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PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 24, 2001

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MEMORANDUM

SUBJECT: Epoxiconazole - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Ayaad Assaad, Toxicologist
Registration Branch 3
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Registration Division (7505C)

The Cancer Assessment Review Committee met on November 8, 2000 to evaluate the carcinogenic potential of Epoxiconazole. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF EPOXICONAZOLE

P.C.Code: 123909

FINAL REPORT

24-JANUARY, 2001

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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DATA PRESENTATION: _____
Ayaad Assaad, Toxicologist

DOCUMENT PREPARATION: _____
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke _____

William Burnam _____

Kerry Dearfield _____

Vicki Dellarco _____

Virginia Dobozy _____

Yiannakis Ioannou _____

Nancy McCarroll _____

Timothy McMahon _____

Esther Rinde _____

Joycelyn Stewart _____

Clark Swentzel _____

Linda Taylor _____

Yin-Tak-Woo See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John M. Pletcher Pathology Consultant See attached sheet

Lori Brunsman, Statistician _____

EPOXICONAZOLE

DATA PRESENTATION: Ayaad Assaad, Toxicologist

DOCUMENT PREPARATION: Sanjivani Diwan, Executive Secretary

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John M. Pletcher Pathology Consultant _____

Lori Brunsman, Statistician _____

CONTENTS

EXECUTIVE SUMMARY

On November 8, 2000, the Cancer Assessment Review Committee (CARC) met to evaluate the carcinogenic potential of epoxiconazole. The studies evaluated included chronic toxicity and combined chronic toxicity and carcinogenicity studies in Wistar rats and a 78 week carcinogenicity study in C57BL/6NCrl BR mice. In carcinogenicity studies, epoxiconazole was administered in the diet to rats (50/sex/group) at 0, 30, 150, 750 or 1500 ppm (0, 2, 7, 40 or 80 mg/kg/day, respectively) and to mice (50/sex/group) at 0, 1, 5, 200 or 500 ppm (males)/1000 ppm (females) (i.e. 0, 0.17, 0.81; 35.3 or 72.2 (for males) and 214.4 mg/kg/day (for females), respectively).

The CARC concluded that:

- **Epoxiconazole was carcinogenic to rats** because: 1) In males, there was a significant positive trend and a significant increase by pairwise comparison of the 1500 ppm dose group with the controls, for adrenal cortex adenomas and combined adenomas/carcinomas. The increase in the combined incidence was driven solely by adenomas and the incidence of adenomas slightly exceeded the range for the spontaneous tumors ; 2) There was a significant positive trend for hepatocellular carcinomas and combined adenomas/carcinomas and a significant increase by pairwise comparison of the 1500 ppm dose group with the controls for hepatocellular carcinomas. No historical control data were available for comparison. 3) Although, there were apparent statistically significant increases in the pituitary and thyroid tumors in the 50, 150 and 750 ppm dose groups and not in the 1500 ppm dose group, the analyses are misleading because not all animals in the low dose groups were examined. If only the organs of animals with gross necropsy abnormalities in these groups were examined microscopically, there could be a falsely increased incidence of tumors. **Therefore, the Committee determined that the adrenal and liver tumors in male rats were treatment-related while pituitary and thyroid tumors were not treatment-related.**

  Among females, 1) there was a significant positive trend for adrenal cortex adenomas, carcinomas, and combined adenomas/carcinomas and a significant increase by pairwise comparison of the 1500 ppm dose group with the controls, for adrenal cortex adenomas and combined adenomas/carcinomas. The increase in the combined incidence was driven by adenomas. Although the incidence of carcinomas was low, and there was no dose-response, this tumor is not commonly found in Wistar rats; therefore, it was of concern to the Committee. The incidences of both adenomas and carcinomas at the high dose exceeded the range for the spontaneous tumor incidence in femaleWistar rats. 2) There was a significant increase by pairwise comparison with the controls, for liver cholangiomas at ≥50 ppm (with borderline increase at 150 ppm). The cholangiomas occurred at all dose levels and the incidences were above concurrent controls; 3) Although there were apparent statistically significant increases in thyroid C-cell tumors in the 50 and 150 ppm dose groups and not in the 1500 ppm dose group, the analyses are

misleading because not all animals in the low dose groups were examined. If only the organs of animals with gross necropsy abnormalities in these groups were examined microscopically, there could be a falsely increased incidence of tumors. **Therefore, the Committee determined that thyroid tumors in female rats were not treatment-related.**

A significant positive trend for ovarian granulosa cell tumors and a significant increase by pair wise comparison with the controls was also noted for ovarian luteomas at ≥150 ppm dose levels and for granulosa cell tumors at ≥750 ppm. Only a borderline significant increase for uterine adenomas was noted at 1500 ppm. The increase in this tumor was not dose-dependent and there was no statistically significant increase by trend analysis. **The CARC determined that the liver, adrenal and ovarian tumors in females were treatment-related.**

The dosing at the highest dose was considered to be adequate and not excessive based on decreases in body weight gains (≥13%) in both sexes in a subchronic study and at terminal sacrifice (≥12%) at ≥750 ppm as well as increased liver and adrenal weights and histopathological changes in the liver, adrenal and ovary in the present study.

- **Epoxiconazole was carcinogenic to mice** because: 1) There was a significant positive trend and a significant increase by pair-wise comparison of the 500 ppm dose group with the controls for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas in both sexes; 2) there was also a statistically significant increasing trend and a significant increase by pairwise comparison of the 500 ppm group with the controls for kidney transitional cell papillomas in males only. However, no dose-response was evident and the occurrence of these tumors was not preceded by pre-neoplastic changes in the kidney. **The CARC determined that only the liver tumors in both sexes were treatment-related.** The dosing at the highest dose was considered by the Committee to be adequate and not excessive based on increased survival, decreases in body weight gains (20%) in both sexes and non-neoplastic changes were not severe.

- **Compared to other triazole compounds the multiplicity and magnitude of tumor response was seen at lower doses and therefore, epoxiconazole was considered by the Committee as one of the more potent triazole compounds studied.**

The genetic toxicology studies indicated that epoxiconazole was not mutagenic in *a battery of mutagenicity assays.* 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. Eight out of ten structurally related compounds tested negative in mutagenicity assays and two were positive only in *in vitro* chromosomal aberration assays.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified epoxiconazole as **"likely to be carcinogenic to humans "** by the oral route based on the occurrence of liver tumors in male and female mice and rats. In addition, treatment-related increases were noted for adrenal tumors in male and female rats and ovarian tumors in female rats. For the quantification of human cancer risk, the Committee recommended a linear low-dose extrapolation approach based on the incidence of liver tumors in male or female mice, whichever is more potent.

II. INTRODUCTION:

On November 8, 2000, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of epoxiconazole. Dr. Ayaad Assaad of the Registration Action Branch 3 presented the results of chronic and carcinogenicity studies in rats and mice, mutagenicity studies, other toxicity studies as well as data on structurally related compounds (Assaad, 2000). The historical control data as well as studies related to the mechanism of toxicity of epoxiconazole were not available for review at the CARC meeting.

II. BACKGROUND INFORMATION:

Epoxiconazole (2RS,3SR)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-2-[(1H-1,2,4-triazol-1-yl)methyl] oxirane , CAS # 106325-08-0; P.C.Code: 123909), is a fungicide intended for use to control Black Sigatoka *(Mycosphaerella fijiensis)*, and Yellow Sigatoka *(Mycosphaerella musicola)* on banana in banana-producing countries of Central and South America (import tolerance). Currently, there are no registered uses for epoxiconazole in the United States.

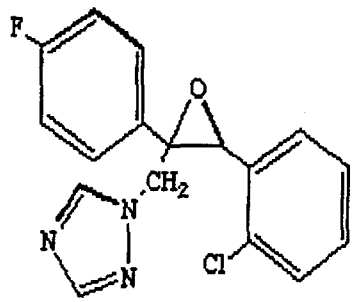

Figure 1: Epoxiconazole

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III. EVALUATION OF CARCINOGENICITY STUDIES:

1. Combined Chronic Toxicity/Carcinogenicity Study with Epoxiconazole in Wistar Rats

Reference:
a) Mellert, W., (1992). Combined Chronic/Oncogenicity Study of Epoxiconazole Administered in Feed to Wistar Rats for 24 Months (1992). Study No. BASF 71S0959/88066, Laboratory Project No. N/A, RD 92/0686. dated July 1992. **MRID** # 44335017. Unpublished.

b) Mellert, W., (1992). Chronic of Epoxiconazole Administered in Feed to Wistar Rats for 24 Months (1992). Study No. BASF 71S0959/88066, Laboratory Project No. N/A, RD 92/10685. dated July 1992. **MRID** # 44335016. Unpublished.

A. Experimental Design:

In a combined chronic/carcinogenicity toxicity study (MRID 44335017), epoxiconazole, 93.2% a.i.] was administered to 500 Wistar Rats 50/sex/dose in the diet at dose levels of 0 (control), 30, 150, 750, 1500 ppm (0, 2, 7, 40, 80 mg/kg/day) for 24 months.

In a chronic oral toxicity study in Wistar rats (MRID No. 44335016), epoxiconazole, 93.2% a.i. was administered to 100 Wistar Rats 20/sex/dose in the diet at dose levels of 0 (control), 30, 150, 750 and 1500 ppm (average dose levels for both sexes: 0, 2 , 8, 38 and 78 mg/kg/day, respectively) for 24 months.

Since the studies were conducted concurrently in the same laboratory, there were no statistically significant differences in mortality between these studies, and the actual doses (mg/kg/day) were comparable, the tumor incidence data were combined for the purposes of statistical analyses (Brunsmann, 2000).

B. Discussion of Tumor Data:

Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 1500 ppm dose group with the controls, for liver carcinomas, adrenal cortex adenomas, and adrenal cortex adenomas/carcinomas combined, all at $p < 0.05$. There was also a significant increasing trend ($p < 0.01$) for combined liver adenomas/carcinomas. Based on a report on spontaneous tumors in 930 Wistar rats from five NTP carcinogenicity bioassays conducted between 1990 and 1995, for male rats the incidence ranges for adrenal cortical tumors were as follows: adenomas: 0%-9.5%, carcinomas: 0%-1% (Pletcher, 2000). Thus, at high dose the incidences of adrenal cortex adenomas (10%) was slightly outside the range for the spontaneous tumors. There were significant differences ($p<0.05$ or 0.01) in the pair-wise comparisons of the 50, 150 and 750 ppm dose groups, for pituitary adenomas. The statistical analyses of the incidence of tumors in the male thyroid and pituitary glands are misleading because not all animals in the 50, 150 and 750 ppm groups were examined. If only the organs of animals with gross necropsy abnormalities in these groups were examined microscopically, there could be a falsely increased incidence of tumors. There were significant differences in the

pair-wise comparisons with the controls for thyroid follicular cell adenomas (at 750 ppm), carcinomas (at 150 ppm), and combined adenomas/carcinomas (at 150 and 750 ppm with p <0.05 or 0.01). However, no increases in thyroid tumors were noted in the 1500 ppm dose group. There were significant differences ($p<0.05$ or 0.01) in the pairwise comparisons of the 50, 150 and 750 ppm dose groups, but not in the 1500 ppm dose group, for pituitary adenomas. There was increase in the prostate adenomas at 1500 ppm (7% vs 1% in controls), however, the increase was not statistically significant by pairwise comparison. The apparent lack of prostate adenomas at ≥50 ppm may have resulted due to limited number of animals examined. The data are presented in tables 1-4.

Female rats had significant increasing trends in adrenal cortex adenomas, carcinomas, and adenomas/carcinomas combined, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 1500 ppm dose group with the controls for adrenal cortex adenomas ($p < 0.05$), and adrenal cortex adenomas/carcinomas combined ($p < 0.01$). Based on a report on spontaneous tumors in 930 Wistar rats from five NTP carcinogenicity bioassays conducted between 1990 and 1995, for female rats the incidence ranges for adrenal cortical tumors were as follows: adenomas: 0%-7%, carcinomas: 0%-1.7% (Pletcher, 2000). Thus, at high dose the incidences of adrenal cortex adenomas (18%) and carcinomas (4%) were outside their respective range for the spontaneous tumors. There were also significant differences in the pair-wise comparisons of the 50 ppm dose group with the controls for liver adenomas, and adenomas/carcinomas combined, both at $p < 0.05$. The incidence of these tumors did not increase at 1500 ppm and therefore, they were not considered to be treatment-related. There were significant differences in the pair-wise comparisons of the 50, 750 and 1500 ppm dose groups with the controls for liver cholangiomas, all at $p < 0.05$. The statistical analyses of the incidence of tumors in the female thyroid C-cell tumors are misleading because not all animals in the 50, 150 and 750 ppm groups were examined. If only the organs of animals with gross necropsy abnormalities in these groups were examined microscopically, there could be a falsely increased incidence of tumors. There was a significant difference in the pair-wise comparison of the 50 and 150 ppm dose groups with the controls for thyroid C-cell adenomas, carcinomas (at 50 ppm; $p < 0.01$) and combined adenomas/carcinomas ($p<0.05$). No increases in thyroid tumors were noted at 1500 ppm.

There was a significant increasing trend, and significant differences in the pair-wise comparisons of the 750 and 1500 ppm dose groups with the controls, for ovarian granulosa cell tumors, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 150, 750 and 1500 ppm dose groups with the controls for ovarian luteomas, all at $p < 0.05$. There was increase (non significant) in the incidence of uterine adenomas at 50 and 1500 ppm dose groups (5% and 6%, respectively, vs 0% in controls). However, these tumors were not seen in the 150 and 750 ppm dose groups. The data for female rats are presented in tables 5-8 .

With the exception of adrenal tumors, the historical control incidences for various tumor types for male and female rats were not available for comparison.

Statistical analysis of survival data in male and female rats showed a significant decreasing trend for mortality with increasing doses of epoxiconazole. Therefore, the statistical analyses of tumor data for male and female rats were based upon the Peto's prevalence test.

Table 1. Epoxiconazole - Wistar Rat Study

Male Adrenal Cortex Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Adenomas (%)	2/60 (3)	3[a]/61 (5)	3/62 (5)	5/66 (8)	7/67 (10)
p =	0.020*	0.387	0.365	0.153	0.047*
Carcinomas (%)	0/59 (0)	1[b]/59 (2)	0/62 (0)	1/65 (2)	0/67 (0)
p=	0.541	0.098	-	0.179	-
Combined (%)	2/60 (3)	4/61 (7)	3/62 (5)	6/66 (9)	7/67 (10)
p =	0.023*	0.242	0.365	0.097	0.047*

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 91, dose 50 ppm.

[b]First carcinoma observed at week 94, dose 50 ppm.

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 2. Epoxiconazole - Wistar Rat Study

Male Liver Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Adenomas (%)	16[a]/60 (27)	9/61 (15)	6/62 (10)	13/66 (20)	18/67 (27)
p =	0.111*	-	-	-	-
Carcinomas (%)	3[b]/62 (5)	4/63 (6)	4/64 (6)	3/66 (5)	10/69 (14)
p=	0.013*	0.427	0.381	0.456	0.040*
Combined (%)	19/62 (31)	13/63 (21)	9[c]/64 (14)	16/66 (24)	27[c]/69 (39)
p =	0.007**	-	-	-	0.221

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 91, dose 0 ppm.

[b]First carcinoma observed at week 87, dose 0 ppm.

[c]One animal in each of the 150 and 1500 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.

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

Table 3. Epoxiconazole - Wistar Rat Study

Male Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Adenomas (%)	1/57 (2)	0/4 (0)	0/8 (0)	2[a]/11 (18)	4/66 (6)
p =	0.096	-	-	0.022*	0.129
Carcinomas (%)	1/50 (2)	0/2 (0)	1/1 (100)	0/6 (0)	1[b]/62 (2)
p=	0.732	-	0.000**	-	-
Combined (%)	2/57 (4)	0/4 (0)	1/8 (12)	2/11 (18)	5/66 (8)
p =	0.234	-	0.000**	0.046*	0.190

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 98, dose 750 ppm.

[b]First carcinoma observed at week 105, dose 1500 ppm.

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. Epoxiconazole - Wistar Rat Study

Male Pituitary and Prostate Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Pituitary Adenomas (%)	26/68 (38)	16[a]/29 (55)	16/29 (55)	17/25 (68)	32/70 (46)
p =	0.854	0.005**	0.012*	0.001**	0.328
Prostate Adenomas (%)	1/70 (1)	0/17 (0)	0/21 (0)	0/17 (0)	5[b]/70 (7)
p=	0.062	-	-	-	0.079

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First pituitary adenoma observed at week 63, dose 50 ppm.

[b]First prostate adenoma observed at week 105, dose 1500 ppm.

Note: There were no pituitary or prostate carcinomas observed.

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 5. Epoxiconazole - Wistar Rat Study

Female Adrenal Cortex Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Adenomas (%)	3/56 (5)	3[a]/60 (5)	2/62 (3)	3/62 (5)	11/60 (18)
p =	0.001**	-	-	-	0.012*
Carcinomas (%)	0/43 (0)	0/44 (0)	0/54 (0)	0/56 (0)	2[b]/54 (4)
p=	0.009**	-	-	-	0.102
Combined (%)	3/56 (5)	3/60 (5)	2/62 (3)	3/62 (5)	13/60 (22)
p =	0.000**	-	-	-	0.005**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 95, dose 50 ppm.

[b]First carcinoma observed at week 105, dose 1500 ppm.

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 6. Epoxiconazole - Wistar Rat Study

Female Liver Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Adenomas (%)	2/66 (3)	8[a]/67 (12)	0/69 (0)	2/67 (3)	2/65 (3)
p =	0.849	0.027*	-	-	-
Carcinomas (%)	0/43 (0)	0/44 (0)	3[b]/54 (6)	0/57 (0)	0/54 (0)
p=	0.876	-	0.059	-	-
Combined (%)	2/66 (3)	8/67 (12)	3/69 (4)	2/67 (3)	2/65 (3)
p =	0.925	0.027*	0.421	-	-
Cholangiomas (%)	0/53 (0)	3[c]/56 (5)	3/59 (5)	4/61 (7)	5/59 (8)
p=	0.054	0.044*	0.059	0.039*	0.021*

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 77, dose 50 ppm.

[b]First carcinoma observed at week 105, dose 150 ppm.

[c]First cholangioma observed at week 97, dose 50 ppm.

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 7. Epoxiconazole - Wistar Rat Study

Female Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Adenomas (%)	8[a]/65 (12)	3/24 (12)	4/18 (22)	2/13 (15)	11/65 (17)
p =	0.662	0.042*	0.028*	0.094	0.357
Carcinomas (%)	0/43 (0)	1[b]/2 (50)	0/3 (0)	0/3 (0)	0/54 (0)
p=	0.846	0.000**	-	-	-
Combined (%)	8/65 (12)	3[c]/24 (12)	4/18 (22)	2/13 (15)	11/65 (17)
p =	0.662	0.042*	0.028*	0.094	0.357

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 79, dose 0 ppm.

[b]First carcinoma observed at week 107, dose 50 ppm.

[c]One animal in the 50 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 8. Epoxiconazole - Wistar Rat Study

Female Ovarian and Uterine Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	50	150	750	1500
Ovarian Granulosa Cell Tumors (%)	2/60 (3)	4/62 (6)	3/65 (5)	13[a]/66 (20)	16/61 (26)
p =	0.000**	0.137	0.294	0.003**	0.001**
Ovarian Luteomas (%)	0/55 (0)	0/57 (0)	3[b]/60 (5)	4/63 (6)	3/60 (5)
p=	0.051	-	0.039*	0.039*	0.033*
Uterine Adenomas (%)	0/43 (0)	2[c]/44 (5)	0/54 (0)	0/57 (0)	3/54 (6)
p =	0.064	0.080	-	-	0.059

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First ovarian granulosa cell tumor observed at week 88, dose 750 ppm.

[b]First ovarian luteoma observed at week 96, dose 150 ppm.

[c]First uterine adenoma observed at week 105, dose 50 ppm.

Note: There were no uterine carcinomas observed.

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 Lesions:

Treatment-related non-neoplastic histopathology findings were seen in males at all dose levels. At ≥50 ppm, increased incidence of adrenal cellular hypertrophy and inflammatory foci in the liver was seen in males. At ≥750 ppm, histopathological findings in the liver consisted of cholangiofibrosis, pericholangitis, hepatocellular hypertrophy; inflammatory cell foci, eosinophilic foci and mixed cell foci. The increased incidences of both eosinophilic and mixed cell foci in the 750 ppm and 1500 ppm males are significant findings because these hepatocellular foci of cellular alteration may represent pre-neoplastic changes that could lead to hepatocellular adenomas and/or carcinomas. Additionally, adrenal angiectasis (abnormal dilation), renal pelvic mineralization, lung perivascular cuffing, and increases in numbers of alveolar macrophages, were also observed.

Among treated female rats, the histopathological changes observed at 50 ppm or above consisted of one or more of the following: increased incidence of hepatocellular and adrenal cellular hypertrophy (at ≥50 ppm), adrenal accessory nodules (at ≥150 ppm) , bile duct proliferation (at ≥150 ppm) , chronic nephropathy (at ≥150 ppm), increased numbers of alveolar histocytosis (at ≥150 ppm) and ovarian cysts (at ≥50 ppm) . Additionally at ≥750 ppm, pituitary hyperplasia, adrenal vascular cell foci, biliary cysts, and adrenal nodular were also observed.

D. Adequacy of the Dosing for Assessment of Carcinogenicity:

There was decreasing trend in survival of males at ≥200 ppm. There was no treatment-related adverse effect on the survival in female mice. Food consumption, food efficiency, and differential blood counts were not altered by exposure to epoxiconazole. The CARC determined that the dosing at the highest dose was adequate and not excessive based on decreases in body weight gains at 750 and 1500 ppm in males (13%) and females (12-14% and 18-19%, respectively), increases in relative liver weights (21% in males and 43% in females) as well as histopathological changes in multiple organs.

2. Carcinogenicity Study in Mice

Reference:

W. Mellert (1992). Study of the potential carcinogenicity of Epoxiconazole feeding study in C57BL/6NCrlBr mice administered via the diet for 78 weeks. Department of Toxicology,BASF Aktiengesellschaft, Ludwigshafen/ Rein, Germany, for BASF Corporation, Agricultural Products, Research Triangle Park, North Carolina, and completed July 1992 (Registration Document No. 92/10699; MRID No. 44335018). Unpublished.

A. Experimental Design

In a mouse carcinogenicity study (MRID # 44335018), epoxiconazole (93.2% a.i) was administered to groups of 50 mice per sex at dose levels of 0, 1, 5, 200 or 500 (males)/1000 (females) ppm (0, 0.17, 0.81, 35.3 or 72.2 (for males) and 214.4 mg/kg/day (for females), respectively) of epoxiconazole for 79 weeks. An additional 10 mice/sex/dose were designated for interim sacrifice at week 53. Since there were no statistically significant differences in mortality between the two control groups, the tumor data have been combined for the purpose of statistical analyses (Brunsmann, 2000).

B. Discussion of Tumor Data

Male mice had significant increasing trends in liver adenomas, carcinomas, and adenomas/carcinomas combined, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 500 ppm dose group with the controls for liver adenomas at $p < 0.05$, and for carcinomas, and adenomas/carcinomas combined, both at $p < 0.01$ (Table 9). There was also a significant increasing trend, and a significant difference in the pair-wise comparison of the 500 ppm dose group with the controls, for kidney transitional cell papillomas, both at $p < 0.01$. There was no treatment-related increase in the incidence of malignant lymphomas (Table 10).

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls, for liver adenomas, carcinomas, and adenomas/carcinomas combined, all at $p < 0.01$ (Table 11). There was no treatment-related increase in the incidence of kidney tumors (Table 12).

Statistical analysis of survival data in male mice showed a significant decreasing trend for mortality with increasing doses of epoxiconazole (Brunsmann, 2000). Therefore, the statistical analyses of tumor data for male mice were based upon the Peto's prevalence test. No treatment-related effect on survival were noted in female mice.

Table 9. Epoxiconazole - C57BL/6NCrlBr Mouse Study

Male Liver Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	1	5	200	500
Adenomas (%)	0/61 (0)	0/30 (0)	0/34 (0)	0/44 (0)	3[a]/42 (7)
p =	0.001**	-	-	-	0.018*
Carcinomas (%)	1/86 (1)	0/40 (0)	0/46 (0)	3/48 (6)	33[b]/50 (66)
p=	0.000**	-	-	0.087	0.000**
Combined (%)	1/86 (1)	0/40 (0)	0/46 (0)	3/48 (6)	34[c]/50 (68)
p =	0.000**	-	-	0.087	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 81, dose 500 ppm.

[b]First carcinoma not in an interim sacrifice animal observed at week 69, dose 500 ppm.

[c]Two animals in the 500 ppm dose group had both an adenoma and a carcinoma.

Note: One animal in the interim sacrifice group of the 5 ppm dose group had a carcinoma. There were no adenomas in any interim sacrifice animals. Interim sacrifice animals are not included in this analysis.

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 10. Epoxiconazole - C57BL/6NCrlBr Mouse Study

Male Kidney and Lymphoma Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	1	5	200	500
Kidney Transitional Cell Papillomas (%)	3[a]/87 (3)	1/42 (2)	1/46 (2)	1/48 (2)	7/50 (14)
p =	0.000**	-	-	-	0.003**
Malignant Lymphoma (all sites) (%)	33[b]/120 (28)	14/60 (23)	15/60 (25)	13/60 (22)	20/60 (33)
p =	0.475	-	-	-	0.466

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First kidney transitional cell papilloma observed at week 68, dose 0 ppm.

[b]First malignant lymphoma (all sites) observed at week 26, dose 0 ppm.

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 11. Epoxiconazole - C57BL/6NCrlBr Mouse Study

Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	1	5	200	1000
Adenomas (%)	0/93 (0)	0/46 (0)	0/48 (0)	0/49 (0)	5[a]/49 (10)
p =	0.000**	1.000	1.000	1.000	0.004**
Carcinomas (%)	1/93 (1)	1/46 (2)	1/48 (2)	1/49 (2)	33[b]/49 (67)
p=	0.000**	0.554	0.567	0.573	0.000**
Combined (%)	1/93 (1)	1/46 (2)	1/48 (2)	1/49 (2)	38/49 (78)
p =	0.000**	0.554	0.567	0.573	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 65, dose 1000 ppm.

[b]First carcinoma not in an interim sacrifice animal observed at week 56, dose 1000 ppm.

Note: One animal in the interim sacrifice group of the 1000 ppm dose group had a carcinoma.

There were no adenomas in any interim sacrifice animals. Interim sacrifice animals are not included in this analysis.

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 12. Epoxiconazole - C57BL/6NCrlBr Mouse Study

Female Kidney Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	1	5	200	1000
Adenomas (%)	4/93 (4)	4/46 (9)	1[a]/48 (2)	4/49 (8)	4/49 (8)
p =	0.191	0.249	0.444[n]	0.279	0.279
Carcinomas (%)	0/93 (0)	1/46 (2)	1/48 (2)	1[b]/49 (2)	0/49 (0)
p=	0.490	0.331	0.340	0.345	1.000
Combined (%)	4/93 (4)	5/46 (11)	2/48 (4)	5/49 (10)	4/49 (8)
p =	0.282	0.134	0.669	0.156	0.279

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 59, dose 5 ppm.

[b]First carcinoma observed at week 75, dose 200 ppm.

[n]Negative change from control.

Note: Interim sacrifice animals are not included in this analysis.

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 Lesions

Satellite group:. In males at ≥200 ppm and/or in females at 1000 ppm treatment-related liver changes consisted of centrilobular hypertrophy of hepatocytes, cellular alteration, focal necrosis, and peripheral fatty liver infiltration. Amyloidosis occurred in all groups of treated and control animals in different organs with different patterns. In males, at the highest dose (500 ppm) amyloid deposition was significantly high in the testes, heart, and in females in liver, glandular stomach, duodenum, cecum, colon, heart, thyroid glands, and parathyroid glands.

Main group:. Treatment related changes seen in the liver consisted of an increased number of clear cell and eosinophilic foci, cellular alterations and diffuse and centrilobular hypertrophy of hepatocytes in both sexes at high dose (♂: 500 and ♀: 1,000 ppm). Histopathologically, most of the macroscopically diagnosed masses and colored foci in liver were diagnosed as carcinomas. Liver necrosis was seen in males at ≥200 ppm. Clear cell foci were increased in high dose group rats (♂: 500 and ♀: 1,000 ppm). Eosinophilic foci and centrilobular hypertrophy of hepatocytes were seen in male at ≥200 ppm and in females at 1,000 ppm.

Among the non-neoplastic findings, amyloidosis was the most common finding in various organs. In males of the main group, only testes showed higher amyloid deposition at ≥200 ppm compared to two controls and was considered treatment-related. In females, amyloidosis was recorded at higher incidences and/or higher grades of severity in various organs at 1,000 ppm and was considered as a treatment-related effect. This included amyloid deposition in liver, mandibular glands, glandular stomach, duodenum, jejunum, colon, pancreas, kidneys, ovaries, uterus, heart, spleen, mandibular lymph node, adrenal cortex, thyroid glands and parathyroid glands.

D. Adequacy of Dosing for Assessment of Carcinogenicity:

The CARC determined that the highest dose was adequate and not excessive based on lack of adverse effect on the survival, decreased body weight of both sexes (≈20%), decreased food consumption during the first year, and increased liver weights accompanied by histopathological changes in various organs in both sexes.

IV. TOXICOLOGY

1. Metabolism

Based on pharmacokinetics and metabolism studies in rats (MRID 44335032, 44401609), epoxiconazole was rapidly absorbed and widely distributed following oral administration as single gavage doses of 3 or 100 mg/kg or 15-day repeated doses of 3 mg/kg/day. During the seven day follow up period, most of the administered radiolabel (77-82%) was in the feces while 12-21% was in the urine and 0.8-1.5% was in the tissues/carcass. The fecal vs. urinary route of excretion did not vary considerably with dose or sex. Excretion via expired air (measured in the high dose males

only) was minimal (<0.1%). Biliary excretion accounted for 31-67% of the administered dose during the first 48 hours following treatment. Assessment of biliary excretion suggested that actual absorption in males and females was 95% and 52% in the low dose group, and 69% and 61% in the high dose, respectively. On a µg/g tissue basis, the greatest amounts of absorbed epoxiconazole were detected in the red blood cells followed by organs associated with metabolic/excretory function and/or are highly perfused, namely the liver, kidney, lung and spleen. Plasma AUC for male and female rats and the blood AUC for male rats between the low and high dose groups was increased approximately 30-fold indicating absorption was not saturated. However, the blood AUC of female rats indicated that some saturation of blood absorption occurred. Elimination T½ of plasma activity was rapid for both sexes, being approximately 5 hours at the low dose and six times that in the high dose. In blood, the C_{max} was reached 8 hours after treatment with the low dose and 24 hours after treatment with the high dose. In both sexes at both doses, blood concentration of epoxiconazole was lower than plasma during the first 24 hours, but thereafter rose to be 2-11 times higher at 168 hours suggesting red cell binding. Following absorption, the test material was extensively metabolized to at least 47 metabolites and excreted predominantly through the bile; urinary metabolite elimination was a minor route. The predominant phase I metabolites resulted from hydrolytic opening of the oxirane ring and hydroxylation of the chlorinated and/or fluorinated aromatic rings. Most urinary metabolites recovered were products of glucuronidation while those in the bile arose from glutathione conjugation. Metabolites identified in the bile and those identified in the feces were completely different; a result of the intestinal bacterial biotransformation.

2. Mutagenicity:

Seven genetic toxicology studies were available for review. The results from these studies indicated that epoxiconazole was not mutagenic in *Salmonella typhimurium, Escherichia coli,* or Chinese hamster ovary cells. There was also no evidence of clastogenicity *in vitro* or *in vivo* and epoxiconazole neither induced unscheduled DNA synthesis in primary rat hepatocytes nor caused DNA adduct formation in rat or mouse livers.

GENE MUTATIONS

1. In a reverse gene mutation assay in bacteria (MRID 44335025), strains TA 100, TA 98, TA 1535, and TA 1537 of *S. typhimurium* were exposed to epoxiconzaole, (93.2% a.i.), in DMSO (dimethyl sulfoxide) at concentrations of 0, 20, 100, 500, 2500, and 5000 µg/plate in the presence and absence of a mammalian metabolic activation system S9. Negative controls consisted of DMSO only. Positive controls were N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine, and 2-aminoanthracene.

Epoxiconazole was tested up to the limit concentration of 5000 µg/plate without inducing an increase in revertants. Additionally, precipitation occurred at both 2500 and 5000 µg/plate. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.** The study is classified as acceptable and satisfies the guideline requirement for gene mutation assay (84-2)

2. In a reverse gene mutation assay in bacteria (MRID 44401608), strain WP2 uvrA of *E. coli* was

exposed to Epoxiconazole, (93.2% a.i.), in DMSO at concentrations of 0, 20, 100, 500, 2500, and 5000 µg/plate in the presence and absence of mammalian metabolic activation system S9. Epoxiconazole was tested up to the limit concentration of 5000 µg/plate **with no evidence of induced mutant colonies over background.** The positive controls did induce the appropriate responses both with and without metabolic activation. The study is classified as acceptable and satisfies the guideline requirement for gene mutation assay (84-2).

3. In mammalian cell gene mutation assays at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus (MRID 44335029), duplicate Chinese hamster ovary (CHO) cell cultures were exposed to epoxiconazole (93.2% a. i.) in DMSO at 6 dose levels (0.05 to 1.0 mg/mL), in the presence and absence of an S9 metabolic activation system. In addition to concurrent vehicle (DMSO) controls, additional cultures were exposed to 5-bromo-2'-deoxyuridine (BrdU) and 3-methylcholanthrene (MCA) to act as positive controls, respectively, for the nonactivation and activation test series.

The test material was neither cytotoxic at any of the concentrations evaluated in either assay up to insoluble levels (0.25 mg/mL and higher) nor had an effect upon the pH or osmolality.

In the original submission, the mutant frequencies of treated cultures varied randomly with dose. The recent revision submitted by the registrant (October 11, 1999) corrected the inconsistencies in the original report. **It was concluded that the test material was not mutagenic in this test system.** This study which was initially classified as unacceptable, is now classified as acceptable and satisfies the guideline requirement for gene mutation assay (84-2).

CHROMOSOMAL ABERRATIONS

4. In a mammalian cell cytogenetic assay (MRID 44335041), CHO cells were exposed for 4 hours in the presence or absence of S9 metabolic activation to a range of concentrations of epoxiconazole (10 to 140 µg/mL), with different harvest times, (7, 24, 30 hrs) and 100 metaphases per culture were scored for structural chromosome aberrations. In addition to concurrent/negative and solvent controls, cultures were treated with ethylmethanesulfonate (EMS), or cyclophosphamide, to serve as positive controls for, respectively, the nonactivation and activation test series.

Epoxiconazole was tested up to a cytotoxic concentration (140 ug/mL +/-S9, as determined by both reduced plating efficiency and mitotic index). Concentrations above 140 mg/mL precipitated in the culture medium. **There was no increase in cells with structural aberrations observed, either in the presence or absence of metabolic activation.** The reference mutagens used as positive controls responded with significant increases in structural aberrations. Hence, it is concluded that epoxiconazole is not mutagenic in this *in vitro* aberration assay. The study is classified as acceptable and satisfies the guideline requirement for cytogenetic assay (84-2).

5. In a mouse bone marrow micronucleus assay (MRID 44335028), NMRI mice (5/sex/dose) were treated orally with epoxiconazole, 95.12% a.i., at doses of 0 (vehicle only), 200, 1000, and 5000 mg/kg. Bone marrow cells were harvested at 24 hr post-treatment in the vehicle control, 200 and

1000 mg/kg test groups and at 16, 24, and 48 hr post-treatment in the 5000 mg/kg test groups. The vehicle was 0.5% carboxymethylcellulose (CMC). Two positive controls (vincristine and cyclophosphamide) and a vehicle control were also run.

There were signs of toxicity which included: irregular respiration in all dose groups; apathy and abnormal abdominal position in the 1000 and 5000 mg/kg groups; and, staggering in the 5000 mg/kg group. All these clinical signs were short-lived as no animal displayed them the day following treatment. However, four of the thirty animals in the 5000 mg/kg group died before study's end, most likely as a result of compound exposure. Epoxiconazole was tested at an adequate dose based on clinical signs of toxicity, including death. Additionally, the high dose tested was 5000 mg/kg which is the maximum dose specified by the OPPTS 870.5395 guidelines. The positive controls induced the appropriate response.

There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any treatment time following epoxiconazole exposure. Nor were there increases in frequencies of micronucleated normochromatic erythrocytes, absolute numbers of polychromatic erythrocytes, or, in the ratio of polychromatic and normochromatic erythrocytes. The is classified as acceptable and satisfies the guideline requirement for micronucleus assay (84-2).

OTHER MUTAGENIC MECHANISMS

6. The potential for DNA adduct formation by epoxiconazole (% a.i.; MRID 44335030) was examined in a rodent assay. Three female Wistar rats and 12 male C57Bl/6 were administered ^{14}C-radio labeled test article by oral gavage [131 mg/kg (2.55×10^9 dpm/kg) - rats) 27.8 mg/kg (2.82×10^9 dpm/kg - mice)] following a 24-day prefeeding period with 1500 ppm (rat) or 500 ppm (mouse) in the diet. Twenty-four hours after the administration of the radioactive test article, treated and vehicle-control animals were sacrificed, and hepatic DNA and chromatin protein were measured for radioactivity. Rat liver samples were processed individually and mouse liver samples were pooled (four animals/pool).

In all DNA samples isolated from treated animals, the radioactivity measured over control was near the limit of detection. Specific activity was 1.4 ± 0.1 dpm/mg DNA in rat liver samples ($n = 3$), and 4.0 ± 0.4 dpm/mg DNA in mouse samples ($n = 3$). Chromatin protein averaged 1240 and 1790 dpm/mg in rats and mice, respectively. Since specific DNA radioactivity derived from protein contamination (presumably) from earlier studies was 0.2%, the radioactivity measured in the DNA samples could be explained entirely by chromatin protein contamination. **There was no evidence of DNA adduct formation in rats and mice treated *in vivo* with epoxiconazole.** The is classified as acceptable/nonguideline.

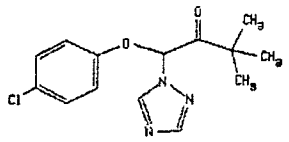
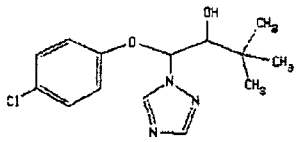
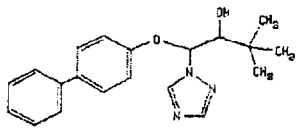
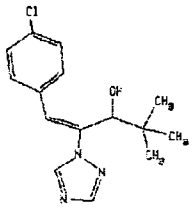
7. In an indenpendently conducted unscheduled DNA synthesis assays (MRID:44335040), primary rat hepatocyte cultures were exposed, in two trials,to epoxiconazole, (93.2% a.i.) dissolved in DMSO at concentrations of 0 (solvent control only), 0.15, 0.5, 1.5, 5.0, 15.0, 50.0, or 150.0 µg/mL in the presence of tritiated thymidine (^{3}HTdR, 5 µCi/mL, specific activity=20 ci/mmol) for

18 hours. Epoxiconazole was tested up to toxic and precipitating concentrations. The positive controls did induce the appropriate response **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced at any concentration in either trial.** The study is classified as acceptable and satisfies the guideline requirement for UDS assay (84-2).

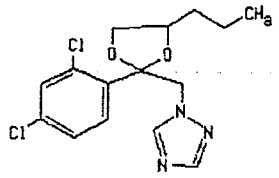
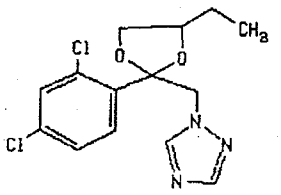
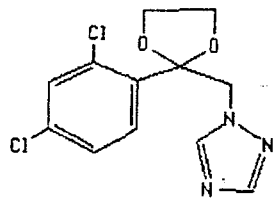
3. Structure-Activity Relationship:

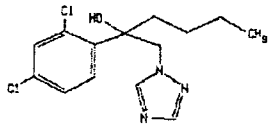
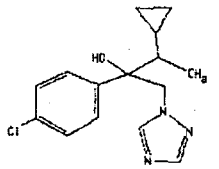
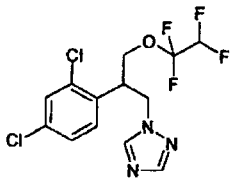
Epoxiconazole is structurally related to a variety of triazole pesticides (the "conazole" class) as shown in Table 17. They all contain or can be hydrolyzed/metabolized to yield a common β-hydroxy (or β-keto) triazole moiety. A number of triazole pesticides (e.g., bayleton, baytan, uniconazole, propiconzole, cyroconazole) have been shown to be carcinogenic in rodents with the liver as the main target organ. Despite the structural similarity, it should be pointed out that epoxiconazole has two unique features that differ from the other triazole pesticides: (a) the presence of an electrophilic epoxide moiety , and (b) the presence of two aryl moieties at both ends of the triazole moiety instead of the alkyl and aryl moieties for the other triazole pesticides shown in Table 17. Although the presence of the epoxide group in epoxiconazole may suggest potential genotoxicity, the steric hindrance around the epoxide group is expected to render it not readily accessible for interaction with DNA. This is consistent with the reports of uniformly negative genotoxicity data on epoxiconazole. Nevertheless, metabolism studies on epoxiconazole indicated the epoxide group is at least accessible for detoxification by glutathione. To some extent, the presence of the two aryl moieties (4-fluorophenyl and 2-chlorophenyl) provides some structural analogy between epoxiconazole and DDT/DDE type pesticides. DDT and DDE have been shown to be hepatocarcinogens in rodents.

Table 17. Structure-Activity of Related Triazole Compounds[1].

Compound	Structure	Carcinogenic Effect	Carcinogen Class/Mutagen
Bayleton PC 109901 Tx.# 862AA		**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% ♀. **Wistar Rat** Dose related trend in thyroid F-cell adenomas in ♂ & combined adenomas/carcinomas with cystic hyperplasia in ♂ & ♀; Pair wise comparisons not significant.	**C NO Q 1*** Negative for mutagenicity
Baytan PC 127201 Tx.# 074A		**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. **Rat**, 125-2000 ppm, increases in thyroid adenomas.	**Weak C** SAP Negative for mutagenicity
Baycor PC 112403 Tx.# 087AA		**Mouse**: up to 500 ppm: (-) **Rat**: up to 500 ppm : (-)	Negative for mutagenicity
Uniconazole PC 128976 Tx.# 207H		**Crl:CD-1(ICR)BR Mouse** Increased incidence of hepatocellular adenomas and carcinomas in males only at HDT . **Crl:CD-1(ICR)SD Rat** No increase in neoplastic findings	**C NO Q 1*** Positive *in vitro* chromosomal aberration with S9

1Abbreviations: ♀: Female; ♂: Male; HDT: Highest dose tested; LDT: Lowest dose tested; SAP: Scientific Advisory Panel

Propiconazole PC 122101 Tx.#323EE		**CD-1 Mouse** Statistically significant trend and pairwise comparisons in liver adenomas and combined. Increases for carcinomas were considered significant by 2 pathologists, but not by the third pathologist.	**C NO Q 1*** Negative for mutagenicity
Etaconazole		No data	Negative in performed genetic toxicity tests: Ames, Dominant Lethal
Azaconazole PC 128882 Tx. # 321A		**Mouse**, 25,100 &400 ppm. There is the question of whether the MTD was reached. No carcinogenic effect.	Negative for mutagenicity except positive *in vitro* CHO aberration (acentric fragments) only with S9

Compound	Structure	Carcinogenic Effect	Carcinogen Class/Mutagen
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 *in vitro* chrom. aberration assay
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

4. Subchronic, and Chronic Toxicity:

Subchronic Toxicity Studies

Dog

In a subchronic toxicity study (MRID 44335008), epoxiconazole (95.12%a.i.) was administered to 40 Beagle dogs (5/sex/dose) in the diet at dose levels of 0 (control), 50, 200 and 800 ppm (0, 1.8, 6.8 and 28.2 mg/kg/day for males and 0, 1.9, 7.8 and 32.4 mg/kg/day for females).

The target organ in this study was the liver. Absolute and relative liver weights were increased (10.8% and 17.6%, respectively) in 800 ppm females compared to controls. Serum alkaline phosphatase levels were increased in both sexes at the high dose. The increase was 13% and 18.7% in males and 16.9% and 47.8% at day 30 and 87/88, respectively. There was an increase in the incidence of inflammatory cell foci of the liver in males but not in females. The incidence of hepatic inflammatory cell foci was 0 in controls, 1 at 50 ppm, 2 at 200 ppm and 4 at 800 ppm.

The LOAEL is 800 ppm (28.2 and 32.4 mg/kg/day in males and females respectively), based on increased incidences of inflammatory cell foci in the liver in males and increased liver weights and serum alkaline phosphatase levels in the female. The NOAEL is 200 ppm (6.8 and 7.8 mg/kg/day in males and females respectively).

Rat

In a subchronic toxicity study (MRID 44401604) epoxiconazole, (95.1% a.i.) was administered to 100 Wistar rats, 10/sex/dose in diet at dose levels of 0, 30 , 90, 270 and 800 ppm (0, 3 ,8 ,21, and 63 mg/kg/day, respectively) for 3 months.

At 800 ppm, male body weights were decreased 3-4% throughout the study in the HDT. At 270 and 800 ppm, the liver toxicity was manifested as increase in relative liver weights in males (6.9 and 13%, respectively) and females (6.7% and 22%, respectively). Female absolute liver weights (28%) and serum cholesterol levels were also increased in high dose females. Centilobular hepatic hypertrophy was increased in both sexes at both dose levels and diffuse hepatic hypertrophy was increased in the females.

The LOAEL in male rats is 30 ppm (2 mg/kg/day) based on adrenal weight decreases. A NOAEL in the male was not identified. The female LOAEL is 270 ppm (22 mg/kg/day) based on histopathology findings in the liver. The female NOAEL is 90 ppm (8 mg/kg/day); the NOAEL for males was not established.

Mouse

In a subchronic toxicity study (MAID 44335009) epoxiconazole, (95.1% a.i.) was administered to 120 C57BL/6Ncr1BR mice, 10/sex/dose in the diet at dose levels of 0, 7.5, 125, 250, 500, 1000 ppm (0, 2, 32 ,67, 123, and 264 mg/kg/day).

There were no compound-related effects on mortality, clinical signs, food efficiency or gross pathology findings. Female body weights and body weight gains were not affected at any dose. Compared to controls, male body weights at the end of the study were decreased 11% ($p<0.05$) in the 1000 ppm group and 7% (not significant) in the 500 ppm group.

No hematology parameters were altered in females. The 500 and 1000 ppm males had platelet counts which were decreased 15.6 and 17.2%, respectively compared to controls ($p<0.05$ in both cases). Mean red blood cell volume measurements were decreased 1.7%, 1.6%, 2.2%, and 1.8% in the 125, 250, 500 and 1000 ppm groups, respectively.

Epoxiconazole was hepatotoxic. Serum levels of the enzyme alanine-amino transferase were elevated in both males and females of the 500 and 1000 ppm groups (males: 175 and 540% and females: 58 and 111% in the 500 and 1000 ppm groups; respectively). Male absolute liver weights were increased 18.3, 28.2, 43.8, and 55% while relative to the body weight, male liver weights were increased 25, 40, 66.6 and 92% in the 125, 250, 500 and 1000 ppm groups, respectively). Female absolute liver weights were increased 17, 34, 44, and 66% while relative weights were increased 17.5, 28.6 42.6 and 65.8% in the 125, 250, 500 and 1000 ppm groups respectively. Eosinophilic degeneration was markedly increased in 500 and 1000 ppm males and in 1000 ppm females. Peripheral-lobular fatty changes of the liver were noted in: 8 males of the 500 ppm group; all 10 males in the 1000 ppm group; and in 5 females of the 1000 ppm group. Centrilobular hypertrophy of the liver was seen in 6, 10, 10, and 10 males (125, 250, 500 and 1000 ppm respectively) and in 8 females (1000 ppm).

The LOAEL is 125 ppm (32 mg/kg/day in both sexes combined), based on hepatotoxicity as indicated by increased relative and absolute liver weights, and hepatic central cellular hypertrophy in males. The NOAEL is 7.5 ppm (2 mg/kg/day).

Reproductive Toxicity - Rat

In a 2-generation reproduction study (MRID 44335024) epoxiconazole, [93.2% a.i.] was administered to 200 Wistar rats, 25/sex/dose, for the F_0 parental generation, and 200 Wistar rats, 25/sex/dose, for the F_1 parental generation. The test compound was administered at dietary concentrations of 0, 10, 25 and 250 ppm [0, 0.85, 2.17, or 22.12 mg/kg/day for F_0 and F_1 generations combined - males; 0, 0.95, 2.41, 31.85 mg/kg/day for F_0 and F_1 combined - females]. Test compound was administered to the parental F_0 animals at least 70 days prior to mating and to the F_1 generation parents their entire life.

Absolute and relative liver weights in F_1 maternal females were increased 11% and 6%, respectively at 250 ppm. Adrenal weights, both absolute and relative, were decreased 28% and 20%, respectively in 250 ppm males of the F_1 generation.

The parental systemic LOAEL is 250 ppm (22.12 mg/kg/day in males, 31.85 mg/kg/day in females), based on reduced body weight, body weight gains and food consumption in males; death of three females; decreased adrenal weights in the male; and increased absolute and relative liver weights in the females of the F_1 parental generation.

Chronic Toxicity

Dog

In a 12-month dietary toxicity study (MRID 44335015), groups of six male and six female Beagle dogs were given epoxiconazole (a.i. 93.2 %) administered in feed at 0, 50, 500 or 1500 ppm (equivalent to 0, 1.5, 14.4 or 46.1 mg/kg/day for males and 0, 1.6, 16.3 or 51.4 mg/kg/day for females). In a supplemental 12-month dietary toxicity study (MAID 44401605), six male Beagle dogs were given epoxiconazole (a.i. 93.2%) administered at 0, 10, 20, 30 or 40 ppm (equivalent to 0, 0.3, 0.6, 0.9 or 1.1 mg/kg/day).

Statistically significant decreases in erythrocytes, hemoglobin, MCHC and hematocrit in 1500 ppm males, and erythrocytes and hemoglobin in 50 ppm males were observed throughout the study. Sporadic statistically significant decreases in erythrocytes and hemoglobin were reported for 500 ppm males. The number of platelets increased in 50, 500 and 1500 ppm males and 1500 ppm females during the study. Changes in clinical chemistry parameters included increased alkaline phosphatase activity in 1500 ppm males and 500 and 1500 ppm females, alanine aminotransferase activity in 500 ppm males and 1500 ppm males and females, decreased total protein, albumin, urea and globulin in 1500 ppm males and decreased cholesterol in 500 ppm females and 1500 ppm males and females.

Changes in organ weight were limited to increased absolute liver weight in 500 ppm males and 1500 ppm males, however statistical significance was achieved in the lower dosed males only. Minimal to severe chronic septal hepatitis was observed in 1500 ppm males (5/6) and females (4/6) and 500 ppm males (1/6).

The LOAEL for male Beagle dogs is 50 ppm (1.5 mg/kg/day) and for female Beagle dogs is 500 ppm (16.3 mg/kg/day) based on decreases of hematologic parameters indicative of anemia. The NOAEL is 40 ppm (1.1 mg/kg/day) for males and 50 ppm (1.6 mg/kg/day) for females.

Rats and Mice

Refer to pages 12 and 18 for non-neoplastic changes seen in chronic/carcinogenicity studies in rats and mice, respectively.

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

1. Carcinogenicity:

The CARC concluded that:

- **Epoxiconazole was carcinogenic to rats** because: 1) In males, there was significant positive trend and a significant ($p<0.05$) increase by pairwise comparison of the 1500 ppm dose group with the controls, for adrenal cortex adenomas and combined adenomas/carcinomas. The increase in the combined incidence was driven solely by adenomas; 2) There was a significant positive trend ($P<0.05$ or 0.001) for hepatocellular

carcinomas and combined adenomas/carcinomas and a significant increase by pairwise comparison of the 1500 ppm dose group with the controls, for carcinomas. No historical control data were available for comparison. 3) Although, there were apparent statistically significant increases in the pituitary and thyroid tumors in the 50, 150 and 750 ppm dose groups and not in the 1500 ppm dose group, the analyses are misleading because not all animals in the low dose groups were examined. If only the organs of animals with gross necropsy abnormalities in these groups were examined microscopically, there could be a falsely increased incidence of tumors. **Therefore, the Committee determined that only adrenal and liver tumors were treatment related while pituitary and thyroid tumors were not treatment-related.**

Among females, there was a significant positive trend ($p<0.001$) for adrenal cortex adenomas, carcinomas, and combined adenomas/carcinomas and a significant increase ($p<0.05$ or 0.001) by pairwise comparison of the 1500 ppm dose group with the controls, for adrenal cortex adenomas and combined adenomas/carcinomas. The increase in the combined incidence was driven by adenomas; the incidence of carcinomas was low and exhibited no dose-response. However, this tumor is not commonly found in Wistar rats and therefore, was of concern to the Committee. The incidences of adrenal cortex adenomas in male and female rats and carcinomas in female rats in the present study were outside the high end of the range (0%-9.5%, 0%-7% and 0%-1.7%, respectively) for Wistar rats (Pletcher, 2000); 2) There was a significant increase ($p<0.05$ or 0.001) by pairwise comparison of the ≥50 ppm (borderline significance at 150 ppm) dose groups with the controls for liver cholangiomas. Although the incidence of cholangiomas was low, these tumors occurred at all dose levels and the incidences were above concurrent controls. 3) There was a significant positive trend for ovarian granulosa cell tumors and a significant increase by pair wise comparison with the controls was also noted for ovarian luteomas at ≥150 ppm dose levels and for granulosa cell tumors at ≥750 ppm. Ovarian luteomas were also seen with tetraconazole, a structurally-related compound. Only a borderline significant increase for uterine adenomas was noted at 1500 ppm. The increase in this tumor was not dose-dependent and there was no statistically significant increase by trend analysis. 4) Although there were apparent statistically significant increases in thyroid C-cell tumors in the 50 and 150 ppm dose groups and not in the 1500 ppm dose group, the analyses are misleading because not all animals in the low dose groups wee examined. If only the organs of animals with gross necropsy abnormalities in these groups were examined microscopically, there could be a falsely increased incidence of tumors. 5) Only a borderline significant ($p=0.059$) increase was noted for uterine adenomas at 1500 ppm. The increase in this tumor was not dose-dependent. There was no statistically significant increase by trend analysis. **Therefore, the Committee determined that thyroid and uterine tumors were not treatment-related while liver, adrenal and ovarian tumors in females were treatment-related.**

The highest dose was considered to be adequate and not excessive based on decreases in body weight gains ((≥13%) in both sexes in a subchronic study and at terminal sacrifice (≥12%) at ≥750 ppm as well as increased liver and adrenal weights and histopathological changes in the liver, adrenal and ovary in the present study.

- **Epoxiconazole was carcinogenic to mice** because: 1) There was a significant ($p<0.001$) positive trend and a significant increase($p<0.05$ or 0.001) by pair-wise comparison of the 500 ppm dose group with the controls for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas in both sexes. There was also a statistically significant increasing trend ($p<0.001$) and a significant increase ($p<0.001$) by pairwise comparison of the 500 ppm group with the controls for kidney transitional cell papillomas in males only. However, no dose-response was evident and the occurrence of these tumors was not preceded by pre-neoplastic changes in the kidney. **Therefore, only the liver tumors in both sexes were considered by the CARC to be treatment-related.** The dosing at the highest dose was considered to be adequate and not excessive based on increased survival, decreases in body weight gains (20%) in both sexes and non-neoplastic changes were not severe.

- **Compared to other triazole compounds, the multiplicity and magnitude of tumor response was seen at lower doses and, therefore, epoxiconazole was considered by the Committee to be one of the more potent triazoles studied.**

2. Mutagenicity

- The submitted genetic toxicology studies indicate that epoxiconazole is not mutagenic in *S. typhimurium*, *E. coli* or Chinese hamster ovary cells.. There is also no evidence of clastogenicity in *in vivo* or *in vitro*. Similarly, epoxiconazole did not induce UDS in primary rat hepatocytes nor cause DNA adduct formation in rat or mouse livers. 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, tetraconazole and cyproconazole. Seven of these structural analogs have been shown to induce hepatocellular tumors in one or both sexes of mice. Eight of these compounds tested negative in mutagenicity assays and two were positive only in *in vitro* chromosomal aberration tests.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

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

1. There were increased incidences of liver tumors in male and female mice and rats. In addition, treatment-related increase were noted for adrenal tumors in male and female rats and ovarian tumors in female rats.

2. The relevance of the observed tumors to human exposure cannot be discounted.

3. The structurally related compounds are largely nonmutagens but 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.

VIII. BIBLIOGRAPHY

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