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014096

Chemical:	**Propiconazole**
PC Code:	**122101**
HED File Code	**13000 Tox Reviews**
Memo Date:	***03/22/2001***
File ID:	**TX014523**
Accession Number:	***412-02-0006***

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12/04/2001

Image 11

014523

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

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: March 22, 2001

SUBJECT: Propiconazole

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

TO: Eric Olson/Robert McNally (PM-60)
Reregistration Section
Special Review and Reregistration Division (7508C)

THRU: Sanjivani Diwan, Ph.D., Senior Toxicologist
and
Susan V. Hummel, Ph.D., Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

TASK ID: DP Code:D270388 Submission: S588082
P.C. Code: 122101 MRID: 45215802 & 45215803
Case: 816533 Chemical: Propiconazole

Registrant: Norvartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

Action Requested: Review hepatic cellular proliferation (MRID 45215802) and hepatic biochemical parameters studies in mice (MRID 45215803).

Agency's Response: HED has reviewed the above two studies (See the attached DERs). Both studies are classified **acceptable/non-guideline**. CGA 64250 or Propiconazole induced liver tumors in male mice only (MRID 00129570; 44381401). These tumors were probably mediated by a non-genotoxic mechanism unique to mouse. An increase in liver weights, and induction of

metabolizing enzymes was reported in mice and rats with CGA 64250 (MRID 45215802). The hepatotrophic effects of CGA 64250 in male mice were characterized later in a 14 day study and compared with those produced by phenobarbital, a known liver tumor promoter (MRID 45215803). After treatment with CGA 64250 for 14 days, there was increase in liver weights. The hepatomegaly was accompanied by induction of microsomal cytochrome P450 isoenzymes of subfamilies Cyp2b and Cyp3a. Simultaneous increase in activities for the microsomal mixed function oxidase reactions 7-pentoxyresorufin O-dependentylation, coumarin 7-hydroxylation, total testosterone oxidation as well as oxidation of testosterone to 2β-, 6 α-, 6β-, 15β-, 16β-hydroxy testosterone and androstenedione were observed. A moderate induction of microsomal epoxide hydrolase as well as a slight induction of UDP-glucuronosyltransferase and cytosolic glutathione S-transferase were also detected. The effects observed were dose-dependent. CGA 64250 appears to be a strong phenobarbital-type inducer of liver metabolizing enzymes. Findings of the hepatic cellular proliferation (MRID 45215802) and hepatic biochemical parameters studies in mice (MRID 45215803) are summarized below:

1. MRID 45215802 EXECUTIVE SUMMARY: In a nonguideline hepatocellular proliferation study conducted to investigate the mechanism of liver tumor induction observed at same dosage levels in oncogenicity studies, groups of 40 male CD-1 mice were given propiconazole (purity 92.4%, Lot. No. OP.303011) in the diet at concentrations of 0.0, 850, or 2500 ppm (0, 127 or 353 mg/kg/day) for up to 60 days. For cell proliferation studies an additional group of 40 mice were fed diets containing 850 ppm of the known tumor promoter phenobarbital (PB). Approximately two hours before sacrifice on days 1, 2, 3, 4, 7, 14, 28, or 60, five mice/group were given a single IP injection of 100 mg/kg BrdU (bromodeoxyuridine) in saline. At sacrifice, the animals were weighed and the liver removed, weighed, and sections prepared for serial review by Hematoxylin and Eosin and BrdU-immunohistochemical staining.

Treatment with the test material did not induce premature deaths, clinical signs of toxicity, or effects on body weight or food efficiency. However within two days, the test material did induce a dose-related increase in absolute and relative liver weights. The amount of the increase stabilized after three days of treatment in the propiconazole 850 ppm group, after 14 days of treatment in the 2500 ppm group, and after 4 days in the PB group. Microscopically, all mice developed time- and dose-related hepatocellular hypertrophy within 24 hours of treatment with propiconazole or PB that persisted through the remainder of the study. The hypertrophic effect for all treatment groups was located primarily in the centrilobular hepatocytes with mild effects in the midzonal hepatocytes. The other treatment-related changes note in the liver consisted of necrosis, and cytoplasmic vacuolation. In addition, increased mitotic activity was observed in all propiconazole and PB groups; the maximum activity was observed following two days of treatment and lasted for up to 25 days. Minimal to moderate hepatocellular necrosis of hypertrophic single or focal cells was found predominately in the 2500 ppm propiconazole and 850 ppm PB treatment groups.

Treatment with propiconazole or PB induced a >1000% increase in BrdU-staining hepatocellular nuclei within 24 hours from the start of the study that peaked at a >3600% increase by 48 hours.

Thereafter, the number of BrdU-stained nuclei decreased dramatically and was not biologically different from controls 7 days after the start and through the remainder of the study. For all treatment groups, the BrdU-staining nuclei were found primarily in the centrilobular/midzonal portions of the liver. These data support the conclusion that propiconazole induced an initial time- and dose-related proliferation in the liver followed by a sustained treatment-related hypertrophy in a manner similar to the known hypertrophic agent PB. The hepatomegaly was attributed to a sharp and transient induction of hepatocellular proliferation as well as to a time- and dose-related increase in the severity of hepatocellular hypertrophy.

2. MRID 45215803 EXECUTIVE SUMMARY: In a nonguideline hepatic biochemical parameter study conducted to determine the type of cytochrome P450 induction, groups of six male CD-1 mice were given propiconazole (purity 92.4%, Lot. No. OP.303011) in the diet at concentrations of 0, 850, or 2500 ppm (0, 149, and 578 mg/kg/day, respectively). An additional group of six male mice were fed diets containing 850 ppm (145 mg/kg/day) phenobarbital (PB). The mice were fed the diets for 14 days, after which they were sacrificed, the livers excised, weighed, and homogenized, and cytosolic and microsomal fractions prepared. The microsomal and cytosolic fractions were then submitted for protein analysis, and the microsomal activities of total cytochrome P450, 7-ethoxyresorufin (EROD) activity, 7-pentoxyresorufin-O-dealkylase (PROD) activity, coumarin 7-hydroxylase activity, lauric acid 11- and 12-hydroxylation activity, UDP-glucuronosyltransferase (UDPGT) activity, and epoxide hydrolase activity determined. In the cytosol, the activity of glutathione S-transferase (GST) was measured. In addition, microsomal regio-and stereoselective testosterone hydroxylation were measured. Finally, the microsomes were analyzed by immunoblot analysis for the proteins associated with the subfamilies of CYP1A, CYP2B, CYP3A, and CYP4A enzymes.

Treatment with the test material did not induce premature deaths, clinical signs of toxicity, or effects on body weight or food efficiency, however, absolute and relative (to body weight) liver weights of all mice treated with propiconazole or PB were increased (150% - 20% of control). Total cytochrome P450 activity was increased significantly (300-390% of the controls) by propiconazole treatment at 850 and 2500 ppm, respectively. EROD activity, indicative of CYP1A1 induction was increased slightly but not to the extent observed following true induction. Lauric acid hydroxylation, specifically a result of peroxisome proliferation, was not induced by propiconazole. However, the activity of PROD, associated with CYP2B or PB-type induction, was clearly increased 30-55-fold by propiconazole. Microsomal coumarin 7-hydroxylase, associated with enzymes belonging to the subfamily CYP2A was also induced by propiconazole treatment consistent with PB-like induction. The microsomal activities of epoxide hydrolase and UDPGT and the cytosolic activity of GST were slightly increased with propiconazole treatment. The pattern of microsomal and cytosolic enzyme induction determined biochemically was entirely consistent with PB-type induction.

Treatment at both levels of propiconazole resulted in a marked increase of total testosterone oxidation. In this study, the greatest amount of hydroxylation induced by propiconazole occurred at the 2β-, 6α-, 6β-, 15β-, 16β-positions with an increased formation of androstenedione.

This pattern of hydroxylation is associated with increased activities in the CYP2B and CYP3A subfamilies and is wholly consistent with PB-like induction.

Immunoblot analyses with monoclonal anti-CYP1A antibody showed that activities associated with CYP1A1 and CYP1A2 proteins (3-methyl cholanthrene (MC)-type induction) were not increased over control in mice treated with propiconazole. Likewise, propiconazole treatment did not induce the proteins associated with the CYP4A family of isozymes (peroxisome proliferator-type induction). However, propiconazole did induce the proteins associated with CYP2B (five isozymes) and the activities of the CYP3A family (two isozymes), which again is wholly consistent with a PB-type induction.

The results from the determination of microsomal and cytosolic enzyme activities, testosterone hydroxylation, and immunoblot analyses clearly show that propiconazole is not a 3-MC or mixed type inducer, but causes a pure PB-type induction of cytochrome P450 activity.

In conclusion, the effects of propiconazole treatment on mouse liver weights and liver enzymes were comparable to those produced by phenobarbital, a known liver enzyme inducer and liver tumor promoter. The authors concluded that propiconazole can thus be considered a strong phenobarbital-type inducer of xenobiotic metabolizing enzymes in the mouse liver.

cc: Ray Kent, Branch chief HED RRB4

DATA EVALUATION RECORD

014523

PROPICONAZOLE
(CGA-64250 TECHNICAL)

Study Type: HEPATIC BIOCHEMICAL PARAMETERS – MOUSE
[NON-GUIDELINE]
MRID 45215803

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-91

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

PROPICONAZOLE

Hepatic Biochemical Parameters, Non-Guideline

EPA Reviewer: A. Khasawinah, Ph.D.
Reregistration Action Branch 4 (7509C)
EPA Secondary Reviewer: Sanjivani Diwan, Ph.D.
Reregistration Action Branch 4 (7509C)

A. Khasawinah, Date 3-22-2001

Sanjivani Diwan, Date 3-22-2001

014523

DATA EVALUATION RECORD

STUDY TYPE: Hepatic Biochemical Parameters - Mouse (Non-guideline)

DP BARCODE: D270388
P.C. CODE: 122101

SUBMISSION CODE: S588082
TOX. CHEM. NO.: 323EE

TEST MATERIAL (PURITY): Propiconazole (92.4%)

SYNONYMS:, CGA-64250 tech., 1-[2-(2,4-dichloro-phenyl)-4-propyl-[1,3]-dioxolan-2-ylmethyl]-1,H,-[1,2,4]triazole

CITATION: Beilstein, P. (1998). CGA-64250 Technical (propiconazole): Final Report – Effects on biochemical parameters in the liver following administration to male mice. Toxicology/Cell Biology, Norvartis Crop Protection AG, CH-4002 Basel, Switzerland. Study No.: CB 97/22. April 7, 1998. MRID 45215803. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a nonguideline hepatic biochemical parameter study conducted to determine the type of cytochrome P450 induction (MRID 45215803), groups of six male CD-1 mice were given propiconazole (purity 92.4%, Lot. No. OP.303011) in the diet at concentrations of 0, 850, or 2500 ppm (0, 149, and 578 mg/kg/day, respectively). An additional group of six male mice were fed diets containing 850 ppm (145 mg/kg/day) phenobarbital (PB). The mice were fed the diets for 14 days, after which they were sacrificed, the livers excised, weighed, and homogenized, and cytosolic and microsomal fractions prepared. The microsomal and cytosolic fractions were then submitted for protein analysis, and the microsomal activities of total cytochrome P450, 7-ethoxyresorufin (EROD) activity, 7-pentoxyresorufin-O-dealkylase (PROD) activity, coumarin 7-hydroxylase activity, lauric acid 11- and 12-hydroxylation activity, UDP-glucuronosyltransferase (UDPGT) activity, and epoxide hydrolase activity determined. In the cytosol, the activity of glutathione S-transferase (GST) was measured. In addition, microsomal regio-and stereoselective testosterone hydroxylation were measured. Finally, the microsomes were analyzed by immunoblot analysis for the proteins associated with the subfamilies of CYP1A, CYP2B, CYP3A, and CYP4A enzymes.

Treatment with the test material did not induce premature deaths, clinical signs of toxicity, or effects on body weight or food efficiency, however, absolute and relative (to body weight) liver weights of all mice treated with propiconazole or PB were increased (150% - 20% of control).

Total cytochrome P450 activity was increased significantly (300-390% of the controls) by propiconazole treatment at 850 and 2500 ppm, respectively. EROD activity, indicative of CYP1A1 induction was increased slightly but not to the extent observed following true induction. Lauric acid hydroxylation, specifically a result of peroxisome proliferation, was not induced by propiconazole. However, the activity of PROD, associated with CYP2B or PB-type induction, was clearly increased 30-55-fold by propiconazole. Microsomal coumarin 7-hydroxylase, associated with enzymes belonging to the subfamily CYP2A was also induced by propiconazole treatment consistent with PB-like induction. The microsomal activities of epoxide hydrolase and UDPGT and the cytosolic activity of GST were slightly increased with propiconazole treatment. The pattern of microsomal and cytosolic enzyme induction determined biochemically was entirely consistent with PB-type induction.

Treatment at both levels of propiconazole resulted in a marked increase of total testosterone oxidation. In this study, the greatest amount of hydroxylation induced by propiconazole occurred at the 2β-, 6α-, 6β-, 15β-, 16β-positions with an increased formation of androstenedione. This pattern of hydroxylation is associated with increased activities in the CYP2B and CYP3A subfamilies and is wholly consistent with PB-like induction.

Immunoblot analyses with monoclonal anti-CYP1A antibody showed that activities associated with CYP1A1 and CYP1A2 proteins (3-methyl cholanthrene (MC)-type induction) were not increased over control in mice treated with propiconazole. Likewise, propiconazole treatment did not induce the proteins associated with the CYP4A family of isozymes (peroxisome proliferator-type induction). However, propiconazole did induce the proteins associated with CYP2B (five isozymes) and the activities of the CYP3A family (two isozymes), which again is wholly consistent with a PB-type induction.

The results from the determination of microsomal and cytosolic enzyme activities, testosterone hydroxylation, and immunoblot analyses clearly show that propiconazole is not a 3-MC or mixed type inducer, but causes a pure PB-type induction of cytochrome P450 activity.

In conclusion, the effects of propiconazole treatment on mouse liver weights and liver enzymes were comparable to those produced by phenobarbital, a known liver enzyme inducer and liver tumor promoter. The authors concluded that propiconazole can thus be considered a strong phenobarbital-type inducer of xenobiotic metabolizing enzymes in the mouse liver.

This study is considered **Acceptable/Non Guideline** for the determination of the mechanism of hepatocellular proliferation in the mouse.

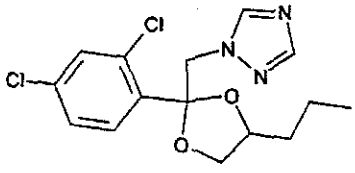
COMPLIANCE: Signed and dated Good Laboratory Practice, Quality Assurance, and Data Confidentiality statements were included with the study. Flagging statement was not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: Propiconazole

Lot No.: OP.303011
Purity: 92.4%
Description: clear, brownish, viscous liquid
Molecular weight: 342.23
Molecular formula: $C_{15}H_{17}Cl_2N_3O_2$
Structure:



2. Positive control

Phenobarbital [PB (purity 99%)]

3. Test animals

Species: mice
Strain: CD-1 (Crl:CD-1 (ICR)BR)
Sex: male
Age and weight at study initiation: 10 weeks; 31.1-37.6 g
Source: Charles River Germany, Niederlassung Sulzfeld, Germany
Housing: individually in macrolon type 2 cages
Food: NAFAG 8900 for GLP, Gossau SG, Switzerland, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 22 ± 2 °C
Humidity: 45-65%
Photoperiod: 12 hour light/dark
Acclimation period: 11 days

4. Preparation of diets

Pelleted diets containing nominal concentrations of 850 ppm and 2500 ppm test material and 850 ppm PB were prepared by Norvartis Crop Protection AG, Stein, Switzerland. During the 14-day study, the diets were stored at room temperature.

Subsamples of the prepared diets were submitted for homogeneity, stability, and dose confirmation analysis for the test material and PB.

Results –
Homogeneity: For each concentration, the deviation of individual values was within ±15% of nominal and was considered homogenous.

Stability: Propiconazole was found to be stable within the diet for the duration of the 14-day study.

Dose confirmation: The actual concentrations of propiconazole in the diets were 942 ppm and 2780 ppm and were within 11% off the nominal concentrations of 850 and 2500 ppm. The actual concentration of PB was 906 ppm, or within 7% of the nominal concentration of 850 ppm.

B. STUDY DESIGN AND METHODS

1. In life dates

Start: July 21, 1997 End: August 4, 1997

2. Animal assignment

Twenty-four mice were randomly divided into four treatment groups of 6 mice and fed diets containing 0, 850, or 2500 ppm propiconazole or 850 ppm PB for a period of 14 days.

3. Dose selection rationale

Increased incidences of hepatocellular neoplasms were found in a carcinogenicity study with male mice fed 2500 ppm propiconazole (MRID 00129570). In a second carcinogenicity study, increased incidences of hepatocellular adenomas were found in male mice fed 850 ppm propiconazole(MRID 44381401). The doses for the present study were chosen so as to cause distinct treatment-related effects without a significant increase in mortality. The phenobarbital dose was selected based on published study (Whysner *et al.* 1996. Pharmacol Therap *71: 153-191*) where doses of 500-1000 ppm (0.048 to 0.083%) in the diet induced liver enzymes strongly.

4. Statistics

The carcass, liver weight, and various biochemical parameters were analyzed by ANOVA followed by Dunnett's test ($p \leq 0.05$) to detect significant differences. Mann-Whitney tests were done to detect significant differences in testosterone hydroxylation between the four treatment groups when control results were below detection limits.

C. METHODS

1. Animal observations

All mice were observed at least once daily for treatment-related effects, moribundity and death.

2. Body weight and food consumption

Body weights and food consumption were recorded daily. In addition, daily food consumption ratios and daily intake of test material were calculated.

3. Sacrifice

Fourteen days after the start of the study, mice in all treatment groups were killed under ether anesthesia, exsanguinated, and the carcass weighed. The liver was then excised, weighed, minced, placed into a vial, quick frozen in liquid nitrogen and stored at -80°C until time of subcellular preparation.

4. Sample preparation/analyses

Subcellular fractionations were made from thawed liver slices homogenized in 10 mMol/L pH 7.5 Tris/HCl buffer. Microsomal and cytosolic liver fractions were prepared by differential centrifugation. The washed microsomal pellets were resuspended in 50 mMol/L pH 7.5 Tris/HCl buffer to a final concentration of ~0.164 g liver equivalent/mL. The cytosolic and microsomal fractions were frozen in liquid nitrogen and stored at -80°C until time of analysis.

The following analyses were done on the prepared subcellular components according to the reference procedures found in Appendix A.

Total Protein was done on cytosolic and microsomal fractions according to the method of Smith et al. (1985).

Total Microsomal Cytochrome P450 was done according to the method of Omura and Sato (1964).

Microsomal 7-Ethoxyresorufin (EROD) and 7-Pentoxyresorufin-O-dealkylase activities were done according to the method of Burke et al. (1985).

Microsomal Coumarin 7-hydroxylase activity was measured according to the method Aitio (1978).

Microsomal Regio-and Stereoselective Testosterone Hydroxylation were done according to the method of Van der Hoeven (1984).

Microsomal Lauric Acid 11- and 12-hydroxylation were measured according to the method of Orton and Parker (1992).

Microsomal UDP-glucuronosyltransferase (UDPGT) was measured using 3-methyl-2-nitrophenol as substrate as described by Mulder and van Doorn (1975).

Cytosolic Glutathione S-transferase (GST) activity was measured according to the method of Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as the substrate.

Microsomal Epoxide Hydrolase activity was measured according to the method of Oesch et al. (1971) with styrene oxide as the substrate.

Immunoblot Analyses of Microsomal Cytochrome P450 Content – Four microsomal mixtures composed of six mice/group were prepared and subjected to SDS-PAGE and monoclonal antibodies against the specific cytochrome P450 families CYP1A, CYP3A, and CYP4A according to the method of Waechter et al. (1988, Biochem. Pharmacol. *138: 57-65*) and Thomas et al. (1994, Toxicol. Appl. Pharmacol. *129:155-162*). A polyclonal antibody for the detection of CYP2B1 was also used. Densitometric scanning was used to evaluate the blots. Liver microsomes from male rats previously induced intraperitoneally with 3-methylcholanthrene (3-MC) (for CYP1a isozymes), PB (for CYP2b isozymes), pregnenolone-16α-carbonitrile (for CYP3a isozymes), and nafenopin (for CYP4A isozymes) were used as positive reference probes.

All methods used were appropriate for the determination of metabolic induction of liver enzymes.

II. RESULTS

A. OBSERVATIONS

None of the animals died and no treatment-related clinical signs of toxicity were observed during the study.

B. BODY WEIGHT AND FOOD CONSUMPTION

Although no significant treatment-related effects on body weight were found, the final carcass weight of male mice fed 850 ppm propiconazole was 7% greater (36.97 g) and male mice fed 850 ppm PB was 9% greater (37.87 g, $p<0.05$) than control animals (34.68 g). No effects were found on the final carcass weight of male mice fed 2500 ppm propiconazole (34.62 g).

The average daily food dose was 149 mg/kg/day and 578 mg/kg/day for the 850 ppm and 2500 ppm treatment groups, respectively. The study authors reported that the average

food consumption by male mice fed 2500 ppm was artificially increased due to food spillage. Mice fed 850 ppm PB received 145 mg/kg/day.

C. ORGAN WEIGHT AND PATHOLOGY

1. Liver weight

As shown in Table 1, there was a dramatic dose-related increase of absolute and relative liver weight over the 14-day study with propiconazole and phenobarbital treatment.

TABLE 1. Absolute and relative liver weights of male rats fed diets containing propiconazole or phenobarbital 14 days

Day of sacrifice	Absolute (g)			Relative to body weight (g%)		
	Mean	SD	% Cont.	Mean	SD	% Cont.
Control	2.11	0.14	–	6.06	0.24	–
850 ppm Propiconazole	2.96***	0.17	140	8.00***	0.29	132
2500 ppm Propiconazole	4.20***	0.33	199	12.15***	1.25	200
850 ppm Phenobarbital	3.44***	0.27	163	9.08***	0.42	150

Data from Table 4, page 36 of MRID 45215803
***$p \leq 0.001$

2. Liver pathology

No gross or microscopic liver pathology was done

3. Liver biochemistry

Although statistical analyses play an important role in evaluating the toxicity of xenobiotics, when it comes to interpreting studies of this type, they must be used in the context of biological relevance. As shown in Table 2, treatment with propiconazole or PB had little effect on hepatic cytosolic or microsomal protein concentrations. However, total cytochrome P450 activity was increased from 239% - 389% by propiconazole and PB. EROD activity, indicative of planar polycyclic aromatic hydrocarbon CYP1A1 induction such as by 3-MC, was increased slightly from 219% to 388%. This is usually observed with strong inducers of CYP2B (PB-type induction). When CYP1A1 is truly induced, activities >700-fold of control are found. The activity of PROD, associated with CYP2B or PB-type induction, was clearly increased by 30-55 fold by propiconazole and PB treatment. Microsomal coumarin 7-hydroxylase belongs to the subfamily CYP2A (PB-type inducer) and was increased 5-24 fold by propiconazole and PB treatment. Hydroxylation of lauric acid is catalyzed by the subfamily CYP4A, or the cytochrome P450 activity associated with peroxisome proliferation. When induced by known peroxisome proliferators such as clofibrate or

nafenopin, the activities associated with CYP4A are dramatically increased several thousand orders of magnitude and not the slight 160-300% increase found in this study. The microsomal activity of UDPGT and cytosolic activity of GST were slightly increased with propiconazole or PB treatment. The activities of these two Phase II enzymes are typically increased 150-200% following treatment with other inducers such as 3-MC and PB. Epoxide hydrolase was increased 170-300%, an increase lower than usually observed with true induction.

Table 3 shows the extent of regio- and stereoselective microsomal cytochrome P450-mediated hydroxylation of testosterone following treatment of male mice with 850 or 2500 ppm propiconazole or 850 ppm PB. Treatment at both levels of propiconazole resulted in a marked increase of total testosterone oxidation (440 & 555% at 850 and 2500 ppm propiconazole, respectively). Total testosterone oxidation was 356% by PB. Oxidation at the 2β-, 6α-, 6β-, and 15β-positions and formation of androstenedione were the most induced reactions with oxidation at the 2α- and 16β- positions near the limit of detection, but increased. Differences between propiconazole and PB induction included the lack of 2α- hydroxylation and an increased oxidation at the 7α-position by PB.

Table 4 shows the relative band intensities recorded from the immunoblot analyses of male mouse microsomes induced with 850 or 2500 ppm propiconazole or 850 ppm PB. Immunoblot analysis with monoclonal anti-CYP1A antibody showed that CYP1A1 and CYP1A2 activities were not increased over control in mice with propiconazole or PB treatment. Likewise, test material and PB treatment did not induce the activities of the CYP4A family of isozymes. However, both propiconazole and PB induced the activities of the CYP2B (five isozymes) and the activities of the CYP3A family (two isozymes).

TABLE 2. Effect of propiconazole or phenobarbital treatment on hepatic microsomal and cytosolic protein and enzyme activities

Parameter	Control	Propiconazole		PB
		850 ppm	2500 ppm	850 ppm
Microsomal Protein (mg/g)	21.57	23.41	24.48	21.02
Cytosolic Protein (mg/g)	109.9	106.8	102.9	101.3*
Cytochrome P450 (nmol/g)	18.46	55.46*** (300)[a]	71.90*** (389)	44.21*** (239)
EROD (nmol/min/g)	1.771	3.885*** (219)	6.871*** (388)	4.111*** (232)
PROD (nmol/min/g)	0.328	9.92*** (3024)	18.13*** (5524)	11.59*** (3534)
Coumarin 7-hydroxylase (nmol/min/g)	0.594	3.17*** (534)	14.16*** (2384)	2.850*** (480)
Lauric Acid 11-hydroxylase (nmol/min/g)	13.57	36.25*** (267)	41.10*** (305)	36.81*** (271)
Lauric Acid 12-hydroxylase (nmol/min/g)	11.72	17.88** (153)	18.82** (161)	19.06** (163)
Microsomal UDPGT (nmol/min/g)	1241	1935*** (156)	1720*** (139)	1942*** (156)
Cytosolic GST (nmol/min/g)	553.0	875*** (158)	1019*** (184)	1036*** (187)
Epoxide Hydrolase (nmol/min/g)	84.6	148.8*** (172)	278.0*** (321)	154.9*** (179)

Data from Tables 5-7 and 9 on pages 37-39 and 41 of MRID 45215803
[a]Results in parentheses are % of control when statistically significant
**p≤0.01, ***p≤0.001

TABLE 3. Effect of propiconazole or phenobarbital treatment on testosterone hydroxylation/oxidation activities by liver microsome fractions

Testosterone Metabolite	Activity (nmol/min/g)			
	Control	Propiconazole		PB
		850 ppm	2500 ppm	850 ppm
2α-OH	ND	2.73** (NA)	2.69** (NA)	ND
2β-OH	2.64	7.88*** (298)	14.01*** (531)	12.30*** (466)
6α-OH	3.36	16.88*** (502)	24.03*** (715)	26.19*** (779)
6β-OH	21.72	79.64*** (366)	113.68*** (524)	108.70*** (500)
7α-OH	5.64	9.85 (175)	7.06 (125)	12.92* (229)
15β-OH	1.45	4.58*** (316)	14.14*** (977)	7.61*** (526)
16α-OH	4.28	9.15*** (214)	11.19*** (262)	8.31*** (194)
16β-OH	ND	5.17** (NA)	6.42** (NA)	5.73** (NA)
Androstenedione	24.05	141.78*** (589)	156.81*** (652)	43.11*** (179)
Total	63.14	277.55*** (440)	350.21*** (555)	224.88*** (356)

Data from page 40 of MRID 45215803
[a]Results in parentheses are % of control
ND=Not detectable
NA=Statistical significance determined nonparametrically
**p≤0.01, ***p≤0.001

TABLE 4. Immunoblot of cytochrome P450 isozymes following treatment with propiconazole or phenobarbital						
	Band Intensity (Relative Area Units) Antibody					
Treatment	Monoclonal Anti-CYP1A	Polyclonal AntiCYP2B1[a]			Monoclonal Anti-CYP3A	Monoclonal Anti-CYP4A
Control	943	90	52	580	179	628
850 ppm Propiconazole	1167	2347	421	2544	1178	880
2500 ppm Propiconazole	838	2744	301	3576	1911	984
850 ppm PB	809	2469	302	2034	1032	811
Reference Microsomes						
3-methylchloranthene	3955/5788	ND	ND	ND	ND	ND
PB	ND	2403	478	1615	ND	ND
Pregnenolone 16α-carbonitrile	ND	ND	ND	ND	1748	ND
Nafenopin	ND	ND	ND	ND	ND	14,524/2104

Data from p. 42 of MRID 45215803
ND=Not done
[a]The polyclonal antiserum recognized 5 isozymes. The third column represents the sum of a larger and two smaller bands.

III. DISCUSSION

A. DISCUSSION

In a study designed to determine the biochemical type of liver induction induced by the test material, none of the male mice fed diets containing 850 ppm or 2500 ppm propiconazole died and there were no treatment-related clinical signs of toxicity or effects on body weight during the 14-day study. But as reported in other studies, the absolute and relative liver weights of male mice fed 850 or 2500 ppm propiconazole or 850 ppm PB were significantly increased 130-200%.

Total cytochrome P450 activity was increased significantly (230% - 390% of the controls) by propiconazole treatment at 850 and 2500 ppm, respectively, suggesting the increase in liver weight was due to hepatocellular hypertrophy. EROD activity, indicative of planar polycyclic aromatic hydrocarbon CYP1A1 induction, was increased slightly but not nearly to the extent observed following true induction. Lauric acid hydroxylation, a specific result of peroxisome proliferation, clearly was not induced by propiconazole. However, the activity of PROD, associated with CYP2B or PB-like induction, was clearly increased 30-55 fold by propiconazole. Microsomal coumarin 7-hydroxylase, associated with enzymes belonging to the subfamily CYP2A, was also induced by propiconazole treatment 5-24 fold consistent with PB-like induction. The microsomal activities of epoxide hydrolase and UDPGT and the cytosolic activity of GST and were slightly

increased with propiconazole. The activities of these Phase II enzymes are typically increased 150-200% following treatment with other inducers such as 3-MC and PB. In conclusion, the pattern of microsomal and cytosolic enzyme induction determined biochemically is entirely consistent with PB-like induction.

Treatment at both levels of propiconazole resulted in a marked increase of total testosterone oxidation (440 & 555% at 850 and 2500 ppm propiconazole, respectively), while that of PB was 356%. Typically, oxidation of testosterone at the 2α-, 7α- and 16β-positions reflect the activities of CYP2C1, CYP2A1, and CYPB1/2, respectively, while hydroxylation at the 2β-, 6β- and 15β-positions reflect the activities of CYP3A1 and/or CYP3A2. In this study, the greatest amount of hydroxylation induced by propiconazole occurred at the 2β-, 6α-, 6β-, 15β-, and 16β-positions and in increased formation of androstenedione. (Androstenedione formation is mediated solely by the CYP2B subfamily of isozymes.) This pattern of hydroxylation is associated with increased activities with the CYP2B and CYP3A subfamilies and is entirely consistent with PB-like induction. The hydroxylation pattern of PB was consistent with propiconazole, with slight discrepancies in the formation of 2α-hydroxylation and in the formation of androstenedione.

Immunoblot analysis with monoclonal anti-CYP1A antibody showed that activities associated with CYP1A1 and CYP1A2 proteins (3-MC-type induction) were not increased over control in mice treated with propiconazole. Likewise, propiconazole treatment did not induce the proteins associated with the CYP4A family of isozymes (peroxisome proliferator-type induction). However, propiconazole did induce the proteins associated with CYP2B (five isozymes) and the activities of the CYP3A family (two isozymes); consistent with a PB-type induction.

The results from the determination of microsomal and cytosolic enzyme activities, testosterone hydroxylation, and immunoblot analyses clearly show that propiconazole is not a 3-MC or mixed type inducer, but induces a pure PB-type induction of cytochrome P450 activity.

In conclusion, the effects of propiconazole treatment on mouse liver weights and liver enzymes were comparable to those produced by phenobarbital, a known liver enzyme inducer and liver tumor promoter. Propiconazole can thus be considered a strong phenobarbital-type inducer of xenobiotic metabolizing enzymes in the mouse liver.

This study is considered **Acceptable/Non Guideline** for the determination of the mechanism of hepatocellular proliferation in the mouse.

B. STUDY DEFICIENCIES

No deficiencies that would affect the interpretation of the study were found.

APPENDIX A

REFERENCES

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DATA EVALUATION RECORD

014523

PROPICONAZOLE

Study Type: HEPATOCELLULAR PROLIFERATION – MOUSE (NON-GUIDELINE)
MRID 45215802

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-91

Primary Reviewer: H. T. Borges, MT(ASCP),Ph.D., D.A.B.T.	Signature: Date:	H T Borges JAN 23 2001
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Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross JAN 23 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson JAN 23 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

PROPICONAZOLE **Hepatocellular Proliferation, Non-Guideline**

EPA Reviewer: Abdallah Khasawinah, Ph.D. _____, Date 3-12-2001
Reregistration Action Branch 4 (7509C)
EPA Secondary Reviewer: Sanjivani Diwan, Ph.D. Sanjivani Diwan Date 3/13/2001
Reregistration Action Branch 4 (7509C)

014523

DATA EVALUATION RECORD

STUDY TYPE: Hepatocellular Proliferation - Mouse (Non-guideline)

DP BARCODE: D270388 SUBMISSION CODE: S588082
P.C. CODE: 122101 TOX. CHEM. NO.: 323EE

TEST MATERIAL (PURITY): Propiconazole (92.4%)

SYNONYMS:, CGA-64250 tech., 1-[2-(2,4-dichloro-phenyl)-4-propyl-[1,3] dioxolan-2-ylmethyl]-1,H,-[1,2,4]triazole

CITATION: Weber, E. (1999). CGA-64250 Assessment of hepatic cell proliferation in male mice. Toxicology/Cell Biology, Norvartis Crop Protection AG, CH-4002 Basel, Switzerland. Study No.: CB 97/23. September 1, 1999. MRID 45215802. Unpublished.

SPONSOR: Norvartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a nonguideline hepatocellular proliferation study (MRID 45215802) conducted to investigate the mechanism of liver tumor induction observed at same dosage levels in oncogenicity studies, groups of 40 male CD-1 mice were given propiconazole (purity 92.4%, Lot. No. OP.303011) in the diet at concentrations of 0.0, 850, or 2500 ppm (0, 127 or 353 mg/kg/day) for up to 60 days. For cell proliferation studies an additional group of 40 mice were fed diets containing 850 ppm of the known tumor promoter phenobarbital (PB). Approximately two hours before sacrifice on days 1, 2, 3, 4, 7, 14, 28, or 60, five mice/group were given a single IP injection of 100 mg/kg BrdU (bromodeoxyuridine) in saline. At sacrifice, the animals were weighed and the liver removed, weighed, and sections prepared for serial review by Hematoxylin and Eosin and BrdU-immunohistochemical staining.

Treatment with the test material did not induce premature deaths, clinical signs of toxicity, or effects on body weight or food efficiency. However within two days, the test material did induce a dose-related increase in absolute and relative liver weights. The amount of the increase stabilized after three days of treatment in the propiconazole 850 ppm group, after 14 days of treatment in the 2500 ppm group, and after 4 days in the PB group. Microscopically, all mice developed time- and dose-related hepatocellular hypertrophy within 24 hours of treatment with propiconazole or PB that persisted through the remainder of the study. The hypertrophic effect for all treatment groups was located primarily in the centrilobular hepatocytes with mild effects in the midzonal hepatocytes. The other treatment-related changes note in the liver consisted of

necrosis, and cytoplasmic vacuolation. In addition, increased mitotic activity was observed in all propiconazole and PB groups; the maximum activity was observed following two days of treatment and lasted for up to 25 days. Minimal to moderate hepatocellular necrosis of hypertrophic single or focal cells was found predominately in the 2500 ppm propiconazole and 850 ppm PB treatment groups.

Treatment with propiconazole or PB induced a >1000% increase in BrdU-staining hepatocellular nuclei within 24 hours from the start of the study that peaked at a >3600% increase by 48 hours. Thereafter, the number of BrdU-stained nuclei decreased dramatically and was not biologically different from controls 7 days after the start and through the remainder of the study. For all treatment groups, the BrdU-staining nuclei were found primarily in the centrilobular/midzonal portions of the liver. These data support the conclusion that propiconazole induced an initial time- and dose-related proliferation in the liver followed by a sustained treatment-related hypertrophy in a manner similar to the known hypertrophic agent PB. The hepatomegaly was attributed to a sharp and transient induction of hepatocellular proliferation as well as to a time- and dose-related increase in the severity of hepatocellular hypertrophy.

This study is considered **Acceptable/Non Guideline** for the determination of the mechanism of hepatocellular proliferation in the mouse.

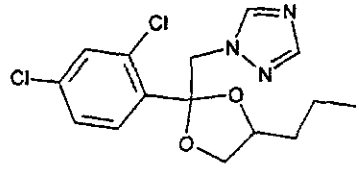
COMPLIANCE: Signed and dated Good Laboratory Practice, Quality Assurance, Flagging, and Data Confidentiality statements were included with the study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: CGA 64250 (Propiconazole)

   Lot No.: OP.303011
   Purity: 92.4%
   Description: clear, brownish, viscous liquid
   Molecular weight: 342.23
   Molecular formula: $C_{15}H_{17}Cl_2N_3O_2$
   Structure:



2. Positive Control

   Phenobarbital [PB (purity >99%)]

3. Test animals

Species: mice
Strain: CD-1
Sex: male
Age and weight at study initiation: young; 32.0-40.0 g
Source: Charles River Germany, Sulzfeld, Germany
Housing: individually in macrolon type 2 cages
Food: NAFAG 8900 for GLP, Gossau SG, Switzerland, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 22 ± 2°C
Humidity: 45-65%
Photoperiod: 12 hour light/dark
Acclimation period: 11 days

4. Preparation diets

Pelleted diets containing nominal concentrations of 850 ppm and 2500 ppm test material and 850 ppm PB were prepared by Novartis Crop Protection AG, Stein, Switzerland. During the study, the diets were stored at room temperature. Three subsamples of the prepared diets were submitted for homogeneity analysis for the test material and PB. Additional samples were sent for stability analyses after 28 and 74 days storage at room temperature.

Results –
Homogeneity: The concentrations of propiconazole and PB were within -3 to 2% of nominal concentrations in three separate samples indicating they were homogeneously distributed throughout the pellets.

Stability: Propiconazole was found to be stable within the pelleted diet for a period of at least 74 days.

Dose confirmation: - All diets were found to be within 107-111% of nominal concentrations.

The prepared diets were suitable for use in the study.

B. STUDY DESIGN AND METHODS

1. In life dates

Start: July 21, 1997 End: September 19, 1997

2. Animal assignment

One hundred sixty mice were randomly divided into 4 treatment groups of 40 mice and fed diets containing 0, 850 ppm, or 2500 ppm propiconazole or 850 ppm PB for a period up to 60 days.

3. Dose selection rationale

Increased incidences of hepatocellular neoplasms were found in a carcinogenicity study with male mice fed 2500 ppm propiconazole (MRID 00129570). In a second carcinogenicity study, increased incidences of hepatocellular adenomas were found in male mice fed 850 ppm propiconazole (MRID 44381401). The doses for the present study were chosen so as to cause distinct treatment-related effects without a significant increase in mortality. The phenobarbital dose was selected based on published study (Whysner *et al.* 1996. Pharmacol Therap *71: 153-191*) where doses of 500-1000 ppm in the diet induced hepatocyte proliferation in rodent livers.

4. Statistics

The carcass and liver weights were analyzed by ANOVA followed by Dunnett's test ($p \leq 0.05$) to detect significant differences. Mann-Whitney tests were done to detect significant differences in labeling indices between groups.

C. METHODS

1. Animal observations

All mice were observed at least once daily for treatment-related effects, moribundity and death.

2. Body weight and food consumption

Body weights and food consumption were recorded daily. In addition, daily food consumption ratios and daily intake of test material were calculated.

3. Animal preparation

Approximately two hours before sacrifice on days 1, 2, 3, 4, 7, 14, 28, and 60, five mice/group were given a single IP injection of 100 mg/kg BrdU (bromodeoxyuridine) in saline.

4. Sacrifice and pathology

At sacrifice, unfasted mice were bled under ether anesthesia, weighed, and a limited necropsy done. In particular, any grossly observable liver abnormalities were noted.

The liver was then quickly removed and weighed. One section from the left (Lobus Sinister Lateralis) and the two from the right (Lobus Dexter Medialis and Lateralis) lobes of the liver, as well as a portion of the small intestine, were excised, fixed in 4% buffered formalin, and embedded in paraffin. Serial sections of each lobe were prepared and stained with Hematoxylin and Eosin or immunohistochemically stained for BrdU-labeled nuclear DNA, both with (test) and without (negative control) the presence of anti-BrdU antibody. The small intestine was processed similarly and served as a BrdU positive control. The assessment of hepatocyte BrdU-labeled nuclear DNA was done using a semi-automated image analysis system (analySIS, Soft Imaging Systems GmbH, Münster, Germany) and the labeling index calculated after review of approximately 17,000 - 60,000 nuclei/animal.

A detailed histopathology was performed on the livers of all animals.

II. RESULTS

A. OBSERVATIONS

None of the animals died during the study and no treatment-related clinical signs of toxicity were observed.

B. BODY WEIGHT AND FOOD CONSUMPTION

No significant treatment-related effects on body weight were found as all body weights were within 7% of control throughout the study. During the first two days of the study, the food consumption of mice treated with 2500 ppm test material was slightly decreased. Thereafter, the food consumption of all propiconazole and PB-treated mice was not significantly different from control mice. During the study, the average intake of test material was 127 and 353 mg/kg/day for the propiconazole 850 and 2500 ppm groups, respectively. Mice treated with PB received ~139 mg/kg/day.

C. ORGAN WEIGHT AND PATHOLOGY

1. Liver weight

As shown in Table 1, there was a rapid and dramatic dose-related increase of absolute and relative liver weight within 1-2 days of treatment with propiconazole. The liver weight gain period was ~3 days for mice treated with 850 ppm test material while ~14 days in mice treated with 2500 ppm. After the weight gain period, the absolute and relative liver weights of both propiconazole treatment groups remained relatively constant. As expected, PB also induced an increase in absolute and relative liver weight with a weight gain period of ~4 days. As with the other treatment groups, the increase was sustained through the remainder of the 60-day study.

Table 1. Absolute and relative liver weights of male rats fed diets containing propiconazole or PB up to 60 days[a]

Day of Sacrifice	Absolute (g)				Relative to body weight (g%)			
	Control	Propiconazole		PB 850 ppm	Control	Propiconazole		PB 850 ppm
		850 ppm	2500 ppm			850 ppm	2500 ppm	
1	1.88	2.08	2.13	2.27**	5.43	5.95	6.36**	6.23*
2	1.90	2.36**	2.45**	2.58***	5.36	6.77***	7.59***	7.42***
3	2.06	2.76***	2.81***	2.96***	5.69	7.47***	8.66***	8.04***
4	1.96	2.67***	3.06***	3.18***	5.54	7.33***	9.42***	8.44***
7	2.08	2.69**	3.74***	3.00***	5.79	7.62***	11.18***	8.39***
14	2.04	2.74**	4.42***	3.20***	5.83	7.32**	12.34***	8.81***
28	1.99	2.93***	4.79***	3.49***	5.40	7.72***	12.45***	8.67***
60	2.24	3.06***	4.67***	3.27***	5.70	7.48***	11.39***	8.32***

Data from Tables 6a and 6b, p 29, MRID 45215802
*p≤0.05, **p≤0.01, ***p≤0.001
[a]Based on 5 mice/group

2. Liver pathology

All mice treated with 2500 ppm propiconazole developed grossly observable hepatomegaly within 7 days of treatment that persisted through the remainder of the study. However, grossly observable hepatomegaly was observed only in one 850 ppm mouse after 28 days of treatment. All mice treated with PB developed hepatomegaly within 60 days of treatment.

From day 7 through the remainder of the study, "speckled" livers were observed in essentially all mice treated with 2500 ppm test material (total incidence = 20). However, the appearance of speckled livers was more random in mice treated with 850 ppm test material with a total incidence of 5. This effect was not found in control mice or mice treated with PB. Liver nodules were found in one mouse from each of the propiconazole treatment groups.

3. Histopathology

Within one day of treatment, all mice treated with 850 ppm propiconazole had developed minimal hepatocellular hypertrophy. The average mean severity of the hypertrophy increased with time, going from minimal on day 1 of treatment to moderate/marked by day 28 and through the remainder of the study. Likewise, all mice treated with 2500 ppm propiconazole developed minimal hepatocellular hypertrophy within one day of treatment. The hypertrophy also increased in severity with time, progressing to moderate/marked by day 28 and through the remainder of the study. For both propiconazole treatment groups, the hypertrophy was most predominate in the centrilobular hepatocytes with mild hypertrophic effects in the midzonal and weak effects

in the periportal hepatocytes. As expected, treatment with 850 ppm PB induced hepatocellular hypertrophy that progressed from minimal to moderate/marked by day 28. In contrast to propiconazole, however, the location of the hypertrophic effect was strictly within centrilobular and midzonal hepatocytes.

Increased mitotic activity was observed in all treated animals primarily within centrilobular and midzonal hepatocytes. The maximum activity was found on day 2 of the study and thereafter decreased to approximately the control activity.

Minimal hepatocellular necrosis (necrotic single or focal hypertrophic hepatocytes often accompanied by granulocytosis) was found in several mice treated with 850 ppm propiconazole, particularly in the latter portion of the study. Minimal to moderate necrosis was found in nearly all mice treated with 2500 ppm propiconazole or PB after day 1 of treatment through the remainder of the study.

The labeling indices for propiconazole and PB are shown in Table 2. Within 24 hours of treatment with propiconazole or PB, the labeling indices for all treatment groups had increased 1000-2000% over control mice. By 48 hours after treatment, labeling indices for all treatment groups peaked at 3660-9500% of control. Thereafter, the labeling indices dramatically declined and after day 7 were not biologically different than control indices. BrdU-labeled hepatocyte nuclei for both propiconazole and PB groups were found primarily in the centrilobular/midzonal portions of the liver. On occasion, the labeled hepatocytes seemed to be arranged in a "ring-like" fashion in the midzonal hepatocytes.

TABLE 2. Percent of BrdU-labeled nuclei in hepatocytes of rats fed propiconazole or PB up to 60 days[a]

Day of Sacrifice	Control	850 ppm Propiconazole	2500 ppm Propiconazole	850 ppm PB
1	0.143 ± 0.145	2.679 (1870) ± 2.443**	1.479 (1033) ± 1.514*	3.180 (2220) ± 0.810**[b]
2	0.061 ± 0.014	2.243 (3662) ± 1.638**	2.978 (4862) ± 1.042**	5.830 (9517) ± 3.061**
3	0.079 ± 0.063	0.453 (571) ± 0.234**	1.497 (1889) ± 0.884**	1.867 (2356) ± 1.423**[b]
4	0.049 ± 0.040	0.260 (535) ± 0.369*	0.534 (1100) ± 0.335**	0.270 (556) ± 0.137**
7	0.097 ± 0.072	0.052 (53) ± 0.011	0.773 (793) ± 0.527**	0.550 (564) ± 0.440**
14	0.091 ± 0.024	0.145 (159) ± 0.092	0.107 (117) ± 0.052[b]	0.125 (137) ± 0.106
28	0.064 ± 0.037[b]	0.059 (92) ± 0.027	0.105 (165) ± 0.062	0.133 (209) ± 0.080
60	0.049 ± 0.021	0.033 (67) ± 0.021	0.062 (128) ± 0.044	0.054 (110) ± 0.050

Data from p. 32 of MRID 45215802
* $p \leq 0.05$, ** $p \leq 0.01$
results in parentheses are percent of control
[a]n=5 for all groups unless otherwise noted.
[b]n=4

III. DISCUSSION

A. DISCUSSION

In this study, none of the male mice fed diets containing 850 ppm or 2500 ppm propiconazole died and there were no treatment-related clinical signs of toxicity, or effects on body weight and food efficiency. Grossly observable hepatomegaly was found at necropsy in mice treated with 2500 ppm propiconazole after 7 days and through the remainder of the 60-day study. Hepatomegaly was observed in only one mouse at 28 days after treatment fed 850 ppm test material. Propiconazole did induce a significant dose-related increase in absolute and relative liver weight within 48 hours of the start of treatment in both groups. The increase stabilized after 3 days for the 850 ppm group and after 14 days for the 2500 ppm group. Similar effects to the 2500 ppm propiconazole group were seen in mice fed 850 ppm PB, the positive control. Both absolute and relative liver weights were increased within 24 hours of the start of treatment with a rapid weight gain period of ~4 days. The increased weights were sustained through the remainder of the treatment period, however, grossly observable hepatomegaly was observed only at the 60-day time point.

Microscopically, all mice treated with 850 ppm or 2500 ppm propiconazole developed minimal hypertrophy within 24 hours of the start of treatment. Thereafter, the hypertrophy progressed to moderate and to marked by day 28 where it persisted through the end of the study. The hypertrophic effect was predominately in the centrilobular hepatocytes with mild effects in the midzonal and weak effects in the periportal hepatocytes. PB also induced hepatocellular hypertrophy, but this effect was limited to the centrilobular and midzonal hepatocytes. In addition, increased mitotic activity was observed in all propiconazole and PB groups; the maximum activity was observed following two days of treatment. Minimal to moderate hepatocellular necrosis of hypertrophic single or focal cells was found predominately in the 2500 ppm propiconazole and 850 ppm PB treatment groups.

Treatment with 850 and 2500 ppm propiconazole or 850 ppm PB induced a >1000% increase in BrdU-staining hepatocellular nuclei within 24 hours from the start of the study. The increase for all groups peaked at >3600% labeling at 48 hours, supporting the increased mitotic activity observed histopathologically on the second day of the study. This finding indicates that not only DNA synthesis was induced but the cell divisions also took place, thereby increasing the number of hepatocytes. Thereafter, the number of BrdU-stained nuclei decreased dramatically and was not biologically different from control 7 days after the start and through the remainder of the study. For all treatment groups, the BrdU-staining nuclei were found primarily in the centrilobular/midzonal portions of the liver. Although hypertrophic single or focal hepatocellular necrosis was observed with 2500 ppm propiconazole treatment, the effect was indistinguishable from that observed by PB treatment. These data support the conclusion that propiconazole induced a time- and dose-related proliferation in the liver followed by a sustained treatment-related hypertrophy in a manner similar to the known hypertrophic agent PB.

The increased absolute and relative to body weight liver size observed in other studies is predominately a result of a sustained hypertrophic effect of the test material on centrilobular and midzonal hepatocytes and not that of a sustained proliferative event.

In conclusion, the results of this study provide evidence of cell proliferation (at carcinogenic doses in mice) as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling under light microscopy.

B. STUDY DEFICIENCIES

No deficiencies were found that would affect the interpretation of the study.

This study is considered **Acceptable/Non Guideline** for the determination of the mechanism of hepatocellular proliferation in the mouse.