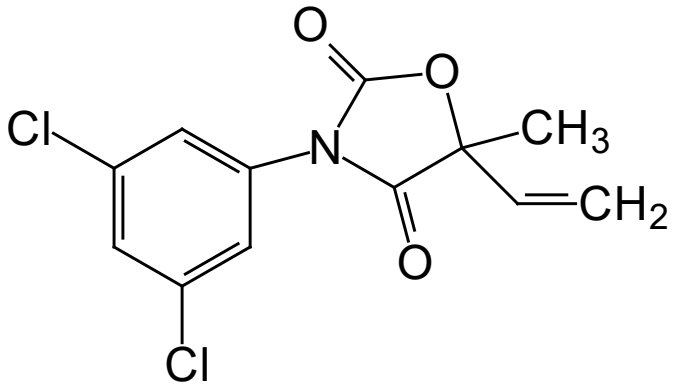
Vinclozolin



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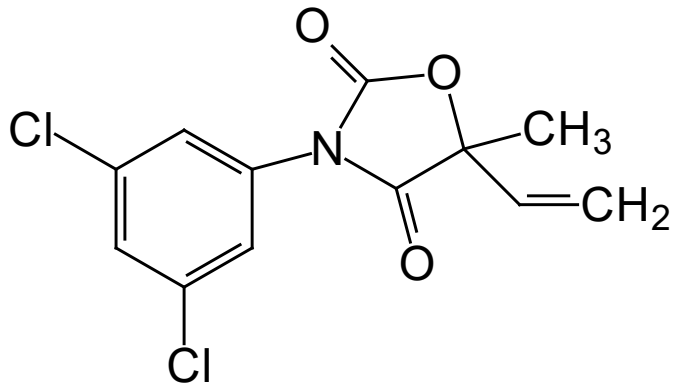
Vinclozolin



LISTED "AS CAUSING CANCER" BY THE AUTHORITATIVE BODIES MECHANISM in 1999

Basis of listing: U.S. EPA Group B2 carcinogen

U.S. EPA Classifications of Carcinogenic Potential

1996: U.S. EPA classified as Group B2 – probable human carcinogen. Based on:

- Testicular Leydig cell adenomas and carcinomas and prostate adenomas in male rats;
- Benign ovarian sex cord stromal tumors, adrenal adenomas and uterine carcinomas in female rats.

2000: U.S. EPA formally accepted a tentative 1997 reclassification to Group C – possible human carcinogen

- Testicular Leydig cell tumors in rats, supported by Leydig cell hyperplasia in mice
- Based on re-evaluation that found prostate and ovarian tumors not significantly increased.

Carcinogenicity studies evaluated by U.S. EPA

- **Two-year oral studies in Wistar rats**
  - —Study 1: BASF report (Mellert, 1994)
    - 50 rats/sex/group
    - Testicular Leydig cell tumors, prostate adenomas in males;
    - Benign ovarian sex cord tumors, adrenal cortical tumors, uterine carcinomas in females.
  - —Study 2: BASF report (Mellert, 1994)
    - 20 rats/sex/group
    - Testicular Leydig cell tumors in males;
    - Benign ovarian sex cord tumors, adrenal cortical tumors in females.
- **18-month oral studies in C57BL mice**

  BASF report (Mellert, 1994)
  - Malignant liver tumors at doses considered excessively toxic.

Tumor Incidence in Study 1
(Evaluated by U.S. EPA in 1996)

Tumor site	Dose (ppm)				Trend Test
	0	50	500	3000	
***Male rats*:**					
Testicular Leydig cell tumors					
Benign	23/48	25/49	47/50**	48/50**	p<0.01
Malignant	0/48	0/49	0/50	2/50	
Combined	23/48	25/49	47/50**	49/50**	p<0.01
Prostate Adenomas	0/48	3/49	7/50**	5/50*	
Hepatocellular tumors					
Adenoma	0/48	1/49	1/50	3/50	
Carcinoma	1/48	1/49	5/50	2/50	
Combined	1/48	2/49	6/50	5/50	
Female rats					
Benign ovarian sex cord stromal tumors	4/39	7/36	10/45	29/45**	p<0.01
Adrenal cortical tumors					
Adenoma	1/42	2/42	1/47	21/48**	p<0.01
Carcinoma	0/37	0/30	0/37	1/42	
Combined	1/42	2/42	1/47	22/48**	p<0.01
Uterine adenocarcinomas	1/41	0/27	1/27	7/47*	p=0.002

**p<0.01;*p<0.05

Tumor Incidence in Study 2
(Evaluated by U.S. EPA in 1996)

Tumor site	Dose (ppm)					Trend Test
	0	150	500	1500	4500	
Male rats:						
Testicular Leydig cell tumors	11/20	12/20	17/20*	19/20**	20/20**	p=0.0003
Hepatocellular carcinomas	0/20	0/20	1/20	1/20	9/20**	p<0.01
Female rats:						
Benign ovarian sex cord stromal tumors	0/20	0/20	2/20	4/20 *(p=0.053)*	10/20**	p<0.01
Adrenal cortical adenoma/carcinoma	0/20	0/20	0/20	1/20	6/20**	p<0.01

**p<0.001;*p<0.05

Summary of Observed Tumors in 1996 Evaluation

Male Rats

Testicular Leydig cell tumors: 2 studies*

Prostate adenomas: 1 study*

Hepatocellular tumors: 2 studies

Female Rats

Benign ovarian sex cord stromal tumors: 2 studies*

Adrenal cortical tumors: 2 studies*

Uterine adenocarcinoma: 1 study*

***Basis of 1996 conclusion that vinclozolin causes cancer (Group B2 – probable human carcinogen)**

U.S. EPA Vinclozolin Re-evaluation (1997)

- Based on re-read of rat ovary and prostate pathology slides by Charles C. Capen, consultant to the Registrant, using different diagnostic criteria than in original evaluation.
- Consultant and original study pathologist met to resolve differences between consultant's diagnosis and original diagnosis.
- Lucas Brennecke, pathology consultant to U.S. EPA at the 1997 meeting, recommended acceptance of the new criteria, but he did not evaluate the pathology slides.

Ovarian Sex Cord Stromal Tumors:
Summary of Re-read Results

- **Capen criteria: classified five different grades of hyperplasia + benign tumors.**
- **Tumor incidence decreased and incidence of hyperplasia increased.**

Benign ovarian sex cord stromal tumors: Study 1

Tumor incidence	Dose (ppm)			
	0	50	500	3000
Original	*4/39**	*7/36*	*10/45*	*29/45***
Capen Re-read	2/50	2/49	2/50	5/50

*p<0.01 trend; **p<0.01 Fisher exact

Benign sex cord stromal tumors: Study 2

Tumor incidence	Dose (ppm)				
	0	150	500	1500	4500
Original	*0/20*	*0/20*	*2/20*	*4/20**	*10/20 **
Capen Re-read	0/20	0/20	0/20	0/20	1/20

*p=0.053; p<0.001

Comparison of Ovarian Tumor Diagnostic Criteria

- **Classification scheme that was used by Dr. Capen (unpublished):**
  - 5 grades of ovarian cortical stromal hyperplasia + benign ovarian sex cord stromal tumors.
- **IARC: International Classification of Rodent Tumors (1997)**
  - "Focal discrete lesions larger than a large corpus luteum are, in the absence of any other morphological criteria…considered to be a tumour."
  - "Diffuse mixed-type lesions occasionally become very large. They may encompass the major part of the ovary and have a size larger than a normal ovary. In these cases they are arbitrarily registered as tumour, sex cord stromal, benign, mixed type."
- **NTP: Guides for Toxicologic Pathology [Dixon et al.,1999]**
  - Hyperplasia is "[d]ifferentiated from tumors of the sex cord-stromal cells by size. Focal lesions up to 2-3 mm are considered hyperplastic, and larger lesions are considered tumors. When change is diffuse and bilateral, a 2- to 3-fold increase in ovary size is used to shift a diagnosis of hyperplasia to neoplasia."

Prostate Tumors: Historical Controls

- Study results
  - 14% (7/50) in mid-dose rats vs. 0% (0/50) in concurrent controls
- Original evaluation historical controls
  - Range 0-12%
  - Based on a 10 year period (29 studies)
- 1997 Capen evaluation historical controls
  - Range 0-15%
  - Based on a 13+ year period (34 studies)

Concurrent and Historical Control Data

Guidelines for Carcinogen Risk Assessment
U.S. EPA (2005)

- **"The standard for determining statistical significance of tumor incidence comes from a comparison of tumors in dosed animals with those in concurrent control animals."**
- **"Generally speaking, statistically significant increases in tumors should not be discounted simply because incidence rates in the treated groups are within the range of historical controls..."**
- **"The most relevant historical data come from the same laboratory and the same supplier and are gathered within 2 or 3 years one way or the other of the study under review; other data should be used only with extreme caution."**

Prostate Tumors: Summary of Re-read Results

- **Capen re-read using original criteria (RENI/IARC):**
  - Tumor incidence same as in original evaluation: 14% (7/50) in mid-dose rats vs. 0% (0/50) in concurrent controls
- **Capen re-read using Bosland criteria:**
  - Tumor incidence decreased and incidence of hyperplasia increased.

Prostate Adenoma: Study 1

Prostate Adenoma	Dose (ppm)			
	0	50	500	3000
Original evaluation	*0/48*	*3/49*	*7/50***	*5/50**
Capen re-read using RENI/IARC criteria	0/48	3/49	7/50**	5/50*
Capen re-read using Bosland criteria***	0	1/3	2/7	2/5

*p<0.05; ** p<0.01

***Only rats previously diagnosed as having a prostate adenoma (RENI criteria) were included in this re-evaluation.

Prostate Hyperplasia: Comparative Descriptions

Morphological features	Bosland	IARC
Size	One to a few adjacent alveoli	Single to a few adjacent alveoli
Obliterated alveolar lumen	No	No
Distorted normal architecture	No	No
Compressed surrounding tissue	No	No
Capsule formation	No	No
Growth pattern	Cribriform	…sometimes papillary or cribriform
Degree of pleomorphism (atypia)	Mild	Minimal
Inflammatory infiltrate	No	No

Prostate Adenoma: Comparative Descriptions

Morphological features	Bosland	IARC
Size	One to several (<10) adjacent alveoli	Partially or completely obliterating the lumen of one or more acini
Obliterated alveolar lumen	Yes	Yes
Distorted normal architecture	Yes	Yes
Compressed surrounding tissue	Yes	Yes
Capsule formation	Sometimes	Sometimes
Growth pattern	Predominantly cribriform, also solid and comedo	Predominantly cribriform, rarely comedo pattern with solid and microglandular areas
Degree of pleomorphism (atypia)	Mild to moderate	Mild, some areas of dysplasia and squamous metaplasia
Inflammatory infiltrate	Occasionally	Usually no

Prostate Adenoma: Comparison of Criteria (cont'd)

Differences described in Dr. Capen's report:

- RENI/IARC criteria: ADENOMA
  - "an intra-acinar epithelial proliferative lesion obliterating only one acinar lumen accompanied by some distortion of normal architecture and obliteration of the lumen of the acinus."

- Bosland criteria: HYPERPLASIA
  - "small focal proliferative lesions of prostatic acinar epithelium limited to the involvement of 1 to 3 adjacent alveoli that do not distort normal alveolar architecture.

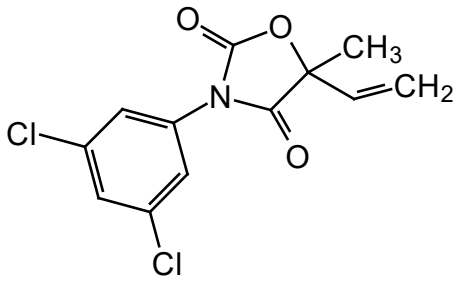
Summary of U.S. EPA Conclusions

Statistically significant tumors in Wistar rats studies	Basis for 1996 U.S. EPA induces cancer call (Group B2)	Basis for 1997/2000 U.S. EPA Reclassification (Group C)
Testicular Leydig cell tumors (adenomas/carcinomas)	✓	✓
Ovarian sex cord stromal tumors	✓	---
Prostate adenomas	✓	---
Adrenal cortical tumors	✓	---
Uterine adenocarcinomas	✓	---
Hepatocellular carcinomas	---	---

Recent findings: Anway et al. (2006)

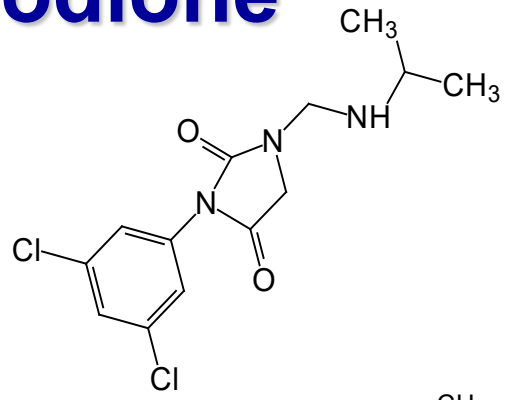
- Female Sprague-Dawley (F_0 generation) were exposed to vinclozolin during gestation.
- F_1 generation and three subsequent unexposed generations were followed for up to 14 months.
- 5/38 males from F_1 – F_4 generations developed mammary tumors. Incidence in comparable control male rats was 0/28.

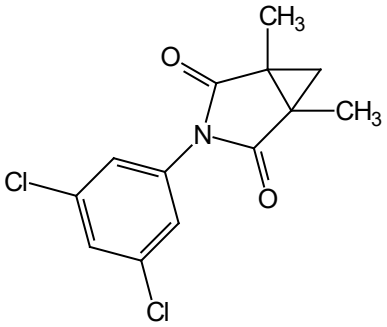
STRUCTURAL ANALOGUES



Vinclozolin

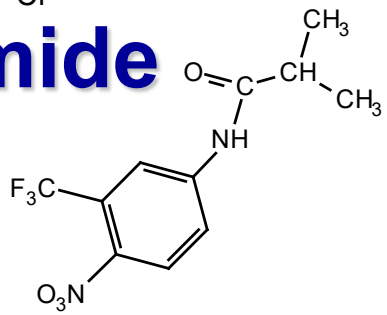
Iprodione





Procymidone

Flutamide



STRUCTURAL ANALOGUES

- **Procymidone**
  - — Testicular Leydig cell tumors in male rats.
  - — Pituitary adenomas in female rats.
  - — Hepatocellular tumors in male and female mice.
- **Iprodione**
  - — Testicular Leydig cell tumors in male rats.
  - — Hepatocellular tumors in male and female mice.
  - — Ovarian luteomas (cells of sex cord origin) in female mice.
- **Flutamide**
  - — Testicular Leydig cell tumors in male rats.
  - — Mammary adenoma, adenocarcinoma, fibroadenoma in male rats (*PDR, 2006*).
  - — Malignant breast tumors in men treated with flutamide (*PDR, 2006*).