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

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010261

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: EPA ID # 007969-00053. Vinclozolin; Review of Reports 90-Day Studies in C57BL (MRID# 418243-01) and B6C3F1 (MRID# 418243-02) Mice, α-Reductase Study (MRID# 418243-04), Hormone Stasis in Rat at 6-Months (MRID# 418243-05), Androgen Receptor Binding (MRID# 418243-06), Metabolism Studies (MRID# 418234-07 and -08) and a Dermal Absorption Study (MRID# 418243-09).

Tox. Chem. No.: 323C.
HED Project No.: 1-1063.
Submission No.: S393896.
Barcode: 163181.
Case: 037677.

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CONCLUSIONS: The conclusions from the DER of each of the study reports reviewed are reproduced below. Two of the submitted reports were not reviewed here because a more detailed report of the same studies were reviewed previously (See below under "3. MRID# 418243-03" and "4. MRID# 418268-01)"

1. MRID# 418243-01.
Hindebrand, B. (1990) Study on the Oral Toxicity of Reg. No. 83 258 (Vinclozolin) in C57BL Mice Administration in the Diet over 3 Months. Conducted at BASF Aktiengesellschaft Depart. of Toxicology 6700 Ludwigshafen, Germany. Lab. Project 90/0421. October 1990, report issued March 4, 1991. (MRID # 418243-01)
The study report was reviewed by CIC and the DER is submitted with this cover memorandum on HED project# 1-1063,

D163181; Submission# S393896.

CONCLUSIONS:

Vinclozolin was administered in the diet to 10 C57BL/6NCrLBR mice per sex per group at 0, 100, 1000, or 5000 ppm (Determined mean for males: ≈ 20, 230, or 940 mg/kg/day, and for females ≈ 30, 310, or 1240 mg/kg/day).

NOEL: 100 ppm (≈ 20 mg/kg/day for males and ≈ 30 mg/kg/day for females).
LEL: 1000 ppm (≈ 230 mg/kg/day for males and 310 mg/kg/day for females). In males and females triglycerides and cholesterol were depressed. Alanine aminotransferase (ALT, also called SGPT, Alkaline phosphatase (SAP)[these latter two values were approximately doubled], total protein, and globulin levels were elevated at the highest dose. Lymphocyte and total leucocyte counts were elevated in high dose males and females. Glucose was depressed at the highest dose level in males. Lipogenic pigment was exhibited in the adrenals at the mid and highest dose tested, but lipid vacuoles were noted only at the high dose. Relative liver weight in males and females was increased at the mid and high dose, but no histological correlates could be detected or ruled out at the mid dose. Hyperplasia and hypertrophy of testicular Leydig cells and ovarian stromal cells were noted at the highest dose tested as well as central lobular hypertrophy of the liver. It was noted that the testes weight increase at the high dose level was probably not due to the Leydig cell hyperplasia. The nominal adrenal weight increase at the mid dose is possibly compound related. The nominal body weight depression occurring in males at the high dose was accompanied by reduced food consumption. This resulted in a minimally reduced (nominal) food efficiency in males. The body weights of females did not change, however there was an apparent increased food efficiency in females (130% of controls). The food efficiency was variable, and thus, the nominal decreased body weight in males has not been shown to be due to toxicity.

Core Classification: Supplementary; this study does not satisfy the Guideline requirement for a 90-day subchronic study (82-1) in mice because individual animal data were not submitted. The data was collected according to OECD GLPs, instead of FIFRA GLP's. However, the study may be unnecessary for registration or reregistration, if an acceptable chronic study (83-1) in rats is submitted..

2. MRID# 418243-02.
Hindebrand, B. (1990) Study on the Oral Toxicity of Reg. No. 83 258 (Vinclozolin) in B6C3F1 Mice

Administration in the Diet over 3 Months. Conducted at BASF Aktiengesellscjaft Depart. of Toxicology 6700 Ludwigshafen, Germany. Lab. Project 90/0422. October 1990, report issued March 4, 1991. (MRID # 418243-02)

The study report was reviewed by CIC and the DER is submitted with this cover memorandum on HED project# 1-1063, D163181; Submission# S393896.

CONCLUSIONS:

Vinclozolin was administered in the diet to 10 B6C3F1/Cr1BR mice per sex per group at 0, 100, 1000, 2500, or 5000 ppm (Determined mean for males: ≈ 17, 170, 390, or 770 mg/kg/day, and for females ≈ 23, 250, 560, or 1170 mg/kg/day).

NOEL: 100 ppm (≈ 17 mg/kg/day for males and ≈ 23 mg/kg/day for females).

LEL: 1000 ppm (≈ 170 mg/kg/day for males and 250 mg/kg/day for females); For males, multifocal Leydig cell hyperplasia of the testes was noted. Adrenal A-cell hyperplasia occurred in males at 1000 ppm and above, and in females in all groups. Decreased cholesterol, triglycerides, and glucose in males, and cholesterol in females occurred at 1000 ppm and above. Testes weights were increased at 1000 ppm and above. At 5000 ppm, stromal cell hyperplasia of the ovaries of females, Triglycerides were depressed in females only at 5000 ppm. Relative and absolute liver weights in males and females were increased at 1000 ppm and above. This weight increase is supported by centrilobular hypertrophy in males at 2500 ppm and above and in females at 5000 ppm, and peripheral fatty infiltration of the liver at 5000 ppm in males and females. Alanine aminotransferase (ALT, also called SGPT) was increased at 5000 ppm in males only, and alkaline phosphatase was increased in males, but decreased in females at 2500 ppm and above. Adrenal weights were increased in males and females at 2500 ppm and above. Adrenal lipid vacuoles were noted in males at 2500 ppm and in all female groups. Lipogenic pigment was increased in females at 1000 ppm and above. There may have been some hematological effects at 5000 ppm because reticulocytes were increased in females. The food efficiency was variable, but slightly less than control in males at 5000 ppm, and thus, the nominal body weight decrement in males may have indicated that the 5000 ppm was close to a toxic dose level. Neither the body weight nor the food efficiency of females were changed at 5000 ppm.

Core Classification: Supplementary; this study does not satisfy the Guideline requirement for a 90-day subchronic study (82-1) in mice because individual animal data were not submitted. However, the study may be unnecessary for registration or reregistration, if acceptable chronic study (83-1) in rats is submitted.

3. MRID# 418243-03; NA. Interim report on the Chronic Toxicity of Vinclozolin in wistar rats; Dietary Administration over 24-Months (Project# 71S0375/88026, Reg. Doc.# 90/0478).

This study report was not reviewed because more detailed interim reports (MRID# 423551-01 and -02) were reviewed and were submitted under HED Project# D179889, Submission# S420450.

4. MRID# 418268-01 Hellwig. Third Preliminary Information on a 2-Generation Reproduction study (Project# 71R0375/88053, Reg. Doc.# 91/10031).

This study report was not reviewed because a more detailed preliminary draft report (MRID# 424127-01) was reviewed and the DER submitted under HED Project# D181325, Submission# S423027.

5. MRID# 418243-04; van Ravenzwaay, B. Report on the effect of vinclozolin (Reg No. 83 258(ZST # 88/375) on 5-α-Reductase Activity *in vitro* (Reg. Doc.# 90/0379).

The study report was reviewed and the DER is submitted with this cover memorandum on HED project# 1-1063, D163181; Submission# S393896.

CONCLUSIONS: 5-α-Reductase was prepared from B6C3F1 mouse liver supernatant and was treated with 0.05 mg vinclozolin and the change in optical density was determined at 340 nm.

Vinclozolin does not act *in vitro* through inhibition of the 5-α-reductase in the conversion of testosterone (T) to dihydro-testosterone (DHT). Although comparability of mouse and rat 5-α-reductase has not been proved, it is reasonable to assume that 5-α-reductase would not be inhibited in treated rats. It was postulated that the prostate atrophy in rats from vinclozolin administration resulted from inhibition of the synthesis of DHT.

Core classification: Supplementary non guideline study because it is a special study on the mechanism of vinclozolin action.

6. MRID# 418243-05; Knuppen, R. Examination of the hormone status associated with the six-month feeding study in Wistar Rats. BASF submitted, for review, a report on androgen binding studies with vinclozolin (Project# 31S0375/88050, Reg. Doc.# 89/0601).

This study report was reviewed and the DER is submitted with this cover memorandum on HED project# 1-1063, D163181; Submission# S393896.

CONCLUSIONS: Ten Wistar rats per sex per group of controls and 10 per sex were administered vinclozolin in the feed at 4500 ppm for 6-months and plasma hormone levels were determined.

In males, ACTH (164% of controls), corticosterone (163% of controls), DHEA (182% of controls), testosterone (276% of controls) and LH (1058% of controls) were statistically significantly and biologically significantly elevated. In females, ACTH (172% of controls) and LH (238% of controls) were statistically significantly and biologically significantly elevated. The failure of corticosterone elevation with ACTH elevation in females is not consistent and may indicate that the serum collections were not timed appropriately for maximal response in females. The LH increase in females may been related to the timing of the blood collection relative to the estrus cycle.

Core classification: Supplementary non guideline study because it is a special study on hormone levels and no details were submitted.

7. MRID# 418243-06; Knuppen, R. Vinclozolin binding to an *in vitro* Androgen Receptor (Project# 21B324/889027, Reg. Doc.# 90/0573).

The study report was reviewed and the DER is submitted with this cover memorandum on HED project# 1-1063, D163181; Submission# S393896.

CONCLUSIONS: Vinclozolin competition with mibolerone binding to the androgen receptor in the nucleus of MCF-7 cells (human mammary carcinoma cells) and in the MCF-7 cell cytosol were studied at 1 to 50 μmoles/l. The data are consistent with the hypothesis that vinclozolin is an antiandrogen, which competes with testosterone for receptors. The authors stated conclusions were supported by insufficient data to evaluate the accuracy of their conclusions. Vinclozolin decomposition in the media was stated to compromise the results. In addition, DES and estradiol were inexplicably competitive with the test androgen,

radiolabeled aibolerone, for the MCF-7 receptors.

Core classification: Supplementary non guideline study because it is a special study on the mechanism of vinclozolin action and supporting details were lacking.

8. MRID# 418243-07; Hawkins, D. R. Biotransformation in Rats (Reg. Doc.# 90/0514).

and MRID# 418243-08; Hawkins, D.R. Biokinetics In Rats (Reg. Doc.# 90/0544).
Both Study reports reviewed by CIC and the DER is submitted with this cover memorandum on HED project# 1-1063, D163181; Submission# S393856.

CONCLUSIONS: Absorption, distribution and excretion were studied after oral or i.v. doses of [C^{14}dichlorophenyl]vinclozolin to male and female Wistar rats. Single oral labeled doses of 10 or 100 mg/kg and single i.v. labeled doses of 1 mg/kg were administered. Multiple doses were administered at 10 mg/kg/day for 14 days, followed by a labeled dose. Pharmacokinetics were conducted on single oral gavage doses at 10, 100, or 200 mg/kg labeled vinclozolin or 5000 ppm of labeled vinclozolin in the diet.

Analyses of the pharmacokinetics indicated that 7 days of dosing was adequate to attain equilibrium dose levels in the plasma with no significant accumulation. Bile cannulation indicated that enterohepatic recirculation of vinclozolin occurred in males and females. Biliary excretion one-half life was 12 hours in males and 18 hours in females.

High and low dose excretion rates are similar in males and females with about 48-55% being excreted in the urine and 43-48% in the feces. The pattern remained similar for males and females after multiple dosing. The excretion curve for vinclozolin was biphasic with the terminal end of the curve indicating a one-half life for vinclozolin of 22 ± 5.7 hours for males and 36 ± 16 hours for females.

In general, the metabolic profile of vinclozolin did not change with sex, high or low dose or repeat dosing. Vinclozolin was extensively metabolized to 15 metabolites, including the major metabolite, N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide glucuronide conjugate and small amounts of the unconjugated product and excreted in the urine. Less than 0.5% of the urinary metabolites was the parent compound. The major components excreted in the feces were parent compound (33-48% of the fecal components) at 10 to 200 mg/kg oral doses and the glucuronide conjugate of N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutramide. Parent compound was excreted at 15% and 42% of the total oral dose levels of 10 mg/kg and 200 mg/kg

dose levels, respectively. The trihydroxybutyramide was excreted at 11% and 4% of the same total respective dose levels. Vinclozolin at high oral doses of 200 mg/kg was more poorly absorbed and a larger percentage of the total dose was excreted as parent vinclozolin in the feces. No vinclozolin was found in feces for i.v. doses.

In the first 6 to 120 hours after dosing male and female rats with labeled vinclozolin, the highest concentrations of label were in the liver and Harderian gland with lower concentrations in the adrenal, kidney, pancreas and fat. Small amounts were distributed throughout the body. Less than 2% of the administered labeled vinclozolin was retained by the carcass and none (< 0.02%) was exhaled as CO_2.

Core grade: Guideline. The study is acceptable under guideline 85-1 for metabolism studies in the rat.

9. MRID# 418243-09; Hawkins, D.R. Dermal absorption in Rats (Req. Doc.# 91/10059).
The study report was reviewed by CIC and the [illegible] submitted with this cover memorandum on HED project# 1-1063, D163181; Submission# S393896.

CONCLUSIONS: Dermal absorption, distribution and excretion of [C^{14}]vinclozolin were studied in the 24 male Wistar rats/dose at 0.002, 0.02, 0.2 or 2.0 mg/cm^2 in 1% carboxymethylcellulose vehicle. Subgroups of 4 rats per dose group were sacrificed at 0.5, 1, 2, 4, 10 and 72 hours post treatment.

Percentage absorption (excluding the treated skin) was 27.3%, 19.8%, 3.16%, 0.91% and (including the treated skin) 28.6%, 23.0%, 3.77%, 2.68% at 72 hours post treatment at the above dose levels, respectively.

Plasma concentrations were maximal after the 10 hour treatment period when the application site was washed; the exception was the 0.2 mg/cm^2 (14 mg/kg Bwt) dose level, where the plasma level continued to increase from 0.021 µeq/g at 10 hours to 0.032 µeq/g at 72 hours. The plasma concentration 10 hours after dosing was 0.0057, 0.018, 0.021 and < 0.2[1] µeq vinclozolin/g plasma tissue at the above dose levels, respectively. The concentration of label at 72 hours after dosing was 0.0012, 0.013, 0.032 and < 0.2[1] µeq vinclozolin/g plasma tissue at the above dose levels, respectively.

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[1] The value of < 0.2 µeq is limit of detection for the specimen and at the specific activity of the radio label used. The specific activity was lowest at the 2.0 mg/cm^2 dose level and most tissue values at this dose level were not accurate.

The highest concentrations of radioactivity were found in the liver, followed by the kidneys, adrenal glands, plasma, brain, blood, and testes generally in that order.

Core grade: Minimum, the study is acceptable under guideline 85-1 for a dermal absorption study. Although, the sponsor should have justified using the 1% carboxymethylcellulose vehicle in the study or characterized the effect on vinclozolin absorption, this single finding is apparently insufficient to down grade the study.

CMemo 90-Day/C57BL/B6C3F1/Mice; Interim Chronic/Rat; Rat
Prel/Repro; 5-Reductase; GM/Hormone/Rats; Androgen Binding;
Metabolism; Dermal Absorption; 01624[illegible] -05, -06, -07,
-08, -09/8; V:\NCLY33.2\C\CM:-
1063.PRO/DAS0282CM, 11/21/92;Edited4/[illegible]

EPA No.: 00282214
DYNAMAC No.: 382-A
TASK No.: 1-82A
July 25, 1991

010261

DATA EVALUATION RECORD

TINCLOSOLIN

Subchronic Oral Toxicity Study in Mice

APPROVED BY:

Robert J. Wolf, Ph.D.
Program Manager
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Signature: William L. McMullen for

Date: July 25, 1991

EPA No.: [illegible]
DYNAMAC No.: 282-A
TASK No.: 3-82A
July 24, 1991

010261

DATA EVALUATION RECORD

FENCLORIM

Subchronic Oral Toxicity Study in Rats

REVIEWED BY:

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DATA EVALUATION RECORD 010261

GUIDELINE § 82-1

STUDY TYPE: Subchronic oral toxicity study in mice.

MRID NUMBER: 418243-01.

TEST MATERIAL: Vinclozolin; 3-(3,5-dichlorophenyl)-5-vinyl-5-methyl-1,3-oxazolidine-2,4-dione.

SYNONYMS: N/A.

STUDY NUMBER(S): 5150375/88054.

SPONSOR: BASF Corporation, Agriculture Chemicals, Research Triangle Park, NC.

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen, Germany.

TITLE OF REPORT: Study on the Oral Toxicity of Reg. No. 83 258 (Vinclozolin) in C57BL mice. Administration in the Diet Over 3 Months.

AUTHOR: B. Hildebrand.

REPORT DATE: March 6, 1991.

CONCLUSIONS:

Vinclozolin was administered in the diet to male and female C57BL mice at dose levels of 0, 100, 1000, or 5000 ppm for 3 months. One low-dose female died during the study, and two high-dose females died prior to study termination; the cause of death was not detectable. Food consumption of high-dose males and females was consistently depressed throughout the study. Lymphocytic and total leukocyte counts were elevated in high-dose males and females. SGPT, alkaline phosphatase, total protein, and globulin levels were elevated, and glucose levels were depressed in high-dose males; triglycerides and cholesterol levels were depressed in mid- and high-dose males and females. Compound-related microscopic findings included peripheral fatty infiltration and centrilobular hypertrophy of the liver, and lesions of the testes and ovaries at the high dose. An increased deposition of lipogenic pigment was exhibited in the adrenals of mid- and high-dose animals; lipid-containing vacuoles were found at the high dose. Liver, testicular, and adrenal weights were increased. The LOEL is 1000 ppm, and the NOEL is 100 ppm vinclozolin.

Classification: Core Supplementary. The study may be upgraded with the submission of individual animal data for all study parameters.

A. MATERIALS:

1. Test Compound: Vinclozolin; description: white solid; batch No.: M183; purity: 99.2%.

2. Test Animals: Species: mouse; strain: C57BL/6NCrLBR; age: 49 days at study initiation; weight: males--19.6 to 22.3 g, females--17.1 to 19.1 g; source: Charles River WIGA GmbH, Sulzfeld, Federal Republic of Germany.

B. STUDY DESIGN:

1. Animal Assignment: Following an acclimation period of 10 days, animals were assigned to the following test groups on the basis of weight using a computerized randomization procedure:

Test group	Dose in diet (ppm)	Main study (3 months) Males	Females
1 Control	0	10	10
2 Low (LDT)	100	10	10
3 Mid (MDT)	1000	10	10
4 High (HDT)	5000	10	10

Mice were housed individually in an environmentally controlled room with temperature and humidity controls set at 20 to 24°C and 30 to 70%, respectively, with a 12-hour light/dark cycle.

Dose levels were based on two previously conducted feeding studies in mice. Vinclozolin was administered to NMRI mice at dose levels of 162, 480, 1458, or 4374 ppm for 112 weeks; body weights and food consumption were significantly reduced at the two highest dose levels. B6C3F1 mice were administered 100, 1000, 2500, or 5000 ppm vinclozolin for 3 months. Body weights were slightly reduced at the high dose; absolute and relative liver weights of males and females administered 1000, 2500, or 5000 ppm were increased. Testicular and adrenal weights of males administered 2500 and 5000 ppm were increased. Changes occurred in the clinical chemistry (triglycerides, cholesterol, glucose, albumin, globulin, SGPT, and alkaline phosphatase) and hematology (hemoglobin, reticulocytes, and calculated red cell parameters) levels of males and females administered 1000, 2500, or 5000 ppm vinclozolin. There were no macroscopic changes in the liver. The study author considers these results to be an indication of potential enzyme induction. Since the B6C3F1 mouse has a high rate of spontaneous liver tumors, the C57BL mouse, with a low spontaneous liver tumor rate, was selected to determine doses for future chronic studies and verify results of the 3-month study with B6C3F1 mice.

2. Diet Preparation: Test diets were prepared at 4-week intervals. A premix was prepared by mixing an appropriate quantity of the test material with a small amount of the basal diet. The premix was adjusted to the appropriate test concentrations with the addition of basal diet and mixed. Analyses of concentratior were reported to have been measured at study initiation and termination. Homogeneity and stability analyses were conducted prior to study initiation; the analysis of stability was conducted

on batch N173 (from a study conducted previously) and also on batch N183 following study termination.

Results: The study authors reported that the test material was stable for 10 to 32 days. Results of homogeneity and concentration of the test material in the feed were not reported; individual data on stability, homogeneity, and concentration were not reported.

3. Food and Water Consumption: Animals received food (Kliba maintenance diet, GLP 343 meal, Klingentalmuhle AG, Switzerland) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights and clinical biochemistry data were analyzed by analysis of variance and Dunnett's test. Organ weight data were compared using Dunnett's test.

5. Quality Assurance: A quality assurance statement was signed and dated October 12, 1990. The study was performed in accordance with OECD Guidelines, Paris, 1981; as indicated by the study author, the study does not meet the requirements of Good Laboratory Practices.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily on weekdays and once daily on weekends and holidays for signs of morbidity and mortality. Detailed clinical observations were performed weekly.

Results: One low-dose female was found dead on day 33 and two high-dose females were found dead at study termination (day 97); the cause of death in these animals was not detectable. The study authors considered sporadic clinical findings to be spontaneous and unrelated to dosing. One low-dose female exhibited a reduced general state of health. Individual findings were not reported.

2. Body Weight: Mice were weighed prior to study initiation, and weekly thereafter.

Results: Table 1 summarizes data on mean body weights. Mean body weights of high-dose males were slightly but significantly ($p < 0.01$) depressed at week 1 and nonsignificantly depressed at week 13 when compared to

TABLE 1. Mean Body Weights (g ± S.D.) at Selected Intervals for Mice Administered Vinclozolin for 13 Weeks[a]

Dose Level (ppm)	Mean Body Weight at Week: 0	1	4	8	13
			Males		
0	21.2 ± 0.8	22.8 ± 0.7	25.7 ± 1.1	28.6 ± 1.4	32.4 ± 2.1
100	21.1 ± 0.6	22.9 ± 0.6	25.2 ± 0.9	27.5 ± 1.1	31.6 ± 2.4
1000	21.1 ± 0.6	22.9 ± 0.7	25.4 ± 1.0	28.0 ± 1.6	31.3 ± 2.1
5000	21.2 ± 0.4	21.1 ± 0.6**	25.1 ± 1.0	28.4 ± 2.7	29.8 ± 2.2
			Females		
0	18.1 ± 0.6	19.5 ± 0.6	22.0 ± 0.6	23.2 ± 0.6	24.7 ± 0.7
100	18.1 ± 0.5	19.6 ± 0.5	22.0 ± 0.7	23.1 ± 0.9	24.2 ± 1.1
1000	18.2 ± 0.6	19.8 ± 0.6	21.6 ± 0.7	22.4 ± 1.5	23.7 ± 0.9
5000	18.4 ± 0.3	19.4 ± 0.7	21.5 ± 0.9	23.0 ± 1.2	24.9 ± 2.0

[a]Based on 10 mice/dose/sex with the exception of low-dose females with 9 animals from study week 5 to study termination.

**Significantly different from controls at $p < 0.01$.

concurrent controls. Depressions were 7.5 and 8.1%, respectively. All other body weights of dosed males and females were similar to concurrent controls. No individual data were presented.

3. Food Consumption and Compound Intake: Food consumption was determined, and mean daily dietary consumption was calculated weekly. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results: Results of food consumption are summarized in Table 2. The study author reported that variations in food consumption were due to food spillage and were not a result of dosing. However, the mean food consumption of high-dose males was depressed 11 to 21% from weeks 2 to 4, week 10, and from weeks 12 to 13; food consumption of high-dose females was consistently depressed from 25 to 40% throughout the study when compared to concurrent controls.

Food efficiency varied sporadically in males and females throughout the study when compared to concurrent controls. The study author considered these changes to be a result of food spillage because of the absence of a dose-response relationship and the intermittent occurrence of the changes. Compound intake was reported as approximately 20, 230, and 940 mg/kg in low-, mid-, and high-dose males, respectively, and approximately 30, 310, and 1240 mg/kg in low-, mid-, and high-dose females, respectively. Compound intake was reported on the basis of mean food consumption, which was considered to vary as a result of food spillage. Individual values were not reported.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from the retroorbital venous plexus following animal sacrifice (96 days for males, 97 days for females) for hematology and clinical analysis from all mice. The CHECKED (X) parameters were examined:

TABLE 2. Food Consumption (g/animal/day) at Selected Intervals for Mice Administered Vinclozolin for 13 Weeks[a]

Dose Level (ppm)	Mean Food Composition at Week: 1	4	8	13
		Males		
0	4.8 ± 0.7	5.6 ± 0.8	6.2 ± 0.8	5.2 ± 0.7
100	5.6 ± 0.9	5.8 ± 0.9	6.0 ± 1.9	5.6 ± 0.7
1000	6.0 ± 0.7	6.3 ± 1.2	7.1 ± 0.9	6.2 ± 0.7
5000	3.8 ± 0.6	4.5 ± 1.2	6.2 ± 0.7	4.6 ± 0.5
		Females		
0	6.7 ± 1.4	8.2 ± 1.5	9.1 ± 1.2	7.7 ± 1.0
100	6.5 ± 1.5	7.7 ± 1.3	8.2 ± 1.8	6.0 ± 0.8
1000	5.9 ± 1.0	6.7 ± 0.8	8.5 ± 1.1	6.6 ± 0.8
5000	4.3 ± 0.6	4.9 ± 1.5	7.5 ± 1.2	5.1 ± 1.1

[a]Based on 10 mice/dose/sex with the exception of low-dose females with 9 animals from study weeks 5 to study termination.

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count†
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†		Coagulation:thromboplastin time (PT)
X	Reticulocyte count (RETIC)		
	Red cell morphology		

Results: Table 3 presents selected hematology data in mice sacrificed at 13 weeks. Erythrocyte counts, hemoglobin concentration, hematocrit levels, and calculated red cell indices (MCH, MCV, MCHC), were slightly increased (3 to 7%) in high-dose males and females; these changes do not appear to be of any toxicological significance. Lymphocyte and leukocyte counts of these animals were increased when compared to concurrent controls; increases were 50 and 57% in males, respectively, and 85 and 55% in females, respectively. The study author considers these increased white cell counts to be a result of dosing and to be associated with the histological changes in the adrenals. Individual data were not provided by the study author.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Albumin/globulin ratio
	Magnesium	X	Blood creatinine†
X	Phosphorus†		Blood urea nitrogen†
X	Potassium†	X	Cholesterol
X	Sodium†	X	Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein†
	Creatine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase	X	Urea
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Selected Mean Hematology Data in Mice Administered Vinclozolin for 13 Weeks[a]

Dose Level (ppm)	Erythrocyte Count (Tera/L)	Hemoglobin (mmol/L)	Hematocrit (L/L)	Lymphocyte Count (Giga/L)[b]	Leukocyte Count (Giga/L)
			Males		
0	10.22 ± 0.22	10.11 ± 0.24	0.43 ± 0.01	4.55 ± 1.04	5.64 ± 1.25
100	10.34 ± 0.24	10.16 ± 0.22	0.44 ± 0.01	5.05 ± 0.85	6.38 ± 1.14
1000	10.22 ± 0.32	10.13 ± 0.30	0.44 ± 0.01	4.47 ± 0.79	6.01 ± 1.32
5000	10.61 ± 0.31**	10.72 ± 0.35**	0.46 ± 0.02**	7.15 ± 1.26	8.45 ± 1.30**
			Females		
0	9.84 ± 0.53	9.65 ± 0.52	0.42 ± 0.02	2.17 ± 1.14	3.09 ± 1.61
100	9.91 ± 0.57	9.87 ± 0.55	0.42 ± 0.03	2.44 ± 1.81	3.25 ± 1.92
1000	10.25 ± 0.42	10.27 ± 0.51*	0.44 ± 0.02	1.50 ± 1.11	2.29 ± 1.18
5000	10.10 ± 0.37	10.27 ± 0.38*	0.45 ± 0.02*	4.01 ± 2.07	4.80 ± 2.13

[a]Based on 10 mice/dose/sex with the exception of low-dose females with 9 animals from study weeks 5 to study termination.

[b]Tera/L = 10^{12}/L; Giga/L = 10^{9}/L.

*Significantly different from controls at $p < 0.05$.

**Significantly different from controls at $p < 0.01$.

Results: Table 4 presents selected clinical chemistry data in animals sacrificed at 13 weeks. Serum alanine aminotransferase (SGPT) and alkaline phosphatase levels of high-dose males were significantly ($p < 0.01$) increased by 1.96-fold and by 18%, respectively, when compared to concurrent controls; SGPT levels of high-dose females were nonsignificantly increased by 58%. These elevated serum enzyme activities were considered to be a result of dosing and associated with changes in liver function. Levels of triglycerides and cholesterol were significantly ($p < 0.01$) depressed in mid- and high-dose males and females. Triglyceride levels were depressed 27 and 42% in mid- and high-dose males, respectively, and 26 and 37% in mid- and high-dose females, respectively; cholesterol levels were depressed 24 and 59% in mid- and high-dose males, respectively, and 30 and 56% in mid- and high-dose females, respectively. These changes were considered a result of dosing and related to changes in lipid metabolism. Total protein (5.5%) and globulin (10.7%) levels of high-dose males were significantly increased ($p < 0.01$ and $p < 0.05$, respectively), and glucose levels of these animals were significantly ($p < 0.01$) depressed (31%); these changes were considered to be a result of compound-related effects on protein and carbohydrate metabolism. Other changes in clinical chemistry parameters were slight and considered to be unrelated to dosing. Individual data were not provided by the study author.

6. Urinalysis: Urinalyses were not conducted.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule (96 days for males, 97 days for females) were subject to gross pathological examination (following a fasting period of 16 hours), and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

TABLE 4. Selected Clinical Chemistry Data in Mice Administered Vinclozolin for 13 Weeks[a]

Dose Level (ppm)	SGPT (μkat/L)[b]	Alkaline Phosphatase (μkat/L)[b]	Glucose (mmol/L)	Total Protein (g/L)	Globulins (g/L)	Triglycerides (mmol/L)	Cholesterol (mmol/L)
				Males			
0	1.49 ± 0.49	2.88 ± 0.30	5.35 ± 0.92	64.97 ± 1.45	27.83 ± 1.81	1.68 ± 0.24	2.99 ± 0.29
100	1.49 ± 0.41	2.84 ± 0.36	4.52 ± 0.63	63.86 ± 3.51	26.86 ± 2.45	1.51 ± 0.19	2.77 ± 0.23
1000	1.49 ± 0.32	2.89 ± 0.27	4.63 ± 0.77	64.11 ± 1.57	27.75 ± 1.84	1.22 ± 0.10**	2.29 ± 0.39**
5000	4.41 ± 1.87**	3.41 ± 0.48**	3.69 ± 0.93**	60.59 ± 2.70**	30.80 ± 2.37*	0.98 ± 0.12**	1.24 ± 0.21**
				Females			
0	1.48 ± 0.51	4.49 ± 0.70	4.72 ± 0.49	59.20 ± 2.46	23.42 ± 2.09	1.10 ± 0.13	2.30 ± 0.32
100	1.88 ± 0.65	4.49 ± 0.81	5.14 ± 0.70	59.21 ± 3.92	22.96 ± 1.71	1.03 ± 0.13	2.24 ± 0.19
1000	1.76 ± 0.38	4.70 ± 0.67	5.59 ± 0.92*	59.40 ± 2.85	23.45 ± 2.81	0.82 ± 0.17**	1.62 ± 0.51**
5000	2.34 ± 1.37	4.44 ± 0.79	4.13 ± 0.80	58.75 ± 2.47	25.39 ± 1.32	0.69 ± 0.11**	1.02 ± 0.17**

[a]Based on 10 mice/dose/sex with the exception of low-dose females with 9 animals from study week 5 to study termination.

[b]μkat = microkatal.

*Significantly different from controls at $p < 0.05$.

**Significantly different from controls at $p < 0.01$.

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta†	X	Brain
X	Salivary glands†	X	Heart†	X	Peripheral nerve (sciatic nerve)•
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes† (mesenteric, mandibular)	X	Pituitary†
X	Duodenum†	X	Spleen	X	Eyes (optic nerve)
X	Jejunum†	X	Thymus		
X	Ileum†				Glandular
X	Cecum†		Urogenital	XX	Adrenals†
X	Colon†	XX	Kidneys†		Lacrimal gland
X	Rectum	X	Urinary bladder†	X	Mammary gland
XX	Liver†	XX	Testes†	X	Thyroids†
X	Gallbladder†	X	Epididymides	X	Parathyroids•
X	Pancreas†	X	Prostate		Harderian glands
		X	Seminal vesicle		
	Respiratory	X	Ovaries		
X	Trachea†	X	Uterus		
X	Lung†				
					Other
				X	Bone (sternum and femur)
				X	Skeletal muscle
				X	Skin
				X	All gross lesions and masses†

Results:

a. Organ Weights: Data for mean organ weights and organ-to-body weight ratios are presented in Table 5. Absolute liver weights of high-dose males and females (64 and 41%, respectively) and relative liver weights of mid- and high-dose mice (15 and 80%, respectively, in males, and 6 and 40%, respectively, in females) were significantly ($p < 0.01$) increased in a dose-related manner when compared to concurrent controls. In addition, absolute and relative adrenal weights (75% and 50% increase, respectively) of high-dose males were significantly increased ($p < 0.01$). These weight changes were considered to be related to dosing, and concurrent changes were found in histopathology and

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Mean Organ Weights and Organ to Body Weight (bw) Ratios in Mice Administered Vinclozolin for 13 Weeks[a]

Dose Level (ppm)	Liver		Adrenals		Kidneys		Testes	
	Absolute (g ± S.D.)	Relative (% of bw ± S.D.)	Absolute (g ± S.D.)	Relative (% of bw ± S.D.)	Absolute (g ± S.D.)	Relative (% of bw ± S.D.)	Absolute (g ± S.D.)	Relative (% of bw ± S.D.)
Males								
0	1.22 ± 0.06	4.28 ± 0.21	0.004 ± 0.0017	0.02 ± 0.005	0.39 ± 0.02	1.36 ± 0.07	0.82 ± 0.008	0.77 ± 0.04
100	1.18 ± 0.04	4.20 ± 0.27	0.004 ± 0.0008	0.01 ± 0.003	0.38 ± 0.02	1.37 ± 0.07	0.82 ± 0.010	0.79 ± 0.05
1000	1.33 ± 0.08	4.91 ± 0.32**	0.005 ± 0.0013	0.02 ± 0.005**	0.36 ± 0.02**	1.32 ± 0.08	0.83 ± 0.007	0.86 ± 0.07
3000	2.00 ± 0.33**	7.69 ± 0.36**	0.007 ± 0.0011**	0.03 ± 0.005**	0.34 ± 0.01**	1.31 ± 0.10	0.83 ± 0.008*	0.89 ± 0.08**
Females								
0	1.03 ± 0.06	5.17 ± 0.23	0.008 ± 0.0017	0.04 ± 0.009	0.31 ± 0.01	1.55 ± 0.08		
100	1.00 ± 0.06	5.07 ± 0.14	0.009 ± 0.0016	0.04 ± 0.008	0.31 ± 0.02	1.59 ± 0.06		
1000	1.07 ± 0.07	5.49 ± 0.20*	0.009 ± 0.0018	0.05 ± 0.008	0.31 ± 0.01	1.62 ± 0.07		
3000	1.48 ± 0.22*	7.24 ± 0.32**	0.009 ± 0.0021	0.04 ± 0.012	0.31 ± 0.02	1.55 ± 0.12		

[a]Based on 10 mice/sex/dose with the exception of low-dose females with 9 animals from study week 5 to study termination.

*Significantly different from controls at $p \leq 0.05$.

**Significantly different from controls at $p \leq 0.01$.

related clinical biochemistry. Testicular weights of high-dose males were slightly but significantly ($p < 0.05$, absolute; $p < 0.01$, relative) increased, and absolute renal weights of mid- and high-dose males were slightly but significantly ($p < 0.01$) depressed when compared to concurrent controls; however, the renal weight depression was not considered to be of toxicological significance since the weight changes were slight and there were no concurrent histopathological lesions. Individual data were not provided.

b. Gross Pathology: There were no macroscopic lesions which were considered to be related to dosing.

c. Microscopic Pathology:

1) Noncompetitive: Table 6 summarizes histological findings in the mice. Centrilobular hypertrophy of the liver was exhibited in 10/10 high-dose males and 4/10 high-dose females; the hypertrophy was slight in 2 males and 2 females and moderate in 8 males and 2 females. Peripheral fatty infiltration of the liver was found in 1/10 high-dose males and females. Diffuse fatty infiltration was exhibited in control and dosed animals. Multifocal Leydig cell hyperplasia of the testes was exhibited in 3/10, 1/10, and 5/10 low-, mid-, and high-dose males; hyperplasia and hypertrophy of stromal cells of the ovaries occurred in 1/10, 6/10, and 9/10 low-, mid-, and high-dose females. Lipogenic pigment was found in the corticoadrenal cells of the adrenals of control and dosed mice; however, the degree of severity of pigment storage was increased in mid- and high-dose males and females. The study author suggested that the deposition of this pigment is indicative of a change in lipometabolism. In addition, lipid-containing vacuoles were exhibited in 10/10 high-dose males and females. All lesions were considered to be related to dosing. Individual data were not provided by the study authors.

2) Neoplastic: There were no neoplastic lesions reported.

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D. STUDY AUTHOR'S CONCLUSIONS:

Vinclozolin was administered to male and female C57BL mice at dose levels of 0, 100, 1000, or 5000 ppm for 3 months. Body weights were depressed in high-dose males (but only statistically significant at week 1). Centrilobular hypertrophy of hepatocytes was exhibited in high-dose males and females; liver weights were increased in mid- and high-dose animals. Multifocal hyperplasia of Leydig cells was found in the testes of high-dose males; hyperplasia of stromal cells was found in the ovaries of mid- and high-dose females. An increased deposition of lipogenic pigment was found in corticoadrenal cells of mid- and high-dose males ar females, and lipid-containing vacuoles were found in the a nals of high-dose animals; adrenal weights of males were i ased as a result. The no-effect level is between 100 and 1 ppm for males and females.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study report was adequate, and the conduct of the study was acceptable with the exception that individual data for all parameters were not available for review. The study report refers to Volume II for individual values, although these data were not provided. The table of contents of the pathology report refers to pages 19-126 for individual weight and histopathology data; however, these pages were omitted from the report. The table of contents of the study report indicates that a supplemental attachment includes data on analyses of the test material in the vehicle; this supplement was not attached. Owing to these omissions, individual data on stability, homogeneity, and concentration of vinclozolin in the diet were not available. Moreover, stability and homogeneity of the test material were indicated to have been tested in a different adjunct study; analyses of test material in the diet should be provided for each study conducted. The study may be upgraded with the submission of individual data for all study parameters.

The study author indicated that this study was to be used to satisfy Guideline §83-1, Chronic Oral Toxicity. The duration of this study is 3 months. According to EPA Pesticide Guidelines, 1984, this study satisfies the requirement for Guideline §82-1, Subchronic Oral Toxicity. In addition, these guidelines suggest the measurement of the blood-urea-nitrogen clinical chemistry level; this parameter was omitted in this study. The study was indicated to have been performed in accordance with OECD Guidelines, Paris, 1981; however, the study author indicated that the study does not meet the requirements of Good Laboratory Practice.

Contrary to the study author, the reviewers do not consider the body weight depressions of high-dose males to be of any toxicological significance, since this weight change occurred only at weeks 1 (statistically significant) and 13 (not statistically significant), were 8% or less, and indicated no weight effects in females. Since food consumption of high-dose males and females was consistently depressed throughout the study, the reviewers consider this effect to be a possible result of dosing. If food consumption changes in high-dose animals were the result of food spillage, an increase rather than a decrease in food consumption would be expected. We agree with the study authors that the target organs of Vinclozolin appear to be the liver, adrenals, testes, and ovaries. The LOEL is 1000 ppm, and the NOEL is 100 ppm for males and females.

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EPA No.: 68D80056
DYNAMAC No.: 382-B
TASK No.: 3-82B
August 21, 1991

DATA EVALUATION RECORD

VINCLOZOLIN

Subchronic Oral Toxicity Study in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: R J Weir

Date: 8/21/91

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Subchronic Oral Toxicity Study in Mice

REVIEWED BY:

Aisar H. Atrakchi, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Aisar Atrakchi
Date: 8/21/91

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(H-7509C)

Signature: K. Hamernik
Date: 10/11/93

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DATA EVALUATION RECORD

GUIDELINE §82-1

STUDY TYPE: Subchronic oral toxicity study in mice.

MRID NUMBER: 418243-02.

TEST MATERIAL: Vinclozolin; 3-(3,5-dichlorophenyl)-5-vinyl-5-methyl-1,3-oxazolidine-2,4-dione.

SYNONYMS: Not provided.

STUDY NUMBER: 53S0375/88025.

SPONSOR: BASF Corporation Agricultural Chemicals, Research Triangle Park, NC.

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, 6700 Ludwigshafen, Germany.

TITLE OF REPORT: Study on the Oral Toxicity of Reg. No. 83 258 (Vinclozolin) in B6C3F1 Mice.

AUTHOR: B. Hildebrand.

REPORT ISSUED: October 1990.

CONCLUSIONS: Dietary concentrations of 0, 100, 1000, 2500, or 5000 ppm (≈17, 170, 390, and 770 mg/kg/day, males; and ≈23, 250, 560, and 1170 mg/kg/day, females) of vinclozolin were fed to 10 B6C3F1/Cr1BR mice/sex/group for 3 months. No deaths or changes in food consumption were noted; there was a slight decrease (8%) in body weight and a 23% depression in weight gain in males fed 5000 ppm at study week 13. Reticulocyte counts were increased in females fed 5000 ppm. SGPT, alkaline phosphatase, and globulin levels were elevated, and triglycerides, cholesterol, albumin, and glucose levels were depressed in dosed males and females. Compound-related microscopic findings included peripheral fatty infiltration and centrilobular hypertrophy of the liver, and lesions of the testes and ovaries in males and females fed 2500 and 5000 ppm. An increased deposition of lipogenic pigment and/or lipid-containing vacuoles were found in animals fed 2500 and 5000 ppm. Liver, testicular, and adrenal weights were increased. Based on compound-related hepatic effects, the LOEL is 1000 ppm (≈170 and 250 mg/kg/day for males and females, respectively), and the NOEL is 100 ppm (≈17 and 23 mg/kg/day for males and females, respectively) of vinclozolin.

Classification: Core Supplementary. The study may be upgraded with the submission of individual animal data for all study parameters.

A. MATERIALS:

1. Test Compound: Vinclozolin; description: white solid; batch No.: N 183; purity: 99.2%.

2. Test Animals: Species: mouse; strain: B6C3F1/Cr1BR; age: 49 days at study initiation; weight: males--22.2 to 25.0 g, females--19.0 to 22.1 g; source: Charles River Breeding Laboratories, Wilmington, MA.

B. STUDY DESIGN:

1. Animal Assignment: Following a 6-day acclimation period, animals were assigned to the following test groups on the basis of weight using a computerized randomization program:

Test group	Dose in diet (ppm)	Main study (90 days) Males	Females
1 Control	0	10	10
2 Low (LDT)	100	10	10
3 Mid A (MDT)	1000	10	10
4 Mid B (MDT)	2500	10	10
5 High (HDT)	5000	10	10

Animals were individually housed in an environment controlled for temperature (20 to 24°C), relative humidity (30-70%), and light (12-hour light/dark cycle).

Dose levels were based on a previously conducted feeding study in mice. Vinclozolin was administered to NMRI mice at dose levels of 162, 486, 1458, or 4374 ppm for 112 weeks; body weight gain and food consumption were significantly reduced at the two highest dose levels; no other adverse compound effects were seen.

2. Diet Preparation: Test diets were prepared weekly. A premix was prepared by mixing an appropriate quantity of the test material with a small amount of the basal diet. The premix was adjusted to the appropriate test concentrations with the addition of basal diet and mixed. Analyses of concentration were reported to have been measured at study initiation and termination. Homogeneity and stability analyses were conducted during unspecified times.

   Results: The study authors reported that the test material was stable for 10 to 32 days. Results of homogeneity and concentration analyses of the test material in the feed were not reported; individual data on stability, homogeneity, and concentration were not reported.

3. Food and Water Consumption: Animals received food (Kliba maintenance diet, GLP 343 meal, Klingentalmuhle AG, Switzerland) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weights and clinical biochemistry data were analyzed by analysis of variance and Dunnett's test. Organ weight data were compared using Dunnett's test.

5. Quality Assurance: A quality assurance statement was signed and dated October 12, 1990. The study was performed in accordance with OECD Guidelines, Paris, 1981; as indicated by the study author, the study does not meet the requirements of Good Laboratory Practices.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily on weekdays and once daily on weekends and holidays for signs of morbidity and mortality. Detailed clinical observations were performed weekly.

   Results: No deaths occurred during the study period. The study author considered sporadic clinical findings to be spontaneous and unrelated to dosing. Individual findings were not reported.

2. Body Weight: Mice were weighed prior to study initiation and weekly thereafter.

   Results: Table 1 summarizes data on mean body weights and body weight changes during selected intervals of the study. Except for a slight (8%) decrease ($p > 0.05$) in body weight and body weight gain (23% depression when compared to concurrent controls) in the 5000-ppm males during the last week of the study, treatment with the test material had no apparent effect on body weights. No individual data were presented.

3. Food Consumption and Compound Intake: Food consumption was determined, and mean daily dietary consumption was calculated weekly. Efficiency and compound intake were calculated from the consumption and body weight gain data.

   Results: Table 2 shows representative daily food consumption data during weeks 1, 6, and 13 of the study, for both male and female mice. There were no significant changes in food consumption between treated and control groups of either sex. The 29% decrease in food consumption shown by males fed the highest dose during the first exposure week may have been related to taste incompatibility. These differences and other random differences during the study were considered unrelated to treatment with the test compound. Food efficiency data

TABLE 1. Representative Results of Mean Body Weights and Body Weight Gain in Mice Fed Vinclozolin for 90 Days[a]

Dose Level (ppm)	Mean Body Weights (g ± S.D.) at Study Weeks: 0	6	13
	Males		
0	23.7 ± 1.0	30.9 ± 1.6 (+7.2)[b]	35.8 ± 2.4 (+12.1)
100	23.6 ± 0.7	29.9 ± 1.9 (+6.3)	34.3 ± 2.3 (+10.7)
1000	23.6 ± 0.9	30.2 ± 1.4 (+6.6)	34.6 ± 2.9 (+11.0)
2500	23.8 ± 0.7	30.8 ± 1.8 (+7.0)	34.1 ± 3.2 (+10.3)
5000	23.7 ± 0.7	30.2 ± 0.9 (+6.5)	33.0 ± 1.0 (+9.3)
	Females		
0	20.3 ± 0.6	25.2 ± 1.7 (+4.9)	27.2 ± 1.8 (+6.9)
100	20.3 ± 0.6	24.4 ± 1.0 (+4.1)	26.9 ± 1.6 (+6.6)
1000	20.2 ± 0.9	24.8 ± 1.4 (+4.6)	26.6 ± 1.9 (+6.4)
2500	20.0 ± 0.7	25.7 ± 1.7 (+5.7)	28.9 ± 3.0 (+8.9)
5000	20.1 ± 0.8	24.6 ± 1.5 (+4.5)	26.9 ± 1.3 (+6.8)

[a]Based on 10 mice/sex/group.

[b]Weight gains/losses from day 0 are presented in ( ); calculated by the reviewers.

TABLE 2. Representative Food Consumption for Mice Fed Vinclozolin for 90 Days[a]

Dose Level (ppm)	Mean Food Consumption (g/week ± S.D.) at Study Weeks: 1	6	13
		Males	
0	5.2 ± 1.0	5.6 ± 0.4	--[b]
100	4.9 ± 0.4	5.6 ± 0.8	--[b]
1000	4.9 ± 0.6	5.2 ± 0.5	--[b]
2500	4.5 ± 0.5	4.8 ± 0.5	--[b]
5000	3.7 ± 0.4	4.7 ± 0.4	--[b]
		Females	
0	5.2 ± 0.4	6.8 ± 0.9	6.4 ± 0.6
100	5.0 ± 0.7	6.0 ± 0.8	6.1 ± 1.2
1000	5.8 ± 0.8	6.5 ± 1.3	6.5 ± 1.3
2500	5.5 ± 1.6	6.0 ± 1.0	6.5 ± 0.8
5000	4.3 ± 0.6	6.1 ± 1.5	6.9 ± 1.4

[a]Values are based on 10 mice/sex/group, except the 13-week value for females of the 5000-ppm dose group; value was based on 9 animals.

[b]The second page of CBI Table 002 was missing; therefore, no data were available after day 56.

varied between and within the treatment groups and the controls. Table 3 shows representative data. The study author suggested that this variability was the result of food spillage, therefore not treatment-related. Table 4 presents the mean daily compound intake during weeks 1, 6, and 13 of the study. The study author attributed weekly variations in compound intake levels to food spillage. However, considering the uniformity of food efficiency in dosed males and females over the 13 weeks of the study, variations in compound intake may have been due to food refusal. Compound intakes were reported as approximately 17, 170, 390, and 770 mg/kg in males administered 100, 1000, 2500, and 5000 ppm, respectively, and approximately 23, 250, 560, and 1170 mg/kg in females administered the same dosages. Compound intake was reported on the basis of mean food consumption, which was considered to vary as a result of food spillage. Table 2, summary table of food consumption in males from days 63 to 91, was missing from the study report. Food consumption and compound intake data were based on 9 (rather than 10) high-dose females at week 13; an explanation was not provided by the study author. Individual values were not reported.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected from the retroorbital venous plexus following animal sacrifice (96 days for males, 97 days for females) for hematology and clinical analysis from all mice. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count†
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†		Coagulation: thrombo-plastin time (PT)
X	Reticulocyte count (RETIC)		
	Red cell morphology		

Results: Hemoglobin concentration, hematocrit levels, reticulocyte counts, and calculated red cell indices (MCH, MCV) were slightly increased in high-dose females. Increases were 3% in all indices with the exception of reticulocyte counts (34% increase) when compared to concurrent controls. Changes were not apparent in males

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Representative Food Efficiency for NicaFed Vinclozolin for 90 Days[a]

Dose Level (ppm)	Mean Food Efficiency (g ± S.D.) at Study Weeks: 1	6	13	1 to 13
		Males		
0	3.5 ± 1.0	4.0 ± 1.3	-0.4 ± 2.2	2.55
100	3.3 ± 1.7	3.3 ± 1.6	-1.4 ± 1.9	2.18
1000	3.2 ± 1.8	2.9 ± 1.0	-0.4 ± 1.3	2.33
2500	3.9 ± 1.2	3.7 ± 2.2	-0.5 ± 1.8	2.45
5000	2.8 ± 2.3	2.1 ± 1.5	-1.0 ± 1.6	2.25
		Females		
0	2.7 ± 1.5	1.8 ± 2.7	-0.7 ± 2.2	1.34
100	1.7 ± 1.1	1.3 ± 1.4	-0.2 ± 1.3	1.35
1000	3.3 ± 1.5	1.2 ± 1.7	-0.8 ± 1.3	1.19
2500	4.5 ± 1.4	2.0 ± 1.2	0.3 ± 0.8	1.78
5000	5.3 ± 1.7	0.9 ± 1.5	0.5 ± 0.6	1.45

[a]Values are based on 10 mice/sex/group, except the 13-week value for females of the 5000-ppm dose group; value was based on 9 animals.

TABLE 4. Representative Daily Intake for Mice Fed Phenacetin for 90 Days[a]

Dose Level (ppm)	Mean Daily Intake (mg/kg/day ± S.D.) at Study Weeks: 1	6	13	Mean Compound Intake (mg/kg/day) (weeks 1 to 13)
		Males		
100	19.8 ± 1.7	18.6 ± 1.9	16.2 ± 1.3	17
1000	197.7 ± 22.8	173.7 ± 14.8	162.4 ± 27.5	170
2500	456.6 ± 49.8	391.0 ± 26.2	373.8 ± 28.4	390
5000	755.8 ± 72.4	775.1 ± 72.4	815.4 ± 146.7	770
		Females		
100	23.8 ± 3.7	24.5 ± 3.9	22.7 ± 5.3	23
1000	268.8 ± 33.5	260.7 ± 47.9	244.8 ± 41.9	250
2500	640.4 ± 196.9	584.7 ± 121.7	568.1 ± 103.5	560
5000	987.2 ± 119.0	1235.0 ± 303.6	1265.3 ± 232.2	1170

[a]Value are based on 10 mice/sex/group, except the 13-week value for females of the 5000-ppm dose group; value was based on 9 animals.

and do not appear to be of any toxicological significance. However, the increase in reticulocyte counts may be the result of a weak hemotoxic potential of the test material. The reviewers would expect a depression in hemoglobin, hematocrit, and erythrocyte counts with such a change in reticulocytes. Individual data were not provided by the study author.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Albumin/globulin ratio
	Magnesium	X	Blood creatinine†
X	Phosphorus†		Blood urea nitrogen†
X	Potassium†	X	Cholesterol
X	Sodium†	X	Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein†
	Creatine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase	X	Urea
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

Results: Tables 5 and 6 present selected clinical chemistry data in animals sacrificed at 13 weeks. Serum alanine aminotransferase (SGPT) and alkaline phosphatase levels of high-dose males were significantly ($p < 0.01$) increased by 62% and by 44%, respectively, when compared to concurrent controls; SGPT levels of high-dose females were nonsignificantly increased by 18%. These elevated serum enzyme activities were considered to be a result of dosing and associated with changes in liver function. Levels of triglycerides and cholesterol were significantly ($p < 0.01$) depressed in a dose-related manner in males fed 1000, 2500, and 5000 ppm when compared to concurrent controls; depressions in triglycerides were 23, 44, and 50% in males fed 1000, 2500, and 5000 ppm, respectively, and depressions in cholesterol were 20, 37, and 44% in these same animals. Levels of triglycerides were nonsignificantly depressed in females fed 1000 (21% depression) and 2500 ppm (23%

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 5. Selected Clinical Chemistry Parameters (± S.D.) in Male Mice Fed Vinclozolin for 90 Days[a]

Dose Level (ppm)	SGPT (μkat/L)[b]	Alkaline Phosphatase (μkat/L)	Triglycerides (mmol/L)	Cholesterol (mmol/L)
0	1.23 ± 0.25	3.25 ± 0.31	2.22 ± 0.38	4.17 ± 0.27
100	1.35 ± 0.39	3.17 ± 0.26	1.99 ± 0.55	3.87 ± 0.25
1000	1.32 ± 0.43	3.26 ± 0.20	1.72 ± 0.33**	3.32 ± 0.22**
3500	1.44 ± 0.30	3.61 ± 0.30*	1.26 ± 0.22**	2.62 ± 0.36**
5000	2.00 ± 0.48**	4.69 ± 0.45**	1.12 ± 0.15**	2.32 ± 0.38**

Dose Level (ppm)	Albumin (g/L)	Globulin (g/L)	Glucose (mmol/L)
0	42.06 ± 1.79	22.55 ± 1.94	5.57 ± 0.87
100	41.84 ± 1.80	22.30 ± 1.38	4.79 ± 0.68
1000	40.89 ± 1.85	24.37 ± 1.90	4.38 ± 0.76**
3500	40.54 ± 1.46	24.76 ± 1.88*	3.91 ± 0.74**
5000	40.18 ± [illegible]	25.36 ± 2.18**	3.69 ± 0.54**

[a]Values are based on 10 mice/sex/group.

[b]μkat = microkatal.

*Significantly different from control value at $p < 0.05$ (Dunnett's test).

**Significantly different from control value at $p < 0.01$ (Dunnett's test).

TABLE 6. Selected Clinical Chemistry Parameters (± S.D.) in Female Mice Fed Vinclozolin for 90 Days[a]

Dose Level (ppm)	SGPT (μkat/L)	Alkaline Phosphatase (μkat/L)	Triglycerides (mmol/L)	Cholesterol (mmol/L)
0	1.77 ± 0.54	5.56 ± 0.82	1.27 ± 0.41	2.85 ± 0.23
100	1.75 ± 0.41	4.98 ± 0.55	1.14 ± 0.36	2.67 ± 0.23
1000	1.92 ± 0.53	5.14 ± 0.46	1.00 ± 0.32	2.11 ± 0.19**
2500	1.66 ± 0.35	4.54 ± 0.63**	0.98 ± 0.21	1.90 ± 0.39**
5000	2.08 ± 0.58	4.74 ± 0.66*	0.85 ± 0.15*	1.72 ± 0.31**

Dose Level (ppm)	Albumin (g/L)	Globulin (g/L)	Glucose (mmol/L)
0	43.83 ± 1.21	18.48 ± 1.10	5.37 ± 1.74
100	43.82 ± 2.05	17.05 ± 2.95	4.79 ± 0.98
1000	41.46 ± 1.40**	18.57 ± 2.07	4.81 ± 1.15
2500	40.34 ± 1.93**	19.82 ± 1.66	4.51 ± 0.79
5000	49.99 ± 1.46**	21.48 ± 1.25**	4.89 ± 0.63

[a]Values are based on 10 mice/sex/group.

[b]μkat = microkatal.

*Significantly different from control value at $p < 0.05$ (Dunnett's test).

**Significantly different from control value at $p < 0.01$ (Dunnett's test).

depression), and significantly ($p < 0.05$) depressed in females fed 5000 ppm (33% depression). Cholesterol levels were significantly ($p < 0.01$) depressed 26, 33, and 40% in females fed 1000, 2500, and 5000 ppm, respectively. Levels of triglycerides and cholesterol were depressed in a dose-related manner; these changes were considered a result of dosing and related to changes in lipid metabolism. Albumin levels of females fed 1000 (5% depression), 2500 (8%), and 5000 ppm (6%) were slightly but significantly ($p < 0.01$) depressed; globulin levels of high-dose males (12% increase) and females (16% increase) and males fed 2500 ppm (10% increase) were significantly increased ($p < 0.05$, $p < 0.01$). Glucose levels of males fed 1000 (24% depression), 2500 (30% depression), and 5000 ppm (34% depression) were significantly ($p < 0.01$) depressed in a dose-related manner. These changes were considered to be a result of compound-related effects on protein and carbohydrate metabolism. Individual data were not provided.

6. Urinalysis: Urinalyses were not conducted.

7. Sacrifice and Pathology: All animals that were sacrificed on schedule were subject to gross pathological examination; the following checked (X) tissues were collected for histological examination. In addition, the (XX) organs were also weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta†	X	Brain
X	Salivary glands†	X	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes† (mesenteric, mandibular)	X	Pituitary†
X	Duodenum†	X	Spleen	X	Eyes (optic nerve)
X	Jejunum†	X	Thymus		
X	Ileum†				
X	Cecum†		Urogenital		Glandular
X	Colon†	XX	Kidneys†	XX	Adrenals†
X	Rectum	X	Urinary bladder†		Lacrimal gland
XX	Liver†	XX	Testes†	X	Mammary gland
X	Gallbladder†	X	Epididymides	X	Thyroids†
X	Pancreas†	X	Prostate	X	Parathyroids†
		X	Seminal vesicle		Harderian glands
	Respiratory	X	Ovaries		
X	Trachea†	X	Uterus		
X	Lung†				
					Other
				X	Bone (sternum and femur)
				X	Skeletal muscle
				X	Skin
				X	All gross lesions and masses†

All tissues were examined histologically in treated and control groups.

Results:

a. Organ Weights: Table 7 presents absolute and relative organ weights for males and females. A significant ($p < 0.01$) dose-related increase in absolute and relative liver weights was found in males (22 to 49% increase) and females (25 to 38% increase) fed the three highest doses (the absolute liver weight in females fed 1000 ppm was not statistically significant). Absolute kidney weights of dosed males were significantly ($p < 0.05$, < 0.01) decreased; relative kidney weights were

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 7. Absolute and Relative Mean Organ Weights (± S.D.) of Mice Fed Vinclozolin for 90 Days[a]

Dose Level (ppm)	Males		Females	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
		Liver		
0	1.211 ± 0.08	3.884 ± 0.19	1.105 ± 0.11	4.923 ± 0.25
100	1.204 ± 0.11	4.024 ± 0.30	1.095 ± 0.13	4.989 ± 0.32
1000	1.358 ± 0.10**	4.53 ± 0.24**	1.206 ± 0.18	5.381 ± 0.52*
2500	1.488 ± 0.14**	5.101 ± 0.46**	1.379 ± 0.10**	5.764 ± 0.39**
5000	1.807 ± 0.11**	6.387 ± 0.18**	1.523 ± 0.15**	6.828 ± 0.39**
		Kidneys		
0	0.507 ± 0.03	1.626 ± 0.11	0.366 ± 0.04	1.633 ± 0.13
100	0.464 ± 0.05*	1.551 ± 0.14	0.36 ± 0.02	1.646 ± 0.07
1000	0.44 ± 0.03**	1.467 ± 0.11**	0.373 ± 0.03	1.674 ± 0.06
2500	0.411 ± 0.04**	1.408 ± 0.11**	0.373 ± 0.02	1.564 ± 0.15
5000	0.429 ± 0.02**	1.519 ± 0.06	0.369 ± 0.03	1.656 ± 0.10
		Testes		
0	0.247 ± 0.02	0.795 ± 0.06		
100	0.244 ± 0.02	0.815 ± 0.04		
1000	0.251 ± 0.01	0.842 ± 0.09		
2500	0.259 ± 0.02	0.889 ± 0.07**		
5000	0.267 ± 0.01**	0.944 ± 0.06**		
		Adrenal Glands		
0	4.1 ± 1.10	0.013 ± 0.003	8.2 ± 1.93	0.037 ± 0.010
100	4.2 ± 0.79	0.014 ± 0.003	8.4 ± 0.97	0.038 ± 0.005
1000	4.7 ± 0.68	0.016 ± 0.003	9.5 ± 1.58	0.042 ± 0.006
2500	5.2 ± 1.23*	0.018 ± 0.004*	10.6 ± 2.07**	0.045 ± 0.011
5000	7.2 ± 1.03**	0.025 ± 0.004**	9.6 ± 1.90	0.043 ± 0.008

[a]Based on 10 mice/sex/group.

*Significantly different from control value at $p < 0.05$ (Dunnett's test).

**Significantly different from control value at $p < 0.01$ (Dunnett's test).

significantly decreased in males fed 1000 and 2500 ppm. Kidney weights of females were similar to concurrent controls. There were no concurrent histological kidney changes. Absolute and relative testicular weights of males fed 2500 and 5000 ppm were slightly but significantly ($p < 0.01$) increased (5 to 8% increase in absolute weights) when compared to concurrent controls; the mean absolute testicular weight of males fed 2500 ppm was nonsignificantly increased. Absolute (27 to 76% increase) and relative adrenal weights of dosed males were increased in a dose-related manner when compared to concurrent controls; increases were significant ($p < 0.05$, $p < 0.01$) at 2500 and 5000 ppm. Absolute (17 to 29% increase) and relative adrenal weights of dosed females were slightly increased; increases were significant ($p < 0.01$) in females fed 2500 ppm. Individual data were not reported.

b. Gross Pathology: Gross pathological examination did not reveal any compound-related changes in either sex.

c. Microscopic Pathology: Table 8 summarizes histological findings in mice fed vinclozolin. Minimal to slight centrilobular hypertrophy of the liver was exhibited in 10/10 high-dose males and 7/10 males fed 2500 ppm. Peripheral fatty infiltration of the liver was observed in 2/10 high-dose males and 8/10 high-dose females; diffuse fatty infiltration was observed in control and dosed animals. Multifocal Leydig cell hyperplasia of the testis was exhibited in 5/10, 10/10, and 10/10 males fed 1000, 2500, and 5000 ppm; minimal to slight hyperplasia of stromal cells of the ovaries occurred in 3/10 control females and 3/10, 1/10, 3/10, and 9/10 females fed 100, 1000, 2500, and 5000 ppm vinclozolin. Lipogenic pigment and/or lipid vacuoles were found in the corticoadrenal cells of the adrenals of control and dosed males and dosed females; however, the degree of severity of pigment storage was increased in mid- and high-dose males and females. A-cell hyperplasia of the adrenals was exhibited in dosed and control males and females; the incidence of this finding was increased in dosed and control females and males fed 1000, 2500, and 5000 ppm. The study author suggested that the deposition of this pigment is indicative of a change in lipometabolism. All lesions were considered to be related to dosing. Individual data were not provided by the study author.

TABLE 8. Representative Histopathological Findings in Mice Fed Vinclozolin for 13 Weeks

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	100	1000	2500	5000	0	100	1000	2500	5000
Liver	(10)[a]	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hypertrophy	0	0	0	7	10	0	0	0	0	0
Fatty infiltration-peripheral	0	0	0	0	2	0	0	0	0	8
Fatty infiltration-diffuse	10	10	10	10	8	7	6	9	10	2
Necrosis, focal	0	1	0	0	0	0	0	1	0	0
Adrenal cortex	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Lipid vacuoles	0	0	0	2	10	9	8	8	10	10
Pigment, lipogenic	9	9	10	8	10	0	1	9	10	10
A-cell hyperplasia	2	4	9	7	8	10	10	10	10	9
Testes	(10)	(10)	(10)	(10)	(10)					
Leydig cell hyperplasia	0	0	5	10	10					
Ovaries						(10)	(10)	(10)	(10)	(10)
Hyperplasia, stromal						3	3	1	3	9

[a]Numbers in parentheses equal number of tissues examined.

D. STUDY AUTHOR'S CONCLUSIONS:

Vinclozolin was administered to male and female B6C3F1 mice at dose levels of 0, 100, 1000, 2500, or 5000 ppm for 3 months. Body weights were depressed in high-dose males. Centrilobular hypertrophy of hepatocytes was exhibited in mid- and high-dose males; liver weights were increased in males and females administered 1000, 2500, or 5000 ppm. Multifocal hyperplasia of Leydig cells was found in the testes of males fed 1000, 2500, or 5000 ppm; hyperplasia of stromal cells was found in the ovaries of high-dose females. An increased deposition of lipogenic pigment was found in corticoadrenal cells of high-dose males and females fed 1000, 2500, or 5000 ppm; lipid-containing vacuoles were found in the adrenals of high-dose males and females and males fed 2500 ppm. Adrenal weights of males were increased as a result. The no-effect level is <1000 ppm for males and females.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study report was adequate, and the conduct of the study was acceptable with the exception that individual data for none of the parameters were available for review. The study report refers to Volume II for individual values, although these data were not provided. Table 2, summary table of food consumption in males from days 63 to 91, was missing from the study report. The table of contents of the pathology report refers to pages 22-145 for individual weight and histopathology data; however, these pages were omitted from the report. The table of contents of the study report indicates that a supplemental attachment includes data on analyses of the test material in the vehicle; this supplement was not attached. Owing to these omissions, individual data on stability, homogeneity, and concentration of vinclozolin in the diet were not available. Food consumption and compound intake data were based on 9 (rather than 10) high-dose females at week 13; an explanation was not provided by the study author. The study may be upgraded with the submission of individual data for all study parameters.

The study author erroneously indicated that this study was to be used to satisfy Guideline §83-1, Chronic Oral Toxicity, even though the duration of this study was 3 months. According to EPA Pesticide Guidelines, 1984, this study satisfies the requirement for Guideline §82-1, Subchronic Oral Toxicity. In addition, these guidelines suggest the measurement of the blood-urea-nitrogen clinical chemistry level; this parameter was omitted in this study. The study was indicated to have been performed in accordance with OECD Guidelines, Paris, 1981; however, the study author indicated that the study does not meet the requirements of Good Laboratory Practices.

We agree with the study author that the hepatic and gonadal effects observed were compound-related. Increased liver weights, enzymatic activity indicative of liver damage, and centrilobular hepatocellular hypertrophy all indicate hepatotoxicity. Gonadal pathology included minimal to slight multifocal hyperplasia of Leydig cells in males and hyperplasia of the ovarian stromal cells in females. We agree that the deposition of pigment in the adrenals may have been the result of changes in lipometabolism. Absolute kidney weights of all dosed males and relative kidney weights of males fed 1000 and 2500 ppm were significantly depressed; there were no pathological findings in the kidneys of these animals. Based on the observed hepatic effects, the LOEL is 1000 ppm (≈170 and 250 mg/kg/day for males and females, respectively), and the NOEL is 100 ppm (≈17 and 23 mg/kg/day for males and females, respectively) vinclozolin.

Primary reviewer: David G Anderson, PhD. David G Anderson 4/19/93
Section 3, Tox. Branch 1 (H7509C).
Secondary reviewer: Karen Hamernik, PhD. K. Hamernik 5/12/93
Section 3, Tox. Branch-1 (H7509C).

010261

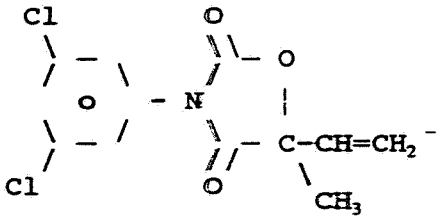
INTERIM DATA EVALUATION REPORT

STUDY TYPE: 5-α-Reductase Activity with vinclozolin, 90/0379 (Special Study).

PC No.: 113201.
TOX. CHEM. No.: 323C
DP Barcode No.: D163181.
Submission No.: S393896.
MRID No.: 418243-04.

TEST MATERIAL: 83 258, Vinclozolin, technical; A.I. is [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedi-2,4-one].

STRUCTURE:



SYNONYMS: Ronilan™.

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.: 90/0379.

REPORT TITLE: Report on the effect of Reg. No. 83 258 (ZST No. 88/375) on 5-α-Reductase Activity in vitro.

AUTHOR(S): Dr. van Ravenzwaay.

REPORT ISSUED: September 4, 1990.

CONCLUSIONS: 5-α-Reductase was prepared from B6C3F1 mouse liver supernatant and treated with 0.05 mg vinclozolin and the change in optical density was determined at 340 nm.

Vinclozolin does not act in vitro through inhibition of the 5-α-reductase in the conversion of testosterone (T) to dihydrotestosterone (DHT). Although comparability of mouse and rat 5-α-reductase was proved, it is reasonable to assume that this enzyme would not be inhibited in treated rats. It was postulated that the prostate atrophy in rats from vinclozolin administration resulted from inhibition of the synthesis of DHT.

Core classification: Supplementary non guideline study because it is a special study on the mechanism of vinclozolin action and insufficient details of the study were given.

A. MATERIALS:

010261

1. Test compound: Vinclozolin.

2. Test livers: Species: Mice, Strain: B6C3F1.

3. Environmental: Not given.

4. Food and Water: NA.

B. STUDY DESIGN: Vinclozolin was postulated to inhibit 5-α-reductase. Testosterone (T) is converted to dihydrotestosterone (DHT) by 5-α-reductase, a cytosol enzyme. DHT is the androgen necessary to stimulate the prostate. The study was conducted on the activity of 5-α-reductase activity in the 104,000 x G fraction of livers from untreated B6C3F1 mice. The study was conducted according to the method of Aumasa and Kochakain (1972) Steroids, 19: 325-355. The method depends on the decrease in absorbance at 254 nm (Maximum absorbance of testosterone).

Interference with a necessary cofactor of the 5-α-reductase reaction forced the investigators to study the reaction at 340 nm, the absorption maximum of the NADPH cofactor for the reaction.

C. RESULTS AND DISCUSSION:

The reaction was studied with 0.05 mg vinclozolin, (absorption decrease = 0.256 units /minute/cm/mg protein), and without, (absorption decrease = 0.262 units /minute/cm/mg protein). vinclozolin The absorption change was within experimental error; the change was similar with and without vinclozolin. This demonstrated that the reaction occurred as rapidly with and without vinclozolin, indicating that vinclozolin did not inhibit the reaction.

Reasonably assuming that 5-α-reductase is the same in the mouse liver and the prostates of rats and other animals, the decrease in the prostate weight occurring in rats, mice and dogs reported in several chronic, subchronic, reproduction and oncogenicity studies was probably not due to failure of the T to DHT conversion by the reductase enzyme. However, the author points out that since vinclozolin is extensively metabolized in vivo, metabolites not present in the in vitro experiment could have still inhibited the T to DHT reaction in rats, mice and dogs.

DER 5-α-Reductase Inhibition by Vinclozolin/90/0379/418243-04/
B:\VINCLV33.23C\D5AREDUC.TAS/DANDERSON/11/16/92;Edited 4/19/93.*

Primary reviewer: David G Anderson, PhD. David G Anderson 4/15/93
Section 3, Tox. Branch 1 (H7509C).
Secondary reviewer: Karen Hamernik, PhD. K. Hamernik 5/12/93
Section 3, Tox. Branch-1 (H7509C).

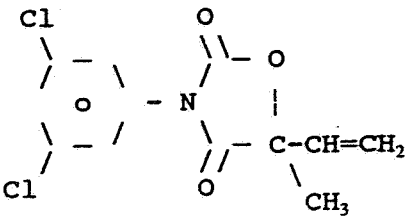
DATA EVALUATION REPORT 010261

STUDY TYPE: Hormone Status of 6-Month Rat Feeding Study with Vinclozolin, 89/0601 (Special Study).

PC No. & Shaughnessy No.: 113201.
ToxChem No.: 323C.
HED Project No.: 1-1063.
DP Barcode No.: D163181.
Submission No.: S393896.
MRID No.: 418243-05.

TEST MATERIAL: 83 258, Vinclozolin, technical; A.I. is [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedi-2,4-one].

STRUCTURE:



SYNONYMS: Ronilan™.

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

TESTING FACILITY: Institute of Biochemical Endocrinology, Medical University of Lübeck, Ratzeburger Allee 160 D-2400 Lübeck, Germany.

STUDY NO.: 89/0601, Project# 31S0375/88050.

REPORT TITLE: Examination of the Hormone Status Associated with the six-Months Feeding Study in Wistar Rats.

AUTHOR(S): Dr. R. Knuppen.

REPORT ISSUED: July 1, 1989.

CONCLUSIONS: Ten Wistar rats per sex per group of controls and 4500 ppm dose group were fed vinclozolin for 6-months and plasma hormone levels were determined.

In males, ACTH (164% of controls), corticosterone (163% of controls), DHEA (182% of controls), testosterone (276% of controls) and LH (1058% of controls) were statistically significantly and biologically significantly elevated. In females, ACTH (172% of controls) and LH (238% of controls) were statistically significantly and biologically significantly

elevated. The failure of corticosterone elevation with ACTH elevation in females is not consistent and may indicate that the serum collections were not timed appropriately for maximal response in females or that the adrenal was unresponsive. The elevated LH in females may be related to the stage of estrus at the time of blood sampling.

Core classification: Supplementary non guideline study because it is a special study on hormone levels with vinclozolin administration and no details were submitted .

010261

A. MATERIALS:

1. Test compound: Vinclozolin.
2. Test livers: Species: Rat, Strain: Wistar.

3. Environmental: Not given.

4. Food and Water: NA.

B. STUDY DESIGN: The effect of vinclozolin on the hormone levels of Wistar rats was conducted. However, no details were given in writing about the method of analysis, study conditions or doses administered. The title implied that vinclozolin was administered for 6-months when the hormone levels were determined, however, this is only a guess because of the complete lack of methods were presented. Evidently hormone levels were determined for ACTH, corticosterone (C), 17α-OH- progesterone (17α-OH-P), dehydroepiandrosterone (DHEA), testosterone (T), estradiol (E2) and luteinizing hormone (LH).

I believe the dose level to the rats was 4500 ppm. Dr. van Ravenzwaay of BASF indicate verbally at a meeting with BASF that a dose level of 4500 ppm was administered for 6 months prior to the hormone determinations.

C. RESULTS:

The values for the hormone levels are presented below in Table A. The investigator expressed concern for the accuracy of some of the values. Some of the sera were lipemic. Several determinations had to be repeated and for some there were insufficient plasma. ACTH values were variable. The relationship between the adrenal and pituitary were questioned because of the variable ACTH and corticosterone. Testosterone in females and FSH were not determined. In addition, prolactin was not determined. In addition, the some of the data in Table A is inconsistent with the data used to analyze statistically (the data on the page just prior to the individual data).

It would appear that the values for females were mislabeled in the submitted report. In the tables in the submitted report on the individual animal data for females, if the column labeled testosterone is relabeled 17α-OH-prog. and the column labeled 17α-OH- prog. is relabeled estradiol (E2), the values would better correlate with known values for controls. The data in the columns in Table A have been renamed accordingly.

In males, ACTH (164% of controls), corticosterone (163% of controls), DHEA (182% of controls), testosterone (276% of controls) and LH (1058% of controls) were statistically significantly and biologically significantly elevated. In females, ACTH (172% of controls) and LH (238% of controls) were statistically significantly and biologically significantly elevated. The failure of corticosterone elevation with ACTH elevation in females is not consistent and may indicate that the timing of the blood collections for females were inappropriate. It is also possible that the adrenal was not responsive to stimulation.

Table A.

Hormone levels in males and females after 6-months treatment.

Hormone determined	Males (hormone levels ± standard deviation)		Females (hormone levels ± standard deviation	
	Control	4500 ppm	Control	4500 ppm
ACTH (pg/ml)	125.9 ± 105.3	206.7 ± 161.9*	171.6 ± 116.1	295.0 ± 160.6*
C (ng/ml)	178.1 ± 104.6	290.9 ± 152.3*	448.9 ± 269.3	417.7 ± 118.7
17α-OH-P (ng/ml)	0.668 ± 0.517	0.701 ± 0.239	2.032 ± 0.578	1.964 ± 0.524
DHEA (ng/ml)	0.997 ± 0.204	1.810 ± 0.419*	0.882 ± 0.177	0.793 ± 0.152
T (ng/ml)	2.364 ± 1.058	6.544 ± 1.596*	ND	ND
E2 (pg/ml)	ND	ND	31.5 ± 14.8	33.1 ± 9.77
LH (ng/ml)	0.245 ± 9.205	2.593 ± 1.138*	0.248 ± 0.316	0.590 ± 0.206*

ND = Not determined
* = Statistically significantly different from control value.

Other Data Submitted in Subsequent Actions:

In addition, data was submitted on two 90-day studies with recovery (MRID# 423551-03 and -04) indicating that the effects (presented below) were largely reversed within 3-months after dosing stopped. For greater detail, see Interim Data Evaluation Report for MRID# 423551-03 and -04.

The effects on the testes, epididymides, seminal vesicles and coagulating gland were probably related to the anti-

androgenicity of vinclozolin. Many of the other effects demonstrated in this study may be related, at least in part, to the hormone imbalance mediated through the pituitary, testes and adrenals and caused by vinclozolin and its metabolites.

Data presented (MRID# 423551-03 and -04) with this interim report on the chronic study indicate that lesions occurring after 90-days of dosing at 4500 ppm were largely reversed after 8 and 12 weeks of untreated recovery. Adrenal, pituitary and testicular hormones were largely returned to normal. Effects on the testes, epididymides, seminal vesicles, prostate and adrenals returned toward control values. There was even some reversal of the cataracts in the eyes. These data will be reviewed in separate DERs on MRID# 423551-03 and -04.

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D. DISCUSSION:

The 6-months feeding study at 4500 ppm indicate[illegible] ACTH, corticosterone, DEHA, testosterone and LH levels were increased in males. In females, ACTH and LH only were increased. The ACTH increase in males and females may be a stress reaction or may indicate that vinclozolin administration results in release of all pituitary hormones. The apparent increase in LH in females could be due to the stage of estrus of some of the females at the time of blood sampling. It could also be related to the ovarian histopathology seen at these dose levels in other studies.

These ACTH and corticosterone levels may be of no significance, since the timing of blood collections were not stated, but are known to be important factors for the determination of these hormone levels. Inconsistent results were obtained for corticosterone and ACTH levels. Corresponding to the increased ACTH levels were increases in corticosterone levels in males but not in females after 6 months of dosing. ACTH in females and males was elevated after 3 months at the same dose level of vinclozolin (MRID# 423551-04), but corticosterone levels were increased in neither males or females. Corticosterone levels in females after 3 months dosing were about 1/2 that of control levels. The latter decrease may not reflect a true decrease with increased ACTH, but it may reflect inappropriate timing for sample collection.

The common release of LH in males and females may indicate that the release of LH in males is more complicated than a simple blockage of testosterone receptors in the pituitary-hypothalamic axis by vinclozolin as hypothesized by BASF. It could also be related to the stage of estrus at the time the blood was sampled.

Several other anti-androgens result in increased LH and testicular Leydig cell hyperplasia in males.

DER 6-months feeding study with vinclozolin on hormone elevation/89/0601/418243-05/ B:\VINCLV33.23C\DHORMONE.6M/ DANDERSON/11/16/92;Edited/4/15/93.*

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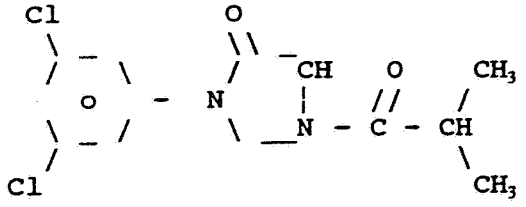
Text for the authors information only. Do not include in the DER.

The increase in LH in males is understandable from the stimulated testicular Leydig cells, but the reason for the increase in females is less clear. Competitively blocking testosterone receptors in the pituitary-hypothalamus axis would lead to greater LH release, but the receptor leading to greater LH release in females is different. In the human female, estrogen increase leads to the LH and FSH increase and progesterone prolongs the release. TSH, prolactin, FSH, somatotrophic hormone and several other hormones may be shown to be elevated if determined.

Several other anti-androgens result in increased LH in males and testicular Leydig cell hyperplasia. Among these chemicals are the following pesticides and drugs,

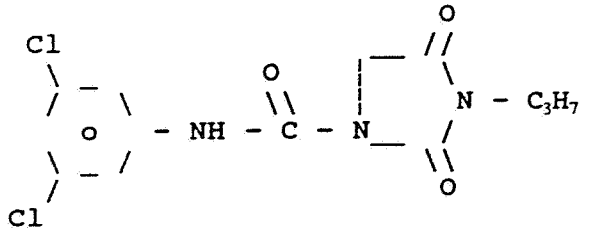
1. Gycophene, iprodione, Anfor, BSI, Chipco and Rovral



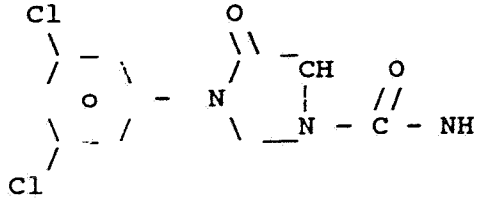
3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinedinecarboxamide.

Metabolites

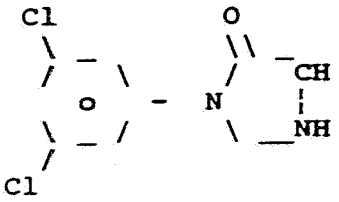
a.



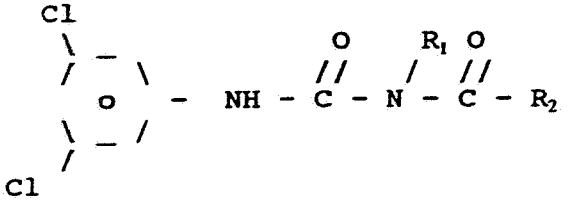
b.



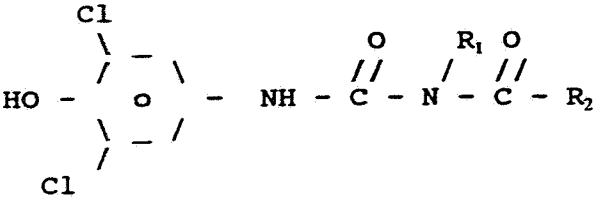
c.



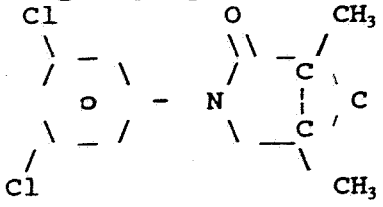
d.



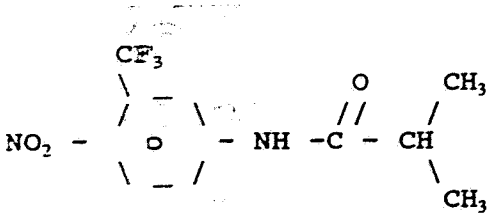
e.



2. Procymidone, [N-(3,5-dichlorophenyl)-1,2-dimethyl-cyclopropane-1,2-dicarboximide



3. Flutamide



2-methyl-N-[4-nitro-3-(trifluromethyl)phenyl]propanamide or 4'-nitro-3'-trifluromethylisobutryanilide; antiandrogenic, pure - Used in treatment of prostatic cancer.

4. A chemical (name unknown) - thought to act through a dopamine receptor. This chemical was discussed with Dr. Ettlan of Sandoz, Switzerland, in October, 1992. This chemical also results in LH increase and testicular Leydig cell hyperplasia in dosed Wistar rats.

Primary reviewer: David G Anderson, PhD. 4/9/93
Section 3, Tox. Branch 1 (H7509C).
Secondary reviewer: Karen Hamernik, PhD. 5/12/93
Section 3, Tox. Branch-1 (H7509C).

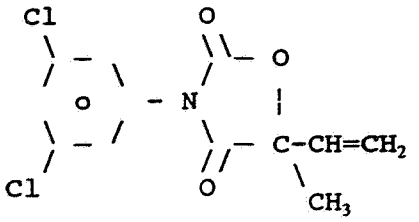
INTERIM DATA EVALUATION REPORT

STUDY TYPE: Androgen Receptor Binding Study; Vinclozolin Competition with Mibolerone binding to MCF-7 Cells. Proj.# 21B0324/889027. (Special Study).

Shaughnessy No.: 113201.
TOX. CHEM. No.: 323C
DP Barcode No.: D163181. 010261
Submission No.: S393896.
MRID No.: 418243-06.

TEST MATERIAL: 83 258, Vinclozolin, technical; A.I. is [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedi-2,4-one] and radiolabeled vinclozolin.

STRUCTURE:



SYNONYMS: Ronilan™.

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

TESTING FACILITY: Institute of Biochemical Endocrinology, Medical University of Lübeck Allee 160, D-2400 Lübeck, FRG.

STUDY NO.: 21B 0324/889027, Reg. Doc.# BASF 90/0573.

REPORT TITLE: Final Report: Study of the Binding of 3-(3,5-Dichlorophenyl)-5-Methyl-Vinyl-2,4-Dion to the Androgen Receptor in MCF-7 Cells.

AUTHOR(S): Dr. R. Knuppen.

REPORT ISSUED: April 27, 1990.

CONCLUSIONS: Vinclozolin competition with mibolerone binding to androgen receptors in the nucleus of MCF-7 cells (human mammary carcinoma cell line) and the MCF-7 cell cytosol were studied at 1 to 50 [illegible]les/l. However, above 10 µmoles/l, vinclozolin was insol[illegible]. The data are consistent with the hypothesis that vinclozolin is an anti-androgen, which competes with testosterone for receptors. The authors stated conclusions were supported by

insufficient data to evaluate the accuracy of their conclusions. Vinclozolin decomposition in the media was stated to compromise the results. In addition, DES and estradiol were inexplicably competitive with the test androgen, radiolabeled mibolerone, for the MCF-7 receptors.

Core classification: Supplementary non guideline study because it is a special study on the mechanism of vinclozolin action. In addition, because supporting details were lacking, the study was not acceptably reported.

A. MATERIALS:

1. Test compound: Vinclozolin and C^{14}-radiolabeled vinclozolin. Solubility in water and cellular cytosol was approximately 10 micromoles/liter (2.9 mg/liter). The following data were extracted from the 1989 Merck Index: Melting point 108°C. Slowly hydrolyzed in dilute alkaline solution (solubility in water stated to be 1 g/liter; may be in error). Mole weight is 289 g.

2. Test livers: Species: Human, MCF-7 Cells (Derived from human mammary carcinoma cells).

3. Environmental: Laboratory conditions.

4. Food and Water: NA.

B. STUDY RATIONALE: Vinclozolin studies on reproduction and development (males with female secondary sexual characteristics, or pseudohermaphroditism) indicated that a mechanism of action could be through hormonal activity similar to anti-androgen effects. To investigate this possibility, vinclozolin binding to the androgen receptors of MCF-7 cells were conducted.

To the best of my information, the vinclozolin binding to the androgen receptor of the cytosol and the nucleus of whole MCF-7 cells was studied through competition with radio-labeled mibolerone (17-hydroxy-7,17-dimethylestr-4-en-3-on, a synthetic anabolic steroid related to testosterone, 17β-hydroxyandrost-4-en-3-one).

C. DISCUSSION AND RESULTS:

The relative binding affinity of vinclozolin to the cytosol fraction of MCF-7 cells was 1/1000 to 1/2000 of mibolerone, and the relative affinity in the nuclear fraction was about 1/4000. No competitive binding occurred with mibolerone and 1 μmole/l vinclozolin; at 10 μmole/l detectable competition occurred, but at 50 μmole/l no further increase in competition was detectable. Unfortunately the relative affinity of testosterone and/or dihydrotestosterone with mibolerone was not presented, thus, the

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meaning of these relative bindings are of unknown biological value. However, the data are consistent with the hypothesis that vinclozolin acts by competing with androgen receptors.

Binding in the intact cell system was considerably reduced, however, the decomposition products were different and more extensive in this system, which complicated the interpretation of the data considerably.

The binding systems were only partially characterized and described. Characteristics of the growth media and portion of the structure of test material labeled were not described. The method used in the binding studies was not described. The systems used were characterized only to limited extent; Org 2058 (stated to be a gestagen, but un-characterized and otherwise un-specified) was not competitive with mibolerone, but DES and estradiol were inexplicably competitive with mibolerone. The report indicates that the known cross reactivity of synthetic androgens with glucocorticoid or progesterone receptors contained in many cells were not a problem (the latter receptors can be masked by triamcinolone acetonide). The report stated that MCF-7 cells do not have these receptors.

Some characteristics of the system were indicated in the summary submission (with no supporting data), such as the solubility of vinclozolin in the cytosol of MCF-7 cells, whole cells, stability of vinclozolin under various conditions, and some of the decomposition products. Suitable incubation conditions were not reported. Vinclozolin was soluble in water, cytosol and cell culture media up to 10 µmole/l (2.86 mg/ml). At higher concentrations from 20 to 50 µmoles/l, vinclozolin was stated to be in suspension form. Vinclozolin stability during extraction with ether and HPLC were determined (no data was submitted). It was stated to be stable during the 1 to 2-hour incubations in the HPLC eluent.

Androgen receptors are characterized by a much narrower specificity range than many receptors, one with which estrogens do not compete. For commercial potential for its androgen activity, mibolerone can be assumed to have a high affinity for androgen receptors. The literature indicates that DES and estradiol do not compete for androgen binding sites. Thus, until the competition with DES and estradiol in the system can be explained, the conclusions about vinclozolin binding to this receptor can not be definitively accepted without further characterization of the mechanism, and further details about the results and data associated with this submission. Without this mechanism, many additional questions about the systems at risk from exposure to vinclozolin should be requested.

The dissociation constant for the androgen receptor is 0.12 nmole/l which is stated to be in good agreement with the literature. The concentration of the receptor in MCF-7 cells was determined to be 160 to 170 fmoles/mg protein, which was stated to be in good agreement with the literature. In addition, the affinity for the androgen receptor was stated to be 100 fold greater than that for the sex-hormone binding globulin. This was stated to remove the possibility that this globulin was responsible for the binding. The vinclozolin was bound less in the cell system than in the cytosol system. It was less certain

that vinclozolin and not a metabolite was the binding material in the cell system because of the greater degradation in the this system. The author also indicated that vinclozolin binding was not definitive in the cell system.

It was speculated that the high loss in recovery was due to metabolite(s) of longer retention times than the chromatographic elution time used, and possibly to the small volumes used.

The system used was only under partial control. Metabolites and degradation products were not identified, and the recovery was so low in some experiments, it was difficult to draw conclusions. The system and test substances used in the studies, were insufficiently characterized, the study results were too variable to be certain of conclusions. It is difficult to be certain about any of the conclusions drawn in this study because of the above deficiencies and the lack of submission of supporting data.

In addition, the study was a translation from an interim report which was stated not be subjected to quality assurance assessment.

The rate of spontaneous decomposition of vinclozolin in solution may preclude conducting meaningful studies of this type.

Unfortunately the study was inadequately reported, referenced, and otherwise documented. The characteristics and source of the MCF-7 cells was not identified. Neither were the characteristics of mibolerone relative to testosterone and dihydrotestosterone identified. Procedures and methods were not described. The submission contained only verbal descriptions of part of the data, and referred to graphs and diagrams not submitted. Many of the problems, stated to occur with the binding study could be neither understood nor evaluated because of the lack of data submitted. Due to the terseness and lack of data submitted, only general conclusions (those presented by the author can be stated, with little to no re-evaluation).

The summary table below of the extractable vinclozolin and decomposition products under various conditions was very difficult to construct because of the lack of clarity and missing data in the report.

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Decomposition occurred during 3 and 18 hour incubations on ice and in the media used for the binding studies. Data on the 1 hour incubations at 37°C in cell culture media are also listed. As possible under necessary assumptions due to the lack of data, the findings reported were assembled in the table below. 010261

3 hour incubation in cytosol	Control	1 μm/l	50 μm/l
Vinclozolin	80%	28%	34%
Decomposition products[1]	-	8%	5%
Total recovery		36%	39%
18 hour incubation			
Vinclozolin	75%	51%	60%
Decomposition products[2]	-	29%	10%
Total recovery	-	80%	70%
1 hour incubation in cell culture medium at 37°C			
Vinclozolin	-	5%	9%
Decomposition products[3]	-	53%	47%
Total recovery	70%	58%	56%

1 Decomposition products, two chromatography peaks.
2 Decomposition products, two chromatography peaks.
3 Decomposition products did not correspond to those of the 3 and 18 hour incubations. They were referred to as metabolic products whose retention times corresponded to compounds 22 and 23 designated in the metabolism reports (MRID# 418243-07 and 418243-08). Structures of compound 22 and 23 are presented below. These same structures were designated M1 and M2 by Szeto et al., J Agric Food Chem. 37, 523-529 (1989).

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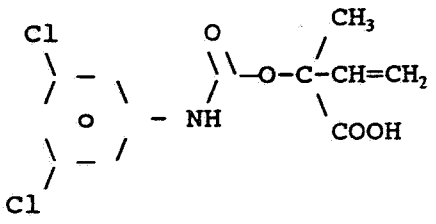
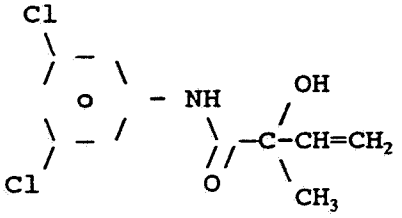
Compound 22, M1



Compound 23, M2



Memo on androgen receptor binding with vinclozolin/90/0573/HED 1-1063/418243-06/B:\VINCLV33.23C\DREPBIN/DANDERSON/11/16/92; edited 4/19/93.*

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EPA No.: 68D80056
DYNAMAC No.: 382-C/D
TASK No.: 3-82C/D
September 4, 1991

DATA EVALUATION RECORD

Vinclozolin

Metabolism in Rats

STUDY IDENTIFICATION: (a) Hawkins, D.R., Kirkpatrick, D., Dean, G.M., et al. The Biokinetics of ^{14}C-Vinclozolin in the Rat. (Unpublished study No. 90/0544, performed by Huntingdon Research Centre, Ltd., Cambridgeshire, England for BASF Aktiengesellschaft, Ludwigschafen, Germany; dated November 28, 1990.) MRID No. 418243-08. (b) Hawkins, D.R., Kirkpatrick, D., Dean, G.M. et al. The Biotransformation of ^{14}C-Vinclozolin in the Rat. (Unpublished study No. 90/0514, performed by Huntingdon Research Centre, Ltd., Cambridgeshire, England, for BASF Aktiengesellschaft, Ludwigschafen, Germany; dated November 29, 1990.) MRID No. 418243-07.

APPROVED BY:

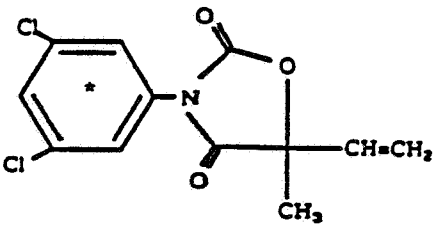
Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 9/4/91

1. CHEMICAL: Vinclozolin; 3-(3,5 dichlorophenyl)-5 methyl-5-vinyl-1,3-oxazolidin-2,4 dione.

2. TEST MATERIAL: A white solid, uniformly labeled with ^{14}C in the phenyl ring; lot 36/38 with a specific activity of 58 mCi/mmol (202.16 Ci/mg); radiochemical purity of >97%. Nonradiolabeled batch No. N183, Lot No. 88/375; 99.2% purity.



3. STUDY/ACTION TYPE: Metabolism/pharmacokinetics-balance.

4. STUDY IDENTIFICATION: (a) Hawkins, D.R., Kirkpatrick, D., Dean, G.M., et al. The Biokinetics of ^{14}C-Vinclozolin in the Rat. (Unpublished study No. 90/0544, performed by Huntingdon Research Centre, Ltd., Cambridgeshire, England, for BASF Aktiengesellschaft, Ludwigschafen, Germany; dated November 28, 1990.) MRID No. 418243-08. (b) Hawkins, D.R., Kirkpatrick, D., Dean, G.M., et al. The biotransformation of ^{14}C-Vinclozolin in the Rat. (Unpublished study No. 90/0514, performed by Huntingdon Research Centre, Ltd., Cambridgeshire, England, for BASF Aktiengesellschaft, Ludwigschafen, Germany; dated November 29, 1990.) MRID No. 418243-07.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L McLellan
Date: Sept 3, 1991

Nicolas P. Hajjar, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: September 4, 1991

010261

6. APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir
Date: 9/4/91

David G. Anderson, Ph.D.
EPA Reviewer
Acting Section Chief
Section III
Toxicology Branch I
HED (H-7509C)

Signature: David G. Anderson
Date: 11/21/92

KAREN HAMERNIK
~~Karl Baetcke, Ph.D.~~
Acting ~~EPA Branch Chief~~ Section Head
Toxicology Branch ~~III~~
(H-7509C)

Signature: K. Hamernik
Date: 5/12/93

7. CONCLUSIONS:

A. Absorption, distribution, and excretion of radioactivity were studied after oral or intravenous administration of [^{14}C-3,5-dichlorophenyl]Vinclozolin to groups of male and female Wistar rats. Single oral doses were administered at nominal levels of 10 and 100 mg/kg, and a single intravenous dose was administered at a level of 1 mg/kg. Non-radiolabeled material was also administered daily for 14 days at 10 mg/kg, followed by a single oral dose of ^{14}C material at 10 mg/kg. For additional pharmacokinetic studies, oral dosing was conducted at 200 mg/kg or by dietary administration of 5000 ppm ^{14}C-Vinclozolin-containing diets.

After single oral doses of 10 or 100 mg/kg, urinary and fecal excretion of radioactivity by both sexes in 5 days ranged between 48 and 54% and 38 and 49% of the dose, respectively. After intravenous dosing at 1 mg/kg, urinary and fecal excretion accounted for 72 and 23% of the dose, respectively. In rats with cannulated bile ducts, excretion of radioactivity in bile accounted for 73.2 and 63.5% of the 10 mg/kg oral dose in males and females, respectively (48 hours), and 62.0 (males) and 38.8% (females) of the 100 mg/kg dose. No radioactivity was excreted in expired air (pilot study). Retained radioactivity 120 hours after dosing accounted for less than 2% of any dose. Excretion of radioactivity in urine and feces was rapid with most of the administered dose (75 to 80%) eliminated by 48 hours for the 100-mg/kg oral dose. Biliary excretion was also rapid with 50% of a 10-mg/kg dose excreted within 12 hours for males and 18 hours for females.

Plasma levels of radioactivity were plotted against time after single oral doses of 10, 100, or 200 mg/kg ^{14}C-Vinclozolin, and pharmacokinetic parameters were determined. The same parameters were determined for rats ingesting diets containing 5000 ppm ^{14}C-Vinclozolin for 24 hours. In the oral gavage studies T_{max}, the time to maximum plasma level, increased with dose from 3.6 to 9.6 hours in males and from 1.8 to 13.2 hours in females with C_{max} values (peak concentration as μg equivalents/mL) between 2.8 and 23.2 in males and 2.0 to 15.6 in females. The areas under the curve (AUC) (μg equivalents/mL·hr) were similar in males and females and increased with dose (averaging approximately 74, 483, and 900 at 10, 100, and 200 mg/kg). After peak levels had been reached, concentrations in plasma declined in a biphasic manner with overall half-lives of 23 and 36 hours for males and females, respectively. Systemic availability of radioactivity appeared equivalent following gavage or dietary dosing.

The dietary doses were equivalent to 439 and 345 mg/kg in males and females, respectively; after withdrawal of diets, plasma levels declined with a half-life of about 40 hours.

Tissue concentrations of ^{14}C were higher in females than in males, with peak levels occurring 2 or 6 hours after dosing. The levels in liver, kidneys, fat, adrenals and Harderian glands were highest, 4 to 10 µg equivalents/g at peak, but the levels declined in a linear manner to about 0.02 to 0.2 µg eq/g by 5 days. After multiple dosing, concentrations in tissues were mainly in the range of 0.1 to 1.2 µg eq/g at 5 days after the ^{14}C dose.

Vinclozolin was extensively metabolized in the rat. HPLC (high performance liquid chromatography) analysis of urine indicated at least 15 metabolites in addition to small amounts of unchanged Vinclozolin. The major urinary metabolite was identified as a glucuronide conjugate of N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide (compound 25). This metabolite is derived from Vinclozolin by both cleavage of the 2,3 bond in the oxazolidine ring and epoxidation and subsequent hydration of the vinyl group. Another metabolite identified in urine had an intact oxazolidine ring but the vinyl group was modified to an ethylene glycol moiety; this compound was excreted as a glucuronide. Cleavage of Vinclozolin to dichloroaniline was a very minor pathway and accounted for less than 1% of the ^{14}C label. Cleavage of the 3,4 bond of the heterocyclic ring also occurred followed by loss of the vinyl group; the two resulting metabolites (12 and 11) accounted for less than 2% of the dose in the urine or feces and about 10% of the biliary metabolites. In the fecal extracts, unchanged Vinclozolin and the butyramide (compound 25) were the major radioactive components. Analysis of extracts of liver also showed that the same compounds were major radioactive components.

B. Both studies are acceptable. The two studies combined fulfill the Guideline Requirements 85-1 for Metabolism studies.

Items 8-10--see footnote 1.

---

[1]Only the items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOL): 010261

I. Study I, Biokinetics of ^{14}C Vinclozolin in the Rat

A. ^{14}C-Vinclozolin had a specific activity of 58 mCi/mmol (202.16 μCi/mg) and a radiochemical purity of >97%. The non-radiolabeled test substance (lot N 183) had a purity of 99.8%. Both were supplied by BASF AG, Ludwigshafen, W. Germany. ^{14}C-Vinclozolin was diluted with non-radiolabeled Vinclozolin in a solution of either acetone or dichloromethane, the solvent removed under reduced pressure, and the substance dried to constant weight. The required amount was weighed out and suspended in the gavage dose vehicle (1% w/v aqueous carboxymethyl cellulose) using a Potter homogenizer. The suspension was stored at 4°C for up to four days. Aliquots were diluted for radioassay. For intravenous dosing, 4.87 mg ^{14}C-Vinclozolin was dissolved in polyethylene glycol 400 using ultrasonic mixing. Aliquots (0.2 mL) were drawn into preweighed 1-mL syringes for dosing and reweighed. For dietary administration, 7.8 g ^{14}C-material was mixed with 100 g powdered diet with a mortar and pestle and portions further diluted with diet and mixed manually by tumbling in a sealed pot. Ten aliquots were taken and combusted and radioassayed to check for homogeneity.

B. Male and female Wistar rats (Charles River, Margate, UK, and Charles River, Portage, MI) were used and weighed approximately 200 g at dosing. The animals received food (LAD 1 pellet or LAD 2 powdered diet) and tap water ad libitum except for those with cannulated bile ducts.

C. Fourteen studies were conducted as outlined in Table 1. ^{14}C-Vinclozolin was administered orally, intravenously, or via the diet. Excretion-balance studies were conducted after low or high oral gavage doses or after intravenous administration. Biliary excretion was also studied after a low or high oral dose and plasma kinetics were determined at 3 oral gavage dose levels as well as during dietary administration. Tissue distribution was studied after a single low oral dose or after repeated administration of unlabeled Vinclozolin followed by a single ^{14}C dose.

D. Single oral dose studies--Urine was collected (5 rats/sex) at 0-6 and 6-24 hours and then at 24-hour intervals for 5 days. Feces were collected every 24 hours. Blood samples were collected prior to sacrifice, and following tissues taken after sacrifice (5 days): stomach, GI-tract, liver, kidneys, heart,

TABLE 1. Study Design

Study type	Dose route	Nominal dose level (mg/kg)	Number of animals		Actual Doses (mg/kg)
			Males	Females	
Excretion balance (pilot)	oral	10	2	2	10.4
Excretion balance	oral	10	5	5	8.5
Excretion balance	oral	100	5	5	85.3
Excretion balance[b]	oral	10	5	5	11.3
Excretion balance	intravenous	1	5	5	1.0
Plasma kinetics	oral	10	5	5	9.6
Plasma kinetics	oral	100	5	5	85.2
Plasma kinetics	dietary	200	5	5	169
Plasma kinetics	oral	5000[a]	21	21	8
Biliary excretion	oral	10	3	3	8.5,11.5
Biliary excretion	oral	100	3	3	91.8,89.8
Whole-body autoradiography	oral	7 x 10[c]	5	5	12.6,12.5
Tissue distribution	oral	10	12	12	9.7,10.7
Tissue distribution	oral	7 x 10[c]	15	15	10.9,11.1

[a]ppm in diet.

[b]Rats were pretreated for 14 days with non-radiolabeled Vinclozolin at 10 mg/kg/day.

[c]Single daily dose of [14]C-Vinclozolin for 7 days.

lungs, brains, spleen, eyes, adrenals, thyroid, gonads, muscle, fat, bone marrow, and remaining carcass.

Biliary excretion--Urine and feces were collected at 0-24 and 24-48 hours (3 rats/sex) and bile samples were collected at 1.5 hour intervals to 48 hours.

Blood kinetics--Following administration by oral gavage at 10, 100, or 200 mg/kg, blood samples were withdrawn from the tail vein into heparinized tubes at pretest, 0.5, 1, 2, 4, 6, 12, and 24 hours and 2, 3, 4, 5, 7, and 10 days. Cells were separated by centrifugation and aliquots of plasma were radioassayed.

Dietary exposure--Seven groups of three rats/sex were housed in a cage and after an eight-hour fast offered food containing 5000 ppm ^{14}C-Vinclozolin. At 24 hours, treated diet was replaced with normal diet. Groups of 3 rats/sex were removed at 2, 4, 6, 9, 12, and 18 hours, blood was removed by cardiac puncture and the animals sacrificed. From the seventh group, blood samples were collected from the tail vein at 24, 43, 67, 91, 120, 168, and 240 hours post initiation.

Tissue distribution--Groups of 3 rats/sex were given a single oral dose of 10 mg/kg ^{14}C-Vinclozolin and sacrificed at 2, 6, 12, and 48 hours. Similar groups were dosed daily with 10 mg/kg unlabeled compound followed by a single ^{14}C-dose and sacrificed at 2, 6, 24, 48, or 120 hours. Rats were bled by cardiac puncture after halothane anesthesia, sacrificed, and tissues collected for radioassay. An additional rat/sex dosed similarly (repeated dose) were sacrificed for whole body radioautography at 2, 6, 24, 48, and 120 hours.

E. Radioassay--Weights of whole organs were recorded. Adrenals, ovaries, prostate, eyes, Harderian glands, thyroid and bone marrow were combusted whole (M2-TriCarb®); samples of other organs were minced and triplicate aliquots combusted. Urine, cagewash, bile, and plasma aliquots were radioassayed in duplicate (MI-31 scintillation cocktail). Feces were homogenized in water and triplicate aliquots radioassayed. Expired air trap aliquots were diluted with methanol (1 mL aliquots plus 1 mL methanol) for radioassay. Carcasses were solubilized for 24 hours at 55°C in NaOH/CH_3OH/Triton X405 (6/3/1), aliquots neutralized with nitric acid, and radioassayed.

F. Whole-body autoradiography--After asphyxiation with CO_2, whole rats were frozen at -80°C and trimmed.

After setting in a block of carboxymethylcellulose (2% w/v) at -80°C they were sectioned in a cryostat (-20°C) and saggital sections of 30 μm cut at several levels were mounted on cellux tape. The sections were exposed to x-ray film at -20°C for 35 days.

G. Pharmacokinetic analysis--C_{max} and T_{max} were determined from radioactivity vs. time plots. Total area under the curve (AUC) used a log-linear trapezoidal method. The AUC curves were adjusted to infinite time based on the concentration (C) at the last sampling time and λZ (the terminal rate constant) determined by linear regression. Half-lives were similarly adjusted. Predicted plasma concentrations during ingestion of ^{14}C-Vinclozolin were derived values assuming a constant rate of input of test compound (zero order) and equivalent bioavailability after dietary or gavage administration.

I. Study II, Biotransformation of ^{14}C-Vinclozolin in the Rat

A. Biotransformation products of ^{14}C-Vinclozolin were analyzed from the following six studies described above (Study I):

Study type	Dose route	Nominal dose level mg/kg	Number of animals	
			Males	Females
Excretion balance	oral	10	5	5
Excretion balance	oral	100	5	5
Excretion balance	oral,repeated	10	5	5
Excretion balance	intravenous	1	5	5
Tissue distribution	oral	10	12	12
Tissue distribution	oral	7 x 10[c]	15	15

Samples from the following additional studies with one rat/sex were analyzed:

- Urine and feces collected at 24, 48, and 72 hours after a single oral dose of 200 mg/kg.
- Urine and feces collected at 6-24, 24-48, and 48-72 hour intervals after dietary dosing for 6 hours at 5000 ppm.

- Bile collected 24 hours after oral doses of 10 or 100 mg/kg to bile duct-cannulated rats.

B. Preparation of biological samples--Urine from male and female rats was analyzed separately. Urine samples (0-24 and 24-48 hours separately) were pooled and processed by absorption on a 500-mg C18 sorbent column. The column was washed with phosphate buffer (0.1 M) and then hexane, followed by elution with acetonitrile/methanol (1/1 by volume).

Urine samples were mixed with pH 5 acetate buffer and incubated at 37°C with ß-glucuronidase for 46 hours or sulfatase for 24 hours. Untreated controls were also incubated. Bile samples were also treated with ß-glucuronidase and sulfatase. Enzyme-treated samples of urine and bile were cleaned up as above on the sorbent column.

Urine samples from male rats 0-24 hours after a single dose of 100 mg/kg ^{14}C-Vinclozolin were also fractionated for metabolites. Urine was extracted with ethylacetate (2 times), acidified to pH 2, and further extracted with ethylacetate. The aqueous phase was adjusted to pH 7, placed on a column of Amberlite XAD 2, washed with water and eluted sequentially with acetonitrile and methanol. All organic fractions were concentrated to dryness and reconstituted to 5 mL.

Fecal samples collected up to 48 hours after dosing were grouped separately by sex and were extracted three times with 10 mL of acetonitrile and the organic phase concentrated.

Tissues (liver and kidney) aliquots were extracted three times with acetonitrile followed by methanol. The separate organic phases were concentrated and an aliquot of the residual tissue was combusted for ^{14}C analysis. Plasma was treated similarly to urine by sorbent extraction.

C. Chromatography and Mass Spectrometry: High performance liquid chromatography (HPLC) was carried out on Spherisorb 5 µm OD52 columns with Waters equipment and gradient elution with acetonitrile/methanol/0.1% formic acid. Radioactivity was measured with a Ramona detector cell and a u.v. detector was included in the eluate line. Samples were collected in scintillation vials. Samples for mass spectral analysis were converted to their trimethylsilyl derivatives and processed by gas chromatography on a fused silica capillary column using a programmed temperature

gradient. The column was interfaced to the ion source for electron impact mass spectrometry. Samples were also analyzed by direct injection into the ion source for liquid secondary mass spectrometry.

13. REPORTED RESULTS:

A. The pilot study showed that more than 95% of the radioactivity was excreted in the urine and feces in 5 days and no detectable radioactivity was eliminated in expired air.

Table 2 summarizes radioactivity retained in the carcass or excreted in urine and feces 5 days after a single oral dose of 10 or 100 mg/kg, repeated oral dosing (14 daily doses of unlabeled Vinclozolin at 10 µg/kg followed by a single ^{14}C-dose), or single intravenous dose (1 mg/kg). Urine and feces accounted for more than 90% of the administered dose. There was no essential difference between males and females. Residue retained in the carcasses after oral dosing ranged from 0.6 to 1.4% of the dose. Most of the dose was excreted within 48 hours (accounting for 86.9 and 81.8% in males and females, respectively, receiving 10 mg/kg orally). After an oral dose of 100 mg/kg, 84.7 and 74.7% were excreted within 48 hours in males and females, respectively. Pretreatment with unlabeled Vinclozolin for 14 days followed by a single labeled 10-mg/kg dose resulted in slightly less excretion of ^{14}C in the urine (5 days--Table 2). At 48 hours, total excretion of radioactivity was 71.8% and 73.4% of the administered dose in males and females, respectively.

B. After an intravenous dose of 1 mg/kg, about 23% of the ^{14}C dose was excreted in the feces by 5 days indicating biliary excretion (Table 2). Total excretion at 48 hours in urine and feces was 80.6 and 78.0% of the administered radioactivity in males and females, respectively.

Table 3 summarizes results of excretion in bile urine and fgtfeces 48 hours after administering single oral doses of 10 or 100 mg/kg ^{14}C-Vinclozolin to bile duct cannulated rats (3/sex). Biliary excretion was rapid with about 50% of the administered 10 mg/kg dose excreted by 12 hours in males and 18 hours in females (Figure 1). At the high dose, 50% of the administered ^{14}C in males was excreted in bile in 30 hours whereas in females the rate and amount (as percent of dose) excreted was less than in males, with only about 39% excreted in the bile at 48 hours. Total recovery of radioactivity ranged from 91 to 102% (Table 3). The retained dose at the high level was 4.9 and 10.6% in males

TABLE 2. Mean Excretion and Retention of Radioactivity by Rats at Five Days After Various Single Doses of ^{14}C-Vinclozolin

Results are expressed as % dose

Dose	Urine	Faeces	Carcass	Total
Oral 10mg/kg				
Male	52.0	46.2	0.9	99.0
Female	52.6	38.3	0.7	91.6
Oral 10mg/kg*				
Male	54.3	35.8	1.4	91.5
Female	55.5	33.8	1.1	90.4
Oral 100mg/kg				
Male	48.1	48.7	0.6	97.4
Female	54.3	39.7	1.1	95.1
I.V 1mg/kg				
Male	72.7	23.1	1.7	97.5
Female	70.5	22.8	1.1	94.5

For results in detail see Tables 3 - 10

* Pretreated with non-radiolabelled vinclozolin for 14 days

Source: Study No. 90/0544, CBI p. 43.

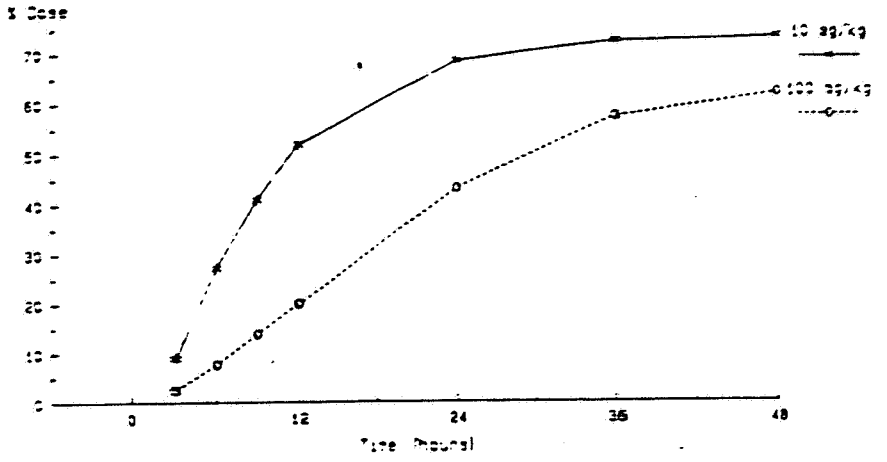
TABLE 3. Excretion and Retention of Radioactivity 48 Hours After a Single Oral Dose in Bile Duct Cannulated Rats

	Mean Percent of Radioactivity (± S.D.)[a]			
	10-mg/kg dose		100-mg/kg dose	
Bile	73.2 ± 4.8	63.5 ± 7.5	62.0 ± 2.3	38.8 ± 3.9
Urine[b]	18.3 ± 1.8	24.1 ± 7.3	14.6 ± 0.5	17.6 ± 2.5
Feces	6.04 ± 1.0	10.8 ± 0.4	16.2 ± 4.1	23.9 ± 1.9
Carcass	1.61 ± 0.2	3.25 ± 1.3	4.85 ± 0.9	10.6 ± 3.4
Total	99.2 ± 4.2	102.0 ± 4.0	97.8 ± 2.0	90.9 ± 2.1

[a]Mean for 3 rats.

[b]Includes cage wash.

(a) Male rats



(b) Female rats

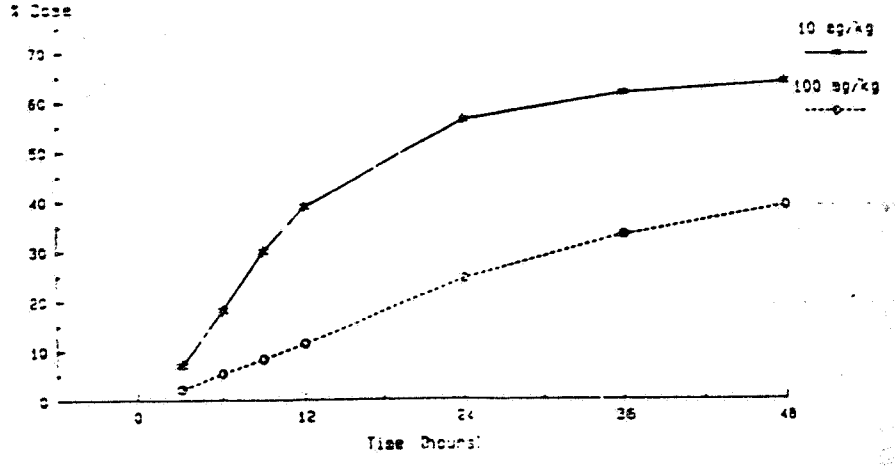


Figure 1. Cumulative excretion of radioactivity in bile by cannulated rats after single oral doses of ^{14}C-Vinclozolin at nominal levels of 10 mg/kg and 100 mg/kg.

Source: Study No. 90/0544, CBI Figure 5, CBI p. 107.

and females, respectively, compared to 1.6 and 3.3% at the low level.

Pharmacokinetics--Plasma concentrations versus time curves (linear scale) are shown in Figures 2 and 3. Expanded time scale curves are presented in Appendix A. The time to reach peak plasma concentrations of ^{14}C (T_{max}) increased as the dose level was increased (single gavage doses). Radioactivity levels in plasma decreased with an apparent biphasic manner and the half-lives for the terminal portion of the curve were longer in females than in males. Table 4 summarizes data for pharmacokinetics parameters with increasing single oral doses.

The increase in C_{max} and AUC in both sexes were less than proportional with dose. Both parameters were higher in males than females. This indicates a lower clearance or greater volume of distribution in females and is consistent with the observed longer half-life in females.

In the dietary study, a diurnal pattern of input was observed as is expected (Table 36 of CBI; data not shown); the doses were equivalent to 439 and 345 mg/kg in males and females. For the first 12 hours, the predicted plasma concentrations based on the rate of ingestion were close to the observed (Table 5). The half-lives of plasma clearance after cessation of feeding labeled material was approximately 40 hours (41.0 and 38.9 in males and females, respectively). Based on AUC x dose, the bioavailability was similar in gavage and dietary studies, the relative bioavailability ratio was approximately 1 (Table 35 of CBI, data not shown). Figure 4 shows that when values for AUC are plotted against dose (mg/kg) for both oral gavage and the dietary route, a straight line relationship was observed up to 400 mg/kg (highest dose; dietary route).

Table 6 shows mean tissue levels of selected tissues in females 2 hours after a single oral 10-mg/kg dose or following repeated dosing for 7 days with labeled compound. After multiple dosing (7 days), tissue levels are approximately double those after a single oral dose. Figure 5 shows the decrease in tissue levels with time (females, multiple dose). The tissue/plasma ratios generally do not change as radioactivity is cleared from tissues (Table 6). This indicates essentially the same half-life for clearance from each tissue as for plasma clearance. Similar data were observed in males, except that tissue levels were slightly lower.

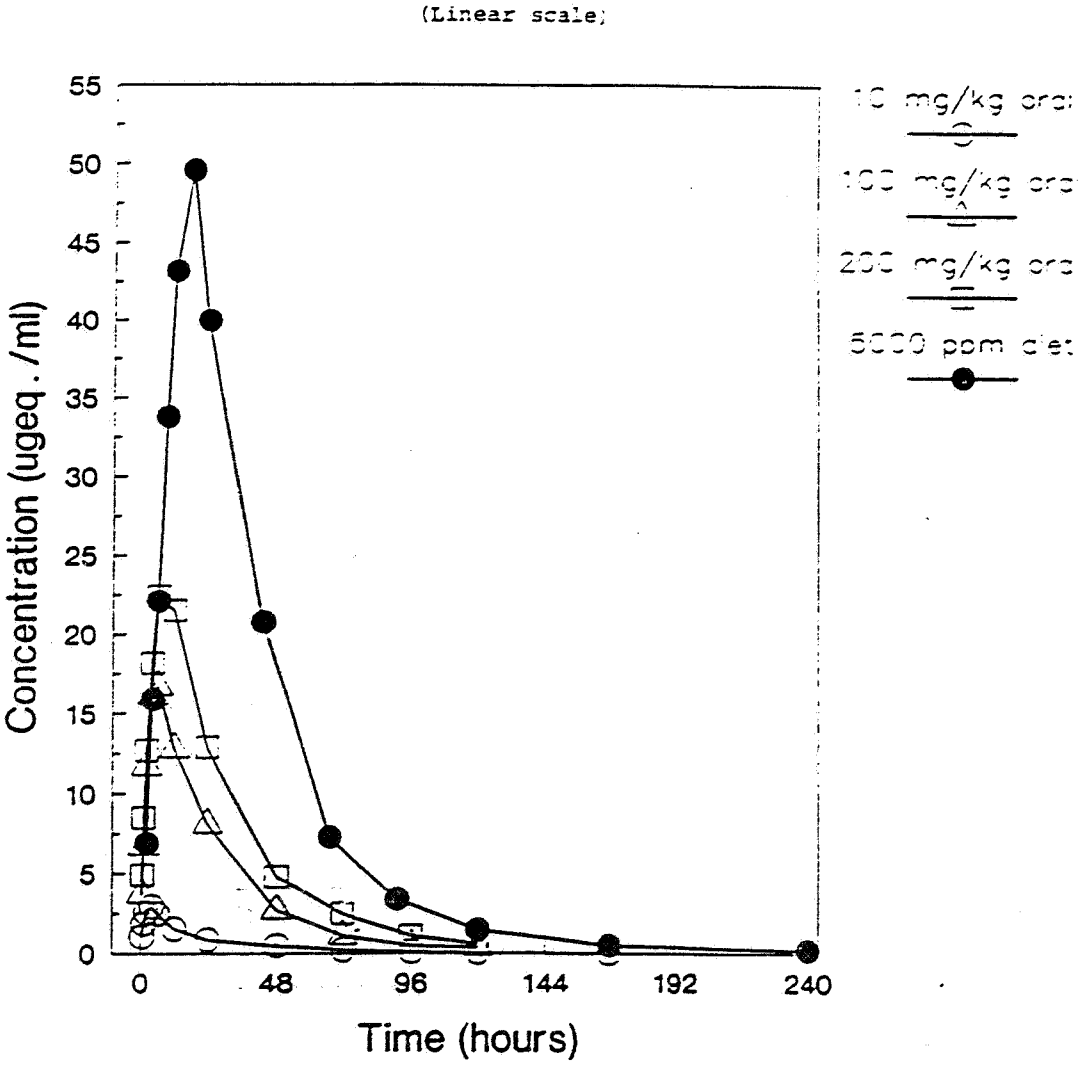


Figure 2. Mean plasma concentrations of radioactivity after various doses of ^{14}C-Vinclozolin administered to male rats.

Source: Study No. 90/0544, CBI Figure 7, CBI p. 109.

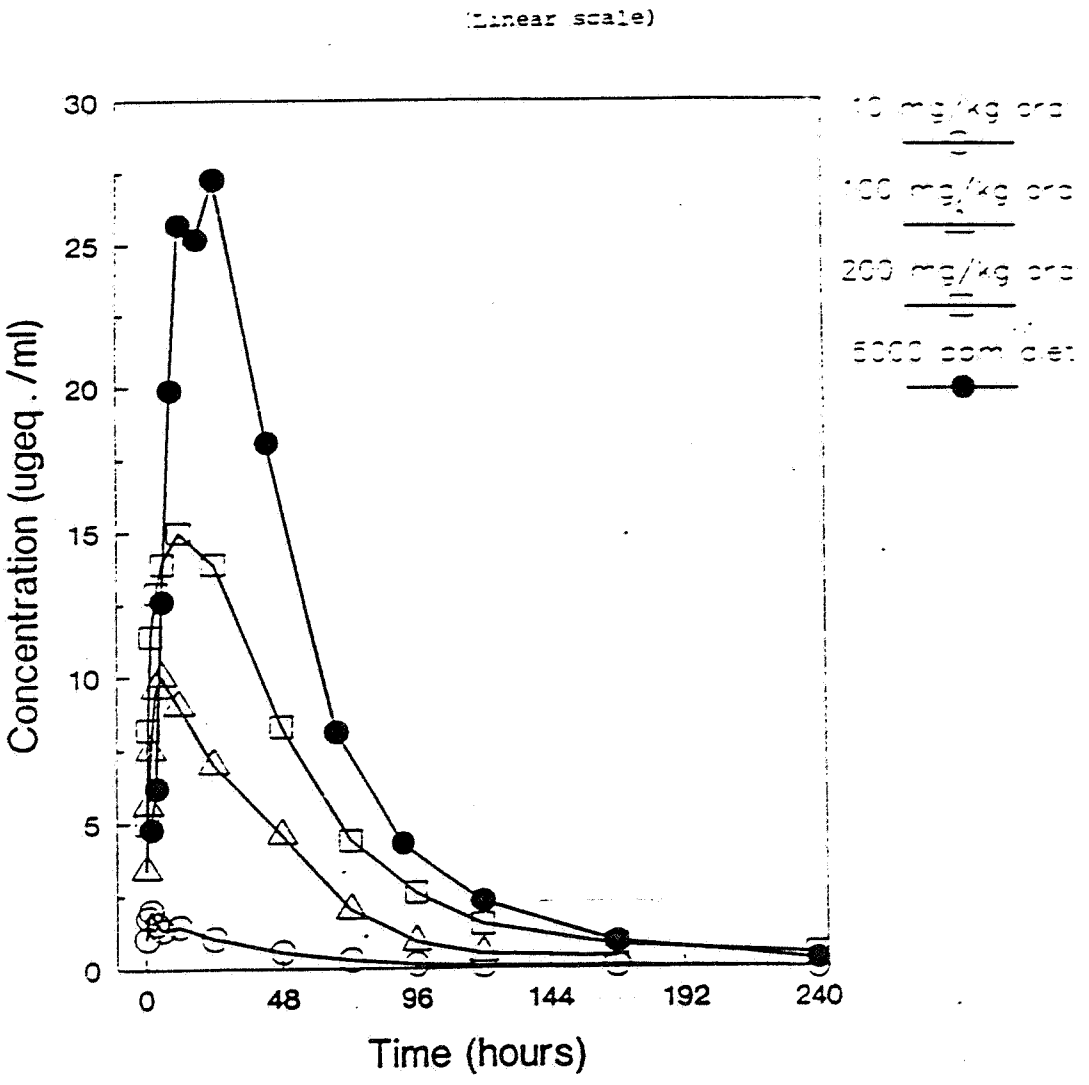


Figure 3. Mean plasma concentrations of radioactivity after various doses of ^{14}C-Vinclozolin administered to female rats.

Source: Study No. 90/0544, CBI Figure 9, CBI p. 111

TABLE 4. Pharmacokinetic Parameters for Single Oral Doses

Dose		C_{max}	T_{max}	AUC	t½
mg/kg	mg/rat	(µg eq/mL)	(hours)	(µg eq/mL•hr)	(hours)
			Males		
10	2.0	2.8 ± 0.5	3.6 ± 1.7	72.7 ± 55	27.3
100	17.9	16.8 ± 2.5	5.2 ± 1.1	482 ± 105	18.0
200	33.8	23.2 ± 3.1	9.6 ± 3.3	788 ± 54	22.6 (21.7)[a]
			Females		
10	2.0	2.0 ± 0.1	1.8 ± 0.4	74.9 ± 9.2	64.4
100	17.9	10.3 ± 2.7	4.8 ± 1.1	485.0 ± 68	26.5
200	33.8	15.6 ± 1.8	13.2 ± 6.6	997.0 ± 90	45.2 (36.1)[a]

[a]The values in parentheses are the mean values for the three dose levels.

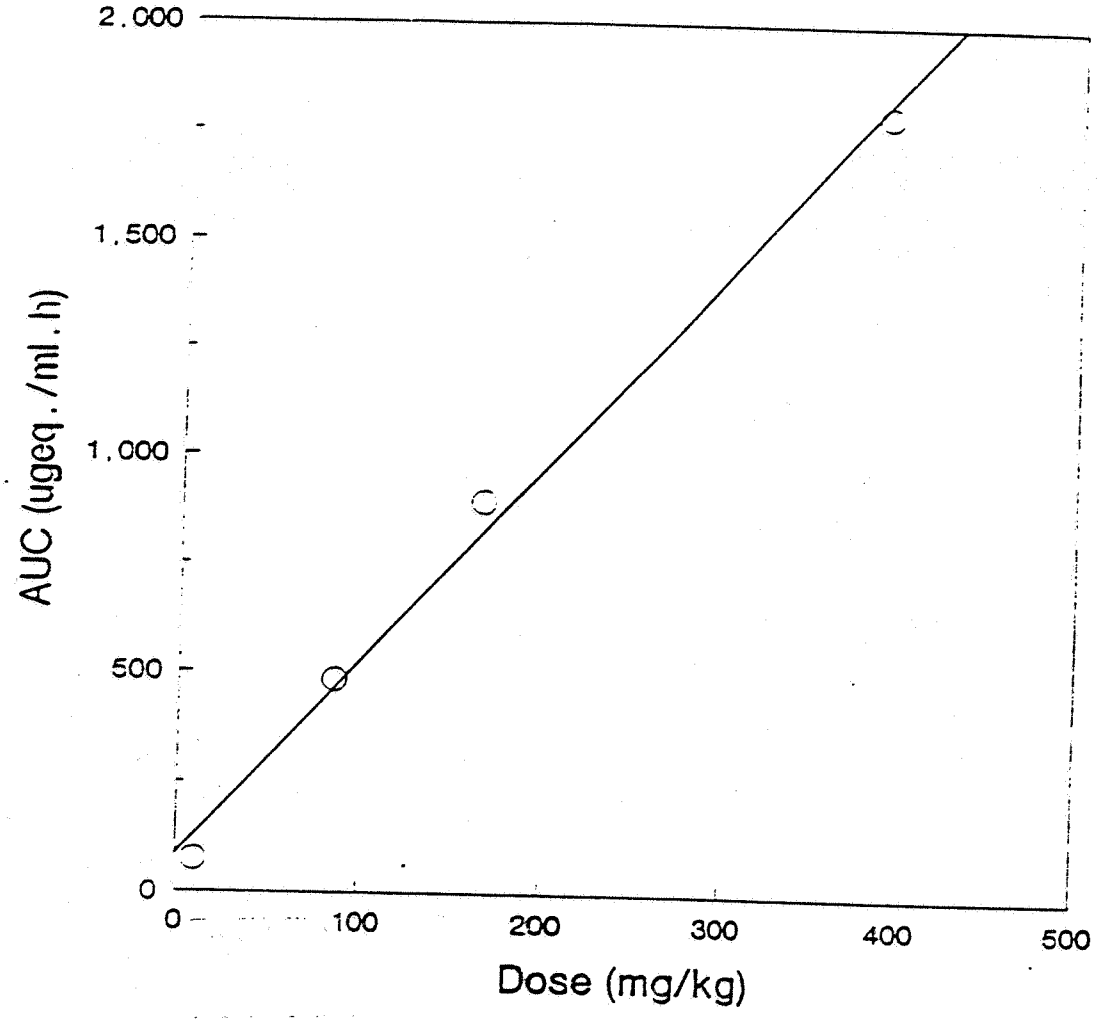
TABLE 5. Plasma Concentrations of Radioactivity During Ingestion of ^{14}C-Vinclozolin With the Diet

Time (hours)	Values expressed as ug eq./ml			
	Males		Females	
	Observed mean plasma concentration	[a]Prediction concentration (Ct)	Observed mean plasma concentration	[a]Predicted concentration (Ct)
2	6.9	8.4	4.3	4.4
4	15.9	16.3	6.2	8.7
6	22.1	23.7	12.6	12.8
9	33.8	34.0	19.9	18.8
12	43.2	43.4	25.7	24.5
18	49.6	-	25.2	-
24	40.0	-	27.3	-

a $C_t = \frac{Ro}{CL} \cdot (1-e^{-\lambda_z t})$

where C_t is the plasma concentration at time t and R_o is the rate of ingestion of ^{14}C-vinclozolin taken as 6.1 and 5.0 mg/h for males and females respectively (Table 36). λ_z is the terminal (elimination) rate constant and CL is the (oral) clearance estimated from dose/AUC (Tables 31 and 32)

Source: Study No. 90/0544, CBI Table 37, CBI p. 78.



Highest dose of approximately 400 mg/kg administered with the diet

Figure 4. Relationship between dose of ^{14}C-Vinclozolin and AUC of radioactivity after single oral doses administered to male and female rats.

Source: Study No. 90/0544, CBI Figure 16, CBI p. 118.

TABLE 6. Radioactivity Levels in Selected Tissues of Female Rats 2 Hours After an Oral Dose of [illegible] mg/kg [illegible]C-Vinclozolin

Tissue	µg eq./g		Ratio tissue/plasma[c]		
	2h[a]	2h[b]	2h	24h	48h
Plasma	2.12	3.91	1 (2.12)[d]	1 (1.10)[d]	1 (0.57)[d]
Harderian gland	6.94	12.3	3.1	2.1	1.9
Adrenal gland	5.77	10.6	2.7	2.3	2.1
Kidney	5.65	10.6	2.7	2.6	2.9
Liver	8.57	16.9	4.3	4.1	4.7
Lung	3.51	6.27	1.6	1.6	1.7
Heart	2.73	5.72	1.4	1.2	1.2
Pancreas	4.28	7.52	1.9	1.8	1.3
Muscle	1.40	2.94	0.75	0.71	0.70

[a]Rats received a single oral dose of [illegible] mg/kg [illegible]C-Vinclozolin (CBI Table 39).

[b]Rats received 7 daily oral doses of [illegible] mg/kg [illegible]C-Vinclozolin (CBI Table 55).

[c]Rats received 7 daily oral doses of [illegible] mg/kg [illegible]C-Vinclozolin (CBI Table 61).

[d]The levels in µg equivalents/mL of plasma are given in parentheses.

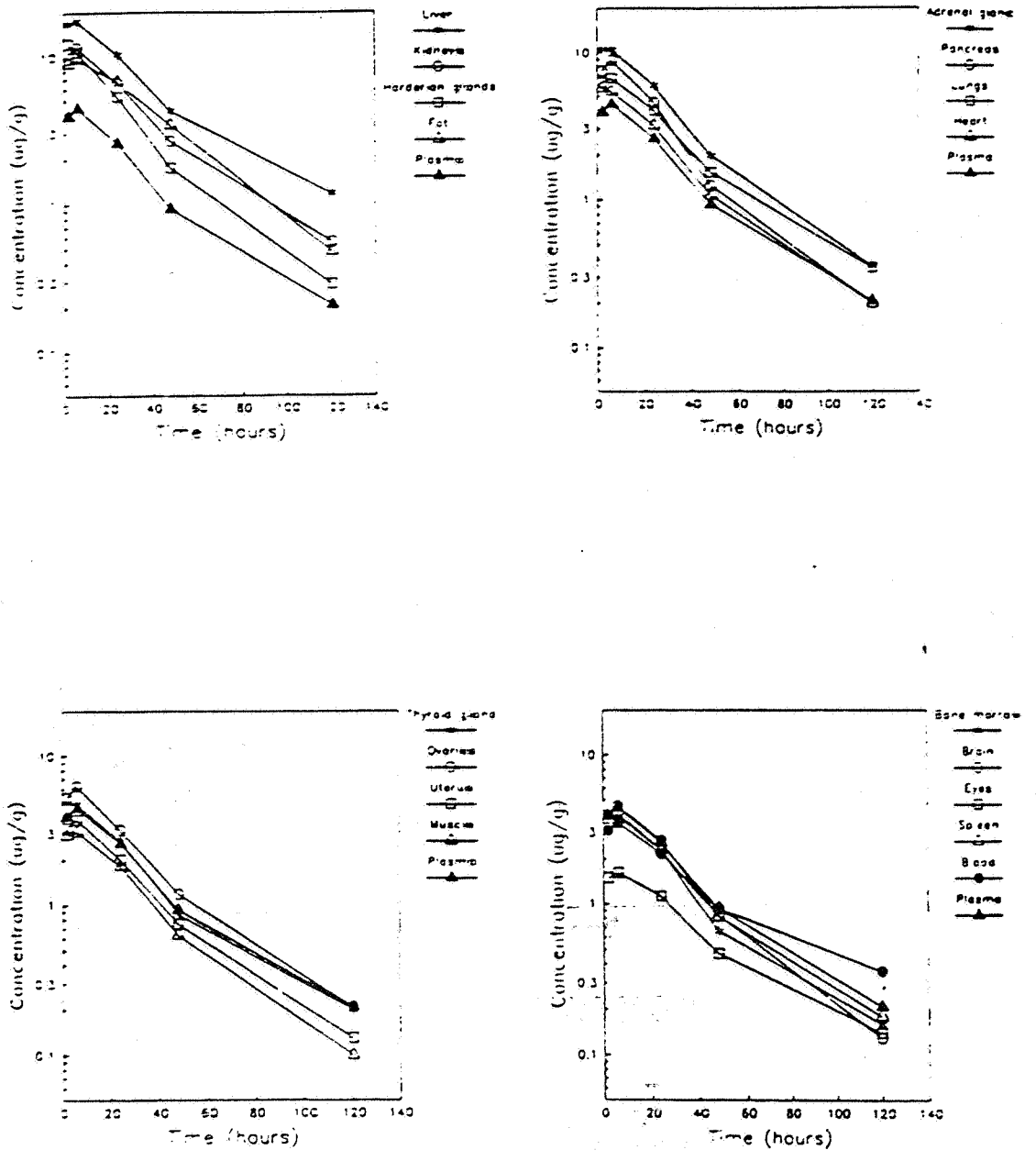


Figure 5. Changes in the mean (n = 3) concentrations of radioactivity with time in tissues of female rats after the last of seven daily oral doses of ^{14}C-vinclozolin at a nominal level of 10 mg/kg/day.

Source: Study No. 90/05-- CBI Figure 25. CBI p. 12.

Tissue levels declined in a generally linear manner with time. After five days levels were mostly in the range of 0.1-3 µg/g with the highest levels present in the liver, kidney, and female fat.

Autoradiography: Whole body autoradiographs from animals dosed for 7 days with ^{14}C-Vinclozolin showed that radioactivity was distributed in all tissues except bone at 48 hours postdosing. There was little variation in distribution with time or between sexes. The highest concentrations were in liver, gastrointestinal tract, kidneys and urinary bladder. Radioactivity in the adrenal was evenly distributed in the cortex. Lacrymal and Harderian glands had relatively high concentrations as well as nasal mucosa, salivary glands, and adrenal glands. The results of radioautography support the quantitative findings of tissue distribution.

II. Biotransformation of ^{14}C-Vinclozolin in the Rat

Table 7 outlines the different groups of animals used, the urinary excretion, extraction efficiency, and percent of dose analyzed by HPLC. Urinary radioactivity extraction efficiency ranged from 93 to 98% for all samples. HPLC separated up to 13 components. Appendix B shows the reference compounds and their structural formulas. In general there were no marked differences in metabolite profiles in urine related to sex, route of administration, low or high dose, or single dose compared to repeated dosing (Table 8). Regardless of route, no more than 0.5% of the dose was unchanged Vinclozolin (component R13). The major radioactive component was R7 which constituted 4 to 23% of the administered dose after oral or dietary doses and up to 32% of the dose after intravenous administration. Component R7 was a conjugate since its level decreased after glucuronidase/sulfatase treatment of urine. For a 10 mg/kg oral dose in male rats, R7 and R8 constituted 14.3 and 2.6% of the dose. After deconjugation, the percent of R7 in urine decreased to 6.1% and R8 increased to 10.9% of the dose. Polar components R1, R2, and R3 decreased and R4, R5, R6 and R9 increased after enzyme treatment indicating that they were conjugates. Components R1, R2, and R3 also decreased after treatment of urine with a specific sulfatase enzyme.

Urinary samples (males 100 mg/kg dose) were pooled and extracted with ethylacetate at pH 7 and at pH 2 and the resulting aqueous phase extracted with methanol. Preparative HPLC on the three fractions (EA1, EA2, and MEL) yielded 10, 12, and 4 fractions, respectively, each of which underwent mass spectral analysis if sufficient material was isolated. Samples were analyzed after

TABLE 7. Excretion of Radioactivity in the Urine of Male and Female Rats After Various Doses of ^{14}C-Vinclozolin and the Proportion of the the Dose Analyzed by HPLC

Nominal dose level (mg/kg)	Dose route	Animal numbers	Time after dosing (hours)	Mean urinary excretion (% dose)	% urinary radioactivity extracted	Mean % dose analysed
10	Oral gavage	21-25♂	0-24	32.2	96.1	30.9
			24-48	12.1	95.4	11.5
		26-30♀	0-24	34.2	95.5	32.7
			24-48	12.2	95.0	11.6
100	Oral gavage	41-45♂	0-24	26.1	95.8	25.0
			24-48	13.8	96.9	13.4
		46-50♀	0-24	19.8	97.5	19.3
			24-48	20.6	98.0	20.2
10*	Oral gavage	61-65♂	0-24	27.5	94.6	26.0
			24-48	14.9	97.1	14.5
		66-70♀	0-24	29.5	97.5	28.8
			24-48	15.1	97.5	14.7
200	Oral gavage	171♂	0-24	14.9	95.0	14.2
			24-48	9.0	97.0	8.7
		172♀	0-24	9.7	95.0	9.2
			24-48	8.7	98.2	8.5
163	Dietary+	215♂	6-24	7.3	95.6	7.0
			24-48	6.8	92.8	6.3
170		216♀	6-24	7.2	95.9	6.9
			24-48	7.7	96.7	7.4
1	Intravenous	71-75♂	0-24	47.2	98.3	46.4
			24-48	15.1	97.8	14.8
		76-80♀	0-24	44.2	95.8	42.3
			24-48	15.4	97.2	15.0

* Rats received 14 consecutive daily oral doses of non-radiolabelled vinclozolin followed by a single oral dose of ^{14}C-vinclozolin at a nominal level of 10 mg/kg

\+ Rats were offered diet for 6 hours containing ^{14}C-vinclozolin at a nominal concentration of 5000 ppm. Urine was collected after the withdrawal of treated diet

Source: Study No. 90/0514, CBI Table 2, CBI p. 38.

TABLE 8. Proportions of Radioactive Components in the Urine (0 - 48 hours) of Rats After Administration of Various Doses of ^{14}C-Vinclozolin

Results are expressed as % dose

010261

Males

Radioactive component	Typical retention time (min)	10 mg/kg oral (21-25♂)	10 mg/kg* oral (61-65♂)	100 mg/kg oral (41-45♂)	200 mg/kg oral (171♂)	163 mg/kg diet+ (215♂)	1 mg/kg intravenous (71-75♂)
R1	6.3	1.0	1.0	1.1	1.0	1.7	5.5
R2	17.8	2.8	4.1	2.7	2.2	1.6	1.0
R3	20.3	2.5	2.9	2.1	1.8	1.0	1.8
R4	22.7	1.5	1.1	0.8	1.0	0.5	1.8
R5	25.7	0.9	1.1	1.0	0.4	0.3	4.3
R6	28.5	1.5	1.7	1.2	0.6	0.5	
R7	32.0	21.2	17.4	20.5	10.1	4.3	32.2
R8	36.3	3.2	2.9	1.9	1.3	1.1	3.7
R9	40.0	3.8	4.2	3.1	2.0	1.4	5.3
R10	43.7	1.3	0.8	0.6	0.4	0.2	1.1
R11	46.2	1.1	1.1	0.8	0.6	0.3	1.6
R12	49.8	0.4	0.5	0.4	0.3	<0.1	0.7
R13	53.2	0.2	0.1	0.2	0.1	<0.1	0.2
Others	-	1.0	1.5	1.8	1.3	0.5	2.2

Females

Radioactive component	Typical retention time (min)	10 mg/kg oral (26-30♀)	10 mg/kg* oral (66-70♀)	100 mg/kg oral (46-50♀)	200 mg/kg oral (172♀)	170 mg/kg diet+ (216♀)	1 mg/kg intravenous (76-80♀)
R1	6.3	0.7	0.8	0.6	0.4	0.6	5.2
R2	17.8	1.7	2.5	1.9	1.2	0.7	0.5
R3	20.3	1.9	2.2	1.3	0.9	0.6	1.4
R4	22.7	1.6	1.5	2.0	1.1	0.4	1.8
R5	25.7	0.7	1.0	0.4	0.2	0.4	4.5
R6	28.5	1.5	1.5	1.3	0.5	0.5	
R7	32.0	21.8	22.2	21.1	8.2	5.9	27.9
R8	36.3	3.3	3.2	2.4	1.0	0.8	3.6
R9	40.0	4.2	3.8	2.6	1.3	1.1	4.0
R10	43.7	1.7	0.8	0.5	0.2	0.2	2.2
R11	46.2	1.4	0.9	0.6	0.2	0.2	1.4
R12	49.8	1.6	1.7	3.2	2.0	2.5	2.2
R13	53.2	0.5	0.3	0.1	<0.1	0.1	0.4
Others	-	1.8	1.0	1.3	0.6	0.6	2.3

Results are the sum of data from separate 0 - 24 and 24 - 48 hour time periods which are shown in Tables 7(a) and 8

* Rats received 14 consecutive daily oral doses of non-radiolabelled vinclozolin followed by a single oral dose of ^{14}C-vinclozolin at a nominal level of 10 mg/kg

\+ Rats were offered diet for 6 hours containing ^{14}C-vinclozolin at a nominal concentration of 5000 ppm. Urine was collected after the withdrawal of treated diet

Source: CBI pp. 36 and 37.

derivatization by GC-MS or underivatized samples were directly injected into the electron impactor. Spectra of reference compounds similarly derivatized were used for comparisons.

Some compounds appeared in more than one fraction. The identity of R4, R5, R7, R8, R9, R11 and R12 were confirmed. Table 9 summarizes the data on characterization.

Fecal and Biliary Metabolites

Extraction efficiencies for fecal samples were generally lower than those for urinary samples, ranging from 73.5 to 97.3% for 12 samples (Table 10). Table 11 summarizes data for HPLC of feces extracts. Following oral dosing (48 hours) at 10, 100 or 200 mg/kg, unchanged ^{14}C-Vinclozolin (R13) accounted for 21.1, 25.5, and 30.3% of the dose in males and 14.7, 21.3, and 42.1% of the dose in females (HPLC); 21.5 and 27.9% of the dietary dose in males and females cochromatographed with Vinclozolin. Only 0.1-0.2 of a 1 mg/kg intravenous dose chromatographed as Vinclozolin. Major metabolites were the N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide (R8) and its intermediate in which the vinyl group underwent epoxidation and hydration and the heterocyclic ring remained intact (R9).

Biliary excretion accounted for 70 and 40% of the administered radioactivity at low or high doses, respectively. Chromatography was not successful prior to glucuronidase/sulfatase digestion. Recovery of ^{14}C was greater than 90% after enzymatic digestion followed by sorbent treatment and elution with acetonitrile/methanol (1/1). The major component was the glucuronide of R8 (the trihydroxybutyramide derivative) which represented about 41% of the low dose and 20% of the high dose. Resolution of other metabolites was poor.

Tissue metabolites--Liver and kidney samples 6 hours after the last of seven daily oral doses of ^{14}C-Vinclozolin (10 mg/kg dose) were analyzed by HPLC after extraction with acetonitrile/methanol (87 and 91% extraction efficiency, liver and kidney, respectively). R8 was the major metabolite in both tissues and accounted for about 26 and 46% of the tissue radioactivity. Some unchanged Vinclozolin was detected (less in kidneys than liver) and variable amounts of more polar metabolites (R1 to R6). R8 was also the major metabolite found in chromatograms of acetonitrile/methanol extracts of plasma. However, only 55 to 72% of the radioactivity of plasma was extracted, suggesting binding of metabolites to plasma proteins.

TABLE 9. Summary of the Characterization of Vinclozolin Biotransformation Products in Rat Urine

Radioactive component	Typical retention time (min)	Characterisation	Corresponding isolated fraction	Supporting evidence for characterisation
R1	6.3	Sulphate conjugate	Me1/F1	Hydrolysis of urine by sulphatase enzyme.
R2	17.8	Sulphate conjugate		Hydrolysis of urine by sulphatase enzyme.
R3	20.3	Sulphate conjugate		Hydrolysis of urine by sulphatase enzyme.
R4	22.7	Reference compound 5	EA2/F2 EA1/F1	Identical mass spectrum to reference compound 5. Co-chromatography of 5 with R4 in rat urine. Co-chromatography of 5 with fraction EA1/F1.
R5	25.7	Ring hydroxylated analogue of reference compound 37	EA2/F4 EA1/F2	Interpretation of mass spectrum.
R6	28.5	Not complete	EA1/F3	Formed by enzyme hydrolysis of urine. Mass spectrum obtained.
R7	32.0	Glucuronic acid conjugate of reference compound 25	EA2/F6 EA2/F7 EA1/F4 EA1/F5 Me1/F3 Me1/F4	Interpretation of mass spectra of EA2/F6 and EA2/F7. Hydrolysed by β-glucuronidase to yield reference compound 25.
		Glucuronic acid conjugate of reference compound 37	EA2/F8	Interpretation of mass spectra.

Source: Study No. 90/0514, CBI Table 11, CBI pp. 47 and 48.

TABLE 9. Summary of the Characterization of Vinclozolin Biotransformation Products in Rat Urine (continued)

Radioactive component	Typical retention time (min)	Characterisation	Corresponding isolated fraction	Supporting evidence for characterisation
R8	36.3	Reference compound 25	EA1/F6	Identical mass spectrum to reference compound 25. Co-chromatography of 25 with R8 in rat urine. Co-chromatography of 25 with EA1/F6. Formed on hydrolysis of the glucuronic acid conjugate R7 by β-glucuronidase enzyme.
R9	40.0	Reference compound 37	EA2/F10	Identical mass spectrum to reference compound 37. Co-chromatography of 37 with R9. Co-chromatography of EA2/F10 with R9.
R10	43.7	None	-	
R11	46.2	Reference compound 31	EA1/F7	Identical mass spectrum to reference compound 31. Co-chromatography of 31 and EA1/F7.
		Reference compound 42	EA1/F8	Identical mass spectrum to reference compound 42. Co-chromatography of 42 with EA1/F8. Co-chromatography of 42 with R11 in rat urine.
R12	49.8	Reference compound 22	EA1/F9	Identical mass spectrum to reference compound 22. Co-chromatography of 22 with EA1/F9. Co-chromatography of 22 with R12 in rat urine.
R13	53.2	Unchanged vinclozolin	EA1/F10	Same HPLC retention time as vinclozolin.

Source: Study No. 90/0514, CBI Table 11, CBI pp. 47 and 48.

TABLE 10. Excretion of Radioactivity in the Feces of Male and Female Rats Up to 48 Hours After Various Doses of ^{14}C-Vinclozolin and the Proportion of the Dose Extracted and Analyzed by HPLC

Nominal dose level mg/kg	Dose route	Animal nos.	Mean faecal excretion (% dose)	% faecal radioactivity extracted	Mean % dose analysed
10	Oral	21-25♂	42.6	90.5	38.6
		26-30♀	35.4	93.8	33.2
10*	Oral	61-65♂	29.4	73.5	21.6
		66-70♀	28.8	81.5	23.5
100	Oral	41-45♂	44.9	90.6	40.7
		46-50♀	34.4	93.8	32.3
200	Oral	171♂	50.0	82.7	41.4
		172♀	49.7	97.3	48.4
163	Diet+	215♂	25.6	88.0	31.3
170		216♀	39.8	89.9	35.8
1	Intravenous	71-75♂	18.4	83.9	15.4
		76-80♀	18.5	79.0	14.6

* Rats received 14 consecutive daily oral doses of non-radiolabelled vinclozolin followed by a single oral dose of ^{14}C-vinclozolin at a nominal level of 10 mg/kg

\+ Rats were offered diet for 6 hours containing ^{14}C-vinclozolin at a nominal concentration of 5000 ppm. Faeces were collected after withdrawal of treated diet

Source: Study No. 90/0514, CBI p. 50.

TABLE 11. Proportions of Radioactive Components in the Feces (0 - 48 Hours) of Rats After Administration of Various Doses of ^{14}C-Vinclozolin

Results are expressed as % dose

Males

Radioactive component	Typical retention time (min)	10 mg/kg oral (21-25♂)	10 mg/kg* oral (61-65♂)	100 mg/kg oral (41-45♂)	200 mg/kg oral (171♂)	163 mg/kg diet+ (215♂)	1 mg/kg intravenous (71-75♂)
R1-R7	-	2.6	1.7	2.3	1.8	1.6	2.1
R8	36.3	9.0	8.9	8.4	6.2	5.4	8.5
R9-R10	-	3.6	3.6	2.6	1.8	1.2	3.5
R11	46.2	1.5	1.4	1.0	0.7	0.9	1.1
R12	49.8	0.5	0.2	0.6	0.3	0.2	0.2
R13	53.2	21.1	5.4	25.5	30.3	21.5	0.2
Others	-	0.3	0.4	0.2	0.2	0.4	-

Females

Radioactive component	Typical retention time (min)	10 mg/kg oral (26-30♀)	10 mg/kg* oral (66-70♀)	100 mg/kg oral (46-50♀)	200 mg/kg oral (172♀)	170 mg/kg diet+ (216♀)	1 mg/kg intravenous (76-80♀)
R1-R7	-	3.2	2.4	2.2	1.7	0.5	3.0
R8	36.3	10.9	9.3	5.9	3.3	3.6	8.9
R9-R10	-	2.1	2.0	1.1	0.7	0.7	1.8
R11	46.2	1.7	1.3	0.7	0.6	0.5	0.9
R12	49.8	0.6	0.5	0.6	0.2	0.2	0.1
R13	53.2	14.7	7.3	21.3	42.1	27.9	<0.1
Others	-	<0.1	0.8	0.6	<0.1	2.3	0.1

* Rats received 14 consecutive daily oral doses of non-radiolabelled vinclozolin followed by a single oral dose of ^{14}C-vinclozolin at a nominal level of 10 mg/kg

\+ Rats were offered diet for 6 hours containing ^{14}C-vinclozolin at a nominal concentration of 5000 ppm. Faeces were collected after the withdrawal of treated diet

Radiochromatograms are shown in Figures 19 - 21

Source: Study No. 90/0514, CBI Tables 14 and 15, CBI pp. 51 and 52.

13. STUDY AUTHORS CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Biokinetics of [U-^{14}C-phenyl]Vinclozolin were studied in male and female Wistar rats. After administration of single oral doses at nominal levels of 10 mg/kg and 100 mg/kg, mean urinary and fecal excretion of radioactivity by both sexes lay in the ranges 48 to 54% and 38 to 49% of the dose, respectively. Retention of radioactivity after 5 days amounted to 0.6 to 1.4% of the dose with similar retention both doses but slightly lower retention in males than in females. After a single intravenous dose of 1 mg/kg excretion (mean of both sexes) after 5 days accounted for 71.6 and 23.0% of the dose in urine and feces respectively. In rats with cannulated bile ducts, biliary excretion accounted for 73.2 and 63.5% of an oral dose of 10 mg/kg and 62.0 and 38.8% of and oral dose of 100 mg/kg in males and females, respectively. Pronounced enterohepatic recirculation of radioactivity occurs. After single oral doses of 10, 100, or 200 mg/kg, peak plasma concentrations (c_{max}) linearly increased with dose level and time to peak also tended to increase c_{max} values were higher in males than females. Plasma concentration decline in an apparent biphasic manner with terminal half-lives of 23 and 36 hours for males and females, respectively.

During dietary ingestion of 5000 ppm ^{14}C-Vinclozolin for 24 hours, the plasma concentrations increased with an apparent zero order absorption model and half lives of decline after removal of labeled diet were apparently 40 hours. Systemic availability of radioactivity appeared equivalent by gavage or dietary administration. ^{14}C-label was generally widely distributed to tissues with peak levels in liver, kidneys, fat, and adrenals and Harderian gland (4-10 µg eq/g at 10 mg/kg) and declined to levels in the range of 0.02 to 0.2 µg eq/g after 5 days.

Vinclozolin was extensively metabolized in the rat; Figure 6 shows the proposed metabolic pathway. Urine contained at least 15 metabolites in addition to low levels of unchanged ^{14}C-Vinclozolin. Metabolic transformation proceeds by epoxidation and hydration of the vinyl side chain and hydrolytic cleavage of the 2,3 bond of the oxazolidine ring to give N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxy-butyramide (R8). This product is conjugated as a glucuronide and excreted in the urine. An intermediate in this pathway with an intact heterocyclic ring is also conjugated as a glucuronide and excreted in the urine. Other metabolites result from aromatic hydroxylation. Minor metabolites result from cleavage of the 3,4 bond of the heterocyclic ring and loss of the vinyl group. 3,5-Dichloroaniline was detected in urine but accounted for only about 1% of the dose. The major urinary metabolite

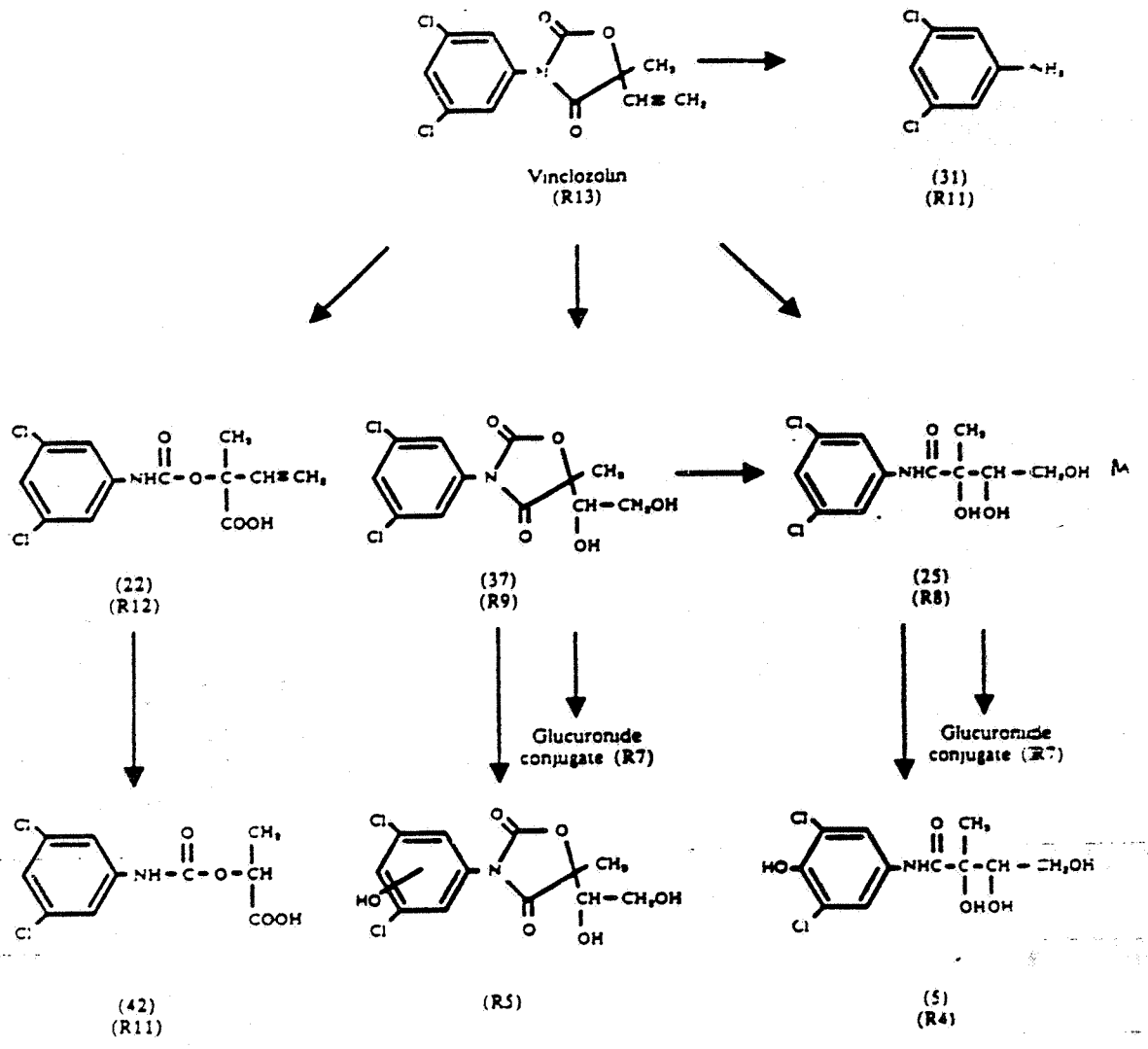


Figure 6. Postulated biotransformation pathway of ^{14}C-Vinclozolin in the rat.

Source: Study No. 90/0514, CBI p. 93.

(R8) was also conjugated with glucuronide and excreted in the bile: The major ^{14}C-compound in feces was unchanged Vinclozolin.

B. Quality Assurance: Quality Assurance statements were present, signed and dated November 28, 1990, for the Biokinetic study and March 4, 1991, for the Biotransformation study.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The design, conduct, and reporting of the two studies were excellent. There were no toxic effects at the high dose. However, the use of a dose approaching the LD_{50} (6400 mg/kg) would have been impractical since the resulting specific activity would have been too low and compromised sensitivity of detection of radioactivity. The reviewers agree with the study authors conclusions. Chromatographic scans and mass spectral data were well presented and all techniques were adequate. Sample calculations were provided and efficiency of counting was well documented.

Items 15-16--see footnote 1.

VINCLOZALIN Sh # 113201

Page_____ is not included in this copy.

Pages 96 through 102 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA No.: 68D80056
DYNAMAC No.: 382-E
TASK No.: 3-82E
September 23, 1991
01926 1

DATA EVALUATION RECORD

VINCLOZOLIN

Dermal Absorption in Rats

STUDY IDENTIFICATION: Hawkins, D.R. et al. Dermal absorption of ^{14}C-vinclozolin in the rat. (Unpublished study No. 91/10059 performed by Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, England, for BASF Aktiengesellschaft, Ludwigshafen, Germany; dated January 3, 1991.) MRID No. 418243-09.

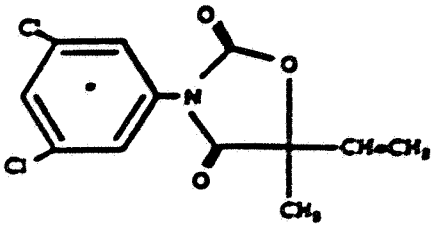
APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____
Date: 9/23/91

1. CHEMICAL: 3-(3,5-Dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidin-2,4-dione; vinclozolin.

2. TEST MATERIAL: Unlabeled vinclozolin (purity 99.2%) and vinclozolin uniformly labeled with ^{14}C in the phenyl ring (specific activity 201.16 µCi/mg, radiochemical purity >97%) were used. The test material was described as a white solid. The structure and radiolabeled carbons (*) of [^{14}C]vinclozolin are shown below:



3. STUDY/ACTION TYPE: Dermal absorption in rats.

4. STUDY IDENTIFICATION: Hawkins, D.R. et al. Dermal absorption of ^{14}C-vinclozolin in the rat. (Unpublished study No. 91/10059 performed by Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, England, for BASF Aktiengesellschaft, Ludwigshafen, Germany; dated January 3, 1991.) MRID No. 418243-09.

5. REVIEWED BY:

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David G Anderson
11/21/92

7. CONCLUSIONS:

A. The metabolism of [^{14}C]Vinclozolin was studied in groups of 24 male rats given a single dermal application of 0.002, 0.02, 0.2, or 2.0 mg [^{14}C]Vinclozolin/cm^2 as a suspension in 1% aqueous carboxymethylcellulose. Test sites were unoccluded for 10 hours, during which time animals were restrained. The test sites were then covered for the remainder of the study (i.e., until 72 hours after application). Subgroups of four rats/dose level were sacrificed at 0.5, 1, 2, 4, 10, and 72 hours posttreatment.

The percent of the applied dose that was absorbed (excluding that retained at the skin, which accounted for 0.6 to 4% of the ^{14}C dose) was inversely related to dose: approximately 29, 24, 5, and 3% of the 0.002-, 0.02-, 0.2-, and 2.0-mg/cm^2 doses of [^{14}C]Vinclozolin, respectively, were absorbed within 72 hours after compound application. In contrast, the total (absolute) amount of radioactivity absorbed increased with dose but not proportionately; 0.04, 0.2, 0.4, and 1.0 to 1.2 mg [^{14}C]Vinclozolin/kg body weight were absorbed by rats in the four ascending dose groups, respectively. Absorbed radioactivity was eliminated in both the urine (0.34 to 16.2% of the ^{14}C dose at 72 hours) and feces (0.22 to 7.9%), with the lower values representing the higher dose levels; in general, the urinary route was favored over the fecal route by a ratio of 2:1. For the two lowest dose groups in particular, the compound was readily absorbed and eliminated; radioactivity was present in the urine within 4 to 10 hours after compound application. Tissue (including plasma) levels of radioactivity indicated that absorption of [^{14}C]Vinclozolin peaked or plateaued at 10 hours after treatment with the 0.002- or 0.02-mg/cm^2 dose, whereas the test material was still being absorbed at 72 hours after application of the 0.2-mg/cm^2 dose. Tissue ^{14}C levels in animals from the highest dose group generally were below the limit of detection, presumably because of the relatively small amounts of radioactivity administered to these rats. Low levels of radioactivity (≤3.2%) were recovered from the tissues and carcasses at 72 hours postdosing; most of this was present in the gastrointestinal tract. At 72 hours after application of all doses, the highest residual ^{14}C levels were found in the liver (0.06 to 0.2 µg/g wet weight); the next highest levels were in the kidneys, adrenals, and plasma. Concentrations of ^{14}C in the brain, blood, and testes were low, and levels in the eyes were generally below detection. The largest proportion of the ^{14}C dose (53 to 94%) was unabsorbed and was recovered from the skin washings. Total mean recoveries of radioactivity for all 24 subgroups were between 82 and 104%; most (19/24) were >90%.

B. The selection of 1% aqueous carboxymethylcellulose to suspend the test material, and the interactions of carboxymethylcellulose with the test material and possible effects on the dermal absorption of Vinclozolin were not described adequately. ~~This study provides supplementary data on the dermal absorption of [^{14}C]Vinclozolin in rats per EPA Guideline 85-3 (see the Appendix of this DER). This study could be upgraded if an adequate explanation is provided by the registrant.~~ However the study is Acceptable and is Core classified Minimum. It satisfies Guideline 85-3. KLH 5/12/93 KLH 5/12/93

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Unlabeled test material (batch No. N183, ZST No. 88/375) was 99.2% pure; this value was reported by the study sponsor. The radiochemical purity of radiolabeled Vinclozolin (preparation No. 36/38, ZST No. 88/342) was at least 95% [as determined by thin-layer chromatography (TLC)] after purification; diluted samples (preparation Nos. 36/38/A and 36/38/D) had radiochemical purities 97% after purification.

2. Male Wistar rats purchased from Charles River U.K. Ltd. (Margate, United Kingdom) and Charles River (Portage, MI) were used. Animals weighed between 162 and 212 g at the time of dosing. A period of acclimation was not described.

3. Four dosing formulations were used. Preparation 36/38 of [^{14}C]Vinclozolin was used undiluted as dose No. 1; this preparation was also diluted to formulate the other dosing solutions. Dose levels 2, 3, and 4 were prepared by diluting radiolabeled Vinclozolin with nonlabeled Vinclozolin in dichloromethane. For batches 36/38/A and 36/38/D (dose levels 3 and 4, respectively), the solvent was removed under reduced pressure on a rotary film evaporator. The diluted test material was then dried in a vacuum desiccator. The solvent in batches 36/38/A3 and 36/38/A4 (both representing dose level 2) was removed under a stream of nitrogen before addition of the dose vehicle. Dosing formulations were freshly prepared prior to application by suspending the appropriate batch of [^{14}C]Vinclozolin in a 1% (w/v)

[1]Only the items appropriate to this DER have been included.

aqueous solution of carboxymethylcellulose to final concentrations of 0.1, 1.0, 10, or 100 mg/mL (dose groups 1 through 4, respectively). The corresponding dose levels were 0.002, 0.02, 0.2 and 2.0 mg/cm^2 or 0.13, 1.3, 13, and 130 mg/kg (Table 1).

4. The absorption, distribution, and elimination of radioactivity were investigated in male rats exposed dermally to one of the four dosing suspensions. Twenty-four rats/dose level were used. One day before application of the test material, an area of skin (approximately 5 cm x 5 cm) on the back of each rat was shaved with electric clippers; care was taken to avoid any abrasion or irritation of the skin. Immediately prior to application of [^{14}C]Vinclozolin suspensions, animals were lightly anaesthetized with fluothane. An application site of approximately 13 cm^2 was marked; the shaved area was covered with nylon mesh secured with adhesive to the flanks of each rat (the nylon mesh was not in contact with the shaved skin). A 0.26-mL aliquot (0.02 mL/cm^2) of the appropriate suspension was applied evenly to the test site using a disposable syringe. The amount of ^{14}C applied to the skin was determined for each subgroup of four rats by radioassaying additional 0.26-mL aliquots diluted to 100 mL with acetone. The treated skin was left uncovered for up to 10 hours, during which time animals were held in restraining cages.

For each dose group, subgroups of four rats were sacrificed at 0.5, 1, 2, 4, and 10 hours after compound application. Urine and feces were collected separately until sacrifice. Immediately before sacrifice, animals were anaesthetized, and the nylon mesh wrappings were removed and retained for washing. The dermal test sites were then washed using cotton wool swabs moistened with water. A blood sample was taken, animals were killed, and the treated skin was removed and placed in dichloromethane. Tissues [gastro-intestinal (GI) tract with contents, liver, kidneys, adrenals, testes, eyes, and brain] were removed, and the urine in the bladder was collected. Excreta and tissue samples and carcasses were held at -15°C until analysis.

Additional subgroups of rats (one subgroup/dose level) were restrained until 10 hours postdosing, during which time urine and feces were collected. At 10 hours, these animals were anaesthetized lightly, the nylon mesh was removed and retained for washing, and the test sites were washed as described above. The treated skin was then covered with gauze, which was secured with an

TABLE 1. Study Design for Male Rats Exposed Dermally to [^{14}C]Vinclozolin

Dose Level	Radioactive Dilution (prep. No.)	Specific Activity (dpm/μg)	Nominal Suspension Concentration (mg/mL)	Nominal Dose Level	
				mg/cm^2 skin	mg/kg bw[a]
1	36/38	448800	0.1	0.002	0.13
2	36/38/A3	53774	1	0.02	1.3
2	36/38/A4	47384			
3	36/38/A	12862	10	0.2	13
4	36/38/D	809	100	2	130

[a]bw, body weight.

Source: CBI pp. 11, 12.

adhesive dressing. Animals were then transferred to metabolism cages; the urine and feces were collected separately between 10 and 24, 24 and 48, and 48 and 72 hours after application of the test material. Rats in these subgroups were sacrificed at 72 hours; blood, tissues, treated skin, and carcasses were taken for analysis as described above. Gauze dressings were also saved and analyzed.

5. Radioactivity was measured using liquid scintillation counting (LSC). Aliquots of urine, cage washes, and water used to moisten swabs were assayed directly. Feces were combusted directly (for small samples) or homogenized first with water and then combusted prior to counting. Blood samples were divided; one portion was centrifuged to separate plasma from cells. The ^{14}C content in aliquots of whole blood and plasma was then determined by LSC. Adrenals and eyes were combusted whole and counted; other tissues were minced, combusted, and radioassayed. Carcasses were solubilized for 24 hours in sodium hydroxide:methanol: Triton X-405 (6:3:1 v/v/v); aliquots were neutralized with nitric acid prior to LSC. The exposed skin samples were extracted twice with dichloromethane; scintillation fluid was added to the extracts, and this mixture was solubilized and then counted, as were the carcasses. Swabs used to remove residual [^{14}C]Vinclozolin were extracted three times with acetone by shaking; the acetone was decanted, the residual solvent was removed by squeezing the swabs, and aliquots were taken for ^{14}C determinations. The nylon mesh wrappings were washed with acetone; washes were radioanalyzed. The occlusive dressings (i.e., gauze) used on animals sacrificed at 72 hours were extracted with acetone; extracts were then analyzed for ^{14}C content. Combustion efficiency was determined to be >95%.

B. Protocol: A protocol was not included in the study report.

12. REPORTED RESULTS

A. The best estimates of absorption were obtained from animals sacrificed at 72 hours; data from these animals were used because relatively large amounts of radioactivity (generally 10 to 18% of the ^{14}C dose) remained at the test sites at earlier sacrifice times, whereas no more than 4%

of the radioactivity was recovered from the test site of rats in the latest sacrifice groups (Tables 2 to 6).

Dermal absorption of [^{14}C]Vinclozolin--expressed as percent of dose applied--was inversely related to dose; the 72-hour absorption was estimated to be approximately 27.3, 19.8, 3.2, and 1% for the 0.002-, 0.02-, 0.2-, and 2.0-mg/cm^2 doses, respectively (Table 2). For the lower two dose levels comparison of results from animals sacrificed at 10 and 72 hours (Tables 3, 4) suggest that radioactivity remaining in the treated skin after washing was in fact ultimately absorbed and excreted. This hypothesis was only partially supported by results from the two higher dose levels (Tables 5, 6). By combining the fraction of radioactivity remaining in the treated skin, total absorption accounts for about 28.6, 24.0, 4.8 and 2.6-2.8%, respectively, for the 0.002, 0.02, 0.2 and 2.0 mg/cm^2 dose concentrations. The total amount of test material--when expressed as mg/kg bw--was related to dose but not proportionately; the respective amounts of test material absorbed by animals in groups 1 through 4 were 0.04, 0.2, 0.4, and 1.0 to 1.2 mg/kg bw. Absorbed radioactivity was eliminated in both the urine and feces; urinary levels of radioactivity were approximately 1.5 to 2 times greater than fecal levels. Recovery of ^{14}C from the tissues and carcasses was inversely related to dose; when combined, these fractions accounted for 0.2 to 3.2% of the dose at 72 hours. Cage washes represented ≤1% of the ^{14}C dose. Unabsorbed radioactivity was generally present in skin washings and accounted for 53% (0.002-mg/cm^2 group) to 94.3% (2-mg/cm^2 group) of the ^{14}C dose. Total recoveries for rats sacrificed at 72 hours were between 82 and 97%. (For the 24 subgroups, total recoveries were between 82 and 104%; all but four of these were >90%.)

Low-dose levels of [^{14}C]Vinclozolin were rapidly absorbed and eliminated by rats. For the two lowest doses (i.e., 0.002 and 0.02 mg/cm^2), approximately 2 and 1% of the radioactivity administered, respectively, was recovered from the urine at 10 hours postapplication (Tables 3, 4); at 24 hours, 7.5 and 3.7% of the ^{14}C dose had been eliminated in the urine, respectively. Dermal absorption of [^{14}C]Vinclozolin was markedly reduced at the two highest dose levels; even at 72 hours after dosing, the urine,

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Page_____ is not included in this copy.

Pages 112 through 116 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__X__ FIFRA registration data.

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____ The document is not responsive to the request.

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feces, and cage washes combined accounted for no more than 2.6% of the administered dose (Tables 5, 6). In addition, most of the radioactivity applied to the skin of these animals was extracted from skin washings.

B. At 72 hours after compound application, only small amounts of radioactivity were found in the body. The tissues and carcass combined accounted for 0.2 to 3.2% of the ^{14}C dose; the lowest levels were associated with the highest dose. For animals in the 2-mg/cm^2 dose group, residual tissue ^{14}C concentrations (expressed as µg [^{14}C]Vinclozolin equivalents/g wet tissue) were below the limit of detection (<0.1 to <0.8 µg/g) (Table 7). For the three other doses, the liver had the highest residual ^{14}C levels (0.06 to 0.2 µg/g); the next highest levels were found in the kidneys, adrenals, and plasma (Table 8). Concentrations of ^{14}C in the brain, blood, and testes were low but generally comparable to each other. In most cases, radioactivity could not be detected in the eyes. Overall, the highest tissue ^{14}C levels at 72 hours postapplication were found in the 0.2-mg/cm^2 group (Figures 1 and 2). Residual tissue radioactivity levels peaked at 10 hours after application to the 0.002- and 0.02-mg/cm^2 groups; tissue concentrations then dropped steadily in the lowest dose group, and plateaued in the 0.02-mg/kg group. In contrast, tissue ^{14}C levels in rats dosed with 0.2 mg [^{14}C]Vinclozolin/kg (in particular, those in the liver and plasma) increased steadily over the 72-hour study, indicating a slow but continual increase in the absorption of the test material. At 72 hours, tissue ^{14}C concentrations of rats in the 0.2-mg/cm^2 dose group were 20 to 50 times higher than those of rats treated with the lowest dose.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES

A. The overall extent of absorption of radioactivity after dermal application of [^{14}C]Vinclozolin was best estimated from the results obtained from animals sacrificed 72 hours after dosing; by that time, there was relatively little radioactivity left in the treated skin. In the groups of rats sacrificed at earlier times, the extent of absorption was more difficult to assess because of the levels of radioactivity remaining in the treated skin after removal of excess dose material with wetted cotton wool swabs. The radioactivity detected in treated skin at these earlier times could be due to inefficient cleansing of the treated area or to material genuinely residing within the skin

TABLE 7. Mean Concentrations of Radioactivity (Expressed As Percent of Dose) in Tissues of Male Rats Sacrificed 72 Hours After Dermal Application of [^{14}C]Vinclozolin

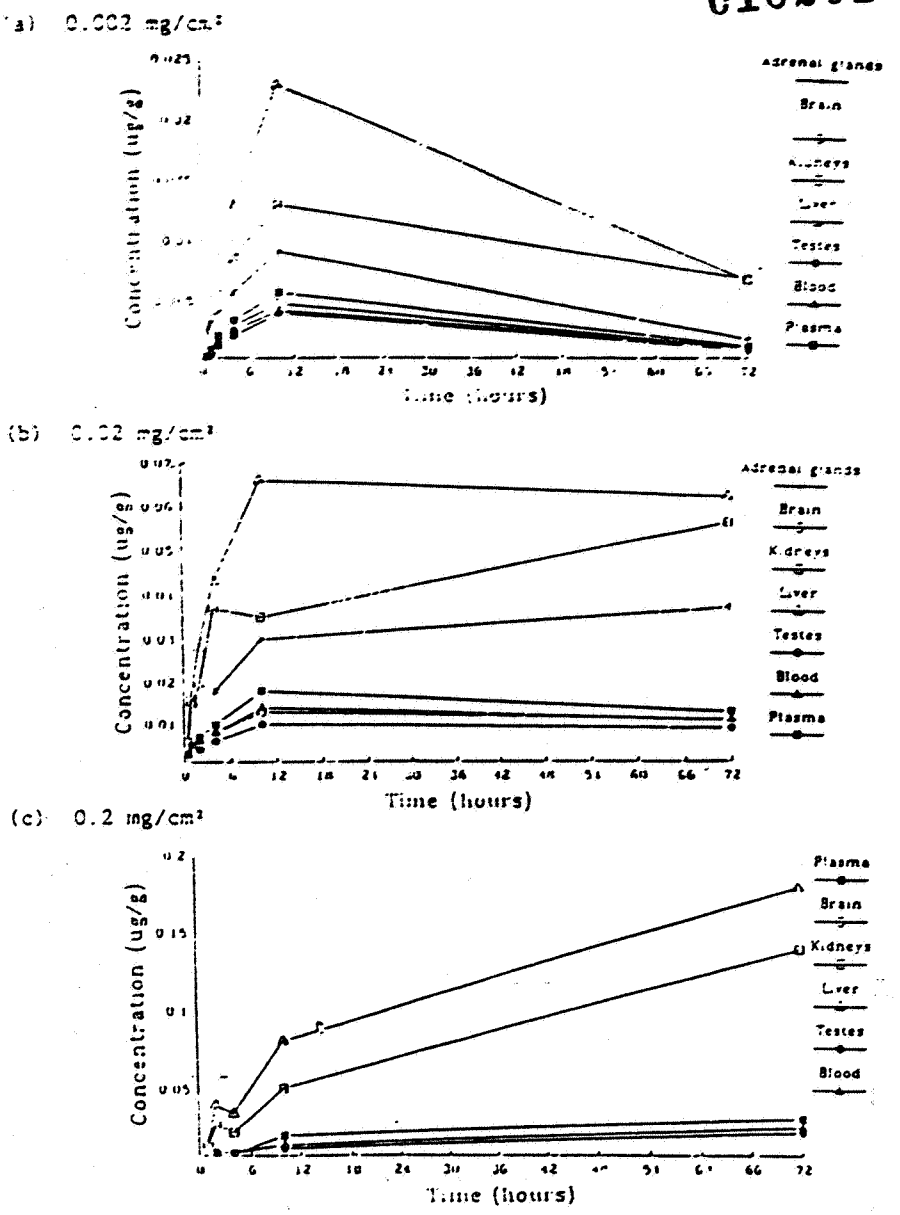
Tissue	Dose Level (mg/cm^2)			
	0.002	0.02	0.2	2.0
Adrenal glands	<0.01	<0.01	<0.01	<0.01
Brain	0.01	0.01	<0.01	<0.01
Eyes	<0.01	<0.01	<0.01	<0.01
Gastrointestinal tract	1.28	1.95	0.36	0.19
Kidneys	0.04	0.04	0.01	<0.01
Liver	0.26	0.24	0.05	0.02
Testes	0.01	0.01	<0.01	<0.01
Carcass	0.53	0.87	0.18	<0.18

Source: CBI Tables 6, 12, 18, and 24; CBI pp. 46, 52, 58, and 64.

TABLE 8. Mean Concentrations of Radioactivity (Expressed As μg ^{14}C Equivalent/g Wet Tissue) in Tissues of Male Rats Sacrificed 72 Hours After Dermal Application of [^{14}C]Vinclozolin

	Dose Level (mg/cm^2)			
Tissue	0.002	0.02	0.2	2.0
Adrenal glands	0.0018	0.037	0.091	<0.8
Brain	0.0011	0.011	0.027	<0.1
Eyes	<0.0017	<0.012	<0.046	<0.6
Kidneys	0.0068	0.056	0.14	0.3
Liver	0.0067	0.062	0.18	0.5
Testes	0.0009	0.009	0.023	<0.2
Whole blood	0.0011	0.011	0.026	<0.1
Plasma	0.0012	0.013	0.032	<0.2

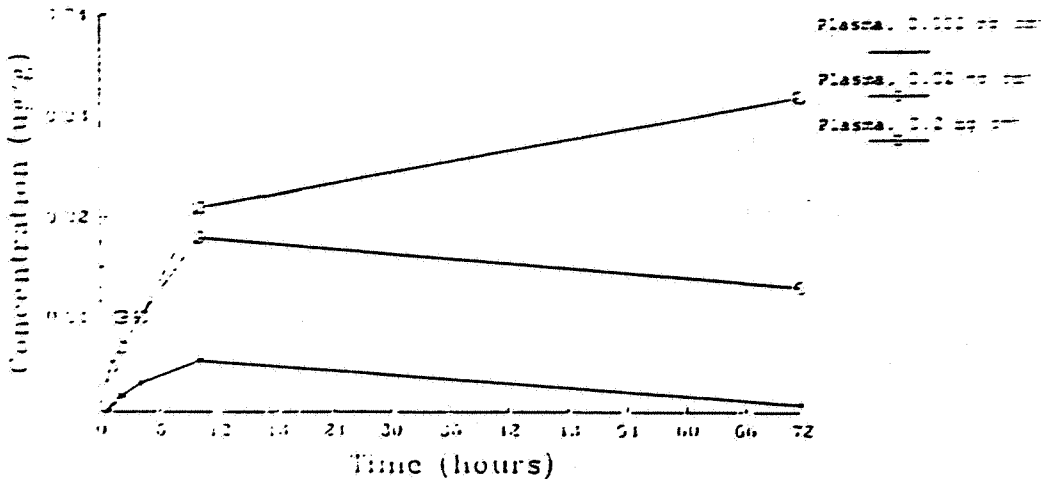
Source: CBI Tables VII-X, CBI pp. 29-32.



N.B. Adrenal glands were below the limit of accurate measurement at 0.2 mg/cm²

Figure 1. Time-related mean concentrations (μg [^{14}C]Vinclozolin equivalents/g tissue) of radioactivity in tissues of male rats treated dermally with [^{14}C]Vinclozolin.

Source: CBI Figure 1, CBI p. 36 (best copy available).



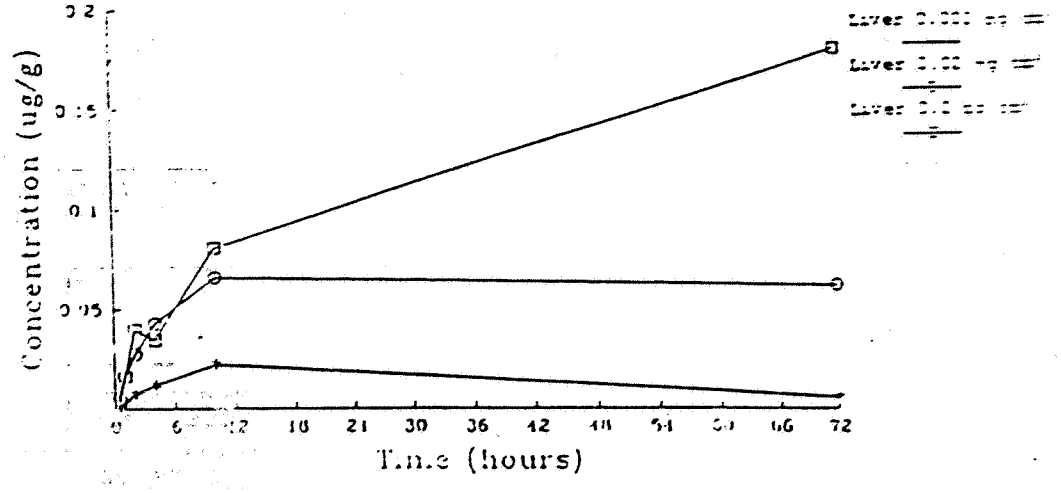


Figure 2. Comparison of mean concentrations of radioactivity in the liver and plasma of male rats treated dermally with [^{14}C]Vinclozolin.

Source: CBI Figure 2, CBI p. 37 (best copy available).

layers. For the two lower dose levels, comparison of results from animals sacrificed at 10 and 72 hours seemed to suggest that radioactivity remaining in the treated skin after washing was in fact ultimately absorbed and excreted. This hypothesis was only partially supported by results from the two higher dose levels.

In terms of percent of applied dose, the absorption of radioactivity decreased as the rate of application of [^{14}C]Vinclozolin was increased. In terms of mg equivalent of Vinclozolin, absorption increased with dose level, but by factors considerably less than the increase in dose. The estimated amounts of the dose absorbed were equivalent to about 0.04, 0.2, 0.4, and 1.1 mg Vinclozolin/kg, respectively, after application of dose formulations 1 to 4. These results represented about a 5-fold increase in the amount of material absorbed, when compared with the 10-fold increase in dose from level 1 to level 2; only a two-fold increase was observed for each of the subsequent 10-fold increases in dose from level 2 to levels 3 and 4. Absorbed radioactivity was excreted in both urine and feces; the urinary route was favored by a ratio of approximately 2:1. This was consistent with patterns of excretion of radioactivity observed after intravenous (iv) administration of [^{14}C]Vinclozolin (results of the iv study were not presented). As a result of reduced absorption, excretion of radioactivity in terms of percent dose was considerably lower in rats treated with [^{14}C]Vinclozolin at the two highest doses. Total retention of radioactivity (excluding the treated skin) by the animals after 72 hours accounted for approximately 2.1, 3.2, 0.6, and between 0.2 and 0.4% of the dose at dose levels 1 to 4, respectively. In each case, most of the retained radioactivity was found in the gastrointestinal tract. Most unabsorbed radioactivity (53 to 94% of the dose) was recovered in the washings of the treated skin at sacrifice or at 10 hours after application of [^{14}C]Vinclozolin. In animals sacrificed up to 10 hours after treatment, the test site (after washing) contained 4 to 23% of the dose. In animals sacrificed after 72 hours, the treated skin contained 0.6 to 4.2% of the dose. The highest tissue concentrations of radioactivity were found in animals sacrificed 10 hours after application of 0.002 mg [^{14}C]Vinclozolin/cm^2 (level 1) and at 72 hours after application of 0.2 mg/cm^2 (level 3). After application of 0.02 mg/cm^2 (level 2), tissue concentrations were generally similar at 10 and 72 hours. Because of the lower specific activity of the applied [^{14}C]Vinclozolin at level 4 (2.0 mg/cm^2), tissue radioactivity concentrations were generally below the limit of accurate measurement; however, it was apparent that maximum concentrations occurred at 72 hours in these rats. At all levels, the liver contained the highest radioactivity

concentrations, followed by kidneys, adrenal glands, plasma, brain, blood, and testes, generally in that order.

B. A quality assurance statement and a statement of compliance with Good Laboratory Practices, both signed and dated January 3, 1991, were provided.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

KLH 5/12/93

~~This study was judged supplementary because~~ It is not clear why Vincolozin was suspended in carboxymethycellulose as the vehicle instead of diluted formulating material as recommended by EPA's proposed guidelines for a dermal absorption study in rats (see Appendix). The interactions of carboxymethylcellulose with Vinclozolin, e.g., binding, and consequently its effects, if any, on the absorption of Vincolozolin are unknown. ~~The registrant is, therefore, required to provide an explanation as to the use and possible effects of carboxymethylcellulose on this study.~~ KLH 5/12/93

Items 15 and 16--see footnote 1.

APPENDIX

Proposed EPA Guidelines for the Conduct of a Dermal Absorption Study in Rats

Procedure for Studying Dermal Absorption

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Introduction

This paper presents a general procedure for dermal absorption studies on pesticides which is applicable to any compound or formulation of a compound. The study requires application of various doses of radiolabeled compound to the shaven skin of male rats followed, at specific intervals after dosing, by total urine and fecal collection, determination of blood concentration, determination of the quantity in the body and determination of the quantity remaining on the skin. It is assumed that a metabolism study of the test compound has been performed in the rat before the dermal absorption study is undertaken.

The rat is used for purely practical reasons, it is not intended as a model of absorption through the human skin but rather as a test system for dermal absorption. The domestic rat is a conveniently sized animal, which is readily available and used for most of the toxicology studies on pesticides including metabolism. Because of its small size, several animals can be used per dose and several dose levels per compound within the constraints of time and resources. Foreign compounds in general pass more rapidly through rat skin than through human skin and thus determination of dermal penetration in the rat offers a built-in safety factor for projection to human exposure.

The study described here combines two different types of dermal absorption studies in a manner which can compensate for their individual deficiencies and simultaneously cover the full range of possible dermal absorption patterns. The first type of study involves placing a measured quantity of compound on the skin for a specific period of time. The animal is then killed and the treated skin is removed. The quantity remaining on the skin is determined and the quantity of compound absorbed is calculated by subtraction. This method works very well for small quantities of a compound which does not fall or vaporize off of the skin. Large quantities, volatile compounds or strange solvents, cannot be used in this procedure.

The second type of study measures what goes into the animal. The compound is applied to the skin in a measured dose and the quantity in the body and the quantity excreted for a specific time period is measured. The procedure has greater possibilities for error in very low doses, for compounds which are not rapidly excreted and for compounds which are completely metabolized to CO_2, water and urea.

Materials

Twenty-four young adult male rats, 225-250 grams in weight, are used at each dose point. It is preferred that the rats be of the same strain used for metabolism studies on the test compound.

The compound should be chemically pure and radiolabeled, usually with carbon-14, in a position which is part of the "core" of the compound. The label should follow the compound and its major metabolites until excreted. The label should not be exchangeable nor should it be metabolically removed to CO_2 or become part of the one-carbon pool of the organism. Double labeling may sometimes be necessary. Unlabeled compound may be used if a sufficiently specific and sensitive test is available.

Methods

Twenty-four hours prior to dosing the back and shoulders of the rats are clipped free of hair and the area washed with acetone. Do not damage the skin.

Twenty-four animals are used per dose. A minimum of three but preferably four doses, at log intervals should be used. The doses should span the range of dose per unit area of skin which can be expected to occur in human exposure. Experience has shown that the highest useful dose is in the order of 10 mg/rat with descending doses of 1, 0.1, and 0.01 mg/rat. If less then four doses are used it is preferred that the lower dose range be used. Doses must be mass/unit area of skin (mg/cm^2) and not mass/body weight (mg/kg) since the rate of absorption is directly related to mass/unit area.

The compound is applied to a measured area of the rat's skin, at least 10 cm^2, in the form applied in the field utilizing the field solvent. Usually the use product (emulsifiable concentrate, flowable powder etc.) is used for the highest dose and is diulted with water for the lower doses. When no solvent is specified, as for the technical material or a dust, the compound is dissolved or suspended in water. Organic solvents should not be used. The material is spread evenly until dry. The spreader should be checked for loss of material. The treated area is covered with a nonocclusive cover to prevent loss by falling or being rubbed off and to prevent the animal eating the test material.

Experience has shown that the application area must be covered. A combination cover consisting of a 'spacer' glued to the skin and a filter paper or gauze glued to the ring appears to be most effective. The 'spacer' will outline the application site and be sufficiently thick to hold the cover from contact with the site.

The treated animals are placed individually in metabolism cages. All urine and feces are collected, a single collection for the entire duration of exposure. At intervals of 1/2, 1, 2, 4, 10 and 24 hours, four animals per dose are anesthetized. The exposed skin and residual compound are collected separately by washing the skin with a mild soap solution followed by several water rinses. Liquid Ivory or Dove for dishwashing is suggested. The skin must be washed before killing the animals, as up to three fold differences have been observed in the ability of skin on the live animal and skin from the killed animal to bind test compounds. The animals are killed, a blood sample taken, and residual urine collected from the bladder and added to the collected urine. Any material on the protective appliance is measured. The remainder of the animal is prepared for determination of the quantity of compound in the carcass.

For each animals the following determinations are made. Results are expressed as quantity or concentration of the parent compound and as percent of applied dose. Metabolites are not separately distinguished.

1) The quantity of the compound in/on the application device and the protective appliance.

2) The quantity of compound that can be washed from the skin.

3) Quantity of compound remaining on/in the skin at the application site which cannot be removed by washing.

4) Concentration of compound in the blood and from this the quantity of compound in the blood.

5) Quantity of compound excreted in the urine and feces.

6) Quantity of material remaining in the carcass.

Results and Conclusions

From the quantity determined in parts 1 and 2 above one may calculate, by subtraction, the quantity absorbed provided that other routes of loss are not significant. Excessive variation of results within groups at the same time and dose will indicate external loss of the dose.

From the quantity in the skin, the quantity excreted, the quantity in the blood and the quantity remaining in the carcass one may obtain directly the quantity absorbed. The quantity which cannot be removed from the skin by washing is considered potentially able to be absorbed and, if the amount is large, special studies may be required to quantitate its potential for absorption.

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The blood concentration of the compound can be used for a direct comparison with other studies on the compound.

Graphs relating dose, time and amount absorbed may be constructed and used to calculate absorption for doses which are not directly studied. Using proper assumptions one may extrapolate to estimate human absorption under conditions of normal exposure.

Additional procedures

1) Procedure to define compounds which are essentially not absorbed.

Results from a study of a compound expected to have little or no dermal absorption have suggested the necessity of treating an additional group of rats. In the study, analysis of the dermal residue indicated no absorption to a limit of 0.1 percent of the dose. This limit was defined by the variability of recovery of compound from the skin. The blood showed no radioactivity at any dose and duration of exposure. The urine showed radioactivity which did not appear to follow the dose and duration of exposure relationship expected. In only one of nine treatment groups were the results internally consistent with all four animals showing similar positive results. In the other eight groups the number of animals having radioactivity in the urine ranged from zero to three with a mean of 1.5. These results appeared indicative of contamination of the urine rather than dermal absorption.

Under such circumstances an additional group of four rats should be treated with the high dose at the 10 and 24 hour durations of exposure. These animals should have their urinary bladders cannulated to avoid contamination of the urine collected during the exposure period. Samples of blood, urine and carcass should be counted for the longest practical time in order to produce the lowest possible limit of dermal absorption. In the case where no absorption occurs under the experimental conditions the limit of dermal absorption will be defined solely by the sensitivity of the method for detecting the radio tracer.

2) Procedure for examining compounds which show a major residue on/in the washed skin.

Several compounds have been tested which show a significant residue on/in the skin despite vigorous washing. The concentration has appeared in short exposures and shows little or no increase with time and often does not appear to increase to any large extent with increase of dose. This suggests a binding process.

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For regulatory purposes one must assume that this material is available for further absorption. However, this may not be true particularly in cases were little or no detectable compound appears in blood, excreta and/or carcass. However, studies such as the one suggested below have shown that absorption of the residue following washing can range from none detectable to essentially all, over a period of two weeks after dosing.

In such cases the following additional study is suggested.

1) Eight rats per dose are treated for the time period which shows the maximum skin concentration (or ten hours).

2) At the end of the exposure period 4 rats per dose are treated as in the basic protocol.

3) The skin of the remaining 4 rats per dose, is washed in the same fashion used in the basic study and the animals followed for at least an additional 72 hours. A study which carried the post-wash period for up to three weeks showed maximum absorption at two weeks. This appears to be a practical limit for observation.

4) The animals are then treated as in the basic protocol.

A balance comparison of the various residues will give some indication as to whether or not the quantity in the washed skin can be absorbed and quantitation of any absorption. If absorption occurs it may be necessary to repeat this process with longer post washed periods to obtain a quantitation of absorption over time.

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Please note. This procedure has been developed by the experimental work performed on pesticides by Registrants in their own or contract laboratories. Their continued work provides valuable and unique information on improving the experimental design and methodology. It is strongly advised that you contact the Agency before performing a dermal absorption study on a pesticide in order to take advantage of the most recent information. You may submit your protocol, through the Registration Division, for evaluation by the author of this document.