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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: EPA ID # 7969-57. Review of a Protocol on a Proposed Oncogenicity Study in the Mice, Study No. 80S0375/88092 (83-2) and the Rat, Study No. 71S0375/88027 (83-2) with Vinclozolin (technical). Sponsor: BASF.

Tox. Chem. No.: 323C.
Project No.: 0-0395/Mice.
Record No.: 257078.
Project No.: 0-0396/Rats.
Record No.: 257079.

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Attachments

CONCLUSIONS:

The data presented do not appear to support the proposed MTD of 5000 ppm for the oncogenicity study in mice (Study number 80S0375/88092). Additional information is needed (See section on Data submitted for Mice). However, the data submitted appear to support a dose level of < 4500 ppm for rats. The proposed MTD for rats is 3000 ppm which may be adequate to demonstrate a MTD. Both of these proposed MTD dose levels in mice and rats are problematical because definitive toxic effects determining a MTD were not produced and the body weight gain decrement occurred in conjunction with reduced food consumption. In the mouse study the sponsor may wish to consider revising the proposed dosage regimen to include a group dosed at the limit dose of approximately 7000 ppm (1 g/kg/day). This may be especially important considering that the 5000 ppm dose level probably would not be a MTD for females and may not be for males. (The dosing regimen for males and females are not required to be the same.)

The registrant should submit the histology results on the livers of the mice from the 90-day study in mice, if they wish to support a MTD of 5000 ppm.

In considering a dosage regimen for an oncogenicity study, it is good practice to include an intermediate dose level of one half the highest dose level. Under these circumstances if there is excess mortality in the highest dosage group the Agency will accept the next lower dose level provided it is no more than one half the highest dose level causing the excess mortality.

In studies where food palatability appears to cause problems with the interpretation of the MTD, the followigng solution is suggested. Mask the taste of the test material by incorporation of microencapsulated test material into the animal feed. Others have successfully used this procedure and published on the method.

ACTION REQUESTED: The Registration Division has requested a review of a protocol and dose selection for an oncogenicity study in mice (Study number 80S0375/88092), (Guideline number 83-2), (HED Project Number 0-0395), and concurrence on the MTD for an oncogenicity study in rats (Study number 71S0375/88027), (Guideline number 83-2), (HED Project Number 0-0396).

DATA SUBMITTED FOR MICE:

The Toxicology Branch does not routinely review protocols, however comments on the MTD are appropriate. The data submitted on a 3-month feeding study at 0, 100, 1000, and 5000 ppm in C57BL mice (Study Number 53S0375/88054) do not support the adequacy of the MTD of 5000 ppm for the proposed oncogenicity study in mice (83-2). The proposed dose levels were 0, 40, 200, 1000 and 5000 ppm. Consideration should be given to revising the proposed dosage regimen to include a dose at the limit dose of approximately 7000 ppm (1 g/kg/day) and one dose at one half the limit dose level besides the required lower dose level(s).

This data and information from the 3-month study included apparently increased final body weight and apparently decreased food consumption including percentage of controls, increased alanine-aminotransferase (ALT) and alkaline phosphatase (AP), decreased triglycerides and cholesterol, increased liver weight and centrilobular hypertrophy of hepatocytes, increased adrenal weight and lipid vacuolation of the adrenals, Leydig cell hyperplasia, and lipogenetic pigment. These data and information submitted are summarized below (In addition, see the attached tables copied from the submitted report).

The body weight gain decrement does not demonstrate sufficient toxicity for the MTD in males or females. Additional data are necessary as indicated below. The body weight data possibly could demonstrate that a MTD would be reached for males but not as presented and it appears that the body weight data

does not demonstrate that a MTD would be reached for females. The centrilobular hypertrophy of the hepatocytes, and the liver weight increase may indicate liver enzyme induction and is not an adequate basis for an estimation of the MTD. The increase in ALT, and decreased triglycerides in males and females would tend to support the possible liver toxicity reported, however, definitive liver pathology would require histological evidence. Dose levels indicating significant lipid infiltration, necrosis or other significant toxicity of the liver is necessary.

The remaining effects noted do not indicate sufficient toxicity to be assured that the MTD would be reached since the adrenal, hormonal, testicular, and ovarian affects apparently were not life threatening, at least from the data presented and other data in the Agency's files on Vinclozolin. If there are data indicating that these effects are life threatening, these data would have a direct bearing on the adequacy of the MTD and should be submitted. The reference to decreased glucose in males and increased total serum protein and globulin in males may not be related to sufficient toxicity. The comment about a toxic interference with lipid metabolism has not been adequately demonstrated.

1. The body weight of males is 92% of control values in males at the 5000 ppm dose level, but food consumption is 88.5% of control values. When weight of the liver is subtracted from the male body weight, the percentage body weight over controls in males is 89%. Female body weights were 100% of control weights at the 5000 ppm dose level. Since weekly food consumption and initial body weights were not presented, it is difficult to determine whether the decrease in body weight gain was because of toxicity or food refusal. Data are needed on weekly food consumption and weekly body weights to determine whether or not the body weight gain decrement is because of reduced efficiency of food utilization or because of food refusal. In addition, initial body weights are necessary to be able to calculate the percentage of the body weight gain decrement. The percentage body weight gain decrement should be 10-15% for each sex in the absence of reduced food consumption, or other definitive toxicity must be demonstrated. The Toxicology Branch calculates percentage body weight gain decrement by the following equation:

% body weight gain decrement = [(BC - BT)/BC] x 100

Where, BC = Fractional body weight gain of the control group (BWtC at 90 days minus BWtC initially)/(BWtC initially).

BT = Body weight gain of the treated group of animals (BWT at 90 days minus BWtT initially)/ (BWtT initially)

BWtC = body weight of the control group.
BWtT = body weight of the treated group.

It should be noted that the food consumption data submitted indicated that the food consumption at the 5000 ppm dose level decreased by a larger percentage of controls than the body weight. This would tend to indicate that efficiency of food utilization in the 5000 ppm dose level is comparable or greater than in controls, and that the body weight gain decrement at the 5000 ppm dose level is the result of reduced food consumption in males.

2. In males, ALT was elevated by 296% and AP was elevated by 118% at termination at the 5000 ppm dose level. In females, ALT was elevated by 158% and AP was normal at termination at the 5000 ppm dose level. A significant elevation in ALT appeared to occur in both males and females.

3. In males and females, triglycerides (58%) and cholesterol (42%) were depressed to the same degree at termination at the 5000 ppm dose level. Lipogenic pigment (moderate) (9/10) was increased to the same extent in males and females at the 5000 ppm dose level.

4. Absolute (164% in males and 141% in females) and relative liver weights (180% in males and 140% in females) were significantly elevated, in addition, to 10/10 males and 4/10 females with centrilobular hypertrophy of the hepatocytes at the 5000 ppm dose level.

5. Lipid vacuoles were increased in the adrenals in both males and females at the 5000 ppm dose level.

6. Leydig cell hypertrophy of the testes (5/10) at the 5000 ppm dose level and stromal cell hyperplasia of the ovaries was increased at the 1000 (6/10) and 5000 (9/10) ppm dose levels.

DATA SUBMITTED FOR RATS:

In January of this year, I telephoned Karen Blundell of BASF who referred me to Mr. Hubert Deissler of BASF, and stated that I could not assure BASF that the 3000 ppm upper dose level would meet the requirements of the Agency for a MTD, and that the adrenal weight increase and adrenal pathology would not be adequate evidence of a MTD. A higher dose level of 4374 ppm was used in a previously submitted oncogenicity study in rats, and there were no life threatening effects at that dose level (No study number or MRID number). I requested more information which could be used as a basis for establishing a probable MTD. Additional information has been submitted and although the 3000 ppm dose level proposed for the MTD appears to be adequate, uncertainty remains, partly because data from the first 7 months of a chronic feeding study in rats used to support the proposed

MTD was not submitted.[1] In addition, the data indicate a reduced food consumption which complicates the interpretation of the percentage body weight gain decrement. However, at 4500 ppm there appears to be a decrease in the relative efficiency of food utilization, and at 1500 ppm there may be a decrease in the relative efficiency of food utilization, although the decrease is less definitive at this lower dose level (See Table A below).

Table A
Relative Efficiency of Food Utilization in the Chronic Study in Rats. Data used: Males - month 7 to 16; Females - month 7 to 15 (The body weights of females at 16 months were unreadable).

Dose Group	Approximate Relative Efficiency of Food Utilization [a]		Approximate Relative Efficiency of Food Utilization [b]	
	Males	Females	Males	Females
1. Control	4.33	3.67	2.70	1.34
2. 50 ppm	4.87	3.87	3.32	1.44
3. 500 ppm	4.56	3.32	2.96	1.16
4. 1500 ppm	3.02	2.30	1.80	0.76
5. 4500 ppm	1.72	0.98	0.89	0.34

[a] (Change in body weight)/(Change in average daily food consumption/animal).
[b] {[Change in body weight(g)]/[Change in average daily food consumption(g)/animal]} X {Average body weight}.

Monthly body weight, food consumption, and water consumption data were presented for day 210 to 490 (about month 7 to 16) from an ongoing chronic feeding study in the same strain of rats (Study Number 71S0375/88026). After 7 months at the 4500 ppm dose level, these data indicate that the body weight was 87% of control values in males and 92% of control values in females, and by 16 months body weight was 78% of controls in males and 77% of controls in females. The data indicated that the body weight gain of the 4500 ppm dose level became progressively smaller than controls as the study progressed in both males and females. The percentage body weight gain at the 4500 ppm dose level between month 7 and month 16 was 62% for males and 74% for females. The percentage body weight gain at the 1500 ppm dose level between month 7 and month 16 was 33% for males and 40% for females. The percentage body weight gain at the 500 ppm dose level between month 7 and month 16 was -6% for males and a body weight gain of 11% for females. These body weight gains and decrements were large because food consumption was also reduced. The monthly

[1] The first 7 months of the study also should have been submitted. These data may have been of help in the determination of the appropriate MTD.

food consumption data during this period indicated that although the food consumption decreased at the 4500 ppm dose level over control values, the relative efficiency of food utilization at the 4500 ppm dose level was about 33% of control values for males and about 25% of control values for females. The relative efficiency of food utilization at the 1500 ppm dose level was about 67% of control values for males and about 57% of control values for females. These data appear to indicate that the 4500 ppm dose level may be too high, and the 3000 ppm dose level may be sufficient for a MTD.

Cover memo on an oncogenicity/mouse protocol and MTD/rat from BASF/A:\VINCLV13.23C\MMOURAT.ONC/D ANDERSON/1/22/90.