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

NOV 8 1994

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Developmental and Reproductive Toxicity Peer Review of Vinclozolin

TO: Sidney Jackson/Leonard Cole, PM-21
Registration Division (H7505C)

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THRU: Penelope A. Fenner-Crisp, Ph.D., Director Penelope A Fenner-Crisp 11/6/94
Health Effects Division (H7509C)

The Health Effects Division Peer Review Committee (PRC) for Developmental and Reproductive Toxicity met on October 20, 1993 to discuss and evaluate the weight-of-evidence for vinclozolin with particular reference to its potential for developmental and reproductive toxicity. This was the first evaluation of vinclozolin by the PRC for developmental and reproductive toxicity.

The PRC concluded that developmental and reproductive toxicity were induced in rats and rabbits following oral administration of vinclozolin. In rats, no NOEL for developmental toxicity (oral route) was determined (gavage, corn oil, postcoital day 14 to postnatal day 3). The LOEL was 3 mg/kg/day based upon reduced anogenital distance. This endpoint was believed to be close to a NOEL. The NOEL for developmental toxicity in rabbits (gavage, 0.5% CMC in water, gestational day 6 through 28) was 200 mg/kg/day. The LOEL in rabbits was 400 mg/kg/day based upon early resorptions, fetal weight increase, decreased live litter size and possible increased skeletal anomalies. The maternal NOEL in rabbits was 50 mg/kg/day with an LOEL of 200 mg/kg/day based upon increased absolute and relative liver weights, reduced defecation and reddish-brown urine. Reproductive effects in rats were decreased epididymal weights and lenticular degeneration at 30 mg/kg/day, with an NOEL of 4.9 mg/kg/day. The parental effect level was 30 mg/kg/day based upon decreased epididymal weights, with an NOEL of 4.9 mg/kg/day.

The critical effect level for risk assessments derived from a developmental toxicity study is 3 mg/kg/day (lowest dose tested) based upon decreased anogenital distance in rats at that dose.

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A. Individuals in Attendance:

1. Peer Review Committee Members in Attendance:

(Signatures indicate concurrence with the peer review unless otherwise stated.)

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2. Other Participants:

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3. Observers:

Kerry L. Dearfield

Elizabeth A. Doyle

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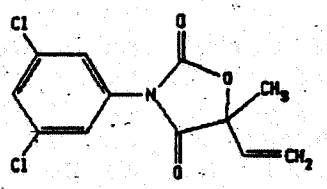
Introduction.

Vinclozolin (Figure 1) is sold under the trade names of Ronilan (various forms; 41 to 50% vinclozolin), Vorlan (41%) and Ornalin (50%) (BASF No. 83 258). It is applied by ground boom spray, aerial spray and hand spray.

Tolerances have been set for endive (5 ppm), cucumbers (1 ppm), stone fruit (25 ppm), grapes (6 ppm), kiwifruit (10 ppm), lettuce (10 ppm), onions (1 ppm), bell peppers (3 ppm), prunes (75 ppm), raisins (30 ppm), raspberries (10 ppm), strawberries (10 ppm) and tomatoes (3 ppm). The sponsor has a pending application for withdrawal of the 75 ppm tolerances on prunes.

Figure 1. Vinclozolin Structure



Solubility: 10.0 μmoles/l of water or 2.86 X 10^{-3} g/l of water.
Vapor pressure: 2.6 X 10^{-6} Torr.
Log Kow: 3.02
Melting Point: 106-108°C
Molecular weight: 286.1

LIST B pesticide.
Case Number: 2740
Chemical Number: 113201
ToxChem Number: 343C.
Chemical Name: 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione.

Historically, the data base for vinclozolin was considered essentially complete prior to 1985. The Carcinogenicity Peer Review (HED Doc. #004475) document dated June 17, 1985, stated that there was no evidence of carcinogenicity in mice through 4374 ppm and the mutagenicity data were all negative. In addition, a chronic study in rats was negative at a MTD of 4374 ppm.

In 1988, inadequacies in the previous data base were discovered. In 1988 BASF, the registrant, submitted a report of a 1979 feeding study from a Japanese subsidiary of BASF that showed evidence of pseudohermaphroditism at 111 mg/kg/day in Sprague Dawley rats when dosed from implantation to the end of gestation. BASF indicated in a series of meetings that they had confirmed the findings and were repeating the chronic, carcinogenicity studies and developmental toxicity studies in addition to the studies on reproduction and metabolism. The chronic and carcinogenicity studies are completed and will be reviewed in 1994. The other studies are complete.

Dr. Earl Gray of HERL confirmed the developmental toxic effects from vinclozolin exposure in 1992. His data showed additional developmental toxic effects in studies where the dams were dosed from gestational day 14 through 3 days after parturition. In addition, he disagreed with the main metabolite reported in the BASF metabolism study. He also confirmed that two metabolites of vinclozolin, but not the parent compound itself, would bind to androgen receptors.

Relevant Data.

A. Developmental Toxicity.

1. Perinatal Developmental Study **Study # 1:** (MRID # 431705-01)

L Earl Gray, JM Ostby and W Kelce (1993) Antiandrogenic Effects of the Fungicide Vinclozolin on Sex Differentiation of the Rat. (Prepublication paper, submitted for publication 12/93 to Toxicology Applied Pharmacology, In Press.) Study conducted at the EPA Laboratories at HERL, RTP as part of a screen for chemicals causing effects on reproduction.

Vinclozolin was administered to Long Evans Hooded rats (Charles River Laboratory, Raleigh, NC) by gavage in corn oil from postcoital day 14 to postnatal day 3 (postcoital day 23 = postnatal day 1). The study was conducted in 3 sets: 1) approximately 5 pregnant rats per dose level at 0, 100 or 200 mg/kg/day; 2) approximately 3 dams per dose level at 0 (5 rats in control group), 3, 6, 12, 25 or 50 mg/kg/day; and 3) 6 dams per dose level at 0, 3 or 6 mg/kg/day. (NOTE: The high dose study with doses of 0, 100 and 200 mg/kg/day has been submitted to Toxicology and Applied Pharmacology (TAP). The low dose study with doses of 0, 3, 6, 12, 25, 50 or 100 mg/kg/day will be submitted to TAP for publication shortly.)

Additional studies are completed, but all the data have not been reported to OPP. Interim reports on the reproductive effects have been made available to OPP for group 1 animals to day 380, group 2 animals to day 56 and for group 3 animals to postnatal day 13 as reported in the above referenced paper. Group 1 animals show the typical hypospadia, reduced sex organ weights, and other effects demonstrated in the study of reproduction conducted by BASF (MRID# 425813-01) and reported in HED Doc# 010380. In addition, cauda epididymal sperm count was statistically significantly lower (52% and 63% of controls) and serum testosterone decreased (64% and 67% of controls) at 100 and 200 mg/kg/day, respectively, at 11 months of age.

The lowest effect level was demonstrated to be 3 mg/kg/day in the combined study 2 and 3 where anogenital distance (AGD) was statistically significantly reduced in males on postnatal day 2. The AGD in experiment 2 was 95.0% and 95.3% of controls at 3 and 6 mg/kg/day, respectively, and in experiment 3 it was 93.6% and 93.0% of controls at 3 and 6 mg/kg/day, respectively. The AGD at 3 and 6 mg/kg/day was shown to be reduced when combined for statistical analysis in a two way ANOVA with dose (2 DF) and block (study 2

versus study 3) as the main parameters. There was a significant block effect, but the block by treatment interaction was not significant. These results indicate that treatment consistently reduced AGD from study 2 to study 3 by the same amount, but the absolute values of AGD for both control and dosed males were higher in study 2 than study 3.

Although, nipple development was nominally increased at 3 and 6 mg/kg/day at day 13, neither were statistically significantly increased.

Androgen receptor binding inhibition in developing males *in utero* is thought to cause the effects in rats from vinclozolin treatment. The effects are similar to those found for flutamide (Imperato-McGinley, 1992) in rats. The antiandrogen metabolite of flutamide, hydroxyflutamide, has a Ki within an order of magnitude of the metabolites/degradation products of vinclozolin and is an excellent model for the effects of vinclozolin in rats and probably in humans.

The use of a corn oil vehicle instead of 0.5% carboxymethylcellulose (CMC) as in the BASF studies may have contributed to the effects at a lower dose levels than in the BASF studies. Corn oil increases absorption from the gut compared with CMC vehicle. The extension of the dosing past gestational day 19 to postnatal day 3, which spans the entire period of sex differentiation, may also have contributed to the effects at lower dose levels than in the BASF studies.

The lowest effect level was 3 mg/kg/day (LDT) for AGD in males, but no NOEL was produced.

2. Rat Gavage Developmental Study #2: (HED Doc. #007909 and 008556).

J Hellwig. Report on the Prenatal Toxicity Studies with Reg. No. 83 258 (Vinclozolin) in Rats After Oral Administration (Gavage) - Consisting of Reports Nos. 89/0090, 89/0091, 89/0092 and 89/0093. Conducted at BASF Aktiengesellschaft, Dept. Toxicology, Germany for BASF and finished on March, 1989. (MRID# 411322-01). (HED Doc. # 007909 is a review of four studies)

Vinclozolin (99.6% pure) was administered by gavage in 5 ml/kg of 0.5% CMC in water to 25 Chbb:THOM-SPF Wistar rats at 0, 15, 50, 100, 150, 200 or 400 mg/kg/day and in 10 ml/kg of 0.5% CMC in water to 10 Chbb:THOM-SPF Wistar rats at 600 or 1000 mg/kg/day from day 6 through day 19 of pregnancy.

Maternal Toxicity (Table 1): NOEL < 600 mg/kg/day; LEL = 600 mg/kg/day for increases in absolute and relative adrenal and liver weights (not studied at doses < 600 mg/kg/day). Body weights were variable, but no definitive dose related body weight decrement occurred through 1000 mg/kg/day. Food consumption and food efficiency were variable, but food consumption was nominally depressed at 1000 mg/kg/day.

Developmental Toxicity (Table 2): NOEL = 15 mg/kg/day; LEL = 50 mg/kg/day for decreased AGD in fetal males, initially stated to be pseudohermaphroditism because males could only be identified by the presence of testes. At 400 mg/kg/day, increased incidence of

dilated renal pelvis, hydroureter and 14th rib appear to be treatment related. At 1000 mg/kg/day, reduced fetal weight occurred.

3. Rat Dermal Developmental Study #3 (HED Doc# 007870 & 008556).

HP Gelbke. Study of Prenatal Toxicity of Reg. No. 83 258 in Rats After Dermal Application. Study No. 34R0375/88074;BASF Reg Doc# 90/0025. Conducted by BASF, Dept. of Toxicology, Germany for BASF, finished by February 1, 1990. (MRID# 414130-01).

Vinclozolin was administered 6 hours/day dermally from day 6 through day 19 of pregnancy to 25 Chbb:THOM (SPF) Wistar rats per group at 0, 60, 180 or 360 mg/kg/day in 0.5% CMC in water vehicle.

Maternal Toxicity (Table 3): NOEL = 60 mg/kg/day. LEL = 180 mg/kg/day based upon increased absolute adrenal weights. Absolute liver weights were increased at 360 mg/kg/day.

Developmental Toxicity (Table 3): NOEL = 60 mg/kg/day. LEL = 180 mg/kg/day based upon statistically significantly decreased AGD in male fetuses. At 360 mg/kg/day, nominally increased incidence of dilated renal pelvis and hydroureter was reported. Dilated renal pelvis incidence was 88% in litters vs. 83% in historical controls for the incidence of dilated renal pelvis and hydroureter combined.

4. Rat Feeding Developmental Study #4 (HED Doc.# 007228).

K Takehara et al. Teratogenicity Study of Vinclozolin (BAS-352F) to Rats in Dietary Administration. Conducted by Nippon Institute for Biological Science, Tokyo. Study report finished December 1979. No project number. MRID# None.

Vinclozolin was administered in the diet to 19 to 20 CD/CRJ Charles River rats per group at 0, 300, 1500 or 7500 ppm (0, 23, 111 or 394 mg/kg/day) day 0 through day 21 of gestation.

Maternal Toxicity (Table 4): NOEL < 300 ppm (23 mg/kg/day). LEL = 300 ppm (23 mg/kg/day) based upon adrenal weight increase. At 7500 ppm, body weights (73% of controls) and pituitary weights (79% of controls) were depressed and adrenal (221% of controls) and ovarian weights (145% of controls) were increased.

Developmental Toxicity: NOEL = 300 ppm (23 mg/kg/day). LEL = 1500 ppm (111 mg/kg/day) based upon shortening of the anogenital distance in male fetuses. At 7500 ppm, male fetuses exhibited external pseudohermaphroditism in 100% of males, fetal weight depression, increased incidence of dilated ureter, hydronephrosis and various delays in ossification of the vertebrae and sternebrae.

Table 1. Study #2: Rat Gavage Developmental Toxicity Study, body weight gain, food consumption and organ weight.

Dose level, mg/kg/day	0	15	50	150[a]	0	400[a]	0	600	1000
Number dams[b]	24	23	24	24	25	24	7	5	8
Body wt. gain, food cons., eff. & organ wt.									
Day 0 thru 6	22.6	24.4	25.2	26.9	27.4	28.5	25.6	32.6*	31.7
Days 6 to 19	98.2	100.3	106.1	107.3	102.3	112.3*	97.8	77.2	93.2
Days 6 to 20	112.9	116.1	122.1	123.5	120.0	130.4	115.3	95.5	108.0
Food cons., day 6 to 20[c]	25.56	25.53	26.31	26.86	26.43	27.27	25.53	25.64	23.56
Relative eff.[d]	4.4	4.5	4.6	4.6	4.5	4.9	4.5	3.7	4.6
Abs./rel.[e] liver wt.	-	-	-	-	-	-	15.1/ 4.51	20.0/6.06	21.8/6.18
Abs./rel[e] adrenal wt.	-	-	-	-	-	-	0.082/0.025	0.18/0.056	0.23/0.067
Reproductive parameters									
Corpora lutea	15.0	15.1	15.3	15.8	15.3	16.2	15.7	17.3	15.8
Implant sites	13.3	13.4	14.7	14.3	14.9	15.0	14.4	12.7	15.0
Post implant loss%	7.4	8.6	9.4	6.1	9.1	5.2	4.9	33.6[f]	5.3

* = Statistically significant at $p \leq 0.05$; ** = Statistically significant at $p \leq 0.01$.

[a] Additional data on the controls, 50, 100 and 200 mg/kg/day dose levels were omitted from the table because the data added nothing to the interpretation.

[b] The number of dams bearing live offspring out of the 25 mated in the groups.

[c] Mean food consumed per animal per day from day 6 to 20 of gestation (g/animal/day).

[d] Relative efficiency = (body wt. gain day 6 to 20)/(Mean daily food consumed from day 6 to 20).

[e] Absolute and relative organ wt. were determined only for the 600 and 1000 mg/kg/day dose groups.

[f] Two of these dams had completely resorbed litters.

Table 2. Study #2: Rat Gavage Developmental Toxicity Study, ano-genital distance (AG distance) in mm and ano-genital index (AG distance/fetal weight) and anomalies.

Dose level (mg/kg)	0	15	50	150[a]	0	200	400	0	600	1000
Litters/fetuses exam., s. tissue & skel.	24/291	23/282	24/320	24/322	25/338	22/296	24/340	7/96	5/66	8/115
Anogenital (AG) distance in mm & AG index										
AG distance, males	2.2	2.2	2.1**	1.8**	2.3	1.5**	1.4**	2.3	1.3**	1.2**
AG distance, females	1.0	1.0	0.9	0.9	1.0	1.0	1.0	1.1	1.1	1.1
AG index, males	0.55	0.55	0.53	0.46**	0.57	0.40**	0.36**	0.58	0.35**	0.32**
AG index, females	0.26	0.25	0.25	0.25	0.26	0.26	0.27	0.29	0.30	0.31
Male fetal wt., g	4.0	4.0	3.9	3.9	4.0	3.9	3.9	3.9	3.9	3.6**
Female fetal wt., g	3.7	3.8	3.8	3.7	3.9	3.8	3.8	3.7	3.8	3.4**
% live males	49.8	51.4	47.5	55.0	51.5	48.6	48.2	47.9	60.6	47.8
% litter anomalies										
Heart, dil. ventricle	0	4.3	0	0	-	-	-	0	0	12.5
Dilated renal pelvis	78	87	83	79	96	95	100	100	100	100
Hydroureter	48	52	42	29	32	59	75**	42.9	100	62.5
14th rib	-	-	-	-	4	14	21*	0	60	37.5
Other skeletal anomalies	No dose related effects				No dose related effects			No dose related effects		
Retardations	No dose related effects				No dose related effects			No dose related effects		

* = Statistically significant at $p \leq 0.05$; ** = Statistically significant at $p \leq 0.01$.
- = Data not collected or no dose related incidence.
[a] = Data from one of the studies at 0, 50, 100 and 200 mg/kg/day were omitted because the data added nothing to the interpretation.

5. Rabbit Gavage Developmental Study #5 (HED Doc# 008311).

HP Gelbe. Report on the Study of the Prenatal/Toxicity of Reg. No. 83 258 (Vinclozolin) in Rabbits After Oral Administration (Gavage). Conducted by BASF Aktiengeschaft, Dept. of Toxicology, Germany, Study No. 38R0375/88062 & 40R0375/88077; Reg. Doc. No. BASF 90/0050 & 90/0051; finished February 14, 1990. MRID# 417093-01.

Vinclozolin was administered by gavage to 15 Himalayan Chbb:HM rabbits per group at 0, 50, 200 or 800 mg/kg/day in 0.5% CMC in water from day 7 through 28 of pregnancy; and to 20 Himalayan Chbb:HM rabbits per group at 0 or 400 mg/kg/day in 0.5% CMC in water from day 6 through 28 of pregnancy. Since the litters were available from only 2 does (1 died and 12 aborted) at the 800 mg/kg/day dose level, additional rabbits were studied at 0 and 400 mg/kg/day to supplement the main study.

Table 3: Study #3, Rat Dermal Devel. Toxicity Study: Body weight, organ weight, effects on reproduction, AGD, index and anomalies from dermal administration of vinclozolin.

Dose level, mg/kg/day	0	60	180	360
Females mated	25	25	25	25
Dams with viable fetuses	25	24	23	24
Litters/fetuses, examined	25/341	24/359	22/331	24/366
Body wt. Day 6 to 20, mean g	38.1	37.1	40.0	41.2
Carcass wt., mean g	306.9	309.1	315.8	314.8
Abs. liver wt., mean g	17.5	17.7	18.6	18.8*
Abs. adrenal wt., mean g	0.103	0.103	0.114**	0.112*
Corpora lutea, mean	16.6	16.8	17.2	17.3
Implant sites, mean	15.1	15.9	15.5	16.3
Post implant loss %	9.5	6.2	6.6	6.5
Resorptions %	9.5	6.2	6.6	6.5
AG distance(mm)/index				
Males	2.3/0.58	2.2/0.59	2.1**/0.54*	2.0**/0.52**
Females	1.0/0.27	1.0/0.28	1.0/0.28	1.0/0.28
Anomalies				
Litters/fetuses, soft tissue Litters/fetuses, skeletal	25/166 25/175	24/175 24/184	22/159 23/172	24/175 24/191
Dilated renal pelvis (%litters/%fetuses)	64.0/19.9	87.5/24.0	68.2/24.5	87.5/30.3*
Hydroureter (%litters/% fetuses)	24.0/5.4	29.2/5.7	27.3/6.3	37.5/7.4
Skeletal anomalies	No dose related effects			
Retardations	No dose related effects			

* = Statistically significant at $p \leq 0.05$; ** = Statistically significant at $p \leq 0.01$.

Maternal Toxicity (Table 5): NOEL = 50 mg/kg/day. LEL = 200 mg/kg/day based upon increases in absolute and relative liver weights and absolute adrenal weights; reduced defecation and reddish-brown urine were observed. Similar findings occurred in the 400 and 800 mg/kg/day groups. Food consumption decreased at 800 and 400 mg/kg/day to 38% and 76% of controls, respectively, from day 7 to 28 of pregnancy. Body weights decreased at 400 mg/kg/day.

Table 4. Study #4: Rat Feeding Developmental Toxicity Study, body weight, food consumption, organ weight, fetal weight, AGD and anomalies.

Dose level (ppm)	0	300	1500	7500
Number of dams mated	22	19	22	22
Number of dams	22	18	22	19
Number of fetuses examined	281	248	295	246
Body wt.				
Day 0 (g)	216	216	217	223
Day 21 (g)	355	355	344	260**
Wt. gain (g)	139	139	127	37
Food consump., day 0-20 (g)	410	423	402	234
Water consump., day 0-20 (ml)	610	606	624	671
Adrenal wt. (mg)	0.388	0.449	0.514*	0.856**
Pituitary wt. (g)	14.0	13.4	13.0	11.0*
Brain wt. (g)	1.93	1.96	1.92	1.93
Ovary wt. (g)	0.1216	0.1335	0.1391	0.1763**
Fetal effects				
Fetal wt.(g): Males- Females-	5.3 5.1	5.2 5.0	5.2 4.9	4.0** 3.8**
Perineal distance, mm: Males Females	3.54 1.97	3.71* 1.94	3.21** 1.98	1.97** 1.76**
Dilated ureter (% litter/ % fetuses	0	0	0	3
Hydronephrosis	0	0	0	15**
Delayed ossification of the vertebrae and sternebrae	-	-	-	Increased

* = statistically significant at $p \leq 0.05$; ** = statistically significant at $p \leq 0.01$.

Developmental Toxicity (Table 5): NOEL = 200 mg/kg/day. LEL = 400 mg/kg/day based upon early resorptions (increased postimplantation loss), fetal weight increase, decreased live litter size and possible increased skeletal anomalies. At 800 mg/kg/day, 1 doe died and 12 aborted after day 11; only 1 had 4 viable fetuses. Of the remaining litter, 1/4 fetuses demonstrated heart defects (traces of interventricular foramen/septum membranaceum) and the others demonstrated sternebrae not ossified and skull incompletely ossified. The study author stated that no antiandrogenic effects were seen.

Reprod ..on

F eproduction **Study #5** (HED Doc #010380).

Hellwig, J. Report Reproduction Study with Reg. No. 83258 (Vinclozolin) in Rats Continuous Dietary Administration over 2-Generations (2 Litters in the First and 2 Litters in the Second Generation), Project No. 71R0375/88053; study conducted at BASF Aktiengesellschaft, Dept. Toxicology, D-W6700 Ludwigshaften, Germany; Reg. Doc. BASF No. 92/11251, 10/21/93 (MRID# 425813-01).

Doses administered in the diet were 0, 50, 300, 1000 or 3000 ppm of vinclozolin (technical, 99.2%) (Males = 0, 4.9, 30, 96 or 290 mg/kg/day; Females = 0, 5.3, 31, 101 or 290 mg/kg/day) to 24 Wistar (Chbb:THOM(SPF) rats per sex per group through the P0, F1 and F2 generations for 14 weeks. Two litters per generation were produced: F1a (F1 adults), F1b (FX adults), F2a (FY adults) and F2b (FZ adults). FY and FZ adults were dosed only at 50 and 300 ppm because no F2 pups were produced at higher dose levels.

<u>Parental Toxicity</u>: NOEL = 50 ppm (4.9 mg/kg/day). LEL = 300 ppm (30 mg/kg/day) based upon epididymal weight reduction (93%** of controls) in males and possibly liver weight increase in females (110%** of controls) (Table 8). A dose related increased incidence of lenticular degeneration occurred in females at the 3 highest dose levels (only 1/24 at 300 ppm). At 1000 and 3000 ppm, testis weights (110%** of controls at 1000 ppm) and Leydig cell hyperplasia increased (10/24 at 1000 ppm), and adrenal weights increased in males (125%** of controls at 1000 ppm) and females (130%** of controls) (Table 8). Lipidosis of the adrenal occurred in females at 1000 (19/24) and 3000 ppm (24/24) and in males at 3000 ppm (24/24).

Pituitary vacuolation cells (castration cells) occurred in all males at 3000 ppm. Single cell necrosis of the liver occurred in most males and females at 3000 ppm; central hypertrophy occurred in both males and female at the rate of 3/24 at 1000 and 24/24 at 3000 ppm. No dose related body weight changes occurred (Table 6). Food efficiency also was unchanged (Table 6). Water consumption increased in P0 and F1 females at 300 ppm and in P0 and F1 males at 1000 ppm (Table 7).

<u>Offspring Toxicity</u>: NOEL = 50 ppm (4.9 mg/kg/day). Absolute epididymal weights (95%*) were statistically significantly lower than controls at 50 ppm in FY adults only, but were nominally lower than controls in PO (97% of controls), F1 (99.8% of controls), FX (96% of controls) and FZ (99% of controls) adults (Table 8). However, the effect at 50 ppm was minimal and not considered to be biologically significant.

Table 5. Study #5: Rabbit Gavage Developmental Toxicity Study, clinical, reproductive and fetal effects of vinclozolin administration to rabbits from day 7 through day 28 of gestation.

Maternal Observation	Dose level (mg/kg/day)					
	0	50	200	800	0	400
#/group	15	15	15	15	20	10
Abortions	0	0	1	12	0	2
Dams with viable fetuses/# fetuses	15/96	15/94	14/94	1/4	19/117	8/42
Mean wt. (g) - Day 16	2811	2843	2802	2569**	2685	2592
Mean wt. (g) - Day 25	2861	2892	2856	2373**	2740	2711
Mean wt. gain - Day 7 to 16	33.1	49.1	32.0	-110**	19.0	-19.6*
Mean wt. gain - Day 7 to 25	43.5	54.7	56.8	-39.1**	44.7	17.7
Carcass wt./wt. gain	2598/ -154	2622/ -152	2651/ -105	-	2488/ -172	2558/ -109
Food consumption - Day 7 to 28	98.7	101.6	91.9	37.5**	90.3	68.6**
Reduced defecation	0	0	1	15	0	15
Discolored urine	0	0	1	13	0	8
Reticulocytes %	16	16	17	67**	16	28**
Absolute liver weight (g)	53.5	57.9	81.5**	144**	50.0	85.6**
Relative liver weight	1.81	1.94	2.69**	5.32**	1.78	3.0**
Absolute adrenal weight (g)	0.20	0.21	0.24*	0.26	0.21	0.23
Relative adrenal weight	0.01	0.01	0.01	0.01	0.01	0.01
Heart dilation & discoloration	0	0	0	3	0	7
Post impl. loss%	4.9	6.1	8.9	42.9*	10.3	32.9*
Fetal effects						
Average litter size	6.4	6.3	6.7	4.0	6.2	5.2
Fetal wt.	40.9	43.7	42.6	-	39.9	44.5*
Total malformations	1.1	3.5	0.9	-	0.7	0
Total variations Acc. 13th rib Flying rib & 12th rib absent	35.8	26.3	31.0	-	28.5	56.6*
Total retardations	57.9	57.3	63.2	-	54.9	33.3

* = Statistically significant at $p \leq 0.05$; ** = $p \leq 0.01$.

LEL = 300 ppm (30 mg/kg/day). Epididymal weights were reduced in the F1 (97% of controls), FX (96% of controls), FY (94%* of controls) and FZ (98% of controls) in males at 300 ppm (Table 8). Dose related lenticular degeneration was noted in 1-2/24 F1 males and 1-3/24 females. These effects occurred in nearly all F1 and FX males and females at the HDT. Absolute testis (106%-107%**, $p \leq 0.001$) and absolute adrenal weights (119%* and 111%** of controls, respectively) were greater than controls in FY and FZ adult males (Table 8); absolute adrenal (107% of controls) and absolute liver weights (109%* of controls) were greater than controls in F1 adult females (Table 9), but not relative adrenal or liver weight. An increased incidence of testicular Leydig cell hyperplasia occurred in F1 (7/24) males at 300 ppm and above.

At 1000 and 3000 ppm pseudohermaphroditism, anomalies and functional deficit occurred in adult male reproductive organs, such as aberrant Wolffian duct, bilateral Muellerian duct, reduced or absent prostate, seminal vesicle and bulbo-urethral gland. In addition, atrophic seminiferous tubules, aspermia/ oligospermia and reduced penis size were noted. Hypospadia occurred in all male offspring only at the 1000 and 3000 ppm dose levels. Increased ovarian lipidosis and ovarian interstitial cell hypertrophy occurred at 1000 and 3000 ppm.

Frequent compound related single cell liver necrosis occurred in F1 and FX adult males (23/29 and 48/49) and in FX female adults (15/40) at 3000 ppm. Central hypertrophy of the liver occurred in the F1 (6/24 at 1000 ppm and 18/26 at 3000 ppm) and in FX females adults (2/24 and 35/40 at 1000 and 3000 ppm, respectively) and single cell liver necrosis occurred in FX female adult offspring (15/40) at 3000 ppm.

Adult male offspring (genital and reproductive tract malformations) sired no offspring at 1000 and 3000 ppm and fertility in adult F1 female offspring may have been reduced at 3000 ppm.

Pinna unfolding, eye opening and auditory canal opening were affected at 3000 ppm and the gripping reflex may have been affected during lactation for F1a and F1b pups. The nominal increase in effects in these parameters at 1000 ppm were within historical control range and may not be biologically significant.

F1a and F1b pup survival was statistically significantly lower than controls at 3000 ppm, at 0-4 days post partum days (47%/92% and 60%/97%, respectively) and 4-21 days post partum (86%/100% and 95%/98%, respectively) (Table 10). Cannibalism during lactation was increased at 3000 ppm. A body weight reduction occurred in F1a and F1b pups by day 1 at 3000 ppm (85% and 73%, both $p \leq 0.01$, respectively) on day 4 and day 21 post partum at 1000 (76% to 81% for F1a and 89% and 84% for F1b, at the respective post partum days) and 3000 ppm (76% and 73% for F1a; 72% and 67% for F1b, at the respective lactational days) (Table 10). A compound related increase in litter incidence over controls occurred in dilated renal pelvis or hydroureter in pups at 3000 ppm (46%/13% for the F1a and 5.6%/0% for the F1b) (Table 10). Nipples were present on male F1a and F1b pups at 1000 and 3000 ppm (Verbal comment by BASF).

Table 6. Study #6: Reproduction - body weight gain, food consumption data and food efficiency calculations for the premating period.

Mean body weight gain (g) over the time period T pre-mating period).	0 ppm	50 ppm	300 ppm	1000 ppm	3000 ppm
PO males	297.8	318.1	313.0	297.4	271.2
PO females	133.2	135.1	146.4	131.1	125.3
F1 males	301.2	306.6	322.3	307.2	275.6
F1 females	144.2	146.3	161.2	146.9	177.4
Mean food consumption in g/animal/day over the time period T.					
PO	26.5	27.6	27.5	26.3	24.6
PO females	19.3	19.8	20.1	18.8	17.8
F1 males	26.0	26.4	27.3	26.3	23.9
F1 females	19.0	19.1	19.9	18.9	19.4
Relative efficiency = (body weight gain over time period T)/(relative food consumed over time period T).*					
PO males	11.2	11.5	11.4	11.3	11.0
PO females	6.90	6.82	7.28	6.97	7.04
F1 males	11.6	11.6	11.8	11.7	11.5
F1 females	7.59	7.66	8.10	7.77	9.14

*Relative food efficiency is only meaningful when compared with controls and other dose groups within a given grouping for PO males or females or F1 males or females because of comparability of the data used in the calculations.

Table 7. Study #6: Reproduction - mean water consumption for the premating period for PO and F1 male and female adults.

Mean water consumption (g/day)	0 ppm	50 ppm	300 ppm	1000 ppm	3000 ppm
PO males	26.1	26.4	27.1	28.2*	28.2*
PO females	19.7	20.0	21.5**	21.4*	24.2**
F1 males	25.9	25.2	27.3	28.1*	29.7**
F1 females	20.3	19.8	21.8*	22.6**	23.0**

* Statistically significant, $p \leq 0.05$. ** Statistically significant, $p \leq 0.01$.

C. Other Developmental/Reproductive Studies.

The following studies are omitted from this review for several reasons. The DERs were short and inadequately characterized the study report and no MRID numbers or accession numbers can be found for the submitted original reports. The rat developmental study apparently does not exist, the DER for the mouse developmental toxicity study reported no antiandrogen effects. The study of reproduction was conducted with highest dose level of 1458 ppm (77.3 mg/kg/day); the slight potential effects occurring at this latter dose level probably went unnoticed. The rabbit study demonstrated a higher NOEL/LEL than the more recent rabbit study. Further discussion of these studies is omitted from this review.

1. A Mouse Developmental Toxicity Dietary Study conducted by BASF Med. Biol Res. Lab from day 0 through day 18, 2/18/75 in 1-liner. NOEL/LEL = 600 ppm (90 mg/kg)/6000 ppm (900 mg/kg) for no implantation sites. At 6000 ppm all mice died on day 9 of gestation. (HED Doc. #000244) No MRID or accession numbers are available.

2. A Rabbit Developmental Toxicity Study conducted by Huntingdon Res. Center, study no. 80/232, 9/4/81. Dosed administered by gavage day 6 through 18 were 0, 20, 80 or 300 mg/kg/day. NOEL/LEL = 80 mg/kg/300 mg/kg for postimplantation loss. (HED Doc. #002409) MRID # 070400.

3. A Rat Study on 3 Generations of Reproduction conducted by Lab. Pharm. Toxik, Germ. 12/9/77, study no.: none. Doses administered in the feed at 0, 162, 486 or 1458 ppm (approximately 0, 8.1, 24.3 or 77.3 mg/kg/day). NOEL/LEL = >1458 ppm/>1458 ppm. (HED Doc. #000244).

D. Other Toxicity Data and Hormone Studies.

1. Subchronic Studies

Subchronic Hormone Recovery Study #7 - 90-day and 2-months recovery hormone studies in 20 Wistar rats per sex per group (MRID# 423551-04) were conducted at 0 or 4500 ppm in the feed. Hormone levels were determined at 3-months in 10 Wistar rats per sex per group. After 2-months undosed, the hormone levels were again determined in 10 rats per sex. Plasma endocrine levels in males and females were analyzed for LH, FSH, ACTH (all three of pituitary origin), testosterone (Testo), estradiol (E2), corticosterone, aldosterone (Aldo), dehydroepiandrosterone (DHEA).

LH, FSH, testosterone and DHEA were elevated in males after 3-months dosing (Table 11), but largely returned to normal values during the recovery. LH in females was elevated (over 2X) after 3-months dosing, but was normal after the 2-months recovery (Table 11). Except for ACTH and corticosterone, all other hormone levels determined were normal.

Table 8. Study #6: Reproduction - effects on body weight and selected target organ weights in the P0 and F1 parental males, FX (F1b adult males), FY (all F2a adult males) and FZ (all F2b adult males).

Dose group→ P0 males, body wt. & absolute organ wt.	Control, 0	50 ppm	300 ppm	1000 ppm	3000 ppm
Body wt., g SD n	530.6 57.0 23	558.4 51.9 24	556.4 44.2 24	530.5 52.7 24	500.0 53.3 22
Liver, g SD n	16.3 2.6 23	17.6 2.9 24	17.4 1.9 24	16.1 2.1 24	18.6** 2.5 22
Testis, g SD n	3.60 0.20 23	3.69 0.24 24	3.75 0.27 24	3.97** 0.26 24	4.13** 0.36 22
Epididymis, mg SD n	1472.2 103.6 23	1433.3 110.6 24	1367.1** 98.8 24	1318.8** 114.4 24	1063.2** 123.2 22
Adrenal gland, mg SD n	73.5 10.2 23	76.2 8.5 24	81.1 13.6 24	91.6** 15.3 24	154.8** 37.5 22
F1 males (adult F1a), body wt. & absolute organ wt.					
Body wt., g SD n	543.0 59.0 24	548.2 79.1 24	568. 66.6 22	494.7* 52.5 24	431.9** 50.2 25
Liver, g SD n	17.0 2.7 24	17.6 3.2 24	18.7 2.8 22	16.0 2.7 24	15.7 3.0 25
Testis, g SD n	3.76 0.24 23	3.80 0.30 24	3.96 0.32 22	3.66 0.49 24	2.90** 0.68 22
Epididymis, mg SD n	1366.9 88.1 23	1363.9 105.9 24	1327.1 75.9 22	1147.3 144.6 24	870.2** 972.0 22
Adrenal gland, mg SD n	66.6 8.1 24	70.9 12.4 23	79. 10.6 22	86.8** 13.7 24	158.6** 34.9 25
FX males (non-mated adult F1b), body wt. & absolute organ wt.					
Body wt., g SD n	432.2 49.6 24	425.1 39.8 24	446.7 44.8 24	430.3 37.3 24	364.6** 30.7 49
Liver, g SD n	15.6 3.5 24	15.3 3.8 24	15.8 2.5 24	14.9 2.6 24	15.5 2.0 49
Testis, g SD n	3.44 0.26 24	3.46 0.27 24	3.69 0.36 24	3.65 0.37 24	2.72** 0.50 44
Epididymis, mg SD n	1302.3 117.4 24	1252.8 91.6 24	1260.8 120.6 24	1099.2** 94.9 24	540.1** 242.9 44
Adrenal gland, mg SD n	70.3 9.6 24	73.0 8.8 24	83.5 12.2 24	104.3** 18.7 24	190.** 38.5 49
Continued next page					

Dose group→ FY males (adult F2a), body wt. & absolute organ wt.	Control, 0	50 ppm	300 ppm	1000 ppm	3000 ppm
Body wt., g SD n	424.8 37.1 24	418.6 30.0 24	437.5 47.6 24	-	-
Liver, g SD n	14.8 2.6 24	14.5 2.1 24	16.0 2.5 24	-	-
Testis, g SD n	3.47 0.32 24	3.45 0.21 24	3.67** 0.31 24	-	-
Epididymis, mg SD n	1269.8 92.5 24	1212.4* 71.0 24	1195.8** 89.3 24	-	-
Adrenal gland, mg SD n	81.2 12.0 24	79.5 10.9 24	89.9* 11.6 24	-	-
FZ males (adult F2b), body wt. & absolute organ wt.					
Body wt., g SD n	388.3 38.2 24	406.6 35.3 24	424.6** 47.3 24	-	-
Liver, g SD n	13.9 2.0 24	14.7 2.3 24	16.3** 3.2 24	-	-
Testis, g SD n	3.33 0.23 24	3.44 0.21 24	3.67** 0.32 24	-	-
Epididymis, mg SD n	1217.2 78.2 24	1200.8 78.9 24	1198.9 107.4 24	-	-
Adrenal gland, mg SD n	78.1 10.0 24	78.5 10.3 24	93.1** 12.0 24	-	-

SD = Standard deviation; n = Number of animals; * = Statistically significant, $p \leq 0.05$; ** = Statistically significant, $p \leq 0.01$; - = Missing data due to reproductive toxicity in the F1 groups at the 1000 and 3000 ppm dose levels.

Table 9. Study #6: Reproduction - effects on selected target organ weights in the P0 and F1 parental females, FX (F1b females adults), FY (all female F2a adults) and FZ (all female F2b adults).

Dose group→ P0 females, body wt. & absolute organ wt.	Control, 0	50 ppm	300 ppm	1000 ppm	3000 ppm
Body wt., g SD n	299.2 20.7 21	301.3 25.2 24	311.6 22.5 24	287.7 20.3 22	285.6 21.3 21
Liver wt., g sd n	9.6 0.8 21	10.0 1.1 24	10.6* 1.8 24	10.5* 0.8 22	13.2** 1.4 21
Adrenal gland wt., mg SD n	110.0 11.2 21	108.8 16.9 24	114.7 15.6 24	143.6** 16.4 24	131.2** 24.5 21

Continued on next page

Dose Group→ F1 female (F1a adults), body wt. & absolute organ wt.	Control, 0	50 ppm	300 ppm	1000 ppm	3000 ppm
Body wt., g SD n	303.5 24.6 23	305.0 23.5 23	319.6 28.1 22	-	-
Liver wt., g sd n	9.5 1.0 23	9.7 1.0 23	10.4* 1.3 22	-	-
Adrenal gland wt., mg SD n	94.2 8.5 23	92. 9.7 23	101.2* 11.7 22	-	-
FX female (non-mate, F1b adults), body wt. & absolute organ wt.					
Body wt., g SD n	247.9 27.6 23	246.3 19.7 24	265.3* 33.2 24	253.3 16.9 23	251.9 18.7 40
Liver wt., g sd n	7.5 1.2 23	7.3 0.6 24	7.8 1.0 24	8.6* 0.6 23	13.3** 1.8 40
Adrenal gland wt., mg SD n	85.7 13.3 23	86.1 8.1 24	92.9 11.8 24	113.5** 16.1 23	132.0** 23.6 40
FY female (F2a adults), body wt. & absolute organ wt.					
Body wt., g SD n	241.8 16.8 24	239.5 17.6 24	247.8 26.0 24	-	-
Liver wt., g sd n	7.3 0.6 24	7.4 0.7 24	8.0* 0.8 24	-	-
Adrenal gland wt., mg SD n	92.3 11.8 24	90.5 13.0 24	100.4* 10.0 24		
FZ female (F2b adults), body wt. & absolute organ wt.					
Body wt., g SD n	232.2 26.2 24	234.4 24.0 24	247.0 27.8 24	-	-
Liver wt., g sd n	7.3 0.9 24	7.4 0.8 24	8.0* 1.1 24	-	-
Adrenal gland wt., mg SD n	93.5 11.7 24	88.6 9.8 24	103.5** 11.6 24	-	-

SD = Standard deviation;
n = Number of animals;
* = Statistically significant, $p \leq 0.05$; ** = Statistically significant, $p \leq 0.01$;
- = Missing data due to reproductive toxicity in the F1 groups at the 1000 and 3000 ppm dose levels.

Table 10. Study #6: Reproduction data during production of the F1a and F1b litters, fetal viability, fetal weight and anomalies in the F1a And F1b litters.

Dose level (ppm)	0	50	300	1000	3000
For F1a litter production					
Males with confirmed mating	24/24	24/24	24/24	24/24	23/24
Males proving fertility	23/24	24/24	24/24	22/24	17*/23
Female proving fertility	23/24	24/24	24/24	22/24	17*/23
F1a pup viability, day 0	13.7	13.8	14.3	15.0	10.1**
F1a pup viability, day 4	12.6	13.0	13.4	13.8	4.7**
F1a pup viability, day 21	12.6	13.0	13.1	13.6	4.1**
F1a pup wt. (g) at day 1: Males Females	6.4 6.1	6.4 6.1	6.4 6.2	6.1 5.8	5.4** 5.2**
F1a pup wt. (g) at day 4: Males Females	9.2 9.1	9.3 8.9	9.3 9.0	8.2* 8.0*	7.0** 6.8**
F1a pup wt. (g) at day 21: Males Females	43.6 42.1	44.2 42.3	44.7 42.6	36.3** 35.2**	31.3** 31.2**
Anomalies: F1a pups/litters					
Heart: dilation of ventricles	0/23	0/24	0/24	1/22	3*/13
Heart: enlarged left ventricle	0/23	0/24	0/24	1/22	1/13
Dilated renal pelvis	3/23	1/24	1/24	4/22	6*/13
Hydroureter	2/23	1/24	0/24	1/22	2/13
For F1b litter production					
Males with confirmed mating	24/24	24/24	24/24	24/24	22/23
Males proving fertility	21/24	23/24	23/24	22/24	19/23
Females proving fertility	21/24	23/24	23/24	22/24	19/23
F1b pup viability, day 0	14.7	15.4	15.2	15.9	11.1**
F1b pup viability, day 4	14.3	14.8	14.5	14.8	6.7**
F1b pup viability, day 21	14.0	14.7	14.3	14.3	6.4**
F1b pup wt. at day 1: Males Females	6.4 6.1	6.4 6.1	6.3 6.0	6.2 5.9	5.3** 5.2**
F1b pup wt. at day 4: Males Females	9.2 9.1	9.3 8.9	9.3 9.0	8.2* 8.0*	6.5** 6.3**
F1b pup wt. at day 21: Males Females	40.8 39.2	40.9 38.5	41.5 38.9	36.3* 34.3*	26.9** 26.8**
Continued on the next page					

Dose level (ppm)	0	50	300	1000	3000
Anomalies: F1b pups/litters					
Heart: dilation of both ventricles	0/21	0/23	0/23	0/22	3/18
Heart: enlarged right ventricle	0/21	0/23	0/23	0/22	5*/18
Heart: enlarged left ventricle	0/21	0/23	0/23	0/22	1/18
Dilated renal pelvis	0/21	2/23	0/23	0/22	6**/18
Hydroureter	0/21	0/23	0/23	0/22	3/18

* = Statistically significant, $p \leq 0.05$; ** = Statistically significant, $p \leq 0.01$

Subchronic Organ Wt. Recovery Study# 7 - A 90-day study was conducted in Wistar rats on organ weights and with 1 and 3 months recovery periods (MRID# 423551-03). Organ weights were determined at 3 months in 30 Wistar rats per group at 0, 1000 and 4500 ppm of vinclozolin in the feed. After 1 and 3 months undosed the organ weights were again determined. Ten rats per group were sacrificed at 3, 4 or 6 months for organ weight determination.

Male body weights were decreased after 3 months of dosing, but returned to normal after 3 months undosed. Female body weights were unchanged after the 3 months of dosing (Table 12). All organ weights which were changed after 3 months of dosing returned toward normal after 3 months of recovery in males and females (Table 12, 13 and 14). Kidney, prostate, spleen and adrenal weights in males (Table 13) and liver, adrenal and spleen weights in females (Table 14) returned toward normal, but were still statistically significantly changed at 4500 ppm after 3 months' recovery.

3. Subchronic Mouse (C57BL) Study #9 (MRID# 418243-01)

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).

The NOEL was 100 ppm (≈ 20 mg/kg/day for males and ≈ 30 mg/kg/day for females). The LEL was 1000 ppm (≈ 230 mg/kg/day for males and 310 mg/kg/day for females). In males and females triglycerides and cholesterol were depressed. Lipogenic pigment was exhibited in the adrenals at the mid and high dose, but lipid vacuoles were noted only at the high dose. Relative liver weights in males and females were increased at the mid and high dose, but no histological correlates could be detected at the mid dose. The nominal adrenal weight increase at the mid dose is possibly compound related.

Alanine aminotransferase (ALT), alkaline phosphatase (SAP) [these latter two values were approximately doubled], total protein, and globulin levels were elevated at the highest dose. Glucose was depressed at the highest dose level in males.

Hyperplasia and hypertrophy of testicular Leydig cells and ovarian stromal cells, and hepatic centrilobular hypertrophy were noted at the highest dose tested. The author noted that the testicular weight increase at the high dose was probably not due to the Leydig cell hyperplasia. 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 food efficiency was variable, and thus, the nominally decreased body weight in males was not demonstrated to be due to toxicity. The body weights of females did not change; however, there was an apparent increase in food efficiency in females (130% of controls).

4. Subchronic (B6C3F1) Mouse Study #10 (MRID# 418243-02)

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).

The NOEL was 100 ppm (≈ 17 mg/kg/day for males and ≈ 23 mg/kg/day for females). The LEL was 1000 ppm (≈ 170 mg/kg/day for males and 250 mg/kg/day for females). The following effects were noted at doses ≥ 1000 ppm: multifocal Leydig cell hyperplasia of the testes; decreased cholesterol, triglycerides, and glucose in males, and cholesterol in females; increased testes; increased relative and absolute liver weights in males and females; and increased lipogenic pigment in females. Adrenal A-cell hyperplasia occurred in males at ≥ 1000 ppm, and in females in all treated groups.

Liver weight increase was accompanied by centrilobular hypertrophy in males at ≥ 2500 ppm and in females at 5000 ppm, and peripheral fatty infiltration of the liver at 5000 ppm in males and females. ALT was increased at 5000 ppm in males only, and SAP was increased in males, but decreased in females at ≥ 2500 ppm. Adrenal weights were increased in males and females at ≥ 2500 ppm. Adrenal lipid vacuoles were noted in males at 2500 ppm and in all treated female groups.

At 5000 ppm, stromal cell hyperplasia of the ovaries occurred. Triglycerides were depressed in females only at 5000 ppm. Reticulocytes were increased in females at 5000 ppm. The food efficiency was variable, but slightly less than control in males at 5000 ppm, and thus, the nominal body weight decrements 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.

Table 11. Study #7: Endocrine values and standard deviation (SD) for 10 animals per sex treated for 3 months and animals treated for 3 months plus 2 months recovery undosed for the 0 and 4500 ppm groups.

Dose group	ACTH SD pg/ml	LH SD ng/ml	FSH SD ng/ml	Testo SD ng/ml	Cortico-sterone SD ng/ml	Aldo SD pg/ml	DHEA SD ng/ml	E2 SD pg/ml
3-Months treatment								
Males								
0	98	0.42	9.5	2.9	236	426	0.75	8.7
SD	31	0.23	2.2	1.0	73	137	0.06	3.4
4500 ppm	174*	3.95*	20.4*	8.0*	381	669	1.42	5.7
SD	83	1.07	2.6	3.3	207	283	0.17	1.3
Females								
0	174	0.53	5.9	0.13	726	1110	0.79	15.4
SD	68	0.22	0.5	0.02	274	518	0.15	2.6
4500 ppm	499*	1.35*	5.7	0.13	383	503	0.78	12.8
SD	142	0.30	0.7	0.03	137	290	0.13	4.7
3-Months treatment + 2-Months recovery								
Males								
0	100	0.64	8.8	4.4	206	475	1.06	5.0
SD	73	0.39	1.0	3.1	115	336	0.31	0.9
4500 ppm	92	0.50	10.5	3.7	174	408	0.87	5.0
SD	47	0.30	1.7	1.6	82	306	0.11	0.9
Females								
0	102	0.64	6.4	0.12	695	962	0.67	13.3
SD	43	0.38	1.0	0.02	209	344	0.13	1.2
4500 ppm	261	0.71	6.6	0.14	611	1179	0.82	19.1
SD	95	0.22	0.7	0.01	95	423	0.11	5.0

* = Biologically significantly elevated.

Table 12. Study #8: Data from MRID# 423551-03. Body weights of males and females at termination in F1, R1 and R2 groups.

Group Body weights	F1 - 3 months dosing			R1 - 1 month recovery			R2 - 3 months recovery group		
	Controls	1000 ppm	4500 ppm	Controls	1000 ppm	4500 ppm	Controls	1000 ppm	4500 ppm
Males	504.3	497.0	407.9**	518.3	512.9	442.7**	547.1	561.0	523.5
SD	52.26	37.3	45.1	52.6	58.8		41.6	19.2	38.4
Females	258.5	259.5	247.8	266.6	266.8	258.0	280.2	286.4	281.0
SD	18.1	24.5	15.7	17.5	9.2	19.8	16.0	13.3	23.2

** = statistically significant at $p < 0.01$.
SD = Standard deviation.

Table 13. Study #8: Absolute and relative organ weight changes (%) from control values in groups dosed 3 months, dosed 3 months + 1 month recovery and dosed 3 months + 3 months recovery in males at 1000 and 4500 ppm.

Males Organ wt. Absolute Relative	Dosed 3 months 1000 ppm	 4500 ppm	Dosed 3 months + 1 month of recovery. 1000 ppm	 4500 ppm	Dosed 3 months + 3 months of recovery. 1000 ppm	 4500 ppm
Liver	↑ ↑	↑ 137%**	- -	↓ -	- -	↓ -
Kidney	- -	85%* -	↓ -	84%* -	- -	92%** -
Testes	117%** 118%**	121%** 149%**	- -	↑ -	- -	- -
Epididymides	88%* 89%*	49%** 60%**	94%* -	77%** ↓	- -	- -
Seminal vesicle	↓ ↓	20%** 24%**	↓ -	70%** ↓	- -	↓ ↓
Prostate	↓ ↓	22%** 27%**	↓ ↓	62%** 73%**	83%* 81%*	77%* 79%*
Spleen	↓ -	75%** -	- -	↓ -	- -	↑ 126%*
Adrenal glands	132%** 129%*	262%** 307%**	- -	138%* 157%**	- -	116%* 117%*
Pituitary	- 150%*	- 150%**	- ↑	- 150%*	- -	- -

* = Statistically significant at $p < 0.05$. ** = statistically significant at $p < 0.001$.
↑ = Nominally increased from control values. ↓ = Nominally decreased from control values.
- = Data equivalent to control values.

Table 14. Study #8: Absolute and relative organ weight changes (%) from control values in groups dosed 3 months, dosed 3 months + 1 month recovery and dosed 3 months + 3 months recovery in females at 1000 and 4500 ppm.

Females Organ Wt. Absolute Relative	Dosed 3 months 1000 ppm	 4500 ppm	Dosed 3 months + 1 month recovery. 1000 ppm	 4500 ppm	Dosed 3 months + 3 months recovery. 1000 ppm	 4500 ppm
Liver	119%** 119%**	183%** 191%**	↑ ↑	↑ 114%**	- -	↑ 108%*
Kidneys	- -	↓ -	- -	- -	- -	- -
Ovaries	121%** 123%**	↓ -	↑ ↑	↑ ↑	- -	- -
Uterus	- -	74%* ↓	- -	- -	- -	- -
Spleen	- -	- -	- -	- -	↑ ↑	- 126%*
Adrenal glands	129%** 129%**	160%** 166%**	- -	- -	87%* 85%**	83%** 82%**
Pituitary	- -	150%** 150%**	- -	78** ↓	- -	- -

* = Statistically significant at $p < 0.05$. ** = statistically significant at $p < 0.001$.
↑ = Nominally increased from control values. ↓ = Nominally decreased from control values.
- = Data equivalent to control values.

E. Chronic Studies

1. Chronic 6-Months Hormone Study #11 (MRID# 418243-05)

Ten Wistar rats per sex per group were fed 0 or 4500 ppm vinclozolin in diet for 6 months. Plasma hormone levels were measured.

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 (Table 15). In females, ACTH (172% of controls) and LH (238% of controls) were statistically significantly and biologically significantly elevated (Table 15). The lack of corticosterone elevation with ACTH elevation in females is not consistent and may indicate some groups were subjected to stress prior to or at blood collection. The elevated LH in females may be related to the stage of estrus at the time of blood sampling.

It would appear that some of 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 (T) is relabeled 17α-OH-progesterone, and the column labeled 17α-OH-progesterone is relabeled estradiol (E2), the values would better correlate with known values for controls. The data in the columns in Table 15 have been renamed accordingly.

Table 15. Study #11: 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.

2. Chronic 6-Month Dog Feeding Study **Study #12** (HED Doc.# 002214, MRID# 248123 and 248124).

Vinclozolin was administered in the feed to Beagle dogs at 0, 100, 300, 600 or 2000 ppm.

NOEL = 100 ppm (2.5 mg/kg/day). LEL = 300 ppm (7.5 mg/kg/day) based upon increased adrenal weight in males and females and increased pituitary weight in females.

3. Chronic 1-Year Dog Feeding **Study # 13** (HED Doc.# 007228 and 007909, MRID# 408595-01)

J Hellwig. Report on the Study of the Toxicity of Vinclozolin in Beagle Dogs After a 12-Month Administration Via the Diet. Study No.: 87/0447, dated October 1987.

Vinclozolin was administered in feed to beagle dogs at concentrations of 0, 35, 75, 150, 1500 ppm (Doses to males equivalent to 0, 1.1, 2.4, 4.8, or 47 mg/kg/day; to females equivalent to 0, 1.1, 2.5, 5.1, or 53 mg/kg/day.)

NOEL = 75 ppm (≈ 2.4 and 2.5 mg/kg/day for males and females, respectively). LEL = 150 ppm (≈ 4.8 and 5.1 mg/kg/day for males and females, respectively) based upon increased bilirubin, increased relative testes weights, and prostate atrophy in males, and increased absolute adrenal weights, adrenal lipid accumulation, and marginally increased liver hemosiderin in females.

In addition, at 1500 ppm in males, increased absolute and relative liver, spleen, testes, adrenal, thyroid weights; increased diffuse hyperplasia of the Leydig cells of the testes, and organ lipid accumulation in the adrenal cortex; and increased platelets were noted. In females at 1500 ppm, increased absolute and relative adrenal weight, and slight increases in MCV and MCH were noted. There was a possible increase in reticulocytes in 1500 ppm males and females.

F. Mutagenicity - Negative in all acceptable studies.
   Ames (HED Doc.# 003181) - Negative with and without S9.
   Mouse lymphoma (HED Doc.# 005054) - Negative.
   Forward mutation (CHO/HGPRT) (HED Doc.# 005853) - Negative.
   Sister Chromatid Exchange (HED Doc.# 003181) - Negative.

G. Pharmacokinetics/Metabolism

DR Hawkins et al. The Biotransformation and Biokinetics of Vinclozolin in the Rat. Conducted by Huntingdon Res. Center, Eng., study no. 90/0514 & 90/0544 for BASF. Studies finished 11/29/1990 (MRID# 418243-07 and 418243-08).

Absorption, distribution and excretion were studied after oral or i.v. doses of [C^{14}-dichlorophenyl]vinclozolin to male and female Wistar rats. Single labeled oral 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. 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 2 major metabolites, N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutramide 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 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 tributramide 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 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.

NOTE (according to Gray): An issue remains to be resolved about the main metabolite determined in the BASF studies (BASF representatives have been requested to resolve this issue). The main metabolite, N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutramide (M2), determined by BASF has been questioned in a memorandum from Dr. Earl Gray, Jr. Dr. W Kelce, a contractor with Dr. Gray's group, stated that 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid (M1) is the major metabolite of vinclozolin and that M1 can not be quantitatively extracted at pH 7.0. BASF extracted urine and plasma samples by a sorbent extraction procedure through washing with aqueous pH 7.4 and hexane and elution with acetonitrile: methanol, 1:1, which according to Dr. Kelce can not quantitatively extract M1.

Resolution of this issue is important because it has a bearing on the nature of the plant residues and possible residual hormonal activity among them. The same memorandum states that vinclozolin may have no anti-androgen activity and that the anti-androgenicity seen is due to M1, while BASF believe that vinclozolin is responsible for the anti-androgenicity and that the main metabolite 119 308 has only 1/20 of the anti-androgen activity of vinclozolin. There is also some ambiguity in nomenclature used to designate some of the compounds in the BASF report.

H. Other Hormone Studies *In Vitro*.

1. Comparison of the binding affinity of vinclozolin and flutamide with glucocorticoid and androgen receptors.

Studies on the interaction of vinclozolin and reg. no. 119 208 (metabolite BF 352-22) with glucocorticoid and androgen receptors from MCF-7 cells and rat prostate and liver tissue. (MRID 425884-01)

Vinclozolin had 39% of the binding affinity of flutamide, an antiandrogen, for MCF-7 cell receptors and no affinity for the glucocorticoid receptors from rat liver. Metabolite 119 208, BF 352-22, had 2% of the binding affinity of flutamide to the androgen receptor from MCF-7 cells and no affinity for the glucocorticoid receptors from rat liver.

2. Interaction of vinclozolin, M1 and M2 with Androgen Receptors.

Kelce, RW, E Monosson, MP Gamcsik, SC Laws and EL Gray, Jr. (1994). An Environmental Antiandrogen: Evidence that Vinclozolin is Hydrolysed to Antiandrogenic Metabolites. Toxicol. Appl. Pharmacol. 126: 276-285. Study conducted at Mantech Environmental Technology, Inc., University of North Carolina-Chapel Hill, Johns Hopkins University and the Health Effects Research Lab., RTP. (MRID 431705-01)

Vinclozolin was postulated to act through androgen receptor binding in a screen for effects on reproduction (Gray et al., 1993). In this report Kelce et al. studied the androgen receptor binding properties of the parent substance, vinclozolin and two metabolites/degradation products, 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1) and 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2). These studies were conducted on epididymal androgen receptors from high-salt nuclear (the most biologically relevant receptors) and low-salt cytosolic extracts.

The M1 metabolite/degradation product is not extracted quantitatively with ethyl acetate or any organic solvent at alkaline pH values, such as those used to extract serum in the metabolic studies by BASF. Only a trace of M1 was identified in the rat serum in the metabolism studies by BASF (MRID# 418243-07

and -08)(HED Doc# 010261). In the current study M1 in rat serum was extracted (90%) with ethyl acetate at pH < 3.0.

None of the three substances, vinclozolin, M1 or M2 interfered with 5α-reductase; thus, the mechanism of action is not through inhibition of the testosterone-dihydroxytestosterone conversion. None of the three substances specifically bound to the estrogen receptors from rat uteri.

The displacement of [17α-H^3-methyl]methyltrienolone ([H^3]R1881), a specific androgen receptor binding agent, from androgen receptors by various concentrations of M1, M2 hydroxyflutamide and cyproterone acetate formed parallel curves indicating that these substances compete for the same binding site as [H^3]R1881. The apparent affinity of R1881 binding in the high-salt (Kd=2.98 nM) and low-salt (Kd=0.53 nM) extracts was comparable to those determined in the literature reports.

Vinclozolin competition with androgen for the androgen receptor was weak (Ki > 700 μM), but the two vinclozolin metabolites/degradation products, M1 and M2, were effective antagonists of androgen receptor binding (Ki = 92 and 9.7 μM, respectively). The vinclozolin binding may have been due to the formation of 10-15% M1 during the 20 hour incubation at pH 7.4 of the receptors with vinclozolin. Vinclozolin, the parent compound, does not appear to be capable of androgen receptor binding at concentrations that are likely to exist *in vivo*. However, M1, and possibly, M2 are present at concentrations around their respective Ki's for inhibition of androgen receptor binding and support the hypothesis that M1 and M2 are responsible for the antiandrogenic effects of vinclozolin exposure. Other metabolites have not been excluded.

The observation by Kelce, et al. of binding of the metabolites/degradation products, M1 and M2, is the first report of pure environmental antiandrogens formed from an environmental chemical and illustrates the caution necessary when interpreting the health risks from the carboximide fungicides.

Dr. Earl Gray of RTP has conducted binding studies with M1 (a decomposition product of vinclozolin, Szeto et al., 1989) and with vinclozolin and finds that M1 binds with equal or higher affinity than vinclozolin. This finding is consistent with the smaller Ki (92 μM) for M1 than for vinclozolin (>700 μM) reported by Kelce, et al. (see above). He also has conducted studies on rats through lactation day 4 and found nipple development in males at 3 and 6 mg/kg/day (not statistically significant). However, since the study was conducted in only 3 litters per dose level, the study needs verification with a larger number of litters.

I. Structure Activity Relationships

Vinclozolin is related structurally to several other antiandrogens. An important part of these structures exhibiting biological activity is electron withdrawing groups on an anilide structure with a branch hydrocarbon attached to the carbonyl of the anilide structure. The branched hydrocarbon may have various groups attached including hydroxyl groups, trifluoro groups and methyl groups attached (Jeffery et al., 1991). Vinclozolin,

procymidone and iprodione (glycophene) are three fungicides with common antiandrogen activity (Figure 2). Flutamide and RU 23 208 are two drugs with antiandrogen activity (Figure 2).

All three of the pesticides cause adrenal enlargement and liver histopathology. The adrenal enlargement occurs at low dose levels and the liver histopathology occurs at the highest or two highest dose levels. All three induce liver carcinomas at high dose levels (at or above a MTD) in mice and Leydig cell hyperplasia at low dose levels at or close to the NOEL in rats.

Antiandrogens generally cause failure of the testosterone suppression of the pituitary release of gonadotropin output. In males the increased LH component (10 times normal) results in Leydig cell hyperplasia and increased testosterone production (3 times normal). Antiandrogens inhibit androgen receptor reaction and consequent stimulation of the male sex organs such as the prostate, seminal vesicles and epididymides resulting in lower organ weight (See Table 8). This inhibition of androgen receptor stimulation is the basis of the use of flutamide therapy and combination therapy for benign prostate hypertrophy, prostate and breast cancer, acne, female hirsutism and therapies reducing sex drive (Sciarra et al., 1990).

Flutamide causes increased LH and testosterone in humans[1], but the degree of increase is much less than in rats and no observations of hyperplasia of the Leydig cells have occurred in humans (Neuman, 1991). In one series in humans, testosterone levels reverted back to normal after a year of treatment (Rasmussen, 1990). Flutamide also causes liver and adrenal problems in some patients. There has been no comment in the literature found so far about cataracts or potential liver carcinoma from flutamide therapy.

Sperm production may or may not be affected. Inhibition of the androgen receptors in the testes may not be sufficient to overcome the increased testosterone output by the Leydig cells adjacent to the seminiferous tubules. The studies of the potential affect on sperm count and motility are held in reserve pending the results from the studies on these parameters being conducted with vinclozolin in rats by Dr. Gray and his group.

Procymidone did not cause ambiguous male sex development if dosed from day 6 to day 16 as required by the developmental guidelines (HED Doc.# 008180). However, procymidone (HED Doc.# 010048) and vinclozolin (HED Doc.# 007909) did cause ambiguous male sex development when rats were dosed from implantation through day 19 of gestation (HED Doc.# 010048 and 007909).[2] It

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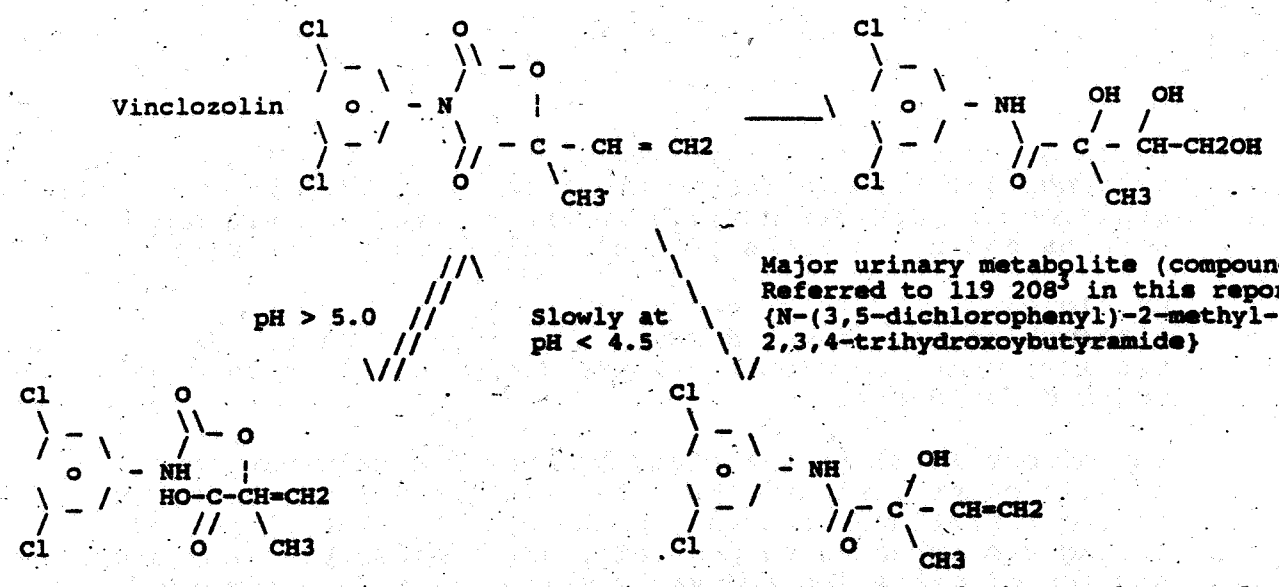
[1] Flutamide is used in treatment of prostate cancer to help prevent the effects of the initial testosterone surge induced by the LHRH analogues which eventually down regulate pituitary hormones and result in decreased testosterone levels.

[2] The current pesticide guidelines are inadequate to test for anti-androgens. It is noteworthy that revisions in the 1978 guidelines included dosing up to day 19-20 of gestation in the rat; these requirements were inexplicably removed before these guidelines went final in 1984. Revisions in the 1993-94 developmental testing guidelines may address these problems.

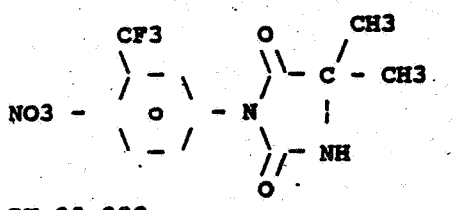
is also interesting that male nipple development was not seen at low dose levels in either of the studies conducted for the registrant(s) with procymidone or vinclozolin; however, when rats were dosed up to day 4 of lactation, vinclozolin may have caused development of nipples in males at 6 and/or 3 mg/kg/day (E Gray, 1993). Testing in additional rats did not confirm the nipple development in perinatal rats.

Figure 2: Analogues: The major metabolite, two major decomposition products of vinclozolin and four other chemicals demonstrating antiandrogenicity (two drugs and two pesticides).

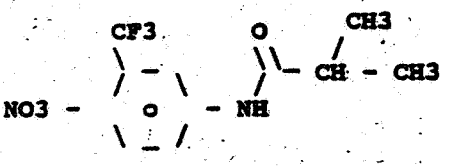


M1 reversible hydrolysis product {2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid}; forms rapidly at alkaline pH > 8.0, Szeto et al., 1989. Also reg no. 119 208& BF 352-22.

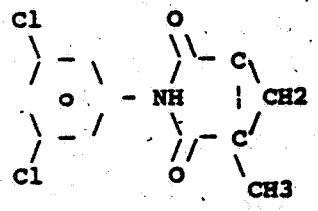
Irreversible degradation product, referred to as Compound 23 {3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide} in the metabolism study and M2 by Szeto et al., 1989.



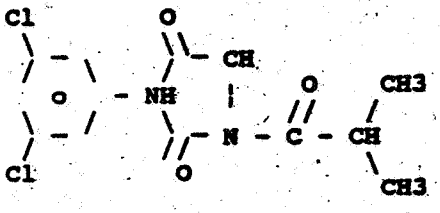
RU 23 908



Flutamide



Procymidone; [N-(3,5-dichlrophenyl)-1,2-dimethyl-cyclopropane-1,2-dicarboximide].



Iprodione, Glycophene, Anfor, BSI, Chipco and Rovral; [3-(3,5-dichlorophenyl)-N-(1-imidazolidinecarboximide].

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3 Substance 119 208 has also been referred to as 352-22 in some reports from BASF.

J. Weight of the Evidence Determination

The Committee considered the following facts regarding the toxicology of vinclozolin to be of importance in a weight of the evidence determination of its potential to cause developmental or reproductive toxicity.

1. Decreased anogenital distance in rats, and other antiandrogen effects due to treatment with vinclozolin were reported in multiple studies in the two rat and two mouse strains and by both the oral and dermal routes in rats.

2. Developmental toxicity occurred in rats and rabbits. Although no anogenital distance decreases occurred in rabbits, other developmental effects occurred at maternally toxic dose levels.

3. The effect level in the study by Gray was markedly lower than in other oral studies. This difference was attributed to two differences in study protocol: 1) The period of dosing was extended to postnatal day 3 by Gray. The later portion of gestation and early postnatal period appears to be critical to the development of vinclozolin mediated developmental effects. 2) The earlier studies used 0.5% CMC in water as the vehicle for dosing; Gray used corn oil as the vehicle. Differences in absorption due to vehicle may influence the severity of the effects observed.

4. Reproductive effects in a rat reproductive toxicity study occurred at doses ≥ 30 mg/kg/day. The LOEL was based, in part, upon decreased epididymal weights. In the same study, fertility was decreased in the highest two doses tested (≈ 96.5 and 286 mg/kg/day for males; ≈ 101.2 and 292 mg/kg/day for females), although the cause could not be determined from the study report. In the same study, pseudohermaphrodism was reported at the two highest doses.

5. In a one-year dog study, males given vinclozolin at doses ≥ 4.8 mg/kg/day exhibited increased relative testes weights and atrophy of the prostate (MRID 408595-01, 411229-01).

6. Vinclozolin binds weakly to the androgen receptor from epididymal tissue. However, two vinclozolin metabolites are effective androgen binding receptor antagonists. They may occur at sufficient quantities *in vivo* to interfere with binding to the androgen receptor of exposed humans, resulting in antiandrogenic effects. (The relative dissociation constant for the most active metabolite, M2, relative to R1881 is about one-half that of flutamide, an antiandrogenic drug used in the treatment of benign prostate hypertrophy.)

7. Vinclozolin is sturcturally related to other compounds exhibiting antiandrogenic activity. In particular,

procymidone, another pesticide, causes reduced anogenital distance in rats.

K. Classification

The PRC concluded that developmental and reproductive toxicity were induced in rats and rabbits following oral administration of vinclozolin. In rats, no NOEL for developmental toxicity (oral route) was determined (gavage, corn oil, postcoital day 14 to postnatal day 3) was identified. The LOEL was 3 mg/kg/day based upon reduced anogenital distance. This endpoint was believed to be close to a NOEL. The NOEL for developmental toxicity in rabbits (gavage, 0.5% CMC in water, gestational day 6 through 28) was 200 mg/kg/day. The LOEL in rabbits was 400 mg/kg/day based upon early resorptions, fetal weight increase, decreased live litter size and possible increased skeletal anomalies. The maternal NOEL in rabbits was 50 mg/kg/day with an LOEL of 200 mg/kg/day based upon increased absolute and relative liver weights, reduced defecation and reddish-brown urine. Reproductive effects in rats were decreased epididymal weights and lenticular degeneration at 30 mg/kg/day, with an NOEL of 4.9 mg/kg/day. The parental effect level was 30 mg/kg/day based upon decreased epididymal weights, with an NOEL of 4.9 mg/kg/day.

The critical effect level for risk assessments derived from a developmental toxicity study is 3 mg/kg/day (lowest dose tested) based upon decreased anogenital distance in rats at that dose.

Summary Table of the Toxicity Evidence.

Study	Developmental/Offspring NOEL/LEL in mg/kg/day	Maternal/Parental NOEL/LEL in mg/kg/day
Rat Gavage Developmental Study #1: (HED Doc# 007909 and 008556)	15/50 - Decreased anogenital distance.	<600/600 - Increases in absolute adrenal and liver wt.
Rat Dermal Developmental Study #2 (HED Doc# 007870 & 008556)	60/180 - Decreased anogenital distance.	60/180 - Increased absolute adrenal wt.
Rat Feeding Developmental Study #3 (HED Doc# 007228)	23/111 - Decreased anogenital distance.	< 23/ 23 - Increased adrenal wt.
Rabbit Gavage Developmental Study #4 (HED Doc# 008311)	200/400 - Resorptions, fetal wt. decrease, reduced litter size.	50/200 - Increased absolute adrenal and liver wt.
Rat Reproduction Study #5 (HED Doc# 010380)	4.9/30 - Epididymal wt decrease, lenticular degeneration and other effects.	4.9/30 - epididymal wt. decrease and other effects.
Older studies conducted prior to 1981 (Not used because Accession/MRID# are unknown and/or reports are unavailable and DERs contain little information).		
Mouse Developmental Toxicity Dietary Study (HED Doc# 00244)	90/900 - No implantation sites	-
Rabbit Developmental Toxicity Study (HED Doc# 002409)	80/300 - Post implantation loss	300/>300
Rat Study on 3-Generations of Reproduction (HED Doc# 000244)	77.3/>77.3 - No effects.	77.3/>77.3 - No effects.

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Knuppen, R. (Orig. Germ. 4/9/91) Interim report: Study of possible binding of Reg. No. 83 258 (vinclozolin), Reg. No. 119 208 (metabolite BF 352-22) to the androgen and glucocorticoid receptors in the cytosol from MCF-7 cells and from prostate and liver tissues of the rat (Proj. No. 21B0375/889033); Oct. 13, 1992 (original German report: April 9, 1991). Conducted at the Medical University of Lübeck, D-W-2400 Lübeck, FRG. Reg. Doc. No. BASF 92/11228.

Knuppen, R. (Orig. Germ. 11/27/91) Study of possible binding of Reg. No. 83 258 (vinclozolin), Reg. No. 119 208 (metabolite BF 352-22) to the androgen and glucocorticoid receptors in the cytosol from MCF-7 cells and from prostate and liver tissues of the rat (Proj. No. 21B0375/889033); Oct. 13, 1992 (original German report: Nov. 27, 1991). Conducted at the Medical University of Lübeck, D-W-2400 Lübeck, FRG. Reg. Doc. No. BASF 92/11229.

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