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DATA EVALUATION REPORT

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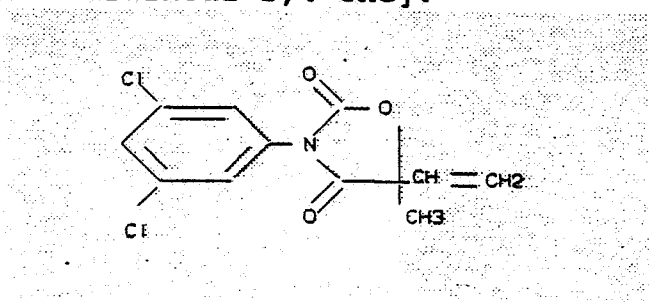
STUDY TYPE: Second Reproduction/Rat/(83-4)/94/10280/ 70R0375/88119/432547-05.

ToxChem No.: 323C. Submission No.: S468567.
PC No.: 113201. MRID No.: 43254705.
DP Barcode No.: D 222842 Case No.: 819455.
Rereg. Case: 2740.

Tox. Review #12053

TEST MATERIAL: Vinclozolin, technical; A.I. is [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedi-2,4-one].

STRUCTURE:



SYNONYMS: Ronilan™ (41 to 50% vinclozolin), Curalan (Turf)™, Ornalin™, Reg no. 83 258.

SPONSOR: BASF Corp. Chemicals Div., Ag. Chem., PO Box 13528, Research Triangle Park, NC 27709-3528.

TESTING FACILITY: BASF Aktiengesellschaft, Dept. Toxicology, 6700 Ludwigshafen, Federal Republic of Germany.

STUDY NO.: BASF Reg.# 94/10280; Lab Proj.# 70R0375/88119. This project was voluntarily conducted because of perceived effects on the epididymal weight at the NOEL in the first study on reproduction 0292/11251 & 71R0375/88053. Reg. Doc. No. BASF 92/10596 (MRID# 425813-01).

REPORT TITLE: Second Reproduction Study with Reg. No. 83 258 (Vinclozolin) in Rats with Continuous Dietary Administration Over 2-Generations (2 litters in Each Generation) Project No.: 70R0375/88119 (MRID# 43254705).

AUTHOR(S): Dr. J Hellwig.

REPORT ISSUED: May 2, 1994.

EXECUTIVE SUMMARY: In a 2-generation study on reproduction (MRID# 43254705) (1994), doses were administered in the diet at 0, 20 or 40 ppm of vinclozolin (technical, 99.2%) (Approximately 0, 2.0 or

4.1 mg/kg/day) to 25 Wistar rats per sex per group through the P0, F1 and F2 generations for 14 weeks. Two litters per generation were produced: F1a (F1 adults), F1b (FX adults), F2a (FY adults) and F2b (FZ adults). The study was specifically conducted to demonstrate a clear NOEL for epididymal weights.

No dose related or biologically significant effects were demonstrated on offspring or parents. Statistically significant effects occasionally occurred, but these effects were either not dose related, appeared incidental to the study or were inconsistent with other studies more clearly showing dose related effects.

The conditional requirement for a sperm parameter study with vinclozolin suggested in the first study on reproduction (42581301) was negated by a study indicating that these parameters were affected only at 100 mg/kg/day (Gray et al. submitted for publication December 1993).

The LOEL for the study is > 40 ppm (HDT).

Core classification: Minimum. This study (MRID# 43254705) along with (MRID# 42581301) is acceptable under guideline 83-4 for reproduction in the rat.

A. MATERIALS:

1. Test material: Vinclozolin, Description: Solid; Batch No.: N 183; Purity - 99.2% a.i.

2. Test animals: Species: Rats, Strain: Wistar (Chbb = THOM(SPF)), Age: 35 days at study initiation, Weight: Males - 126.8 (115 - 137) g, Females - 115.0 (106 - 126) g at study initiation, Source: Karl Thomae, Biberach an der Riss, FRG. Animals were acclimatized for 7 days after receipt.

3. Environment: The animal room was maintained at 20 to 24° C; Relative humidity was 30-70%; Light:dark = 12:12, starting at 6:00 AM. Rooms were disinfected with Autex apparatus, fully automatic. Final disinfecting used formaldehyde and ammonia. Each week walls and floor were disinfected with 0.5% Mikro-Quat.

Pre-mated animals were housed individually in stainless steel wire mesh cages (800 cm^2).

B. STUDY DESIGN:

1. Animal Assignment - Twenty-five animals per sex were assigned randomly to each group using a randomization program software, which randomizes according to body weight. F1a animals used for mating were also randomly assigned. Neither P0 nor F1 litter mates were mated.

2. Study Purpose and Protocol - The objective of the study was to clearly define a NOEL for epididymal weight reduction because a statistically significant epididymal weight reduction occurred in the first study on reproduction (MRID# 42581301) in FY males only, but was nominally reduced in F1, FX and FZ adults. Although this latter study was given a core minimum grade, this repeat study was conducted to unequivocally determine the NOEL.

The second study was conducted essentially the same as the first study, however, only 2 dose levels were used at 20 and 40 ppm. Behavioral tests of offspring were conducted, such as pinna unfolding on day 4 post partum, opening of the auditory canal on day 13 and eye opening on day 15, acoustical startle at day 21 and gripping reflex on post partum day 13, pupillary reflex on day 20 postpartum and a hearing test on day 21 post partum. Litters were standardized at day 4 and randomly selected pups were raised to adulthood in both litters.

3. Diet preparation - Diet was prepared at least every 32 days, and stored at room temperature until used. Samples of the diet were collected and showed satisfactory concentrations when analyzed. Samples of treated food were analyzed for homogeneity, stability and concentration at ≈ 22° C.

Results - The overall homogeneity analyses was satisfactory. Stability determinations indicated that test material was stable within the diet for at least 32 days when new diets were prepared. Analyses of dietary concentrations were satisfactory.

4. Animals receive food and water ad libitum. Rats were fed Kliba maintenance diet rat/mouse/hamster GLP 343 meal supplied by Klingentalmühle AG, CH-4303 Kaiseraugst, Switzerland. Tap water was also supplied.

5. Statistics - The data were evaluated statistically using the computer systems of the Department of Toxicology of BASF, Aktiengesellschaft.

Dunnett's test was used for all parametric data and Fisher's Exact Test was used for developmental stages, mating and fertility indexes, gestation, live birth, viability and lactation indexes.

6. Data presented in the submitted report was quality assurance audited throughout the study and signed by H Fleig, Head of QA on 5/2/94.

C. METHODS AND RESULT FOR P0, F1 and F2 GENERATIONS: (Lettered tables were constructed from data in the submitted report)

1. Observations - Animals were inspected daily for signs of toxicity and mortality.

Results - Toxicity - No biologically significant signs were observed.

Mortality (Survival) - No dose related mortality occurred during the study.

2. Body Weight, Food and Water Consumption - Body weights and body weight gain were determined weekly for P0, F1a, (selected for the F1), F1b (selected for the FX), F2a (selected for the FY) and F2b (selected for the FZ) males and females from initiation of dosing after weaning.

Results for P0, F1, FX, FY and FY Adult Males and Females - No biologically significant changes occurred in the body weight or body weight gain in P0, F1, FX, FY or FZ males and females or pups. No significant changes occurred in food of water consumption in P0. F1, FX, FY and FZ males or females.

3. Reproductive Parameters and Litter Data - No significant changes occurred in any reproductive parameter.

a. Results on Fertility and Mating - No significant changes occurred in any parameter.

b. Pup weights for the F1a, F1b, F2a and F2b during Lactation (reported only as litter means) - Pup weights were recorded individually but reported as litter means for day 1, 4, 7, 14, and 21 (weaning).

Results - No biologically significant changes occurred.

c. Average Number of Pups at Birth and Pup Viability No biologically significant changes occurred.

d. Developmental Stages and Behavior for the F1a, F1b, F2a and F2b Offspring - Developmental stages such as pinna unfolding (PU), auditory canal opening (ACO), eye opening (EO) and pupil constriction (PC) and behavior such as gripping reflex (GR), pupillary reflex and acoustical startle.

Results - No statistically significant or biologically significant changes occurred.

5. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule in all P0, F1, FX, FY and FZ animals were subject to gross pathological examination. Gross lesions were fixed in 4%

buffered formalin and examined grossly. The epididymides, seminal vesicles, coagulating gland, prostate, pituitary, eyes, liver, adrenals and bone marrow were examined microscopically in the male. The ovaries, cervix and vagina, uterus, pituitary, eyes, liver, adrenals and bone marrow were examined microscopically in females. In addition, the testes, epididymides, liver and adrenals were weighed.

Results - a. Organ weights - No dose related or treatment related absolute organ weights changes occurred in the study. Statistically significantly elevated (13.6%, $p \leq 0.01$) absolute and relative (15.4%, $p \leq 0.01$) adrenal weights in F1 males and reduced (4.9%, $p \leq 0.05$) epididymal weights in FX males were noted, respectively at 40 ppm (Table A). However, this elevation in the adrenal gland showed a clear NOEL at 50 ppm in F1 adults and the reduction in epididymal weight showed a clear NOEL at 50 ppm in the FX adults in the first reproduction study (MRID# 42581301). An increasing trend in the adrenal weights was seen only in the F1 and FZ males, but not in P0 or FY males. In addition, a clear NOEL was demonstrated at 50 ppm in adrenal weights in P0, F1, FX, FY and FZ males in the first study on reproduction (MRID# 42581301).

The decreasing trend seen in the epididymal weights for the FX ($p \leq 0.05$) and FZ ($p \geq 0.05$) animals was not seen in P0, F1 and FY animals and was probably incidental to the dosage used. In addition, the epididymal weights in the P0, F1 and FY did not show a decreasing dose relationship which lends support to the incidental nature of the nominal weight reduction. Other statistically significant changes occurred, but these were either clearly not dose related or were inconsistent with the direction of change from other studies at higher dose levels and were considered incidental to the dosage administered.

The statistically significant decrease in liver weights (11.1%, $p \leq 0.01$) was inconsistent with the statistically significant increases in liver weight seen in other studies showing a dose related response. The liver weight decrease seen in the current study were considered incidental to the study.

Table A. Effects on terminal body weight and selected target organ weights in the P0 and F1 parental males, FX (F1b adult males), FY (all F2a adult males) and FZ (all F2b adult males).

	Males			Females		
Dose group→ P0 males, body wt. & absolute organ wt.	Control	20 ppm	40 ppm	Control	20 ppm	40 ppm
Body wt., g SD n	546.7 50.7 24	540.1 39.3 24	546.3 44.9 24	304.9 25.2 25	316.6 21.8 24	307.8 25.5 24
Liver, g SD n	17.8 2.0 23	17.1 1.9 24	17.6 12.2 24	9.48 0.8 25	9.99 0.9 24	9.63 1.2 24
Testis, g SD n	3.89 0.32 23	3.73 0.30 24	3.92 0.34 24	-	-	-
Epididymis, mg SD n	1413.2 108.5 24	1349.5* 79.8 24	1374.8 90.3 24	-	-	-
Adrenal gland, mg SD n	78.8 8.7 24	79.9 8.2 24	82.5 9.6 24	107.6 8.8 25	113.9 14.8 24	105.1 15.2 24
Dose group→ F1 (adult F1a), body wt. & absolute organ wt.	Control	20 ppm	40 ppm	Control	20 ppm	40 ppm
Body wt., g SD n	565.2 49.7 24	569.6 43.8 25	562.8 48.1 25	321.0 17.4 25	317.9 19.2 25	314.8 28.1 24
Liver, g SD n	18.8 3.0 24	19.2 2.6 25	18.3 2.1 25	10.4 0.7 25	10.4 1.0 25	10.3 1.0 24
Testis, g SD n	4.17 0.36 24	4.20 0.76 25	4.15 0.35 25	-	-	-
Epididymis, mg SD n	1443.0 -112.0 24	1420.8 124.7 25	1422.2 105.6 25	-	-	-
Adrenal gland, mg SD n	74.8 8.3 24	80.3 8.6 25	85.0** 10.33 25	106.2 13.2 25	106.6 14.1 25	110 14.8 24

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Dose group→ FX males (non-mated adult F1b), body wt. & absolute organ wt.	Males			Females		
	Control	20 ppm	40 ppm	Control	20 ppm	40 ppm
Body wt., g SD n	496.0 41.2 25	484.0 38.6 25	476.7 48.2 25	275.8 22.6 25	277.7 21.5 25	272.7 18.4 25
Liver, g SD n	17.6 2.8 25	16.8 2.3 25	16.2 3.0 25	8.0 0.94 25	8.1 0.88 25	8.1 0.71 24
Testis, g SD n	3.84 0.28 25	3.73 0.36 25	3.75 0.24 25	-	-	-
Epididymis, mg SD n	1353.3 84.7 25	1299.1 108.8 25	1287.3* 93.9 25	-	-	-
Adrenal gland, mg SD n	75.0 10.4 25	85.4** 11.6 25	77.4 14.0 25	105.8 14.1 25	106.8 14.1 25	107.1 12.1 25
Dose group→ FY (adult F2a), body wt. & absolute organ wt.	Control	20 ppm	40 ppm	Control	20 ppm	40 ppm
Body wt., g SD n	449.4 47.1 25	435.2 34.7 25	442.9 41.6 25	264.2 19.5 25	259.1 25.2 25	265.8 28.5 25
Liver, g SD n	16.9 2.6 25	15. 8 2.0 25	16.7 2.6 25	8.3 1.0 25	8.1 1.1 25	8.2 1.2 25
Testis, g SD n	3.71 0.3 25	3.58 0.26 25	3.66 0.27 25	-	-	-
Epididymis, mg SD n	1265.8 92.6 25	1208.6* 94.6 25	1221.6 67.0 25	-	-	-
Adrenal gland, mg SD n	93.5 15.3 25	85.9 15.3 25	91.6 14.4 25	107.8 14.4	113.3 14.9 25	111.3 16.2 25

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Dose group→ FZ (adult F2b), body wt. & absolute organ wt.	Males			Females		
	Control	20 ppm	40 ppm	Control	20 ppm	40 ppm
Body wt., g SD n	476.2 46.8 25	459.1 36.7 25	452.9 38.0 25	273.0 21.1 25	272.6 17.4 25	272.3 19.3 25
Liver, g SD n	18.0 3.2 25	16.6 1.9 25	16.0** 2.2 25	8.4 0.7 25	8.4 0.9 25	8.4 1.1 25
Testis, g SD n	3.75 0.33 25	3.75 0.31 25	3.7 0.25 25	-	-	-
Epididymis, mg SD n	1296.9 114.6 25	1283.1 99.5 25	1240.7 78.1 25	-	-	-
Adrenal gland, mg SD n	84.6 11.7 25	87.8 13.6 25	88.8 12.9 25	112.4 14.9 25	106.2 14.4 25	105.8 18.5 25

SD = Standard deviation; n = Number of animals; * = Statistically significance, $p \leq 0.05$; ** = Statistically significance, $p \leq 0.01$; - = Missing data.

b. Gross Necropsy and Microscopic Examination -

i. Results from Gross pathology - Gross examination was conducted.

ii. Microscopic examination - No test material related findings occurred in males and females of any dose level or generation.

D. **Summary**: No dose related changes occurred in any parameter, including the statistical significant changes that occurred in some organ weights.

The absolute and relative adrenal weight changes were considered to be incidental to dosing. Absolute (13.6%, $p \leq 0.01$) and relative adrenal weights (15.4%, $p \leq 0.01$) were statistically significantly increased in F1 adult males at 40 ppm, however, this elevation was not statistically significantly in P0, F1, FX, FY or FZ adults at 50 ppm in the first study on reproduction (MRID# 42581301). An increasing trend in the adrenal weights was seen only in the F1 and FZ males. In addition, a clear NOEL was demonstrated at 50 ppm in the first study on reproduction.

Absolute epididymal weights (4.9%) were statistically significantly reduced only in FX males at 40 ppm. A decreasing trend was seen in the epididymal weights for the FX and FZ animals, but the trend was not seen in P0, F1 and FY animals and no statistically significant increases occurred at 40 ppm. The increase in FX males seen at 40 ppm was probably incidental to the dosage used. The failure of the epididymal weights in the P0, F1

and FY to show a decreasing trend lend additional support to the incidental nature of the weight reduction in FX adults. In addition the statistically significant reduction in epididymal weights in the first study on reproduction (MRID# 42581301) were seen only in the FY animals and a decreasing trend was not seen in all the animals of that study either. These results tend to confirm that the statistically significant reduction in the epididymal weights seen in the FY animals from the first study of reproduction was also incidental to the study and that the NOEL for the study was indeed 50 ppm (LDT). In addition, no dose related histological or gross findings were noted in this 2nd reproduction study (MRID# 43254705).

The conditional requirement for a sperm parameter study with vinclozolin suggested in the first study on reproduction (42581301) was negated by a study indicating that these parameters were affected only at 100 mg/kg/day (Gray et al. submitted for publication December 1993).

The statistically significant decrease in liver weights (11.1%, $p \leq 0.01$) was inconsistent with the statistically significant increases in liver weight seen in other studies showing a dose related response. The liver weight decrease seen in the current study were considered incidental to the study.

Other statistically significant changes occurred, but these were either clearly not dose related or were inconsistent with the direction of change from other studies at higher dose levels and were considered incidental to the dosage administered.

<u>One-Liner</u>:

In a 2-generation study on reproduction (1994), doses were administered in the diet at 0, 20 or 40 ppm of vinclozolin (technical, 99.2%) (Approximately 0, 2.0 or 4.1 mg/kg/day) to 25 Wistar rats per sex per group through the P0, F1 and F2 generations for 14 weeks. Two litters per generation were produced: F1a (F1 adults), F1b (FX adults), F2a (FY adults) and F2b (FZ adults). The study was specifically conducted to demonstrate a clear NOEL for epididymal weights.

NOEL: > 40 ppm (4.1 mg/kg/day).

LOEL: > 40 ppm (4.1 mg/kg/day) No dose related or biologically significant effects were demonstrated on offspring or parents. Statistically significant effects occasionally occurred, but these effects were either not dose related, appeared incidental to the study or were inconsistent with other studies clearly showing dose related effects.

The conditional requirement for a sperm parameter study with vinclozolin suggested in the first study on reproduction (425813-01) was negated by a study indicating that these parameters were affected only at 100 mg/kg/day (Gray et al. submitted for publication December 1993).

<u>Core classification</u>: Minimum. This study (MRID# 432547-05) along with (MRID# 425813-01) are acceptable under guideline 83-4 for reproduction in the rat.