ATTACHMENT I

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Vinclozolin (2nd)

FROM: David Anderson, Ph.D. 9/5/96
Review Section III
Toxicology Branch I
Health Effects Division (7509C)
and
Esther Rinde, Ph.D. 9/5/96
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

TO: Connie Welch PM 21
Fungicide/Herbicide Branch
Registration Division (7505C)
and
Bruce Sidwell PM 72
Accelerated Reregistration Branch
Special Review and Reregistration Division (7508W)

THROUGH: Stephanie R. Irene Ph.D. 9/18/96
Acting Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on August 30, 1995 and April 17, 1996 to discuss and evaluate the weight-of-the-evidence on vinclozolin with particular reference to its carcinogenic potential. The majority of the CPRC agreed that vinclozolin should be classified as a Group B2 - probable human carcinogen - and that for the purpose of risk characterization, a non-linear approach - Margin of Exposure (MOE) should be used for quantitation of human risk. This classification of Group B2 was based on statistically significant increases in multiple tumor types in male Wistar rats and ovarian tumors in female Wistar rats at a dose which was not excessive. The MOE approach was chosen because the tumors were benign at dose levels which were not considered to be excessive, and there was little concern for mutagenicity of vinclozolin. Mechanistic data for the Leydig cell tumors also provided further support for the use of the MOE approach.

SUMMARY

1. Administration of vinclozolin in the diet to C57 mice resulted in statistically significant increases in liver tumors in both sexes of the C57 mouse only at a dose which was excessively toxic, based on body weight gain reductions of >>15% and statistically significant increased mortality. Because of the excessive dosing, the CPRC questioned the relevancy of the hepatocellular tumors in the mice.

2. Administration of vinclozolin in the diet to Wistar rats in a carcinogenicity study resulted in statistically significant increases in testicular Leydig cell and prostate adenomas in male rats and ovarian adenomas in female rats at both the mid- and highest doses (500 and 3000 ppm, respectively). Statistically significant increases in ovarian sex cord stromal tumors (benign) occurred at the highest dose only; however, there were also increases at the low and mid-doses with borderline significance (p=0.053) at the mid-dose. Statistically significant increases in uterine adenocarcinomas and adrenal cortical tumors in female rats were found at the highest dose only.

The majority of the CPRC considered the 3000 ppm dose to be excessively toxic in both sexes, based on weight gain depressions >>15% and other histological signs. The CPRC agreed that the next highest dose (500 ppm) was adequate for assessing the carcinogenic potential of vinclozolin in both sexes and considered the tumors which occurred at that dose with statistically significant increases to be relevant: Leydig cell and prostate adenomas in male rats and ovarian sex cord stromal benign tumors in female rats.

3. In a chronic study with Wistar rats (which used only 20 animals/sex/dose); (the carcinogenicity study used 50 animals/sex/dose), male rats administered vinclozolin had a statistically significant increase in hepatocellular carcinomas at the highest dose (4500 ppm) and a statistically significant positive trend. Male rats also had statistically significant increases in benign testicular Leydig cell tumors at 500, 1500 and 4500 ppm (p<.01 at the two 2 top doses) and a statistically significant positive trend. Female rats administered vinclozolin in this chronic study had statistically significant increases in adrenal combined adenoma/carcinoma and ovarian sex cord (benign) "tumors" at the highest dose (4500 ppm) and there were statistically significant positive trends (p<.01) for both tumor types.

The CPRC concluded that the results from this chronic study were consistent with that in the carcinogenicity study, in terms of the Leydig cell and ovarian sex cord tumors (however not for the prostate or uterine tumors) and signs of toxicity (body weight gain reductions >>15% and histological signs). The highest dose (4500 ppm) was considered to be excessively toxic, and the next highest

dose (1500 ppm) was considered to be adequate (not excessively toxic).

Vinclozolin was tested in a variety of mutagenicity assays and found to be mostly negative. One in vitro study in the mouse lymphoma assay with activation (negative without activation) was considered uncertain by the sponsor, but positive by the OPP reviewer. Overall there is little concern for mutagenicity for vinclozolin.

Vinclozolin is structurally related to chlorpropham, procymidone, iprodione and linuron, which are also associated with testicular Leydig cell tumors in rats.

The evidence from humans taking pharmacologic doses of antiandrogenic drugs (cyproterone acetate and flutamide) was discussed. The CPRC concluded that the association of liver cancer in humans with the therapeutic use of these drugs could not be determined from references in the literature. In addition, a study of vinclozolin workers (67) was conducted (physical examination, ultrasonography, endocrine studies were conducted) by the registrant which suggested that no statistically significant endocrine or other effects were noted in these men exposed to vinclozolin at 0.0067 to 0.134 mg/kg/day for 1 to 13 years. According to the registrant, this study should have been adequate to detect hormone disturbances, if they had occurred at these exposure levels. The study is inadequate to determine effects from life-time exposure.

The classification of Group B2 was based on the multiple tumors seen in the rat (benign testicular Leydig cell and prostate adenomas in males, and benign ovarian sex cord stromal tumors in females) even at a dose which was not excessively toxic. Hormonal disruption as a basis for the Leydig cell tumors was considered, and the CPRC agreed that a mode of action (antiandrogenic) for these tumors in rodents appears to have been demonstrated. The consensus of the CPRC was that the relevance of Leydig cell tumors to humans could not be dismissed. The CPRC also agreed that hormonal mechanisms for prostate tumors and ovarian sex cord stromal tumors have not yet been developed. There are several chemicals structurally related to vinclozolin also associated with Leydig cell tumors in rats.

The tumors were mainly benign and there was little concern for mutagenicity of vinclozolin; therefore, a non-linear approach (MOE) was recommended for estimating risk.

A. Individuals in Attendance at one or both meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene _____

William Burnam _____

Karl Baetcke _____

Elizabeth Doyle _____

Hugh Pettigrew _____

Esther Rinde _____

Kerry Dearfield _____

Richard Hill _____

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

David Anderson[1] _____

Karen Hamernik _____

Lori Brunsman _____

Lucas Brennecke[2] (PAI/ORNL) _____

3. Other Attendees:

Bernice Fisher, Byong Chin, Linnea Hansen, Kathryn Boyle, Karen Whitby and Roger Gardner, Kit Farwell (HED)

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[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report

B. Material Reviewed:

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. David Anderson, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information:

Vinclozolin is a fungicide used on various flowers, vegetables and strawberries, raspberries and stone fruit to control molds. Synonyms include Ronilan™, Oronalin™, BAS 35202-F™ and BAS 35204-F™. The chemical name is 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione and the structure illustrated in Figure 1.

LIST B
Case Number: 2740
Chemical Number: 113201
CAS Reg. Number: 50471-44-8
Chemical Name: Vinclozolin

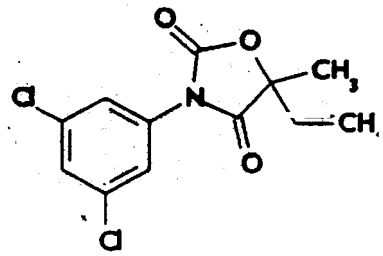


Figure 1
Vinclozolin

D. Evaluation of Carcinogenic Evidence:

1. Carcinogenicity Study in C57BL/6/JICO Mice:

Reference: Mellert , W : "Vinclozolin, technical; 18-month oral feeding oncogenicity study in mice.", May 4, 1994. MRID# 432547-04, Lab. Study# 80S0375/88112. Testing facility BASF Akiengesellenschaft Crop Protection, Product Safety, Dept. Toxicology, Ludwigshafen/ Rhein, Germany.

a. Experimental Design

In a 18-month oncogenicity feeding study, vinclozolin was administered in the diet to 50 male and 50 female C57BL/6/JICO mice

per group at 0, 15, 150, 3000, or 8000 ppm. Groups of 10 animals per sex were added and sacrificed after 12 months. The doses corresponded to average doses of about 0, 2.1, 20.6, 432, and 1225 mg/kg/day for males; and to 0, 2.8, 28.5, 557, and 1411 mg/kg/day for females. Two control groups each containing 50 mice per sex for the main study and 10 mice per sex for the satellite study were maintained to obtain more historical data for this strain of mouse. The treated groups were compared to each control group and, in some cases, to the combined control groups.

b. Discussion of Tumor Data

The incidence of hepatocellular carcinomas was significantly increased in females ($p < 0.001$) at 8000 ppm (22/50) when compared to either control group, 0/50 in control group 0 and 2/50 in control group 1. Most of the carcinomas (17/26) were observed at terminal sacrifice. Only one female developed hepatocellular carcinoma at 8000 ppm in the 12-month satellite study. In surviving males, the incidence of hepatocellular carcinomas was 3/20, $p \leq 0.05$, at 8000 ppm and 0/90 in combined controls.

Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls, for hepatocellular carcinomas, and hemangiomas and/or hemangiosarcomas combined, all at $p < 0.01$ (Table 1). Male mice also had a significant increasing trend, and a significant difference in the pair-wise comparison of the 8000 ppm dose group with the controls, for hepatocellular hemangiomas at $p < 0.05$. There was a significant difference in the pair-wise comparison of the 8000 ppm dose group with the controls for hepatocellular hemangiosarcomas at $p < 0.05$ (Table 1).

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls, for hepatocellular carcinomas, and adenomas and/or carcinomas combined, all at $p < 0.01$ (Table 2). There were also significant increasing trends in hepatocellular adenomas and hemangiosarcomas at $p < 0.01$, and significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls for these same tumors at $p < 0.05$ (Table 2).

The statistical analyses for the mice were based upon Peto's Prevalence Test since there were statistically significant positive trends in male and female mice for mortality with increasing doses of Vinclozolin.

Historical Control Data

Historical control data were not submitted nor requested

Table 1. Vinclozolin - C57BL/6/JICO Mouse Study

Male Liver Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	15	150	3000	8000
Carcinoma (%)	0/92 (0)	0/41 (0)	0/40 (0)	1[a]/42 (2)	3/20 (15)
p =	0.000**		-	0.282	0.000**
Hemangioma (%)	2/95 (2)	0/42 (0)	1[b]/42 (2)	1/42 (2)	2/22 (9)
p =	0.042*		0.502	0.453	0.047*
Hemangio-sarcoma (%)	0/93 (0)	0/41 (0)	1[c]/41 (2)	2/42 (5)	1/21 (5)
p =	0.062		0.079	0.051	0.017*
Hemangioma and/or Hemangiosarcoma Combined (%)	2/95 (2)	0/42 (0)	2/42 (5)	3/42 (7)	3/22 (14)
p =	0.007**		0.250	0.124	0.007**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First carcinoma observed at week 71, dose 3000 ppm.

[b]First hemangioma observed at week 62, dose 150 ppm.

[c]First hemangiosarcoma observed at week 68, dose 150 ppm

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Vinclozolin - C57BL/6/JICO Mouse Study

Female Liver Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	15	150	3000	8000
Hemangio-sarcoma	0/92	0/47	0/47	0/40	2[a]/30
(%)	(0)	(0)	(0)	(0)	(7)
p =	0.008**				0.039
Adenoma	0/99	0/48	0/50	0/43	5[b]/43
(%)	(0)	(0)	(0)	(0)	(12)
p =	0.003**				0.029
Carcinoma	2[c]/96	0/47	0/47	0/41	22/33
(%)	(2)	(0)	(0)	(0)	(67)
p =	0.000**				0.000**
Adenoma and/or Carcinoma Combined	2/99	0/48	0/50	0/43	27/43
(%)	(2)	(0)	(0)	(0)	(63)
p =	0.000**				0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First hemangiosarcoma observed at week 77, dose 8000 ppm.
[b]First adenoma observed at week 38, dose 8000 ppm.
[c]First carcinoma observed at week 53, dose 8000 ppm, in an interim sacrifice animal. Second carcinoma observed at week 64, dose 0 ppm, in an animal that died on study.

Note: One animal in the 8000 ppm dose group of the interim sacrifice group had an hepatocellular carcinoma. Interim sacrifice animals are not included in this analysis.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Non-neoplastic Lesions

The most commonly reported effect in premature decedents was erosion and ulceration of the glandular stomach (highest in the 8000 ppm group) that occurred in all groups including the controls. Increased incidences of focal necrosis, pigment storage and bile duct proliferation in the liver were seen at 3000 ppm in both sexes. These findings at the 8000 ppm dose were increased in incidence and severity; additionally, increased incidences of basophilic foci, focal hyperplasia, and cellular alterations were also reported. Male and female absolute and relative liver, kidney, brain and adrenal organ weights were increased at 3000 and 8000 ppm, but the increase was frequently equivocal at 15 and 150 ppm. The absolute and relative adrenal weights were significantly ($p<0.01$) increased in both sexes at 3000 and 8000 ppm. An increased incidence of lipoidosis in the adrenal cortex and testicular Leydig cell hyperplasia, seminal vesicle, epididymis, prostate and uterine atrophy and/or reduced size were seen at 3000 and 8000 ppm and at 8000 ppm ovarian stromal hyperplasia and absence of follicles were seen. An increase in polymorphonuclear neutrophils and monocytes, and a decrease in the percent of lymphocytes were seen at 3000 and 8000 ppm. These hematological changes, however, were seen primarily at 8000 ppm, are close to the normal range of values for mice.

The Lowest-Effect-Level (LEL) of 150 ppm (20.6 mg/kg/day for males; 28.5 mg/kg/day for females was based on significantly decreased mean terminal body weight gains (decrements were greater than 10% of control values). A No-Observable- Effect-Level (NOEL) of 15 ppm (2.1 mg/kg/day for males; 2.8 mg/kg/day for females) was identified.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dosing at 8000 ppm was excessive and even the dosing at 3000 ppm may have been in excess of that required for testing for carcinogenic potential.

Mortality at 8000 ppm was 64% in males ($p<0.01$) and 52% in females ($p<0.01$) when compared with the combined control group (combined control group of 101 mice/sex showed 11% mortality for each sex). Mortality of males in the 18 month study at 3000 ppm was increased significantly when compared to the combined control groups (24%, $p \leq 0.05$) and in females it was 25%, $p \leq 0.01$, compared with respective combined controls. In addition, based on Petro's Prevalence Test, male and female mice showed a positive trend ($p \leq 0.01$ for both) for mortality with increasing dose levels of vinclozolin.

Decreased body weight and gain was seen in both sexes at all dose levels, and the mean weights were significantly less than control animals from the first week of the experiment at the 8000 ppm dose. At termination and 0, 15, 150 3000 or 8000 ppm dose levels, respectively, male body weight gain was 100%, 67%, 79%, 56% and 19% and female body weight gain was 100%, 85, 56%, 61% and 33% of control. The decreased body weight gain was accompanied by

decreased food intake especially at the beginning of the study; however, a decrease in overall food efficiency was seen, especially at the 2 highest doses. Food efficiency at 0, 15, 150, 3000 or 8000 ppm was 0.375, 0.272, 0.315, 0.229 and 0.082, respectively for males and 0.273, 0.252, 0.166, 0.190 and 0.120, respectively for females.

In addition, increased liver focal necrosis, bile duct proliferation, pigment storage and lipidosis of the adrenal cortex were seen in males and females at 3000 and 8000 ppm. Other possibly endocrine related histological findings in addition to testicular Leydig cell hyperplasia occurred at these 2 dose levels.

2. Carcinogenicity Study in Wistar Rats

Reference: Mellert, W.: "Vinclozolin, technical; 24-month oral feeding carcinogenicity study in rats." May 2, 1994. MRID# 43254703. Lab Study# 71S0375/88105. Testing facility BASF Akiengesellenschaft Crop Protection, Product Safety, Dept. Toxicology, Ludwigshafen/ Rhein, Germany.

a. Experimental Design

In this carcinogenicity toxicity study, groups of 50 male and 50 female Wistar rats were administered 0, 50, 500, or 3000 ppm (0, 2.3, 23, or 143 mg/kg/day, respectively, for males and 0, 3.0, 30, or 180 mg/kg/day, respectively, for females) of vinclozolin in their diets for 104 weeks.

b. Discussion of Tumor Data

Male rats had significant increasing trends, as well as significant differences in the pair-wise comparisons of the 500 and 3000 ppm dose groups with the controls, for benign and benign and/or malignant testicular Leydig cell tumors combined, all at $p < 0.01$ (Table 3). There was also a significant increasing trend in liver adenomas at $p < 0.05$ (Table 4). There were significant differences in the pair-wise comparisons of the 500 and 3000 ppm dose groups with the controls for prostate adenomas, with $p < 0.01$ at 500 ppm and $p < 0.05$ at 3000 ppm (Table 5).

Female rats had significant increasing trends, in addition to significant differences in the pair-wise comparisons of the 3000 ppm dose group with the controls, for adrenal cortical adenomas and adenomas and/or carcinomas combined and benign ovarian sex cord stromal tumors, all at $p < 0.01$ (Table 7). Female rats also had a significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 3000 ppm dose group with the controls at $p < 0.05$, for uterine adenocarcinomas (Table 7).

The statistical analyses of the male rats were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical analyses of the female rats were based upon Peto's prevalence test since there was a statistically significant negative trend in female rats for mortality with increasing doses of Vinclozolin.

Historical control tumor data for the Wistar rat are presented in Table 8.

Table 3. Vinclozolin - Wistar (Chbb:THOM (SPF Rat Study

Male Testicular Leydig Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	50	500	3000
Benign (%)	23[a]/48 (48)	25/49 (51)	47/50 (94)	48/50 (96)
p =	0.000	0.459	0.000**	0.000**
Malignant (%)	0/48 (0)	0/49 (0)	0/50 (0)	2[b]/50 (4)
	0.063	1.000	1.000	0.258
Combined (%)	23/48 (48)	25/49 (51)	47/50 (94)	49[c]/50 (98)
	0.000**	0.459	0.000**	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[a]First benign tumor observed at week 65, dose 0 ppm.

[b]First malignant tumor observed at week 102, dose 3000 ppm.

[c]One animal in the 3000 ppm dose group had both a benign and a malignant Leydig cell tumor.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If then $p < 0.05$. If ** then $p < 0.01$.

Table 4. Vinclozolin - Wistar (Chbb:THOM (SPF)) Rat Study

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	50	500	3000
Adenoma	0/48	1/49	1/50	3[a]/50
(%)	(0)	(2)	(2)	(6)
p =	0.046*	0.505	0.510	0.129
Carcinoma	1/48	1[b]/49	5/50	2/50
(%)	(2)	(2)	(10)	(4)
p =	0.421	0.747	0.112	0.516
Combined	1/48	2/49	6/50	5/50
(%)	(2)	(4)	(12)	(10)
p =	0.114	0.508	0.062	0.112

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[a]First adenoma observed at week 96, dose 3000 ppm.

[b]First carcinoma observed at week 71, dose 50 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Vinclozolin - Wistar (Chbb:THOM (SPF)) Rat Study

Male Prostate Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	50	500	3000
Adenoma[#]	0/48	3/49	7/50	5[a]/50
(%)	(0)	(6)	(14)	(10)
p =	0.146	0.125	0.007**	0.031*

[+]Number of tumor bearing animals/Number of animals examined excluding those that died before week 53.

[a]First adenoma observed at week 96, dose 3000 ppm.

[#]There were no carcinomas diagnosed.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Vinclozolin - Wistar (Chbb:THOM (SPF)) Rat Study

Female Adrenal Cortical Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	50	500	3000
Adenoma (%)	1/42 (2)	2/42 (5)	1/47 (2)	21[a]/48 (44)
p =	0.000**	0.427		0.000**
Carcinoma (%)	0/37 (0)	0/30 (0)	0/37 (0)	1[b]/42 (2)
p =	0.060	-		0.174
Combined (%)	1/42 (2)	2/42 (5)	1/47 (2)	22/48 (46)
p =	0.000**	0.427		0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 91, dose 3000 ppm.

[b]First carcinoma observed at week 105, dose 3000 ppm

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If * then $p < 0.05$. If ** then $p < 0.01$.

Table 7. Vinclozolin - Wistar (Chbb:THOM (SPF) Rat Study

Female Ovarian and Uterine Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	50	500	3000
Ovarian Benign Sex Cord Stromal Tumor (%)	4/39 (10)	7/36 (19)	10/45 (22)	29[a]/45 (64)
	0.000**	0.135	0.053	0.000**
Uterine Adenocarcinoma (%)	1/41 (2)	0/27 (0)	1/27 (4)	7[b]/47 (15)
	0.002**		0.441	0.026*

[+]Number of tumor bearing animals/Number of animals examined excluding those that died before observation of the first tumor.

[a]First ovarian benign sex cord stromal tumor observed at week 99, dose 3000 ppm.

[b]First uterine adenocarcinoma observed at week 97, dose 3000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$

Historical Control Data for the Wistar Rat

Historical control data from the testing laboratory on the Wistar rat and from RITA, a European data base for the Wistar rat. The means are presented along with the range as shown by the controls from the approximately 24 to 29 individual studies in the two data bases (Table 8).

Table 8. Historical Control Data - Data from 2-year studies in the Wistar rat conducted from 1984 to 1994 and submitted in support of the carcinogenicity study in rats.

TABLE 8. HISTORICAL CONTROL DATA FROM BASF; APPROX. 1250 WISTAR RATS/SEX IN APPROX. 29 2-YEAR STUDIES.				
Tumor type	Males		Females	
	Mean	Range	Mean	Range
Hepatocellular adenomas	8.3%	0 to 30%	2.8%	0 to 20%
Hepatocellular carcinomas	3.5%	0 to 10%	0.7%	0 to 5%
Adrenal cortical adenomas	1.8%	0 to 5.1%	2.7%	0 to 15%
Adrenal cortical adenocarcinomas	0.4%	0 to 2.0%	0.4%	0 to 4.0%
Prostate adenomas	1.0%	0 to 12%	-	-
Prostate carcinomas	0.24%	0 to 5%	-	-
DATA FROM HANOVER (RITA) DATA BASE (EUROPEAN); APPROX. 1300 WISTAR RATS/SEX IN 24 2-YEAR STUDIES.				
Testicular Leydig cell adenomas	17.7%	2.0 to 52.5%	-	-
Uterine adenocarcinomas	-	-	1.9%	0 to 10%
Ovarian sex cord, stromal, mixed, benign	-	-	0.4%	0 to 2.0%
Ovarian granulosa cell, benign	-	-	1.9%	0 to 5.2%
Ovarian granulosa cell, malignant	-	-	0.4%	0 to 2.0%

c. Non-neoplastic Lesions

Liver cellular hypertrophy was not seen in either male or female controls, but the incidence was increased in treated male (0/50, 2/50, 12/50*[3], 44/50*) and female rats (0/50, 0/50, 11/50*, 44/50*). Eosinophilic foci, which were not seen in male controls and in only one female control animal, showed a significantly increased incidence at all doses in males (0/50, 15/50*, 33/50*, 38/50*) and at the high dose in females (1/50, 0/50, 5/50, 38/50*).

[3]The asterisk (*) denotes statistical significance at $p \leq 0.05$ or 0.01.

Basophilic and clear cell foci showed dose related decreases. The incidence of biliary cysts was increased in males (4/50, 5/50, 11/50*, 15/50*) and females (8/50, 10/50, 20/50*, 36/50*). Foam cell aggregates in the lungs also showed a significantly increased incidence in male rats at all doses (14/50, 27/50*, 24/50*, 40/50*). The incidence of lenticular degeneration was significantly increased in female rats at all doses (9/50, 23/50*, 49/50*, 50/50*) and at the two highest doses in male rats (11/50, 15/50, 39/50*, 50/50*). This lesion occurred bilaterally in almost all animals receiving 500 and 3000 ppm of the test material. In addition, lenticular calcification (males: 0/50, 1/50, 4/50, 46/50*; females: 0/50, 0/50, 12/50*, 48/50*) occurred in almost all high-dose animals.

The absolute and relative weights of the testes were slightly decreased at 50 ppm and significantly increased ($p<0.01$) at 500 ppm (168 and 163%, respectively) and 3000 ppm (188 and 244%, respectively). Absolute and relative weights of the epididymides were depressed at all doses, but were significant ($p<0.01$) only at 500 ppm (66 and 68%, respectively) and 3000 ppm (45 and 63%, respectively). The adrenal glands weights were elevated at all doses, but were significant ($p<0.01$) at 3000 ppm (191 and 262%, respectively). In females rats, organ weights did not show a clear dose-response or the effects were not biologically or statistically significant, except for the liver weights and the relative weight of the ovaries. The absolute (129%) and relative weights (156%) of the liver were increased ($p<0.01$) at 3000 ppm compared with the control group; the effect appeared to be dose-related. The absolute (123 to 156%) and relative weights (121 to 190%) of the ovaries were increased at all three doses compared with the control group, but only relative weight at 3000 ppm was significant ($p<0.01$). The absolute weights of the adrenal gland fluctuated: decreased at 50 ppm (94% of control, N.S.) and 500 ppm (69%, $p<0.01$) and increased at 3000 ppm (117%, N.S.).

Vinclozolin had an effect on multiple organs including the eyes, testes and male accessory organs, ovaries, adrenal gland, liver, lungs, pancreas, kidneys (males only), and skeletal muscle in both sexes. The lesions discussed below are considered to be treatment-related. Incidences or numbers of animals developing lesions are listed in the following order: control, 50, 500, and 3000 ppm.

Testicular lesions showing increased incidences included interstitial edema (5/50, 6/50, 7/50, 12/50*), diffuse tubular atrophy (15/50, 14/50, 36/50*, 45/50*), tubular calcification (16/50, 20/50, 37/50*, 42/50*), cystic rete testis (3/50, 5/50, 3/50, 16/50*), and hyperplastic rete testis (1/15, 0/50, 0/50, 13/50*). There were decreases in the incidence of focal tubular atrophy (37/50, 29/50, 22/50*, 9/50*) and focal Leydig cell hyperplasia (36/50, 39/50, 34/50, 11/50*). Accompanying the testicular lesions were azoospermia and oligospermia in the epididymides (13/50, 14/50, 41/50*, 49/50*), which occurred bilaterally in most 500- and 3000-ppm animals and degenerative lesions in accessory organs. Atrophy was noted in the seminal vesicle (3/50, 5/50, 16/50*, 31/50*) and coagulation gland (3/50,

5/50, 16/50*, 30/50*). Prostate effects included reduced secretion (4/50, 7/50, 12/50*, 21/50*), interstitial fibrosis (4/50, 9/50, 16/50*, 31/50*), and focal hyperplasia (11/50, 17/50, 25/50*, and 20/50*). In female rats, statistically significant increased incidences of interstitial lipidosis in the ovaries occurred at all doses (2/50, 15/49*, 35/50*, 43/50*). There was also a dose-related increase in the severity of this lesion. Adrenal cortical lesions occurred in both sexes with incidences reaching statistical significance at 500 and 3000 ppm. Lipidosis occurred in males (1/50, 2/50, 33/50*, 50/50*) and females (0/50, 3/50, 12/50*, 50/50*) as did extra cortical nodules (males: 25/50, 32/50, 21/50, 40/50*; females: 19/50, 24/50, 40/50*, 45/50*). The occurrence of lipogenic pigment did not show an increase in incidence, which was very high in all groups, but an increase in the severity rating at 3000 ppm was noted for both sexes. There was a decrease in the incidence of cystic degeneration in the adrenal cortex of female rats (50/50, 48/50, 49/50, 13/50*).

Other organs showing dose related and statistically significant increased incidences of lesions at one or more doses are the pancreas of both sexes (vacuolated acinar cells, 500 and 3000 ppm, skeletal muscle (focal fiber atrophy: 3000 ppm in males, dose-related increase in females), and iliac lymph nodes in males (pigment storage: 500 and 3000 ppm).

Lesions showing statistically significant decreased incidences were basophilic foci (males and females, 500 and 500 ppm) in the liver, clear cell foci in the liver (males 3000 ppm; females, 3000 ppm), myocardial fibrosis (males, 3000 ppm; females, all doses), and cystic degeneration of the adrenal cortex (females, 3000 ppm).

The LEL for systemic toxicity is 50 ppm (2.3 mg/kg/day for males and 3 mg/kg/day for females) based on eosinophilic foci in the liver and foam cell aggregates in the lung of male rats and lenticular degeneration of the eyes and interstitial cell lipidosis in the ovaries of female rats. There is no corresponding NOEL, because the lowest dose tested is the LEL.

Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Vinclozolin in male rats. Female rats indicated a significant decreasing trend for mortality with increasing doses of Vinclozolin.

Body weight

The body weights of both sexes receiving 3000 ppm were significantly depressed throughout the study. The males weighed 25% less than the controls and females weighed 17% less. Net body weight gain (week 0 to week 104) showed a dose-related decrease that was statistically significant throughout the study in animals receiving 3000 ppm. At termination, the high-dose males had gained 34% less weight than controls and females had gained 27% less than control.

At the high-dose food consumption showed a significant

decrease ($p<0.01$, student's t-test) throughout the study, with decreases ranging from 12 to 16% in males and 8 to 16% in females during the second year.

The relative efficiency of food utilization over several time periods was calculated and differences were found for the last half of the study. A distinct decrease in relative efficiency of food utilization occurred in males (negative) and females (84% less than control) at 3000 ppm from study weeks 52 to termination. Decreases may have been seen in males (39%), but not in females at 500 ppm. Marginal decreases to no decreases in efficiency (especially in females) were seen at 3000 ppm in males and females during weeks 0 to 13, 13 to 52 and overall efficiency from weeks 0 to 102. These findings are similar to those seen in the 2-year chronic study in rats (MRID# 43254701), which were calculated on food efficiency per animal rather than per kg rat weight.

The decrease in relative efficiency of food utilization at 3000 ppm in males and females indicate that the body weight decrease may have been due, at least in part, to toxicity rather than decreased food consumption only.

Histological lesions

There were microscopic lesions in the liver, lungs, kidneys pancreas, eyes, adrenals and pigment storage in males at the 500 and 3000 ppm dose levels and liver, pancreas, heart, eyes and adrenals in females at the 500 and 3000 ppm dose levels.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

While it was noted that survival was enhanced in female rats at the highest dose (3000 ppm) relative to controls, and survival in males was comparable to that of controls, the majority of the CPRC considered this dose to be excessively toxic in both sexes, based on weight gain depressions >>15% and other clinical signs. The CPRC agreed that the next highest dose (500 ppm) was adequate for assessing the carcinogenic potential of vinclozolin in both sexes.

3. Chronic Feeding Study in Wistar Rats

Reference: Mellert , W : "Vinclozolin, technical; 24-month oral feeding chronic study in rats."(Dosed at 0, 150, 500,1500 or 4500 ppm), May 3, 1994. MRID# 432547-01, Lab. Study# 71S0375/88026. Testing facility BASF Akiengesellenschaft Crop Protection, Product Safety, Dept. Toxicology, Ludwigshafen/ Rhein, Germany.

and

Reference: Mellert, W.: "Vinclozolin, technical; Second 24-month oral feeding chronic study in rats."(Dosed at 0, 25 or 50 ppm), May 4, 1994. MRID# 432547-02, Lab. Study# 71S0375/88109. Testing facility BASF Akiengesellenschaft Crop Protection, Product Safety, Dept. Toxicology, Ludwigshafen/ Rhein, Germany.

a. Experimental Design

In a chronic toxicity study, groups of 20 male and 20 female Wistar rats were administered 0, 150, 500, 1500, or 4500 ppm (0, 7, 23, 71, or 221 mg/kg/day, respectively, for males and 0, 9, 29, 88, or 257 mg/kg/day, respectively, for females) of vinclozolin in their diets for 104 weeks.

A second chronic toxicity study was initiated because no NOEL was seen at 150 ppm in the above 4 dose group study. Groups on 20 male and female Wistar rats were administered at 0, 25 or 50 ppm ((0, 1.2 or 2.4 mg/kg/day for males and 0, 2.4 or 3.2 mg/kg/day for females, respectively) of vinclozolin in the diet for 104 weeks.

b. Discussion of Tumor Data

In males, hepatocellular carcinoma was statistically significantly increased in males at 4500 ppm by pair-wise comparison with control and in trend (Table 9). In addition, testicular Leydig cell tumors were statistically significantly increased by pair-wise comparison with control at 500, 1500 and 4500 ppm and for trend. In females, adrenal cortical adenoma(5/20)/carcinoma(1/20) and ovarian sex cord tumors were statistically significantly increased by pair-wise comparison with control at 4500 ppm and in trend (Table 10).

Leydig cell tumors (mostly benign) occurred in male rats at ≥ 500 ppm. Hepatocellular carcinomas at incidences of 0/20, 0/20, 1/20, 1/20, 9/20* occurred in controls, 150, 500, 1500, and 4500 ppm, respectively. Females had significantly increased incidences of adrenal cortical tumors (0/20, 0/20, 0/20, 1/20, 6/20*), benign sex cord tumors in the ovaries (0/20, 0/20, 2/20, 4/20*, 10/20*), and all ovarian tumors combined (4/20, 3/20, 4/20, 7/20, 11/20*). The incidences of pituitary adenomas in females rats (14/20, 12/20, 12/20, 6/18*, 5/20**) and mammary fibroadenomas (5/20, 5/9, 3/10, 6/9, 1/18) were decreased.

The incidences of pituitary adenomas in females rats (14/20, 12/20, 12/20, 6/18*, 5/20**) and mammary gland adenocarcinoma (7/50, 0/27, 3/23 and 0/49**) were decreased.

The induction of hepatocellular carcinomas was not confirmed in the carcinogenicity study (MRID No. 432547-03) where male rats fed 3000 ppm for 104 weeks did not develop hepatocellular carcinomas at a significantly increased incidence. Except for one male rat in the 1500 ppm group, hepatocellular carcinomas developed only in male rats fed 4500 ppm.

The second chronic two year study in Wistar rats was conducted at 25 and 50 ppm of vinclozolin to define a NOEL. The study showed no dose related effects. However, a statistically significantly (* = $p \leq 0.05$) increase at 50 ppm in thymoma in males (0/20, 2/7, 4*/20) and benign thyroid C-cell tumor in females (1/20, 4/20 and 6*/20) and benign/malignant C-cell tumor (1/20, 4/20 and 7*/20) in control, 25 and 50 ppm, respectively. Since no dose relationship in these tumor types were seen up to and including 4500 ppm in the

first chronic study, these tumors in the second chronic study were considered incidental and were not considered further.

Table 9: Vinclozolin - First Chronic (Cbb:THOM (SPF)) Rat Study (MRID# 43254701).
Tumor incidence (%)

Dose (ppm)	0	150 ppm	500 ppm	1500 ppm	4500 ppm
Tumor type (%)	Tumors in males				
Hepatocellular carcinoma (%)	0/20 (0)	0/20 (0)	1/20 (5)	1/20 (5)	9/20 (45)
p value[a]	0.000**	1.000	0.5000	0.5000	0.0006**
Testicular Leydig cell tumors (%)	11/20 (55)	12/20 (60)	17/20 (85)	19/20 (95)	20/20 (100)
p value[a]	0.0003**	0.5000	0.0412*	0.0042**	0.0006**
	Tumors in Females				
Adrenal cortical adenoma/carcinoma (%)	0/20 (0)	0/20 (0)	0/20 (0)	1/20 (5)	6/20 (30)
p value[a]	0.000**	1.000	1.000	0.5000	0.0101*
Benign ovarian sex cord tumors (%)	0/20 (0)	0/20 (0)	2/20 (10)	4/20 (20)	10/20 (50)
p value[a]	0.000**	1.000	0.2436	0.0530	0.0002**

[a] = Fisher's Exact Test/Cochran-Armitage trend test. Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. *, ** = Statistically significant at $p < 0.05$ and $p < 0.01$., respectively. Statistical analyses was conducted on the data from the chronic 2-year studies in Wistar rats (MRID# 43254701 & 43254702) by Lori Brunsman of the Statistics Section of HED at the request of David Anderson for inclusion into the CRPC document.

c. Non-neoplastic Lesions

Organ weight changes in male and female rats fed 4500 ppm of the test material included statistically significant increases in absolute and relative liver and adrenal weights. Increases in absolute and relative testes weights occurred at all doses, but relative weights were statistically significant at 4500 ppm (Tables of data not presented).

At various times during treatment bilateral lesions such as cataracts, and focal opacity were seen in both sexes receiving doses ≥ 500 ppm and in males at all dose levels. The incidences of lenticular microscopic lesions were significantly elevated in males at all doses and in females at doses ≥500 ppm. Serous fluid accumulation in the anterior chamber of the eyes was noted in males and females at 4500 ppm.

Microscopically, several lesions were statistically significantly increased in the testes and accessory organs; tubular calcification at all dose levels and diffuse tubular atrophy was increased at ≥ 500 ppm. There was a significant decrease in the incidence of focal Leydig cell hyperplasia at ≥ 1500 and a significant increase in hyperplasia in the rete testes at 4500 ppm. Microscopic lesions were noted in the epididymis (notably reduced size or atrophy and azoospermia/oligospermia at doses ≥500 ppm), seminal vesicle (reduced size or atrophy at doses ≥1500 ppm), coagulation gland (atrophy at doses ≥1500 ppm), and prostate (reduced size or reduced secretion at doses ≥500 ppm). In addition, interstitial fibrosis was a notable lesion in the prostate, showing a dose-related increase in incidence and severity at all dose levels. In female rats, a statistically significant increase in the incidence of interstitial lipidosis in the ovaries occurred at all doses. Test material related atrophy of skeletal muscle fibers was significant in males and females at 4500 ppm.

Increased incidences of lesions were seen at one or more dose levels in the liver of both sexes (cellular hypertrophy, single cell necrosis, and eosinophilic foci (1500 and 4500 ppm), kidney of male rats (urothelial hyperplasia, ≥500 ppm; renal pelvis calcification, 4500 ppm), lungs of both sexes (foam cell aggregates, 4500 ppm), pancreas of both sexes (vacuolated acinar cells, ≥500 ppm), skeletal muscle of both sexes (focal fiber atrophy, 4500 ppm), and adrenal gland of both sexes (lipidosis, ≥500 ppm for males and ≥1500 ppm for females, extra-cortical nodules, 4500 ppm).

Incidences of lesions showing significant **decreases** were clear cell foci in the liver (male and female, 4500 ppm), basophilic foci in the liver (females, 4500 ppm), interstitial nephritis (females, 4500 ppm), cystic degeneration of the adrenal cortex (females, ≥500 ppm), myocardial fibrosis (male, 4500 ppm; females, ≥1500 ppm), glandular cysts in mammary tissue (females, ≥1500 ppm), focal hyperplasia of the pituitary (males, ≥500 ppm), interstitial edema in the testes (males, ≥1500 ppm), and acinar concretions in the prostate (males, 4500 ppm).

The LEL for systemic toxicity is 150 ppm (7 mg/kg/day for males and 9 mg/kg/day for females) based on bilateral lenticular degeneration of the eyes, seminiferous tubular calcification in the testes, and interstitial fibrosis in the prostate of male rats and interstitial cell lipidosis in the ovaries of female rats. There is no corresponding NOEL, because the lowest dose tested is the LEL.

A NOEL was determined from the second chronic 2 year study rats (20 per sex per group) at 25 ppm (1.2M/1.6F mg/kg/day) with a LEL of 50 ppm in the oncogenicity study (MRID# 43254703) in 50 rats per sex per dose level and based on eosinophilic liver foci, lung foam cell aggregates, lenticular degeneration and intersitial cell lipidosis in ovaries (See RfD/QA Report on Vinclozolin, dated 01/30/96).

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Survival was not significantly affected in either male or female rats fed vinclozolin. Growth was markedly reduced in both sexes fed the test material. During the second year of treatment with 4500 ppm, male and female rats weighed 17-33% and 14-30%, respectively, less than controls. At termination body weight gain was reduced by about 45% in both sexes receiving 4500 ppm, due in part to reduced food consumption (Table 11). At termination and 1500 ppm, body weight gain in females was 76% ($p<0.01$) and in males 82% ($p>0.05$, N.S.) of control values.

The overall relative efficiency of food utilization from week 52 to 102 in males and females showed a biologically significant dose related decrease at 1500 and 4500 ppm. In control, 1500 and 4500 ppm, respectively, the values for males are 4.1, -0.03 and -3.3 and for females the values are 6.9, 4.5 and -0.2.

Water consumption (g/kg) appeared to be increased week 0-13, 0-52 and 0-102 in males and females. Water consumption from week 0-102 was increased at 1500 ppm in males (13%) and females (15%) and at 4500 ppm in males (50%) and females (17%).

In addition, liver cellular hypertrophy, altered liver eosinophilic foci, biliary cysts, vacuolated pancreatic acinar cells, eye lens degeneration and adrenal lipidosis occurred in males and females at 1500 and 4500 ppm. The liver enzyme, serum γ-glutamyltransferase (SGGT) was elevated at various intervals during the study in females and/or males and at termination at 1500 and 4500 ppm.

In addition, the level of liver P450 enzymes was about twice that in control Wistar rats after 3-months of treatment with vinclozolin at 4500 ppm (MRID# 427061-02, Study# 92/11548, 12/9/92). In the same study, female estrus cyclicity studies showed the cycles were short and long, showed continuous diestrus, continuous estrus and general disruption at 4500 ppm after 3-months of treatment.

The CPRC considered the highest dose (4500 ppm) to be excessively toxic, and the next highest dose (1500 ppm) was considered to be adequate (not excessively toxic).

E. Additional Toxicology Data on Vinclozolin:

1. Pharmacokinetics and 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 entero-hepatic 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-tributramide. 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.

2. Mutagenicity

All categories of mutagenicity studies have been conducted and are acceptable. Six of the seven studies conducted were negative for gene mutations, structural chromosomal aberrations and other genotoxic effects, including an *in vivo* study in hamsters for sister chromatid exchange. One *in vitro* study in the mouse lymphoma assay with activation was considered uncertain by the

sponsor, but considered positive at insoluble concentrations by the reviewer. The study was negative without activation.

(a) Salmonella assay (MRID# 00128319. HED Doc.# 003181) - Vinclozolin, technical was tested with the following strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100 as well as with E. coli WP2, with and without activation with a liver S9 preparation. The study was negative in all strains with and without activation at concentrations up to 3000 ug/plate.

The study is acceptable for a reverse gene mutation assay in Salmonella.

(b) Salmonella assay (MRID# 00156861, also 41471005. No HED Doc.# given) - negative up to concentrations of 10,000 ug/plate.

(c) Mutagenicity, forward mutation in mouse lymphoma L5178Y TK=/- 3.72 clone cells assay (MRID# 00147733, Study# E-431, 6/84, HED Doc.# 005054). Negative without activation, however, in the presence of activation, a reproducible increased mutation frequency with activation was seen at insoluble concentrations. The sponsor therefore considers this evidence of mutagenicity to be uncertain. The reviewer considered vinclozolin to be mutagenic in this study. The secondary reviewer considered there to be come sporadic increases with activation, but these were usually marginal and not consistent between 3 experiments at approximate toxicities >10% relative growth.

The study is acceptable for a gene mutation assay in mouse lymphoma cells.

(d) Mutagenicity in Chinese hamster ovary (CHO/HGPRT) forward gene mutation assay (MRID# 00156048, Study# 85/352, 10/85, HED Doc.# 005853) - Vinclozolin, technical was tested at cytotoxic concentrations with and without S9 activation, which resulted in no indication of mutagenicity with or without S9 activation.

The study is acceptable for a forward gene mutation in mammalian cells.

(e) Mutagenicity in mammalian cells in culture cytogenetic assay in Chinese hamster ovary cells (MRID# 41496902, Study# 10536-0-437, 31M0375/889128, 3/7/89,HED Doc.# 010774). The study was negative for chromosomal aberrations in Chinese hamster ovary cells at soluble and cytotoxic concentrations of vinclozolin.

The study is acceptable for chromosomal aberrations in mammalian cells in culture.

(f) Mutagenicity studies *in vitro* rat hepatocyte unscheduled DNA synthesis (HPC-UDS) assay (MRID# 00147732, Study# 20991, 1/84, HED Doc.# 005054). The study was negative for UDS up to cytotoxic concentrations.

The study is acceptable for a DNA repair study.

(g) Mutagenicity in Chinese hamsters, an *in vivo* study for sister chromatid exchange (MRID# 00128318, Study# 16M0232/8013, 12/81, HED

Doc.# 003181). The study was negative for inducing sister chromatid exchange up to a toxic dose level of 5260 mg/kg in bone marrow. Piloerection, irregular respiration and atony were observed.

The study is acceptable for sister chromatid exchange.

(h) Mutagenicity in *Bacillus subtilis* for DNA repair (ACC/MRID# 250114, Study# ?, 2/78, HED Doc.# 003181). The study was negative for DNA repair in strains M45 rec- and H17.

The study is unacceptable for DNA repair because the concentration of the test material was insufficient, no S9 activation was used and the number of organisms per strain was not standardized.

Other Studies:

1) B. Subtilis rec assay (MRID# 005156862. No HED Doc.# given) -negative to 10,000 ug/well.

2) Host mediated assay with Salmonella (MRID# 00128319. HED Doc.# 003181 - negative at 1000 mg/kg/day for 2 days.

3) Mouse dominant lethal (MRID# 00062646. HED Doc.# 000244) - negative at 2000 mg/kg/day for 5 days (noted no apparent toxicity at this currently accepted limit dose and no positive control).

3. Structure-Activity Relationships (In some cases only abstracts of references were reviewed. This is indicated at the citation.)

Several pesticides and drugs are especially relevant to vinclozolin because the parent compound and/or rat metabolites result in Leydig cell hyperplasia/tumors, ovarian tumors, adrenal hypertrophy at mid and low dose levels and liver carcinoma/ adenoma at high dose levels (some studies were conducted at or above a maximum tolerated dose (MTD)). The structures also have structural features in common and with other antiandrogen activity and inhibitors of enzymes converting steroids to testosterone and dihydro-testosterone and other mechanisms resulting in Leydig cell hyperplasia. 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 (Morris et al., 1993). Vinclozolin (Figure 2) and procymidone (Figure 4) are two fungicides with common antiandrogen activity. Flutamide/hydroxyflutamide (Figure 5 and 6) and RU 23 208 (Figure 14) are two drugs with antiandrogen activity (Neumann, 1991). Benzimidazole (Figure 8) is a potential drug that appears to inhibit cholesterol transfer to the cholesterol side chain cleaving enzyme, thus resulting lower serum testosterone levels (Fort et al., 1995). Iprodione or glycophene (Figure 3) also appears to lower androgen levels in treated rats (data in

review). All the above substances are negative in most mutagenicity studies.

Antiandrogens generally cause failure of the androgen suppression of the pituitary release of gonadotropin. In male rats, vinclozolin administration was associated with increased serum LH (10 times normal) and resulted in Leydig cell hyperplasia/tumors and increased testosterone production (nearly 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. This inhibition of androgen-receptor complex 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/hydroxyflutamide causes increased LH and testosterone in humans, but the degree of increase is much less than in rats and no problems with hyperplasia of the Leydig cells have occurred in humans (Neumann, F, 1991). In one series in humans, testosterone levels reverted back to normal after a year of treatment (Rasmussen, F, 1990). There has been no comment in the literature found so far about cataracts or adrenal hypertrophy from flutamide.

Flutamide was reported to cause severe liver damage. In a study of 1019 prostate cancer patients, severe liver damage was reported with an incidence of about 0.36% (Gomez et al., 1992, abstract), but it does not cause liver cancer in two year rat studies at an MTD of 50 mg/kg/day and its involvement in potential liver cancer in humans is undetermined. Methemoglobinemia has been reported in some humans at therapeutic dose levels of flutamide of ≈10 mg/kg/day [Since anilides can cause methemoglobinemia, this may indicate that the 4-nitro, 3-(trifluromethyl) anilide is a significant metabolite of flutamide in patients with methemoglobinemia]. Methemoglobinemia was not reported in chronic or carcinogenicity studies in rats treated with vinclozolin. However, reduced hematocrit, RBC count and hemoglobin concentration were reported in both rat sexes treated with vinclozolin at 80 and 239 mg/kg/day.

There is a possibility that antiandrogens may affect sperm production in humans at lower relative dose levels that in rats. Flutamide causes reduced spermatogenesis in rats at dose levels as low as ≈30 mg/kg/day for 52 weeks and in dogs at ≈14 mg/kg/day (product information insert for flutamide from Schering Co.). Reduced sperm counts were noted in a 6-week study in normal humans, presumably at therapeutic doses levels (≈10 mg/kg/day).

These findings are consistent with the hypothesis that testosterone synthesis by Leydig cells (possibly hyperplastic Leydig cells) in rats is greater than in humans resulting in relatively higher testosterone levels in rats. As a consequence, the Leydig cells adjacent to the seminiferous tubules in testes produce sufficient testosterone to overcome the flutamide

inhibition of the androgen receptors and secondarily subsequent sperm production. At the dose levels causing these effects, obvious androgen receptors and secondarily subsequent sperm production. At the dose levels causing these effects, obvious androgen deficiency occur in rat organs outside of the testes, such as the epididymides, prostates and seminal vesicles. In humans, a lower testosterone production and probably lower Leydig cell stimulation results in relatively low levels of testosterone in the internal environment of the testes and seminiferous tubules, thus, reducing sperm production to a greater degree than in rats at comparable dose levels. According to this hypothesis vinclozolin may also be associated with reduced sperm production in humans at lower dose levels than in rats. Vinclozolin caused reduced sperm in rats at ≥ 50 mg/kg/day in modified 1-generation studies (Gray Jr. et al., 1994).

Other antiandrogens such as cyproterone acetate (Figure 6) have been reported to cause liver cancer in humans at therapeutic dose levels (Drakos et al., 1992, abstract; Hirsch et al., 1994, abstract; Kattan et al., 1994, abstract; Ohri et al., 1991, abstract; Rudiger et al., 1995, abstract; Watanabe et al., 1994, abstract).

Vinclozolin - (Figure 2) Antiandrogen activity, 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione PC# 113201, Caswell# 323C. Vinclozolin results in testicular benign Leydig cell, liver and ovarian tumors in rats. Leydig cell hyperplasia/tumors and liver tumors are seen in mice at an excessively high dose level. Metabolites/degradation products (at least M1 and M2) binding to androgen receptors are probably responsible for antiandrogenic activity of vinclozolin. There is little concern for mutagenicity for vinclozolin. In older carcinogenicity studies (conducted prior to 1981) in the rat and mouse, vinclozolin was classified as a group E carcinogen; since then newer studies have been submitted, as cited in this document.

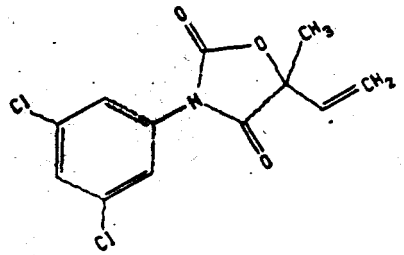


Figure 2 Vinclozolin

Iprodione (Figure 3) - Lowers androgen levels [3-(3,5-dichlorophenyl)-N-(1-imidazolidinecarboximide]
Iprodione (PC# 109801, Caswell# 470A) differs from vinclozolin in several ways as can be seen in figures 2 & 3. Iprodione has a substituted imidazole ring and vinclozolin has a substituted oxazolidine ring. Iprodione causes testicular Leydig cell tumors in rats and testicular Leydig cell and liver tumors in mice. Iprodione was positive for DNA repair in B. subtils., but negative in the Ames test, SCE test in CHO cells, forward mutation in mammalian cells, and chromosomal aberrations in CHO cells. Iprodione was classified as a Group B2 carcinogen by the CPRC.

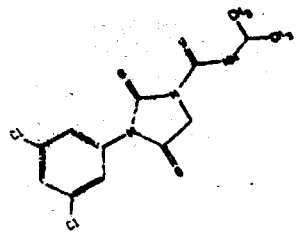


Figure 3 Iprodione

Procymidone (Figure 4) (PC# 129044, Caswell# 704J) - Antiandrogen activity - [N-(3,5-dichlorophenyl)-1,2-dimethyl-cyclopropane-1,2-dicarboximide].
Procymidone causes testicular Leydig cell and liver tumors in rats and binds androgen receptors, but was negative in a battery of mutagenicity tests. Procymidone (Figure 4) differs from vinclozolin in several ways as can be seen in figures 2 & 4. Procymidone was classified as a Group B2 carcinogen by the CPRC, however SAP on 11/30/90 considered it as Group C.

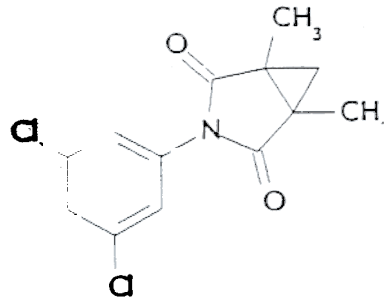


Figure 4: Procymidone

Flutamide (Figure 5) - Antiandrogen activity - **Hydroxyflutamide (Figure 6)**, a metabolite of flutamide is a close analog of the active metabolites of vinclozolin, differing by only by the phenyl ring substituents and the isobutanoate side chain. Flutamide/ hydroxyflutamide cause testicular Leydig cell tumors in rats and have been used to treat prostate cancer in humans [Labrie et al. (1990), Neumann (1991), Pavone-Macaluso et al. (1990), Petrangeli et al. (1988), Rasmussen (1990), Roberts et al. (1989) and The Merck Index (1989)].

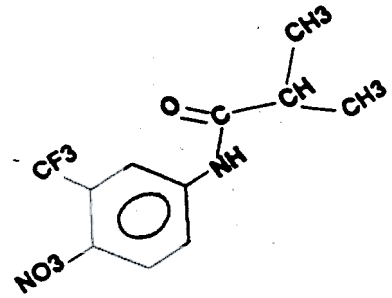


Figure 5 Flutamide

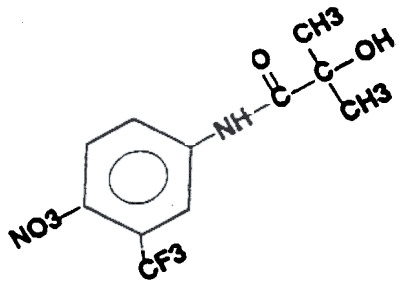


Figure 6: Hydroxy-flutamide

Cyproterone acetate (Figure 7) (CP) - is an steroidal antiandrogen that has been used in the treatment of prostate cancer and hypertrophy and hirsutism. CP is considered to be a promoter of liver carcinoma (Neumann et al., 1992, abstract). It is reported to cause liver carcinoma and other liver toxicity in rats (Neumann et al., 1992, abstract) and humans (Drakos et al., 1992, abstract; Hirsch et al., 1994, abstract; Kattan et al., 1994, abstract; Ohri et al., 1991, abstract; Rudiger et al., 1995, abstract; Watanabe et al., 1994, abstract). Bosland et al.(1990) reported that CP and testosterone propionate promoted dorsolateral prostate carcinomas in Wistar rats.

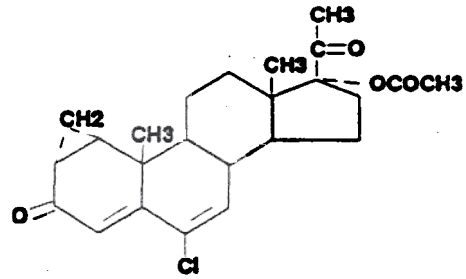


Figure 7 Cyproterone acetate

Cyproterone acetate was negative in the Ames or HGRT assay with V79 cells with or without liver microsomes, but positive for DNA repair/synthesis and formed DNA adducts in rat hepatocytes (Schering, 1993; Topinka et al., 1993, abstract).

Benzimidazole (Figure 8) (Lansoprazole) - is an inhibitor of cholesterol transfer to cholesterol side chain cleaving enzyme. Several rat metabolites of benzimidazole are much more active than the parent. Benzimidazole treatment results in testicular Leydig cell hyperplasia in two year rat studies. No other tumor data were reported in the paper reviewed (Fort et al., 1995). A battery of mutagenicity studies were reported to be negative (Fort et al., 1995).

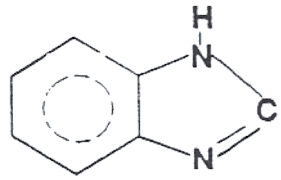


Figure 8
Benzimidazole
(Lansoproazole)

Linuron (Figure 9) (PC# 035506, Caswell# 528) differs from vinclozolin by the substituent on the carbanilate group and 1-methyl-1-oxymethyl urea side chain. Linuron causes Leydig cell adenomas in rats and hepatocellular adenomas in mice. Increased RBC destruction occurred in both studies which were acceptable. Linuron was negative for gene mutation in CHO cells, in the Ames test, for chromosomal aberrations and for unscheduled DNA synthesis. Linuron was classified as a Group C carcinogen with no Q* by the CPRC.

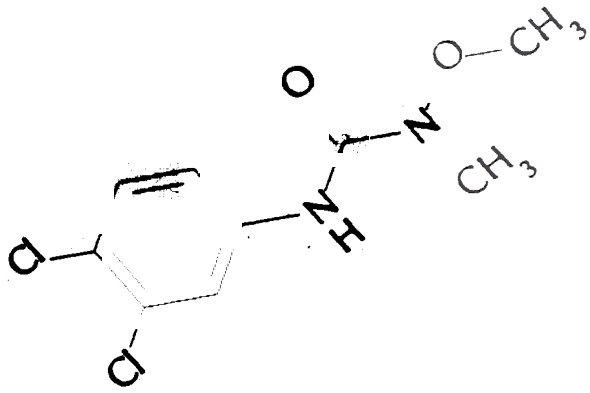


Figure 9: Linuron

Chlorpropham (Figure 10) (PC# 018301, Caswell# 510A) - Antiandrogenic activity unknown. Chlorpropham administration results in Leydig cell hyperplasia in rats, but not mice in two year studies. The pesticide was not mutagenic in a battery of mutagenicity assays. The CPRC classified Chlorpropham as group E.

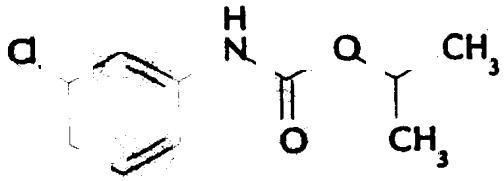


Figure 10: Chlorpropham

Propham (Figure 11) (PC# 047601, Caswell# 510) has no known antiandrogen activity and is only remotely structurally related to vinclozolin. It lacks 2 chloro groups on the benzene ring and differs in the side chain. There is no mutagenicity or acceptable chronic studies for this pesticide. An unacceptable two year rat study (reviewed in 1967) at 45, 135 and 450 mg/kg/day indicated that random subcutaneous fibromas or adenomas occurred during the study but that test animals did not differ from controls other than a "mild reduction in weight gain". No record could be found of a RfD/Peer Review or CPRC review of propham.

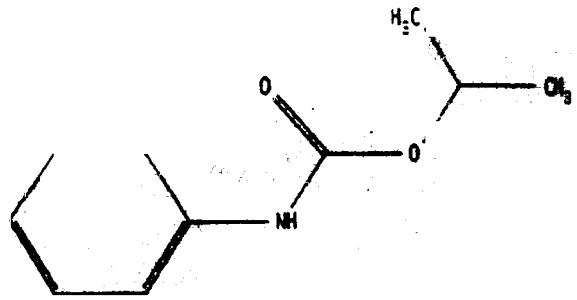


Figure 11: Propham

Fluometuron (Figure 12) (PC# 035503, Caswell# 460A) differs from vinclozolin by substitution of a meta trifluoro group for the a dichloro group on the phenyl ring and the dimethyl urea side chain. Fluometuron is negative for carcinogenicity in the rat and mouse according to the reviewer of the studies. Only the rat study was adequate. Fluometuron was negative in an Ames test, micronucleus test and for Unscheduled DNA synthesis, but it has not been reviewed by the CPRC.

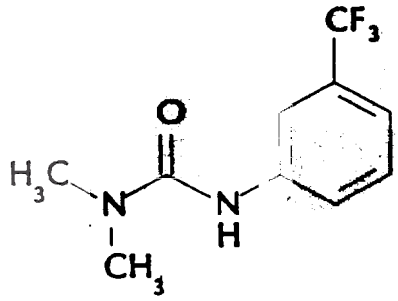


Figure 12: Fluometuron

Diuron (Figure 13) (PC# 035505, Caswell# 410) differs from vinclozolin by a dimethylurea side chain. As stated by the reviewer of the studies, both the rat and mouse studies are supplementary with the rat study showing an increase urinary bladder and renal pelvis epithelial papillomas and carcinomas at 250 ppm (HDT) and the mouse study showing increases in ovarian luteomas and mammary gland adenomas. Diuron was clastogenic in an in vitro cytogenetic assay, but negative for unscheduled DNA synthesis, in a HGPRT (CHO) assay and an Ames (S. typh.) assay, all acceptable. No report on the assessment of Diuron could be found from the CPRC nor the RfD/Peer Review Committee.

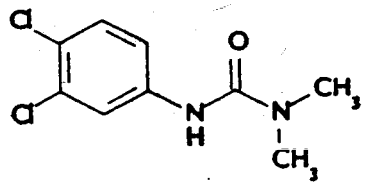
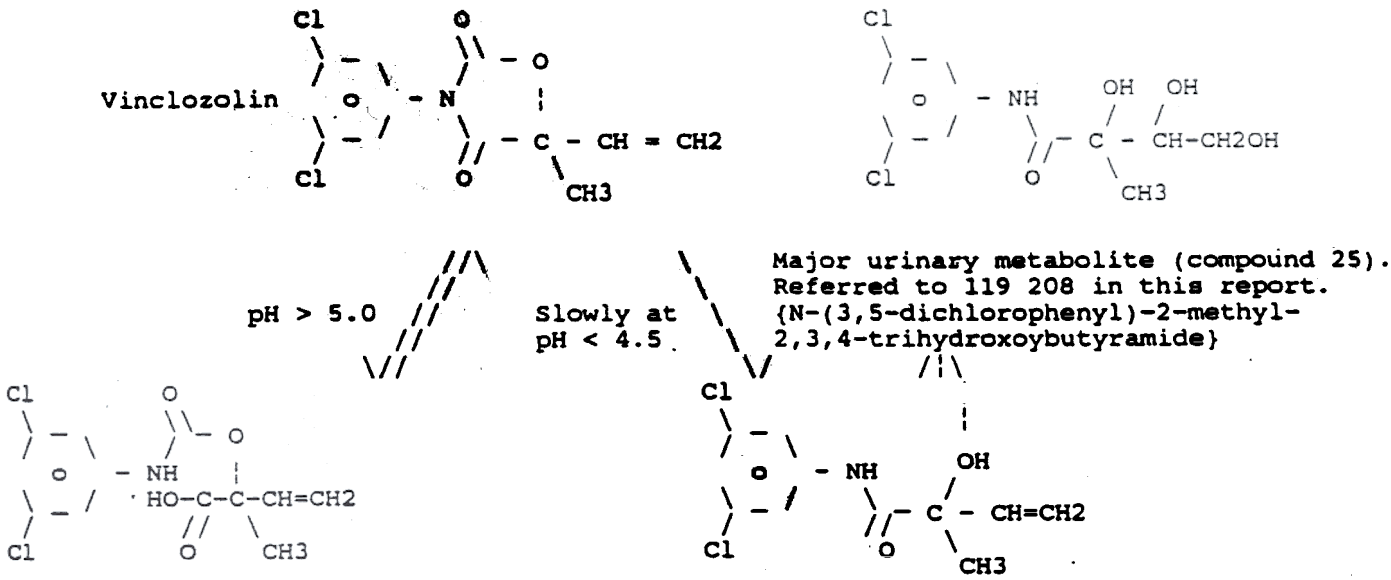


Figure 13: Diuron

Figure 14: Analogues: The major metabolite, two major decomposition products of vinclozolin

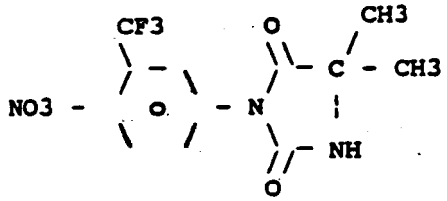


M1 reversible hydrolysis product {2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid}; forms rapidly at alkaline pH > 8.0, Szeto et al., 1989. Also reg no. 119 208& BF 352-22.

M1 binds to the androgen receptor and has antiandrogen activity. M1 has not been tested directly for mutagenicity or carcinogenicity.

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.

M2 is probably the most active antiandrogenic metabolite M2 of Vinclozolin. M2 has not been directly tested for mutagenicity or carcinogenicity.



RU 23 908 is another experimental antiandrogen

4. Acute, Subchronic and Chronic Toxicity Studies

Acute Toxicity (Data from 1-liner)

The oral and dermal LD50 are >10 g/kg in the rat and >2.0 g/kg in the rabbit, respectively. The acute LC50 for inhalation is 29.1 mg/l. It was considered moderately irritating to skin of rabbits and caused corneal opacity and conjunctivitis in rabbits. It was considered to be a potential sensitizer in guinea pigs. The skin sensitizing of vinclozolin was based on less pure test material than is currently being used.

(b) 90-Day Oral Feeding Toxicity in Rats (MRID# 42728801 & 42728802, Study# 31S0375/88034 & 31S0375/990110, 3/5/93 & 3/9/93, HED Doc.# 010999). Doses were 0, 50, (MRID# 42728802), 0, 300, 1000 or 3000 ppm (M: 0, 3.6, 20, 66 or 200 mg/kg/day; F: 0, 4.4, 24, 77 or 219 mg/kg/day.

At 300 ppm, females had an increased incidence of enlarged white discolored adrenal glands and acinar vacuolation of the pancreas. At 1000 ppm, male and female adrenal weights were 133% of control values; adrenal hypertrophy, vacuolation, lipid storage of the zona fasciculata and cloudy swelling single cell necrosis of the liver were also noted. Testes weight increased to 113% of control, Leydig cell hyperplasia and clinical chemistry variations were observed. Body weight decreases occurred at 3000 ppm in males and females (94% of control) and as did food consumption decreases (81% and 74% of controls in males and females, respectively). Cataracts were also seen. The NOEL/LOEL = 50/300 ppm.

(c) Organ Weight and Hormone Reversibility Subchronic Studies

a. Subchronic Hormone Recovery - 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, testosterone (Testo), estradiol (E2), corticosterone, aldosterone (Aldo) and dehydroepiandrosterone (DEHA).

LH, FSH, testosterone and dehydroepiandrosterone were elevated in males after 3-months dosing (Table 10), 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 10). All other hormone levels determined were normal if ACTH and corticosterone levels are ignored (both values may be stress related rather than compound related).

Table 10: 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. Data extracted from MRID# 42355104.

b. Subchronic Organ Wt. - A 90-day study was conducted in Wistar rats on organ weights and after 1 and 3-months recovery undosed (MRID# 423551-03). Organ weights were determined at 3-months in 30 Wistar rats per group after administration of 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.

Body weights, and all organ weights, which were changed after 3-months of dosing returned toward normal after 3-months of recovery in males and females. Kidney, prostate, spleen and adrenal weights in males and liver, adrenal and spleen weights in females returned toward normal, but were still statistically significantly changed at 4500 ppm after 3-months recovery.

Male body weights, which were decreased after 3-month of dosing had returned to normal after 3-months undosed. Female body weights were unchanged after the 3-months of dosing.

Most of the organ weights determined were statistically significantly changed after the 3-months of dosing in males (F1 group) and many in females. After 1-month recovery, all the organs exhibiting weight changes tended to return toward normal. After 3-months recovery in males, all organs except the prostate had returned to normal at 1000 ppm and at 4500 ppm, all except the prostate, kidney, spleen and adrenal had returned to normal, but which tended toward recovery. After 3-months recovery in females at 4500 ppm, all organs had returned to normal except the liver, spleen and adrenals, which tended toward recovery. Summary histological data have also indicated testicular Leydig cell hyperplasia, ovarian and adrenal lipidosis were reversible or approached reversiblity (BASF Draft Report# 39S0375/88116 by W Mellert (1996) Vinclozolin - Reversibility of Selective Findings in Wistar Rats Dietary Administration for 3-Months and 1-Month and 3-Months Recovery Periods).

c. Subchronic Mouse (C57BL) (MRID# 41824301

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. 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. The nominal adrenal weight increase at the mid dose is possibly compound related.

- 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. Glucose was depressed at the highest dose level in males. 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 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 nominal decreased body weight in males has not been shown to be due to toxicity. The body weights of females did not change, however there was an apparent increased food efficiency in females (130% of controls). Core Classification: Supplementary because individual animal data were absent.

d. Subchronic B6C3F1) Mouse (MRID# 41824302)

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. Relative and absolute liver weights in males and females were increased at 1000 ppm and above. Lipogenic pigment was increased in females at 1000 ppm and above.

- This liver 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.
- At 5000 ppm, stromal cell hyperplasia of the ovaries of females. Triglycerides were depressed in females only at 5000 ppm. 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 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.

1. Chronic Studies

a. Chronic 6-Months Hormone (MRID# 41824305) (HED Doc.#) - 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 (Table 14). In females, ACTH (172% of controls) and LH (238% of controls) were statistically significantly and biologically significantly elevated (Table 14). The failure of corticosterone elevation with ACTH elevation in females is not consistent and may indicate some groups were subjected to stress prior to the blood collection. The elevated LH in females may be real since it was statistically significantly elevated in 2 studies (MRID# 41824305, Table 14 and MRID# 42355104, Table 10).

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 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 11 have been renamed accordingly.

Table 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. Data extracted from MRID# 41824305.

2. Other Hormone Studies *In Vitro*. (MRID# 425884-01)

Other 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. Vinclozolin had 39% of the binding affinity of flutamide 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. The binding studies to the rat prostate receptor were failures.

Dr. William Kelce of RTP has conducted androgen receptor binding studies with M1 and M2 (Kelce et al., 1994), metabolic/hydrolysis products of vinclozolin, Szeto et al., 1989) and with vinclozolin and finds that M1 and M2 binds with higher affinity than vinclozolin. Dr. Earl Gray Jr of RTP has conducted studies on rats through to lactation day 4 and found decreased ano-genital distance and nipple development in males at 3 and 6 mg/kg/day. The decreased ano-genital-distance was verified in 6 litter per group at 3 mg/kg/day in postnatal studies (Gray Jr et al., 1994). Continuing studies on the effects of vinclozolin in rats have confirmed the postnatal effects a 3 mg/kg/day, indicated that puberty was delayed at 15 mg/kg/day and that LH was increased at 10 mg/kg/day. In addition, after 15 weeks of dosing with vinclozolin, rats showed a LH increase at 5 mg/kg/day (Telephone call from LE Gray to David Anderson, 3/25/96).

F. Weight of the Evidence Considerations: (In some cases only abstracts of references were reviewed. This is indicated at the citation.)

The Committee considered the following observations regarding the toxicology of vinclozolin for a weight-of-the-evidence determination of its carcinogenic potential.

1. Male and female C57/6/JICO mice were fed 0, 15, 150, 3000 or 8000 ppm of vinclozolin, technical for 78-weeks. The highest dose tested (HDT) in the mouse study, 8000 was determined to be excessively high for carcinogenicity testing. This was based on statistically significant increases in mortality and body weight gain decrement and reduced relative efficiency of food utilization; liver focal necrosis, bile duct proliferation, pigment storage and lipidosis of the adrenal cortex and obvious endocrine disruption were also observed in males and females. Because of the excessive dosing in this study, the relevance to carcinogenicity in humans of tumors occurring at the HDT was questioned by CPRC.

Hepatocellular carcinoma/adenoma and hemangioma/hemangio-sarcoma were statistical significantly increased by pair-wise comparison with controls at 8000 ppm and for increased trend in males and females.

The incidence in testicular Leydig cell hyperplasia in male mice was increased at the 8000 ppm dose level only.

2. Male and female Wistar rats were fed vinclozolin at 0, 50, 500, or 3000 ppm for 104 weeks (Carcinogenicity Study). Clinical signs of toxicity were observed in both sexes and in males, there was also a 35% body weight decrement accompanied by a reduced food efficiency; in females, there was a 27% reduction in body weight and reduced food efficiency.

There were statistically significant increases in testicular leydig cell and prostate adenomas in male rats and in ovarian adenomas in female rats at both the mid- and highest doses (500 and 3000 ppm, respectively). Statistically significant increases in ovarian sex cord stromal tumors (benign) occurred at the highest dose only; however, there were also increases at the low and mid-doses with borderline significance ($p=0.053$) at the mid-dose. Statistically significant increases in uterine adenocarcinomas and adrenal cortical tumors in female rats were found at the highest dose only.

The majority of the CPRC considered the 3000 ppm dose to be excessively toxic based on the weight gain depressions and other clinical signs. However, it was noted that survival was enhanced in female rats at this dose relative to controls, and survival in males was comparable to that of controls. The CPRC agreed that the next highest dose (500 ppm) was adequate for assessing the carcinogenic potential of vinclozolin in both sexes and considered the tumors which occurred at that dose with statistically

significant increases to be relevant: leydig cell and prostate adenomas in male rats and ovarian sex cord stromal benign tumors in female rats.

3. Male and female Wistar rats were fed vinclozolin at 0, 150, 500, or 4500 ppm (Chronic Study). Body weight gain decrements of >>15% and other clinical signs of toxicity were noted at the highest dose.

Male rats had a statistically significant increase in hepatocellular carcinomas at the highest dose (4500 ppm) and a statistically significant positive trend. Male rats also had statistically significant increases in benign testicular leydig cell tumors at 500, 1500 and 4500 ppm (p<.01 at 2 top doses) and a statistically significant positive trend. Female rats administered vinclozolin in this chronic study had statistically significant increases in adrenal combined adenoma/carcinoma and ovarian sex cord (benign) "tumors" at the highest dose (4500 ppm) and there were statistically significant positive trends (p<.01) for both tumor types.

The CPRC concluded that the results from this chronic study were consistent with that in the carcinogenicity study, in terms of both the testicular Leydig cell and ovarian tumors and signs of toxicity (body weight gain reductions >>15% and other histological signs). The highest dose (4500 ppm) was considered to be excessively toxic, and the next highest dose (1500 ppm) was considered to be adequate (not excessively toxic).

4. From the submitted studies, vinclozolin was not mutagenic in most of the mutagenicity assays with and without activation where applicable. These studies were the Salmonella assay, Chinese hamster ovary (CHO/HGPRT) forward gene mutation assay, mammalian cells in culture cytogenetic assay, rat hepatocyte UDS assay and in the Chinese hamster *in vivo* sister chromatid exchange assay. One study, forward mutation in the mouse lymphoma L5178Y TK+/- 3.72 clone assay, was negative without activation, but equivocal with activation because of test material precipitation. These results indicate little concern for mutagenicity of vinclozolin. The results of a dominant lethal study (HED Doc# 000244) suggest no concern for heritable effects to follow-up at this time.

5. Vinclozolin and/or its metabolic products are structurally related and related by biological activity to procymidone, flutamide, cyproterone acetate and/or their related metabolic products. All result in androgen deficiency to androgen sensitive organs, cause liver toxicity at high dose levels, and all cause testicular Leydig cell hyperplasia/adenomas and liver carcinomas (liver carcinoma has not been verified for flutamide) at high dose levels. Unlike vinclozolin and procymidone, which have antiandrogenic properties, iprodione administration resulted in decreased plasma androgens possibly by a different mechanism (data

in review). Note: procymidone, a weaker antiandrogen than vinclozolin, causes ovarian stromal cell hyperplasia at 2000 ppm. These data were unavailable for consideration by the CPRC.

Vinclozolin and procymidone are negative in a battery of mutagenicity studies and iprodione is positive for unscheduled DNA synthesis (vinclozolin was negative and procymidone was studied and negative for unscheduled DNA synthesis, but the study was inadequate). The literature considers cyproterone acetate a weak mutagen and discusses the possibility that flutamide may be a weak mutagen because a metabolite was found that covalently binds to liver proteins (Fau et al., 1994, abstract).[4] The CPRC indicated that iprodione and procymidone were B2 carcinogens and suggested that flutamide and/or cyproterone acetate may result in liver carcinoma in humans. The evidence taken together (see also Appendix IIIA) was not considered as a basis of support for potential carcinogenicity of vinclozolin for humans.

6. Hormonal disruption as a basis for the Leydig cell tumors was considered, and the CPRC agreed that a mode of action (antiandrogenic) for these tumors in rodents appears to have been demonstrated (see Appendix for a detailed discussion). The consensus of the CPRC was that the relevance of Leydig cell tumors to humans cannot be dismissed. The CPRC also agreed that hormonal mechanisms for prostate and ovarian sex cord stromal tumors have not yet been developed.

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4 Fau-D, Eugene-D, Berson-A, Letteron-P, Fromentry-B, Fish-C and Pessayre-D. (1994) Toxicity of the antiandrogen flutamide in isolated hepatocyte. J. Pharmacol. Exp. Ther. 269:954-962. Only abstract seen. 4/17

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The majority of the CPRC recommended that vinclozolin should be classified as a Group B2 - probable human carcinogen - and that for the purpose of risk characterization, a non-linear approach (MOE) should be used for quantitation of human risk. This was based on statistically significant increases in multiple tumor types in the Wistar rat even at a dose which the CPRC agreed was not excessive: testicular Leydig cell tumors and prostate adenomas in male rats and ovarian adenomas in female rats. (There also were statistically significant increases in liver tumors in both sexes of the C57 mouse and adrenal adenomas and uterine carcinomas in female rats, the relevancy of which were questioned by the CPRC because of excessive dosing).

Hormonal disruption as a basis for the Leydig cell tumors was considered, and the CPRC agreed that a mode of action (antiandrogenic) for these tumors in rodents appears to have been demonstrated. However, the consensus of the CPRC was that the relevance of Leydig cell tumors to humans could not be dismissed. The CPRC also agreed that hormonal mechanisms for prostate tumors and ovarian sex cord stromal tumors have not yet been developed.

There are several chemicals structurally related to vinclozolin also associated with Leydig cell tumors in rats.

The tumors were mainly benign and there was little concern for mutagenicity of vinclozolin; therefore, a non-linear approach (MOE) was recommended for estimating risk. In addition, as discussed later, mechanistic data for the Leydig cell tumors provided further support for the use of the MOE approach.

Some members of the CPRC felt that the evidence for vinclozolin warranted only a Group C classification because the tumors were mainly benign and due to the absence of concern for mutagenicity. It was also felt that the human LH and testosterone response to antiandrogens is sufficiently different from the rat to raise questions about the applicability of the rat as a model for human Leydig cell tumors.

The majority opinion however was for a B2 classification for vinclozolin.

The MOE approach was selected because there was good evidence for an androgen receptor inhibition mechanism for the testicular Leydig cell tumors and that receptor interaction by its nature is a threshold phenomenon. The lowest effect level for androgen receptor binding was probably exhibited by the epididymal weight decrement in the study on reproduction with a NOEL/LOEL of 4.9/30 mg/kg/day (MRID# 42581301 & 42581302, HED Doc.# 010380),and the NOEL/LOEL of <3/3 mg/kg/day from a developmental/post natal study (Gray et al.); the LOEL of 3 mg/kg/day was subsequently changed to a NOEL of 3 mg/kg/day (Developmental/Reproductive Peer Review for Vinclozolin, the RfD/QA Peer Review Committee and the Toxicology Endpoint Selection Committee based on the low numbers of litters tested, the sensitivity of the AGD in the rat and possibly greater absorption of vinclozolin due to use of a corn oil vehicle). The RfD is based on data at even lower dose levels with a NOEL/LOEL of 25/50 ppm (1.2/2.3 mg/kg/day). The RfD is based on the NOEL, which in turn is based on lung foam cell aggregates in males and lens degeneration and ovarian lipidosis and cysts in females (The RfD/QA Peer Review Committee). Both the carcinogenicity study and chronic 2-year study in rats indicated evidence of nominal antiandrogen activity at dose levels as low as (50 ppm) 2.3 mg/kg/day based on nominally increased incidence of reduced prostatic secretions, coagulating gland atrophy and seminal vesicle atrophy in the rat carcinogenicity study (MRID# 43254703), that were statistically significant at higher dose levels.

Endpoints based on effects in the prostate and ovaries were not selected because the Committee did not believe sufficient evidence was available to show a mechanism for tumor formation in either organ. However, the NOEL/LOEL of 500/3000 ppm (23/143 mg/kg/day) for benign ovarian tumors seen in the rat carcinogenicity study (MRID# 43254703) and the NOEL/LOEL of 1500/4500 ppm (71/221 mg/kg/day) for the ovarian tumors (same tumor type) were seen in the 2-year rat chronic study (MRID# 43254701). The NOEL/LOEL for benign prostate tumor were 50/500 ppm (2.3/23 mg/kg/day) in the rat carcinogenicity study (MRID# 43254703) and no trends nor dose related effects even at 4500 ppm (221 mg/kg/day) were seen in the 2-year chronic rat study (43254701). These data are summarized in a Table on the following page.

Note: Stromal hyperplasia of the ovaries occurred with a NOEL/LOEL of 1000/2000 ppm (61/121 mg/kg/day)(5/50 at 2000 ppm vs. 0/49 in control) in rats dosed with procymidone (MRID# 41477703, HED Doc# 008074), a less potent analogue of vinclozolin resulting in androgen deficiency. No effects were seen in the prostate in the rat carcinogenicity study with procymidone, however, the lack of effects on the epididymal weight, seminal vesicle weight and prostate weight indicates that these organs were not well studied. These organs showed weight decrements at 250 and 750 ppm in adult rats from a 2-generation study on reproduction with procymidone (MRID# 41477708, HED Doc# 008074).

Study	Endpoints related to antiandrogen activity	NOEL (ppm)/(mg/kg/day)	LOEL (ppm)/(mg/kg/day)
Developmental, Gray et al.	AGD decrease	3[a] mg/kg/day	3 mg/kg/day
Reproduction MRID#42581301/ 42581302/43254705	Epididymal wt. decrement	50/4.9	300/30
Carc./rat/MRID# 43254703	Nominal dec. prostatic secretions, coagulating gland atrophy, seminal vesicle atrophy	25/1.2	50/23
Carc./rat/MRID# 43254703	Significant dec. epidid. sperm, seminal vesicle atrophy, coagulating gland atrophy, reduced prostate size & secretions	50/2.3	500/23
Chr/rat/MRID# 43254701	Epid. sperm dec. & reduced prostate secretions	150/7	500/23
Study	**Endpoints related to toxicity of unknown mechanism**	**NOEL (ppm)/(mg/kg/day)**	**LOEL (ppm)/(mg/kg/day)**
RfD bases/Chr&Carc./ MRID# 43254701/ 43254702/43254703	M:Lung foam cell aggreg.; F:lens degen., ovarian lipidosis & cysts	25/1.2	50/2.3
Carc./rat/ MRID# 43254703	Inc. prostatic benign adenoma	50/2.3	500/23
Chr./rat/MRID# 43254701	No inc. prostatic adenomas	(-)/(-)	>4500/>221
Carc./rat/MRID# 43254703	Inc. ovarian benign sex chord tumors	500/23	3000/143
Chr./rat/MRID# 43254701	Inc. ovarian benign sex chord tumors	1500/71	4500/221
Preliminary results from Dr. E. Leon Gray, Jr as of 4/23/96; (work complete, but not cleared for publication yet, thus undocumented)			
Study	**Endpoints related to antiandrogen activity**	**NOEL (ppm)/(mg/kg/day)**	**LOEL (ppm)/(mg/kg/day)**
Developmental/post natal segment	Delayed puberty/dec. epidi wt.	(-)/(-)	(-)/15
Developmental/post natal segment	LH increase males	(-)/(-)	(-)/10
15-weeks/rats at RTI (contract)	LH increase in males	(-)/(-)	(-)/5

No data.

H. Induces Cancer Call -- Vinclozolin

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to vinclozolin resulted in an increased incidence of testicular Leydig cell tumors (adenomas and carcinomas) and prostate adenomas in male Wistar rats and adrenal adenomas, ovarian adenomas and uterine carcinomas in female Wistar rats. Liver tumors (benign and malignant) in both sexes of the C57 mouse, were only seen at a dose which was excessively toxic.

Although there appears to be little concern for mutagenicity vinclozolin is structurally related to other chemicals associated with similar tumor types.

The Committee agrees that vinclozolin induces cancer in animals.

APPENDIX

IA. Mechanism of Antiandrogen Action: Accepted information - Summary of Endocrine Relationship between the Hypothalamus/pituitary and Testicular Leydig Cell Axis

Leydig cell tumors:

1. Testicular Leydig cell hyperplasia/tumors can occur in the rat and mouse from luteinizing hormone (LH) stimulation. LH is released through reduced androgen activity. The pituitary may see reduced androgen activity from reduced rate of synthesis or androgen receptor inhibition (mostly testosterone (T) and dihydrotestosterone(DHT))(androgen receptor inhibition - also known as antiandrogen activity) (Simard et al., 1986; Neumann, 1991). There are androgen receptors on the pituitary and hypothalamus. Increased dihydrotestosterone (DHT) reduces the pituitary LH pulse, but the major inhibitory effect of androgens on the hypothalamus appears to be mediated through locally derived estradiol from testosterone (Brunstein, 1991).

2. Testicular Leydig cells have LH receptors that bind LH when released by the pituitary (Saez, 1994). The LH is released from the pituitary through receptors and negative feed back from androgens, i.e., as plasma androgens (DHT & testosterone) are lowered, LH is released from the pituitary. When LH binds to testicular Leydig cell LH receptors, testosterone synthesis is stimulated and DHT is increased (i.e., T + 5α-reductase → DHT). They feed back to the pituitary and lower the rate of LH release and the cycle begins again (Saez, 1994).

3. LH stimulates the growth and proliferation of Leydig cells in rodents and to a lesser extent in humans by a poorly understood mechanism (Workshop on Leydig cell hyperplasia and tumor formation: Mechanisms and relevance to humans, 1995). However, one of the claimed reasons that testicular Leydig cell hyperplasia is more frequent in rodents than in humans is due to the larger numbers of LH receptors per Leydig cell in rodents than in humans (Workshop on Leydig cell hyperplasia and tumor formation: Mechanisms and relevance to humans, 1995) and to a decreased response to antiandrogens (Schepper et al., 1991). Experimentally, it is found that the antiandrogen flutamide stimulates the release of LH in rats and in humans, but the data in humans are conflicting (Knuth et al., 1984). LH was slightly elevated in a 2 weeks study, but the amount of LH released by GnRH stimulation was blunted in this 2 week study (Knuth et al., 1994). However, LH levels remain elevated in rats and to a lesser extent in mice (Murikami et al., 1995). There probably is no human data from long term studies on humans treated only with flutamide where LH levels have been measured, but there may be data on flutamide patients that have stopped therapy and endocrine measurements have been made.

4. There are several ways to reduce androgen end organ activity; it can be reduced by reducing androgen synthesis, accelerating

degradation or blocking androgen receptors; (a) inhibition of 5α-reductase conversion of testosterone to dihydrotestosterone (finasteride, Gray Jr. et al., 1994), (b) several other mechanisms that reduce steroidogenesis and result in reduced testosterone synthesis (benzimidazole and possibly iprodione, Fort et al., 1995; Rhone Poulenc, 1996), (c) Increased P450 enzymes (possibly peroxisome proliferators) that metabolize testosterone sufficiently fast to reduce it's circulating concentration (gemfibrozil and maybe linuron, or may be linuron by antiandrogen activity)(Alison et al., 1994; Cook et al., 1993), (d) inhibiting androgen receptors (vinclozolin and probably procymidone)(Kelce et al., 1994 & Murikami et al., 1995), (e) other mechanisms such as dopamine agonists that prevent the prolactin release that is needed to maintain LH receptors on the Leydig cells (Alison et al., 1994). With reduced numbers of LH receptors per Leydig cell, testosterone synthesis is stimulated less, which results in increased pituitary LH, which then is postulated to result in Leydig cell hyperplasia/tumors. Experimentally it is found that parbiosed rats develop Leydig cell hyperplasia/ tumors in the intact rat (Brown et al., 1979). If Fisher 344 rats are given silastic testosterone implants, they do no develop Leydig cell hyperplasia/tumors at 6 months where as in normal un-implanted rats, nearly 100% develop the tumors (Fort et al., 1995). The histamine H2-receptor antagonist that causes Leydig cell hyperplasia/tumors in rats, has been used in the treatment of 30,000,000 patients secreting excess gastric acid and ulcer patients safely since its introduction in 1976 (Brimblecombe et al., 1985).

5. Nearly all tissue in animals and humans contain androgen receptors. The function of the androgen receptors in many tissues is unknown, but it is suspected of playing some role. The function of androgen receptors, in and on androgen sensitive cells (male sex and accessary sex organs), is better understood than other tissue. The stimulating effects of a hormones are relative to the number of receptors per cell and the degree to which the receptors are occupied. Some receptors appear to be fully functional with only 5 to 10% occupancy while others appear to require about 50% occupancy.

6. Androgen deficit (i.e., by reduced synthesis or receptor inhibition) in rats results in decreased weight of the most sensitive androgen dependent organs and tissue, such as epididymides, seminal vesicles, prostates, testes, spermatogenesis. In addition, the secretory process in these organs is reduced. Thus, whether androgen synthesis is inhibited or androgen receptors are blocked in rats, androgen sensitive organs undergo a reduction in weight and plasma LH will increase (Alison et al., 1994). In the case of receptor inhibition, plasma androgens may increase because of the increased LH stimulated synthesis, however, since the pituitary androgen receptor is blocked from sending the signal

to the pituitary to stop releasing LH. In rats, testosterone and LH are continually released and elevated with antiandrogen administration, but testosterone decreases to pretreatment level in humans (Schroeder, 1990). LH levels were not reported, if conducted. LH levels were reported to slightly increase in 14 day studies (Knuth et al., 1984). Roberts et al., 1989 suggest that LH is not elevated in humans treated with SDZ-200-110 (a calcium channel blocker), but that LH is elevated in rats and Leydig cell hyperplasia/tumors are produced by the drug administered to rats. Provone-Macaluso et al., 1990, suggest that there is a small increase in LH and testosterone in older men treated with flutamide, which are much less than treated rats and states that no Leydig cell hyperplasia was seen in these patients.

Possible involvement of antiandrogen activity in the ovary:

1 There are also androgen receptors in the ovary.

2. In the ovary androgens are necessary for follicular growth and ovulation. They appear to play an important role in regulating follicular development in both immature and mature cycling rats (Beyer et al., 1974; Kumari et al., 1978). They induce atresia of the preantral follicle and play a role in HCG induced ovulation (Louvet et al., 1975).

3. Important to the discussion of antiandrogen exposure to the female, is the experimental finding that cyproterone acetate has been reported to accelerate the rate of atresia and subsequent transformation of the atretic preovulatory follicle into an ovarian cyst (Peluso et al., 1979).

IIA. Mechanistic work done on vinclozolin:

1. Summary: The preponderance of the evidence indicates the Leydig cell hyperplasia/tumors in rats from antiandrogen activity results from increased tonic LH resulting in Leydig cell hyperplasia and longer LH stimulation results in Leydig cell adenomas. Without the antiandrogen inhibition and LH release, Leydig cell hyperplasia and adenomas would be very unlikely. If this mechanism can occur in humans, it is not apparent that it has to date (Alison et al., 1994; Brimblecombe et al, 1985). However, there are differing opinions on the circumstances under which potential induction of Leydig cell hyperplasia/tumors may be a human problem. In addition, the effect of small increases in LH, if it occurs in preadolescent children, is not known.

The prostate adenomas seen in the carcinogenicity study and not seen in the chronic study may not be treatment related. On the other hand, the prostate is an androgen

sensitive organ that showed androgen deprivation, decreased secretions and fibrosis at the same dose levels as the adenomas, all of which may be related. Some antiandrogens (WIN49596, Descotes et al., 1996), about to be marketed, are claimed to inhibit prostate carcinoma in rats while some are claimed to promote prostate carcinomas (cyproterone acetate) in rats.

The benign ovarian sex chord tumors may also be related to antiandrogenic activity on the preantral follicles, however, data supporting this relationship is not well defined.

The liver and adrenal tumors are mostly benign occurred only at excessive dose levels.

2. W. Kelce (1994) presented strong evidence that vinclozolin and/or its metabolites is a pure antiandrogen with little to no other endocrine activity at relevant dose levels. He also stated that metabolites of vinclozolin in rat plasma are at a concentration around the pKi for androgen receptor binding, which means, at probable plasma concentrations, androgen receptor inhibition is probable (his conclusion).

3. E. Gray Jr (1993 and 1994) confirmed the antiandrogen effects in developmental toxicity studies seen by the registrant (Hellwig, 1989, MRID# 41132201) on anogenital distance (AGD) decrease, nipple development in male pups, undescended testicles and malformed male accessory organs at higher dose levels. He also confirmed the registrant's findings of decreased organ weight for the seminal vesicles, epididymides, prostate and spermatogenesis, which illustrate the decreased androgen stimulation resulting from androgen receptor binding (His attribution). E.L. Gray, Jr. has also reported additional data on AGD in a total of 16 litters, which confirm the decrease seen in two 3 litters studies at 3 mg/kg/day (4/25/96 CC:Mail message to David G Anderson from E.L. Gray, Jr.).

4. The registrant has shown decreased weight for seminal vesicles, epididymides, prostate and spermatogenesis in the chronic and the carcinogenicity studies in rats and increased Leydig cell hyperplasia/adenomas (Mellert, 1993, MRID# 42728801; Mellert, 1994, MRID# 43254701; Mellert, 1994, MRID# 43254703).

5. There are citations in the literature that suggest that prolonged excessive stimulation of any endocrine sensitive organ will result in hyperplasia, which will continue to tumor development and malignancy (Neumann, 1991). It has also been reported in the literature that if the stimulation is stopped soon enough, the hyperplasia is reversible and without hyperplasia, adenomas and malignancies probably will not develop from temporary excessive stimulation (Neumann, 1993; Alison et al., 1994; Niwa et al., 1993; Terada et al. 1993;

Yamada et al., 1994; Paynter et al., 1988)

6. Hormone levels and androgen sensitive organ weights that were changed after 3 months of vinclozolin administration at 4500 ppm were reversed completely or tended to be reversed after 1 or 3 months undosed (Mellert, 1992, MRID# 42706102, 42355103, 42355104).

7 Hormone levels were not studied below 4500 ppm by the registrant, however, unless the mechanism of hormonal action and organ responses changed drastically at lower dose levels, similar hormone perturbations may have occurred to a lesser extent at lower dose levels of vinclozolin. Although androgen levels below 4500 ppm were not studied, androgen deficiency was shown in androgen sensitive organ weights, which were shown to be decreased ≥ 500 ppm. The carcinogenicity and the chronic studies showed test material related tumors only at ≥ 500 ppm (Mellert, 1994, MRID# 43254701 and 43254703). In addition, the androgen sensitive epididymal weight was decreased in the 2-generation study on reproduction at ≥ 300 ppm (30 mg/kg/day)(Hellwig, 1989, MRID# 42581301) and in the developmental toxicity study in the rat (Gray Jr et al, 1993, 1994), the anogenital distance (another sensitive androgen indicator) was decreased at ≥ 3 mg/kg/day in male offspring.

8. LH levels were increased at 4500 ppm, but LH levels were not determined by the registrant at dose levels lower than 4500 ppm (Kuppen, 1989, MRID# 41824305). However, E. Gray, Jr. (personal communication, March 25, 1996) indicated that they had detected elevated plasma LH levels in rats as low as 10 mg vinclozolin/kg/day (approximately equivalent to 200 ppm). He also reported that RTI had conducted a study, soon to be published, where they found dose related LH increases that correlated with the Leydig cell hyperplasia/tumors in a 15 month study in rats. It is assumed that plasma LH levels may be detectably elevated at ≥ 500 ppm in the carcinogenicity and chronic studies in rats conducted by BASF (Mellert, 1994, MRID# 43254701, 43254703) but the elevation at 3 mg/kg per day may be impossible to detect. In additional studies reported by telephone 4/23/96 to David G Anderson and 4/3/96 by CC:Mail, E.L. Gray, Jr. reported that delayed puberty and decreased epididymal weight were seen in postnatally at 15 mg/kg/day. An LH increase was seen postnatally at 10 mg/kg/day and in contract research conducted at RTI, an LH increase was seen in male rats after 15-weeks of exposure to vinclozolin. The studies are complete, but they have not been cleared for publication as yet; thus, they are undocumented. The amount of LH elevation in rats necessary to produce detectable Leydig cell hyperplasia in long term studies appears to have been determined by the studies with vinclozolin, conducted at RTI (private communication from Earl Gray Jr.).

9. Prostate adenomas were noted at the same dose levels that antiandrogen activity was noted ≥ 500 ppm (Mellert, 1994, MRID# 43254703). The relationship between the significant decreases in prostatic secretion, increases in prostatic fibrosis and prostatic adenomas all at the same dose levels is not known.

10. The adrenal and ovarian tumors may be related to the lipid accumulation in these organs. Steroidogenesis and/or function of androgens may be affected in the adrenal and possibly in the ovary. Ovarian lipidosis was noted at all dose levels.

    The lipid accumulation in the adrenal may have interfered with the normal hormonal activity in this organ. The rat chronic study (Mellert, 1994, MRID# 43254701) indicated statistical significantly increased water consumption after prolonged treatment that may have been related to adrenal hormonal perturbation.

    It should be noted that procymidone a weaker antiandrogen than vinclozolin, causes ovarian stromal hyperplasia in rats at 2000 ppm.

    Other effects may be related to the antiandrogen activity in the female, however, the hormone studies in females were not as well studied because the male appeared to be more sensitive and resulted in antiandrogenic effects at a lower dose levels than in the female.

    Antiandrogen activity may have been involved in the development of the ovarian tumors, however, the data to support this is lacking. Decreased number of follicles, increased cysts in ovaries and atrophic uteri were seen in mice (MRID# 43254704) and disrupted estrus cycles were noted in the 90-day study in rats at 4500 ppm (MRID# 42706102). However, a statistically significant increase in females with cysts in ovaries were seen at all dose levels at termination in the carcinogenicity study in rats (MRID# 43254703), all of which may indicate a hormonal imbalance, that could be related to the sex chord tumors.

    Lipid accumulation in the ovary also may have resulted in interference with normal steroidogenesis in this organ.

IIIA. Human Data

A case study, Kattan et al. (1994, abstract) claims that a 65-year-old man developed hepatocellular carcinoma 3 months after the initiation of a hormonal treatment with cyproterone acetate (for 1 month) and LH-RH agonist and that a cause and effect relationship between cyproterone acetate and hepatocellular carcinoma has been suggested by the literature (Ohri et al., 1991, abstract; Rudiger et al., 1995, abstract; Watanabe et al., 1994, abstract). Flutamide has been reported to cause abnormal liver function tests in about 0.38% and liver disease in about 0.13% of 1091 flutamide patients (Gomez et al., 1992 abstract).

Vinclozolin workers in a plant were exposed for 1 to 13 years to approximately 0.0067 to 0.134 mg of vinclozolin/kg/day. Sixty seven workers associated with the synthesis and formulation operations at the BASF plant for 1 to 13 years were medically examined, which included ultrasonography of the liver and prostate, a detailed eye examination and routine spirometry for lung evaluation (BASF Doc# 94/11016 and Zober et al., 1994). In addition to a general physical examination that included examination for loss of pubic hair, breast enlargement, fertility problems and clinical chemistry deviations, hormone level changes and excreted urinary 3,5-dichloroaniline containing compounds were determined. A control group of fifty two workers with similar demographic characteristics and social habits were followed similarly.

Unilateral and bilateral atrophy of the testes (7 workers) were the most frequent findings, four of which were attributed to preexposure childhood conditions. Although FSH was significantly increased in exposed groups, as would be expected from the probable decreased spermatogenesis associated with the testicular atrophy seen, after removal of the men with atrophy ascribed to preexisting conditions, the significance disappeared. The men excluded included two with FSH and LH levels out of the range indicated for men with childhood acquired testicular conditions.

No differences were noted at ophthalmologic examination, or in prostate, lung or hematological parameters. One benign liver tumor in one individual could not be related to treatment. According to the registrant, this study should have been adequate to detect hormone disturbances, if they had occurred at these exposure levels. The study is inadequate to determine effects from life-time exposure.

References:

References to submitted studies:

90-Day Subchronic Study (Rat).
W. Mellert (March 5, 1993) Study on the Oral Toxicity of Reg. No 83 258 (vinclozolin) in Wistar Rats: Administration in the Diet Over 3 Months. Conducted by BASF Aktiengesellschaft, Dept. Toxicology, D-W46700 Ludwigshafen/Rhein Germany, for BASF Corp., Agricultural Products. Project No. 31S0375/88034, Registration Doc. No. BASF 93/10191. MRID# 427288-01.

2-Year Chronic Feeding Study in Rats.
Mellert, W. (1994) Chronic Toxicity Study with Reg. No. 83 258 - Vinclozolin in Rats Administration in the Diet for 24 months. Study conducted by BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/ Rhein, FRG, study no. 71S0375/88026, BASF no. 94/10287, May 3, 1994. MRID# 43254701. Unpublished report submitted to USEPA, OPP.

83-1/2-Year Chronic Feeding Study in Rats (Supplementary Study).
Mellert, W. (1994) Second Chronic Toxicity Study with Reg. No. 83 258 - Vinclozolin in Rats Administration in the Diet for 24 months. Study conducted by BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/Rhein, FRG, study no. 71S375/88109, BASF no. 94/10288, May 4, 1994. Unpublished report submitted to USEPA, OPP. MRID# 43254702

83-2/2-Year Carcinogenicity Study in Rats.
Mellert, W. (1994) Toxicology Study Report: Carcinogenicity Study with Reg. No. 83 258-Vinclozolin in Wistar Rats Administration in the Diet for 24 Months. Study conducted by BASF Aktiengesellschaft Crop Protection, Product Safety, Dept. of Toxicology, Ludwigshafen/Rhein, Germany, study no. 71S0375/88105, BASF no. 94/10279, May 2, 1994. MRID# 43254703. Unpublished report submitted to USEPA, OPP.

83-2/18-Months Carcinogenicity Study in Mice.
Mellert, W. (1994) Toxicology Study Report: Carcinogenicity Study with Reg. No. 83 258-Vinclozolin in C57BL Mice Administration in the Diet for 18 Months. Study conducted by BASF Aktiengesellschaft Crop Protection, Product Safety, Dept. of Toxicology, Ludwigshafen/Rhein, Germany, study no. 80S0375/88112, BASF no. 94/10278, May 4, 1994. MRID# 43254704. Unpublished report submitted to USEPA, OPP.

83-3/Developmental Toxicity Study in Rats:
Hellwig, J. (1989) Report on the Prenatal Toxicity Studies with Reg. No. 83 258 (Vinclozolin) in Rats After Oral Administration (Gavage) - Consisting of BASF Report Nos. 89/0091, (89/0092) (This latter number was omitted from title), 89/0093. Conducted by BASF

Aktiengesellshaft, Dept. Toxicology, 6700 Ludwigshafen, Federal Republic of Germany, for BASF Corp., Project# 34R0165/84084, 34R0165/84085, 34R0165/84086, and 92R0165/84088, respectively, for BASF Corp, dated March, 1989. BASF Proj. # 89-0190. MRID# 41132201. Unpublished reports submitted to USEPA, OPP.

83-4/Two-Generation Study of Reproduction in Rats.
Hellwig, J. (1989) Report on the Reproduction Study with Reg. No. 83 258 (Vinclozolin) in Rats with Continuous Dietary Administration Over 2-Generations (2 litters in the First and 2 litters in the Second Generation). Conducted by BASF Aktiengesellschaft, Dept. Toxicology, 6700 Ludwigshafen, Federal Republic of Germany, Project No.: 71R0375/88053, BASF Reg. # 92/10596 for BASF Corp.; Dated October 21, 1989. (MRID# 425813-01). 92/11251 & 71R0375/88053. Reg. Doc. No. BASF 92/10596 (For MRID# 425813-01). Unpublished report submitted to USEPA, OPP.

Other References:

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