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MEMORANDUM

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

SUBJECT: Tetraconazole - Report of the Cancer Assessment Review Committee

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The Cancer Assessment Review Committee met on November 10, 1999 to evaluate the carcinogenic potential of Tetraconazole. Attached please find the Final Cancer Assessment Document.

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**CANCER ASSESSMENT DOCUMENT**

**EVALUATION OF THE CARCINOGENIC POTENTIAL OF  
TETRACONAZOLE**

**FINAL REPORT**

**11-JANUARY, 2000**

**CANCER ASSESSMENT REVIEW COMMITTEE  
HEALTH EFFECTS DIVISION  
OFFICE OF PESTICIDE PROGRAMS**

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## EXECUTIVE SUMMARY

On November 10, 1999, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of tetraconazole. The studies evaluated included a 2-year combined chronic toxicity/carcinogenicity study in Crl:CD (SD) rats and a 20-month carcinogenicity study in Crl:CD-1 (ICR) mice as well as mechanistic studies submitted by the Registrant to support the mode of action for the induction of liver tumors in mice. In carcinogenicity studies, tetraconazole was administered in the diet to rats (70/sex/group) at 0, 10, 80, 640 or 1280 (males only) ppm (0, 0.4, 3.4, 27.7 or 59.4 mg/kg/day for males, and 0, 0.6, 4.4 or 39.4 mg/kg/day for females; respectively) and to mice (60/sex/group) at 0, 1, 10, 90, 800 or 1250 ppm (0, 1.4, 12, 118 or 217 mg/kg/day for males and 0, 1.6, 14.8, 140 or 224 mg/kg/day for females, respectively).

The CARC concluded that:

- **Tetraconazole was not carcinogenic to rats** because: 1) In males, although there was an increase in the incidence of thyroid follicular cell adenomas compared with control at  $\geq 80$  ppm ( $\geq 3.4$  mg/kg/day), the increase was not statistically significant, lacked dose-response and was within the range for the historical controls; 2) There was no increase in thyroid or other tumors in females. The dosing at the highest dose in both sexes was considered to be adequate based on a decrease in body weight gains in females in a subchronic study, decreases in body weights at terminal sacrifice in both sexes at the highest dose, increased adrenal and pituitary weights and histopathological changes in bone and liver in the present study. The CARC determined that the thyroid tumors in males were not treatment-related.
- **Tetraconazole was carcinogenic to mice** because: 1) There was a statistically significant ( $p < 0.01$ ) increase by pair-wise comparisons of the 800 ppm (118 and 140 mg/kg/day, in males and females, respectively) and 1250 ppm dose groups (217 and 224 mg/kg/day, for males and females, respectively) with the controls for hepatocellular adenomas, carcinomas (1250 ppm groups only) and combined adenomas/carcinomas in both sexes. The incidences of these tumors exceeded the range of historical controls. There were also statistically significant ( $p < 0.01$ ) increasing trends in both sexes for hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas; 2) There was a numerical increase in the incidence of ovarian luteomas in high-dose females. A statistically significant ( $p < 0.01$ ) increasing trend for luteomas was also evident. The incidence of luteomas (12%) was barely outside the historical control range (0%-8%). Nevertheless, the incidence of 4 such tumors at the highest dose along with a positive trend was considered by some members to be biologically significant and supportive of carcinogenic potential of tetraconazole in mice. The dosing at 1250 ppm was considered by the Committee to

be excessive due to increased mortality in both sexes. However, 800 ppm was considered to be adequate since mortality was comparable to controls and the tumor incidences were significantly increased in both sexes. In addition, there was a decrease in body weight gain, increased liver and kidney weights and the presence of non-neoplastic changes in various organs including bone, liver, lungs, kidney, testes, epididymides and ovaries.

The genetic toxicology studies indicated that tetraconazole was not mutagenic in *Salmonella typhimurium* and mouse lymphoma assays. It was not clastogenic *in vitro* and *in vivo* and did not induce unscheduled DNA synthesis (UDS) in human HeLa cells.

Structurally related compounds including bayleton, baytan, baycor, uniconazole, propiconazole, hexaconazole and cyproconazole as well as tetraconazole have been shown to induce hepatocellular tumors in one or both sexes of mice. Seven out of nine structurally related compounds tested negative in mutagenicity assays and three are positive only in *in vitro* chromosomal aberration tests.

The Committee determined that the mechanistic studies do not support the proposed mode of action for the induction of liver tumors in mice.

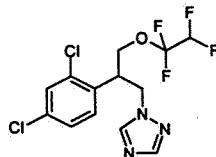
In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified tetraconazole as "**likely to be carcinogenic to humans**" by the oral route based on the occurrence of liver tumors in male and female mice. For the quantification of human cancer risk, the Committee recommended a linear low-dose extrapolation approach based on the incidence of combined liver tumors in male or female mice, whichever is more potent. This approach is supported by the lack of confirmation of the mode of action of tetraconazole.

## I. INTRODUCTION

On November 10, 1999, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of tetraconazole.

## II. BACKGROUND INFORMATION

Tetraconazole is a triazole fungicide. The PC Code is 120603 and the CAS Number is 112281-77-3. It is recommended for agricultural use on sugar beets and turf and has an import tolerance set for bananas. Future uses may be included on grapes, peanuts, and wheat.



## III. EVALUATION OF CARCINOGENICITY STUDIES

### 1. Combined Chronic Toxicity/Carcinogenicity Study with tetraconazole in Crl CD (SD) rats

Reference: Crome, S. J, *et al.* (1992) M 14360 Potential tumorigenic and toxic effects in prolonged dietary administration to rats. Laboratory name: Huntingdon Research Centre Ltd. Laboratory report number: AGR 74/911683. December 10, 1992. MRID 44305304. Unpublished.

#### A. Experimental Design

In a carcinogenicity toxicity study (MRID 44305304), tetraconazole (94.6% a.i.) was administered to Crl:CD (SD) rats (50/sex/dose), in the diet at dose levels of 0 (control) 10, 80, 640 or 1280 ppm for males and 0 (control), 10, 80 or 640 ppm for females for 2 years. This corresponds to 0, 0.6, 4.4 or 39.4 mg/kg/day for females and 0, 0.4, 3.4, 27.7 or 59 mg/kg/day for males. An additional 20 rats/sex/dose were sacrificed and examined at 12 months.

#### B. Discussion of Tumor Data

The statistical evaluation of mortality indicated significant decreasing trends with increasing doses of tetraconazole in male and female rats. The dietary administration of tetraconazole up to 1280 ppm in males and 640 ppm in females did not result in an overall treatment-related increased incidence of tumor formation in Crl:CD (SD) rats. The statistical analyses of tumors in male rats are presented in Table 1. Among males, although an increase in the incidence of thyroid adenomas was noted at doses  $\geq$  80 ppm (4.2%, 9.1%, 13.8% and 11.6% at 0, 10, 80, 640 and 1260 ppm, respectively), these values did not achieve statistical significance and were

well within the historical control range (mean of 9.7%; range of 0-19.6%).

**Table 1. Male Rats: Thyroid Follicular Tumor Rates<sup>+</sup> and Peto's Prevalence Test Results.**

ppm	0	10	80	640	1280
mg/kg/day	0	0.4	3.4	27.7	59
Adenoma	1/24	1/29	3/33	4/29	5/43
%	(4)	(3)	(9)	(14)	(12)
p =	0.103	-	0.238	0.119	0.154
Carcinoma	0/24	1/29	0/33	2/29	1/43
%	(0)	(3)	(0)	(7)	(2)
p =	0.276	0.181	-	0.097	0.228
Combined	1/24	2/29	3/33	4 <sup>a</sup> /29	6/43
%	(4)	(7)	(9)	(14)	(14)
p =	0.089	0.336	0.238	0.119	0.106

<sup>+</sup>Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

First thyroid follicular cell adenoma and carcinoma observed at Week 105, in final sacrifice animals, concurrently in all dose groups.

<sup>a</sup>Two animals in the 640-ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no thyroid follicular cell adenomas or carcinomas in any interim sacrifice animals.

Significance of trend denoted at control.

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

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

### C. Non-Neoplastic Changes

Packed cell volume, red blood cell counts and hemoglobin levels were reduced in both sexes at their respective high doses and also in males at 640 ppm. Absolute adrenal weights were decreased, compared to controls, 18% and 33.6% in the 640 and 1280 ppm males, respectively, but only 3.4% in the 640 ppm females. Adrenal weights, relative to body weights, were decreased 12.8% and 25.65% in 640 and 1280 ppm males and 17.5% in 640 ppm females. Absolute pituitary weights were decreased, compared to controls, 34.2% and 46.8% in 640 and 1280 ppm males but only 9.4% in 640 ppm females. Pituitary weights, relative to body weights, were decreased 13.4% and 23% at 640 and 1280 ppm in males but were not greatly altered in females. Both sexes did show a statistically significant increase in liver weights at 640 and 1280 ppm for the males and 640 ppm for the females. Males, but not females, showed at the



1280 ppm dose (and to a much lesser extent the 640 ppm dose) necropsy findings of white cranium, thickened cranium and thickened parietal bones. Liver findings at necropsy consisted of pale subcapsular areas in both sexes (incidences were 6, 7, 8, 24 and 29 males in control through 1280 ppm groups and 5, 9, 11, and 17 females in control through 640 ppm female groups) as well as accentuated lobular markings in the males only (incidences were 2, 4, 2, 14, 25 in the control through 1280 ppm groups). Histopathology findings supported the liver findings at necropsy for both sexes. Hepatic findings at histopathology were indicative of cellular proliferation and hypertrophy. Some findings, such as centrilobular hypertrophy, (incidences were 0, 0, 17, 34, and 44 in control through 1280 ppm males and 0, 0, 17 and 39 in control through 640 ppm females) were increased in the 80 ppm groups. Most hepatic findings however, were confined to the 640 and 1280 ppm males and 640 ppm females. Fine vacuolation of hepatocytes and inflammatory cell foci in males were not seen at all in controls but were seen in 18% to 62% of the 640 and 1280 ppm males. The skull bone findings at necropsy were supported by the histopathology findings which found an increased incidence of osseous hypertrophy of the parietal bones (incidences were 8 and 43 at 640 and 1280 compared to zero in controls) and osseous hypertrophy of the cranium (incidences were 8 and 45 at 640 and 1280 ppm compared to zero in controls). Additionally, an increased incidence of cystic follicular atrophy in the thyroid was seen in males only (6 incidences in 1280 ppm and zero in all other groups).

The findings in the liver at doses less than 1280 ppm (cellular proliferation/hypertrophy) are indicative of an adaptive response rather than a toxicologic response. Only at the 1280 ppm dose, where increases in inflammatory foci are seen, can the liver responses be called toxicologically significant. The alterations in the skeletal system at 640 ppm in males can be considered toxicologically relevant.

#### D. Adequacy of the Dosing for Assessment of Carcinogenicity

The dosing at the highest level was considered by the CARC to be adequate and not excessive based on decreased body weight gain (24% in both sexes at terminal sacrifice) in females at 640 ppm and in males at 1280 ppm (decreased absolute and relative adrenal and pituitary weights in males at 640 and 1280 ppm and histopathological changes in the bone (osseous hypertrophy of the cranium/parietal bone) and liver (cellular proliferation, centrilobular hypertrophy, vacuolization of hepatocytes and inflammatory foci).

#### 2. Carcinogenicity Study in Mice

Reference: Crome, S., M.E. Bellringer, *et al.* (1992) M 14360 Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice. Huntingdon Research Centre, Ltd., Cambridgeshire, England. Report # AGR73/920469, December 10, 1998. MRID # 44305305. Unpublished.

### A. Experimental Design

In a carcinogenicity study (MRID 44305305) M14360 (95.05% a.i.) was administered to 50 Crl:CD-1 (ICR) mice/sex/dose in their diet at dose levels of 0, 10, 90, 800, 1250 ppm (for males: 0, 1.4, 12, 118, 217 mg/kg/day; for females: 0, 1.6, 14.8, 140, 224 mg/kg/day) for 80 weeks.

### B. Discussion of Tumor Data

The statistical evaluation of mortality indicated significant increasing trends with increasing doses of tetraconazole in male and female mice. The results of tumor analyses for male and female mice are presented in Tables 2 and 3, respectively. Statistical evaluation of liver tumors in both sexes revealed a significant increasing trend with differences in the pair-wise comparisons of the 1250 ppm dose group with the controls for benign, malignant and benign and/or malignant tumors combined, all at  $p < 0.01$ . A statistically significant increased incidence of combined benign and malignant liver tumors was observed at 1250 ppm (86% for males and 65% for females) and 800 ppm (49% for males and 22% for females) compared to the control (20% for males and 0% for females). The tumor incidence in animals receiving  $\leq 90$  ppm was found to be similar to that of controls. 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, both at  $p < 0.01$  (Tables 2 and 3). The increased incidence of liver tumors at both 800 and 1250 ppm was well outside the range for the historical controls (the incidence of hepatocellular adenomas in historical controls ranged from 7.7% - 24% in males and 0% - 1.9% in females and the incidence of hepatocellular carcinomas ranged from 0% - 14% in males and was 0% in females).

Female mice had a significant increasing trend in ovarian benign luteomas at  $p < 0.01$ , but no significant differences in the pair-wise comparisons of any dose group (Table 4). The incidence of ovarian tumors was 5 % and 12 % at 800 ppm and 1250 ppm, respectively. The incidence at 1250 ppm was slightly outside the historical control range (0% to 8 %). However, if the denominator of 50 instead of 32 was used, the incidence at high dose would be 8% (4/50) and within the historical control range. Nevertheless, the incidence of 4 such tumors at the highest dose along with a positive trend was considered by some members to be biologically significant and supportive of carcinogenic potential of tetraconazole in mice.

**Table 2. Male Mice: Liver Tumor Rates<sup>+</sup> and Peto's Prevalence Test Results.**

ppm	0	10	90	800	1250
mg/kg/day	0	1.4	12	118	217
Benign % p =	9/49 (18) 0.000**	8/50 (16) -	6/49 (12) -	22/49 (45) 0.003**	34 <sup>a</sup> /49 (69) 0.000**
Malignant % p =	1/48 (2) 0.000**	2/47 (4) 0.398	2/47 (4) 0.059	4/48 (8) 0.134	20 <sup>b</sup> /45 (44) 0.000**
Combined % p =	10/49 (20) 0.000**	9 <sup>c</sup> /50 (18) -	7 <sup>c</sup> /33 (14) -	24 <sup>d</sup> /49 (49) 0.002**	42 <sup>e</sup> /49 (86) 0.000**

\*Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

<sup>a</sup>First liver benign tumor observed at Week 34, 1250 ppm.

<sup>b</sup>First liver malignant tumor observed at Week 50, dose 1250 ppm.

<sup>c</sup>One animal in each of the 10 and 90 ppm dose groups had both an adenoma and a carcinoma.

<sup>d</sup>Two animals in the 800 ppm dose group had both an adenoma and a carcinoma.

<sup>e</sup>Twelve animals in the 1250 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  $p < 0.01$ .

**Table 3. Female Mice: Liver Tumor Rates<sup>+</sup> and Peto's Prevalence Test Results.**

ppm	0	10	90	800	1250
mg/kg/day	0	1.6	14.8	140	224
Benign %	0/49 (0)	0/49 (0)	0/50 (0)	11/49 (22)	26 <sup>a</sup> /49 (53)
p =	0.000**	-	-	0.000**	0.000**
Malignant %	0/48 (0)	0/47 (0)	0/50 (0)	1/49 (2)	17 <sup>b</sup> /44 (39)
p =	0.000**	-	-	0.162	0.000**
Combined %	0/49 (0)	0/49 (0)	0/50 (0)	11 <sup>c</sup> /49 (22)	32 <sup>d</sup> /49 (65)
p =	0.000**	-	-	0.000**	0.000**

<sup>+</sup>Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

<sup>a</sup>First liver benign tumor observed at Week 57, 1250 ppm.

<sup>b</sup>First liver malignant tumor observed at Week 64, dose 1250 ppm.

<sup>c</sup>One animal in the 800 ppm dose group had both an adenoma and a carcinoma.

<sup>d</sup>Eleven animals in the 1250 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  $p < 0.01$ .

**Table 4. Female Mice: Ovarian Tumor (Luteomas) Rates<sup>+</sup> and Peto's Prevalence Test Results.**

ppm	0	10	90	800	1250
mg/kg/day	0	1.6	14.8	140	224
Benign %	2/41 (5)	0/39 (0)	0/41 (0)	2/42 (5)	4/32 (12)
p =	0.006**	-	-	-	0.121

<sup>+</sup>Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

First ovarian benign luteomas observed at Week 81, in final sacrifice animals, concurrently at all doses.

### C. Non-neoplastic Changes

Gross pathology revealed slight to severe changes in the liver which correlated with the dose given. At 90 ppm, the liver appeared pale with accentuated lobular markings. At higher concentrations (800-1250 ppm), masses were found with raised, pale, or dark subcapsular areas. Masses were also found in the kidneys of males receiving 1250 ppm. Dose-related increases in liver weights were noted in mice of both sexes given  $\geq 90$  ppm of the test material. Kidney weights were also found to increase slightly (9-11 % above controls) in males receiving  $\geq 90$  ppm.

Histopathological findings revealed liver toxicity including hepatocyte vacuolation, fat deposition, granulomatous inflammation, pigmented macrophages, generalized hepatocyte enlargement, and bile duct hyperplasia in mice receiving 800 and 1250 ppm of the test material. In addition, at the high dose of 1250 ppm, non-neoplastic changes were noted in the brain, lungs, kidneys, testes, epididymides, and ovaries. Thickening of compact bone of the cranium, in the ribs, collar bone (females only), and femur (females only) at 800 and 1250 ppm also appeared to be treatment related.

### D. Adequacy of Dosing for Assessment of Carcinogenicity

The dosing at the highest dose (1250 ppm) was considered by the CARC to be excessive due to significantly increased mortality in both sexes. However, 800 ppm was considered to be adequate since mortality was comparable to controls. In addition, there was a decrease in body weight gain, increased liver and kidney weights and the presence of non-neoplastic changes in various organs including bone, liver, lungs, kidney, testes, epididymides or ovaries. The CARC considered the liver tumors to be treatment-related.

## IV. TOXICOLOGY

### 1. Metabolism

Groups of ten Sprague-Dawley rats of each sex were given single oral doses of [ $^{14}$ C] triazole or phenyl ring labeled M-14360 in a 0.75% (w/v) methylcellulose/HPLC water suspension or single oral doses of non-radiolabeled M-14360 at 5 or 60 mg/kg/for 14 days followed by single oral dose of labeled M-41360 in 1% (w/v) methylcellulose/saline suspension (MRID 44268114 to 44268119).

Results of study with M-14360 labeled on triazole ring: Average total recovered radioactivity for either high or low single oral dose in males or females ranged from 95% to 102% of the administered dose (AD). Most of the radioactivity (75%) was recovered in the urine after 7 days. Recovered radioactivity in the feces ranged from 15% (high dose) to 18% (low dose) of the AD. In the urine and fecal samples, triazole was the major metabolite for both dose levels and sexes. M-14360 acid along with minor metabolites of M-14360 alcohol and its

glucuronide conjugate (M3) were also isolated from the urine. In the feces minor amounts of the parent material M-14360, the acid and alcohol were also isolated.

Radioactivity in the tissues was minimal after 7 days and accounted for less than 1.5% of the AD. The data indicate that M-14360 or its metabolites are not accumulated in rat tissues following a single oral dose.

Following a single oral low or high dose, male rats produced in the urine more triazole than females (65-67% of the AD vs 48% in females), while urine from females had more of M-14360 acid (7-13% vs 3.5-4% for males), M-3 (2-4% vs 0.0-0.6% for males), and M-14360 alcohol (1.4-2.4% vs 0.0% for males). The same pattern, though not as pronounced, was also seen in the multiple dosing study.

There were also dose and sex differences in the quantitative and qualitative nature of the metabolites in the feces from single and repeated dose animals. In the multiple dosing, triazole (3.2-3.9% of the AD for males and females at the low dose vs 6.5-6.8% for the high dose), M-14360 acid, M-14360 alcohol, M-14360, M6 and others were reported while in the single dosing only triazole (5.6 - 10.4% of the administered low and high doses in both sexes), M-14360 acid and M-14360 were reported.

The metabolic cleavage of M-14360 to yield triazole appears to be the major step in M-14360 metabolism. The study authors postulate that this step is glutathione mediated. A metabolic pathway was proposed where the formation of an aldehyde intermediate of M-14360 occurs following dealkylation of the fluoro-alkyl group of the molecule.

Results of study with M-14360 labeled on phenyl ring: Urine was the major route of excretion accounting for 57-75% of the AD after 7 days of exposure following a single or multiple doses. Most of the urinary radioactivity was excreted during the first 48 hours. Fecal radioactivity was next major route accounting for 27-39% of the AD after 7 days of exposure following single or multiple dosing. The test material was depurated from the body within 7 days and very little residual radioactivity was detected in the tissues representing less than 1.5% of the AD. Very minor sex differences and between dose levels were seen.

The majority of radioactivity was excreted in urine (51 to 62% AD) and feces (23 to 36% AD) in 48 hours. Most of the radioactivity in the feces was extractable (19 to 28% AD), only 4 to 8% AD remained bound. The major metabolite of the <sup>14</sup>C-phenyl M-14360 isolated from urine were P1 and P4 along with the minor metabolite M 14360 acid. No parent compound was identified in urine. In feces, the major metabolites were P4 and M 14360 alcohol along with a small amount of parent. Unknowns typically amounting to less than 10% of AD were P2 and P3 from urine and P5 from feces.

There were also dose and sex differences in the quantitative and qualitative nature of the metabolites. Males excreted higher levels of radioactivity in feces than females. Rats appeared to become acclimated by the repeated dose of M 14360 since multiple dose rats excreted more radioactivity and metabolites in urine than single dose rats.

The study authors postulate that the major metabolic pathways include oxidation and reduction involving the ether function, and displacement of triazole by glutathione with subsequent metabolism. M-14360 is metabolized in rats predominately by glutathione conjugation. The further metabolism of these conjugates generated end metabolites P1 and P4.

## 2. Mutagenicity:

Five acceptable genetic toxicology studies were available for review. The results from these studies indicate that tetraconazole was not mutagenic in *Salmonella typhimurium* (MRID 44335511) or in mouse lymphoma cells (MRID 44335508). There was also no evidence of clastogenicity *in vitro* (MRID 44335507) or *in vivo* (MRID 44335509) and tetraconazole did not induce unscheduled DNA synthesis (UDS) in human HeLa cells (MRID 44335510).

### GENE MUTATIONS

In microbial reverse gene mutation assays (MRID No. 44335511), *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to tetraconazole as M 14360 (95.0%) at doses ranging from 125-2000  $\mu\text{g}/\text{plate}$  with or without S9 activation in both trials. The test material was delivered to the test system in dimethylsulfoxide. Cytotoxicity was evident for the majority of strains at  $\geq 1000 \mu\text{g}/\text{plate}$  -S9 and at 2000  $\mu\text{g}/\text{plate}$  +S9. There was no evidence that M 14360 induced a mutagenic effect under any test condition in either assay. This study is classified as **Acceptable** and satisfies the guideline requirements for a bacterial gene mutation assay (84-2).

In *in vitro* mammalian cell gene mutation assays (MRID No. 44335508), L5178Y mouse lymphoma cells were exposed to tetraconazole as M 14360 (94.6%) at eight concentrations ranging from 5 to 125  $\mu\text{g}/\text{mL}$  with or without S9 activation (Trial 1) or 10 to 100  $\mu\text{g}/\text{mL}$  +/- S9 (Trial 2). The test material was delivered to the test system in dimethylsulfoxide. Cytotoxicity was observed in all trials at  $\geq 100 \mu\text{g}/\text{mL}$  +/-S9. Findings with the positive controls confirmed the sensitivity of the test system to detect mutagenesis. There was, however, no indication that M 14360 induced a mutagenic response, either in the presence or absence of S9 activation. The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian forward gene mutation study (84-2).

### CHROMOSOME ABERRATIONS

In *in vitro* chromosome aberration assays (MRID No. 44335507), Chinese hamster ovary (CHO) cells were exposed for 6 hours to Tetraconazole as M 14360 (94.0 $\pm$ 0.5%) at doses ranging from 0.5-250  $\mu\text{g}/\text{mL}$  in both the absence and presence of S9 activation. The test material was delivered to the test system in dimethylsulfoxide. The positive controls induced the expected high yield of cells with structural and/or numerical chromosome aberrations. There was, however, no evidence that M 41360 induced a clastogenic response at any nonactivated or S9-activated dose. Similarly, there was no evidence that M 41360 had an adverse effect on the number of chromosomes (i.e., polyploidy). The study is classified as

**Acceptable** and satisfies the guideline requirement for an in vitro mammalian cell cytogenetic assay (84-2).

In an in vivo mouse micronucleus assay (MRID No. 44335509), groups of five male and five female CD-1 mice received single oral gavage administrations of 185, 370 or 740 mg/kg Tetraconazole as M 14360 (94.6%). Bone marrow cells were collected 24, 48 or 72 hours after compound administration and were examined for micronucleated polychromatic erythrocytes (MPEs). The test material was delivered to the test animals in aqueous 1% methylcellulose.

One male and four females in the high-dose group succumbed to treatment prior to the scheduled sacrifice; other toxic signs at this concentration included piloerection, hunched posture, lethargy, decreased respiration and pallor of extremities. The test material was not cytotoxic to the target tissue. The positive control induced the expected high yield of MPEs. There was, however, no evidence that M 14360 was clastogenic or aneugenic at any dose or harvest time. The study is classified as **Acceptable** and satisfies the guideline requirement for a mouse micronucleus assay (84-2).

#### OTHER MUTAGENIC MECHANISMS

In unscheduled DNA synthesis (UDS) assays (MRID No. 44335510), cultured HeLa cells were exposed to 12 concentrations of M 14360 (94.6%) ranging from 0.25-512 µg/mL in both the absence and the presence of S9 activation in both trials. The test material was delivered to the test system in dimethylsulfoxide. Cytotoxicity was evident in all trials at  $\geq 64$  µg/mL +/-S9. The positive controls induced significant and dose-related increases in UDS. No evidence that M 14360 induced a genotoxic effect under any test condition in either assay. The study is classified as **Acceptable** and satisfies the guideline requirement for an unscheduled DNA synthesis assay (84-2).

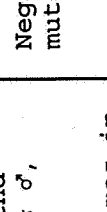
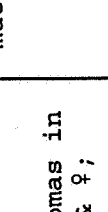
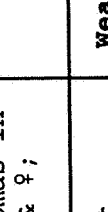
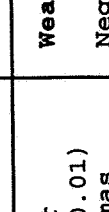
Overall, the data indicate that tetraconazole is negative for mutagenicity *in vitro* and *in vivo*.  
\* The acceptable studies satisfy the 1991 mutagenicity guideline requirements.

### **3. Structure-Activity Relationship**

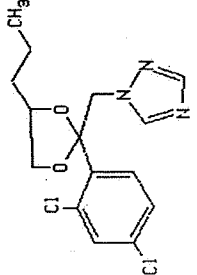
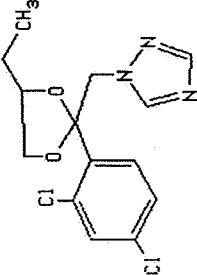
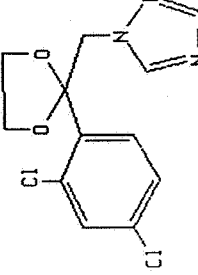
Structurally related compounds include bayleton, baytan, baycor, uniconazole, propiconazole, etaconazole, azaconazole, hexaconazole and cyproconazole. Six of these structural analogs as well as tetraconazole have been shown to induce hepatocellular tumors in one or both sexes of mice (Table 5).



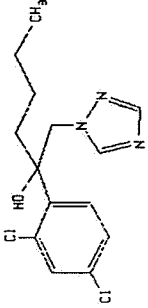
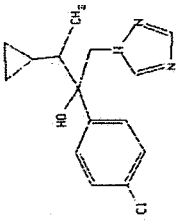
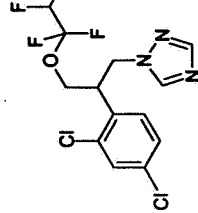
Table 5 Structure-Activity of Related Triazole Compounds.

Compound	Structure	Carcinogenic Effect	Carcinogen Class/Mutagen
Bayleton PC 109901 Tx.# 862AA		<p><b>NMRI Mouse</b> Only hepatocellular adenoma, at 1800 in (22%) ♂ &amp; (18%) ♀ p&lt;0.05 for trend and paired comps. Hist. Conts.: 18.4% ♂, and 2.0% ♀.</p> <p><b>Wistar rat</b> Do. rel. trend in TFC adenomas in ♂ &amp; comb. w. cystic hyperplasia in ♂ &amp; ♀; Pair-wise comparisons not significant.</p>	<p><b>C NO Q</b>                      Negative for mutagenicity</p>
Baytan PC 127201 Tx.# 074A		<p><b>F1-W74 mouse</b>, 2000 ppm: Hepatocellular adenomas and hyperplastic nodules (p&lt;0.01) in ♀. No increase in ♂. Adrenal adenomas noted in ♀ LDT and HDT but not in hist. conts. No elevation in carcinomas.</p> <p><b>Rat</b>, 125-2000 ppm, increases in thyroid adenoma.</p>	<p><b>Weak C SAP</b>                      Negative for mutagenicity</p>
Baycor PC 112403 Tx.# 087AA		<p><b>Mouse</b>: up to 500 ppm: (-)  <b>Rat</b>: up to 500 ppm: (-)</p>	<p>Negative for mutagenicity</p>
Uniconazole PC 128976 Tx.# 207H		<p><b>Cr1:CD-1(ICR)BR mouse</b> Incr. incidence of hepatocell. adenomas and carcinomas in HDT males only.</p> <p><b>Cr1:CD-1(ICR)SD rat</b> No increase in neoplastic findings</p>	<p><b>C NO Q</b>                      Positive in vitro chrom. aberration with S9</p>

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<p>Propiconazole  PC 122101  Tx. #323EE</p>		<p>CD-1 mouse Statistically significant trend and pair-wise comparisons in liver adenomas and combined. For carcinomas 2 pathologists were significant, the third was not.</p>	<p>C NO Q  Negative for mutagenicity</p>
<p>Etaconazole</p>		<p>No data</p>	<p>Negative in performed gene tox tests:  Ames,  dominant lethal</p>
<p>Azaconazole  PC 12882  Tx. # 321A</p>		<p>Mouse, 25,100 &amp; 400 ppm. There is the question of whether the MTD was reached. No oncogenicity effect.</p>	<p>Negative for mutagenicity except positive in vitro CHO aberration (acentric fragments) only with S9</p>

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Compound	Structure	Carcinogenic Effect	Carcinogen Class/Mutagenicity
Hexaconazole PC 128925 Tx. # 480G		<p><b>CD-1/Alpk mouse, 5, 40 &amp; 200 ppm.</b> No oncogenicity effect. Should be seen with caution because MTD was not reached. No oncogenicity effect.</p> <p><b>Alpk:AFfSD (Wistar derived) rats, 10, 100, 1000 ppm.</b> There was a significant (<math>p &lt; 0.01</math>) dose-related trend and a significant pair-wise 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%</p>	<p><b>C with Q</b></p> <p>Mutagenicity Negative: Ames Microsomal UDS HL</p>
Cyproconazole PC 128993 Tx. # 272E		<p><b>CD-1 mouse, 5, 15, 100 &amp; 200 ppm.</b> Significant incidence of adenomas &amp; carcinomas at the MDT and HDT in males and at the HDT in females.</p>	<p><b>B2</b></p> <p>Negative for mutagenicity except positive in vitro chrom. aberration (dicentric)</p>
Tetraconazole PC 120603		<p><b>CD-1 mouse, 10, 90, 800 &amp; 1250 ppm.</b> Significant increasing trend in both sexes with differences in the pair-wise comparisons of the 1250-ppm dose group with the controls for benign, malignant and benign and/or malignant tumors combined, all at <math>p &lt; 0.01</math>. 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 <math>p &lt; 0.01</math>.</p>	<p><b>Not Classified</b></p> <p>Mutagenicity Negative: Ames Micronucleus UDS assays</p>

#### **4. Subchronic Toxicity**

##### Rat

In a subchronic oral toxicity study (MRID 44335504), tetraconazole caused increases in liver weights relative to body weight were seen in 60 ppm (females only) and in 360 ppm treated males and females. Kidney weight relative to body weight was also significantly increased in the 360 ppm females rats. Macroscopic post mortem examination revealed two males at 360 ppm with enlarged livers and two males at 360 ppm and a single female at 10 ppm with swollen liver. Histological examinations revealed minimal centrilobular hepatocyte enlargement in all rats receiving 360 ppm and 5/10 males and a single female receiving 60 ppm.

##### Mouse

In a subchronic mouse range-finding oral toxicity study (MRID 44778701), increased serum SGPT (+165% above controls) and SGOT (+56%), decreased serum BUN (-19%), slightly increased mean/absolute liver weights (15%/15%) and microscopic liver lesions (4/10 single cell necrosis, 1/10 necrosis, and 1/10 single cell degeneration vs. 0 in controls) were observed in females at 125 ppm (most of these findings showed a dose-related increase at 625 ppm). Two of ten males had single liver cell degeneration and only a slight (+39%; not significant) increase in SGPT. At 625 ppm, increased serum SGPT (+103%), decreased BUN (-15%) and increased absolute/relative liver weights (+77%/+75%), along with single cell necrosis and areas of necrosis (each 2/10), were also observed in males. In addition, midzonal hepatocyte hypertrophy and vacuolization in females (4/10 and 3/10, respectively) and liver cell degeneration in 1/10 females were also observed. Although the incidence of centrilobular hepatocyte hypertrophy was increased in both sexes at 25 ppm and higher, it was not considered toxicologically significant at that dose level, based on minimal severity and lack of other liver effects. Females appeared to be more sensitive at 125 ppm while significant changes in liver parameters and histopathology occurred in males at 625 ppm only.

#### **5) Chronic Toxicity**

##### Dog

In a chronic toxicity study (MRID No. 44305303), Tetraconazole as M 14360 (94.6%) was administered to groups of four male and four female Beagle dogs/dose in the diet, at dose levels of 0, 22.5, 90, or 360 ppm (equivalent to achieved intakes of 0, 0.73, 2.95 or 12.97 for males or 0, 0.82, 3.33 or 14.50 mg/kg/day for females) for 52 weeks.

Exposure to M 14360 had no effect on feed consumption, hematological parameters or urinalysis. Treatment-related effects at the high dose included slight but nonsignificant body weight reductions in both sexes from study week 3 to termination; significantly increased alkaline phosphatase,  $\gamma$ -glutamyltransferase, alanine aminotransferase and ornithine

carbamoyl transferase in both sexes from study week 13 to 52, increased absolute and relative liver and kidney weights for both sexes, and histopathological changes in both organs. In the mid-dose group, effects were manifested as increased absolute and relative kidney weights for males correlated with histopathological findings in the males (apparent hypertrophy in cortical tubules of the kidneys-1 male). No adverse effects were seen at the low dose.

Based on these findings, the NOAEL is 22.5 ppm (equivalent to achieved intakes of 0.73 mg/kg/day for males or 0.82 mg/kg/day for females) and the LOAEL is 90 ppm (equivalent to achieved intakes of 2.95 mg/kg/day for males or 3.33 mg/kg/day for females), based on increased absolute and relative kidney weights and histopathological changes in the male kidney.

## 5. Mode of Action Studies

### Liver Enzyme Induction

In a mouse subchronic toxicity study (MRID 44751309), tetraconazole (96.3% ai) was administered to 18 male and female Crl:CD-1 (ICR)BR mice per dose level at levels of 0, 20, 800, or 1250 ppm (0, 3.9, 150, or 225 mg/kg/day in males, 0, 4.6, 175, or 293 mg/kg/day in females) in the diet. The positive control was Phenobarbital (Na) salt, 75 mg/kg/day.

The results of this study indicate that tetraconazole administration for 4 weeks results in liver enzyme induction. Statistically significant increases were apparent in females at the 20 ppm dose level based on increases in microsomal protein, cytochrome P-450, and ethylmorphine N-demethylase. At all dose levels in males and females, 7-pentoxoresorufin O-depentyase values were statistically elevated. At 800 and 1250 ppm, statistically significant findings were typically noted. However, dose response increases were not apparent in these findings at the 1250 ppm level as compared to the lower 800 ppm level.

In a rat subchronic toxicity study (MRID 44751310), tetraconazole (95.2%) was administered to 6 male and female Crl:CD BR rats per dose level at levels of 0, 10, 80, or 640 ppm (0, 0.8, 6.6, or 54.6 mg/kg/day in males, 0, 0.9, 7.6, or 57.6 mg/kg/day in females) in the diet. The positive control was phenobarbital (Na) salt, 75 mg/kg/day.

Tetraconazole administration for 4 weeks results in liver enzyme induction at dose levels of 80 and 640 ppm. Induction at the 640 ppm dose level was similar to that induced by phenobarbital at 75 mg/kg/day.

Neither of these studies demonstrates a threshold level for initiation of hepatic carcinogenesis in the mouse as a result of oral administration of tetraconazole. Even though they show induction of Phase I and Phase II enzymes in the rat liver, no mechanism was demonstrated to explain the progression from enzyme induction to tumor formation in the mouse.

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

### 1. Carcinogenicity:

- **The CARC concluded that tetraconazole was not carcinogenic to male and female rats** because: 1) Although, males had increased incidences of thyroid follicular cell adenomas at  $\geq 80$  ppm (3/33, 9%; 4/29, 14%; and 5/43, 12%; at 80, 640 and 1250 ppm or 3.4, 27.7 and 59 mg/kg/day, respectively, vs 1/24, 4% in controls), the increase was not statistically significant when analyzed by pair-wise comparison. Moreover, the incidences of tumors were within the range for the historical controls (0%-19.6%; mean: 9.7%); 2) There was no increase in thyroid tumors in females. The highest dose in both sexes was considered to be adequate based on decrease (32%) in body weight gains in females in a subchronic study, decreased body weights (24%) at terminal sacrifice in both sexes at the highest dose, increased adrenal and pituitary weights and histopathological changes in bone and liver in the present study. The CARC determined that the thyroid tumors in males were not treatment-related.
- **Tetraconazole was carcinogenic to mice** because: 1) Males and females had significant increases in the pair-wise comparisons of the 800 and 1250 ppm (males: 118 and 217 mg/kg/day, respectively; females: 140 and 224 mg/kg/day, respectively) dose groups with the controls for hepatocellular adenomas (males: 22/49, 45%, and 34/49, 69%,  $p < 0.01$  vs 9/49, 18%; females: 11/49, 22% and 26/49, 53%,  $p < 0.01$  vs 0/49 0% in controls). There were also significant increases in carcinomas (at 1250 ppm only: males: 20/45, 44%,  $p < 0.01$  vs 1/48, 2% in controls; females: 17/44, 39%,  $p < 0.01$  vs 0/48, 0% in controls) and for combined hepatocellular adenomas/ carcinomas (males: 24/49, 49% and 42/49, 86%,  $p < 0.01$  vs 10/49, 20% in controls; females: 11/49, 22% and 32/49, 65%,  $p < 0.01$  vs 0/49, 0% in controls). There were significant ( $p < 0.01$ ) increasing trends for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas in both sexes. The increased incidence of carcinomas in males at 800 ppm (4/48, 8% vs 1/48, 2% in controls) was considered by the CARC to be biologically significant. The incidences of hepatocellular adenomas and carcinomas in males were outside the historical control ranges (adenomas: males: 7.7%-24%; females: 0%-1.9%; carcinomas: males: 0%-14%; females: 0%); 2) There was a numerical increase in the incidence of ovarian luteomas (12%) in high-dose females. A statistically significant ( $p < 0.01$ ) increasing trend was also evident. The incidence at the highest dose was slightly outside the historical control range (0%-8%). These tumors were thought to be biologically significant by some members of the Committee because of the significant trend and the occurrence of 4 luteomas at the highest dose. Although there was significantly increased mortality in both sexes at 1250 ppm, sufficient number of animals survived at 15 and 18 months to develop tumors. The highest dose was considered to be excessive by the CARC based on significantly increased mortality in both sexes. However, tumors were also present at dose (800 ppm) that was not excessively toxic. The dose level of 800 ppm was considered to be adequate since mortality was comparable to controls. In addition, there was a decrease in body weight gain, increased liver and kidney weights and presence of non-neoplastic changes in

various organs including bone, liver, lungs, kidney, testes, epididymides or ovaries. The CARC determined that the liver tumors in male and female mice were treatment-related.

## 2. Mutagenicity

- The submitted genetic toxicology studies indicate that tetraconazole is not mutagenic in bacteria (*Salmonella typhimurium*) or cultured mammalian cells (mouse lymphoma cells). There is also no evidence of clastogenicity in *in vivo* or *in vitro* assays. Similarly, tetraconazole did not induce unscheduled DNA synthesis (UDS) in human HeLa cells. These submitted studies satisfy the 1991 mutagenicity guideline requirements and, therefore, no additional studies were recommended by the CARC.

## 3. Structure Activity Relationship

- Structurally related compounds include bayleton, baytan, baycor, uniconazole, propiconazole, etaconazole, azaconazole, hexaconazole and cyproconazole. Six of these structural analogs as well as tetraconazole have been shown to induce hepatocellular tumors in one or both sexes of mice. Seven of these compounds tested negative in mutagenicity assays and three were positive only in *in vitro* chromosomal aberration tests.

## 4. Mode of Action

- The CARC evaluated the mechanistic studies submitted by the Registrant. The Registrant contended that these studies demonstrate the presence of a threshold level for hepatocarcinogenesis in mice. In a mouse subchronic study, tetraconazole induced microsomal proteins, cytochrome P-450 (especially as measured by pentoxyresorufin O-depentylase), and ethylmorphine N-demethylase similar to Phenobarbital, a known tumor promoter. In another subchronic study, Tetraconazole was an inducer of Phase I and II liver enzymes in the rat liver.

The CARC determined that the submitted studies do not support the proposed mode of action for liver carcinogenicity for tetraconazole based on the fact that although enzymes were induced in rats and mice, liver tumors were observed in mice only which raises an uncertainty about the mechanism of tumorigenesis in mice.

The CARC understands that induction of cytochrome p-450 enzymes may be involved in altering the liver metabolism leading to cell proliferation and resulting in liver tumor induction, but the Registrant did not provide information to support their hypothesis. The lack of liver tumors in rats raises uncertainty regarding the role of liver enzyme induction in mouse hepatocarcinogenesis. No dose-related association of liver enzyme activity with increase in liver tumors in mice was demonstrated. The CARC, therefore,

concluded that these studies do not support the proposed mode of action for the occurrence of liver tumors in mice.

## **VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL**

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified tetraconazole as "**likely to be carcinogenic to humans**" by the oral route based on the following weight-of-the-evidence considerations:

1. Increased incidences of liver tumors in male and female mice.
2. The relevance of the observed tumors to human exposure cannot be discounted.
3. Structurally related compounds are hepatocarcinogens in mice.

## **VIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL**

For human cancer risk assessment, the Committee recommended a linear low-dose extrapolation approach based on the combined hepatocellular tumors in male or female mice, whichever is more potent. This approach is supported by the lack of confirmation of the mode of action of tetraconazole.



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