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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

Subject: EPA ID No.: 111901-043813; Imazalil - Company Response to Upgrade (MRID# 429494-02) Data on a Study of Reproduction in the Rat/2337 (83-4).

ToxChem. No.: 497AB. Submission No.: S451271.
PC Code: 111901. Case No.: 816389.
Cas Registry No.: 35554-44-0. MRID No.: 429494-02.
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From: David G Anderson, PhD. David G Anderson 5/26/94
Toxicologist, Section 3
Toxicology Branch-1
Health Effects Division (7509C)

To: Kathryn Davis/Katheen Depukat PM 52
Special Review Branch
Special Review and
Registration Division (7508C)

Thru: Karen Hamernik, PhD. 5/26/94 KB 5/21/94
Section Head, Section 3
Toxicology Branch-1
Health Effects Division (7509C)

CONCLUSIONS:

The problems associated with effects of imazalil on 2-generations of reproduction in the rat are resolved and the NOEL/LEL can be set. The NOEL/LEL for parental toxicity is 5/20 mg/kg/day based of body weight decrement in gestating and lactating mothers. The reproductive NOEL/LEL is 20/80 mg/kg/day based on increased gestation time in the F1 females and reduced litter sizes at birth and increased pup mortality from birth to day 4 in the F1 and F2 generations.

All responses were not adequate. However, since most of the information requested was an attempt to understand the statistically significant apparent effects on pups at all dose levels and this problem has been resolved, the information requested is not necessary. The apparent decreased F1 pup survival at all dose levels was not real because there was a strong litter effect in the data and the registrant based the statistical analysis on the fetus. A NOEL can be set for the study because additional statistical analyses on pup mortality by litter was significant only at HDT.

(1)

The variation in the survival of pups in the study and in the historical control data submitted is excessive. The testing laboratory should consider determining the cause of this excessive variability.

BASES FOR THE CONCLUSIONS:

The registrant has submitted a 2-generation study on reproduction in the rat (83-4) with imazalil {1-[2-((2,4-dichlorophenyl)-2-propenyloxy-ethyl]-1H-imidazole}. Toxicology Branch-1 (TB-1) raised questions about this study impacting on the NOEL/LEL. The sponsor has responded to these questions in MRID# 429494-02. The question will be presented first, followed by the sponsor's response and Toxicology Branch-1 response to the response. TB-1 responses to the questions are for sponsor informational purposes only, since the study is accepted as core minimum. The questions refer to the following report.

> P Dirkx and Herman Van Cauteren. R23979 - Imazalil: 2-Generation Reproduction Study with 1 litter per Generation in Wistar Rats, conducted for Jenssen Pharmaceutica, William Goodwine, Agent at Department of Toxicology, Janssen Research Foundation, 2340 Breese, Belgium, study date-October 26, 1992, study No. 2337 (MRID# 425707-01).

Question 1: Historical control data on the non-inbred strain of Wistar rats used is needed on ring-tail in pups, on the pup survival and body weight to weaning. The data may include data after 1991, but no more than 5 years before 1991.

Sponsor's Response 1a to Question 1:

There are no historical control data for ring tail in rat pups from the testing laboratory, however, the cause is unknown since the literature states that ring tail is caused by a < 20% or < 40% humidity, depending on the source, and the monitored humidity was never below 40%. In addition, the ring tail was decreased with increasing dose level and thus it was not compound related and was limited to the first generation.

> Toxicology Branch-1 (TB-1) Response:
>
> The explanation is not accepted. Information on the temperature and humidity was submitted for only 1 of the 2 animal rooms used in the 2-generation reproduction study (See response to Question 2). For additional TB-1 response to Question 2, see below. For some reason, the sponsor has failed to supply part of the information requested.
>
> TB-1 is aware of the literature reports on ring-tail. The request for historical data on ring tail was requested in an attempt to understand some of the variables in the treatment of the groups, such as environmental conditions, that may have resulted in the statistical significant

increased mortality at 5 mg/kg/day, especially in the F1 generation pups. Unfortunately the sponsor has not supplied this information.

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Sponsor's Response 1b to Question 1:

(a) The historical control data on 10 segment III studies (peri and postnatal studies) and 2 reproduction capacity studies (RCS) (combined Segment I, II and III studies with a non-dosed 2nd generation). The historical control data indicated that pup body weight varied by a small amount, but that pup survival on day 4, 14 and 21 varied considerably. (b) The F1 pup survival was low in the 5 and 20 mg/kg/day dose levels compared with concurrent controls, but this data should not be over estimated in view of the wide range of the historical control data. The survival rate at 5 mg/kg/day was within historical control limits and is not considered a test material related effect. Similar effects were not seen at 20 mg/kg/day in F2 pups. Abnormal mean litter size (decreased), live and stillborn were seen only at 80 mg/kg/day in F1 pups. (c) Furthermore, if it were a test material related effect it should be more pronounced in the second generation since the dams were dosed longer than the first generation.

(d) There were no signs of disease, genetic factors or incidental random variation in the conduct of the study. The stability and homogeneity of the test material and concentration in the test diet were checked and the response is under Question 5.

Toxicology Branch-1 Response:

A higher LEL for F1 pup survival on day 4, 14 and 21 at 5 mg/kg/day is accepted, although the explanation is only partially accepted.

The submitted historical data submitted is of marginal value in evaluating the variability of the data from the 2-generation study on reproduction under consideration because the data base for historical control are from segment III studies that cover day 15 of gestation and lactation only. Thus, the studies are not exposed for a sufficient duration to the testing laboratories environmental conditions and other variables that may affect control values and results of a study.

Other differences occurred in the mean body weight of the animals used. The mean body weight of the animals in the historical control data were 200.2 g on day 1 of pregnancy (mean of 10 sets of historical controls) compared with a body weight of 323.7 g on day 1 of pregnancy from the current control group in the 2-generation study of reproduction under consideration.

After reconsideration of the statistical treatment of the pup survival data by the registrant, TB-1 found that the variances among groups were not equal and that the statistical treatment of the data was inappropriate. The statistical treatment was inappropriate because the incidence of survival (mortality) was analyzed without consideration of the litter, i.e., the data should be

analyzed by litter because a fetus from one litter may have a different level of risk than a fetus from another litter. TB-1 (Roger Gardner) recalculated the data using F1 and F2 pup mortality at day 0-4, 4-14 and 14-21 on a litter basis using the more appropriate Mann-Whitney U and Kruskal Wallis tests for statistical significance of the proportion of each litter not surviving. These latter tests indicated that the only statistically significant increase in pup mortality occurred in F1 and F2 pups at 80 mg/kg/day between birth and day 4 of lactation. Thus, the NOEL is 20 mg/kg/day for pup mortality, but since the pregnant and lactating mothers demonstrated a statistically significant body weight decrease, the 20 mg/kg dose level is considered an effect level. Thus, the revised NOEL/LEL is 5/20 mg/kg for the 2-generation study on reproduction with imazalil (MRID# 425701-01).

In addition, the effects on pup mortality at the 5 mg/kg were not consistent with effects at this dose level in the F2 generation, although the reduced litters sizes at the F1 HDT probably caused more resistent F1 animal selection at this dose level. Additionally as the registrant pointed out, the F1 adults were dosed longer than the P0 adults and it would be more reasonable to expect effects at lower dose levels in the F2 than in F1 pups, which was not shown by the data in the submitted report.

Question 2. The temperature, humidity and lighting in each animal room used through out the P0 and F1 generations, the groups housed in separate rooms (animals and groups identified with the room used) and the number of animal rooms used for the study must be submitted.

Sponsor's Response to Question 2:

Two animal rooms were used (Nos. 005 and 007). Exhibit 2, presented the data on the temperature and humidity in room 005, which varied from a minimum of 19 °C to a maximum of 27 °C and 40% to 64%, respectively. Lighting was 12 hours light and 12 hours dark from 6:00 AM to 18:00 PM as described in SOP/GEN/086/2 on page 7.

Toxicology Branch-1 Response:

The data requested is not complete. Conditions in only 1 of the 2 animal rooms used were submitted and data on the room identified with animals housed was not submitted for either room. However, the data was primarily requested to help understand the decreased pup survival at the 5 mg/kg dose level. Since TB-1 no longer believes effects occurred at this dose level, the requested information is no longer necessary. Perhaps the conditions in the animal room 007 with no data submitted is the reason for the variability in pup survival.

Question 3. The rationale for not mating some selected F1 males for the F1 generation must be submitted. How were the males selected for the mating trials? This is especially important because the fertility of males dosed in utero were not adequately studied since some F1 offspring selected as parents were not selected for breeding and some of the selected males were bred more than once. The rationale and an explanation of the selection method must be submitted.

Sponsor's Response to Question 3:

For mating the F1 parental animals, 4 F1 males and 4 F1 females were randomly selected per litter and raised to maturity. After a 3-month growth period animals were mated until 24 females were sperm positive per dose. In the high dose level group, only 14 matings occurred because of the poor survival in this group. This was also the same reason males were bred twice and three times in this group.

Toxicology Branch-1 Response:

The response is accepted.

Question 4: Please supply the body weight data, food consumption data (if available), clinical observational data and summary tables for the F1 males throughout dosing until sacrificed. It is recognized that the data in the highest dose group may not be meaningful because of food wastage.

Sponsor's Response to Question 4:

All F1 males were weighed at birth and on day 4, 14 and 21 after birth. Afterwards no further data were recorded, except for F1 females from Day 1 of pregnancy till day 21 of lactation. Food consumption data was not recorded. Clinical observations were done daily (see initialed activities by biotechnicians as shown in exhibit No. 3). Records for F1 males were only made if abnormal behavior or occurrence of untoward clinical effects were noted. Since none occurred none were noted.

Toxicology Branch-1 Response:

The response is not complete, however, since a NOEL/LEL can be set the data are not necessary.

For informational purposes, the guidelines require at least weekly body weights on all parental animals and daily observations. The failure to obtain weekly body weights of F1 males used for mating is a violation of the guidelines. No initialed activities of any biotechnicians were submitted in exhibit no. 3. Thus, the only evidence that observations were conducted on F1 animals are the written sponsor's response to the question.

Question 5: Please submit the analyses for homogeneity and stability of the test material in the dietary preparations used. Please indicate the dates that each dietary preparation was administered to the animals and analytical concentration data for that dietary preparation.

Sponsor's Response to Question 5:
In exhibit No. 4, a list of the preparation dates of all test article/food admixtures, together with the first day of administration, are given. Analysis on homogeneity and stability was performed on samples of mixtures taken periodically through out the study. The results are included in the report. Homogeneity was not performed on each mixture.

Toxicology Branch-1 Response:
The response is accepted. The dates of the preparation and administration of the diets and diets analyzed were submitted. The results of the dietary analyses were submitted in the original report. Diets were prepared 13 times and analyzed 5 times during the study. Analyses were within an acceptable 91 to 118% of nominal. Only 2 analyses were conducted about the time of gestation and lactation for the F1 litters and once during early lactation for the F2 litters, and no significant deviations of the concentration were noted. Thus, the comparison of dosing during these periods appears to be acceptable.

Question 6: Please clarify the Tables 12 through 19, page 000060 through 000067. There is a discrepancy in the designated sex between Table 20 and the text at the top of the page for Tables 12 through 19. Animal numbers 1 through 114 refer to female animals whereas the Tables 12 through 19 indicate the animal numbers refer to male animals. Also the mean body weight gain within Table 10, page 000058 indicates female animal numbers when these numbers are referred to as male animal numbers outside Table 10 and in the text, page 000024. Please clarify these tables and any other discrepancies occurring.

Sponsor's Response to Question 6:
The discrepancies in Tables 10 and 12-19 concerning the sexes mentioned were based upon typing errors. The corrected Tables are corrected and enclosed as exhibit No. 5.

Toxicology Branch-1 Response:
The requested corrected Tables requiring clarification were submitted and are accepted.

Question 7. There appears to discrepancies in histological mean scores between Tables 56, 57, 58, 59 and 60 and the Tables on individual animals data in Tables A 8.1 through A 8.36, page

000532 to 000567. Perhaps the apparent discrepancy would be explained if the sponsor would please explain the method used to compute the mean histological scores in Tables 56, 57, 58, 59 and 60.

Sponsor's Response to Question 7:
The mean histological scores per dosage group were calculated by counting the individual scores of the animals and dividing by the number of animals in the group.

Toxicology Branch-1 Response:
The explanation is acknowledged and accepted.

8. Other information and data may be requested depending on the response to the requirements in 1 to 6.

The study of the effects of imazalil on reproduction (MRID# 425707-01) is upgraded from core supplementary to core minimum. The revised summary for study follows.

Revised Conclusions on the Two-generation Study on Reproduction (The submitted 6(a)(2) Data : Imazalil was administered in the diet to a non-inbred strain of 24 Wistar rats per sex at approximately 0, 5, 20 or 80 mg/kg/day for 60 days prior to mating, through mating, gestation and lactation. The F1 generation was administered the same dietary concentrations for similar periods. Mating was approximately 1 male to 3 females only in the second generation at the HDT. Twenty-four F1 females were mated at the lower dose level, but only 14 matings were conducted for the mating producing the F2 pups at the 80 mg/kg/day dose level.
Study starting date was 1/31/91 and completion date was 9/30/91.

Parental toxicity:
NOEL: 5 mg/kg/day.
LEL: 20 mg/kg/day (HDT) for body weight decrement in F1 gestating females (93% of controls at day 1, $p \leq 0.05$, and 93% of controls at day 22 of pregnancy, $p \leq 0.01$) females. At 80 mg/kg, P0 male body weight gain decrease (90% of controls, $p \leq 0.05$) and body weight decrease (95% of control, $p \leq 0.05$) and P0 (94% of controls) and F1 female body weight gain decrement during gestation (76% to 80% of controls) and lactation (94% to 92% of controls, $p \leq 0.05$ and $p \leq 0.0.01$). Food scattering (wastage) by females at the HDT negates food efficiency calculations. Increased liver vacuolation occurred in P0 males (11/24 vs. 0/24 in controls, mean score 0.5, $p \leq 0.05$) and possibly in F1 males (1/7 vs. 0/20 in controls, mean score 0.14, $p \geq 0.05$).

Reproductive toxicity:
NOEL: 20 mg/kg/day

LEL: 80 mg/kg/day for increased duration of gestation for the P0 (104% of controls) and F1 females (105% of controls).

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Offspring toxicity:
NOEL: 20 mg/kg/day
LEL: 80 mg/kg/day (MDT) for mortality of the F1 and F2 pups from birth to day 4 of lactation.

Core classification: Minimum. The study is acceptable for a guideline (83-4) study for effects on reproduction in the rat.

Memo on Sponsor's responses and Toxicology Branch-1 responses on data on Repro/Imazalil/2337/D185810/425707-01/A:\IMAZAL49.7AB\MCOREREP.RAT/DANDERSON/1/21/94.*(Edited 2/24/94& 5/26/94)*.