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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: April 14, 2006

SUBJECT: **METCONAZOLE**: Report of the Cancer Assessment Review Committee
PC Code: 125619

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)



TO: Gregory Akerman, Toxicologist (TB)
Barry O'Keefe, Risk Assessor (RAB3)
Health Effects Division (7509C)
and
Lana Coppolino/Cynthia Giles-Parker (Fungicide Branch)
Anthony Britten/Dan Rosenblatt (MUIERB)
Registration Division (7505C)

The Cancer Assessment Review Committee met on November 16, 2005 to evaluate the carcinogenic potential of Metconazole. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
METCONAZOLE

PC CODE 125619

FINAL
April 14, 2006

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

METCONAZOLE

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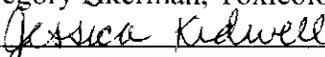
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DATA PRESENTATION:



Gregory Akerman, Toxicologist

DOCUMENT PREPARATION:

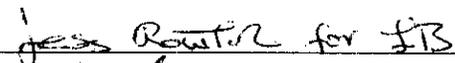


Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE:

(Signature indicates concurrence with the assessment unless otherwise stated).

Lori Brunsman, Statistician



Lori Brunsman for TB

William Burnam, Chair



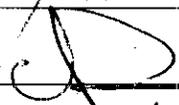
William Burnam

Marion Copley



Marion Copley

Vicki Dellarco



Vicki Dellarco

Nancy McCarroll



Nancy McCarroll

Tim McMahon



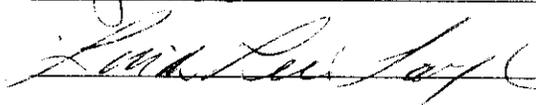
Tim McMahon

Jess Rowland



Jess Rowland

Linda Taylor

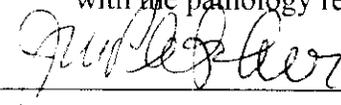


Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE

(Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist



John Pletcher

OTHER ATTENDEES: Doug Wolf (ORD/NHEERL/RTP), Stephen Dapson (HED/RAB3), Gino Scarano (HED/TB), Whang Phang (HED/RRB1), Barry O'Keefe (HED/RAB3), Alberto Protzel (HED/TB)

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EXECUTIVE SUMMARY

On November 16, 2005, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Metconazole.

Greg Akerman of the Toxicology Branch presented the chronic toxicity and carcinogenicity studies in Fischer 344 rats and the carcinogenicity study in CD-1 mice. In the rat chronic toxicity study, metconazole was administered in the diet to groups of 20 Fischer 344 rats per sex at dose levels of 10, 100, 300 or 1000 ppm (0, 0.4, 4.3, 13.1 or 43.9 mg/kg/day for males; 0, 0.5, 5.3, 16.0 or 53.8 mg/kg/day for females) for 104 weeks. A group of 40 rats/sex were designated to the control group. In the rat carcinogenicity study, metconazole was administered in the diet to groups of 50 rats per sex at dose levels of 0, 100, 300 or 1000 ppm (0, 4.6, 13.8 or 46.5 mg/kg/day for males; 0, 5.5, 16.6 or 56.2 mg/kg/day for females) for 104 weeks. In the mouse carcinogenicity study, metconazole was administered to groups of 51 mice per sex at dose levels of 0, 30, 300 or 1000 ppm (0, 4.4, 43.6 or 144.9 mg/kg/day for males; 0, 5.2, 53.0 or 179.2 mg/kg/day for females) for 92 weeks. He also presented information on mutagenicity, structure activity relationship, and mode of action data for liver tumors.

The CARC concluded the following:

Carcinogenicity

Rat

- ▶ In female rats, the incidence of mononuclear cell leukemia (MCL) (at all sites) was 10/89 (11%), 2/19 (11%), 10/41 (24%), 12/40 (30%), and 16/70 (23%) for the control, 10, 100, 300 and 1000 ppm dose groups for both the chronic toxicity and carcinogenicity study combined. There was a significant difference in the pair-wise comparison of the 300 and 1000 ppm dose group with controls for MCL at all sites, however, the increased incidences were within the historical control range for the testing laboratory. The CARC concluded that the increased incidence of mononuclear cell leukemia seen in female rats did not contribute to the weight-of-evidence since it was difficult to interpret the biological significance of the response since not all animals in the 100 and 300 ppm dose groups were examined for this tumor (only the decedents were examined for all tissues).
- ▶ No treatment related tumors were seen in male rats.
- ▶ Adequacy of Dosing: The CARC considered the highest dose tested, 1000 ppm, to be adequate, but not excessive, to assess the carcinogenicity of metconazole in male and female rats. This was based on decreased body weight (14% males, 16% females) and

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body weight gain (6% male, 9% female), increased organ weights (liver, kidney, spleen), and adrenal and liver histopathology seen in males and/or females at 1000 ppm.

Mouse

► Liver tumors noted at the highest dose tested (1000 ppm) in both sexes were considered to be treatment-related since:

- In male mice, there were significant differences in the trends and pair-wise comparisons of the 1000 ppm dose group with the controls for liver adenomas (35/55 (64%) vs 11/53 (21%), controls) and adenomas and/or carcinomas combined (38/55 (69%) vs 13/53 (25%), controls), all at $p < 0.01$.
- In female mice, there were significant differences in the trends and pair-wise comparisons of the 1000 ppm dose group with the controls for liver adenomas (50/61 (82%) vs 0/56 (0%), controls), carcinomas (20/61 (33%) vs 0/56 (0%), controls), and adenomas and/or carcinomas combined (52/61 (85%) vs 0/56 (0%), controls), all at $p < 0.01$.
- Both the incidence of liver adenomas and carcinomas in both sexes at the high dose exceeded the historical control ranges for the testing laboratory.

► Adequacy of Dosing: The CARC considered the highest dose tested, 1000 ppm, to be adequate, but not excessive, to assess the carcinogenicity of metconazole in male and female mice. This was based on decreased body weight (↓7% males, ↓13% females), body weight gain (↓25% males, ↓32% females), increased liver weight, elevated aminotransferase enzyme levels, liver vacuolation, hypertrophy and necrosis.

Mutagenicity

► There is no mutagenicity concern for metconazole.

Structure-Activity Relationship

► Metconazole is structurally related to other triazole ("conazole") fungicides. Several parent triazole pesticides have been shown to be carcinogenic in rodents, specifically liver tumors in the mouse and/or thyroid tumors in rats. The triazole class of compounds generally lack genotoxic potential, while some compounds (including metconazole) test positive only in chromosomal aberration assays.

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Mode of Action

▶The CARC agreed with the registrant that a plausible non-genotoxic mode of action involving mitogenesis was established for the development of liver tumors in a mouse bioassay with metconazole. This conclusion was based on the following:

- Data from *in vivo* and *in vitro* genetic toxicology studies are largely negative (with the exception of one *in vitro* test for clastogenicity) and, therefore, indicate that mutagenicity is not a key event in the mode of action;
- There is dose-concordance between liver tumors, cell proliferation, and hepatic microsomal enzyme induction. The threshold level is a NOAEL of 4.3 mg/kg/day; this would be protective of early liver disturbances;
- A temporal relationship supporting the MOA was demonstrated. The mitogenic proliferative response was identified as early as 3 days after the onset of treatment, which declined after 14 days of treatment.

Although evidence of hepatotoxicity was also seen in the mouse liver, this does not discount a threshold mode of action. The hepatotoxicity would contribute, along with the mitogenicity of the compound, to the tumor promotion.

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified Metconazole as "**Not Likely to be Carcinogenic to Humans**" based on convincing evidence that a non-genotoxic mode of action for liver tumors was established in the mouse and that the carcinogenic effects were not likely below a defined dose that doesn't cause mitogenesis. There was evidence of liver effects (microsomal induction, liver weight increases, hypertrophy) at 300 ppm (47.6 mg/kg/day), but no effects at 30 ppm (4.5 mg/kg/day) in the mode of action studies in the mouse. The chronic Reference Dose of 0.04 mg/kg/day based on the 2-year chronic rat study with a NOAEL of 4.3 mg/kg/day would be protective of early liver disturbances seen in the mouse studies. The quantification of carcinogenic potential is not required.

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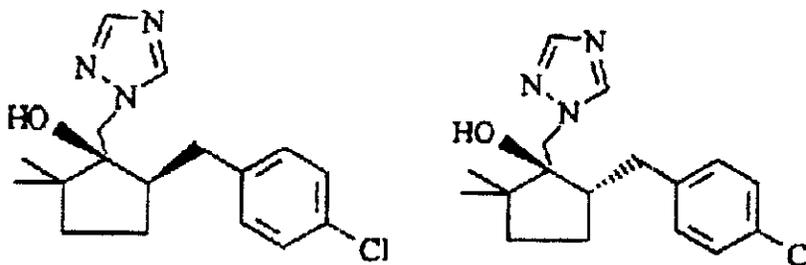
I. INTRODUCTION

On November 16, 2005, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Metconazole.

II. BACKGROUND INFORMATION

Metconazole (5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol) belongs to the triazole class of fungicides, acting primarily as an inhibitor of ergosterol biosynthesis. The registrant (BASF) has requested an import tolerance for its use on bananas for the target fungal pest Black Sigatoka (*Mycosphaerella fijiensis*).

The toxicology studies were conducted with the *cis* only isomer metconazole (approximately 95% *cis*) and/or a *cis*:*trans* isomer mix (approximately 85% *cis*:15% *trans*). The PC Code is 125619 and the CAS # is 125116-23-6.

cis Metconazole (CL 354,801)*trans* Metconazole (CL 354,802)**III. EVALUATION OF CARCINOGENICITY STUDIES****1. Carcinogenicity Study and Chronic Toxicity Study in Rats**

The 104-Week Fischer 344 Rat Carcinogenicity Study (MRID 44721611)

Reference: A carcinogenicity study in Fischer 344 rats was conducted by Shell Research Limited, Sittingbourne Research Center, Sittingbourne, Kent, England, for BASF Corporation, Research Triangle Park, North Carolina, and dated June 30, 1992 (Laboratory Report No. SBGR.91.192, MRID No. 44721611).

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The 104-Week Fischer 344 Rat Chronic Toxicity Study (MRID 44721609)

Reference: A chronic toxicity study in Fischer 344 rats was conducted by Sittingbourne Research Centre, Sittingbourne, Kent, England, for BASF Corporation, Research Triangle Park, North Carolina, and dated September 16, 1992 (Laboratory Report No. SBGR.91.193, MRID No. 44721609).

A. Experimental Design

1. Rat Carcinogenicity Study

The study design allocated groups of 50 rats per sex to dose levels of 0, 100, 300 or 1000 ppm (0, 4.6, 13.8 or 46.5 mg/kg/day for males; 0, 5.5, 16.6 or 56.2 mg/kg/day for females) of Metconazole via the diet for 104 weeks.

2. Rat Chronic Toxicity Study

The study design allocated groups of 20 rats per sex to dose levels of 10, 100, 300 or 1000 ppm (0, 0.4, 4.3, 13.1 or 43.9 mg/kg/day for males; 0, 0.5, 5.3, 16.0 or 53.8 mg/kg/day for females) of Metconazole via the diet for 104 weeks. A group of 40 rats/sex were designated to the control group. Study termination occurred at weeks 107 and 108. Another 20 rats per sex in the control group and 10 rats per sex for the dosed levels were designated for interim sacrifice at week 52.

B. Survival and Tumor Analyses

There were no compound-related tumors observed in male rats so this section only contains statistical analyses of the females.

Survival Analyses

There were no statistically significant incremental changes in mortality with increasing doses of Metconazole in female rats of either study. The mortality analyses of the carcinogenicity study is presented in Table 1. The chronic study indicated no survival disparities among the female rats.

Tumor Analyses

At the request of Bill Burnam, chairman of the CARC committee, tumor tables from the carcinogenicity and chronic studies have been combined into one table (Table 2a) to fully assess the extent of the mononuclear cell leukemia in female rats. For the convenience of the reader, the respective original tables from the carcinogenicity and chronic studies are included as Tables 2b and 2c.

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As shown in Table 2a, female rats had significant differences in the pair-wise comparisons of the 300 and 1000 ppm dose groups with the controls for mononuclear cell leukemia at all sites, both at $p < 0.05$. There was no statistically significant trend. The statistical analyses of the female rats were based upon *ad hoc* Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Table 2a).

Table 1. Metconazole - Fischer 344 Rat Carcinogenicity Study (MRID 44721611)

Female Mortality Rates¹ and Cox or Generalized K/W Test Results

Dose (ppm)	<u>Weeks</u>				Total
	1-26	27-52	53-78	79-106 [†]	
0	0/50	0/50	0/50	17/50	17/50 (34)
100	0/50	0/50	1/50	19/49	20/50 (40)
300	0/50	0/50	1/50	18/49	19/50 (38)
1000	0/50	0/50	0/50	16/50	16/50 (32)

¹Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[†]Final sacrifice at weeks 104-106.

() Percent.

Note:

Time intervals were selected for display purposes only.

Significance of trend denoted at control.

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

If ^{*}, then $p < 0.05$. If ^{**}, then $p < 0.01$.

(Taken from Qualitative Risk Assessment Memo, L. Brunzman, 08/31/05, TXR No. 0053709)

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Table 2a. Metconazole - Fischer 344 Rat Carcinogenicity (MRID 44721611) and Chronic Toxicity (MRID 44721609) Combined Studies

Female Mononuclear Cell Leukemia Rates¹ and *ad hoc* Fisher's Exact Test and Exact Test for Trend Results

	Dose (ppm)				
	0	10 ^a	100	300	1000
Mononuclear Cell Leukemia – All Sites (%)	10/89 (11)	2/19 (11)	10/41# (24)	12/40# (30)	16/70 (23)
p =	0.07181	0.66605	0.05026	0.01054*	0.04032*

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

^aThe carcinogenicity study had a control and 3 dose groups. The chronic study had a control and 4 dose groups. Only the chronic study had a 10 ppm dose group.

#In the 100 and 300 ppm dose groups, all tissues were examined only in decedents.

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

(Taken from Qualitative Risk Assessment Memo, L. Brunzman, 10/19/05, TXR No. 0053807)

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The incidences of mononuclear cell leukemia in the individual rat carcinogenicity and chronic studies are presented in Tables 2b and 2c, respectively.

TABLE 2b. Neoplastic incidences^a in carcinogenicity study in rats dosed with metconzaole .

Dose (ppm)	Male				Female			
	0	100#	300#	1000	0	100#	300#	1000
Hematopoietic Tissue								
mononuclear cell leukemia	17/50	22/32** (69%)	21/31** (68%)	14/50	5/50	8/22* (17%)	7/20* (35%)	15/50* (30%)

^aTotal number of incidences; (frequency, %): MRID 44721612

*Statistically different ($p < 0.05$) from the control, (Fisher's test).

** Statistically different ($p < 0.01$) from the control, (Fisher's test).

#In the 100 and 300 ppm dose groups, all tissues were examined only in decedents.

TABLE 2c. Neoplastic incidences^a in 2-year chronic study in rats dosed with metconzaole.

Dose (ppm)	Male					Female				
	0	10	100	300	1000	0	10	100	300	1000
Hematopoietic Tissue										
mononuclear cell leukemia	21/40	8/20	10/20	10/20	10/20	5/40	2/20	2/20	5/20	1/20

^a Total number of incidences, 2 year sacrifice: MRID 44721609.

Historical control data for mononuclear cell leukemia

Mononuclear cell leukemia is a frequently occurring neoplasm in untreated Fischer 344 rats. The frequency of mononuclear cell leukemia in the test facility historical control animals for males is 5-44% (mean 25%) and for females is 5-31% (mean 13%) (MRID 44721611). The frequency of spontaneous mononuclear cell leukemia as reported by NTP in Fischer 344 rats:

1991 Males: 10-72% (mean 34%) Females: 6-31% (mean 20%)

1998 Males: 32-74% (mean 50%) Females: 14-52% (mean 28%)

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C. Non-Neoplastic Lesions in the Rat

The incidence of non-neoplastic lesions observed in the rat are presented in a Table 3.

Table 3. Non-Neoplastic Lesions in F344 Rats Fed Metconazole

Dose (ppm) Histopathology		Male				Females			
		0	100	300	1000	0	100	300	3000
Adrenals									
cortical vacuolation		3/50	17/50	25/49* (51%)	38/50** (76%)	11/50	7/50	8/50	7/50
medullary hyperplasia foci		4/50	13/50* (26%)	18/49** (37%)	10/50	4/50	2/50	3/50	1/50
Kidney									
chronic nephropathy	severe	7/50	6/50	15/50	10/50	5/50	3/50	4/50	2/50
	very severe	3/50	7/50	10/50	11/50* (22%)	0/50	3/50	1/50	1/50
Liver									
basophilic foci		32/50	27/50	34/50	38/50	36/50	34/50	33/50	23/50* (46%)
clear cell foci		4/50	7/50	9/50	28/50** (56%)	1/50	3/50	3/50	15/50** (30%)
eosinophilic foci		1/50	3/50	4/50	12/50** (24%)	0/50	1/50	0/50	2/50
fatty vacuolated foci/areas		8/50	1/50* (2%)	11/50	18/50* (36%)	19/50	7/50* (14%)	6/50* (12%)	16/50
fatty centrilobular vacuolation		3/50	3/50	4/50	13/50* (26%)	0/50	1/50	2/50	7/50* (14%)
centrilobular hypertrophy		0/50	2/50	9/50** (18%)	31/50*** (62%)	0/50	0/50	0/50	1/50
pigment deposits		2/50	6/50	14/50** (28%)	35/50*** (70%)	0/50	0/50	1/50	4/50
Spleen									
histiocytic foci		2/50	1/50	1/50	20/50*** (40%)	11/50	5/50	7/50	31/50*** (62%)
Testes									

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Dose (ppm) Histopathology	Male				Females			
	0	100	300	1000	0	100	300	3000
focal interstitial cell hyperplasia	6/50	9/48	10/50	17/50* (34%)				
arteritis	3/50	7/48	9/50	11/50* (22%)				
Urinary Bladder								
transitional cell hyperplasia	3/49	3/22	6/25* (25%)	3/50	1/50	0/50	1/50	1/50

^a Total number of incidences: (frequency, %)

* Statistically different ($p < 0.05$) from the control, (Fisher's test).

** Statistically different ($p < 0.01$) from the control, (Fisher's test).

*** Statistically different ($p < 0.001$) from the control, (Fisher's test).

D. Adequacy of the Dosing for Assessment of Carcinogenicity

The CARC considered the highest dose tested, 1000 ppm, to be adequate, but not excessive, to assess the carcinogenicity of metconazole in the rat. This was based on decreased body weight/body weight gain, increased organ weights, and liver histopathology. In the carcinogenicity study in the rat, decreased food consumption (6% male, 5% female) and body weight (4% male, 6% female, $p < 0.01$) were observed at the high dose. Overall body weight gain was decreased in both sexes at 1000 ppm (6% male, 9% female). Increased liver, kidney and spleen weights were reported in both sexes at 1000 ppm and increased adrenal weight was observed in males at the high dose. Histopathology revealed an increased incidence of adrenal cortical vacuolation and hyperplastic foci in males at 300 ppm and above. An increased incidence of hepatocellular hypertrophy and pigment deposit in liver parenchymal cells was also observed in males at 300 ppm. Liver eosinophilic foci were reported in males at the high dose and clear-cell foci were observed in both sexes at 1000 ppm.

In addition, there was no treatment-related effect on survival for either sex. Survival percentages at 0, 100, 300 and 1000 ppm were 60, 56, 50 and 66% for males and 66, 60, 62 and 68% for females. The dose levels were selected based on the results from a 90-day feeding study with WL148271 in the Fischer 344 rat (MRID 44721518) where animals, fed a diet of up to 4050 ppm cis metconazole, showed decreased food consumption, decreased body weight, increased spleen and liver weights, elevated AST/ALT and -GT, decreased RBC parameters and hepatocellular vacuolation at 1350 ppm and greater.

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2. Carcinogenicity Study in Mice

The 91-Week Crl:CD-1(ICR)BR Mouse Carcinogenicity Study (MRID 44721612)

Reference: A carcinogenicity study in Crl:CD-1(ICR)BR mice was conducted by Hazelton UK, North Yorkshire, England, for BASF Corporation, Research Triangle Park, North Carolina, and dated August 26, 1992 (Laboratory Report No. 579/26, MRID No. 44721612).

A. Experimental Design

The study design allocated groups of 51 mice per sex to dose levels of 0, 30, 300 or 1000 ppm (0, 4.4, 43.6 or 144.9 mg/kg/day for males; 0, 5.2, 53.0 or 179.2 mg/kg/day for females) of Metconazole via the diet for 92 weeks. An additional 12 mice per sex per dose were designated for interim sacrifice at week 53.

B. Survival and Tumor Analyses

Survival Analyses

There were no statistically significant incremental changes in mortality with increasing doses of Metconazole in male or female mice (Tables 4 and 5) (Memo, L. Brunsmann, 8/31/05, TXR No. 0053709). The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male mice had significant increasing trends, and significant differences in the pair-wise comparison of the 1000 ppm dose group with the controls, for liver adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$. The statistical analyses of the male mice were based upon Fisher's Exact test for pair-wise comparisons and the Exact test for trend (Table 6).

Female mice had significant increasing trends, and significant differences in the pair-wise comparison of the 1000 ppm dose group with the controls, for liver adenomas, carcinomas and adenomas and/or carcinomas combined, all at $p < 0.01$. The statistical analyses of the female mice were based upon Fisher's Exact test for pair-wise comparisons and the Exact test for trend (Table 7).

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Table 4. Metconazole - Crl:CD-1(ICR)BR Mouse Study (MRID 44721612)Male Mortality Rates¹ and Cox or Generalized K/W Test ResultsWeeks

Dose (ppm)	1-26	27-52	53 ⁱ	53-78	79-92 ^f	Total
0	1/63	9/62	11/53	10/42	8/32	28/52 (54)
30	4/63	11/59	11/48	7/37	8/30	30/52 (58)
300	1/63	11/62	10/51	7/41	11/34	30/53 (57)
1000	4/63	4/59	9/55	14/46	9/32	31/54 (57)

¹Number of animals that died during interval/Number of animals alive at the beginning of the interval.ⁱ Interim sacrifice at week 53.^f Final sacrifice at week 92.

() Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

(Taken from memo, L. Brunzman, 8/31/05, TXR No. 0053709)

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Table 5. Metconazole - CrI:CD-1(ICR)BR Mouse Study (MRID 44721612)Female Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose (ppm)	<u>Weeks</u>					Total
	1-26	27-52	53 ⁱ	53-78	79-93 ^f	
0	1/62	6/61	12/55	11/43	6/32	24/50 (48)
30	0/63	3/63	12/60	12/48	10/36	25/51 (49)
300	0/63	2/63	12/61	5/49	8/44	15/51 (29) ^{*n}
1000	1/63	2/62	11/60	10/49	7/39	20/52 (38)

⁺Number of animals that died during interval/Number of animals alive at the beginning of the interval.

ⁱInterim sacrifice at week 53.

^fFinal sacrifice at weeks 92-93.

ⁿNegative change from control.

()Percent.

Note:

Time intervals were selected for display purposes only.

Significance of trend denoted at control.

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

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

(Taken from memo, L. Brunzman, 8/31/05, TXR No. 0053709)

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Table 6. Metconazole - CrI:CD-1(ICR)BR Mouse Study (MRID 44721612)Male Liver Tumor Rates^a and Fisher's Exact Test and Exact Test for Trend Test Results

	Dose (ppm)			
	0	30	300	1000
Adenomas (%)	11 ^a /53 (21)	17 ^a /48 (35)	16 ^a /51 (31)	35 ^a /55 (64)
p =	0.00000**	0.07753	0.15604	0.00001**
Carcinomas (%)	4 ^b /53 (8)	4/48 (8)	7/51 (14)	7/55 (13)
p =	0.1952	0.58527	0.24092	0.28516
Combined (%)	13 ^c /53 (25)	17 ^d /48 (35)	19 ^d /51 (37)	38 ^d /55 (69)
p =	0.00000**	0.16409	0.11631	0.00000**

^aNumber of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

^aFirst adenoma observed simultaneously in interim sacrifice animals at week 53 in all dose groups.

^bFirst carcinoma observed at week 68, dose 0 ppm.

^cTwo animals in the control group had both an adenoma and a carcinoma.

^dFour animals in each of the 30, 300 and 1000 ppm dose groups had both an adenoma and a carcinoma.

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

(Taken from memo, L. Brunsmann, 8/31/05, TXR No. 0053709)

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Table 7. Metconazole - Crl:CD-1(ICR)BR Mouse Study (MRID 44721612)Female Liver Tumor Rates[†] and Fisher's Exact Test and Exact Test for Trend Test Results

	Dose (ppm)			
	0	30	300	1000
Adenomas (%)	0/56 (0)	1/61 (2)	4/61 (7)	50 ^a /61 (82)
p =	0.00000**	0.52137	0.07039	0.00000**
Carcinomas (%)	0/56 (0)	1/61 (2)	0/61 (0)	20 ^b /61 (33)
p =	0.00000**	0.52137	1.00000	0.00000**
Combined (%)	0/56 (0)	2/61 (3)	4/61 (7)	52 ^c /61 (85)
p =	0.00000**	0.26967	0.07039	0.00000**

[†]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 46.

^aFirst adenoma observed at week 46, dose 1000 ppm.

^bFirst carcinoma observed at week 46, dose 1000 ppm.

^cEighteen animals in the 1000 ppm dose group had both an adenoma and a carcinoma.

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

(Taken from memo, L. Brunsmann, 8/31/05, TXR No. 0053709)

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Historical Control for Liver Tumors

When compared to the historical data from eight studies of comparable duration from 1988-1991 (n= 407 males/females; source: page 44 of study report MRID 44721612), the highest incidence of liver adenoma in the CD-1 strain was 33% in males and 2% in females. The highest incidences of liver carcinoma in this historical control data set were 18% for males and 2% for females. The incidence of adenomas and carcinomas in males and female mice in this study exceeded that of historical controls.

Adenoma: 10-33%, mean 21.6% (males)	0-2%, mean 1.5% (females)
Carcinoma: 2-18%, mean 1.5% (males)	0-2%, mean 0.5% (females)

C. Non-Neoplastic Lesions

The non-neoplastic lesions of male and female mice are presented in Tables 8a and 8b, respectively.

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Table 8a. Non-Neoplastic Lesions in Male CD-1 Mice Fed Metconazole.

Dose (ppm)		0	30	300	1000	
Adrenals						
corticomedullary pigmentation	wk 52	2/12	2/11	5/12	9/11	
	wk 93#	2/49	4/51	9/51	35/51	
Liver						
vacuolation	wk 52	3/12	4/12	5/12	6/11	
	wk 93	11/50	10/51	20/51*	37/51**	
hypertrophy	wk 52	0/12	0/12	9/12	11/11	
	wk 93	0/50	0/51	13/51*	44/51**	
sinusoidal hypercellularity/ single cell necrosis/ pigment deposition	wk 52	total	0/12	0/12	2/12	9/11
		minimal	0	0	1	5
		slight	0	0	1	4
		moderate	0	0	0	0
	wk 93	total	0/50	1/51	10/51*	37/51**
		minimal	0	0	5	17
		slight	0	1	3	17
		moderate	0	0	2	3
oval cell hyperplasia	wk 52	0/12	0/12	0/12	1/11	
	wk 93	0/50	0/51	0/51	12/51**	
biliary proliferation	wk 52	0/12	0/12	1/12	3/11	
	wk 93	0/50	1/51	1/51	8/51*	
multifocal hyperplasia	wk 52	0/12	0/12	0/12	1/11	
	wk 93	0/50	1/51	0/51	31/51**	
Spleen						
atrophy/ prominent trabeculae and stroma	wk 52	0/12	0/12	1/12	8/11	
	wk 93	1/49	1/51	9/49	39/50	

* p<0.05 (Fisher's Exact Test)

**p<0.01 (Fisher's Exact Test)

Week 93 values include unscheduled sacrifice animals

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Table 8b. Non-Neoplastic Lesions in Female CD-1 Mice Fed Metconazole.

Dose (ppm)		0	30	300	1000	
Adrenals						
corticomedullary pigmentation	wk 52	4/12	3/12	11/11	12/12	
	wk 93#	17/51	13/51	49/51	50/51	
Liver						
vacuolation	wk 52	5/12	4/12	10/12	10/12	
	wk 93	11/50	12/51	36/51**	44/51**	
hypertrophy	wk 52	0/12	0/12	3/12	10/12	
	wk 93	0/50	0/51	8/51*	38/51**	
sinusoidal hypercellularity/single cell necrosis/pigment deposition	wk 52	total	0/12	0/12	3/12	11/12
		minimal	0	0	3	5
		slight	0	0	0	5
		moderate	0	0	0	1
	wk 93	total	0/50	0/51	18/51**	35/51**
		minimal	0	0	12	18
		slight	0	0	5	14
		moderate	0	0	1	3
oval cell hyperplasia	wk 52	0/12	0/12	0/12	2/12	
	wk 93	0/50	0/51	0/51	14/51**	
biliary proliferation	wk 52	0/12	0/12	0/12	1/12	
	wk 93	0/50	1/51	0/51	13/51**	
multifocal hyperplasia	wk 52	0/12	0/12	0/12	9/12	
	wk 93	0/50	0/51	0/51	44/51**	
Spleen						
atrophy/ prominent trabeculae and stroma	wk 52	0/12	1/12	1/12	10/12	
	wk 93	0/50	0/51	15/50	41/49	

* p<0.05 (Fisher's Exact Test)

**p<0.01 (Fisher's Exact Test)

Week 93 values include unscheduled sacrifice animals.

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D. Adequacy of Dosing for Assessment of Carcinogenicity

The CARC considered the highest dose tested, 1000 ppm, to be adequate, but not excessive, to assess the carcinogenicity of metconazole. This was based on decreased body weight/body weight gain, increased liver weight, elevated aminotransferase enzyme levels, liver vacuolation, hypertrophy and necrosis. Decreased body weight (-7% males, -13% females) and food consumption (-3% males, -6% females) were observed at the high dose in both sexes compared to the control animals. Female mice at 300 ppm showed an 8% decrease in body weight relative to the control animals. The overall body weight gain was significantly less in males ($p < 0.05$) and females ($p < 0.01$) at 1000 ppm, compared to the controls. Hematology revealed increased leukocyte counts in the males at 300 ppm (35%, $p < 0.05$) and in both sexes at 1000 ppm (males 68%, $p < 0.001$; females 230%, $p < 0.001$). At the high dose, an increase in neutrophils (males 91%, $p < 0.01$; females 318%, $p < 0.001$) and lymphocytes (males 40%, $p < 0.05$; females 264%, $p > 0.05$) were also observed. Increased liver weights and decreased spleen weights were reported at the high dose. Decreased triglyceride levels were reported in both sexes at 300 and 1000 ppm (weeks 52 and 91). Significantly increased AST, ALT levels were observed in the male at 300 ppm and in both sexes at 1000 ppm. Histopathology revealed evidence of hepatotoxicity at 300 ppm and 1000 ppm with increased incidences of hepatocellular vacuolation, hypertrophy, inflammation and single cell necrosis.

In addition, there were no treatment-related effects on survival for either sex. The survival percentages for males at 0, 30, 300 and 1000 ppm were 47%, 44%, 42% and 43% respectively. In the females, the survival rates at 0, 30, 300 and 1000 ppm were 51%, 54%, 69% and 56%.

IV. TOXICOLOGY**1. Metabolism**

(MRID Nos. 44721622, 44721623, 44721624, 44721625)

In a series of metabolism studies, the fate of metconazole was studied in Fischer 344 rats by administering WL136184 (cis metconazole) and/or WL148271 (cis:trans metconazole) ¹⁴C-radiolabeled at either the cyclopentyl or triazole moieties.

In a metabolism study (**MRID 44721625**), labeled [cyclopentyl-¹⁴C]WL136184 (98.2% a.i., batch # S.1190:2), was administered via stomach cannula as a single dose to three bile-cannulated Fischer 344 rats/sex in at dose level of 2 mg/kg. Biliary excretion was rapid with at least 50% of the dose excreted in the bile within the first 6 hours with 79% (male) and 83% (female) of the administered dose excreted in the bile at 48 hours. Urinary excretion accounted for 4.3% dose in males and 12.1% dose in females. Fecal excretion of radioactivity was low in both sexes accounting for 0.2% and 0.3% dose in male and females, respectively. The retention of radioactivity in the gastrointestinal (GI) tract and carcass was notably higher in male rats

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(combined retention 12.1%) than in females (1.2%). Total mean total recovery of the radiolabeled dose was 95.5% and 97.2% in male and female rats respectively.

In a metabolism study (**MRID 44721623**) metconazole (>99 % a.i., batch S1106/1, [cyclopentyl-¹⁴C] WL148271) was administered as a single dose of 2 mg/kg by gavage to two Fischer 344 rats/sex in the preliminary CO₂ study and 5 rats/sex in the main study. One rat/sex was administered DMSO as a vehicle control.. Radioanalysis of the CO₂ expired during the first 24 hours after dosing yielded ¹⁴C values (0.05%) that were below the level of reliable measurement. Greater than 90% of the administered dose was eliminated in three days by urine and feces. Overall, the mean recovery of the administered radioactivity for the 7 days was 97.6% (including cage wash). Whole body autoradiography was performed on the four preliminary study animals (one male, one female each at day 1 and day 7 post-dose). Radioactivity at day 1 was concentrated in the GI tract, liver and adrenal glands in the male and female. By day 7, the radioactivity in the GI tract and liver were greatly diminished, however appreciable levels of radioactivity in the adrenal glands were detected. Specifically, the radioactivity was focused primarily in the adrenal cortex. Generally, the radioactivity was more pronounced in the female than in the male. In the main study, 93-96% of the administered dose was excreted by day 3, with 67-80% of the radioactivity eliminated in feces and 15-26% via the urine. A higher percentage of the administered dose was eliminated in urine in females than males. Elimination was rapid with 54-61% of the dose excreted by day 1. Marked levels of radioactivity were detected in the GI tract, liver and adrenals. Sex differences included higher levels of radioactivity in the female liver and male adrenals.

In a metabolism study (**MRID 44721622**) [cyclopentyl-¹⁴C]WL148271 (cis:trans metconazole, >99% a.i.; SCP/1, batch # S1164/1) was administered to 5 Fischer 344 strain rats/sex by gavage in a single dose at 164 mg/kg (target dose was 200 mg/kg). Two Fischer 344 strain rats/sex were treated with dose vehicle (DMSO) only. In the male rats, fecal elimination accounted for 81% of the radioactivity with 14% dose excreted in the urine. In females, 65% of the dose was eliminated in the feces and 28% in urine. The elimination was rapid, although slightly longer relative to the low (2 mg/kg) dose administration. Radioactive residues were elevated in the adrenals, liver and GI tract. No sex-related differences in the level of radioactivity retained in the organs/tissues were observed (contrary to the 2 mg/kg dose study). The major metabolites identified in the feces and urine were hydroxy- and carboxy-metabolites. The metconazole metabolites were further characterized in study (**MRID 44721624**) in which [triazole-¹⁴C] metconazole (>98% a.i., batch 1084/1, WL136184) was administered to six male Fischer 344 rats by a single oral gavage dose of 200 mg/kg. Elimination of the radioactivity was rapid with 70% of the administered dose excreted within 72 hours. Little or no labeled parent compound was detected in the excreta. Greater than 95% of the dose was eliminated by day 7 and major route of excretion (75%) was via the feces. The total recovery of radioactivity in the urine was 20%. The metabolites identified in the excreta primarily represent products of the oxidation of the methyl groups on the cyclopentane ring. Some cleavage of the compound was also observed.

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The six classes of metabolites identified were:

1. Monohydroxy-metabolites (oxidation site at benzylic methylene group or methyl, methylene or methine groups of cyclopentane ring). Examples M1 and M2.
2. Hydroxyphenyl-metabolites such as M15 and M19
3. Carboxy-metabolites (from further oxidation of methyl groups) such as M12 and M13
4. Multihydroxy-metabolites- (dihydroxy- and trihydroxy-metabolites) such as M18
5. Mixed function metabolites (e.g. hydroxycarboxy- or hydroxyphenylcarboxy-)
6. Sulfate conjugates of metabolites in classes 1 and 5 such as M22.

The metabolites representing the highest estimated percentage of administered radioactivity in the excreta were M12 (15%), M1 (14%), M19 (6%), **M20 [1,2,4-triazole](5%)**, M2 (5%), M13 (4%), M21 (2%) and M16 (1%). The other metabolites identified represented less than 1% of the radioactive dose. The M20 urine metabolite (1,2,4-triazole) is likely a product of the oxidation and subsequent hydrolysis of the methylene group adjacent to the triazole group. These metabolites suggest that the primary metabolic pathway involves the oxidation of methyl groups on the cyclopentane ring.

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2. Mutagenicity

When the genotoxic potential of cis and/or cis:trans metconazole was tested in several *in vitro* and *in vivo* mutagenicity assays, all tests were negative with the exception of the chromosomal aberration assay with cis/trans metconazole in the presence of S-9 mix. Overall, both cis and cis/trans metconazole are considered to be non-genotoxic. These assays satisfy the Subdivision F Guideline requirements for mutagenicity testing.

(i) In an Ames assay, cis metconazole and cis/trans metconazole were not mutagenic with and without metabolic activation when tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* WP2 uvrA at concentrations up to 5000 g/plate (MRID Nos. 44721613 and 44721614).

(ii) In a mutagenicity study, mouse lymphoma cells L5178Y/TK+/- treated with cis metconazole up to 70 g/ml (without S9) showed no statistical increase in the mutation frequency in two separate experiments. In the presence of S9, a statistically significant increase in the mutation frequency was observed at 50 g/ml, but not at higher doses (90 g/ml). No increase in mutation frequency was observed when the experiment was repeated. Since the results were not reproducible, cis metconazole is not mutagenic in this assay (MRID 44721615).

(iii) In an *in vitro* chromosome study, the exposure of CHO-K1 cells with cis/trans metconazole in the absence of S9 mix did not induce chromosomal aberrations at a dose up to 50 g/ml after 24 or 48 hours of exposure. In the presence of S9, an increase in chromosomal aberrations relative to the negative controls was observed at 50 g/ml at 24 hours in two experiments. The increase in aberrations was observed in the absence of cytotoxicity. Cis/trans metconazole is clastogenic in CHO cells in the presence of S9 (MRID 44721616).

(iv) In an *in vitro* chromosome studies, the exposure of human lymphocytes to cis metconazole up to 750 g/ml for 3, 24 or 48 hours in the presence and absence of S9 mix showed no increase in metaphase chromosome damage relative to the negative controls (MRID No. 44721617).

(v) In an *in vivo* test bone marrow micronucleus test, mice were exposed to either cis metconazole or cis/trans metconazole. No statistically or biologically significant increase in micronucleated polychromatic erythrocytes was observed at dose levels exhibiting bone marrow toxicity. (MRID Nos. 44721618 and 44721619).

(vi) In studies to evaluate the potential to induce *in vivo* /*in vitro* unscheduled DNA synthesis, rats were treated with cis metconazole or cis/trans metconazole at doses up to 1400 or 2000 mg/kg bw, respectively. Hepatocytes were isolated, cultured and labeled in

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the presence of ^3H -thymidine. Analysis revealed no significant dose-related increase in nuclear grain count (associated with induced unscheduled DNA synthesis) with cis or cis/trans metconazole (**MRID Nos. 44721621 and 44721622**).

There was evidence of a clastogenic effect in the absence of cytotoxicity in CHO cells, but not in human lymphocytes. The test material was also negative in two whole animal studies (micronucleus up to a cytotoxic dose with bone marrow cytotoxicity and *in vivo* unscheduled DNA synthesis up to the limit dose). It was concluded, therefore, that metconazole has intrinsic clastogenic activity which is not expressed in the whole animal. Consequently, there is no concern for mutagenicity.

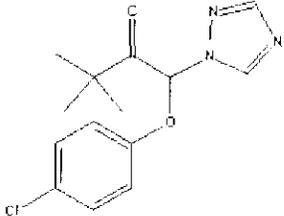
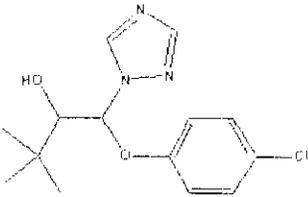
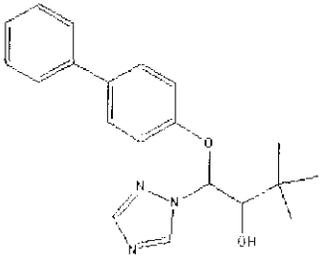
3. Structure-Activity Relationship

Metconazole is structurally related to other triazole ("conazole") fungicides as illustrated in Table 9. Several parent triazole pesticides have been shown to be carcinogenic in rodents, specifically liver tumors in the mouse and/or thyroid tumors in rats. The triazole class of compounds generally lack genotoxic potential, while some compounds (including metconazole) test positive only in *in vitro* chromosomal aberration assays. A common mammalian metabolite of the conazole fungicides is 1,2,4-triazole. The degree of *in vivo* conversion of the parent compound to free triazole varies greatly (0-77% in the rat) and appears to be parent compound-dependent.

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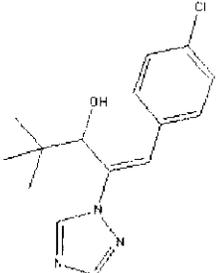
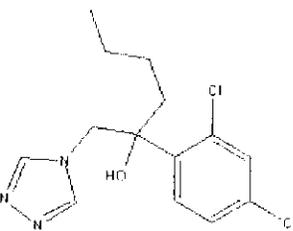
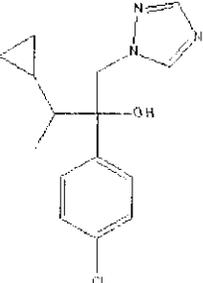
TABLE 9. Structure-Activity Relationship of Related Triazole Compounds ^{a,b}

Compound	Structure	Carcinogenic Effect	Carcinogen Class/Mutagen
Bayleton PC 109901 Tx.# 862AA		<p>NMRI Mouse Only hepatocellular adenoma, at 1800 in (22%)♂ & (18%)♀ p<0.05 for trend and pairwise comparisons. Historical Control incidence: 18.4% ♂, and 2.0% ♀.</p> <p>Wistar Rat Dose related trend in thyroid F-cell adenomas in ♂ & combined adenomas/carcinomas with cystic hyperplasia in ♂ & ♀; Pair wise comparisons not significant.</p>	<p>C NO Q 1*</p> <p>Negative for mutagenicity</p>
Baytan PC 127201 Tx.# 074A		<p>F1-W74 Mouse, 2000 ppm: Hepatocellular adenomas and hyperplastic nodules (p<0.01) in ♀. No increase in ♂. Adrenal adenomas noted in ♀ LDT and HDT but not in historical controls. No elevation in carcinomas.</p> <p>Rat, 125-2000 ppm, increases in thyroid adenomas.</p>	<p>Weak C SAP</p> <p>Negative for mutagenicity</p>
Baycor PC 112403 Tx.# 087AA		<p>Mouse: up to 500 ppm: (-)</p> <p>Rat: up to 500 ppm : (-)</p>	<p>Negative for mutagenicity</p>

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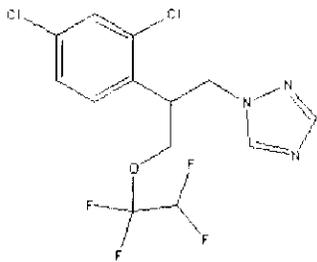
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Compound	Structure	Carcinogenic Effect	Carcinogen Class/Mutagen
Uniconazole PC 128976 Tx.# 207H		CrI:CD-1(ICR)BR Mouse Increased incidence of hepatocellular adenomas and carcinomas in males only at HDT . CrI:CD-1(ICR)SD Rat No increase in neoplastic findings	C NO Q 1* Positive <i>in vitro</i> chromosomal aberration with S9
Hexaconazole PC 128925 Tx. # 480G		CD-1/Alpk Mouse , 5, 40 & 200 ppm. No oncogenic effect. Should be seen with caution because MTD was not reached. No oncogenic effect. ALpk:APfSD (Wistar derived) Rat , 10, 100, 1000 ppm. There was a significant ($p < 0.01$) dose-related trend and a significant pairwise comparison with controls at the HDT for benign Leydig cell tumors in the testes. The incidence at the HDT (16%) exceeded historical control values of up to 6.0%	C with Q 1* Mutagenicity Negative: Ames Microsomal, Unscheduled DNA synthesis and human lymphocyte assays
Cyproconazole PC 128993 Tx. # 272E		CD-1 Mouse , 5, 15, 100 & 200 ppm. Significant incidence of liver adenomas and carcinomas at the MDT and HDT in males and at the HDT in females.	B2 Negative for mutagenicity except positive <i>in vitro</i> chrom. aberration assay

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Compound	Structure	Carcinogenic Effect	Carcinogen Class/Mutagen
Tetraconazole PC 120603		CD-1 Mouse , 10, 90, 800 & 1250 ppm. Significant increasing trend in both sexes with differences in the pair-wise comparisons of the 1250-ppm dose group with the controls for liver adenomas, carcinomas and combined adenomas/carcinomas, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 800-ppm dose group with the controls for liver benign tumors and for benign and/or malignant tumors combined in both sexes, both at $p < 0.01$.	Not Classified Mutagenicity Negative: Ames Micronucleus UDS assays

a Taken from Cancer Assessment Document: Evaluation of the Carcinogenic Potential of Epoxiconazole. HED Document No. 014451

b Abbreviations: ♀: Female; ♂: Male; HDT: Highest dose tested; LDT: Lowest dose tested; SAP: Scientific Advisory Panel

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

Similar to the chronic rat studies, decreased body weight, increased liver weight, hepatocellular hypertrophy were observed in the 28 day (**MRID 44721515**) and 90 day (**MRID 44721517**) subchronic rat studies at 1000 ppm. In the 90 day mouse study, decreased cholesterol, increased liver and spleen weights, elevated AST and ALT levels, and increased incidence of hepatocellular hypertrophy were observed at 300 ppm (**MRID 44721519**).

i) 28-day oral toxicity study in rats

In a 28-day oral toxicity study (MRID 44721515), seven Fisher F344 rats/sex/dose were fed a diet of Metconazole (94.5% a.i.; 85% cis:15% trans; nominal 80:20, batch 88-10) at dose levels of 0, 30, 100, 1000 or 3000 ppm (equivalent to (m/f): 0, 2.7/3.1, 9.1/10.1, 90.2/97.0, and 261.2/287.4 mg/kg bw/day). Hematology and blood chemistry parameters were measured at 28 days. Urine samples were analyzed after a water loading in week 4. Gross examinations were performed on all animals and nine tissues were examined microscopically in the control and high dose groups. Only the livers and adrenals were examined in all dose groups.

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Administration of metconazole had no effect on survival during the 28 day study period. Overall body weight gain was decreased in males at 1000 ppm (↓27%) and in both sexes at 3000 ppm (males ↓71%, females ↓58%). At week 4, bodyweight in males at 1000 and 3000 ppm were statistically significantly reduced compared to the controls (↓14% and ↓34%, respectively) and in females at 3000 ppm, the mean bodyweight was 19% lower than the control group. Food consumption was reduced 10-30% in these groups. At 3000 ppm, a slight reduction in mean cell hemoglobin concentration and erythrocyte mean diameter was observed. Platelet counts at 3000 ppm were reduced 20%. Increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (-GT) activities were observed at the highest dose. Glucose levels were reduced 15-30% in both sexes at the high dose. Cholesterol decreased 25% in both sexes at 3000 ppm and a 23% reduction in cholesterol was observed in males at 1000 ppm. Urinalysis revealed no treatment-related changes. Dose-related changes in absolute liver weights were observed in females at 1000 ppm and 3000 ppm (↑21% and ↑67%, respectively). Slight increases in absolute liver weights were observed in males at 1000 and 3000 ppm (↑4% and ↑8%, respectively). Relative spleen weights (↑23%, ↑33%) and relative kidney weights (↑5.8%, ↑11.8%) were statistically significantly higher at 3000 ppm than the control group in males and females, respectively. Macroscopic examination showed enlarged and pale livers in both sexes at 1000 ppm and 3000 ppm. Enlarged livers were also observed in 3/7 males at 100 ppm. Junctional ridge thickening and/or ulceration of the forestomach was observed in 4/7 males at the highest dose. The primary target organ was the liver as revealed by increased incidences of fatty vacuolation and hepatocellular hypertrophy in both sexes at 1000 and 3000 ppm. No microscopic abnormalities were observed in the liver at 100 ppm. Increased incidences of hyperkeratosis was observed in the forestomach of males at 3000 ppm which correlated with the macroscopic findings. Most animals at 3000 ppm showed adrenal cortical vacuolation. **The LOAEL is 1000 ppm (90.5 mg/kg/day), based on decreased body weight, increased liver and kidney weights, hepatocellular vacuolation and hypertrophy. The NOAEL is 100 ppm (9.1 mg/kg/day).**

This 28-day oral toxicity study in the rat is classified as acceptable/guideline and satisfies the guideline requirement for a 28-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats

ii) 90-day oral toxicity study in rats

In a 90-day oral toxicity study (MRID 44721517), WL148271 (94.5% pure with a cis/trans ratio of 81:19, batch # 88-10) was administered in diet to 10 F344 rats/sex/dose at dose levels of 0, 30, 100, 300, 1000 or 3000 ppm (equivalent to 0, 1.9, 6.4, 19.2, 64.3 and 192.7 mg/kg/day in males and 0, 2.1, 7.2, 22.1, 71.4, and 208.0 mg/kg bw/day in females). Two satellite groups (10/sex/group) were fed 0 or 3000 ppm for 13 weeks, followed by a 7 week recovery period.

There were no treatment-related effects on mortality. Increased food spillage was observed at the high dose in both sexes and occasionally at 1000 ppm. Food consumption was reduced in both sexes at 1000 ppm (males 12%, females 9%, both $p < 0.01$) and 3000 ppm (males 33%, females

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22%, both $p < 0.01$). Overall body weight was reduced in males at 1000 ppm (9%, $p < 0.01$) and in both sexes at 3000 ppm (males 35%, females 19%, both $p < 0.01$). Discharge and alopecia were observed in most high dose (3000 ppm) animals in both sexes, but not in the control animals. The eyes of lower dose animals were not examined. Hematological findings revealed changes in parameters consistent with mild hypochromic microcytic anemia at the high dose in both sexes. Decreased hemoglobin (4%, $p < 0.01$), mean corpuscular hemoglobin (2.7%, $p < 0.01$) and mean corpuscular hemoglobin concentration (2%, $p < 0.01$) were also observed in females at 1000 ppm. Elevated white blood cell counts were observed in females at 300 ppm and above. Some recovery was observed in the satellite high dose group, however mean corpuscular volume, platelet count and plateletcrit remained low in males. Clinical chemistry revealed statistically significant ($p < 0.01$) increases in plasma alkaline phosphatase (m 35%, f 42%), gamma-glutamyl transpeptidase, aspartate aminotransferase (m 77%, f 70%) and alanine aminotransferase (m 136%, f 94%) in both sexes at 3000 ppm. Alanine aminotransferase was also elevated in males (13.6%, $p < 0.01$) at 1000 ppm. Cholesterol was decreased in males at 1000 ppm (36%, $p < 0.01$) and 3000 ppm (48%, $p < 0.01$) and triglyceride levels were down 69% ($p < 0.01$) in males at 1000 ppm and in both sexes (m 96% and f 70%) at 3000 ppm. The decreased cholesterol and triglycerides is indicative of perturbations in hepatic lipid metabolism. Increased adjusted liver weight ($p < 0.01$) was observed in the male and female at 1000 ppm (31% and 20%, respectively) and at 3000 ppm (11% and 53%, respectively). Increased spleen weights were reported in females at 300 and above and in both sexes at 3000 ppm. Decreased relative adrenal weights were also observed in males at 3000 ppm (11%, $p < 0.05$). Histopathological changes in the liver were observed in all males at 1000 and in both sexes 3000 ppm including centrilobular hepatocyte hypertrophy and fatty vacuolation. An increased incidence of fatty vacuolation (4/10, $p < 0.05$) was observed in males at 300 ppm and a single incidence of centrilobular hypertrophy was in this group. In the satellite group, a reduction in the incidence of fatty vacuolation was observed in high-dose males (4/10, $p < 0.01$), but remained statistically significant in females, 6/10 ($p < 0.05$). Increased incidences of pigmented Kupffer cells was also observed in the high dose group (9/10 males, 10/10 females). In the spleen, decreased hematopoiesis was observed in all high dose animals. Pigment deposit was observed in 9/10 males and all females at 3000 ppm and white pulp was reduced in the high-dose males (3/10, $p < 0.001$) and females (9/10, $p < 0.001$). Forestomach focal hyperplasia was observed in the forestomach of 6 males and 2 females at the high dose. Moderate or slight adrenal cortical vacuolation was observed in all males and 6 females at 3000 ppm. **The NOAEL for this study is 100 ppm (6.4 mg/kg/day) and the LOAEL is 300 ppm (19.2 mg/kg/day) based on increased hepatocellular fatty vacuolation and increased spleen weight in females.**

This 90-day oral toxicity study in the rat is acceptable/guideline and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

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iii) 90-day oral toxicity study in mice

In a 90-day oral toxicity study (MRID 44721519, Metconazole (95.3% pure, WL148271, Batch 89-01) was administered to 12 Crl:CD-1 (ICR)BR mice/sex/dose in diet at dose levels of 0, 30, 300, 3000 ppm (equivalent to 0, 4.6, 50.5 and 341.1 mg/kg bw/day in males and 6.5, 60.7 and 446.2 mg/kg bw/day in females). The high dose was reduced from 3000 ppm to 2000 ppm beginning on week two, due to significantly reduced food consumption and body weight in this dose group.

There were no compound related effects on mortality or clinical signs. Overall food consumption was impaired in the high dose group in both sexes (-9% males, -14% females, $p < 0.05$). This resulted in reduced body weight gain of 61% ($p < 0.001$) and 51% ($p < 0.001$) in males and females respectively at 2000 ppm. Hematological findings showed slightly decreased values in hematocrit, mean cell volume and cell hemoglobin at 2000 ppm indicative of mild microcytic anemia. In high-dose females, increased leukocytes and neutrophils, and decreased lymphocyte counts were observed. The primary target organ at the high dose was the liver corroborated by increased liver weight (males 113% $p < 0.001$, females 121%, $p < 0.001$), hepatocellular hypertrophy and vacuolation, increased alanine aminotransferase (ALT; males 455%, $p < 0.001$, females 508%, $p < 0.001$) and aspartate aminotransferase levels (AST; males 172%, $p < 0.001$, females 146%, $p < 0.001$) decreased bilirubin (males 46%, females 28%, both $p < 0.001$) and cholesterol (males 65%, females 60%, both $p < 0.001$). At 300 ppm, increased relative liver weight was observed at 300 ppm in both sexes (males 22%, females 24%, both $p < 0.001$) and increased spleen weight (30%, $p < 0.01$) in females. In males at 300 ppm, increased AST (61, $p < 0.001$) and ALT (61%, $p < 0.01$) levels were reported. Elevated AST levels were reported in males at 30 ppm (32%, $p < 0.05$), however no other clinical or histopathological findings support evidence of toxicity at this dose level. **The LOAEL is 50.5 mg/kg/day, based on increased aminotransferase (ALT and AST) levels, increased liver and spleen weight, hepatocellular hypertrophy and decreased cholesterol. The NOAEL is 4.6 mg/kg/day.**

This 90-day oral toxicity study in the mouse is acceptable (guideline) and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse.

b) Chronic Toxicity

In a chronic toxicity study (MRID 44721609) Metconazole (WL148271, 95.3% pure, 79.8% cis: 15.5% trans, batch # 89-01) was administered to 20 Fischer 344 rats/sex/dose in diet at dose levels of 10, 100, 300 or 1000 ppm (equivalent to 0.4, 4.3, 13.1 and 43.9 mg/kg bw/day in males and 0.5, 5.3, 16.0 and 53.8 mg/kg bw/day in females) for 2 years. A group of 40 Fischer 344 rats/sex were fed untreated diet and served as controls. A second group of 10 rats/sex/dose were fed the test material at the doses above and 20 rats/sex were fed the control diet for 1 year for an interim sacrifice.

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There were no compound related effects on the incidence or cause of mortality among the treatment groups and no relevant clinical signs were observed. Food consumption was reduced in the high dose group (1000 ppm) in both sexes (10% male, 5% female) during the first 13 weeks of treatment. In the high-dose animals, a reduction in mean body weight gain was observed throughout the study with an overall reduction of 9% in males and 6% in females. Mean (overall) body weight gain was also reduced in female rats at 300 ppm. No abnormal leukocyte cell types were observed in the blood films at any dose. Statistically significant decreases in total cholesterol, triglycerides, bilirubin, albumin (females only), alkaline phosphatase (males only) and alanine aminotransferase (both sexes at 1000 ppm and females at 300 ppm) were observed at sampling periods throughout the study in the high dose groups, but not at termination. Decreased total cholesterol (11%, $p < 0.05$) in females at 300 ppm was observed on week 26 and decreased albumin (3%) was observed in this group on weeks 26 ($p < 0.01$) and 51 ($p < 0.05$). Urine osmolarity was higher (25-32%, $p < 0.01$) in males at 1000 ppm and urine volume was decreased (18-39%, $p < 0.01$) in this group. At termination, absolute and relative spleen weights were increased in males at 300 ppm (39%) and in both sexes (56% male and 21% female) at the high dose. Relative spleen weights were also elevated in females at 52 week (9%, $p < 0.05$) at 1000 ppm. Increased relative liver weight was observed at week 52 in males at 300 ppm (5%, $p < 0.01$) and 1000 ppm (20%, $p < 0.01$). Relative liver weight were increased 12% ($p < 0.05$) at week 104 in high-dose females. At week 52 sacrifice, livers with enlarged and/or mottled appearance were reported in males at 300 and 1000 ppm. Hepatocellular hypertrophy and hepatocellular lipid vacuolation were observed at week 52 in males at 300 and in both sexes at 1000 ppm. At termination sacrifice, hepatocellular hypertrophy and vacuolation were restricted to the high dose animals. Males in the high dose group also exhibited an increased incidence of eosinophilic and clear-cell hepatocellular foci at termination. An increased incidence of splenic histiocytic foci was observed in both sexes at the high dose. **The LOAEL is 300 ppm (13.1 mg/kg/day), based on increased spleen and liver weights and increased hepatocellular vacuolation and hypertrophy. The NOAEL is 100 ppm (4.3 mg/kg/day).**

This chronic study in the rat is acceptable (guideline) and satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in the rat.

5. Mode of Action for Mouse Liver Tumors

There are two fundamental ways in which a chemical may result in hepatocarcinogenesis. It may either increase the amount of DNA damage that occurs every time the cell replicates (i.e., via a mutagenic mode of action) or it can increase the number of times the DNA replicates (i.e., enhance cell proliferation) leading to the clonal growth of spontaneous initiated cells. Increased cell replication can occur either by direct mitogenesis (i.e., directly stimulating cells to divide via certain hormones or growth factors) or increased cell proliferation may be the result of toxicity (cell death) with consequent regenerative proliferation. Because metconazole is predominantly negative in a wide range of genotoxicity tests and is not overtly cytotoxic, it appears that this compound is a rodent hepatocarcinogen via a mitogenic mode of action (MOA). Consequently, the registrant has submitted two mechanistic studies to support this MOA:

a) 28-day mouse and rat hepatic xenobiotic metabolizing enzyme study

In a mechanistic study (MRID 44721626), metconazole (94.2% a.i., *cis* isomer, ST90/369, batch 12) was administered to 16 male CD1 mice/dose in diet at dose levels of 0 or 300 ppm (equivalent to 0 and 58.2 mg/kg/day) and was administered to 16 male Fisher 344 rats/dose at a dose level of 0 or 1000 ppm (equivalent to 0 and 86.7 mg/kg/day). Animals were sacrificed 7 or 28 days after initiation of treatment. The livers were isolated, weighed and the liver homogenates and microsomal fractions were analyzed for xenobiotic metabolizing enzymes. Liver sections were examined by light and electron microscopy for treatment-related organelle and ultrastructural changes. Phenobarbitone is a well-known non-genotoxic rodent carcinogen and was used in this study as a positive control in mice and rats. Phenobarbitone produced the anticipated effects including increased liver weight, induction of microsomal protein, cytochrome P450 and increased the activities of ethoxycoumarin O-deethylase, ethoxyresorufin O-deethylase, ethylmorphine N-demethylase and lauric acid 11-hydroxylase. Phenobarbitone-treated animals showed no evidence of changes in peroxisome numbers or morphology and the positive control article had no effect on palmitoyl-CoA oxidation.

Mouse

There were no significant changes in body weight or food consumption in mice treated with 300 ppm metconazole compared to the control animals. Other relevant findings:

- **Liver weights:** Relative (adjusted) and absolute mouse liver weights were significantly higher at 300 ppm on day 7 (↑22.4%) and day 28 (↑12.7%) than in the control group.

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- **Hepatic mixed function oxidases (MFO):** Significant increases in microsomal protein and CYP450 content were observed at days 7 and 28 (↑14% and ↑24%, respectively). Ethylmorphine N-demethylase (EMND) and 7-Ethoxycoumarin O-deethylase (ECOD) activity increased 1.4- and 1.6-fold at day 7 and 1.6- and 1.4-fold at day 28, respectively. Lauric acid 11-hydroxylase increased to 140% of control after 28 days ($p<0.01$). No difference in liver protein content or homogenate palmitoyl-CoA oxidation activity was observed
- **Hepatocellular hypertrophy and vacuolation:** Midzonal vacuolation was observed in 3/6 mice at day 28. In addition, single incidences of cytoplasmic vacuolation were observed at day 7 and day 28 and a single incidence of (slight) centrilobular hypertrophy was observed in the mouse at day 28.
- **Electron microscopy:** Electron microscopy of liver sections showed no evidence of an increase in peroxisome numbers or morphology in mice treated with metconazole.

Rat

Body weight was slightly reduced on day 7 (↓5%, $p<0.05$) and food consumption was lower (↓6%, $p<0.05$) on days 3-7 in metconazole-treated animals.

- **Liver weights:** Relative liver weights were higher at 1000 ppm at day 7 (↑9%, $p<0.01$) and day 28 (↑4%, $p<0.05$) compared to the control animals.
- **Hepatic mixed function oxidases:** Statistically significant increases in rat microsomal protein levels were seen in treated animals at days 7 and 28 (↑117% and ↑139% control, respectively). Increased levels of CYP450 were statistically significant ($p<0.001$) at day 7 (154% of control) and day 28 (140% of control) as were activity levels of microsomal enzymes EMND (149% control, day 7; 141% control, day 28) and ECOD (173% control, day 7; 178% control, day 28). No statistically significant changes in the activity levels of hydroxylases LA-11 or LA-12 were observed. No difference in liver protein or DNA content or homogenate palmitoyl-CoA oxidation activity was observed.
- **Hepatocellular hypertrophy and vacuolation:** No treatment-related histopathological findings in the liver were observed at day 7. At day 28, 5/6 rats showed slight or moderate midzonal vacuolation, which was statistically significant ($p<0.05$) when incidences of differing severities of vacuolation were compared to the controls.

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- **Electron microscopy:** Electron microscopy of liver sections showed no evidence of an increase in peroxisome numbers or morphology in rats treated with metconazole

Tabular presentation of these data on measurable liver parameters from the comparative analysis of the metconazole and phenobarbitone induction in mice and rats appears in Table 10. As shown, after 7 and 28 days of treatment, a general trend of significantly increased induction of microsomal P-450 content and the P-450 subset of MFO (e.g., EMND and ECOD) occurred in both rats and mice treated with metconazole. This activity is similar to the MFO induction pattern for phenobarbitone (PB). The only exception was the increased level of LA-12-H in mice (140 % vs 265% for PB in mice) contrasted with no increase in metconazole-treated rats. Additional data presented in Table 11 indicate that both metconazole and PB caused significant increases in the absolute and relative liver weight. Weight changes in livers from rats treated with metconazole were not as clearly increased and conflict with the highly significant effects in PB-induced rat livers. These findings support the observations of Grasso et al, (1991)¹ that increases in the activity of cytochrome P-450 and associated enzymes (principally MFO) may occur in the livers of rodents treated with agents that produce liver growth and liver tumors. These authors also cited PB as a model compound for this liver tumor induction. It is of note that the possibility of peroxisome proliferation as the MOA is rule out for metconazole and PB because of the lack of an effect on palmitoyl-CoA oxidation (Table 10). Furthermore, the lack of ultrastructural changes in peroxisome numbers or size in either rodent exposed to metconazole argues against this MOA. Overall, these data indicate that metconazole in both rodent species can induce a pattern of hepatic metabolism, accompanied by liver enlargement similar to PB with no evidence of peroxisome proliferation.

¹Grasso, P., Sharrat, M., Cohen, A.J (1991). Role of persistent non-genotoxic tissue damage in rodent cancer and relevance to humans.

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TABLE 10. Liver biochemistry findings (presented as % control) in male mice fed 300 ppm metconazole and male rats fed 1000 ppm metconazole for 7 or 28 days in a study to investigate the effects of *cis* Metconazole on male rat and male mouse hepatic xenobiotic metabolizing enzymes.^a

Findings (% Control)	Mouse			Rat		
	Metconazole (300 ppm)		PB (0.05%)	Metconazole (1000 ppm)		PB (0.05%)
	Day 7	Day 28	Day 28	Day 7	Day 28	Day 28
Protein, mg/g liver	93	102	97	105	103	104
DNA content, mg/g liver	81*	99	90*	99	96	92
Palmitoyl-CoA oxidation, nmol/min/mg protein	104	114	89	94	98	91
Microsomal protein content, mg/g liver	114*	124***	128**	117*	139***	146***
Microsomal CYP450 content nmol/mg protein	152***	135***	146***	154***	140***	197***
EMND	139***	159***	213***	149***	141***	172***
ECOD	156**	143***	277***	173***	178***	440***
EROD	102	117	184***	114*	86	300***
LA-11-H	96	140**	265***	95	91	189***
LA-12-H	74	124	148	103	89	130*

^a Data from pages 34 and 53-60 of study report. MRID 44721626.

* Statistically different (p < 0.05) from the control

** Statistically different (p < 0.01) from the control

*** Statistically different (p < 0.001) from the control

PB-- Phenobarbitone

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TABLE 11. Mean absolute and relative liver weights (g) of male mice fed 0 or 300 ppm metconazole and male rats fed 0 or 1000 ppm metconazole for 7 or 28 days in a study to investigate the effects of *cis* Metconazole on male rat and male mouse hepatic xenobiotic metabolizing enzymes.^a

Dose Group	Day 7		Day 28	
	Absolute	Relative	Absolute	Relative
Liver weights- MOUSE				
0 ppm metconazole	1.983 ± 0.1347	5.955 ± 0.2071	2.003 ± 0.1633	5.698 ± 0.2618
300 ppm metconazole	2.387 ± 0.2908* (117%)	7.287 ± 0.5381*** (118%)	2.260 ± 0.3254 (111%)	6.420 ± 0.4834** (111%)
0.05% phenobarbitone	--	--	2.825 ± 0.2863*** (129%)	7.497 ± 0.3950*** (124%)
Liver weights- RAT				
0 ppm metconazole	8.587 ± 0.9637	4.793 ± 0.2788	10.958 ± 0.4840	4.108 ± 0.1061
1000 ppm metconazole	8.815 ± 0.5032	5.230 ± 0.1914** (18%)	10.755 ± 1.0554	4.287 ± 0.1113* (14%)
0.05% phenobarbitone	--	--	14.505 ± 0.7236*** (124%)	5.173 ± 0.0644*** (121%)

^a Data from pages 49 and 50 of study report. MRID 44721626.

* Statistically different (p <0.05) from the control

** Statistically different (p <0.01) from the control

*** Statistically different (p <0.001) from the control

Additional mechanistic data were provided in the following study:

b) 14-day hepatic drug-metabolizing enzyme induction, cell proliferation and reactive oxygen species production study in mice.

In a 14-day mechanistic study (MRID 46665403, Metconazole (98.53% pure; *cis* 82.68%, *trans* 15.85%) was administered to 18 Crj:CD-1 (ICR) female mice/dose in diet at dose levels of 0, 30, 300, 1000 ppm (equivalent to 0, 4.49, 47.6 or 151 mg/kg bw/day). The animals were evaluated at 3, 7 or 14 days for the effects of the test material on drug-metabolizing enzyme induction, cell proliferation, and reactive oxygen species (ROS) production. There were no treatment-related deaths or clinical signs

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during the study. Body weight and food consumption were similar to the controls in all treatment groups.

Liver weights: Enlarged livers were observed in nearly all animals at 1000 ppm after 3, 7 and 14 days of treatment. Absolute and relative liver weights were significantly higher (35-52% and 46-58%, respectively) in the 1000 ppm group at all time points compared to the controls. Liver weights increased 11-18% at 300 ppm compared to the control groups, but did not reach statistical significance.

- **Hepatic mixed function oxidases:** Ethoxycoumarin O-dealkylase (ECOD) activity was elevated 2.1- and 3.1-fold at 300 and 1000 ppm, respectively compared to the control group after 7 days of treatment. Marked elevation in pentoxoresorufin O-dealkylase (PROD) activity was observed at 300 and 1000 ppm (↑3.8- and 14.5-fold, respectively) at day 7 compared to the controls. Western blot analysis showed a significant induction in the levels of the P-450 isozymes CYP1A (↑4.5-fold), CYP2B (↑11.5-fold) and CYP3A (↑3.9-fold) at 1000 ppm relative to the controls. CYP2B and CYP3A were also significantly elevated at 300 and 1000 ppm compared to the control group (↑3.0- and 112-fold, respectively).
- **Hepatic cell proliferation:** An increase in PCNA labeling index after 3 and 7 days of treatment (↑8.5- and 6-fold, respectively) at 1000 ppm. The increase in PCNA labeling index at 1000 ppm was not statistically significant at day 14. No significant differences in PCNA labeling index were observed at the lower doses.
- **Clinical Chemistry alterations:** Significantly elevated glutamic oxaloacetic transaminase (↑94%) and glutamic pyruvic transaminase (↑154%) activity was observed at 1000 ppm. Other blood chemistry findings included dose-related decreases in total cholesterol (↓26% and ↓56%) and total bilirubin (↓22% and ↓33%) at 300 and 1000 ppm, respectively. After 14 days of treatment, lipid peroxide (LPO) levels were significantly higher in the 300 and 1000 ppm groups (↑2.6- and 2.3-fold) compared to the control group indicating membrane lipid peroxidation. No significant changes in 8-hydroxydeoxyguanosine (8-OHdG) levels were observed at any dose.
- **Hepatocellular hypertrophy and vacuolation:** Histopathology of the liver showed hepatocellular hypertrophy and hepatocellular vacuolation in nearly every animal at 1000 ppm. Similar, but less severe histopathology findings were observed in the 300 ppm group

The NOAEL for this study is 30 ppm (4.49 mg/kg/day) based on increased incidence of hepatocellular hypertrophy, increased P-450 content, increased ECOD and PROD activities and increased LPO observed at 300 ppm (47.6 mg/kg/day).

In agreement with the earlier findings, data in the 14-day mechanistic study show significantly ($p < 0.05$, 0.01) increased microsomal activity, ethoxycoumarin O-dealkylase (ECOD) and pentoxyresorufin O-dealkylase (PROD) levels after 7 days of treatment with the tumorigenic dosage of 1000 ppm in the diet (Table 12). Significant effects were also noted at 300 ppm. These increases are reflective of CYP2B and CYP3A subfamily activity which were similarly increased ($p < 0.05$, 0.01) 7 days after treatment with either 300 or 1000 ppm. These findings were accompanied by enlarged livers in 6/6 animals commencing at 3 days and continuing through day 14 (1000 ppm) and increased hepatocellular hypertrophy ($p < 0.01$) at 300 and 1000 ppm, (Table 13). It is of further note that the increased incidence of hepatocellular hypertrophy and enlarged livers is consistent with the profile developed by Grasso et al (1991)² for compounds causing liver enlargement without initial liver damage. As expected, absolute and relative liver weights for the 1000- ppm group were also significantly ($p < 0.01$) increased starting at day 3 and continuing through day 14 (Table 14). Liver weight changes are supported by similar findings in other subchronic and chronic mouse studies. It is of additional note that the liver enlargement and the increased liver weight only occur at a dose where tumors do develop, illustrating the significance of liver enlargement. Taken together, these data provide clear supporting evidence that metconazole has the potential to induce hepatic drug-metabolizing enzymes early in the course of treatment with enhancement of cell volume causing enlarged livers, mirrored by significantly increased liver weight.

Concomitant with the above effects is a mild hepatotoxic response at 1000 ppm associated with hepatocellular vacuolation in 5 of 6 animals by day 14 (Table 13). This is a consequence of enzyme induction; nevertheless, it does suggest a persistent demand on liver output that is further supported by hepatotoxicity manifested as significantly increased glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) values (suggestive of hepatocyte necrosis) at 1000 ppm starting by treatment day 14 and single cell necrosis evident at 52 weeks but persisting through week 93 (Table 15), all of which contribute to cell death.

As a consequence of cell death, increased hyperplasia is evident but only in the high-dose group at the terminal sacrifice. As part of the mechanistic study, however, hepatic cell proliferation was also examined at days 3, 7 and 14 after treatment; these data are presented in Table 16. As shown, marked and significant ($p < 0.01$) increases in the proliferating cell nuclear antigen (PCNA) labeling index of mouse livers occurred at 3 and 7 days but only in the high- dose group. The net result of increased cell proliferation would, therefore, be regenerative proliferation leading to tumor formation.

²Grasso et al, (1991).

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Thus, the overall findings of this analysis are in good accord with Grasso et al.(1991) observations regarding agents associated with liver enlargement and tumor induction:

"...With some compounds a mild toxic process and a prolonged episode of increased cell turnover occurs."

"...the liver remains enlarged from the early stages until the appearance of tumors, suggesting that the original demands made on the liver persist. This type of "stress" may involve episodes of proliferative activity that could easily be missed in commonly undertaken experiments...'

It is also noteworthy that while a similar pattern of MFO induction: increased liver weights (marginally increased, 8% by day 7), hypertrophy (slight centrilobular hypertrophy by day 28 in 5 of 6 rats and 62% at 104 weeks), and hepatotoxicity (e.g. vacuolation slight to moderate in 4 of 6 rats at 28 days; other elements of hepatotoxicity at 104 weeks (↑basophilic and clear cell foci) were noted in rats fed dietary concentrations up to 1000 ppm in males and 3000 ppm in females. Despite these similarities, there was no indication of hyperplasia or tumors in male or female rats receiving a high dose stated above for 104 weeks.

Accordingly, the CARC concluded that the available data are sufficient to support a plausible non-linear, non-genotoxic mode of action for liver carcinogenicity of metconazole in mice. The CARC agrees with the registrant that a series of steps involved in the mitogenic response have been identified that form the basis for the MOA for liver tumors induction in mice. The initial key event in the induction of liver neoplasms in female ICR mice treated with metconazole is enhancement of P-450 microsomal enzymes, leading to increased cell proliferation, and ultimately resulting in liver adenomas and carcinomas.

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Table 12. Data supporting mitogenesis as the mode of action for metconazole induced mouse liver tumors- biochemical markers^a (female mice)

Effect	Treatment Time (days)	Dose (ppm)			
		0	30	300	1000
Microsomal Enzyme Activity					
Microsoma: Protein content (mg/g liver)	7	44 ± 5	49 ± 5	56 ± 4* (+127%)	55 ± 11** (+25%)
Ethoxycoumarin O-dealkylase activity (nmol/min/mg protein)	7	0.53 ± 0.03	0.61 ± 0.12	1.09 ± 0.18* (+106%)	1.65 ± 0.31** (+211%)
Pentoxoresorufin O-dealkylase (nmol/min/mg protein)	7	33 ± 4	45 ± 8	125 ± 17** (+279%)	148 ± 18** (+348%)
P-450 content (nmol/mg protein)	7	0.42 ± 0.03	0.49 ± 0.09	0.87 ± 0.10* (+107%)	1.29 ± 0.12** (+207%)
CYP1A (%)	7	0.96 ± 0.43	0.95 ± 0.53	2.13 ± 1.89 (+122%)	4.33 ± 2.25** (+351%)
CYP2B (%)	7	23.6 ± 6.0	38.3 ± 11.8	93.5 ± 10.8* (+296%)	271.2 ± 66.4** (+1049%)
CYP3A (%)	7	18.2 ± 2.6	15.0 ± 4.5	45.6 ± 7.3** (+151%)	71.8 ± 6.3** (+295%)

^a Data were extracted from MRID 46665403.

*Significantly different (p<0.05) than control.

** Significantly different (p<0.01) than control.

Table 13. Incidence of macroscopic findings and microscopic liver lesions in female mice for each dose group after treatment with metconazole.^a

Dose (ppm):		0	30	300	1000
Enlarged liver	Day 3	0/6	0/6	0/6	6/6**
	Day 7	0/6	0/6	0/6	6/6**
	Day 14	0/6	0/6	0/6	5/6**
Hepatocellular hypertrophy	Day 14	0/6	0/6	6/6**	6/6**
Hepatocellular vacuolation	Day 14	0/6	0/6	2/6	5/6**

^a Data from MRID 46665403.

** Statistically different (p < 0.01) from the control

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Table 14. Absolute and relative female mouse liver weights for each dose group after 3, 7 or 14 days of treatment with metconazole.^a

Dose (ppm) n=6/group	Treatment period (days)	Body weight	Liver weights	
			Absolute	Relative#
0	3	29.7±2.0	1.36±0.18	4.55±0.33
	7	32.4±2.1	1.51±0.06	4.65±0.16
	14	33.8±2.3	1.44±0.14	4.27±0.36
30	3	29.2±3.7	1.36±0.31	4.61±0.65
	7	31.7±2.4	1.43±0.19	4.52±0.38
	14	32.4±1.6	1.46±0.11	4.52±0.43
300	3	31.4±2.4	1.61±0.15 (+18%)	5.11±0.18 (+12%)
	7	31.7±1.8	1.68±0.22 (+11%)	5.31±0.63 (+14%)
	14	31.4±2.6	1.57±0.18 (+12%)	4.99±0.27 (+17%)
1000	3	30.8±1.7	2.07±0.15** (+52%)	6.75±0.54** (+48%)
	7	30.1±1.8	2.04±0.21** (+35%)	6.79±0.59** (+46%)
	14	30.6±5.7	2.07±0.55 (+44%)	6.67±0.78** (+56%)

^a Data from MRID 46665403.

Relative liver weight to body weight (%)

** Statistically different (p < 0.01) from the control

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Table 15. Summary of Hepatotoxicity Data for Mouse Liver Tumors in Females Fed Metconazole in the Diet

Dose (ppm)	Treatment Time	0	30	300	1000	Source (MRID)
EFFECTS						
G01 (U/L)	14 days	48-7	55±12	55±8	93±20* (194%)	46665403
	90 days	107-42	91±48	93±22	263±88** (508%)	44721519
G21 (U/L)	14 days	24-3	31±15	31±7	61±26* (154%)	46665403
	90 days	39±23	33±14	41±16	237±84** (146%)	44721519
Single Cell Necrosis (%)	52 weeks	0	0	25	92	44721612
	93 weeks	0	2	20	73	
Oval Cell Hyperplasia (%)	93 weeks	0	0	0	27	
Multifocal Hyperplasia (%)	93	0	0	0	86	

*Significantly different (p<0.01) than control.

** Significantly different (p<0.001) than control.

Table 16. Hepatic cell proliferation activity in female mouse livers treated with metconazole^a

Effect	Treatment Time (days)	Dose (ppm)			
		0	30	300	1000
Hepatic Cell Proliferation Activity					
Proliferating Cell Nuclear Antigen (PCNA) Labeling Index (%)	3	0.2±0.2	0.2±0.2	0.3±0.1	1.7±0.4** (750%)
	7	0.1±0.1	0.1±0.1	0.2±0.1	0.6±0.1** (500%)
	14	0.1±0.1	0.4±0.5	0.1±0.1	0.3±0.3

^a Data were extracted from MRID 446665403.

*Significantly different (p<0.05) than control.

** Significantly different (p<0.01) than control.

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Summarized Key Events

Metconazole appears to induce P450 expression. It should be stressed that CYP induction may reflect a broader pleiotropic response, and, therefore, may not be causal in the carcinogenic process. The induction of microsomal enzymes may be mediated by nuclear receptors (such as CAR or PXR). The key events in the non-genotoxic mode of action include the following:

- *Differential induction of hepatic P450 microsomal enzymes.* The induction of P450-dependent mixed function oxidases was seen at 7 days (300 and 1000 ppm) and 28 days (300 ppm, only dose tested). There are a number of characteristic morphological changes that are associated with CYP induction including an increase in liver weight, proliferation of the smooth endoplasmic reticulum, centrilobular hypertrophy and hyperplasia, as follows:
 - Cellular hypertrophy: Microscopic evidence of hypertrophy was observed at 14 days (300 and 1000 ppm), 28 days (300 ppm, only dose tested), 90 days (300 and 2000 ppm), 52 and 91 weeks (300 and 1000 ppm). A dose-related increase in incidence and severity of hepatocellular hypertrophy was observed.
 - Enlarged livers and increased relative liver weights. Increased relative liver organ weights were observed at 3, 7 and 14 days (300 and 1000 ppm, MRID 46665403), at 28 days (300 ppm, only dose tested, MRID 44721626), 90 days (300 and 2000 ppm, MRID 44721519) 52 weeks and 91 weeks (300 and 1000 ppm, MRID 44721612).
- *Hepatic cell proliferation.* Metconazole showed early mitogenic activity in the liver with increased cell proliferation (PCNA labeling index) at 3, 7 and 14 days at 1000 ppm.

Accompanying this mitogenic response is hepatotoxicity. The hepatotoxicity would contribute to the promotion of the liver tumor response. Evidence for hepatotoxicity includes:

- *Hepatocellular vacuolation.* Hepatotoxicity as evidenced by hepatocellular degeneration/vacuolation was observed at 14 days (1000 ppm), 28 days (300 ppm, only dose tested), 90 days (300 and 2000 ppm), 52 and 91 weeks (300 and 1000 ppm).
- *Hepatocellular (single cell) necrosis/pigmented Kupffer cells.* Hepatotoxic changes of single cell necrosis and pigment deposition were observed at the highest dose tested (2000 ppm) at 90 days and at 300 and 1000 ppm at weeks 51 and 91.

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- *Clinical chemistry alterations.* Elevated ALT, AST were observed at 1000 ppm at 14 days, 90 days, 51 weeks and 91 weeks. Increased lipid peroxide was seen at 300 and 1000 ppm at 14 days, 90 days, 51 weeks and 91 weeks

As consequence of cell death, the sequence of event that follows are:

- *Hepatocellular hyperplasia.* Hepatocellular hyperplasia, indicative of compensatory cell renewal, was evident at weeks 51 and 91 following persistent long-term dietary administration of 1000 ppm metconazole. This is supported by liver data showing highly significant increased levels of PCNA labeling commencing on day 3 and continuing through day 14 in females dosed with 1000 ppm metconazole.
- *Hepatocellular tumors.* Progression to hepatocellular adenomas (male and females) and carcinomas (females) was observed at the highest dose tested, 1000 ppm.

B. Alternative Modes of Action

Two alternative MOAs that could be operative in liver tumors results from 1) oxidative damage and 2) peroxisome proliferation. Oxidative damage as suggested by the induction of lipid peroxidation can, however, be ruled out because there was no significant change in 8-hydroxydeoxyguanosine (8-OHdG) adducts or increased palmitoyl-CoA oxidation activity. Similarly, peroxisome proliferation is eliminated because of the lack of data showing an effect on palmitoyl-CoA oxidation. Furthermore, the lack of ultrastructural changes in peroxisome numbers or size in either rodent exposed to metconazole argues against this MOA

C. Reversibility

Although no studies were submitted to address the reversibility of effects, CARC concluded, based on experience with other liver mitogens, that these data were not critical because of the clear evidence that metconazole fits the model for compounds causing liver enlargement (without initial liver damage) developed by Grasso et al. (1991). Similarly, the data fit the profile of no tumors in the absence of liver enlargement. Although these data are desirable, the lack of a reversibility study does not discount the proposed MOA.

D. Relevance to humans

The key events in the non-genotoxic MOA are plausible in humans. One would anticipate the downstream events of increased cell proliferation and tumor response if a dose was reached that produced similar liver perturbations. The MOA is applicable to all populations, including children. Although metabolic enzyme systems in children do not reach adult levels of activity until 6 months to 1 year of age, we should still assume this MOA is operational in general. The NOAEL is protective.

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V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

The Committee's assessment of the weight-of-the-evidence is discussed below:

I. Carcinogenicity

Rat

► In female rats, the incidence of mononuclear cell leukemia (MCL) (at all sites) was 10/89 (11%), 2/19 (11%), 10/41 (24%), 12/40 (30%), and 16/70 (23%) for the control, 10, 100, 300 and 1000 ppm dose groups for both the chronic toxicity and carcinogenicity study combined. There was a significant difference in the pair-wise comparison of the 300 and 1000 ppm dose group with controls for MCL at all sites, however, the increased incidences were within the historical control range for the testing laboratory. The CARC concluded that the increased incidence of mononuclear cell leukemia seen in female rats did not contribute to the weight-of-evidence since it was difficult to interpret the biological significance of the response since not all animals in the 100 and 300 ppm dose groups were examined for this tumor (only the decedents were examined for all tissues).

► No treatment related tumors were seen in male rats.

► Adequacy of Dosing: The CARC considered the highest dose tested, 1000 ppm, to be adequate, but not excessive, to assess the carcinogenicity of metconazole in male and female rats. This was based on decreased body weight (14% males, 16% females) and body weight gain (6% male, 9% female), increased organ weights (liver, kidney, spleen), and adrenal and liver histopathology seen in males and/or females at 1000 ppm.

Mouse

► Liver tumors noted at the highest dose tested (1000 ppm) in both sexes were considered to be treatment-related since:

- In male mice, there were significant differences in the trends and pair-wise comparisons of the 1000 ppm dose group with the controls for liver adenomas (35/55 (64%) vs 11/53 (21%), controls) and adenomas and/or carcinomas combined (38/55 (69%) vs 13/53 (25%), controls), all at $p < 0.01$.

- In female mice, there were significant differences in the trends and pair-wise comparisons of the 1000 ppm dose group with the controls for liver adenomas (50/61 (82%) vs 0/56 (0%), controls), carcinomas (20/61 (33%) vs 0/56 (0%),

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controls), and adenomas and/or carcinomas combined (52/61 (85%) vs 0/56 (0%), controls), all at $p < 0.01$.

- Both the incidence of liver adenomas and carcinomas in both sexes at the high dose exceeded the historical control ranges for the testing laboratory.

- ▶ Adequacy of Dosing: The CARC considered the highest dose tested, 1000 ppm, to be adequate, but not excessive, to assess the carcinogenicity of metconazole in male and female mice. This was based on decreased body weight (↓7% males, ↓13% females), body weight gain (↓25% males, ↓32% females), increased liver weight, elevated aminotransferase enzyme levels, liver vacuolation, hypertrophy and necrosis.

2. Mutagenicity

- ▶ There is no mutagenicity concern for metconazole.

3. Structure-Activity Relationship

- ▶ Metconazole is structurally related to other triazole ("conazole") fungicides. Several parent triazole pesticides have been shown to be carcinogenic in rodents, specifically liver tumors in the mouse and/or thyroid tumors in rats. The triazole class of compounds generally lack genotoxic potential, while some compounds (including metconazole) test positive only in chromosomal aberration assays.

4. Mode of Action

- ▶ The CARC agreed with the registrant that a plausible non-genotoxic mode of action involving mitogenesis was established for the development of liver tumors in a mouse bioassay with metconazole. This conclusion was based on the following:

- Data from *in vivo* and *in vitro* genetic toxicology studies are largely negative (with the exception of one *in vitro* test for clastogenicity) and, therefore, indicate that mutagenicity is not a key event in the mode of action;
- There is dose-concordance between liver tumors, cell proliferation, and hepatic microsomal enzyme induction. The threshold level is a NOAEL of 4.3 mg/kg/day; this would be protective of early liver disturbances;

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- A temporal relationship supporting the MOA was demonstrated. The mitogenic proliferative response was identified as early as 3 days after the onset of treatment, which declined after 14 days of treatment.

Although evidence of hepatotoxicity was also seen in the mouse liver, this does not discount a threshold mode of action. The hepatotoxicity would contribute, along with the mitogenicity of the compound, to the tumor promotion.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified Metconazole as "**Not Likely to be Carcinogenic to Humans**" based on convincing evidence that a non-genotoxic mode of action for liver tumors was established in the mouse and that the carcinogenic effects were not likely below a defined dose that doesn't cause mitogenesis. There was evidence of liver effects (microsomal induction, liver weight increases, hypertrophy) at 300 ppm (47.6 mg/kg/day), but no effects at 30 ppm (4.5 mg/kg/day) in the mode of action studies in the mouse. The chronic Reference Dose of 0.04 mg/kg/day based on the 2-year chronic rat study with a NOAEL of 4.3 mg/kg/day would be protective of early liver disturbances seen in the mouse studies.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The quantification of carcinogenic potential is not required.

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