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

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

TXR: 0052913

DATE: March 29, 2005

SUBJECT: **Propiconazole:** Oncogenicity in Male Mice
P.C. Code: 122101 Reregistration Case #: 3125

FROM: Abdallah Khasawinah, Ph.D., Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

TO: Jacqueline Guerry
Reregistration Section
Special Review and Reregistration Division (7508C)

THRU: Susan V. Hummel, Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

TASK ID: DP Code: D276672, D279111
MRID: 45215804 , 45215805 , 45516501

Registrant: Syngenta (formerly Norvartis Crop Protection, Inc.) , 410 Swing Road, Greensboro, NC 27419

Action Requested: Review of:

1) 18-month oncogenicity study in mice - amendment (MRID 45215804)
2) Report on rationalefor the use of MOE approach for regulation (MRID 45215805).
3) Triazoles: The inhibition of isozymes of cytochrome P-450 (MRID 45516501).

Agency's Response: HED has reviewed these reports and the findings are presented below:

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1. **18-Month Oncogenicity Study in Mice - Amendment**

Gerspach, R. 1999. CGA-64250 Technical: Final Report Amendment - 18-Month Oncogenicity Study in Mice. Novartis Crop Protection (Switzerland), Study No. 943126. Novartis No. 800-97, November 11, 1999. MRID 45215804. Unpublished. No DER was prepared for this report.

This is an amendment to the final report of the 18-month oncogenicity study in mice (MRID 44381401) concerning the spontaneous occurrence of liver tumors in CD-1 mice. In the 18-month oncogenicity study (conducted November 23, 1994 - June 21, 1996), it was concluded that CGA 64250 (propiconazole) resulted in an increased liver tumor incidence at the dietary dose of 850 ppm (highest dose tested) when compared to concurrent controls (20% adenomas and 4% carcinomas vs 2% adenomas and 2% carcinomas, respectively in the concurrent control in the male mice). The historical control data submitted with the study at the time was considered inadequate by the HED reviewers because "the collection dates were not specified and were not collected in the testing facility". In the current submission (MRID 45315804, conducted April 21, 1997 - November 9, 1998), additional historical control data for the CD-1 mice was generated under the same experimental and environmental conditions of the 18-month oncogenicity study (MRID 44381401), and the incidence of hepatocellular adenomas and carcinomas was reported. The new historical control data was generated from CD-1 mice obtained from IFFA-Credo France while in the propiconazole study, the mice were obtained from Charles River Deutschland.

Five groups of 70 CD-1 mice (IFFA-Credo France)/sex were kept under standard laboratory conditions on control diet for 18 months. 10 mice/sex/group were used for evaluation of hematological parameters and 10 mice/sex/group were used for evaluation of clinical chemistry and urinary parameters. 50 mice/group/sex were used for the evaluation of the natural incidence of liver neoplasia at the end of 18 months. Additional groups of 10 mice/sex/group were kept under similar conditions for 3 month interim sacrifice. A variety of different primary neoplastic lesions including hepatocellular adenoma (6-18%) and hepatocellular carcinoma (8-16%) corresponding to 14-30% male CD-1 mice bearing hepatocellular neoplasia, hemangioma (2%) and hemangiosarcoma (2%), and foci of cellular alteration (2-4%) were observed in the livers of males. A summary table is presented below (Table 1). Neither primary neoplasia nor foci of cellular alteration were observed in the female livers. "All livers in the all animals were reevaluated by an internal peer review procedure, performed by the reviewing pathologist. The findings reflect the mutually agreed on diagnosis".

The report is described as "preliminary histopathology subreport " and does not contain detailed results. "The detailed experimental procedure will be described in the final report of the study". Individual animal data is not reported. No clinical or gross pathological data is presented. A determination on this report acceptability cannot be determined at this time pending the submission of a final and complete report.

The report's introduction refers to published data from the USA and Europe regarding the incidence of hepatocellular lesions in CD-1 male mice (Table 2).

The new data, though preliminary, demonstrated spontaneous neoplastic lesions including hepatocellular adenoma (6-18%) and hepatocellular carcinoma (8-16%) in the livers of males that are more consistent with previous historical control data for the CD-1 mouse. It also indicates that the incidence of spontaneous neoplasm in the control of the second carcinogenicity propiconazole mouse study (MRID 44381401) may be low. The tumor incidence observed in male livers at the 850 ppm dose is within the range of the new historical data.

Table 1. Pathology data in historical control male CD-1 mice in % (MRID 45215805)

Organ: Liver	Control 1	Control 2	Control 3	Control 4	Control 5
Focus of alteration	4	4	4	4	2
Hepatocelluar adenomas	18	14	6	8	14
Hepatocellular adenocarcinomas	12	16	10	8	14
Hepatocelluair neoplasia	30	24	14	14	26
Hemangioma					2
Hemangiosacrcoma	2			2	

Table 2. Published Historical Control Data

	France[1]	Germany[2]	USA[3]	USA[4]
Hepatocelluar adenomas	6-18	0-19.4	0-19.2	5.6-26.4
Hepatocellular adenocarcinomas	0-12	4-17.6	1.3-11.5	5.7-19.1

[1] Vandenberghe, J. 1993. Historical control data of toxicity studies with SPF Swiss mice (CD-1); Charle River.
[2] Registry for Industrial Toxicology Animal Data. 1991-1997. Fraunhofer Inst. of Tox. and Aerosol Research, Hanover, FRG. WHO/IARC, Lyon, France Version 2.231
[3] Lang, PL. 1995. Spontaneous neoplastic lesions in the Crl:CD-1 (ICR)BR mouse; Charles River.
[4] Harada t *et al.* 1996. Pathobiology of the aging mice, vol II, ILSI press, Washington DC

2. **Rationale for the Use of Margin-of-Exposure Approach for Regulation**

Chow E. 2000. Propiconazole Technical (CGA-64250): Final Report. Rationale for the Continued Use of the Margin-of-Exposure Approach for Regulation. Toxicology and Human Safety Assessment, Novartis Crop Protection, Inc. Greensboro, NC. Novartis No. 1185-00. MRID 452158-05. September 15, 2000. Unpublished Report. 303 pages. No DER was prepared for this report.

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This report presents the Registrant's rationale for using the margin-of exposure approach for regulation of propiconazole. The Registrant discusses several studies on propiconaxole (oncogenicity, mutagenicity, hepatocellular proliferation, enzyme induction, subchronic toxicity, toxicokinetics) that have been submitted and reviewed previously by HED in addition to many published studies. The key points in this report are:

- Increased liver adenoma and carcinoma incidence occurred in male mice at a dose of 2500 ppm causing excessive liver toxicity and mortality.

- Increased liver adenoma incidence in male mice in a second oncogenicity study at 850 ppm dose causing excessive effects on liver (necrosis) and body weight gain. The Registrant contends that although this incidence was higher than the historically low liver tumor incidence in the concurrent control group, it was not biologically significant.

- In a 17-week and 13-week studies in mice, there was a dose dependent liver toxicity (necrosis) at 500 ppm or higher with a NOAEL of 20 ppm.

- In an enzyme induction study in rats and mice, P450 enzyme induction was much more marked in mice than in rats and no induction of P450 isozyme was seen at 20 mg/kg.

- In a 14-enzyme induction study in mice, marked induction of cytochrome P450 isozymes of up to 5000% at 2500 ppm. The magnitude and profile of P450 isozyme induction was identical tho that of phenobarbital.

- In a 60-day hepatocyte proliferation study in mice, there was a marked increase in hepatocyte prolifiration especially over the first two weeks of the study. The time course and magnitude of effects were very similar to that caused by phenobarbital. Liver necrosis and hypertrophy also appeared almost immediately upon exposure.

A mechanism for the tumor induction in male mice is presented in this report. It is based on the combined mitogenic, excessive P450 inductive and hepatotoxic effects of propoiconazole leading to early hepatocyte damage (accumulation of irreversibly altered cells) leading to progressive pre-neoplastic changes (clonal expansion of pre-neoplastic cells) and eventually leading to hepatocellular tumor formation (finding at excessive dose).

The registrant concludes that propiconazole induces liver tumors in male mice by threshold-based mechanism because it is not mutagenic and it induces liver tumors in male mice only, which is "known to be uniquely susceptible to liver tumor induction". Propiconazole hepatotoxicity profile is compared to that of phenobarbital and is considered to be identical to it - a rodent carcinogen that is not carcinogenic to humans. The report also concludes that a "threshold-based mode of action is of negligible relevance to humans" and that it "should continue to be regulated by a margin-of exposure approach based on the most sensitive toxicity endpoint (stomach irritation in a chronic dog study), which has a NOAEL of 1.25 mg/kg/day

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which is more than two orders of magnitude lower than the spurious tumor response in male mice" yielding a chronic RfD of 0.013 mg/kg/day. In support of the registrant conclusions, the Registrant included in this report the "expert opinion of three independent hepatocarcinogenesis experts". These are Dr. Byron E. Butterworth Iconsultant), Dr. James E. Klauing (Profesor and Director of Toxicology-Indiana University) and Dr. Randy L Jirtle (Duke University Medical Center).

It should be noted that in the latest HED review of propiconazole (HIARC 2003), HIARC selected an RfD of 0.1 mg/kg/day based on a NOAEL of 10 mg/kg/day derived from the 24 month mouse oncogenicity study where the endpoint of toxicity was based on liver toxicity which is 10 times higher than RfD proposed by the Registrant based on a one year dog study. The one year dog study was considered by HIARC but was not selected because the stomach irritations were considered to be local effects and not systemic toxicity and the target organ of liver toxicity seen in rats and mice was not seen in dogs.

Based on the findings in these studies and the scientific published literature, the Registrant advanced a mechanism for the tumor induction in male mice based on the combined mitogenic, excessive P450 inductive and hepatotoxic effects of propoiconazole leading to early hepatocyte damage (accumulation of irreversibly altered cells) leading to progressive pre-neoplastic changes (clonal expansion of pre-neoplastic cells) and eventually leading to hepatocellular tumor formation at excessive doses and that propiconazole should continue to be regulated by a margin-of-exposure approach (MRID 45215805). While we have not referred completely this new data to our CARC, the rationale supported by the registrant seems reasonable and consistent with our previous classification of possible human carcinogen using the RfD for risk assessment and as being protective of the carcinogenic effect.

3. **Triazoles: The Inhibition of Isozymes of Cytochrome P-450**

Stevens JT. 2001. Triazoles: The Inhibition of Isozymes of Cytochrome P-450. Syngenta Crop Protection. 370 p. with the appendix. Oct. 5, 2001. MRID 45516501. No DER was prepared for this report.

This report is a 15 page literature analysis by the Registrant's scientist of 17 published articles with an appendix of these articles to demonstrate a relationship between the *in vitro* inhibition potency of Cytochrome P450 isozymes by certain azole compound and their *in vivo* toxicity. This review is not specific to propiconazole, it includes three triazoles (penconazole, propiconazole and anastrozole) and two imidazoles (imazalil and ketoconazole). According to this review, a number of cytochrome P450 dependent enzymes are major targets for inhibitors for sterol biosynthesis such as the triazole and imidazole compounds. The review lists *in vitro* screening systems used for evaluating the inhibitory effects of azoles on different P450 isozymes and provides comparative I_{50} values for this inhibition by the 5 azoles listed. Conclusions regarding the differences in the inhibition specificity by these 5 chemicals were not possible based on this *in vitro* data according to the author. A summary table of the *in vivo* hazard profile

(developmental, reproductive toxicity, genotoxicity and carcinogenicity) of the 5 compounds is presented to correlate if the *in vitro* inhibition of any of the desirable isozymes or non-target enzymes manifest themselves as an adverse *in vivo* effect. For propiconazole and peconazole the liver was the target organ of toxicity with propiconazole producing liver tumors in male mice at high doses, yet propiconazole was not a potent liver microsomal (CYP_{14DM}) inhibitor. However, ketoconazole, an *in vitro* inhibitor of testicular 17 α-hydroxylase and placental aromatase (CYP_{19}) exhibited developmental toxicity but no indication of genotoxicty or carcinogenicity. Imazalil, an *in vitro* inhibitor of testicular 17 α-hydroxylase, adrenal mitochondrial 11 β-hydroxylase and liver CYP_{14DM} exhibited liver toxicity including tumors in mice, and fetal mortality and implantation loss. Anastrozole, a potent and specific placental aromatase (CYP_{19}) inhibitor and a weakly testicular 17 α-hydroxylase inhibitor was not surprising to find it manifesting developmental effects in rats and rabbits and reproductive effects of survival impairment of the litters of rats and causing endocrine tumors in rats and mice though it is not genotoxic. The author's conclusion was that the type of *in vitro* cytochrome P450 inhibition by an azole compound might be associated to its *in vivo* hazard profile and vice versa.

The probable association between the in vitro P450 inhibition by the azole compounds and their in vivo toxicity as described by the author seems to be weak at best. The significance of such association does not seem to be relevant to the hazard assessment of a particular azole compound. It may be useful in providing understanding some structural/activity relationship.

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