VINCLOZOLIN

A CHEMICAL LISTED "AS CAUSING CANCER" BY THE AUTHORITATIVE BODIES MECHANISM AND UNDER REVIEW BY THE CARCINOGEN IDENTIFICATION COMMITTEE

September 2006

PREFACE

The California Environmental Protection Agency's (Cal/EPA) Office of Environmental Health Hazard Assessment (OEHHA), as lead agency for the implementation of the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq*.) maintains the Proposition 65 list of chemicals that have been identified by the State to cause cancer, birth defects or other reproductive harm. One of the mechanisms by which a chemical can be put on the Proposition 65 list is when the chemical has been identified as causing cancer by an organization that has been designated as "authoritative" for purposes of Proposition 65. The authoritative bodies for identifying agents as causing cancer are: U.S. Environmental Protection Agency (U.S. EPA), U.S. Food and Drug Administration, National Institute of Occupational Safety and Health, National Toxicology Program, and the International Agency for Research on Cancer.

If the lead agency finds that a chemical is no longer identified by the authoritative body as causing cancer or reproductive toxicity, the listing under the Proposition can be reconsidered (Title 22, Cal. Code Regs. §12306(j)). Chemicals listed "as causing cancer" which are under reconsideration and which have been placed on the list by the authoritative bodies mechanism are referred to the Carcinogen Identification Committee (CIC), the state's qualified experts for carcinogenicity determinations under the Proposition (Title 22 Cal. Code Regs. §12306(j)). The CIC then makes a recommendation regarding whether the chemical has been "clearly shown, through scientifically valid testing according to generally accepted principles to cause cancer"(Title 22 Cal. Code Regs. §12305 (a)(1)).

Vinclozolin (CAS No. 50471-44-8) was listed "as causing cancer" under Proposition 65 on August 20, 1999, based upon its classification by the U.S. EPA (1996) as a probable human carcinogen (Group B2). The U.S. EPA's classification as Group B2 was based on statistically significant increases in multiple tumor types in male Wistar rats and ovarian tumors in female Wistar rats.

In 1997, the U.S. EPA revised the classification of vinclozolin to Group C, possible human carcinogen (U.S. EPA, 1997). This re-classification was based on preliminary results of a re-evaluation of pathology slides from the ovary of female rats and the prostate of male rats. In 2000, the U.S. EPA confirmed the conclusions of the 1997 re-evaluation and concluded that increases in ovarian adenomas or prostate adenomas in the rat carcinogenicity studies were not statistically significant (U.S. EPA, 2000).

These hazard identification materials were compiled to provide the Committee with relevant information for use in its deliberations. A public meeting of the Committee to discuss this evidence is scheduled for November 16, 2006. At this meeting it is expected that the Committee will render an opinion on whether vinclozolin has been clearly shown to cause cancer. Written public comments should be submitted to OEHHA by October 30, 2006, in order to be considered by the Committee in advance of the meeting. During the November 2006 meeting, the public will have an opportunity to present verbal comments to the Committee.

SUMMARY OF AVAILABLE CARCINOGENICITY INFORMATION ON VINCLOZOLIN

Vinclozolin is a fungicide used on various flowers, vegetables (especially lettuce), strawberries, raspberries and stone fruits to control molds. The California Department of Pesticide Regulation reports that 18,568 pounds of vinclozolin were applied in California in 2003 (http://www.cdpr.ca.gov/docs/pur/pur03rep/chmrpt03.pdf).

As discussed in more detail in Attachment I (U.S. EPA, 1996), vinclozolin was classified as a Group B2 – probable human carcinogen, based on statistically significant increases in testicular Leydig cell tumors and prostate adenomas in male Wistar rats and benign ovarian sex cord stromal tumors in female Wistar rats. Significant increases in adrenal cortical adenomas and carcinomas (combined) and uterine adenocarcinomas were also observed in high-dose female rats, but the U.S. EPA considered the high dose to be excessively toxic, based on weight gain depressions greater than 15%. These tumors were observed in two-year carcinogenicity studies in male and female rats (50 rats/sex/dose group). In chronic two-year studies with Wistar rats using smaller group sizes (20 rats/sex/dose group) testicular Leydig cell tumors were observed in males and benign ovarian sex cord tumors were observed in females. In addition, significant increases in hepatocellular carcinoma were observed in high-dose male rats, and adrenal cortical adenomas and carcinomas (combined) were observed in high-dose female rats. U.S. EPA discounted these findings because of toxicity in the high dose group. Carcinogenicity studies in mice resulted in statistically significant increases in benign and malignant liver tumors. U.S. EPA also discounted these findings because of toxicity in the high dose groups.

As discussed in Attachment II (U.S. EPA, 1997), the U.S. EPA reclassification of vinclozolin as a Group C – possible human carcinogen was based on preliminary findings from the Registrant's re-evaluation of the pathology slides for tumors of the ovary and prostate. Ovarian tumors were re-evaluated using revised criteria for grades of ovarian cortical stromal hyperplasia and for benign ovarian sex cord/stromal tumors. Re-evaluation of prostate adenomas involved both the inclusion of additional historical control data as well as a re-analysis of tumor slides by revised criteria. The Registrant's re-evaluation of these findings was subsequently confirmed by a Pathology Working Group (PWG). A brief summary of the PWG report is included in Attachment III, which is the U.S. EPA's Cancer Assessment Document for vinclozolin (U.S. EPA, 2000).

Additional studies that provide data relevant to the carcinogenicity of vinclozolin include studies investigating induction of genotoxicity, markers of cell damage and cell transformation, and disruption of normal hormonal activities. Studies identified by OEHHA through a search of the published scientific literature, that are not included in the U.S. EPA carcinogenicity reviews of 1996, 1997, or 2000, are included as Attachment IV. These include studies in which vinclozolin was tested for the ability to induce increases in chromosomal aberrations, sister chromatid exchanges, mitotic index, micronucleated erythrocytes, glucose 6-phosphate dehydrogenase enzyme activity, 8-OH-2-deoxyguanosine, cell transformation foci, and GST-P positive foci. Also included

in Attachment IV are studies evaluating the antiandrogenicity of vinclozolin (Kojima et al., 2004) and its metabolites (Wong et al., 1995) and the *in utero* effects of vinclozolin on progesterone and estrogen activities (Buckley et al., 2006). U.S. EPA evaluations of two other dichlorophenyl dicarboximide fungicides, procymidone and iprodione, are also included in Attachment IV (U.S. EPA, 1994; U.S. EPA, 1991). Procymidone and iprodione are also listed as causing cancer under Proposition 65. Both induced testicular tumors in male rats and hepatocellular tumors in mice. Also, iprodione induced ovarian tumors in female mice. Both vinclozolin and procymidone are potent antiandrogens and appear to exert their effects by interfering with the androgen receptor (Kojima et al., 2004). Iprodione also has antiandrogenic activity but appears to act through a different mechanism (Wolf et al. 1999).

ATTACHMENTS

In order to provide the Committee with materials relevant to the evaluation of the carcinogenic potential of vinclozolin, attached are: [NOTE: Documents are posted in the form they were provided to OEHHA. If you are unable to access or read these documents, please contact Cynthia Oshita with the Proposition 65 Implementation office at coshita@oehha.ca.gov or (916) 445-6900 to request a copy in an alternative format. OEHHA will provide documents in alternative formats when possible.]

Attachment I. *Memorandum on the Carcinogenicity Peer Review of Vinclozolin (2nd).* September 18, 1996. U.S. EPA Office of Pesticide Programs Health Effects Division Carcinogenicity Peer Review Committee (CPRC). This memorandum documents the CPRC's evaluations of vinclozolin on August 30, 1995 and April 17, 1996 and includes data summaries from two sets of rat studies (two-year carcinogenicity studies and two-year chronic toxicity studies) and from 18-month mouse carcinogenicity studies. It reports results of genotoxicity studies and reviews structure-activity relationships.

Attachment II. *Memorandum on the Carcinogenicity Peer Review of Vinclozolin (3rd).* April 3, 1997. U.S. EPA's Office of Pesticides Programs Health Effects Division CPRC. This memorandum reports on the preliminary findings from the Registrant's re-evaluation of the pathology slides for tumors of the ovary and prostate, and the CPRC's re-evaluation of vinclozolin's carcinogenic potential on January 15, 1997. It also includes a summary of recommendations from a joint FIFRA Scientific Advisory Panel and Scientific Advisory Board meeting on vinclozolin.

Attachment III. Cancer Assessment Document. *Evaluation of the Carcinogenic Potential of Vinclozolin (Fourth Review)* P.C. Code: 113201. Final Report. June 20, 2000. Cancer Assessment Review Committee. Health Effects Division. Office of Pesticide Programs. This document includes a summary of the Pathology Working Group's evaluation of the re-examination of the ovary and prostate tumor pathology.

Attachment IV. The following articles are attached:

Hoshiya et al. 1993. Enhancement by non-mutagenic pesticides of GST-P positive hepatic foci development initiated with diethylnitrosamine in the rat. *Cancer Letters* 72:59-64.

Hrelia et al. 1996. The genetic and non-genetic toxicity of the fungicide vinclozolin. *Mutagenesis* 5:445-453.

Ito et al. 1994. Medium-term rat liver bioassay for rapid detection of carcinogens and modifiers of hepatocarcinogenesis. *Drug Metabolism Reviews* 26: 431-442.

Kevekordes et al. 1996. Genotoxicity of selected pesticides in the mouse bone-marrow micronucleus test and in the sister-chromatid exchange test with human lymphocytes in vitro. *Toxicology Letters* 89:35-42.

Kojima et al. 2004. Screening of estrogen and androgen receptor activities in 200 pesticides by *in vitro* reporter gene assays using Chinese hamster ovary cells. *Environ Health Perspect* 112:524-531.

Lioi et al**.** 1998. Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to gliphosate, vinclozolin, atrazine, and DPX-E9636. *Environmental and Molecular Mutagenesis* 32:39-46.

Lioi et al. 1998. Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. *Mutation Research* 403:13-20.

Lodovici et al. 1997. Oxidative liver DNA damage in rats treated with pesticide mixtures. *Toxicology* 117:55-60.

Perocco et al. 1993. In vitro cytotoxic and cell transforming activities exerted by the pesticides cyanazine, dithianon, diflubenzuron, procymidone and vinclozolin on BALB/c 3T3 cells. *Environmental and Molecular Mutagenesis* 21:81-86.

Radice et al. 1998. Adaptation to oxidative stress: effects of vinclozolin and iprodione on the HepG2 cell line. *Toxicology* 129:183-191.

U.S. Environmental Protection Agency (U.S. EPA) 1994. *Carcinogenicity Peer Review of Iprodione*. Health Effects Division, Office of Prevention, Pesticides and Toxic Substances, Washington, DC.

U.S. Environmental Protection Agency (U.S. EPA) 1991. *Peer Review of Procymidone: Consideration of Science Advisory Panel Recommendations.* Health Effects Division Peer Review Committee, Health Effects Division, Office of Pesticides and Toxic Substances. Washington DC.

Wolf et al. 1999. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p′-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicol Ind Health* 15:94-118.

Wong et al. 1995. Androgen receptor antagonist versus agonist activities of the fungicide vinclozolin relative to hydroxyflutamide. *J Biol Chem* 34:19998-20003.

Wu et al. 2005. Vinclozolin, a widely used fungizide, enhanced BaP-induced micronucleus formation in human derived hepatoma cells by increasing CYP1A1 expression. *Toxicology Letters* 159:83-88.