated in the Registration Standard prepared for TPTH.

TB Response:

The specific comments for each study are as follows.

1. 90-day Dog Study with 28-day Recovery Period. [Protocol dated May 13, 1985 and prepared by WIL Research Laboratories]

The registrant should be informed that the proposed 90 day dog study will not fulfill the requirement for a chronic feeding study in a nonrodent species in support of permanent tolerances on major crops for human and livestock consumption. TB recommends that the registrant conduct the study, with appropriate modifications, for one year or longer in order to satisfy this requirement since they apparently intend to request establishment of permanent tolerances for TPTH.

This one year or longer chronic feeding study should clarify the

potential of TPTH to cause brain and spinal cord edema. Dr. Louis Kasza, Pathologist, TB has made the following recommendations to assess the potential of TPTH to cause edema in the nervous system:

i. The study should include interim sacrifices of 2 dogs per sex per group at 1, 3 and 6 months in addition to the terminal sacrifice at 1 year (or later).

   Thus, 10 dogs of each sex per dose group should be available at the initiation of the study so that after the interim sacrifices there will be 4 dogs of each sex at each dose level scheduled for terminal sacrifice.

   [Note: Inclusion of additional dogs for a recovery phase is not required by TB. The sponsor is welcome to include this aspect of the study if they choose. TB currently gives equal weight when setting the ADI to lesions whether or not they are reversible after discontinuing dosing.]

ii. The nervous system should be evaluated by a neuropathologist with experience in assessing edema of the nervous system. The neuropathologist should also assist in designing the aspects of the study involved with assessing the edema status.

iii. The nervous system should have sufficient slides prepared for microscopic analysis to indicate that the spinal cord and brain (including the ventricular system) were carefully assessed for edema.

Other comments

1. Dosing the dogs via gelatine capsule is preferable to incorporating the test material into the diet.

   If the registrant chooses to administer the test material in the diet, it is suggested that daily determinations of the amount of food and test material consumed should be made for each dog. The average daily dose in mg/kg/day per dog should also be calculated and presented.

   No mention was made in the protocol as submitted regarding which dose levels would be used in the suggested 90-day dog study. It is the study sponsor's responsibility to select dose levels so that the highest test dose level will show signs of toxicity and the lowest test dose level no signs of toxicity.

2. TB requests that the tissue levels of tin be determined for each dog at each sacrifice time including interim and recovery (if included) groups. In particular, at least the following tissues should be included: brain, liver, lung, body fat,

kidney, thymus, spleen, testis, ovaries, uterus, bone and bone marrow, blood, and any organ showing pathology and other organs/tissues which the sponsor or tester feels appropriate. Urinary levels of tin should also be monitored periodically.

3. Lastly, if a 90 day dog study is conducted, similar examinations of the nervous system and determinations of tissue levels of tin should be made at the end of the first and second months and at termination of the study on similar numbers of test animals.

2. 13-Week Rat Study with a 13-Week Recovery Period. [Protocol dated January 24, 1985 and prepared by International Research Development Corporation].

The registrant should be informed that a 2-year chronic feeding/oncogenicity study in rats is required to support establishment of permanent tolerances for TPTH. This proposed 90-day subchronic feeding study in rats will not fulfill this requirement. The 2-year chronic feeding/oncogenicity study should be performed in accordance with EPA Subpart F Guidelines for this study type. Additional considerations for this study include:

i. Systematic examination of the brain and spinal cord by a neuropathologist. See comments on the dog study above. The neuropathologist should assist in designing the protocol.

ii. The organs/tissues and urinary levels of tin should be determined. See comments on the dog study above.

iii. Regarding the proposed 90-day rat study:

a. The recovery phase is not required by TB but the sponsor is welcome to include this aspect of the study if they choose.

b. The study protocol contains an inconsistency regarding dosing the rats. On page 3 it is stated that the test material will be mixed into the diet. This implies that the rats will be dosed at a constant ppm level in their diets but that the quantity of TPTH received by each rat will depend upon the amount of food each rat consumes.

On page 4 of the protocol, however, it is stated that the "test article will be administered at dosage related to body weight throughout the 13 week study". Still later in the protocol (page 6) it is stated that the dosage will be in "mg/kg/day". Rats that are dosed at a constant ppm in the feed will not at the same time be dosed at a constant mg/kg/day.

The sponsor or testing laboratory should clarify their procedure for dosing the rats.

c. The dose levels to be used in this proposed study were not provided. It is the sponsors responsibility to select the appropriate dose levels.

3. A Dietary 2-Generation Reproduction Study in Rats with Triphenyltin Hydroxide. [Protocol dated March 8, 1985 and prepared by WIL Research Laboratories].

i. This study also has inconsistencies in describing the dosing procedures. On page 5 it is stated that the route of administration will be dietary (oral). On page 6 the dosage levels are listed as "mg/kg/day" in the protocol table. Part 3 (page 6) states that the diets will be administered ad libitum. Part 4 (again page 6) states that the test article will be administered as a constant mg/kg in the diet.

The sponsor (or testing laboratory) should clarify how they intend to dose the rats.

ii. A previous rat reproduction study indicated that the testis and/or spleen of the pups are possible target sites for TPTH. Because there may be organ weight and/or pathological changes in the pups, TB requests that the major organs (especially including but not limited to the testes and spleens) of at least 20 pups of each sex per dose group be assessed for organ weight changes and histologically examined. Pertinent data which should be submitted for each rat pup include body weight, organ weights, organ to body weight ratios, and histopathological examination of the organs.

iii. Several of the rat pups from each generation should be assessed for tin content in selected organs to determine if TPTH (or its metabolites) passes the placenta and accumulate in the pups.

iv. It is the sponsors responsibility to select the appropriate dose levels.

4. Mutagenicity [These comments were made in conjunction with Dr. I. Mauer, Geneticist, TB].

i. Review of the protocol for the study entitled "Chromosome Aberration in Chinese Hamster Ovary (CHO) Cells". [Protocol dated May 9, 1985 and prepared by Microbiological Associates].

Dr. Mauer made the following comments:

"Protocol appears to be generally adequate to generate valid results, except I recommend:

(1) More than one harvest time, especially if significant cell cycle delay is anticipated or evident".

ii. In response to the reference in Mr. Lawatsch's letter regarding other mutagenicity studies, TB requests that a study assessing primary DNA damage (i.e. sister chromatid exchange or unscheduled DNA synthesis) be conducted and submitted.

5. Immunotoxicity. Mr. Lawatsch's letter (May 16, 1985) refers to discussing a study design for the "immuno tox study".

At the present time TB does not have a specific protocol for an immunotoxicity study. The immunotoxicity potential of TPTH is being reevaluated by another Division of the Office of Pesticides and Toxic Substances at TB's request. Additional studies (if any) will be requested pending receipt of the reevaluation.