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OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: November 15, 2011

SUBJECT: PICOXYSTROBIN: Report of the Cancer Assessment Review Committee

PC Code: 129200	DP Barcode: N/A
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FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509P)

THROUGH: Jess Rowland, Chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

TO: Whang Phang, Toxicologist
RAB III, Health Effects Division (7509P)

Kathryn Montague, RM 23
Herbicide Branch, Registration Division (7505P)

The Cancer Assessment Review Committee met on September 14, 2011 to evaluate the cancer classification of Picoxystrobin in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

NOV 18 REC'D, 2011

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

PICOXYSTROBIN

PC CODE 129200

FINAL
November 15, 2011

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

PICOXYSTROBIN CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: Whang Phang, Toxicologist

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

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

Gregory Akerman

Lori Brunsman, Statistician

Marion Copley

Kit Farwell

Nancy McCarroll

Karlyn Middleton

Jess Rowland (Chair)

P.V. Shah

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist

OTHER ATTENDEES: PMRA: Kimberley Lowe and Catherine Adcock (by telephone); HED: Jack Fowle, Meheret Negussie, Cassi Walls, Jessica Ryman, Paula Deschamp; ORD: Charles Wood

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EXECUTIVE SUMMARY

On September 14, 2011, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met to evaluate the carcinogenic potential of picoxystrobin. This is the first assessment of picoxystrobin by the CARC. Picoxystrobin is a NAFTA joint review chemical in partnership with PMRA of Canada. US EPA has the work share lead for review of toxicology data.

Whang Phang of Risk Assessment Branch III presented the chronic toxicity/carcinogenicity study in rats and mice. There are two sets of carcinogenicity studies available. The first set of carcinogenicity studies were completed in 1999 with Alpk:AP$_f$ SD rats and C57BL/10J$_f$ AP Alpk mice. Both of these studies were conducted in the UK. The dose levels employed in these studies were too low (highest concentration for rat, 750 ppm; highest concentration for mice 800 ppm), and they were determined to be insufficient for evaluating the carcinogenic potential of picoxystrobin. A repeated set of carcinogenicity studies were completed in 2011. In evaluating the carcinogenicity of picoxystrobin, the CARC focused on the 2011 studies.

For the 2011 rat chronic/carcinogenicity study, groups of CD® [Crl:CD®(SD)] rats (80/sex/dose) received picoxystrobin in the diet for up to 24 months at concentrations of 0, 50, 200, 1000, or 3500 ppm (equivalent to 0, 2.2, 8.8, 45.3, and 162.1 mg/kg/day for males and 2.8, 11.0, 57.1, and 203.3 mg/kg/day for females). An interim sacrifice (10 rats/sex/group) was conducted on Day 365. For the 2011 mouse carcinogenicity study, five groups of Crlj:CD1 (ICR) mice (60/sex/group) received picoxystrobin in the diet at concentrations of 0, 100, 600, 2400, or 4800 ppm (males: 0, 12, 71, 293, and 583 mg/kg/day; females: 0, 16, 99, 412, and 799 mg/kg/day) for 18 months.

Information on mutagenicity and structure activity relationship were also presented. No tumor mode of action studies are available.

Carcinogenicity

Rat (2011 Study)

- *Testicular Tumors:* Administration of picoxystrobin resulted in the induction of testicular interstitial cell tumors in male CD® [Crl:CD®(SD)] rats. The testicular interstitial cell tumors in male rats exhibited a statistically significant trend ($p<0.01$) and a significant pair-wise comparison ($p<0.05$) of the high dose (3500 ppm; 162.1 mg/kg/day) with the controls. When compared to historical control data from the testing laboratory, the concurrent control incidence is within the adjusted historical control range (0 - 8.3%). Although the concurrent control is considered the most relevant with which to compare the tumor incidences of the dosed groups, it is also noted that the tumor incidence (7/70, 10%) at the high dose was above the laboratory historical control range (0 - 8.3%) as well as the Charles River Laboratory historical control

mean (2.4%) and range (1.1-9.3%) for CD® [Crl:CD®(SD)] rats. Additionally, the testicular tumors are also supported by pre-neoplastic lesions (interstitial cell hyperplasia) at the high dose. **Consequently, the CARC considered the testicular tumors in male CD® [Crl:CD®(SD)] rats to be treatment-related.**

• *Adequacy of Dosing*: The highest dose tested (3500 ppm) was considered to be adequate, and not excessive, to assess the carcinogenic potential of picoxystrobin in both sexes. At the high dose (3500 ppm), both males and females demonstrated body weight reduction (males: 11 %, female: 17 % at the end of the study), while survival rates were significantly increased ($p<0.05$) relative to the controls (control: 25%, 3500 ppm: 49%). Treatment also produced an increased incidence of testicular cell interstitial cell hyperplasia.

Mouse (2011 Study)

• *Liver Tumors*: Male mice had a statistically significant trend ($p<0.05$) only for liver adenomas. When compared to the controls, none of the increases in liver tumors (adenomas, carcinomas, or combined adenomas/carcinomas) at any dose level showed pair-wise significance. Additionally, there was no dose-response for the incidence of this common tumor in male mice. Except for the presence of mixed foci, there were no corroborative pre-neoplastic lesions. No increase in liver tumor incidence was seen in female mice which received a higher dose (799 mg/kg/day) than male mice (583 mg/kg/day). **Therefore, the CARC determined that the liver tumors in male Crlj:CD1 (ICR) mice were NOT treatment-related.**

• *Adequacy of Dosing*: The high dose of 4800 ppm was considered to be adequate, and not excessive, to assess carcinogenic potential in both male and female mice. This was based on treatment- related increase in the incidence of duodenual mucosal hyperplasia in 2400 ppm or above in males and in 4800 ppm in females. In addition, stomach glandular mucosal hyperplasia was also found in 2400 and 4800 ppm males.

Mutagenicity: There is no concern for mutagenicity.

Structure-Activity Relationship: The SAR data are of limited support for picoxystrobin.

Mode of Action: There are no mode of action data available.

Classification and Quantification of Carcinogenic Potential

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified picoxystrobin as **"Suggestive Evidence of Carcinogenic Potential"** based on tumors observed in one species (rat) and one sex (males): a treatment-related increase in testicular interstitial cell benign tumors in the high dose only. No tumors were seen in female rats. The highest dose tested was considered to be adequate, but not excessive, to assess carcinogenicity in rats.-No treatment-related increase in tumor incidence was seen in male or

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female mice at doses that were considered to be adequate for the assessment of carcinogenicity of picoxystrobin. There is no mutagenic concern.

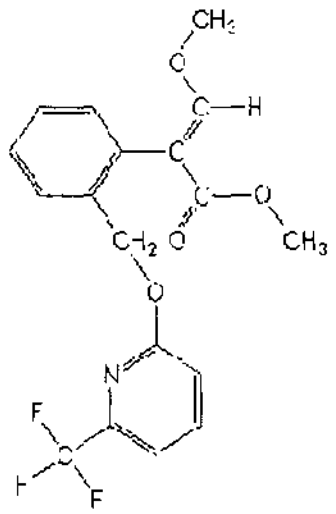
The evidence from animal data is suggestive of carcinogenicity which raises a concern for carcinogenic effects but is judged not sufficient for quantification of cancer risk in humans. Also, when there is suggestive evidence, the Agency does not attempt a linear dose-response assessment as the nature of the data generally would not support one. Therefore, the Agency has determined that quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to picoxystrobin, The NOAEL (4.6 mg/kg/day) used for establishing the Chronic RfD is approximately 30-fold lower than the dose (162 mg/kg/day) that induced testicular tumors in male rats.

I. INTRODUCTION

On September 14, 2011, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of picoxystrobin.

II. BACKGROUND INFORMATION

Picoxystrobin is a NAFTA joint review chemical in partnership with PMRA of Canada. UA EPA has the work share lead for review of toxicology data. The chemical structure of picoxystrobin is presented below:



Picoxystrobin is a fungicide and a member of strobilurins, which are natural products isolated from specific fungi. It blocks fungal growth by disrupting mitrochondria respiration through inhibition of Complex III of the electron transport system. In the available toxicological studies, picoxystrobin produced three frequently seen effects: (1) decreased body weights and food consumption (found across species, gender, and treatment durations), (2) liver effects (increased liver weight and hypertrophy), and (3) gastrointestinal disturbances (increased incidence of diarrhea, soft feces, hyperplasia of the GI mucosal glands, and erosion of the stomach).

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

There are two combined chronic toxicity/carcinogenicity studies in rats available. The first study was conducted in 1999 and did not employ high enough dose levels (highest dietary concentration, 750 ppm). It was judged to be insufficient to assess the carcinogenic potential of picoxystrobin. A repeat study was completed in 2011. For the 1999 study, the relevant

information was presented along with the executive summary, but the details were not presented. The focus was on the 2011 study.

1999 Combined Chronic/Carcinogenicity Study in Rats (MRID: 48073746)

Reference: Rattray, NJ **(1999).** ZA1963: 2-Year Dietary Toxicity and Oncogenicity Study in Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield Cheshire, UK. Laboratory report number: CTL/P/5963. Feb 1, 1999. Unpublished. MRID 48073746.

In the 1999 combined chronic /carcinogenicity study, ZA1963 (picoxystrobin) (99.3% purity) was administered to Alpk:AP_f SD rats (64/sex/dose) in the diet at concentrations of 0, 50, 200, or 750 ppm (equivalent to 0, 3.1, 12.2, or 45.6 mg/kg bw/day for males; 0, 3.8, 14.8, or 57.8 mg/kg bw/day for females) for 2 years. 12 rats/sex/dose group were designated for interim sacrifice at 53 weeks.

Under the conditions of this study picoxystrobin produced no treatment-related changes in mortality, clinical signs, hematological parameters, clinical chemistry parameters, urinalyses, organ weights, and non-neoplastic histopathology. The survival rates in the mid and high dose males were higher than the concurrent controls. In females, the survival rates in all the treated groups were higher than the controls (Table 1). There was no apparent finding to support a specific reason for this discrepancy. However, picoxystrobin at 750 ppm consistently produced slight decreases (<10%) in body weight and food consumption in males and females. Food efficiency was slightly reduced in 750 ppm males. The decrease in body weights might positively influence the survival rates at high dose males and females.

Table 1. Survival Rate (%)

Sex	Concentration (ppm)			
	0	50	200	750
Males	23	17.3	26.9	40.4
Females	38.5	46.2	51.9	51.9

Although the body weight decreases and reduced food consumption showed statistically significant difference from controls, these two effects, in this study, appeared to have a positive impact on the survival rates in high dose groups of both males and females. It is notable that the reduction in body weight is one of the consistent effects seen in other studies with picoxystrobin; however, in this study, this finding is slight and not seems to be adverse in the context of survival rates and the general health of the picoxystrobin treated animals. Therefore, the LOAEL for this chronic study can not be established. The NOAEL is 750 ppm (57.8 mg/kg/day) (HDT). The dietary concentrations were not high enough to evaluate the carcinogenic potential in rats.

2011 Combined Chronic/Carcinogenicity in Rats (MRID 48457402)

Reference: Craig, L. **(2011)** Picoxystrobin (DPX-YT669) Technical: Combined Chronic Toxicity/Carcinogenicity 2-Year Feeding Study in Rats. MPI Research, Inc. 54943 North Main Street Mattawan, Michigan 49071-8353. Laboratory Study No.: 125-093, April 11, 2011. Unpublished.

A. Experimental Design

Picoxystrobin(99.3% a.i) was administered in the diet to CD® [Crl:CD®(SD)] rats (80/sex/dose) for up to 24 months at concentrations of 0, 50, 200, 1000, or 3500 ppm (0, 2.2, 8.8, 45.3, and 162.1 mg/kg/day for males and 2.8, 11.0, 57.1, and 203.3 mg/kg/day for females). An interim sacrifice (10 rats/sex/group) was conducted on Day 365 (Table 2).

TABLE 2: STUDY DESIGN[a]

Test group	Dietary concentions (ppm)	Average Dose (mg/kg/day; M/F)	Main Study (Start of Study)		Interim Sacrifice 12 months	
			Male	Female	Male	Female
1	0	0/0	80	80	10	10
2	50	2.2/2.8	80	80	10	10
3	200	8.8/11.0	80	80	10	10
4	1000	45.3/57.1	80	80	10	10
5	3500[b]	164.1/203.3	80	80	10	10

[a] Ten animals/sex/group were submitted to necropsy following 12 months of treatment. The remaining animals continued treatment for up to approximately 12 additional months

B. Discussion of Mortality, Testicular Interstitial Cell Adenomas and Hyperplasia

The mortality data showed that there was a statistically significant negative trend in mortality and a statistically significant negative pair-wise comparison of the high dose group with the controls, with increasing doses of picoxystrobin in male rats, at $p<0.01$ (Table 3). Therefore, a Peto's Prevalence test was conducted to rule out any survival influence on the observed tumor incidence. The testicular interstitial cell tumors in male rats demonstrated a statistically significant trend at $p < 0.01$ and a statistically significant pair-wise comparison of the 3500 ppm dose group with the controls at $p < 0.05$ (Table 4).

The incidence of interstitial cell hyperplasia in 3500 ppm males (11.4%) showed a statistically significant trend and significant pair-wise comparison (Table 4). In addition, the increased incidence was outside the laboratory historical control range (0 to 1.5%) for interstitial cell hyperplasia and was considered to be treatment-related.

Table 3. Male Rat Mortality Rates[+] and Cox or Generalized K/W Test Results

ppm	1-26 wk	27-52 wk	52 wk[i]	53-78 wk	79-104 wk[f]	Total
0	0/79[a]	2/79	10/77	11/67	39/56	52/69 (75)**n
50	0/80	3/80	10/77	15/67	30/52	48/70 (69)
200	0/80	5/80	10/75	10/65	37/55	52/70 (74)
1000	0/80	4/80	10/76	13/66	33/53	50/70 (71)
3500	2/80	2/78	10/76	10/66	22/56	36/70 (51)**n

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[a]One accidental death at week 13, dose 0 ppm. [i]Interim sacrifice at week 52.
[f]Final sacrifice at week 104. n: Negative trend or negative change from control.
( )Percent. Wk: Week
Time intervals were selected for display purposes only. Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
* $p < 0.05$. ** $p < 0.01$.

Table 4. Male Rat Testicular Tumor Rates[+] and Peto's Prevalence Test Results

Dietary concentration(ppm)	0	50	200	1000	3500
Interstitial Cell Tumors[a] (%) p =	1/38 (3) 0.00100**	1/44 (2) 0.57349	0/40 (0) 0.84826	2/43 (5) 0.33201	7[b]/52 (13) 0.03461*
Testicular interstitial cell Hyperplasia[c]	1/70 (1.4%)**	2/70 (2.3%)	1/70 (1.43%)	1/70 (1.4%)	8/70 (11.4%)*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a] Statistical analysis conducted HED/EPA (L. Brunsman, Aug. 25, 2011)
[b]First interstitial cell tumor observed at week 88, dose 3500 ppm.
[c] Statistically analysis was performed by the study author using Fisher's Exact Test and Cochran-Armitage Trend Test.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
* = $p < 0.05$. ** = $p < 0.01$.

Historical Control Data for Testicular Tumors:

The MPI Research historical data submitted by the registrant included a total of 48 studies dating from 1997 to 2009 (Attachment A). The historical control data for testicular interstitial cell adenomas in rats showed a range of 0 to 14%. However, the 14% incidence was seen in only a single study with the starting and ending dates of 1/11/1999 and 1/10/2001, respectively. The majority of the incidence fell between 0 to 8.3%. The submission also mentioned that the most recent Charles River Laboratory publication summarized the background incidence for the overall testicular interstitial adenomas in Crl:CD(SD) rats was 2.4% with a range from 1.11% to

9.3%.

C. Non-Neoplastic Lesions: A statistically significant increase in testicular interstitial cell hyperplasia was seen at the high dose (see Table 3). No other treatment-related non-neoplastic lesions were seen.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

The highest dose tested (3500 ppm) was considered to be adequate, but not excessive, for assessing the carcinogenicity of picoxystrobin. This was based on body weight reductions (males: 11 %, female: 17 % at the end of the study) at 3500 ppm and decreased food efficiency, as well as increased incidence of testicular cell interstitial cell hyperplasia.

2. Carcinogenicity Study in Mice

Similar to the carcinogenicity studies in rats, there are two mouse bioassays. The first study was conducted in 1999 in England using C57BL/10J$_f$ AP Alpk mice. The highest dietary concentration was 800 ppm, but it was judged to be inadequate to evaluate the carcinogenic potential of picoxystrobin. The registrant completed a second study in 2011. Again, for the purpose of this document, the details of the 1999 study are not presented. The executive summary and relevant information are summarized below. The discussion of carcinogenicity in mice focuses on the 2011 study.

1999 Mouse Carcinogenicity Study (MRID 48073744)

Reference: Rattray, NJ **(1999).** ZA1963: 80 Week Carcinogenicity study in mice. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK. January 22, 1999. Report No. CTL/P/5962. Unpublished.

In this study, picoxystrobin (ZA1963) (93.3%) was administered to C57BL/10J$_f$ AP Alpk mice (50/sex/dose) in the diet at concentrations of 0, 50, 200 or 800 ppm (M/F: 0/0, 6.6/8.8, 26.2/35.6, or 108.8/144.7 mg/kg/day) for at least 80 weeks.

Under the conditions of the study, picoxystrobin produced no treatment-related effects on survival, clinical signs of toxicity, hematology, or organ weights. It appeared to cause, at best, a marginal decrease in body weight (4%) in 800 ppm males only. There was a slight increase in the incidence of macroscopic and microscopic changes in the stomach of females only. The macroscopic finding was described as ulceration. The microscopic finding was inflammation (control: 4/50; 800 ppm: 8/50) and erosion of the stomach (control: 3/50; 800 ppm: 7/50) (Table 5). The LOAEL was 800 ppm (144.7 mg/kg/day) based on slight increase in the incidence ulceration and inflammation of the stomach. The NOAEL was 200 ppm (35.9 mg/kg/day). Under the condition of the study, there was no increase in treatment-related tumor incidence. However,

the test animals could have tolerated higher dose levels, and this study was classified as **unacceptable/guideline.**

Table 5. Histopathology Findings in the Stomach of Female Mice				
Concentration (ppm)	0	50	200	800
Inflammation –non-glandular	4/50	2/50	4/50	8/50
Erosion non-glandular	3/50	1/50	2/50	7/50

2011 Oncogenicity Study in Mice (MRID 48457501)

Reference: Moon K.S. (**2011**). Picoxystrobin (DPX-YT669) Technical: Oncogenicity 18-Month Feeding Study in Mice. Korea Institute of Toxicology, 100 Jang-dong, Yuseong-gu, Daejeon 305-600, Republic of Korea. Laboratory ID # IG08070, April 11, 2011. Unpublished.

A. Experimental Design

Picoxystrobin (DPX-YT669) Technical (Picoxystrobin) (99.3%) was administered to five groups of young adult male and female Crlj:CD1 (ICR) mice (60/sex/group) in the diet at concentrations of 0, 100, 600, 2400, or 4800 ppm (males: 0, 12, 71, 293, and 583 mg/kg/day; females: 0, 16, 99, 412, and 799 mg/kg/day). Body weights and food consumption were evaluated weekly for the first 13 weeks, then every other week thereafter. After approximately 18 months of dietary exposure, mice were sacrificed and gross and microscopic pathological examinations were performed

B. Discussion of Mortality and Tumor Data

There was a statistically significant increase in survival in males as indicated by a statistically significant negative trend in mortality and a statistically significant negative pair-wise comparison of the high dose group with the controls, with increasing doses of picoxystrobin in male mice ($p < 0.01$) (Table 6).

As shown in Table 7, male mice had a statistically significant trend in liver adenomas at $p < 0.05$. There were no other statistically significant findings in male mice (L. Brunsman, August 25, 2011).

An increase in the incidence of mixed foci of cellular alteration in the liver was seen in the 4800 ppm male (Table 8).

Table 6. Male Mouse Mortality Rates[+] and Cox or Generalized K/W Test Results				
ppm	**1-26 wk**	**27-52 wk**	**53-79 wk[f]**	**Total**
0	1/60	4/59	12/55	17/60 (28)**n
100	0/59[a]	4/59	14/55	18/59 (31)
600	1/60	2/59	10/57	13/60 (22)
2400	0/60	2/60	8/58	10/60 (17)
4800	0/60	2/60	2/58	4/60 (7)**n

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[a]One accidental death at week 3, dose 100 ppm. [f]Final sacrifice at weeks 78-79.
n: Negative trend or negative change from control. ( ) Percent.
Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
* = $p < 0.05$; ** = $p < 0.01$.

Table 7. Male Mouse Liver Tumor Rates[+] and Peto's Prevalence Test Results					
ppm	**0**	**100**	**600**	**2400**	**4800**
Adenomas (%) p =	6/52 (12) 0.03794*	5[a]/54 (9) 0.63074	9/56 (16) 0.27858	9/58 (16) 0.40703	13/58 (22) 0.12427
Carcinomas (%) p =	6[b]/49 (12) 0.14743	6/51 (12) 0.54436	6/54 (11) 0.65701	12/57 (21) 0.16942	9/57 (16) 0.26574
Combined (%) p =	12/52 (23) 0.06733	11/54 (20) 0.60579	15/56 (27) 0.39425	20[c]/58 (34) 0.18677	19[d]/58 (33) 0.18640

+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 56, dose 100 ppm. [b]First carcinoma observed at week 64, dose 0 ppm.
[c]One animal in the 2400 ppm dose group had both an adenoma and a carcinoma.
[d]Three animals in the 4800 ppm dose group had both an adenoma and a carcinoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
* $p < 0.05$; ** $p < 0.01$.

TABLE 8. Incidence of Liver Mixed Foci in Male Mice					
	Dose Level (ppm)				
	0	**100**	**600**	**2400**	**4800**
Liver foci, mixed	1/60 (2%)	0/60	3/60 (5%)	1/60 (2%)	7/60 (12%)*

*Statistically significant by Cochran Armitage trend test, $p<0.05$

Historical Control Data on Liver Tumor in Male Crl:CD-1 (1CR)BR Mice

The registrant submitted the limited historical control data from the performing laboratory. The submission also included the general historical control data from Charles River (United States). In comparison to the laboratory historical control data (range: 0-15%) and to the Charles River historical control data on Crl:CD-1 (1CR)BR mice (5-20%), the hepatocellular adenoma finding in the current study (22%) was slightly above the historical control values.

C. Non-Neoplastic Lesions

Treatment-related non-neoplastic findings were present in the duodenum, stomach, and liver.

Duodenum: In males, an increased incidence and severity of duodenal mucosal hyperplasia was present in the 2400 and 4800 ppm groups compared to control. This increase was statistically significant by the Cochran-Armitage trend test; $p<0.05$ in the 4800 ppm male group only (Table 9). A pair-wise comparison by Fischer's exact test, as well as survival adjusted statistics (Poly-3 test and Peto analysis), showed no significant difference in the incidence of duodenal hyperplasia in 2400 ppm and 4800 ppm males as compared to controls. Twenty-one of the 23 males with duodenal mucosal hyperplasia were among the terminal sacrifice mice. Nevertheless, based on the dose response for the severity grade of this lesion, duodenal hyperplasia was considered to be test substance related and an adverse finding in the 2400 and 4800 ppm males. **In females**, fewer test animals were examined for duodenum effects in the low and middle dose groups. Consequently, a dose-related effect could not be adequately assessed. Nevertheless, the 4800 ppm group appeared to show a slightly higher incidence of duodenum effects (Table 9).

Table 9. Test Substance-Related Microscopic Findings in Duodenum & Stomach in Mice					
Findings	Dose Level (ppm)				
	0	100	600	2400	4800
Males					
Number of Duodenum Examined	50	58	54	59	58
Hyperplasia, Mucosal	3	3	1	6	10*
Minimal	2	1	0	1	1
Mild	1	1	0	2	4
Moderate	0	1	1	3	2
Severe	0	0	0	0	3
Dilatation, mucosal glands	2	2	0	5	7 *
Minimal Mild	2	0	0	4	5
Mild	0	2	0	1	2
Stomach glandular mucosal hyperplasia	10/53	22/58	22/58	26/59*	18/58
Females					
Number of Duodenum Examined	56	7	12	13	58
Hyperplasia, mucosal	5	0	2	5	7
Dilatation, mucosal glands	2	0	2	3	5
Stomach glandular mucosal hyperplasia	10/57	2/11	5/13	5/14	4/60

* Statistically significant ($p<0.05$) by Cochran –Armitage trend Test.
Microscopic findings were graded on a 4-point scale: minimal, mild, moderate, severe.

Microscopically, duodenal hyperplasia was characterized by thickened areas of duodenal mucosa with irregular luminal borders and increased basophilic staining compared to normal mucosa. These thickened mucosal areas consisted of densely arranged crypts and tubular glands lined by proliferating epithelium. In the more severely affected animals, duodenal hyperplasia was often associated with dilation of mucosal glands. There was no evidence of invasion of proliferating epithelium into the submucosa.

Stomach: In males, there was an increase in the incidence of stomach glandular mucosal hyperplasia at all dose levels relative to the controls (Table 8). The increase did not show a dose-related response; the severity of effect did not show a clear progression with increasing dose levels. In addition, similar effects were not found in treated females (Table 8). However, given the effects seen in the duodenum and the stomach (stomach inflammation and erosion) of the 1999 carcinogenicity study (MRID 58073744) using a different strain of mice, the increased incidence of stomach glandular mucosal hyperplasia observed at 2400 and 4800 ppm should not be dismissed as unrelated to treatment. This is because intestinal disturbances (increased incidences of diarrhea and soft stools) were demonstrated at relatively high dose levels (~>800 ppm) in rats, mice and dogs. Furthermore, the highest tested dose in the previous carcinogenicity study (1999) was 800 ppm.

Liver: In the liver, test substance-related microscopic findings consisted of increased incidences of hepatocellular hypertrophy in females fed ≥ 2400 ppm (Table 10) and mixed foci of cellular alterations in males at 4800 ppm (Table 10).

Hepatocellular Hypertrophy, Centrilobular: In female mice, centrilobular hepatocellular hypertrophy was present in 2/58, 0/59, 3/60, 5/60, and 28/60 mice in 0, 100, 600, 2400 or 4800 ppm, respectively (Table 10). All were graded as minimal except for two 4800 ppm female mice (numbers 5213 and 5239), which were graded as mild. Thirty-four of the 38 females with hepatocellular hypertrophy were among the terminal sacrifice mice. Hepatocellular hypertrophy can be observed in aging mice as spontaneous background finding. In this study, a low incidence was observed in both male and female control mice (Table 10). An incidence of 3/60 minimal hepatocellular hypertrophy in 600 ppm females was not considered biologically different from control females, and therefore was considered unrelated to treatment. However, the dose related increased incidences and degree of hepatocellular hypertrophy in the 2400 and 4800 ppm female mice (incidences were only statistically significant in the 4800 ppm group), were treatment related.

Table 10. Incidences of Hepatocellular Hypertrophy in the Liver of Male and Female Mice

Findings	Dose Level (ppm)				
	0	100	600	2400	4800
Male					
Number of Liver Examined	58	59	60	60	60
Hypertrophy, Hepatocellular	2	2	1	2	2
Minimal	0	2	0	2	2
Mild	2	0	1	0	0
Female					
Number of Liver Examined	60	60	60	60	60
Hypertrophy, Hepatocellular	2	0	3	5	28*
Minimal	2	0	3	5	26
Mild	0	0	0	0	2

* Statistically significant ($p<0.05$) by Cochran –Armitage trend Test.
Underlined values were interpreted to be test-substance related differences from control values.
Microscopic findings were graded on a 4-point scale: minimal, mild, moderate, severe.
Data excerpted from 2152 of the report or page 14 of the Annex3 (Pathology Report).

The hepatocellular hypertrophy diagnosed in this study was characterized by enlarged centrilobular hepatocytes. The enlargement was due to an increase in cytoplasmic volume rather than nuclear size. The increase in the incidence and degree of centrilobular hepatocellular hypertrophy, as observed in this study, was consistent with the induction of hepatocellular enzyme systems secondary to xenobiotic exposure and was not associated with microscopic evidence of hepatotoxicity. Therefore, hepatocellular hypertrophy was considered adaptive and non-adverse. Treatment- related hepatocellular hypertrophy was not observed in male mice at any concentration.

Liver weights: There was a test substance-related increase in liver weights in males and females fed ≥2400 ppm for 18 months (Table 11). In males exposed to 4800 ppm of the test article, mean absolute and relative (% body weight and % brain weight) liver weights were increased approximately 20%, 22% and 20%, respectively as compared to control. The

differences in mean absolute and mean relative (% body weight) liver weights in 4800 ppm were statistically significant ($p < 0.05$). In 2400 ppm males, mean absolute and relative (% body weight and % brain weight) liver weights were increased approximately 13%, 16% and 12%, respectively. Although none of the increases in these liver weight parameters in 2400 ppm males were statistically significant, these weight increases were considered test article related.

In females fed the 4800 ppm diet, mean absolute and relative (% body and % brain weight) liver weights were increased approximately 25%, 32% and 26%, respectively compared to control (variable statistical significance). In females fed 2400 ppm, mean absolute and relative (% body and % brain weight) liver weights were increased 23%, 26% and 21%, respectively compared to control. Only the difference in mean relative (% body weight) liver weight was statistically significant ($p < 0.01$) in 2400 ppm females. The liver weight changes at both 2400 and 4800 ppm were considered test article related.

The liver weight increases noted above in males were not associated with detectable microscopic hepatocellular hypertrophy. Liver weight effects in females were correlated with the microscopic finding of hepatocellular hypertrophy at the same exposure levels (see microscopic findings). These changes in liver weight parameters were not associated with other microscopic findings indicative of liver toxicity. Therefore, these liver weight changes were considered to represent an adaptive response associated with metabolism of the test-substance and thus were not considered adverse.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The dietary concentrations tested in the current study were considered to be adequate and not excessive in both sexes. The highest dose tested (4800 ppm) was sufficient for assessing the carcinogenic potential of picoxystrobin based on treatment-related effects in mucosal gland of the duodenum and of the stomach in the 2400 ppm group or above.

TABLE 11. Test Substance-Related Liver Weight Effects in Males and Females					
Parameter	Dose Level (ppm)				
	0	100	600	2400	4800
Male					
Number of Animals	43	41	47	50	56
Mean final body weight (g)	51.9±4.7	51.4±5.8	54.2±6.1	51.1±4.8	50.9±4.9
Absolute weight (g)	2.90±0.91	2.94±1.09	3.09±0.92	3.28±1.09	3.47±1.22*(↑20%)
Liver weight/body weight	5.58±1.67	5.74±2.08	5.73±1.77	6.47±2.32	6.79±1.99*(↑22%)
Liver weight/brain weight	561.90	581.06	598.35	631.86	676.29 (↑20%)
Female					
Number of Animals	47	51	48	43	45
Mean final body weight (g)	44.7±6.9	41.6±6.2	43.0±7.9	42.4±8.8	42.2±7.4
Absolute weight (g)	2.12±035	2.02±0.36	2.23±0.42	2.60±1.28	2.65±0.68**(↑25%)
Liver weight/body weight	4.79±0.74	4.87±0.66	5.25±0.85	6.05±1.92**(↑26%)	6.30±1.10**(↑32%)
Liver weight/brain weight	405.78	383.02	422.95	490.27	509.77**(↑26%)

* Significant difference from control group ($p<0.05$). ** Significant difference from control group ($p<0.01$).
Values for liver weight/body weight or brain weight are percentages.

IV. TOXICOLOGY

1. Metabolism

Absorption, distribution, metabolism and elimination of picoxystrobin were evaluated in male and female rats following single or repeated low-dose (10 mg/kg) administration or a single high-dose (100 mg/kg) administration. Bile was collected from rats administered a single high-dose (100mg/kg). In both sexes of rats, approximately 77-82% of the orally administered dose was absorbed. The peak plasma concentration was reached after 1 and 12 hours post dosing for males and females, respectively, following a single low-dose and 24 hours for both sexes following a single high-dose.

The distribution results indicated that liver and kidney received the highest concentration of the administered radioactivity; the liver and the kidneys were found to contain 2.5-4.7 % and1.8-2.3% of the administered dose, respectively.

Picoxystrobin was extensively metabolized and a total of 42 metabolites were isolated from the excreta. Thirty four out of the 42 isolated metabolites were identified. Approximately 9 identified compounds were major metabolites accounting for >5% of the administered dose. The general metabolic profile was similar regardless of sex, dose level, radiolabel, or number of doses administered. The major route of metabolism for picoxystrobin in rats was via ester hydrolysis and glucuronide conjugation.

Within 120 hours, 73-89% of the administered dose was eliminated. Biliary excretion was the primary route of elimination and accounted for 66-72% of the administered radioactivity in the high dose rats. Feces and bile together accounted for 74-78% and 61-65% of the administered dose in males and females, respectively. In comparison, elimination via urine in all dose groups accounted for 18-21% and 26-34% of the dose in males and females, respective. Together, elimination through feces and urine accounted for 92-99% of the parent compound and its metabolites within 120 hours of dosing. As indicated before, orally administered picoxystrobin was not completely absorbed. The elimination data showed that 5-19% of the administered dose was the parent compound. The mean recovery of radioactivity in tissues and carcass at the time of sacrifice (120 hours post dosing) was <1% of the administered dose indicating little potential for bioaccumulation (MRID: 48073755, 48073756, 48073757, 48073758, & 48073759).

2. Mutagenicity

Picoxystrobin was tested in a battery of standard *in vitro* and *in vivo* genotoxicity and mutagenicity tests (Table 12). These studies indicate that picoxystrobin has no genotoxic potential. There was no indication of gene mutation either in the presence or absence of metabolic activation in both the bacterial reverse mutation and mammalian gene mutation tests. The *in vitro* chromosome aberration test and the *in vivo* mouse micronucleus test were both negative and indicate a lack of clastogenic potential for picoxystrobin. Therefore, there is no mutagenic concern for picoxystrobin.

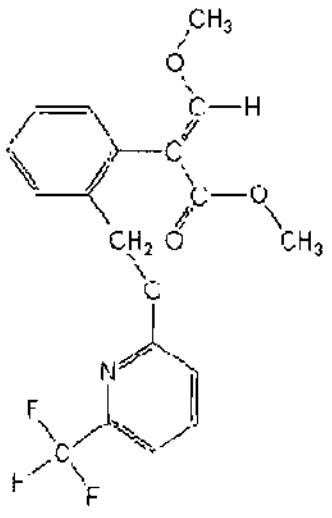
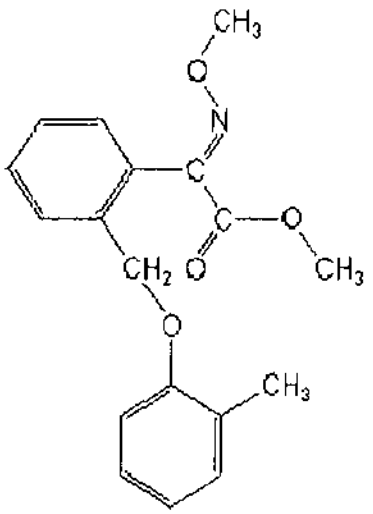
Table 12. Mutagenicity Data

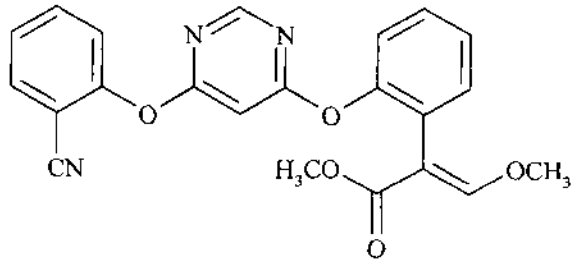
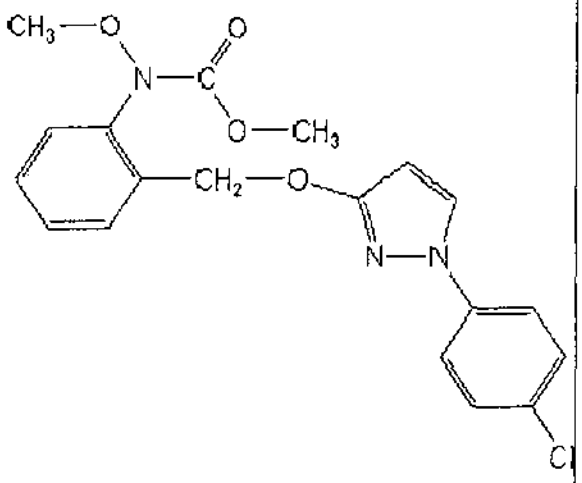
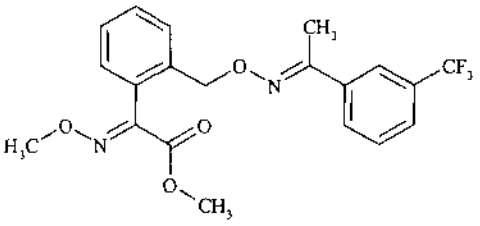
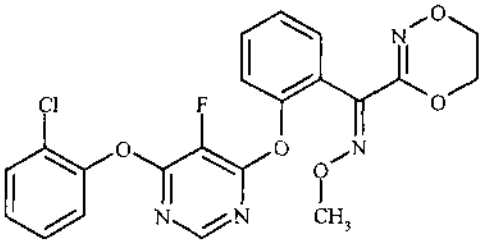
Study Type	Metabolic Activation	Concentrations that could be tested	Results	Classification
***In vitro* test**				
Ames Test with strains TA 1535, TA 100, TA 1537, TA 98 and TA 102 ; E. Coli WP2 & WP2*uvrA* Callander, RD, 1996; CTL/P/4827; MRID 48073747	+/-	100, 200, 500, 1000, 25000 & 5000 µg/plate	Negative	Acceptable / Guideline
Mouse lymphoma cell forward gene mutation assay Clay, P (1996); CTL/P/4993; MRID 48073748	+/-	1st Trial: 4, 8, 16, 32, or 64 µg/ml. 2nd Trial: 24, 32, 42, 56, or 75 µg/ml.	Negative	Acceptable / Guideline
Chromosomal aberration assay (human lymphocytes) Fox, V & Wildgoose, J (1996); CTL/P/4973; MRID 48073749	+/-	**20 hrs exposure**: 0.5-20.0 µg/ml -S9. **20 hrs exposure**: 1-60 µg/ml, +S9 for 3 hrs. **20 hrs exposure**: 2.5, 5.0, 7.5, or 10.0 µg/ml, -S9. **20 hrs exposure**: 30, 40, 50,or 60 µg/ml, +S9 for 3 hrs.	Negative	Acceptable / Guideline
In vivo tests				
Bone marrow micronucleus assay in Cd-1 mice (5/sex/dose) Fox, DA & Mackay, JM (1996); CTL/P/5008; MRID 48073750	NA	0, 2000, 3200, & 5000 mg/kg/day (gavage) (Bone marrow cells were harvested 24 or 48 hrs post dosing)	Negative	Acceptable / Guideline
Unscheduled DNA synthesis assay 1-3 male Alpk:AP$_f$SD rats/group. Mackay, JM (1996); CTL/P/5007; MRID 48073751	NA	0, 3200 & 5000 mg/kg (gavage)	Negative	Acceptable / Guideline

3. Structure Activity Relationship

There are several structurally related fungicides, and they all belong to the same class of strobilurins. They all produced similar effects commonly found in this class of fungicides; these effects are reduced body weight, liver effects with similar characteristics (increased liver weight and hepatocellular hypertrophy), gastro-intestinal effects characterized by diarrhea, soft stool, and hyperplasia of mucosal gland of duodenum (picoxystrobin). Currently EPA has toxicological data on 6 strobilurins (Figure 1); among these 6 strobilurin fungicides only picoxystrobin and kresoxim-methyl show an increase in the tumor incidence. Kresoxim-methyl produced an increase in liver tumor in male and female rats in two studies. The CARC in 1999 evaluated the relevant data on kresoxim-methyl and classified it as **"likely to be carcinogenic to humans"** by the oral route because (1) liver tumors were seen in male and female rats in two studies and (2) tumors in both sexes had a malignant component (Cancer Assessment Document: Kresoxim-Methyl, Aug 19, 1999). Like picoxystrobin, kresoxim-methyl was also shown to be negative in the *in vitro* and *in vivo* battery of mutagenicity/genotoxicity studies. DEREK analyses showed no alerts. Overall these data are of limited support for picoxystrobin.

Figure 1. Structurally Related Compounds (Strobilurins)

Picoxystrobin	Kresoxim-methyl
	

Azoxystrobin 	Pyraclostrobin 
Trifloxystrobin 	Fluoxastobin 

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

90-Day Oral Toxicity Study in Rats [MRID 48073731 (1999)]

In a subchronic oral toxicity study (MRID 48073731), picoxystrobin (ZA1963; 93.3% a.i.) was administered in the diet to Wistar rats (12/sex/dose) at doses of 0, 100, 500, or 1250 ppm (equivalent to 0/0, 8.5/9.7, 41.7/48.1, and 104.9/120.1 mg/kg/day for males/females) for 13 weeks.

No adverse, treatment-related effects were observed on mortality, clinical signs,

ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, organ weights, or gross or microscopic pathology.

At 1250 ppm, decreased ($p<=0.05$) body weights were observed throughout the study in males (decr. 7-10%) and females (decr. 5-9%). Body weight gain during the initial week of treatment was decreased by 27-28%, and overall (Weeks 1-14) body weight gains were decreased by 14-15%. Decreased food consumption was observed each week during Weeks 1-10 in males (decr. 7-15%) and during Weeks 3-7 and 10 in females (10-15%). Food utilization was decreased during Weeks 1-4 in the females (11.9% treated vs 14.1% controls).

The LOAEL is 1250 ppm (104.9 mg/kg/day in males/females) based on decreased body weight, body weight gains and food consumption. The NOAEL is 500 ppm (41.7 mg/kg/day in males/females).

90-Day Oral Toxicity Study in Mice [MRID 48073732 (1996)]

In a subchronic oral toxicity study (MRID 48073732), picoxystrobin (ZA1963; 99% a.i.) was administered in the diet to C57BL/10J$_f$AP/Alpk mice (10/sex/dose) at doses of 0, 200, 800, 1600, or 2400 ppm (equivalent to 0/0, 33.2/43.8, 137.3/176.1, 290.8/358.5, and 421.6/534.8 mg/kg/day for males/females) for 13 weeks.

No adverse, treatment-related effects were observed on mortality, clinical signs, food consumption, organ weights, or gross or microscopic pathology.

Body weights were decreased ($p<=0.05$; except as noted) as follows: (i) sporadically throughout the study in the 800 ppm males (not statistically significant [NS]) and in the females (decr 3-7%); (ii) sporadically throughout the study in the 1600 ppm males (decr 2-7%) and females (decr 3-5%); and (iii) in the 2400 ppm males sporadically throughout the study (decr 2-6%) and females often throughout the study (decr 4-10%). At 800 ppm and above, body weight gains were decreased in males and in females during the initial week, contributing to decreased overall (Days 1-92) body weight gains of 10-28% in males and 18-34% in females.

At 1600 and 2400 ppm, food utilization (g food/100 g body weight gain) was decreased ($p<=0.05$; except as noted) during Days 1-28 in males by 38-52% and in females by 15-44%. The initial effects on food utilization in 1600 and 2400 ppm groups contributed to an overall (Days 1-91) decrease of 25-32% in males and 10-25% in females. At 800 ppm, a slight decrease in food utilization was also found demonstrating a threshold treatment-related effect.

The LOAEL is 800 ppm (137.3 mg/kg/day) based on decreases in body weights, body weight gains and food utilization. The NOAEL is 200 ppm (33.2 mg/kg/day).

b) Chronic Toxicity

Carcinogenicity Study in Mice [MRID 48073744 (1999)]

In a carcinogenicity study (MRID48073744), picoxystrobin (ZA1963) (93.3%) was administered to C57BL/10J$_f$ AP Alpk mice (50/sex/dose) in the diet at concentrations of 0, 50, 200 or 800 ppm (M/F: 0/0, 6.6/8.8, 26.2/35.6, or 108.8/144.7 mg/kg/day) for at least 80 weeks.

Under the conditions of the study picoxystrobin produced no treatment-related effects on survival, clinical signs of toxicity, hematology, or organ weights. It appeared to cause, at best, a marginal decrease in body weight (4%) in 800 ppm males only. There was a slight increase in the incidence of macroscopic and microscopic changes in the stomach of females only. The macroscopic finding was described as ulceration. The microscopic finding was inflammation (control: 4/50; 800 ppm: 8/50) and erosion of the stomach (control: 3/50; 800 ppm: 7/50). The LOAEL might be established at 800 ppm (144.7 mg/kg/day) based on slight increase in the incidence ulceration and inflammation of the stomach. NOAEL was 200 ppm (35.9 mg/kg/day). Under the condition of the study, there was no increase in treatment-related tumor incidence. However, the test animals could have tolerated higher dose levels.

This study is classified as unacceptable/guideline as a carcinogenicity study in mice because the dose levels employed in this study are insufficient in evaluating the carcinogenic potential of picoxystrobin.

Combined Chronic Toxicity/Carcinogenicity Study in Rats [MRID 48073746 (1999)]

In a combined chronic /carcinogenicity study (MRID 48073746) ZA1963 (picoxystrobin) (99.3% purity) was administered to Alpk:AP$_f$ SD rats (64/sex/dose) in the diet at concentrations of 0, 50, 200, or 750 ppm (equivalent to 0, 3.1, 12.2, or 45.6 mg/kg bw/day for males; 0, 3.8, 14.8, or 57.8 mg/kg bw/day for females) for 2 years. 12 rats/sex/dose group were designated for interim sacrifice at 53 weeks.

Under the conditions of this study picoxystrobin produced no treatment-related changes in mortality, clinical signs, hematological parameters, clinical chemistry parameters, urinalyses, organ weights, and non-neoplastic histopathology. The survival rates in the mid and high dose males were better than the concurrent controls. In females, the survival rates in all the treated groups were better than the controls. There was no apparent finding to support a specific reason for this discrepancy. However, picoxystrobin at 750 ppm consistently produced slight decreases (<10%) in body weight and food consumption in males and females. Food efficiency was slightly reduced in 750 ppm males. The decrease in body weights might positively influence the survival in rats at high dose males and females.

There was a slight increase in the incidence of large granular lymphocyte leukemia in 750 ppm males (7/52) relative to the controls (2/52). The increase showed a statistically significant trend.

The individual animal data showed that the first incidence occurred at week 97, and all of the tumors were observed between 97 weeks and 105 weeks. There was no decrease in time to tumor occurrence in treated males relative to the controls. This increase was marginally above the historical background range for males (0/52 - 5/52) while it was within control range for females (0/52 - 11/52). There was no increase in tumor incidence in females, and there were no associated changes in other hematological parameters. Therefore, a slight increase in the incidence of large granular lymphocyte leukemia seen in 750 ppm males only was not considered to be treatment-related. Since the highest dose tested (750 ppm) produced marginal effects in body weight, food consumption, and food utilization, it was not high enough to assess the carcinogenic potential of picoxystrobin.

It should be noted that recently this rat chronic/carcinogenicity study has been repeated at higher concentrations (3500 ppm, highest concentration), and 12-month interim sacrifice data do not show any tumor incidence in treated-animals at any dose group (MRID 48073745). Consistent with the 1999 study, no changes in any of the hematological parameters are found in the 12-month interim results.

Although the body weight decreases and reduced food consumption showed statistically significant difference from controls, these two effects, in this study, appeared to have a positive impact on the survival in rats in high dose groups of both males and females. It is notable that the reduction in body weight is one of the consistent effects seen in other studies with picoxystrobin; however, in this study, this finding is slight and not seems to be adverse in the context of survival rates and the general health of picoxystrobin treated animals. Therefore, the LOAEL for this chronic study cannot be established. The NOAEL is 750 ppm (57.8 mg/kg/day) (HDT).

The study is considered acceptable/guideline for the chronic toxicity part of the combined study, but it is unacceptable for the carcinogenicity part of the study because the highest dose tested is not sufficient to properly assess the carcinogenic potential of the chemical.

Combined Chronic Toxicity/Carcinogenicity Study in Rats [MRID 48073745 interim study (2011)]

In a combined chronic / carcinogenicity study (MRID: 48073745), picoxystrobin (99.3% a.i; batch #: SEP07A013).) was administered to 70 CD® [Crl:CD®(SD)] rats/sex/dose in the diet at the concentrations of 0, 50, 200, 1000, & 3500 ppm (equivalent to 0, 2.6, 10.4, 52.3, & 186.3 mg/kg bw/day for males; 0, 3.2, 13.1, 65.0, & 229.6 mg/kg bw/day for females; respectively) for 2 years. The current report presents the results of test animals treated for 12-months.

At 12-month, 10 rats/sex/dose were sacrificed and examined grossly and microscopically. The data showed no treatment-related effects on mortality, hematology, clinical chemistry, organ weights, gross exanimation and histological changes. No increase in tumor incidence. There appeared to be treatment-related findings in clinical observations characterized by increased incidence of soft feces (i.e., increases in frequency of occurrence/# of animals) at 1000 and 3500

ppm groups (control: 13/8; 1000 ppm: 97/25; 3500 ppm: 257/39). However, It was not associated with any histological or clinical chemistry changes; the adversity if this observation could not be determined. The body weight, body weight gains, food consumption, and food efficiency were consistently decreased at various intervals of examination in 3500 ppm males and females. The decreases also showed statistical significance. Based on the 12-months results on body weights, body weight gains, food consumption, and food efficiency, the LOAEL was 3500 ppm (M/F: 186.3/229.6mg/kg/day). The NOAEL was 1000 ppm (M/F: 52.3/65.0 mg/kg/day).

Mouse Carcinogenicity Study [MRID 48457401 (2011)]

In a carcinogenicity study (MRID 48457501) picoxystrobin (DPX-YT669) Technical (Picoxystrobin) (99.3% purity, batch/lot # SEP07AS013) was administered to five groups of young adult male and female Crlj:CD1 (ICR) mice (60/sex/group) in diet at concentrations of 0, 100, 600, 2400, or 4800 ppm (males: 0, 12, 71, 293, and 583 mg/kg/day; females: 0, 16, 99, 412, and 799 mg/kg/day) for approximately 18 months. Body weights and food consumption were evaluated weekly for the first 13 weeks, then every other week thereafter. Detailed clinical observations were evaluated weekly. Ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. White blood cell differential counts were evaluated in surviving mice at the end of the exposure period and in mice that were sacrificed in extremis. After approximately 18 months of dietary exposure, mice were sacrificed and gross and microscopic pathological examinations were performed

Under the conditions of this study, picoxystrobin did not produce compound-related changes in clinical observation, body weights, food consumption, ophthalmology, white blood cell differential counts, mortality, or gross pathology. At 2400 ppm and above, picoxystrobin produced dose-related increases in the incidence of duodenal mucosal hyperplasia and dilatation of mucosal gland in male mice. Another related effect was an increase in stomach glandular mucosal hyperplasia which did not show a dose-related response, but this effect might be related to the picoxystobin's ability to affect the upper gastrointestinal tract.

At 4800 pm, picoxystrobin produced liver effects characterized by increases in absolute liver weight in male s and females and increased incidence of hepatocellular hypertrophy in females. The liver weight increase and hepatocellular hypertrophy could be considered as adaptive effects. Picoxystrobin also caused an increase in the incidence of hepatocelluar adenomas and carcinomas and combined heptocellular adenomas/carcinomas in 2400 and 4800 ppm males.

The NOAEL was 600 ppm (71 mg/kg/day); LOAEL was 2400 ppm (293 mg/kg/day) based on duodenal mucosal hyperplasia and mucosal gland dilatation in male mice.

Based on the treatment-related effects found in duodenum, the highest concentration, 4800 ppm, tested was sufficiently high for evaluating the carcinogenic potential of picoxystrobin.

Combined chronic/carcinogenicity study in rats [MRID 48457402 (2011)]

In a combined chronic toxicity/carcinogenicity study (MRID 48457402), Picoxystrobin; 99.3% a.i) was administered in the diet to CD® [Crl:CD®(SD)] rats (80/sex/dose) for up to 24 months at concentrations of 0, 50, 200, 1000, or 3500 ppm (0, 2.2, 8.8, 45.3, and 162.1 mg/kg/day for males and 2.8, 11.0, 57.1, and 203.3 mg/kg/day for females). An interim sacrifice (10 rats/sex/group) was conducted on Day 365. A sentinel group consisted of 15 rats/sex was also included in the study for monitoring the health status of the animals, and are not considered part of the chronic toxicity/carcinogenicity study. They did not receive the standard parameter evaluations conducted on study animals, and the only data reported on these animals are those from the serological health screens.

The results showed that there were no adverse clinical or ophthalmological observations attributed to test article exposure. There was an increase in the incidence of soft feces at 1000 and 3500 ppm primarily in males that was considered possibly test article-related, but it might not be adverse in the context of the overall results of this study. Survival in the 3500 ppm male and female groups and in the 1000 ppm female group was significantly greater than in the controls, This increase is likely due to lower body weight in these groups (not statistically significant in females at 1000 ppm).

Mean body weight and body weight gain were reduced during the study in both sexes at 3500 ppm. In males and females, mean body weight was 9% and 17% below control, respectively, at Week 49, and 11% and 17% below control, respectively, on the last weigh day before final sacrifice. Mean body weight gain in this group was 15% and 33% below control for males and females, respectively, over weeks 1 to 49, and 18% and 28% below control, respectively, over the two year exposure period. All of these differences were statistically significant except the male final body weight and overall body weight gain. However, these values were significantly different from control for most of the study. These body weight findings were associated with significantly lower mean food consumption and food efficiency over the first year at this exposure level which continued for the duration of the study (variable statistical significance). Body weight and nutritional parameters in lower concentration groups were generally comparable to control over the study.

No test article-related effects were noted on any clinical pathology parameters, organ weights, macroscopic findings, or incidence of masses. There were no treatment-related microscopic findings following 1 year of treatment. Towards the end of the study (2 years), statistically significant increases in the incidences of interstitial cell hyperplasia and adenoma in the testes were observed in male rats at 3500 ppm. The majority of the incidence of adenomas and hyperplasia was found in the terminal or near terminal animals, and the percent incidence of adenomas fell marginally within the historical limits of the laboratory. It was likely that the increases in testicular interstitial cell adenoma and hyperplasia in the 3500 ppm males were due to exposure to the test article.

Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was 1000 ppm, equivalent to 45.3 and 57.1 mg/kg/day picoxystrobin in males and females, respectively. The LOAEL is 3500 ppm (equivalent to 162.1 and 203.3 mg/kg/day for males and females, respectively) based on reduced body weight and food consumption parameters observed in both sexes and increased incidence of interstitial cell hyperplasia in male rat.
At the comparable dose levels, the results of this study are consistent with those of the 1999 study (MRID 48073746) (i.e., no adverse effect was found at or below the concentration level of 1000 ppm). The highest tested dose (750 ppm) in the 1999 study was considered to be insufficient for evaluating the carcinogenic potential of picoxystrobin.

5. Mode of Action Studies

There are no mode of action studies available. However, the registrant submitted a position paper, *Picoxystrobin: Justification for the Use of Margin of Exposure Approach for Human Risk Assessment* (MRID 48546901), which mainly provides the rationale for the proposed method of performing the human health risk assessment. In this position paper, the registrant cited some of the published information and attempted to draw a connection between the picoxystrobin's fungicidal mode of action (inhibition of mitochondrial respiration by blocking electron transport through cytochrome b-c1 of mitochondrial complex III, which is necessary for the production of ATP) and the increase in the incidence of testicular interstitial cell tumor. The relevant part of the position paper is reproduced in the following paragraphs.

Mitochondria function is a critical point of control for the synthesis and secretion of steroid hormones by the Leydig cell (Allen 2006). The rate limiting regulatory role played by mitochondria is in the transport of cholesterol to the inner mitochondrial membrane, a process that is dependent on the actions of steroidogenic acute regulatory protein (StAR) and the peripheral-type benzodiazepine receptor in mitochondria (Midzak 2007). The biochemical mechanism responsible for the fungicidal activity of picoxystrobin is identical to the biochemical mechanism by which other complex III inhibitors (e..g., myxothiazol, a natural strobiliurin fungicide produced by the bacterium Myxococcus fluvus) inhibit LH-stimulated testosterone production by Leydig cells.

Inhibition of complex III via binding of myxothiazol to the Q_o site of cytochrome b in the inner mitochondrial membrane inhibits LH-stimulated testosterone production by isolated Leydig cells from Brown Norway rats and in MA-10 human Leydig cell cultures (Midzak 2007; Allen 2006). LH-stimulated testosterone production by the Leydig cell requires energized, polarized and actively respiring mitochondria (Allen 2006). Inhibition of peak levels of LH-stimulated testosterone production lead to a compensatory increase in gonadotropin-releasing hormone (GnRH) and LH via homeostatic feedback loops through the HPG axis. Myxothiazol, as well as two other strobilurin fungicides azoxystrobin and kresoxim methyl, have been shown to induce modest increases in basal testosterone levels in cultured Leydig cells (Midzak 2007). It is presumed that a similar increase in basal testosterone production was induced by picoxystrobin and was sufficient to maintain sex accessory organ weights and fertility as no compound-related effects on reproductive endpoints were observed in the two reproduction studies conducted with this product. Since increased LH levels are

known to produce Leydig cell hyperplasia and adenomas in rats if sustained (Bar 1992; Ewing 1992; Neumann 1991; Prentice 1995; Walsh 1977), this mechanistic information provides strong support for a hormonally-mediated mechanism of hyperplasia and adenoma formation.

It is known that homeostatic mechanisms deteriorate in aging animals (Lin 1980). This deterioration leads to the loss of the ability to overcome the inhibition of testosterone biosynthesis without an increase in Leydig cell mass, which some authors have coined "decompensation" (Fort 1995). The resulting decompensation would result in Leydig cell hyperplasia in aging animals and eventually progress to Leydig cell adenomas as observed in the rat 2-year carcinogenicity study with picoxystrobin. This pattern of hormonal changes (inhibition-compensation-decompensation) has also been seen with two other inhibitors of testosterone biosynthesis, isradipine (Roberts 1989) and a triazole herbicide (Foster 1992). The fact that rat Leydig cell hyperplasia and adenomas were not observed at the one year interim sacrifice, but only following a nearly lifetime of chronic daily exposure to picoxystrobin, also supports a hormonally-mediated mechanism.

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

The Committee considered the following for a weight-of-evidence determination of the carcinogenic potential of picoxystrobin:

1. Carcinogenicity

Rat

• *Testicular Tumors:* Administration of picoxystrobin resulted in the induction of testicular interstitial cell tumors in male CD® [Crl:CD®(SD)] rats. The testicular interstitial cell tumors in male rats exhibited a statistically significant trend ($p<0.01$) and a significant pair-wise comparison ($p<0.05$) of the high dose group (3500 ppm; 162.1 mg/kg/day) with the controls. When compared to historical control data from the testing laboratory, the concurrent control incidence is within the adjusted historical control range (0 - 8.3%). Although the concurrent control is considered the most relevant with which to compare the tumor incidences of the dosed groups, it is also noted that the tumor incidence (7/70, 10%) at the high dose was above the laboratory historical control range (0 - 8.3%) as well as the Charles River Laboratory historical control mean (2.4%) and range (1.1-9.3%) for CD® [Crl:CD®(SD)] rats. Additionally, the testicular tumors are also supported by pre-neoplastic lesions (interstitial cell hyperplasia) at the high dose. **Consequently, the CARC considered the testicular tumors in male CD® [Crl:CD®(SD)] rats to be treatment-related.**

• *Adequacy of Dosing*: The highest dose tested (3500 ppm) was considered to be adequate, and not excessive, to assess the carcinogenic potential of picoxystrobin in both sexes. At the high dose (3500 ppm), both males and females demonstrated body weight reduction (males: 11 %, female: 17 % at the end of the study), while survival rates were significantly increased ($p<0.05$) relative to the controls (control: 25%, 3500 ppm: 49%). It also produced increased incidence of testicular cell interstitial cell hyperplasia.

Mouse

• *Liver Tumors*: Male mice had a statistically significant trend ($p<0.05$) only for liver adenomas. When compared to the controls, none of the increases in liver tumors (adenomas, carcinomas, or combined adenomas/carcinomas) at any dose level showed pair-wise significance. Additionally, there was no dose-response for the incidence of this common tumor in male mice. Except for the presence of mixed foci, there were no corroborative pre-neoplastic lesions. No increase in liver tumor incidence was seen in female mice which received a higher dose (799 mg/kg/day) than male mice (583 mg/kg/day). **Therefore, the CARC determined that the liver tumors in male Crlj:CD1(ICR) mice were NOT treatment-related.**

• *Adequacy of Dosing*: The high dose of 4800 ppm was considered to be adequate, and not excessive, to assess carcinogenic potential in both male and female mice. This was based on treatment- related increase in the incidence of duodenual mucosal hyperplasia in 2400 ppm or above in males and in 4800 ppm in females. In addition, stomach glandular mucosal hyperplasia was also found in 2400 and 4800 ppm males.

2. Mutagenicity

There is no concern for mutagenicity.

3. Structure-Activity Relationship

The SAR data are of limited support for picoxystrobin.

4. Mode of Action

There are no mode of action studies available.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified picoxystrobin as **"Suggestive Evidence of Carcinogenic Potential"** based on tumors observed in one species (rat) and one sex (males): a treatment-related increase in testicular interstitial cell benign tumors in the high dose only. No tumors were seen in female rats. The highest dose tested was considered to be adequate, but not excessive, to assess carcinogenicity in rats. No treatment-related increase in tumor incidence was seen in male or female mice at doses that were considered to be adequate for the assessment of carcinogenicity of picoxystrobin. There is no mutagenic concern.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The evidence from animal data is suggestive of carcinogenicity which raises a concern for carcinogenic effects but is judged not sufficient for quantification of cancer risk in humans. Also, when there is suggestive evidence, the Agency does not attempt a linear dose-response assessment as the nature of the data generally would not support one. Therefore, the Agency has determined that quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to picoxystrobin, The NOAEL (4.6 mg/kg/day) used for establishing the Chronic RfD is approximately 30-fold lower than the dose (162 mg/kg/day) that induced testicular tumors in male rats.

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