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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Date: September 13, 2001

MEMORANDUM

SUBJECT: ***PYRACLOSTROBIN*** - Report of the Hazard Identification Assessment Review Committee.

FROM: Ghazi Dannan Ghazi A. Dannan
Registration Action Branch 3
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair 9/17/01
and
Elizabeth Doyle, Co-Chair E A. Doyle
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: William Wassell, Risk Assessor
Registration Action Branch 3
Health Effects Division (7509C)

PC Code: 099100

On July 31, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Pyraclostrobin with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Pyraclostrobin was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. This meeting was held with the participation of representatives from Canada's PMRA and California's CDPR via teleconference call. The conclusions drawn at the meeting are presented in this report.

Committee Members in Attendance

Members present were: Ayaad Assaad, William Burnam, Pamela Hurley, Elizabeth Mendez, David Nixon, Jess Rowland, and Yung Yang

Member(s) in absentia were: Jonathan Chen, Elizabeth Doyle, Brenda Tarplee

Also in attendance were: Leung Cheng, William Wassell, Kathleen Raffaele, Clark Swentzel, all from RAB3/HED, and John Bazuin from RD

Data evaluation prepared by: Ghazi Dannan of the Registration Action Branch 3

Data Evaluation / Report Presentation

Ghazi A. Dannan

Ghazi A. Dannan, Ph.D.
Toxicologist

1. INTRODUCTION

On July 31, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Pyraclostrobin with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Pyraclostrobin was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996.

2. HAZARD IDENTIFICATION

2.1 Acute Reference Dose (RfD)

a) Sub Population: Females 13-50

Study Selected: Rabbit Prenatal Developmental Toxicity §870.3700

MRID No.: 45118326/45437001

Executive Summary: In a prenatal developmental toxicity study (Teratology) (MRID# 45118326), sexually mature, virgin Chbb:HM (outbred strain) Himalayan rabbits (Supplier: BOEHRINGER INGELHEIM PHARMA KG received either 0, 5, 10, or 20 mg/kg/day BAS 500 F (Purity: 98.9%; Batch No.: CP028719) in 0.5% Tylose CB 30.000 (in doubly distilled water) by oral gavage from days 7 through 28 p.i., inclusive. A check was made twice daily on working days or once daily (Saturday, Sunday or on public holidays) (days 0 - 29 p.i.). The maternal animals were examined for clinical symptoms with all animals weighed on days 0, 2, 4, 7, 9, 11, 14, 16, 19, 21, 23, 25, 28 and 29 p.i. along with consumption of food determined daily during the entire study period. On day 29 p.i., the surviving dams were sacrificed and the fetuses were removed from the uterus, the dams were then necropsied and assessed by gross pathology, the uterus and the ovaries were removed and weighed with the number of corpora lutea, the number and distribution of implantation sites recorded. The fetuses were examined for external, visceral and skeletal anomalies.

No treatment related mortality was noted. Reduced fecal output was seen in 1 mid dose (day 10 p.i.) and 10 high-dose animals (days 10-14 p.i.). Two mid-dose and 4 high-dose animals showed blood in the bedding (between days 16-29 p.i.). No other relevant clinical observations were noted. All treated groups had lower body weight gains during the dosing period (days 7-28) and the overall gestation period (day 0-29) while the mid and high dose groups had lower body weight gains during the post dosing period (days 28-29). The decreased body weight gain, among all treated groups, can mainly be attributed to the earliest post-treatment period, namely gestation days 7-9 (treatment days 0-2). As seen with the body weights and body weight gains, all treated groups had reduced food consumption during the treatment period (days 7-28), and the overall gestation period (days 0-29). Food efficiency was lower in all treated groups during the same periods as food consumption and during the post dosing period (days 28-29). No treatment related

pathological observations were noted in the data provided. There was reduced litter size, increased resorptions per dam and increased post implantation loss in the high dose group. Maternal toxicity was further evaluated in another study (MRID 45437001, review in Appendix I) and no significant maternal toxicity was observed a 1, 3, or 5 mg/kg/day. Therefore when these two studies are used together, they support a Maternal Toxicity NOAEL of 5 mg/kg/day. **The maternal toxicity NOAEL was 5 mg/kg/day and the maternal toxicity LOAEL was 10 mg/kg/day based on body weight gains, reduced food consumption and reduced food efficiency.**

There was increased resorptions per dam, increased post implantation loss and a dose related increase in dams with total resorptions in the mid and high dose groups. There was also an increased incidence of the anomaly: lumbar vertebrae absent in the high dose group as well as reduced litter size. **The developmental toxicity NOAEL was 5 mg/kg/day and the developmental toxicity LOAEL was 10 mg/kg/day based on increased resorptions per litter, increased post-implantation loss and dams with total resorptions.**

This study is classified as Acceptable-Guideline and satisfies the guideline requirements (§ 83-3a) for a Prenatal Developmental Toxicity Study (Teratology) in rabbits.

Dose and Endpoint for Establishing RfD: Developmental Toxicity NOAEL of 5.0 mg/kg/day based on increased resorptions/litter and increased total resorptions (i.e., dams with complete litter loss).

Uncertainty Factor (UF): 100

Comments about Study/Endpoint/Uncertainty Factor: The developmental effects are presumed to occur following a single exposure of females of child-bearing age and, therefore, are appropriate for this risk assessment.

Acute RfD (Females 13-50) =	$\frac{5.0 \text{ mg/kg (NOAEL)}}{100 \text{ (UF)}}$ =	**0.05 mg/kg**

b) **General population including infants and children:**

Study Selected: Rat Acute Oral Neurotoxicity §870.6100

MRID No.: 45118337

Executive Summary: In an acute neurotoxicity study (MRID # 45118337), groups of 10 Chbb:THOM (SPF) Wistar rats/sex were given a single oral dose of BAS 500 F (purity 99.0%) in a 0.5% aqueous solution of carboxymethylcellulose at dose levels of 0, 100, 300, and 1000 mg/kg bw and observed for 14 days. Neurobehavioral assessments were performed on all animals at ~4 to 6 hours, 7 days and 14 days post-dosing. At study

termination, 5 animals/sex/group were euthanized, perfused in situ and subjected to histopathological evaluation of central and peripheral nervous system tissues. The Systemic Toxicity LOAEL for males was 1000 mg/kg bw based on 33% decreased body weight gain ($p \leq 0.01$) on days 0-7; no similar effect was detected on days 0-14. The Systemic Toxicity NOAEL was 300 mg/kg bw. The Systemic Toxicity LOAEL for females could not be determined since there were no adverse, treatment-related effects noted at any dose level tested. The Systemic Toxicity NOAEL for females was 1000 mg/kg bw. The Neurotoxicity LOAEL could not be determined since there were no treatment-related neurotoxic effects noted at any dose level tested. The Neurotoxicity NOAEL was 1000 mg/kg bw.

This acute neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study [OPPTS 870.6100(§81-7)] in rats. Also note that the highest dose tested was 1000 mg/kg and not 2000 mg/kg as suggested by the guidelines. However, this deficiency is not considered serious enough to render the study unacceptable because there were no changes in any of the neurotoxicity indices up to the highest tested dose.

Dose and Endpoint for Establishing RfD: The systemic toxicity NOAEL of 300 mg/kg based on decreased body weight gain in males at 1000 mg/kg.

Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor: The decreased body weight gain was seen after a single oral dose during the first week after exposure and therefore is appropriate for the exposure scenario.

Acute RfD (General Population) = $\frac{\text{300 mg/kg (NOAEL)}}{\text{100 (UF)}}$ = **3.0 mg/kg**

2.2 Chronic Reference Dose (RfD)

Study Selected: Rat Oral Carcinogenicity Guideline #: 870.4200

MRID No.: 45118331

Executive Summary: In a rat carcinogenicity study (MRID 45118331), pyraclostrobin (97.09% a.i., Lot/Batch # J.-Nr. 27882/191/c) was administered in the diet to Wistar rats (50/sex/group) for up to 104 weeks at nominal doses of 0, 25, 75, or 200 ppm, equivalent to 0/0, 1.2/1.5, 3.4/4.7, and 9.2/12.6 mg/kg/day [M/F], respectively.

Mortality, clinical signs, food efficiency, and hematology findings for both sexes at all doses were unaffected by treatment. No treatment-related findings were observed in the 25 or 75 ppm dose groups.

In the 200 ppm group, decreased ($p \leq 0.05$ or 0.01) body weights were observed in the males (↓3-7%) at weeks 1 through 81 and in the females (↓4-14%) at weeks 21 through 104; body weights in the males at 104 weeks was decreased (↓4%; not statistically significant [NS]). Decreased ($p \leq 0.05$ or 0.01) body weight gains were observed in the males (↓6-10%) at weeks 1 through 81 and in the females (↓7-22%) at weeks 21 through 104; cumulative body weight gain in the males at week 104 was decreased (↓5%; NS). Decreased ($p \leq 0.05$ or 0.01) food consumption was observed in the males (↓3-7%) sporadically during weeks 1 to 13. Increased ($p \leq 0.05$) relative kidney weights were observed in the males (↑9%) and females (↑19%). In addition, increased incidences of kidney tubular casts in the males (15/50 treated vs 5/50) and females (14/50 treated vs 10/50 controls) and kidney tubular atrophy in the males (16/50 treated vs 5/50 controls) and females (19/50 treated vs 12/50 controls) were observed. In the males, an increased incidence of necrosis of the liver was observed microscopically (10/50 treated vs 1/50 control). Additionally, erosion/ulcer of the glandular stomach was observed grossly (12/50 treated vs 7/50 controls) in the males. Microscopically, an increased incidence of acanthosis (6/50 treated vs 0/50 controls) and ulcers (4/50 treated vs 2/50 controls) of the forestomach and ulcers (7/50 treated vs 2/50 controls) and erosion of the glandular stomach (10/50 treated vs 2/50 controls) were also observed in the males.

The LOAEL is 200 ppm for males and females (equivalent to 9.2/12.6 mg/kg/day [M/F]) based on differences in body weight and body weight gains, increased incidences of kidney tubular casts and atrophy in males and females, and in males, an increased incidence of necrosis of the liver, gross and microscopic evidence of erosion/ulcer of the glandular stomach and an increased incidence of acanthosis and ulcers of the forestomach. The NOAEL is 75 ppm (equivalent to 3.4/4.7 mg/kg/day [M/F]).

Histiocytic sarcoma and lymphoma of the hemolymphoreticular system was observed in males at 25 (2/9 vs 1/50 controls), 75 (2/10 vs 1/50 controls), and 200 (6/50 [12%] vs 1/50 [2%]) ppm. Historical data were submitted on 6/14/2001 (MRID 45429902). The incidence of hemolymphoreticular tumors in male wistar rats from 4/95- 7/00 at the testing laboratory is 4% (mean) and 0-10% (range). There was also an increased incidence of mammary gland adenocarcinoma in the females at 200 ppm (16% vs 4% controls). Historical data showed an incidence of female mammary gland adenocarcinoma in wistar rats from 4/95- 7/00 to be 6% (mean) and 0-14% (range). Testicular leydig cell tumor were observed in all male groups but were slightly higher in each of the three treated groups (48-50%) than the control group (38%). Historical control data for this tumor were submitted separately (MRID 45443001). The incidence of benign testicular leydig cell tumors in male wistar rats from 4/95- 7/00 at the testing laboratory is 44% (mean) with a range of 30-60%. Neoplasms were not analyzed for statistical significance.

Under the conditions of this study, there is evidence that pyraclostrobin may be carcinogenic.

The submitted study is classified as **Acceptable/guideline(§83-2[a])** and satisfies the requirements for a carcinogenicity study in rats.

Dose and Endpoint for Establishing RfD: The NOAEL of 3.4 mg/kg/day (75 ppm) based on decreased body weight and body weight gains, increased incidences of kidney tubular casts and atrophy in males and females, and in males, an increased incidence of liver necrosis, gross and microscopic evidence of erosion/ulcer of the glandular stomach and an increased incidence of acanthosis (hyperplasia) and ulcers of the forestomach at 9.2 mg/kg/day (200 ppm).

Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor: The lowest NOAEL in the most sensitive species following chronic exposure.

Chronic RfD = $\frac{\text{3.4 mg/kg/day (NOAEL)}}{\text{100 (UF)}}$ = **0.034 mg/kg/day**

2.3 Occupational/Residential Exposure

2.3.1 Short-Term (1-30 days) Incidental Oral Exposure

Study Selected: 13-Week Feeding Dog Study
§870.3150

MRID No.: 45118323

Executive Summary: In a subchronic toxicity study (MRID #45118323), BAS 500 F, purity 97.09%, was administered to 5 beagles/sex/dose in the diet at dose levels of 0, 100, 200 and 450 ppm (equal to 0, 2.8, 5.8 and 12.9 mg/kg bw/day for males, and 0, 3.0, 6.2 and 13.6 mg/kg bw/day for females) for a 90-day period.

No treatment-related deaths were observed at any dose level. All animals in the 450 ppm group vomited for the first 1 to 3 weeks, which was considered to be a transient aversion to the test material. In addition, all animals in the high dose group had diarrhea throughout the study period. A slight increase in the incidence of diarrhea noted in the 200 ppm group was not considered to be toxicologically significant because of its scattered/ isolated occurrence. Decreased food intake (-9%), a net loss in body weight (-12%) and decreased food efficiency (-1.05% vs 5.26% in controls) were seen in the 450 ppm group, females only. Clinical chemistry findings included a slight decrease at 450 ppm in total protein, albumin, globulin (all three parameters in both sexes ranged from -7% to -12%; only total protein was significantly different from controls) and in glucose on days 41/43 and 90 among the 450 ppm females (-9.4 % to -13%, $p \leq 0.01$). These findings are considered to reflect a trend towards lower values indicative of a marginal treatment-related effect. Gross and histopathological examination revealed that the

duodenum is a target organ in both sexes at 450 ppm as evidenced by thickening of the duodenal wall (each sex: 2/5 vs 0/5 controls) and mucosal hypertrophy (males: 2/5 vs 0/5 controls and females: 1/5 vs 0/5 controls).

The LOAEL is 450 ppm (equal to 12.9 mg/kg bw/day for males and 13.6 mg/kg bw/day for females), based on an increased incidence of diarrhea, clinical chemistry changes and mucosal hypertrophy of the duodenum (both sexes) and body weight loss, decreased food intake and decreased food efficiency (females only). The NOAEL is 200 ppm (equal to 5.8 mg/kg bw/day for males and 6.2 mg/kg bw/day for females).

This subchronic toxicity study in the dog is acceptable and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3150; OECD 409) in dogs.

Dose and Endpoint for Risk Assessment: The NOAEL of 6 mg/kg/day based on increased incidence of diarrhea, clinical chemistry changes, duodenum mucosal hypertrophy, and effects on body weight and food intake/efficiency at 13 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The NOAEL/LOAEL in this study are comparable to the maternal NOAEL/LOAEL (5/10 mg/kg/day) established in the rabbit developmental toxicity study, and the end-point is appropriate for the population of concern (toddlers).

2.3.2 Intermediate-Term (1 - 6 Months) Incidental Oral Exposure

Study Selected: 13-Week Feeding Dog Study §870.3150

MRID No.: 45118323

Executive Summary: under above item 2.3.1

Dose and Endpoint for Risk Assessment: The NOAEL of 6 mg/kg/day based on increased incidence of diarrhea, clinical chemistry changes, duodenum mucosal hypertrophy, and effects on body weight and food intake/efficiency at 13 mg/kg/day.

Comments about Study/Endpoint: The NOAEL/LOAEL in this study are lower than the NOAEL/LOAEL in the 13-week feeding toxicity studies in the rat (11.7/37.8 mg/kg/day; MRID 45118321) and mouse (11.1/35.4 mg/kg/day; MRID 45118320), and the end-point seen after 13 weeks is appropriate for the exposure scenario and the population of concern (toddlers).

For the short- and intermediate-term incidental oral exposures, the HIARC noted that the 13-week and 1-year feeding dog studies had similar or comparable NOAEL/LOAEL and that the effects at 13 weeks did not seem to progress in severity after the longer 1-year exposure period and, therefore, appropriate to use

for the short-term scenario. Therefore, the HIARC recommended using the 13-week feeding dog study for both the short- and intermediate-term incidental oral exposure scenarios. It should be noted, however, that the observed duodenal thickening (2/5/sex) and mucosal hypertrophy (2/5 males and 1/5 female) in the high dose (450 ppm) group at 13-weeks but not at 1-year does not necessarily mean that effects on the duodenum are reversible over time. In the 1-year dog study, the highest dose was 400 ppm (M/F: 10.8/11.2 mg/kg/day), nearly 21% lower (on mg/kg/day basis) than the highest dose of 450 ppm (M/F: 12.9/13.6 mg/kg/day) in the 13-week dog study. Other studies in rats and mice indicate that a threshold dose is needed for the duodenum to be a target organ (for additional detail, refer to section 6 below).

2.3.3 Dermal Absorption

Dermal Absorption Factor: The available dermal absorption study in rats (MRID 45118402) is classified as **unacceptable/guideline [§85-3]** and **cannot** be upgraded because most of the test material was retained on the dressings and was unavailable for absorption; therefore, the actual dose cannot be determined. However, the HIARC discussed the Canada-PMRA suggestion to calculate the dermal absorption rate based on the percentage of the available dose rather than the total administered dose. Using this approach, the dermal absorption rate is calculated at nearly 50%. (At 8 hours after initiating the dermal exposure to a nominal dose of 0.375 mg/cm^2, 76.4% was retained on the dressing and the remaining 23.6% was available for absorption. Of the administered dose, 11.97% was in the skin at the test site after washing, and 0.5% was in the carcass and excreta. The % absorption = % of dose available at skin site (11.97%) + % of dose in carcass/excreta (0.5%) ÷ % of dose available for absorption (23.6%). Therefore, % absorption = 12.47% ÷ 23.6% = 52.8%.) The HIARC agreed with this approach and concluded that the dermal absorption rate of 50% is sufficiently conservative and is the best available estimate.

2.3.4 Short-Term Dermal (1-30 days) Exposure

Study Selected: Rabbit Prenatal Developmental Toxicity §870.3700

MRID No.: 45118326

Executive Summary: under above item 2.1a

Dose/Endpoint for Risk Assessment: Developmental Toxicity NOAEL of 5.0 mg/kg/day based on increased resorption/litter and increased total resorptions (i.e., dams with complete litter loss) at 10 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The HIARC noted that the 28-day dermal toxicity study in rats (MRID 45118324), showed no effects at the highest tested dose of 250 mg/kg/day, which is well below the limit-dose of 1000 mg/kg/day. Therefore, it is not known if there are systemic effects at higher doses via the

dermal route. The HIARC selected an oral study because of the concern for the developmental toxicity seen in the rabbits which are not assessed in the dermal study. Furthermore, the NOAEL/LOAEL in the selected study are supported by the NOAEL/LOAEL of the 13-week dog (6/13 mg/kg/day) and 1-year dog (5.4/10.8 mg/kg/day) studies. The dermal absorption factor of 50% should be applied to extrapolate from the oral route to the dermal route.

2.3.5 Intermediate-Term Dermal (1 - 6 Months) Exposure

Study Selected: Rabbit Prenatal Developmental Toxicity §870.3700

MRID No.: 45118326

Executive Summary: under above item 2.1a

Dose/Endpoint for Risk Assessment: Developmental Toxicity NOAEL of 5.0 mg/kg/day based on increased resorptions/litter and increased total resorptions (i.e., dams with complete litter loss) at 10 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The HIARC noted that the 28-day dermal toxicity study in rats (MRID 45118324), showed no effects at the highest tested dose of 250 mg/kg/day, which is well below the limit-dose of 1000 mg/kg/day. Therefore, it is not known if there are systemic effects at higher doses via the dermal route. The HIARC selected an oral study because of the concern for the developmental toxicity seen in the rabbits which are not assessed in the dermal study. Furthermore, the NOAEL/LOAEL in the selected study are supported by the NOAEL/LOAEL of the 13-week dog (6/13 mg/kg/day) and 1-year dog (5.4/10.8 mg/kg/day) studies. The dermal absorption factor of 50% should be applied to extrapolate from the oral route to the dermal route.

2.3.6 Long-Term Dermal (Several Months to Life-Time) Exposure

Study Selected: Rat Oral Carcinogenicity §870.4200

MRID No.: 45118331

Executive Summary: under above item 2.2

Dose and Endpoint for Risk Assessment: The NOAEL of 3.4 mg/kg/day based on decreased body weight and body weight gains, increased incidences of kidney tubular casts and atrophy in males and females, and in males, an increased incidence of liver necrosis, gross and microscopic evidence of erosion/ulcer of the glandular stomach and an increased incidence of acanthosis (hyperplasia) and ulcers of the forestomach at 9.2 mg/kg/day.

Comments about Study/Endpoint: This study/dose/endpoint was also used for deriving the chronic RfD. The dermal absorption factor of 50% should be applied

Comments about Study/Endpoint: This study/dose/endpoint was also used for deriving the chronic RfD. The dermal absorption factor of 50% should be applied to extrapolate from the oral route to the dermal route.

2.3.7 Inhalation Exposure

a) Short- and Intermediate-Terms

Study Selected: Rabbit Prenatal Developmental Toxicity §870.3700

MRID No.: 45118326/45437001

Executive Summary: under above item 2.1a

Dose/Endpoint for Risk Assessment: Developmental Toxicity NOAEL of 5.0 mg/kg/day based on increased resorptions/litter and increased total resorptions (i.e., dams with complete litter loss).

Comments about Study/Endpoint: Pyraclostrobin is considered Toxicity Category II ($0.31<LC_{50}<1.07$ mg/L) based on an acute rat inhalation toxicity study of a four hour nose-only exposure to an aerosol of the chemical that was diluted (1:2, w/w) with acetone (MRID 45118308). However, there is no inhalation toxicity study available for this risk assessment. **The HIARC recommended the submission of a 28-day inhalation toxicity study using the same form of Pyraclostrobin to which workers are exposed; the a.i. should not be diluted in acetone as done in the acute inhalation study.** The HIARC also recommended using the same studies/end-points that are outlined above for the short- and intermediate-term dermal exposure scenarios for the short- and intermediate-term inhalation exposures using route-to-route extrapolation and a 100% absorption rate (default value).

b) Long-Term

Study Selected: Rat Oral Carcinogenicity §870.4200

MRID No.:45118331

Executive Summary: under above item 2.2

Dose and Endpoint for Risk Assessment: The NOAEL of 3.4 mg/kg/day (75 ppm) based on decreased body weight and body weight gains, increased incidences of kidney tubular casts and atrophy in males and females, and in males, an increased incidence of liver necrosis, gross and microscopic evidence of erosion/ulcer of the glandular stomach and an increased incidence of acanthosis (hyperplasia) and ulcers of the forestomach at 9.2 mg/kg/day (200 ppm).

Comments about Study/Endpoint: The HIARC recommended using the same study/end-point pertaining to the long-term dermal exposure scenario using route-to-route extrapolation and a 100% absorption rate (default value).

Since oral NOAELs were selected, the following steps should be used for route-to route extrapolation:

Step I. Convert the inhalation exposure component (i.e., μg a.i./day) using a 100% absorption rate (default value) and an application rate to an **equivalent oral dose** (mg/kg/day).

Step II. Convert the dermal exposure component (i.e. mg/kg/day) using a 50% dermal absorption rate and an application rate to an **equivalent oral dose** (mg/kg/day).

Step III. Combine the oral equivalent doses (steps I and II) to obtain a total dose for each of the exposure duration scenarios. For the Short- and Intermediate-term exposure scenarios, compare the combined oral equivalent dose to the oral developmental NOAEL of 5.0 mg/kg/day to calculate the MOE. For the Long-term exposure scenario, compare the combined oral equivalent dose to the oral NOAEL of 3.4 mg/kg/day from the rat carcinogenicity study to calculate the MOE.

2.3.8 Margins of Exposure for Occupational/Residential Risk Assessments

Margin of exposure of 100 is adequate for occupational dermal and inhalation exposure risk assessments. The acceptable MOEs for residential exposure will be determined by the FQPA SF committee.

2.4 Recommendation for Aggregate Exposure Risk Assessments

A common toxicity endpoint (developmental toxicity effects) was selected for the dermal (oral equivalent) and inhalation (oral equivalent) routes for **short-** and **intermediate-**term exposure durations. Therefore, these routes can be combined for the appropriate population. However, the incidental oral can not be combined with these routes due to different toxicity end-point (systemic toxicity in the dog study).

A common toxicity endpoint (kidney tubular casts/atrophy, increased liver necrosis, erosions/ulcers in the fore- and glandular stomachs, and increased incidences of tumors) was selected for **long-**term exposure by oral, dermal (oral equivalent), and inhalation (oral equivalent) routes. These routes can be aggregated for the appropriate population.

3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

3.1 Two Year Carcinogenicity Feeding Study in Rats

MRID No. 45118331

Executive Summary: under chronic RfD, section 2.2

Discussion of Tumor Data: **Evidence that the test material produced neoplasms in the high dose rats in the study:** The males of the high dose group (200 ppm) had increased incidences of histiocytic sarcoma and lymphoma of the hemolymphoreticular system (6/50 [12%] vs 1/50 [2%]). The incidence of hemolympohreticular tumors in male Wistar rats from 4/95- 7/00 at the testing laboratory is 4% (mean) and 0-10% (range). There was also an increased incidence of mammary gland adenocarcinoma in the females at 200 ppm (16% vs 4% controls). Historical data showed an incidence of female mammary gland adenocarcinoma in Wistar rats from 4/95- 7/00 to be 6% (mean) and 0-14% (range). Testicular leydig cell tumor were observed in all male groups but were slightly higher in each of the three treated groups (48-50%) than the control group (38%). Historical control data were not submitted for this tumor. Neoplasms were not analyzed for statistical significance.

Adequacy of the Dose Levels Tested: The animals in this study were adequately dosed as evidenced by decreased body weight (↓~ 5-10%) and body weight gain (↓ 6-22%), and increased incidences of kidney tubular casts and atrophy in males and females of the high dose group, and increased incidence of necrosis of the liver in addition to gross and microscopic evidence of erosion/ulcer of the glandular and forestomach in the high dose males.

3.2 Carcinogenicity Study in Mice

MRID No. 45118330

Executive Summary: In a mouse oncogenicity study (MRID 45118330), pyraclostrobin (97.09% a.i., Lot/Batch # J.-Nr. 27882/191/c) was administered in the diet to B6C3F1/CrlBR mice (50/sex/group) for up to 80 weeks at nominal doses of 0, 10, 30 or 120 ppm in males and 0, 10, 30, 120, or 180 ppm in females, equivalent to 0/0, 1.4/1.6, 4.1/4.8, 17.2/20.5, and 32.8 (females only) mg/kg/day [M/F], respectively).

Mortality, clinical signs, body weight, body weight gain, food consumption, food efficiency, hematology, organ weights, and gross and microscopic findings for both sexes at all doses were unaffected by treatment.

Among all treated male and female groups, there were statistically significant decreased mean body weights (↓4-13%) and body weight gains (4-28%, excluding week 1). However, the magnitude of these effects was not clearly dose-related despite the fact that, during the study period, these effects were more consistently observed among the high dose animals than among the mid- or low dose mice. Nonetheless, in the analysis of the

body weight and body weight gain data, one should compare the results obtained in the high dose groups (120 ppm-M; 180 ppm-F) with the results obtained in the lowest dose group (10 ppm). Since no adverse effects are expected in the 10 ppm group, the 10 ppm group could serve as a second control group to which comparisons could be made. [It is noted that the 10 ppm group in the mouse oncogenicity group is 5X lower than the NOAEL obtained for mice in the 90-day subchronic study-MRID 45118320.] When the comparisons are made between body weight and body weight gain data obtained for both sexes in the high dose groups and the body weight and body weight gain data obtained for both sexes in the low dose (10 ppm) group, the overall differences become biologically insignificant.

The LOAEL is >120 ppm for males (equivalent to >17.2 mg/kg/day) and >180 ppm for females (equivalent to >32.8 mg/kg/day). The NOAEL is ≥120 ppm for males (equivalent to ≥17.2 mg/kg/day) and ≥180 ppm for females (equivalent to ≥32.8 mg/kg/day).

Under the conditions of this study, there was no evidence of carcinogenic potential.

The submitted study is classified as **Unacceptable/guideline (§83-2[b])** and does not satisfy the requirements for a carcinogenicity study in mice. Dose levels were too low.

Discussion of Tumor Data There were no increased incidence of tumors.

Adequacy of the Dose Levels Tested The dose levels tested were considered to be not adequate to assess the carcinogenic potential of pyraclostrobin. Mortality, clinical signs, body weight, body weight gain, food consumption, food efficiency, hematology, organ weights, and gross and microscopic findings for both sexes at all doses were unaffected by treatment. The study was classified **Unacceptable/guideline (§83-2[b])** and does not satisfy the requirements for a carcinogenicity study in mice.

3.3 Classification of Carcinogenic Potential

Based on the evidence of carcinogenicity in rats, the HIARC recommended that the carcinogenic potential of pyraclostrobin should be evaluated by the Cancer Assessment Review Committee. At present, the CARC meeting is scheduled for October 10, 2001.

4 MUTAGENICITY

Pyraclostrobin is not mutagenic or genotoxic based on a battery of guideline/acceptable tests.

***Salmonella typhimurium* and *Escherichia coli*/mammalian activation gene mutation assay; OPPTS 870.5100 (84-2)**

MRID 45118332: EXECUTIVE SUMMARY (from PMRA Primary Review):

In a reverse gene mutation assay in bacteria (MRID 45118332), strains, TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain *WP2 uvrA* of *E. coli* were exposed to BAS 500F (Batch No. 026063, 98.2% a.i.) dissolved in dimethylsulfoxide (DMSO), at concentrations of 0, 20, 100, 500, 2500 and 5000 μg/plate in the presence and absence of an Aroclor 1254-stimulated rat liver metabolic activation system using the standard plate test. A second assay was conducted at the same concentrations using the preincubation test.

BAS 500F was tested up to precipitating concentrations. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of treatment-induced mutant colonies above background levels.**

This study is classified as acceptable. This study satisfies the requirement of FIFRA Test Guideline 84-2; OECD 471/472 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

***In Vitro* mammalian chromosome aberrations in Chinese hamster V79 cells ; OPPTS 870.5375 (84-2)**

MRID 45118333: EXECUTIVE SUMMARY (from PMRA Primary Review):

In a mammalian cell cytogenetics chromosomal aberration assay (MRID 45118333), V79 cell cultures were exposed to BAS 500 F [Batch No. 026063], purity 98.2%, dissolved in dimethylsulfoxide (DMSO), at concentrations of 0.0, 6.25, 12.5 and 25.0 μg/mL in the presence and absence of an Aroclor 1254-stimulated rat liver metabolic activation system. A second assay was conducted at concentrations of 0, 3.125, 6.25 and 12.5 μg/mL in the presence of metabolic activation, and concentrations of 0, 0.005, 0.010, 0.050 and 0.100 μg/mL in the absence of metabolic activation.

BAS 500 F was tested up to precipitating concentrations. Positive controls induced the appropriate response. There was no evidence of an increase in the number of structural or numerical chromosomal aberrations induced over background. It is therefore concluded that BAS 500 F is not a clastogenic agent under the conditions of this study.

This study is classified as acceptable. This study satisfies the requirement for FIFRA Test Guideline [*In Vitro* mammalian cytogenetics chromosomal aberration assay] OPPTS 870.5375; OECD 473 for cytogenetic mutagenicity data.

***In vivo* mammalian cytogenetics - micronucleus assay in NMRI mice: OPPTS 870.5395 (84-2)**

MRID 45118334: EXECUTIVE SUMMARY (from PMRA primary reviewer):

In a mouse bone marrow micronucleus assay (MRID 45118334), NMRI mice were dosed once by oral gavage with BAS 500 F [Batch No. CP026063], purity 98.2% at doses of 0 (vehicle control) and 300 mg/kg bw, 10 per sex per group and 75 and 150 mg/kg bw, 5

per sex per group. Bone marrow cells were harvested at 24 hours post-treatment, 5 per sex per group, at all dose levels, and at 48 hours post-treatment, 5 per sex per group in the 0 and 300 mg/kg bw groups. The vehicle was olive oil.

One male in the 300 mg/kg bw group died on study day 2. Clinical signs of toxicity included piloerection and squatting posture in all dose group ~ 30 minutes post-dosing, with complete recovery within 24 hours. There was no evidence of target cell cytotoxicity. The positive controls induced the appropriate responses. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any dose level tested, at any time after treatment. It is therefore concluded that BAS 500 F did not induce a clastogenic effect in either sex at any sacrifice time.**

This study is classified as acceptable. This study satisfies the requirement for FIFRA Test Guideline 84-2; OECD 474 for *in vivo* cytogenetic mutagenicity data.

Mammalian cells in culture gene mutation assay in Chinese hamster ovary (CHO) cells; OPPTS 870.5300 (84-2)

MRID 45118335: EXECUTIVE SUMMARY (from PMRA primary review):

In a mammalian cell gene mutation assay (MRID 45118335), Chinese Hamster Ovary (CHO) cells cultured *in vitro* were exposed to BAS 500 F [Batch No. CP026063], purity 98.2% a.i., dissolved in DMSO, at concentrations of 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 μg/mL in the presence and absence of metabolic activation. A second experiment was conducted in the absence of metabolic activation only at concentrations of 3, 4, 5, 6, 7 and 8 μg/mL. A third experiment tested concentrations of 1.25, 2.5, 5.0, 10.0 and 20.0 μg/mL, both in the presence and absence of metabolic activation.

BAS 500 F was tested up to cytotoxic and solubility limit concentrations. The positive controls induced the appropriate response. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

Unscheduled DNA Syntheses in Primary Rat Hepatocytes/Mammalian Cell Cultures; OPPTS 870.5550 (84-2)

MRID 45118336: EXECUTIVE SUMMARY (from PMRA primary review):

In an unscheduled DNA synthesis assay (MRID 45118336), primary rat hepatocyte cultures were exposed to BAS 500 F (Batch No. CP026063), purity 98.2% a.i., dissolved and diluted in DMSO [dimethylsulfoxide] at concentrations of 0.01, 0.03, 0.1, 0.3 and 1.0 μg/mL for 18 hours. A second experiment was conducted at concentrations of 0.004, 0.02, and 0.5 μg/mL.

BAS 500 F was tested up to cytotoxic concentrations. The positive controls induced the appropriate response. **There was no evidence that BAS 500 F induced unscheduled DNA synthesis, as determined by net nuclear silver grain counts.**

This study is classified as acceptable. This study satisfies the requirement for FIFRA Test Guideline 84-2; OECD 482 for other genotoxic mutagenicity data.

5 FQPA CONSIDERATIONS

5.1 Adequacy of the Data Base

The toxicology data base is complete but is inadequate for an FQPA assessment. The two-generation rat reproduction study (MRID 45118327) is unacceptable because no parental systemic toxicity was seen at the highest dose tested indicating that the animals could have tolerated higher doses.

5.2 Neurotoxicity

-- *Acute Neurotoxicity -§81-8* (MRID 45118337)

Executive Summary (based on PMRA primary review and modifications by EPA/HED):

In an acute neurotoxicity study (MRID # 45118337), groups of 10 Chbb:THOM (SPF) Wistar rats/sex were given a single oral dose of BAS 500 F (purity 99.0%) in a 0.5% aqueous solution of carboxymethylcellulose at dose levels of 0, 100, 300, and 1000 mg/kg bw and observed for 14 days. Neurobehavioral assessments were performed on all animals at ~4 to 6 hours, 7 days and 14 days post-dosing. At study termination, 5 animals/sex/group were euthanized, perfused in situ and subjected to histopathological evaluation of central and peripheral nervous system tissues. The Systemic Toxicity LOAEL for males was 1000 mg/kg bw based on 33% decreased body weight gain ($p \leq 0.01$) on days 0-7; no similar effect was detected on days 0-14. The Systemic Toxicity NOAEL was 300 mg/kg bw. The Systemic Toxicity LOAEL for females could not be determined since there were no adverse, treatment-related effects noted at any dose level tested. The Systemic Toxicity NOAEL for females was 1000 mg/kg bw. The Neurotoxicity LOAEL could not be determined since there were no treatment-related neurotoxic effects noted at any dose level tested. The Neurotoxicity NOAEL was 1000 mg/kg bw.

This acute neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study [OPPTS 870.6100(§81-7)] in rats. Also note that the highest dose tested was 1000 mg/kg and not 2000 mg/kg as suggested by the guidelines. However, this deficiency is not considered serious enough to render the study unacceptable because there were no changes in any of the neurotoxicity indices up to the highest tested dose.

-- *Subchronic Neurotoxicity- §82-7* (MRID 45118401)

Executive Summary (based on PMRA primary review and concurrence by EPA/HED):

In an subchronic neurotoxicity study (MRID # 45118401), BAS 500 F purity 97.09%, was administered to 10 Wistar rats/sex/group in the diet at dose levels of 0, 50, 250, and 750 (m) / 1500 (f) ppm (equal to 0, 3.5, 16.9 and 49.9 mg/kg bw/day for males, and 0, 4.0, 20.4, and 111.9 mg/kg bw/day for females) for a 3 month period. Neurobehavioral assessments (functional observation battery and motor activity testing) were performed on all animals at 22, 50, and 85 days post-dosing. At study termination, 5 animals/sex/group were euthanized, perfused in situ and subjected to histopathological evaluation of central and peripheral nervous system tissues. The Systemic Toxicity LOAEL was 750 ppm (equal to 49.9 mg/kg bw/day) for males and 1500 ppm (equal to 111.9 mg/kg bw/day) for females based on decreased body weight gain, food intake and food efficiency (both sexes) and decreased water intake (males only). The Systemic Toxicity NOAEL was 250 ppm (equal to 16.9 mg/kg bw/day for males and 20.4 mg/kg bw/day for females. The Neurotoxicity LOAEL could not be determined since there were no treatment-related neurotoxic effects noted at any dose level tested. The Neurotoxicity NOAEL was 750 ppm mg/kg bw (equal to 49.9 mg/kg bw/day) for males and 1500 ppm (equal to 111.9 mg/kg bw/day) for females.

This subchronic neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic neurotoxicity study [OPPTS 870.6200 (§82-7)] in rats.

5.3 Developmental Toxicity

-- *Developmental Toxicity - Rat- §83-3a* (MRID 45118325)

Executive Summary: In a prenatal developmental toxicity study (Teratology) (MRID# 45118325), sexually mature, virgin Chbb:THOM (SPF) Wistar rats (supplier: BOEHRINGER INGELHEIM PHARMA KG received either 0, 10, 25, or 50 mg/kg/day BAS 500 F (Purity: 98.9%; Batch No.: CP028719) in 0.5% Tylose CB 30.000 (in doubly distilled water) by oral gavage from gestation days 6 through 19, inclusive. The animals were examined for clinical symptoms, weighed on days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20 p.c. Also with the exception of day 0, the consumption of food was determined on the same days as was body weight. The animals were sacrificed on day 20 p.c., were necropsied and assessed by gross pathology, the uterus and the ovaries were removed and weighed with the number of corpora lutea, the number and distribution of implantation sites were recorded. The fetuses were weighed and examined for external, visceral and skeletal anomalies.

No deaths, clinical signs of toxicity or gross pathological observations were noted in the maternal animals. The 25 and 50 mg/kg/day dose groups had lower overall body weights at gestation days 19/20 and gained less weight than the control during the dosing period (gestation days 6-19), for the post dosing period (19-20), for the overall gestation period

(0-20) and for the calculated period of gestation days 6-20, also for corrected body weight gains from gestation days 6-20. As seen with the body weights and body weight gains, the 25 and 50 mg/kg/day dose groups had reduced food consumption during the dosing period (gestation days 6-19), for the post dosing period (19-20) and for the overall gestation period (0-20). There was reduced food efficiency in the 50 mg/kg/day dose group during the dosing period (gestation days 6-19) and in the 25 and 50 mg/kg/day dose groups for the post dosing period (19-20), for the overall gestation period (0-20) and for the calculated period of gestation days 6-20. **The maternal toxicity NOAEL was 10 mg/kg/day and the maternal toxicity LOAEL of 25 mg/kg/day based on decreased body weights and body weight gains and reduced food consumption and reduced food efficiency.**

There was an increase in fetal and litter incidence of cervical ribs with cartilage not present in the high dose group. **The developmental toxicity NOAEL was 25 mg/kg/day and the developmental toxicity LOAEL was 50 mg/kg/day based on increased incidence of cervical ribs with cartilage not present.**

This study is classified as Acceptable-Guideline and satisfies the guideline requirements (OPPTS 870.3700; OPP§ 83-3a) for a prenatal developmental toxicity (teratology) study in rats.

-- *Developmental Toxicity - Rabbit- §83-3b* (MRID 45118326/45437001)

Executive Summary: under above item 2.1a

5.4 Reproductive Toxicity

-- *Multigeneration Reproduction - Rat- §83-4* (MRID 45118327)

Executive Summary: In a multigeneration reproduction study (MRID# 45118327), groups of male and female Wistar rats (Chbb = THOM (SPF) from Boehringer Ingelheim, Pharma KG, Biberach/Riss, FRG received 0, 25, 75 or 300 ppm BAS 500 F (Purity: 98.7%; Batch No.: J.-No. 27882/199/b) in the diet for two successive generations [Mean intakes for the 25, 75 and 300 ppm dose groups in mg/kg/day were 2.5 for males and 2.6 for females, 7.4 for males and 7.8 for females, and 29.0 for males and 30.4 for females, respectively, in the F0 generation; from 2.8 for males and 3.0 for females, 8.6 for males and 9.0 for females and 35.0 for males and 36.0 for females, respectively, in the F1 generation]. Maternal and paternal recordings and measurements included daily clinical observations, weekly body weights (individual pup weights on days 0, 4, 7, 14, and 21 postpartum), weekly feed consumption, mating, gestation and delivery parameters, pup survival and sexual maturation landmarks, and gross necropsy (macroscopic pathological examination) and histopathological observations in organs of parental animals showing gross pathological changes, as well as representative organs from all control and high dose F0 and F1 animals.

No substance related mortality or clinical signs of toxicity was noted in any of the F0 or F1 parental animals. Further, there was no treatment related effects noted in the body

weights, body weight gains, food consumption, reproductive performance (all measured parameters), sexual maturation landmarks, necropsy results, both gross and microscopic including assessment of differential ovarian follicle counts and organ weights of the parental animals and the pups. It should be noted, that there was a slight delay (5 % above concurrent control) in vaginal opening among the F1 pups at the highest dose tested but this finding was not considered to be toxicologically significant; however, a new study conducted with higher doses might provide more information on this observation.

The parental systemic/reproductive and offspring toxicity NOAEL is ≥ 300 ppm (29.0-35.0 mg/kg/day for males and 30.4-36.0 mg/kg/day for females), the highest dose tested; a LOAEL was not established.

This study is classified as Unacceptable-Guideline and does not satisfy the guideline requirements (OPPTS 870.3800, OPP §83-4) for a multigeneration reproduction study in rats. The HIARC determined that a new study that uses higher doses is required to assess the potential of pyraclostrobin to cause reproductive toxicity.

5.5 Additional Information from Literature Sources (*if available*)

N/A

5.6 Determination of Susceptibility

There was no evidence of increased susceptibility following in utero exposure to rats; developmental effects were seen at a higher dose than that which caused maternal toxicity. However, in the rabbit developmental toxicity study, there was qualitative evidence of in utero susceptibility; increases in resorptions/litter and post-implantation losses were seen in the presence of maternal toxicity (decrease in body weight gain and food consumption). The HIARC could not assess susceptibility in the two-generation reproduction study since the highest dose tested did not cause maternal systemic toxicity, nor did it elicit reproductive or offspring toxicity. However, it was noted that there was a slight delay (5% above concurrent control) in vaginal opening at the highest dose tested and that a new study conducted with higher doses might provide more data on the significance of this finding.

5.7 Recommendation for a Developmental Neurotoxicity Study

Based on the following weight-of-evidence considerations, the HIARC **did not recommended** a Developmental Neurotoxicity study in rats for Pyraclostrobin.

5.7.1 Evidence that suggest requiring a Developmental Neurotoxicity study:

- Serum cholinesterase (CHE) activity was decreased (by 41-56%) among the females of the 1000 ppm and 1500 ppm (80 and 119 mg/kg/day) groups in the 90-day rat study and among the 1500 ppm (126 mg/kg/day) females in the 28-day rat study. On the other hand, erythrocyte CHE in the 28 and 90-day rat studies and brain CHE in the 28-day were all unchanged. It is possible that, at relatively high

doses, this is a compound specific effect that might not be toxicologically relevant.

5.7.2 Evidence that **do not** support a need for a Developmental Neurotoxicity study:

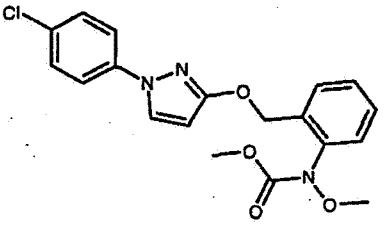
- In all studies, including the acute and subchronic guideline acceptable neurotoxicity studies, there were no signs of neurotoxicity or neuropathology.

- There was no evidence of increased susceptibility following in utero exposure to rats.

- In the acute and subchronic toxicity studies, there were no physiological or behavioral effects to corroborate the serum CHE inhibition seen in female rats

6 HAZARD CHARACTERIZATION

Pyraclostrobin has been designated a "reduced risk" new active ingredient that is undergoing "joint review" by the US-EPA and Canada-PMRA. Pyraclostrobin is a fungicidal β-methacrylate compound that is structurally related to the naturally occurring strobilurins, compounds derived from some fungal species. Pyraclostrobin is also in the same chemical class as Azoxystrobin (PC 128810), registered for several crops and turf/lawn, and Trifloxystrobin (PC 129112) which recently was granted a "reduced risk" status as a fungicide on several crops. The biochemical mode of action of these compounds is inhibition of electron transport in pathogenic fungi. There are several food and nonfood uses including grass grown for seed and golf course turf. The primary routes of concern are dermal and inhalation, with time frames varying from days to many months (as in the case of golf course maintenance).



The toxicology data base is complete; however, five of the guideline studies are unacceptable. These studies are rat dermal absorption, 2-year rat feeding, mouse carcinogenicity, 2-generation rat reproduction, and 28-day rat dermal. The dermal absorption study was designated unacceptable because most of the administered dose remained in the dressing and, therefore, was unavailable for possible dermal uptake; in the remaining four unacceptable studies, the doses were not properly selected because the highest tested dose did not elicit a toxic response.

Pyraclostrobin has a low to moderate order of acute toxicity based on its classification in Toxicity Category IV via the oral route, Toxicity Category III by the dermal route, and Toxicity Category

II by the inhalation route of exposure. It produces minimal eye irritation (Toxicity Category III), is a moderate dermal irritant (Toxicity Category III), and is not a dermal sensitizer.

Based on findings in repeated dosing oral studies in more than one species, the main target organs for Pyraclostrobin are the upper gastrointestinal tract (mainly the duodenum and stomach), the spleen/hematopoiesis, the immune system, and the liver. In the 90-day dietary rat, mouse, and dog feeding studies, one or more of the following GI changes were noted: thickening of the duodenal wall, duodenum mucosal hypertrophy or hyperplasia, as well as gross and microscopic ulceration/erosion in the glandular stomach. Mucosal hyperplasia in the duodenum was also observed in rats of both sexes after 28-day administration of 500 and 1500 ppm Pyraclostrobin. The upper GI-tract effects might, at least partly, explain some of the adverse effects on food consumption/utilization, and possibly, body weight.

It is important to note that the carcinogenicity/chronic rat and mouse studies caused no treatment-related duodenal changes, due, most likely, to inadequate dosing rather than to adaptation or reversibility over time. In the oral feeding subchronic studies, the duodenum effects, were triggered at threshold dose levels of 500 ppm (~35 mg/kg/day in the rat and ~120 mg/kg/day in the mouse). In the chronic/ carcinogenicity studies, however, the top dose was 200 ppm in the rat studies and 120 ppm/180 ppm (M/F) in the mouse study, both of which were below the dose levels in the 90-day studies that triggered duodenum changes (500 ppm) in both species and glandular stomach ulcer/erosion in the female mouse (150 ppm). Likewise, the reported duodenal changes in the 90-day dog study, but not in the 1-year dog study, is not likely due to adaptation or reversibility but is rather due to selecting a lower top dose in the 1-year study than in the 90-day study. The top dose (400 ppm or M/F: 10.8/11.2 mg/kg/day) in the 1-year dog study was nearly 21% lower than the top dose (450 ppm or M/F: 12.9/13.6 mg/kg/day) in the 90-day dog study which, in the 450 ppm dose group, reported increased thickening of duodenal wall (2/5/sex vs 0/5 in all other groups) and duodenum mucosal hypertrophy (2/5 males and 1/5 female vs 0/5 in all other groups).

On the other hand, there were increased macro- and microscopic ulcerations/lesions of the glandular- and fore-stomachs among the mid- (75 ppm) and high- (200 ppm) dose treated males in the rat carcinogenicity study. However, most of these findings were among the dead/moribund animals in the control and treated groups and, therefore, the possibility of post-mortem autolysis could not be ruled out. Interestingly, no similar stomach lesions were reported in the rat chronic toxicity study despite the fact that both studies had identical dosing regimens. This could be due to using fewer animals/group/sex (50 in the carcinogenicity vs. 20 in the chronic), or other factors, including possibly, being less rigorous in the macro- and microscopic examinations in the chronic toxicity study compared to the carcinogenicity study.

The hematopoietic toxicity in the rat and mouse 90-day studies and the immunotoxicity in the mouse 90-day study were evident at or above doses of around 100-150 mg/kg/day (500 ppm in mice and 1000 ppm in rats). In rats, these effects included mild hemolytic anemia accompanied by increased reticulocytes, increased spleen weight, spleen microscopic changes, and increased total leukocytes, neutrophils, and lymphocytes. In mice, there were mild hemolytic anemia accompanied by a large decline (≥50%) in leukocytes (leukopenia), neutrophils, and lymphocytes. The large decline in WBC populations was accompanied by dose-dependent thymus atrophy and increased lymph node apoptosis. (The incidences of thymus atrophy ranged from 3/10 to 8/10 in

each of the top four dose groups of both sexes compared to 0/10 in the control and lowest dose groups; the severity also increased dose-dependently.) Collectively, these changes strongly suggest that Pyraclostrobin is immunotoxic in the mouse. As discussed above for the GI-tract toxicity, the absence of similar hematopoietic- and immuno-toxicity in the chronic/carcinogenicity feeding studies, where the top doses were 120-200 ppm, is most likely due to inadequate dosing.

The liver also was a target organ in the 28-day rat and the 90-day rat and mouse dietary feeding studies based on slight to moderate increased relative liver weight in both species and hepatocellular hypertrophy in the rat studies. There was also mild or moderate sporadic changes in some clinical chemistry parameters (e.g., decreased triglycerides, decreased or increased cholesterol, and decreased proteins/globulins) possibly reflecting a compromised nutritional status, or hemolytic anemia (e.g., increased total bilirubin in rats) rather than an adverse effect on the liver. Occasionally, there were also diminished hepatocyte lipid vacuoles due to possibly a compromised nutritional status. Following chronic oral administration of pyraclostrobin, the only reported liver effect was in the form of increased hepatic necrosis (type unspecified) among the high dose males (10/50 vs 1-2/50 in all other groups) of the 2-year rat carcinogenicity study but not in the accompanying 2-year rat chronic study. The lack of additional liver effects or more severe toxicity in this organ is also likely due to using far lower doses in the chronic studies than in the subchronic study.

Other common findings were decreased body weight/weight gain, and decreased food intake/efficiency in a variety of species and studies. The threshold dose for these effects seemed to coincide with the threshold for the GI-tract effects but was, generally, lower than that needed to elicit the other toxic responses summarized above.

The pre- and post-natal toxicology data base for pyraclostrobin includes the rat and rabbit developmental toxicity studies and the 2-generation reproduction toxicity study in rats. There was no evidence of increased susceptibility following in utero exposure to rats; developmental effects were seen at a higher dose than that which caused maternal toxicity. However, in the rabbit developmental toxicity study, there was qualitative evidence of in utero susceptibility; increases in resorptions/litter and post-implantation losses were seen in the presence of maternal toxicity (decrease in body weight gain and food consumption). The HIARC could not assess susceptibility in the two-generation reproduction study since the highest dose tested did not cause maternal systemic toxicity, nor did it elicit reproductive or offspring toxicity. However, it was noted that there was a slight delay (5% above concurrent control) in vaginal opening at the highest dose tested and that a new study conducted with higher doses might provide more data on the significance of this finding.

In both the acute and subchronic neurotoxicity studies, there were no indications of treatment-related neurotoxicity including clinical signs, qualitative or quantitative neurobehavioral effects, brain weight, or gross/microscopic pathology. None of the other guideline studies reported treatment-related effects on any of these parameters. However, there was a large decrease (↓ ~ 50%) in serum cholinesterase (but not in erythrocyte or brain cholinesterases) among the females in the rat 28- and 90-day dietary studies at relatively large doses (1000/1500 ppm). It is possible that, at relatively high doses, this is a compound specific effect that might not be toxicologically relevant. Cholinesterase was not measured in any other study.

Based on pharmacokinetics/metabolism studies in the rat, at least 34.5-37.7% of an orally administered low- or high-dose (5 or 50 mg/kg) or repeated doses of pyraclostrobin was absorbed. Regardless of sex, dose, radiolabel (^{14}C-tolyl or ^{14}C-chlorophenyl) or pretreatment, urinary excretion accounted for 10.8-16.0% of the administered dose, with the majority of urinary excretion occurring within 24 hours. Fecal excretion accounted for nearly 85% of an administered dose of which nearly 30% was from biliary excretion. Plasma concentrations were highest in all groups at 0.5-1 hours and 8 hours (two peaks); males had lower plasma concentrations than females (↓16-38%) during the earlier time points. Elimination was biphasic at the low dose with plasma half lives of nearly 10/35 hours and monophasic at the high dose with a half life of nearly 20 hours. The distribution pattern of radioactivity in tissues was similar between sexes (typically higher among females) reaching peak levels at 0.5 hours post-dosing; some of the highest concentrations were found in the liver, thyroid, kidney, lung, adrenal glands, and pancreas. There was no evidence of tissue accumulation since levels at 42-72 hours dropped greater than 20-fold relative to the earliest measurement at 0.5 hour. Nearly 33 metabolites were isolated and identified in the urine, feces, and bile; there were no sex- or dose-related differences in the metabolite profile in urine or feces but the position of the label seemed to alter the profile, particularly in the urine. The metabolic pathway included phase-I reactions such as N-demethoxylation, various hydroxylations, and cleavage of the ether bond with subsequent oxidation; these were followed by the phase II glucuronidation and sulfation reactions.

7 DATA GAPS

- 28-Day inhalation toxicity study: Required due to the potential for occupational/residential exposure via this route. The Registrant is required to follow the 90-day inhalation toxicity protocol using 28-day exposure duration and no organic solvents should be used.

- 28-Day dermal toxicity study: Required since the highest tested dose in the available study was determined to be inadequate to assess dermal/systemic toxicity.

- 2-Generation reproduction toxicity study: Required to assess the reproductive/ offspring toxicity as well as for assessment of susceptibility following pre-/post-natal exposure since the doses in the available study were considered inadequate.

- Carcinogenicity study in mice: The doses tested in the available study were determined inadequate to assess the carcinogenic potential since no systemic toxicity was seen at the HDT.

8 ACUTE TOXICITY

Acute Toxicity of Pyraclostrobin

Guideline No.	Study Type	MRID #	Results	Toxicity Category
81-1	Acute Oral	45118302	LD_{50} = > 5000 mg/kg	IV
81-2	Acute Dermal	45118305	LD_{50} = > 2000 mg/kg	III
81-3	Acute Inhalation	45118308	$0.31 < LC_{50} < 1.07$ mg/L	II
81-4	Primary Eye Irritation	45118311	minimal eye irritation; MAS 4.6/110	III
81-5	Primary Skin Irritation	45118314	moderate skin irritation; MAS 2.2/8.0	III
81-6	Dermal Sensitization	45118317	not a dermal sensitizer	N/A

9 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Females 13-50)	NOAEL= 5 UF = 100	Developmental toxicity findings of increased resorptions/litter and increased total resorptions (i.e., dams with complete litter loss) at 10 mg/kg/day (LOAEL).	Rabbit Prenatal Developmental Toxicity (MRID 45118326/45437001)
	Acute RfD (Females 13-50 years old) = 0.05 mg/kg		
(General Population)	NOAEL= 300 UF = 100	The systemic toxicity NOAEL of 300 mg/kg based on decreased body weight gain in males at 1000 mg/kg (LOAEL).	Rat Acute Oral Neurotoxicity (MRID 45118337)
	Acute RfD (General Population) = 3 mg/kg		
Chronic Dietary	NOAEL = 3.4 UF = 100	Decreased body weight/gain, kidney tubular casts and atrophy in both sexes; increased incidence of liver necrosis and erosion/ulceration of the glandular stomach and forestomach in males in addition to hemolymphoreticular tumors in males and mammary adenocarcinoma in females at 9.2 mg/kg/day (LOAEL).	Rat Oral Carcinogenicity (MRID 45118331)
		Chronic RfD = 0.034 mg/kg/day	
Incidental Oral, Short and Intermediate-Term	NOAEL= 5.8	Increased incidence of diarrhea, clinical chemistry changes, duodenum mucosal hypertrophy, and decreased body weight and food intake/efficiency at 12.9 mg/kg/day (LOAEL).	13-Week Feeding Dog Study (MRID 45118323)
Dermal, Short- and Intermediate-Term	Oral NOAEL= 5.0	Developmental toxicity findings of increased resorptions at 10.0 mg/kg/day (LOAEL).[1]	Rabbit Prenatal Developmental Toxicity (MRID 45118326)

Dermal, Long-Term	Oral NOAEL= 3.4	Decreased body weight/gain, kidney tubular casts and atrophy in both sexes; increased incidence of liver necrosis and erosion/ulceration of the glandular stomach and forestomach in males in addition to hemolymphoreticular tumors in males and mammary adenocarcinoma in females at 9.2 mg/kg/day (LOAEL).[1]	Rat Oral Carcinogenicity (MRID 45118331)
Inhalation, Short- and Intermediate-Term	Oral NOAEL= 5.0	Developmental toxicity findings of increased resorptions at 10.0 mg/kg/day (LOAEL).[2]	Rabbit Prenatal Developmental Toxicity (MRID 45118326)
Inhalation, Long-Term	Oral NOAEL= 3.4	Decreased body weight/gain, kidney tubular casts and atrophy in both sexes; increased incidence of liver necrosis and erosion/ulceration of the glandular stomach and forestomach in males in addition to hemolymphoreticular tumors in males and mammary adenocarcinoma in females at 9.2 mg/kg/day (LOAEL).[2]	Rat Oral Carcinogenicity (MRID 45118331)

[1] The dermal absorption factor of 50% should be applied to extrapolate from the oral route to the dermal route.

[2] 100% absorption rate (default value) should be used to extrapolate from the oral route to the inhalation route.