

US EPA ARCHIVE DOCUMENT



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

APR 12 1995

#272E

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Cyproconazole: 13-Week Feeding Study in Rats
Registrant's Response to TB II Review

TO: Carl Grable
PM Team Reviewer (21)
Registration Division (7509C)

FROM: Linda L. Taylor, Ph.D. *Linda L. Taylor 4/5/95*
Toxicology Branch II, Section II
Health Effects Division (7509C)

THRU: K. Clark Swentzel *K. Clark Swentzel 4/6/95*
Section Head II, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. *Marcia van Gemert 4/11/95*
Chief, Toxicology Branch II, HED (7509C)
Sandoz Crop Protection Corporation
 α -(chlorophenyl)- α -(1-cyclopropylethyl)-1H-
1,2,4-triazole-1-ethanol
Cyproconazole Technical

Registrant:

Chemical:

Synonyms:

Caswell No.:

Case:

Submission:

Barcode:

Identifying No.:

Shaughnessey No.:

MRID No.:

Action Requested: Please review company response to review of
second 13 week rat subchronic study [MRID # 430786-01].

Comment: The Registrant has submitted a response to the questions
raised in the TB II review [DER dated 3/28/94] of the 13-week rat
feeding study. The study was classified Core Minimum.

The Registrant addresses three issues: (1) "Inadvertent Fasting";
(2) Terminology Regarding Microscopic Findings; and (3) Dose
Selection for a Repeat Chronic Study.

(1) TB II requested additional information regarding the
"inadvertent fasting" that occurred on several occasions during the
study. There is no discussion of why the quantities of food



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

provided were not adequate on several occasions nor why this occurred at four different intervals during the study. The rats were housed 2 of one sex per cage, and each cage was provided with feed once a week. In the Methods section of the final report [MRID #430786-01], it states that food consumption was calculated as the difference in food provided and food remaining at week's end. Tables 9 [σσ] and 10 [ϑϑ] of the submission list the quantity of food provided/cage/week, and the * next to the values signifies that the amount was less than 300 grams. It is not apparent to this reviewer how the amount of food to be provided each week was determined, but it seems to vary each week and among the groups for both sexes. It appears that after the first "inadvertent fasting" at the 3-4 week interval 300+ grams were provided to all groups, which resulted in body-weight gains in all groups. The Registrant does not explain why amounts of food of less than 300 grams/cage/week were provided subsequently to all groups during an additional 3 weeks. Additionally, Tables 11 and 12 of the submission [Estimated Mean Duration of Fasting] list the number of hours/week that food was not available for the 4 time intervals of the "inadvertent fasting", but it is not apparent whether these were consecutive hours without food or the total number of hours without food within the 168-hour week. To illustrate, 16 hours is listed for the control males during the interval week 1-2, which if consecutive would be like an overnight fast. For the high-dose females [Table 12], 52 hours are listed, which if consecutive would be greater than 2 days. Since the rats were observed twice a day during the week and once a day during the weekend, one or more empty food containers should have been noticed. Additionally, it is not apparent why all of the cages were provided with inadequate amounts of food on three separate occasions, and the "duration" of fasting among the groups/sex during any one of these occurrences varied by as much as 22 hours. Another unknown is the relationship between the fasting and the measurement of body weights. The intervals weeks 1-2 [controls/both sexes], and weeks 3-4, 9-10, and 11-12 [all groups/both sexes] are listed as the intervals in which the "inadvertent fasting" occurred, and the grams of food/cage/week values for these time intervals range from ≈261-291 [σσ]/≈254-288 [ϑϑ]. For the interval weeks 10-11 for both sexes, the amount of food/cage/week for all groups/both sexes is also below 300 grams, but this interval was not included in the "inadvertent fasting" because "bodyweight development and mean food conversion were not affected"; the duration of the "fasting" for this time period is not provided. Since this was only a 13-week study, this lack of attention to detail [observation of food hoppers during the week to ensure an adequate supply of food; the occurrence of more than one interval where adequate food was not provided] compromises the **quality** of the data being relied on to support the use of lower dose levels than considered appropriate by the Agency for the repeat **carcinogenicity** study. Without the added stress of fasting, the body-weight decrements in the 700 and 1400 ppm dose groups may not have been of as great a magnitude as were observed in this 13-week study.

(2) With regard to the inconsistency in the terminology used by the Study Director and the pathologist, it is stated that the Study Director compiled the histopathological findings in a "toxicological context", choosing necrosis with centrilobular bridging as the important primary event, which was followed by post necrotic scarring [term used by pathologist]. The terms bridging and scarring were used synonymously to describe post necrotic formation of fibrous tissue.

The slightly different terms used by the pathologist [e.g., foamy macrophages, vascular macrophages, etc.] were used synonymously. It was also noted that no results of the immunostaining of selected slides for Factor VIII were obtained because the available antibodies did not work in the formalin-fixed tissues and no frozen or adequately-fixed tissues are available from this study.

(3) The Registrant discussed dose-level selection for a repeat "chronic" study. TB II points out that a repeat carcinogenicity study is the required study. As part of the discussion, comparison tables of data from the rat carcinogenicity study and the 13-week study in question were provided, the purpose of which [apparently] is to demonstrate that for several of the measured parameters comparable values were observed among the control, the 20 ppm, and the 350 ppm dose groups in both studies.

The bottom line is that, due to the unfortunate occurrence of "inadvertent fasting" at several intervals during the 13-week study, which was performed to justify dosage levels used in the original rat carcinogenicity study and to justify the choice of dose levels for the repeat carcinogenicity study, little confidence can be placed in the body-weight effects observed at 700 and 1400 ppm; i.e., the added stress to the high-dose rats due to "inadvertent fasting" may have accentuated their condition and the magnitude of the body-weight effects. Fasting can have a wide range of effects on the body, and although all dose levels as well as the controls experienced "inadvertent fasting", the controls and lower dose level groups were not subject to the additional stress placed on the body by doses of test material that induced adaptive reactions. This 13-week study does not justify the choice of 700 ppm as the high-dose level to be used in the repeat carcinogenicity study.

DISCUSSION: The effects observed in the 13-week study in question, as well as in previous studies, are mainly adaptive processes. Toxic effects per se are not observed. The magnitude of the body-weight deficit at the high-dose level [1400 ppm] at termination in males [\approx 83% of control] and females [\approx 88% of control] is, for the males, slightly above the high-end of the range [10-15%] considered appropriate for MTD assessments. But, given the uncertainty regarding the effect of the added stress ["inadvertent fasting"] at the high-dose levels, especially in males, a convincing case against the use of the 1400 ppm dose level as the high dose has not been established.

A factor that needs to be addressed is that of palatability. All previous feeding studies have incorporated dose levels only as high as 500 ppm in the diet. During the first week of dosing in the 13-week study in question, at least at the 1400 ppm dose level, the rats were ingesting less food than the control groups, which may have been due to palatability.

With regard to the repeat of the rat carcinogenicity study, as an alternative approach, a fourth dose level could be incorporated [e.g., 1000 ppm] to ensure an adequately high dose were the 1400 ppm dose level to prove to be excessive. Or, an additional group of pair-feed rats could be run for comparison with the 1400 ppm dose level rats. Another option is to repeat the 90-day study, paying close attention to details, including but not limited to providing adequate food every day to each rat.

CONCLUSION: The Registrant has addressed the questions raised in the TB II review of the rat 13-week study. Given the uncertainty regarding the effect of the added stress ["inadvertent fasting"] at the high-dose levels, especially in males, a convincing case against the use of the 1400 ppm dose level as the high dose for the repeat rat carcinogenicity study has not been established. The 13-week study does not justify the choice of 700 ppm as the high-dose level to be used. The Registrant has several options as to how to fulfill the data requirement, which include (1) incorporating a fourth dose level such as 1000 ppm to ensure an adequately high dose were the 1400 ppm dose level to prove to be excessive; (2) incorporating an additional group of pair-feed rats for comparison with the 1400 ppm dose level rats; (3) repeating the 90-day study, paying close attention to details and assessing palatability.