**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
**WASHINGTON, D.C. 20460**

**OFFICE OF CHEMICAL SAFETY**
**AND POLLUTION PREVENTION**

**MEMORANDUM**

**DATE:** October 25, 2021

**SUBJECT:** **Pyraclonil:** Report of the Cancer Assessment Review Committee

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**FROM:** Ruthanne Louden, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509P)

**THRU:** Greg Akerman, Ph.D., Chair
Anwar Dunbar, Ph.D., Co-Chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

**TO:** Minerva Mercado-Feliciano, Ph.D., DABT, Toxicologist
Risk Assessment Branch IV
Health Effects Division (7509P)

On February 17, 2021, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) evaluated the carcinogenic potential of pyraclonil in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

*CANCER ASSESSMENT DOCUMENT*

# FOR COMMITTEE DELIBERATION

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
**PYRACLONIL**
**PC: 104502**

October 25, 2021

Submitted by: Minerva Mercado-Feliciano, DABT, PhD

**C**ANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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## EXECUTIVE SUMMARY

On February 17, 2021, 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 pyraclonil.

Pyraclonil is currently undergoing registration as a new active ingredient. It is a pyrazole herbicide proposed for weed control in water-seeded rice via inhibition of protoporphyrinogen oxidase (PPO).

Carcinogenicity studies in CD-1 mice (MRID 50971035 and 50971036) and Sprague Dawley rats (MRID 50971034 and 50971037) were presented to the CARC. In both species, the initial studies did not adequately challenge the animals to assess carcinogenic potential. A second study was thus conducted for each species at higher doses based on consultations with the Agency.

For the **carcinogenicity studies with mice**, pyraclonil was administered in the diet to groups of 52 mice per sex at dose levels of 0, 5, 50 or 500 ppm for 83 weeks in the first study (equivalent to 0, 0.68, 6.7 or 68 mg/kg/day in males, and 0, 0.83, 8.6, and 87 mg/kg/day in females). In the second study, the mice were administered dose levels of 0, 1000 or 2000 ppm for 78 weeks (equivalent to 0, 102, and 210 mg/kg/day in males and 0, 100, and 204 mg/kg/day in the females). The second study focused on thyroid and liver which were identified as potential target organs in the first study. The results of both studies were considered together.

For the **combined chronic/carcinogenicity studies with rats**, pyraclonil was administered in the diet to groups of 50 rats per sex at dose levels of 0, 10, 100 or 1000 ppm for 104 weeks in the first study (equivalent to 0, 0.4, 4.4 or 44 mg/kg in males, and 0, 0.6, 5.8 or 58 mg/kg day in females). In the second study, rats were administered 0, 2000 or 4000 ppm (equivalent to 0, 77 or 159 mg/kg/day in males, and 0, 99 or 208 mg/kg in females) for 104 weeks. The second study focused on thyroid and liver identified as potential target organs in the first study. The results of both studies were considered together.

Information on mutagenicity and structure-activity relationships (SAR) to similar chemicals were also presented.

The CARC concluded the following:

<u>*Mice*</u>

- *Malignant Lymphomas*: The CARC identified observed increases in malignant lymphomas in female mice in the second study. Since lymphomas are systemic tumors and only the liver and thyroid were evaluated in this study, CARC concluded that the malignant lymphoma findings were inconclusive.

- *Liver Tumors:* In the second study (MRID 50971035), females had a statistically significant trend, and statistically significant pair-wise comparison at 2000 ppm or 204 mg/kg/day with

the controls, for hepatocellular adenomas (19% vs. 2% controls), and the combined hepatocellular adenomas and carcinomas combined (21% vs. 2% controls), all at $p < 0.01$. The incidence of adenomas at the high dose exceeded both the concurrent control and the historical control range for the preforming laboratory (0-3.9%; 2013-2015). Liver histopathology was fully evaluated in this study, and the tumor findings were supported by gross and non-neoplastic findings of the liver (increased incidence of enlarged livers, of liver masses and eosinophilic foci of cellular alteration). **The CARC concluded that the liver adenomas and adenomas/carcinomas combined to be treatment related in females at the high dose (2000 ppm or 204 mg/kg/day). No treatment related tumors were seen in male mice.**

- *Adequacy of Dosing:* The doses were considered adequate when the two mouse studies are considered together, and the studies are acceptable/guideline. The first carcinogenicity study in mice (MRID 50971036) did not show any effects of treatment up to 500 ppm (68 mg/kg/day in males, 87 mg/kg/day in females). The second mouse carcinogenicity study (MRID 50971035) was conducted following the Dose Adequacy Review Team (DART) recommendations. The highest dose tested of 2000 ppm (210 mg/kg/day in males, 204 mg/kg/day in females) was considered adequate and not excessive in both sexes in the mouse. Liver weights and the incidence of dark colored livers were increased in males at 2000 ppm (2010 mg/kg/day) compared to controls. In females, treatment related macroscopic findings in the liver included increased incidences of liver mass(es), spot(s), and enlarged livers. In some females these changes correlated with incidences of hepatic eosinophilic foci of cellular alteration at 1000 ppm (100 mg/kg/day) and 2000 ppm (204 mg/kg/day), which was also increased in incidence at the same doses. There was no increased mortality in treatment groups compared to controls.

<u>Rats</u>

- *Mesenteric Lymph Node Tumors:* In the first study (MRID 50971034), hemangiomas of the mesenteric lymph node were evaluated in male rats, but there were no statistically significant trends or pair-wise comparisons from 10 to 1000 ppm pyraclonil. There were also no compound-related tumors observed in female rats. The doses used in the first study were not adequate (based upon DART conclusions). The DART determined that an additional study with limited evaluation of liver and thyroid should be generated similar to the study design for the mouse study (MRID 50971037). **The CARC determined the mesenteric lymph node hemangiomas to not be treatment-related at the high dose in males (1000 ppm or 44 mg/kg/day).**

- *Histiocytic Sarcomas:* In the second study (MRID 50971037), male rats had a statistically significant trend for systemic histiocytic sarcomas at $p < 0.05$. There were no pairwise significance, and no effect for mononuclear leukemia at the high dose. There was low incidence and no statistical significance for tumors in females. **The CARC determined the mesenteric systemic histiocytic sarcomas were not treatment related at the high dose in either sex (4000 ppm, 159 mg/kg/day in males, 208 mg/kg/day in females).**

- *Liver Tumors:* In the second study, liver tumors were evaluated in female rats. There were no statistically significant trends or pair-wise comparisons up to the highest dose tested. All adenomas and carcinomas in females were observed at final sacrifice. **The CARC determined the liver tumors to be not treatment related in females at the high dose (4000 ppm, 208 mg/kg/day).**

- *Thyroid Tumors:* In the second study (MRID 50971037), there was a statistically significant trend, and a statistically significant pair-wise comparison of the high dose group with the controls, for thyroid follicular cell adenomas in males, both at $p < 0.05$. There was also a statistically significant trend at $p < 0.01$, and a statistically significant pair-wise comparison of the high dose group with the controls at $p < 0.05$, for the combined thyroid follicular cell adenomas and/or carcinomas combined in males. Thyroid histopathology was fully evaluated in this study, and the tumor findings were supported by non-neoplastic findings of the thyroid (slight to severe thyroid colloid degeneration). **The CARC determined the thyroid follicular cell tumors to be treatment related in males at the high dose (159 mg/kg/day).** Thyroid follicular cell tumors were evaluated in female rats, but there were no statistically significant trends or pair-wise comparisons in the data analyzed, therefore, the thyroid tumors in females were not considered to be treatment-related.

- *Adequacy of Dosing:* The doses were considered adequate when the two studies are considered together, and the studies are acceptable/guideline. The first carcinogenicity study in rats (MRID 50971034) did not show any effects of treatment. The second rat carcinogenicity study (MRID 50971037) was conducted following the DART recommendations. The highest dose tested of 2000 ppm was considered adequate and not excessive in both sexes in the rat. Increased incidences of thyroid colloid degeneration were observed at the lower doses in both sexes. In addition, females exposed to 4000 ppm in diet showed significant decreases in mean body weight, and both sexes showed increases in γ-glutamyl transpeptidase levels.

*Mutagenicity*

The was no evidence of increased gene mutations in bacteria or mammalian cells *in vitro*. Potential clastogenicity was seen in two in vitro chromosomal aberrations assays; however, pyraclonil was negative when tested in two *in vivo* micronucleus assays indicating a low potential for clastogenicity *in vivo*.

*Structure Activity Relationships*

Chemicals with available carcinogenicity studies had low structural similarity to pyraclonil (≤61%) and their cancer classifications ranged from "not likely to be carcinogenic to humans" to "possible human carcinogen" based on liver tumors in both sexes of mice (bromoxynil) or male rats (dichlobenil).

*Classification of Carcinogenic Potential*

In accordance with EPA's *Final Guidelines for Carcinogen Risk Assessment* (March 2005), the CARC classified pyraclonil as "Likely to be Carcinogenic to Humans" this classification was based on an increased incidence of hepatocellular adenomas and combined adenomas and carcinomas in female mice, and thyroid follicular cell adenomas and combined adenomas/carcinomas in male rats.

*Quantification of Carcinogenic Potential*

For the purpose of risk assessment, the CARC recommends a low dose extrapolation model applied to the experimental animal tumor data be used for quantification of human cancer risk (Q1*).

## I. INTRODUCTION

On February 17, 2021, 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 pyraclonil. On March 4, 2021, the CARC revisited the conclusions from the initial meeting via electronic mail communication with all committee members.

## II. BACKGROUND INFORMATION

Pyraclonil (PC: 104502; CAS#: 158353-15-2), is currently undergoing registration as a new active ingredient. It is a pyrazole herbicide proposed for weed control in water-seeded rice via inhibition of protoporphyrinogen oxidase (PPO).

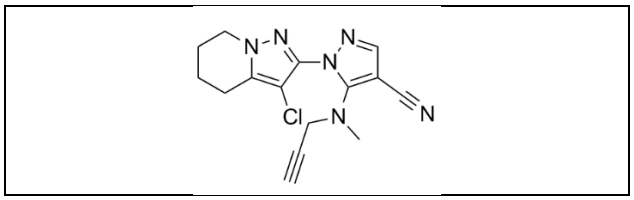



**Figure 1. Structure of pyraclonil.**

## III. EVALUATION OF CARCINOGENICITY STUDIES

### 1. Carcinogenicity Studies in Mice

#### A. Carcinogenicity Study in Mice – MRID 50971036

*Reference:* Mallyon, B.A. (2001) AE B172391: Mouse 18-month dietary oncogenicity study. Aventis CropScience UK Limited, Essex, England. Laboratory Project ID.: TOX 95338, January 26, 2001. MRID 50971036. Unpublished.

*i. Experimental Design*

In a carcinogenicity study (MRID 50971036), groups of 52 CD-1 mice/sex/dose were administered AE B172391 Technical (pyraclonil; ≥98.0% a.i.; lot # AE B172391 00 1C99 0003) in the diet at dose levels of 0, 5, 50, or 500 ppm (equivalent to 0, 0.68, 6.7 or 68 mg/kg/day in males, and 0, 0.83, 8.6, or 87 mg/kg/day in females) for 83 weeks. All tissues were examined microscopically.

*ii. Survival Analysis*

The statistical survival analysis for this study was taken from the original study report (not conducted by HED). No significant increase in mortality was noted for male or female mice of any dose group, as compared to concurrent controls. Survival through week 78 was 56%, 62%, 63%, and 63% for males, and 71%, 73%, 73%, and 81% for females in the 0, 5, 50, and 500 ppm groups, respectively.

### iii. *Discussion of Tumor Data*

No significant treatment related tumor incidences were observed in male mice. A positive trend was noted in the study report for the combined incidence of Harderian gland tumors in females. The CARC does not consider Harderian gland tumors to be relevant to humans for assessing cancer risk, and as such, the Harderian gland observations were not discussed. The second study (MRID 50971035), which tested at higher dose levels, did not find this type of tumor.

### iv. *Discussion of Non-Neoplastic Lesions*

Several microscopic parameters demonstrated a marginally significant (two-tailed, $p \leq 0.05$) positive trend in incidence (Table 1). Except for the increasing trend in the incidence of squamous keratinizing epithelium of the vagina, these trends were due to small changes in incidence. Since none of the changes, including the vaginal findings, were confirmed in a later carcinogenicity study in mice using higher doses (MRID 50971035), the effects observed in the current study are considered incidental.

Table 1. Pyraclonil – Sprague Dawley CD-1 Mouse Study
(MRID No. 50971036)

Non-Neoplastic Findings After Up to 84 weeks of Treatment; N=52. [a]

| Dose (ppm) | 0 | 5 | 50 | 500 |
|---|---|---|---|---|
| Males | | | | |
| Eye, iridial mineralization | 1* | 0 | 0 | 4 |
| Females | | | | |
| Lymph node, mesenteric, sinus erythrophagia | 0* | 0 | 1 | 3 |
| Thymus, atrophy | 0* | 0 | 0 | 3 |
| Vagina, squamous keratinizing epithelium | 8* | 7 | 12 | 16 |

a Data were obtained from pages 94-142 and 345 of MRID 50971036; total data includes survivors through Week 84 and mice euthanized *in extremis* or found dead.

* Positive trend; $p \leq 0.05$ as indicated in MRID 50971036

### v. *Adequacy of the Dosing for Assessment of Carcinogenicity*

There were no significant treatment-related effects on mortality, clinical signs, palpable masses, body weight, food consumption or conversion, hematology parameters, organ weights, or macroscopic or microscopic pathology findings in either sex. Because of a concern that the animals were not adequately challenged for assessing carcinogenicity, the agency was consulted, and a second study in CD-1 mice was conducted testing higher doses of 1000 and 2000 ppm (MRID 50971035).

In 2013, the DART recommended a modified mouse carcinogenicity study with a control and two dose levels higher than those tested in the original study: 0, 1000, and 2000 ppm (TXR 0056681, J. Kidwell, 6/06/2013). This recommendation was based on the existing 90-day study where a dose of 2000 ppm showed decreases in body weight (↓13%) and body weight gain (↓35%) in males, increased liver weights in both sexes and corresponding histopathological

changes in the liver (hepatocyte hypertrophy, slight focal necrosis, and centrilobular fat deposition). The 1000 ppm dose would likely not exceed the maximum tolerated dose (MTD) and would ensure an adequate top dose in case the dose of 2000 ppm proved to be excessive. After the DART meeting, the registrant requested that the parameters be limited to the thyroid and liver. The agency responded that the supplemental carcinogenicity study with control, mid-, and high dose groups would need to evaluate the following parameters: food consumption, body weight (absolute and body weight gain), and liver effects (organ weight, enzymes, gross pathology and histopathology).

A second mouse carcinogenicity study (MRID 50971035) was thus conducted following the DART recommendations. When considered together, the first (MRID 50971036) and second (MRID 50971035) mouse carcinogenicity studies are classified as acceptable/guideline.

## B. Carcinogenicity Study in Mice – MRID 50971035

*Reference:* Ito, T. (2018) Pyraclonil technical: Carcinogenicity study in mice. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID: IET 15-0004, October 17, 2018. MRID 50971035. Unpublished.

### *i. Experimental Design*

In a carcinogenicity study (MRID 50971035), groups of 52 CD-1 mice/sex/dose were administered AE B172391 Technical (pyraclonil; 97.81% a.i.; lot # ANBP02Y01) in the diet at dose levels of 0, 1000, or 2000 ppm (equivalent to 0, 102 or 210 mg/kg/day in males, and 0, 100, or 204 mg/kg/day in females) for 78 weeks. Additionally, groups of 16 CD-1 mice/sex/dose were dosed in the same manner for 52 weeks. Microscopic examination was conducted on the liver and thyroid only from all mice. This study was conducted as recommended by the DART (TXR 0056681, J. Kidwell, 6/06/2013).

### *ii. Survival Analysis*

No significant increase in mortality were noted for male mice of any dose group. The statistical analysis of survival data for females is summarized in Table 2 (TXR 0058144, L. Brunsman, 1/27/2021). There were no survival disparities among the dose groups for female mice.

Table 2. Pyraclonil – ICR [Crl:CD1(ICR)] Mouse Study
(MRID No. 50971035)
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | |
|---|---|---|---|---|---|
| Dose (ppm) | 1-26 | 27-52 | 52[i] | 53-78[f] | Total |
| 0 | 0/68 | 7/68 | 14/61 | 13/47 | 20/54 (37) |
| 1000 | 1/68 | 2/67 | 16/65 | 15/49 | 18/52 (35) |

| Dose (ppm) | 1-26 | 27-52 | 52[i] | 53-78[f] | Total |
|---|---|---|---|---|---|
| 2000 | 0/68 | 7/68 | 13/61 | 5/48 | 12/55 (22) |

+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
i Interim sacrifice at week 52.
f Final sacrifice at week 78.

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

iii. Discussion of Tumor Data

There were no compound-related tumors observed in male mice. The CARC initially evaluated observed malignant lymphomas in female mice. It was later determined that based upon only one tissue type being examined, a conclusion could not be made on the lymphomas. Unless all tissues are examined the origins of lymphoma cannot be determined.

For females there were statistically significant trends, and statistically significant pair-wise comparisons of the 2000 ppm dose group with the controls, for hepatocellular adenomas, and hepatocellular adenomas and/or carcinomas combined, all at $p < 0.01$. The combined incidences are driven by adenomas (Table 3). The historical control range for liver adenomas is 0-3.9%. There were no liver carcinomas in the historical controls (Table 4). In MRID 50971036, no adenomas or carcinomas were reported in the pathology report, only one liver hemangioma at the mid-dose. The statistical analyses of the tumors in female mice were based upon Fisher's Exact Test and the Exact Test for Trend (Tables 3; TXR 0058144, L. Brunsman, 1/27/2021).

Table 3. Pyraclonil – ICR [Crl:CD1(ICR)] Mouse Study
(MRID No. 50971035)

Female Hepatocellular Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

| Dose (ppm) | 0 | 1000 | 2000 |
|---|---|---|---|
| Adenomas[a] (%) | 1/47 (2) | 5/49 (10) | 9/48 (19) |
| P = | 0.0058** | 0.1118 | 0.0085** |
| Carcinomas[a] (%) | 0/47 (0) | 0/49 (0) | 1/48 (2) |
| P = | 0.3333 | 1.0000 | 0.5053 |
| Combined (%) | 1/47 (2) | 5/49 (10) | 10/48 (21) |
| P = | 0.0026** | 0.1118 | 0.0042** |

+ Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
a All tumors were observed at week 78.

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

Table 4. Pyraclonil – ICR [Crl:CD1(ICR)] Mouse Study
(MRID No. 50971035)

Selected Historical Controls for Females

| **Lesion** | **Num. of Studies** | **Years** | **N per study** | **Range of incidence** |
|---|---|---|---|---|
| Malignant lymphoma | 5 | 2013-2015 | 52 | 5-11 (9.6-21.2%) |
| Hepatocellular adenoma | 5 | 2013-2015 | 52 | 0-2 (0-3.9%) |
| Hepatocellular carcinoma | 5 | 2013-2015 | 52 | 0 |

a Data from MRID 50971035 page 827.

*iv. Discussion of Non-Neoplastic*

There were no significant treatment-related, non-neoplastic microscopic findings at 52 weeks or in early decedents. At 78 weeks, an increased incidence of enlarged livers (12%) was observed at 2000 ppm. Liver masses (17% and 23%) (macroscopic) and eosinophilic foci of cellular alteration in the liver (10% and 23%) were observed in female mice at 1000 and 2000 ppm, respectively (Table 5). The same effects were not seen in treated males.

Table 5. Pyraclonil – ICR [Crl:CD1(ICR)] Mouse Study
(MRID No. 50971035)

Female Non-Neoplastic Findings in the Liver After Up to 84 weeks of Treatment; N=52. [a]

| **Dose (ppm)** | **0** | **1000** | **2000** |
|---|---|---|---|
| Enlarged (macroscopic) | 0 | 2 | 6* (12%) |
| Mass(es) (macroscopic) | 2 | 9* (17%) | 12** (23%) |
| Foci of cellular alteration, eosinophilic | 0 | 5* (10%) | 12** (23%) |
| Minimal | | 2 | 5 |
| Moderate | | 2 | 2 |
| Severe | | 1 | 5 |

a Data were obtained from pages 135, 156 and 568-771 of MRID 50971035.

*v. Adequacy of the Dosing for Assessment of Carcinogenicity*

This study was conducted in conjunction with the previous study (MRID 50971036) which did not find adverse effects up to the highest dose tested (500 ppm). The high dose tested in this study of 2000 ppm was considered adequate and not excessive in both sexes. Liver weights were increased in males (↑40% absolute and ↑27% relative to body weight) at 2000 ppm compared to

controls. Treatment-related macroscopic findings included an increased incidence of dark colored livers in the 2000 ppm male mice at 52 and 78 weeks. In the 2000 ppm females treated for 78 weeks, treatment-related macroscopic findings in the liver included increased incidences of liver mass(es), spot(s), and enlarged livers. In some females these changes correlated with incidences of hepatic eosinophilic foci of cellular alteration at 1000 ppm and 2000 ppm (main group), which was also increased in incidence at the same doses. There was no increased mortality in treatment groups compared to controls.

### C. Carcinogenicity Studies in Mice - Conclusions

There were no compound-related tumors observed in male mice. Female mice had a statistically significant increase in systemic malignant lymphomas at 2000 ppm in the tissues evaluated. Since the study design focused on the liver and thyroid, the total incidence of this systemic tumor type could not be determined.

Female mice had a statistically significant increase in hepatocellular adenomas, and hepatocellular adenomas and/or carcinomas combined, at 2000 ppm, that were outside the historical control ranges. An increased incidence (compared to concurrent control) of liver masses and eosinophilic foci of cellular alteration in the liver were observed in female mice at 1000 and 2000 ppm, while increased incidence of enlarged livers was observed at 2000 ppm. **The CARC considered the hepatocellular adenomas, and hepatocellular adenomas and/or carcinomas combined in female mice to be treatment related at 2000 ppm (204 mg/kg/day).**

## 2. Carcinogenicity Studies in Rats

### A. Carcinogenicity Study in Rats - MRID 50971034

*Reference:* Mallyon, B.A. (2000) AE B172391: Rat dietary combined chronic toxicity and oncogenicity study. Aventis CropScience UK, Ltd., Toxicology, Chesterford Park, Saffron Walden Essex, England. Laboratory Study ID: TOX 95337, December 11, 2000. MRID 50971034. Unpublished.

#### *i. Experimental Design*

In a carcinogenicity study (MRID 50971034), groups of 50 Sprague Dawley rats/sex/dose were administered AE B172391 (pyraclonil; 97.0-98.5% a.i.; lot # AE B172391 00 1C99 0003) in the diet at dose levels of 0, 10, 100 or 1000 ppm (equivalent to 0, 0.4, 4.4 or 44 mg/kg/day in males, and 0, 0.56, 5.8, or 58 mg/kg/day in females) for 104 weeks. Additionally, groups of 20 Sprague Dawley rats /sex/dose were dosed in the same manner for 52 weeks. All tissues were examined microscopically.

#### ii. Survival Analysis

No significant increases in mortality were noted for female rats of any dose group. The statistical analysis of survival data for males is summarized in Table 6 (TXR 0058144, L. Brunsman, 1/27/2021). There were no survival disparities among the dose groups for male rats.

Table 6. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971034)
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose (ppm) | 1-26 | 27-54 | 54[i] | 55-78 | 79-106[f] | Total |
| 0 | 0/70 | 2/70 | 18/68 | 5/50 | 15/45 | 22/52 (42) |
| 10 | 0/70 | 3/70 | 20/67 | 1/47 | 12/46 | 16/50 (32) |
| 100 | 0/70 | 0/70 | 20/70 | 3/50 | 14/47 | 17/50 (34) |
| 1000 | 0/70 | 2/70 | 20/68 | 2/48 | 15/46 | 19/50 (38) |

\+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
i Interim sacrifice at weeks 53-54.
f Final sacrifice at weeks 105-106.

iii. Discussion of Tumor Data

Hemangiomas of the mesenteric lymph node were evaluated in male rats, but there were no statistically significant trends or pair-wise comparisons in the data analyzed. The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test and the Exact Test for Trend (Table 7; TXR 0058144, L. Brunsman, 1/27/2021). There were no compound-related tumors observed in female rats. Historical controls were not provided.

Table 7. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971034)

Male Mesenteric Lymph Node Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

| Dose (ppm) | 0 | 10 | 100 | 1000 |
|---|---|---|---|---|
| Hemangiomas (%) | 3[a]/69 (4) | 6/68 (9) | 6[a]/70 (9) | 7/68 (10) |
| P = | 0.1798 | 0.2392 | 0.2538 | 0.1566 |

\+ Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
a First hemangiomas observed at interim sacrifice in the control and 100 ppm dose groups.

*iv. Discussion of Non-Neoplastic*

There were no treatment-related non-neoplastic findings at 52 weeks. Incidence of significant histopathological findings at 104 weeks are presented in Table 8. In the 1000 ppm males, minimal centrilobular hypertrophy was observed in an apparent dose-dependent manner (18%), however;

the trend is not statistically significant (per analysis provided in the study report submitted by the registrant).

Table 8. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971034)

Microscopic Findings After Up to 104 weeks of Treatment; N=50. [a]

| **Dose (ppm)** | **0** | **10** | **100** | **1000** |
|---|---|---|---|---|
| **Males** | | | | |
| Liver, hepatocellular hypertrophy, centrilobular | 1/50 | 1/50 | 2/50 | 9/50 (18%) |

a Data were obtained from pages 223 and 252 of MRID 50971034.

*v. Adequacy of Dosing for Assessment of Carcinogenicity*

There were no significant treatment-related effects on mortality, clinical signs, palpable masses, body weight, food consumption or conversion, hematology parameters, organ weights, or macroscopic or microscopic pathology findings in either sex. Because the maximum tolerated dose was not reached in this study, a second study in Sprague Dawley rats was conducted including higher doses of 2000 and 4000 ppm (MRID 50971037).

The DART recommended a modified rat chronic toxicity/carcinogenicity study with a control and two dose levels: 0, 2000, and 4000 ppm. The proposed 2000 ppm dose would likely not exceed the MTD and would ensure an adequate top dose in case the 4000 ppm dose proves to be excessive. This recommendation is based on the existing 90-day study (MRID 50971020) where body weights were decreased 18/14% in males/female at 4000 ppm and 5.8/8.5% in males/females at 2000 ppm. In that study, absolute thyroid weights and relative thyroid weights were increased in males and females at 4000 ppm and non-significant increases in relative thyroid weights were reported at 2000 ppm. Thyroid histopathology (increase in the severity and incidence of colloid depletion and follicular cell hypertrophy) was reported in males at 4000 ppm and females at 2000 and 4000 ppm. Additional support for the proposed dose levels included an additional 90-day study (MRID 50971018) which showed significantly decreased absolute body weight (↓11%) at the highest dose of 4000 ppm in female rats only. Decreases in absolute body weight in males did not exceed 5% at 4000 ppm. Statistically significant increased absolute thyroid weight and thyroid weight relative to body weight were noted in males at 4000 ppm and in females in the 2000 and 4000 ppm dose groups. Statistically significant increases in the incidences of thyroid follicular cell hypertrophy were reported in females at 2000 and 4000 ppm and in males at 4000 ppm. In response to additional questions from the registrant regarding the limiting of evaluation to thyroid and liver parameters, the DART recommended that control, mid, and high dose groups will need to evaluate the following parameters: food consumption, body weight (absolute and body weight gain), liver effects (organ weight, enzymes, gross pathology and histopathology), thyroid effects (organ weight, thyroid hormones (interim and termination); gross pathology, and histopathology).

## B. Carcinogenicity study in rats – MRID 50971037

*Reference:* Takahashi, N. (2018) Pyraclonil technical: combined chronic toxicity and carcinogenicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID: IET 15-0002, October 17, 2018. MRID 50971037. Unpublished.

### i. *Experimental Design*

In a combined chronic toxicity/carcinogenicity study (MRID 50971037), groups of 50 Sprague Dawley rats/sex/dose were administered pyraclonil (97.81% a.i.; lot # ANBP02Y01) in the diet at dose levels of 0, 2000, or 4000 ppm (equivalent to 0, 77 or 159 mg/kg/day in males, and 0, 99, or 208 mg/kg/day in females) for 104 weeks. Additionally, groups of 12 Sprague Dawley rats/sex/dose were dosed in the same manner for 52 weeks. Thyroid hormone levels were measured. The thyroid, liver, and macroscopic masses from the liver of all rats were examined microscopically. This study was conducted as recommended by the DART (TXR 0056681, J. Kidwell, 6/06/2013).

### ii. Survival Analysis

The statistical analysis of survival data for males and females are summarized in Tables 9 and 10, respectively (TXR 0058144, L. Brunsman, 1/27/2021). There were no survival disparities among the dose groups for male or female rats.

Table 9. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose (ppm) | 1-26 | 27-53 | 53[i] | 54-78 | 79-105[f] | Total |
| 0 | 0/62 | 3/62 | 11/59 | 6/48 | 18/42 | 27/51 (53) |
| 2000 | 1/62 | 3/61 | 12/58 | 3/46 | 15/43 | 22/50 (44) |
| 4000 | 2/61[a] | 3/59 | 11/56 | 3/45 | 21/42 | 29/50 (58) |

\+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
i Interim sacrifice at week 53.
f Final sacrifice at week 105.
a One accidental death at week 8, dose 4000 ppm.

Table 10. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| Dose (ppm) | 1-26 | 27-53 | 53[i] | 54-78 | 79-105[f] | Total |
| 0 | 0/62 | 0/62 | 12/62 | 6/50 | 19/44 | 25/50 (50) |
| 2000 | 1/62 | 1/61 | 12/60 | 2/48 | 18/46 | 22/50 (44) |
| 4000 | 0/62 | 0/62 | 12/62 | 6/50 | 16/44 | 22/50 (44) |

\+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
i Interim sacrifice at week 53.
f Final sacrifice at week 105.

### *iii. Discussion of Tumor Data*

Male rats had a statistically significant trend for systemic histiocytic sarcomas at $p < 0.05$ (Table 11). There was no pairwise significance for any of the other tumor types or the combined incidences. There was no trend or pairwise comparisons in females for these tumor types (Table 12).

In males, there was a statistically significant trend, and a statistically significant pair-wise comparison of the 4000 ppm group with the controls, for thyroid follicular cell adenomas, both at $p < 0.05$ (Table 13). There was also a statistically significant trend at $p < 0.01$, and a statistically significant pair-wise comparison of the 4000 ppm group with the controls at $p < 0.05$, for the combined thyroid follicular cell adenomas and carcinomas combined (Table 13). The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test and the Exact Test for Trend (Tables 11 and 13). In study MRID 50971034 the incidences for thyroid follicular cell adenomas from 0-1000 ppm are: 2/50, 1/50, 1/50 and 0/50. Thyroid follicular cell and liver tumors were evaluated in female rats, but there were no statistically significant trends or pair-wise comparisons in the data analyzed (Table 14). The statistical analyses of the tumors in female rats were based upon Fisher's Exact Test and the Exact Test for Trend (Tables 12, 14 and 15; TXR 0058144, L. Brunsman, 1/27/2021,).

Historical controls from the performing laboratory were provided. Most of the historical studies were conducted 11 years or more before the current study, and therefore are not applicable. Only one of the historical studies (2017) was comparable to the current study and had the following control tumor incidences: histiocytic sarcoma 1/50 males (2%), 0/50 females; malignant lymphoma 1/50 males (2%), 0/50 females; myelogenous leukemia 0/50 males, 0/50 females; thyroid follicular cell adenoma 0/50 males, 1/50 females (2%), thyroid follicular cell carcinoma 0/50 males, 0/50 females.

Table 11. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)

Male Systemic Tumor Rates+ and Fisher's Exact Test and Exact Trend Test Results

| Dose (ppm) | 0 | 2000 | 4000 |
|---|---|---|---|
| Histiocytic Sarcomas<br>(%)<br><br>P = | 0/48<br>(0)<br><br>0.0324* | 0/46<br>(0)<br><br>1.0000 | 3[a]/45<br>(7)<br><br>0.1094 |
| Mononuclear Cell Leukemia<br>(%)<br><br>P = | 1[b]/48<br>(0)<br><br>0.3453 | 0/46<br>(0)<br><br>1.0000 | 0/45<br>(0)<br><br>1.0000 |
| Malignant Lymphomas<br>(%)<br><br>P = | 1/62<br>(2)<br><br>0.1755 | 0/61<br>(0)<br><br>1.0000 | 3[c]/60<br>(5)<br><br>0.2971 |
| Combined<br>(%)<br><br>P = | 2/62<br>(3)<br><br>0.0560 | 0/61<br>(0)<br><br>1.0000 | 6/60<br>(10)<br><br>0.1258 |

\+ Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54 for histiocytic sarcomas and mononuclear cell leukemia, and before week 19 for malignant lymphomas and combined.

a First histiocytic sarcoma observed at week 92, dose 4000 ppm.

b All mononuclear cell leukemia observed at final sacrifice in the control group.

c First malignant lymphoma observed at 19 weeks, dose 4000 ppm.

Table 12. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)

Female Systemic Tumor Rates+ and Fisher's Exact Test and Exact Trend Test Results

| Dose (ppm) | 0 | 2000 | 4000 |
|---|---|---|---|
| Histiocytic Sarcomas<br>(%)<br><br>P = | 1/50<br>(2)<br><br>0.6176 | 2/48<br>(4)<br><br>0.4845 | 1[a]/50<br>(2)<br><br>0.7525 |
| Mononuclear Cell Leukemia<br>(%)<br><br>P = | 0/50<br>(0)<br><br>0.1481 | 1/48<br>(2)<br><br>0.4898 | 2[b]/50<br>(4)<br><br>0.2475 |
| Malignant Lymphomas<br>(%)<br><br>P = | 0/62<br>(2)<br><br>0.1469 | 1[c]/62<br>(2)<br><br>0.5000 | 2/62<br>(3)<br><br>0.2480 |
| Combined<br>(%)<br><br>P = | 1/62<br>(2)<br><br>0.0823 | 4/62<br>(6)<br><br>0.1824 | 5/62<br>(8)<br><br>0.1036 |

\+ Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed

before week 54 for histiocytic sarcomas and mononuclear cell leukemia, and before week 17 for malignant lymphomas and combined.

a First histiocytic sarcoma observed at week 63, dose 4000 ppm.

b First mononuclear cell leukemia observed at week 65, dose 4000 ppm.

c First malignant lymphoma observed at 17 weeks, dose 2000 ppm.

Table 13. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)

Male Thyroid Follicular Cell Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

| Dose (ppm) | 0 | 2000 | 4000 |
|---|---|---|---|
| Adenomas [a]<br>(%)<br><br>P = | 1/59<br>(2)<br><br>0.0125* | 0/59<br>(0)<br><br>1.0000 | 6[b]/56<br>(11)<br><br>0.0490* |
| Carcinomas<br>(%)<br><br>P = | 0/59<br>(0)<br><br>0.1701 | 2/59<br>(3)<br><br>0.2479 | 2[c]/56<br>(4)<br><br>0.2349 |
| Combined<br>(%)<br><br>P = | 1/59<br>(2)<br><br>0.0044** | 2/59<br>(3)<br><br>0.5000 | 8/56<br>(14)<br><br>0.0130* |

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

a For comparison, in the first study (MRID 50971034) the incidence of adenomas at 0, 10, 100 and 1000 ppm was 2/50, 1/50, 1/50 and 0/50 respectively. No carcinomas were found.

b First adenoma observed at interim sacrifice, dose 4000 ppm.

c First carcinoma observed at interim sacrifice, dose 4000 ppm.

Table 14. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)

Female Thyroid Follicular Cell Tumor Rates+ and
Fisher's Exact Test and Exact Trend Test Results

| Dose (ppm) | 0 | 2000 | 4000 |
|---|---|---|---|
| Adenomas<br>(%)<br><br>P = | 0/50<br>(0)<br><br>0.3332 | 1[a]/48<br>(2)<br><br>0.4898 | 1/50<br>(2)<br><br>0.5000 |
| Carcinomas<br>(%)<br><br>P = | 0/50<br>(0)<br><br>0.1126 | 0/48<br>(0)<br><br>1.0000 | 2[b]/50<br>(4)<br><br>0.2475 |
| Combined<br>(%)<br><br>P = | 0/50<br>(0)<br><br>0.0610 | 1/48<br>(2)<br><br>0.4898 | 3/50<br>(6)<br><br>0.1212 |

+ Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed

before week 54.
a First adenoma observed at week 96, dose 2000 ppm.
b All carcinomas observed at final sacrifice, dose 4000 ppm.

Table 15. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)

Female Hepatocellular Tumor Rates+ and Fisher's Exact Test and Exact Trend Test Results

| Dose (ppm) | 0 | 2000 | 4000 |
|---|---|---|---|
| Adenomas<br>(%)<br><br>P = | 1[a]/50<br>(2)<br><br>0.3703 | 0/48<br>(0)<br><br>1.0000 | 2[a]/50<br>(4)<br><br>0.5000 |
| Carcinomas<br>(%)<br><br>P = | 0/50<br>(0)<br><br>0.1126 | 0/48<br>(0)<br><br>1.0000 | 2[b]/50<br>(4)<br><br>0.2475 |
| Combined<br>(%)<br><br>P = | 1/50<br>(2)<br><br>0.0847 | 0/48<br>(0)<br><br>1.0000 | 4/50<br>(8)<br><br>0.1811 |

+ Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
a All adenomas observed at final sacrifice in the control and 4000 ppm dose groups.
b All carcinomas observed at final sacrifice, dose 4000 ppm.

iv. *Discussion of Non-Neoplastic*

Selected histopathological findings are presented in Table 16 (52 weeks assay) and Table 17 (104 weeks assay). At 52 weeks, the incidence of slight to moderate periportal hepatocyte hypertrophy was significantly increased in males at 4000 ppm (63%) compared to control (0%), but not in females. Also, at 52 weeks, the incidence of slight thyroid follicular cell hypertrophy was increased in 4000 ppm females (67%) compared to controls (0%), but not in males. Males also showed a significant increase in the incidence of slight to moderate colloid degeneration at 2000 ppm, but this was not dose-dependent and was not seen in females at 52 weeks.

At 104 weeks, females showed significantly increased incidences of slight to severe brown pigment deposition and eosinophilic foci of cellular alteration in the liver as well as bile duct hyperplasia at 4000 ppm. Pigments were positive for the Schmorl reaction and negative for Prussian blue stain and were identified as lipofuscin. Males did not show any significant liver effects at 104 weeks.

In the thyroid at 104 weeks, both sexes showed a dose-dependent, increased incidence of colloid degeneration at 2000 ppm and 4000 ppm, while a significant increase in follicular cell hypertrophy occurred in females only at 4000 ppm.

Table 16. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)

Microscopic Findings After Up to 52 weeks of Treatment. [a]

| Dose (ppm) | **0** | **2000** | **4000** |
|---|---|---|---|
| **Males** | | | |
| **Number of tissues examined** | **11** | **12** | **11** |
| Liver, hypertrophy, hepatocyte, periportal, slight to moderate | 0 | 0 | 7** (64%) |
| Thyroid, degeneration, colloid, slight to moderate | 1 | 6* | 5 |
| Thyroid, hypertrophy, follicular cell, slight | 0 | 0 | 3 |
| Thyroid, follicular cell, hyperplasia | 0 | 0 | 0 |
| **Females** | | | |
| **Number of tissues examined** | **12** | **12** | **12** |
| Liver, hypertrophy, hepatocyte, centrilobular, slight | 0 | 0 | 2 |
| Thyroid, degeneration, colloid, slight | 2 | 1 | 4 |
| Thyroid, hypertrophy, follicular cell, slight | 0 | 3 | 8** (67%) |
| Thyroid, follicular cell, hyperplasia | 0 | 0 | 0 |

a Data were obtained from pages 43, 163, 563-598 and 749-784 of MRID 50971037.
Significantly different from control: * $p \leq 0.05$; ** $p \leq 0.01$.

Table 17. Pyraclonil – Sprague Dawley [Crl:CD(SD)BR] Rat Study
(MRID No. 50971037)

Microscopic Findings After Up to 104 weeks of Treatment. [a]

| Dose (ppm) | **0** | **2000** | **4000** |
|---|---|---|---|
| **Males** | | | |
| *Number of tissues examined* | *50* | *50* | *49* |
| Thyroid, follicular cell, hypertrophy | 0 | 0 | 0 |
| Thyroid, follicular cell, hyperplasia | 1 | 0 | 1 |
| Thyroid, degeneration, colloid, any severity | 16 | 30** (60%) | 37** (76%) |
| " " " , unilateral, slight | 2 | 3 | 3 |
| " " " , bilateral, slight | 8 | 15 | 6 |
| " " " , " , slight/moderate | 2 | 4 | 9 |
| " " " , " , moderate | 3 | 5 | 10 |
| " " " , " , slight/severe | | 1 | |
| " " " , " , moderate/severe | | | 1 |
| " " " , " , severe | 1 | 2 | 8 |

| Dose (ppm) | 0 | 2000 | 4000 |
|---|---|---|---|
| **Females** | | | |
| *Number of tissues examined* | *50* | *50* | *50* |
| Liver, deposition, brown pigment (lipofuscin), hepatocyte | 0 | 0 | 12** (24%) |
| Liver, foci of cellular alteration, eosinophilic cell type | 2 | 6 | 25** (50%) |
| Liver, hyperplasia, bile duct | 22 | 30 | 39** (78%) |
| Thyroid, degeneration, colloid, slight | 2 | 12** (24%) | 22** (44%) |
| " " " , unilateral, slight to moderate | 1 | 4 | 3 |
| " " " , bilateral, slight | 1 | 4 | 6 |
| " " " , " , slight/moderate | | 3 | 3 |
| " " " , " , moderate | | 1 | 8 |
| " " " , " , severe | | | 2 |
| Thyroid, hypertrophy, follicular cell, bilateral, slight | 0 | 0 | 10** (20%) |
| Thyroid, follicular cell, hyperplasia | 0 | 1 | 1 |

a Data were obtained from pages 168-169, 412-562, and 599-748 of MRID 50971037.
* $p < 0.05$, ** $p < 0.01$; significantly different as indicated by investigator.

v. *Adequacy of the Dosing for Assessment of Carcinogenicity*

The high doses tested were considered adequate and not excessive in both sexes in the rat, based on increased incidence and severity of thyroid colloid degeneration in both sexes, and increased incidence and severity of thyroid hypertrophy in females at 104 weeks. Increased incidences of thyroid colloid degeneration was also observed at the lower dose in both sexes. In addition, females exposed to 4000 ppm in diet showed significant decreases in mean absolute body weight starting on week 52 (10% compared to controls) and through week 104 (17%). GGT (γ-glutamyl transpeptidase) was increased at the highest dose at 104 weeks in both sexes (288% males, 400% females), and in both dose groups at 52 weeks (78-211% males, 233-467% females). There was no excessive decrease in survival in either sex.

## C. Carcinogenicity Studies in Rats - Conclusions

In male rats, an apparent increased incidence of mesenteric lymph node hemangiomas in the first study (lower doses) was not statistically significant. In the second study (higher doses) male rats had a statistically significant trend (but not pairwise) for systemic histiocytic sarcomas. **The CARC determined the mesenteric lymph node hemangiomas and systemic histiocytic sarcomas in males are not treatment related.**

Also, in male rats, there was a statistically significant increase (trend and pairwise at 4000 ppm) for thyroid follicular cell adenomas and adenomas and/or carcinomas combined. **The CARC determined the thyroid follicular cell tumors are treatment related in males at 4000 ppm (159 mg/kg/day).**

## IV. TOXICOLOGY

### 1. Metabolism

Absorption of a low oral dose (25 mg/kg dose) was rapid, since > 50% was excreted in urine by 6 hours and > 70% by 24 hours, and the concentration in the GI tract (including contents) decreased steadily over the first 12 hours. However, absorption was not complete, since residues could still be detected in the GI tract 72 hours after a low dose. Additionally, after a high oral dose (500 mg/kg/day) the residue concentration in the GI tract was quite high compared to most other tissues, up to 96 hours post dose. Based on urinary and bile excretion data the estimated total absorption is ≥ 84% in males and ≥ 94% in females.

Most of the dose was excreted in the urine. In both sexes, about 70% of the dose was excreted in urine and 25% in feces, regardless of sex or dose level. Data from bile-cannulated rats indicates that 14% (males) to 27% (females) of the dose was excreted in bile. Two peaks in the blood concentration suggest enterohepatic recirculation of the residues. The timing of excretion was different between the sexes. Males excreted a larger percent of the dose during the first 6 hours after exposure. While female excretion was slower, it tended to reach levels similar to those in males by 8 hours following a 25 mg/kg dose, and after 48 hours following a 500 mg/kg dose. Excretion data after 14 daily doses of 25 mg pyraclonil is available, however it is still being reviewed by the agency.

After a low oral dose (25 mg/kg) in both sexes, the GI tract (including contents) had the highest concentration of radioactivity at 0.5, 1 and 4 hours, however by 12 hour the whole blood had the highest concentration. Plasma concentrations were lower than whole blood, indicating some affinity of the test article for the cellular components of blood. Furthermore, the concentration in whole blood displayed two peaks (suggesting enterohepatic recirculation): one at 1 hour and another one at 12 hours. In contrast, plasma concentration peaked only once, at 0.5 hours in males, and at 1 hour in females. The difference in plasma peaks for the sexes is consistent with the differences in excretion, and together the excretion and plasma concentration time courses indicate that female excretion was delayed in the first hour after exposure, compared to males.

Tissue concentrations were also high in the metabolic/excretory tissues (ME tissues; i.e., kidney and liver) of both sexes for the first 4 hours after the oral low dose (25 mg/kg/day). Outside the ME tissues and the GI tract, there was a slight sex difference in which tissues had the highest residues at 4 hours: adrenals in both sexes; prostate, spleen and epididymis in males; thyroid and ovaries in females. By 12 hours post dose, concentrations in most tissues of both sexes (including fat) were similar to each other and quite low, indicating that pyraclonil residues do not accumulate in tissues.

Similar to the lower dose, with a higher dose (500 mg/kg) the GI tract (including contents) had the highest concentration of radioactivity in both sexes at early time points. However, differently from the low dose, whole blood did not have the highest concentration at any time. Instead, the GI tract had the highest concentration of any tissue through the end of the assay, albeit concentrations decreased steadily, suggesting continuous absorption. Whole blood and plasma concentrations were similar to each other and to most other low concentration tissues. Plasma

and liver concentrations peaked at 2 hours in both sexes, as also did concentrations in most other non-ME tissues. Whole blood concentrations peaked at 2 hours in males, and at 8 hours in females.

While at the low dose, tissue concentrations were higher in the ME tissues of both sexes than other tissues, after a high oral dose the concentration in the thyroid was higher than in the ME tissues, with some sex-specific differences. In males, the residue concentration in the thyroid peaked at 0.5 hours and was higher than any other tissue (except GI tract) at 0.5 and 2 hours, and still higher than in most tissues (except ME and GI tract) thru 30 hours post dose. In female thyroid, residue concentrations peaked at 2 hours and were higher or similar to the concentration in ME tissues thru 16 hours post dose.

As with the low oral dose, the adrenal gland of both sexes also had a high concentration of residues, as did the prostate and seminal vesicles in males, and the ovaries in females. Differently from the low dose, at the high dose, relatively high concentrations were detected also in the pituitary and lungs of both sexes. These tissues retained higher concentrations than blood or plasma up to 30 hours post dose. By 54 hours post dose, concentrations in most tissues of both sexes (including fat) were similar and quite low, indicating that pyraclonil residues do not accumulate in tissues. A significant exception was in females, were the GI tract still showed much higher concentrations than other tissues up to the last time point measured (96 hours). Also in females, the ME tissues and the carcass had higher concentrations that other tissues at 54 hours. This data suggests that at a high oral dose, absorption may be delayed in females, compared to males.

Pharmacokinetic parameters for pyraclonil were estimated in two independent studies, however the results from each study seem to be only partially in agreement with each other. Further review of this data is needed. Tissue distribution data indicates two concentration peaks, therefore the time of maximum blood concentration ($T_{max}$) occurred at 1 hour and 12 hours in both sexes.

The major metabolites were similar in both sexes, however there was some difference between the sexes in minor metabolites. Structures of major metabolites were determined using nuclear magnetic resonance (NMR). There was no evidence of cleavage of the pyraclonil molecule between its tetrahydropyrazole ring (THP) side and its N-(methyl)propargyl (PG) side. In general, pyraclonil was mono-, di- or tri-hydroxylated at its THP side of the molecule, and in some cases the THP ring was opened into a carboxylic acid. In some metabolites, the PG chain was removed. Of the major urinary metabolites, 5 were found in both sexes, while one was a major metabolite in females, but a minor one in males. The overall fecal metabolite profile was similar to urine with an additional non-polar peak that co-chromatographed with unmetabolized pyraclonil and three additional peaks containing two unknowns and a trihydroxy without the PG chain (metabolite J). Most of the fecal metabolites were found in both males and females, with few exceptions.

**2. Mutagenicity**

The database of genotoxicity and mutagenicity studies is extensive, and most of the studies did

not indicate a mutagenic concern for pyraclonil, as indicated by the study summaries in Table 18. There was evidence of chromosomal aberrations in two *in vitro* studies conducted in human lymphocytes (MRIDs 50971047 and 50971048) and one study conducted in Chinese hamster cells (50971049). However, there was no evidence of chromosomal aberrations was observed in another *in vitro* study conducted in Chinese hamster cells (50971046). When pyraclonil was tested *in vivo*, no evidence of clastogenicity was seen in two *in vivo* micronucleus assays. Overall, the concern for mutagenicity in vivo is low.

Table 18. Pyraclonil – Summary of Genotoxicity and Mutagenicity Studies

| Guideline No. | Study Type | MRID No. (year)/ Classification/Doses | Results |
|---|---|---|---|
| 870.5100 | Bacterial Reverse Mutation Test (*Salmonella typhimurium, Escherichia coli*) | 50971043 (1996)<br>Acceptable/guideline<br>0, 4, 20, 100, 500, 2500, or 5000 µg/plate (±S9) | There was no evidence of induced mutant colonies over background. |
| 870.5300 | *In Vitro* Mammalian Cell Gene Mutation Test (Chinese hamster ovary (CHO) cells) | 50971045 (2018)<br>Acceptable/guideline<br>0, 125, 250, 500, 1000, 1500, and 2000 µg/mL (±S9) | There was no evidence of induced mutant colonies over background in the presence or absence of S9 activation. |
| 870.5375 | *In Vitro* Mammalian Chromosome Aberration Test (Chinese hamster lung (CHL) cells) | 50971049 (2003)<br>Acceptable/guideline<br>0, 193, 231 or 278 µg/mL +S9 or -S9 for 6 h exposure, 18 h unexposed<br>0, 13, 25, 50 or 100 µg/mL -S9 for 24 h continuous exposure<br>0, 6.3, 13, 25 or 50 µg/mL -S9 for 48 h continuous exposure | There was evidence of chromosome aberrations induced over background in the presence and absence of S9 activation.<br>In the presence and the absence of S9 activation, increases in the percentage of cells with aberrations were observed at 231 and 278 µg/mL compared to the solvent control. However, at 278 µg/mL aberrations co-occurred with cytotoxicity. |
| 870.5375 | *In Vitro* Mammalian Chromosome Aberration Test (Chinese hamster lung (CHL) cells) | 50971046 (2018)<br>Acceptable/guideline<br>0, 120, 140, 160, 180, 200, and 220 µg/mL -S9 for 6 h exposure, 18 h unexposed<br>0, 180, 220, 260, 300, 340, and 380 µg/mL +S9 for 6 h exposure, 18 h unexposed<br>0, 10, 20, 40, 60, and 80 µg/mL −S9 for 24 h continuous exposure | There was no evidence of chromosome aberrations induced over background. |

| Guideline No. | Study Type | MRID No. (year)/ Classification/Doses | Results |
|---|---|---|---|
| 870.5375 | *In Vitro* Mammalian Chromosome Aberration Test (human lymphocytes) | 50971047 (2000)<br>Acceptable/guideline<br>0, 800, 1600 or 3148 µg/mL +S9 for 6 h exposure, 18 h unexposed<br>0, 100, 500 or 1000 µg/mL -S9 for 24 continuous exposure<br>Batch # R001512 | There was evidence of chromosome aberrations induced over background in the absence of S9 activation.<br>In the absence of S9 and excluding gaps, percent aberrant cells was significantly increased at 100 µg/mL (8.0%) compared to the solvent control (1.5%), in the absence of precipitation and with a relative mitotic index above 50%. Other doses showed no increase in aberrant cells or showed precipitation and/or a relative mitotic index > 50%. |
| 870.5375 | *In Vitro* Mammalian Chromosome Aberration Test (human lymphocytes) | 50971048 (2000)<br>Acceptable/guideline<br>0, 800, 1600 or 3148 µg/mL +S9; 6 h exposure, 18 h unexposed<br>0, 100, 200 or 400 µg/mL -S9 for 6 h exposure, 18 h unexposed<br>0, 25, 100 or 200 µg/mL -S9 for 24 h continuous exposure<br>0, 50, 100 or 200 µg/mL -S9 for 48 h continuous exposure<br>Batch # 960202 | There was evidence of chromosome aberrations induced over background in the presence and absence of S9 activation.<br>In the presence of S9, increases in the percentage of cells with aberrations were observed at 800 µg/mL. In the absence of S9 activation, increases in the percentage of cells with aberrations were observed at 100 and 400 µg/mL. Other doses showed cytotoxicity, precipitation, or were within historical control ranges. |
| 870.5395 | *In Vivo* Mammalian Erythrocyte Micronucleus Test (mouse) | 50971050 (1997)<br>Acceptable/guideline<br>0, 150, 300 or 600 mg/kg | There was no increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time. |
| 870.5395 | *In Vivo* Mammalian Erythrocyte Micronucleus Test (mouse) | 50971051(1996)<br>Acceptable/guideline<br>0 or 900 mg/kg | There was no significant increase in the frequency of micronucleated erythrocytes at either sampling time. |
| 870.5500 | Bacterial DNA Damage or Repair Tests (*Bacillus subtilis*) | 50971052 (1997)<br>Acceptable/guideline<br>50, 150, 500, 1500 or 5000 µg/disk ± S9 | There was no evidence that DNA damage was induced. |
| 870.5550 | Unscheduled DNA Synthesis in Mammalian Cells in Culture (rat hepatocytes) | 50971053 (1996)<br>Acceptable/guideline<br>0, 600 or 2000 mg/kg by gavage to live rats; hepatocytes harvested at 2 or 14 hours after dose | There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures, was induced. |

## 3. Structure-Activity Relationship

Structure activity relationship data were not submitted for the registration of pyraclonil. A search of the ChemID*plus* database showed that 97 chemicals had between 60% and 66% structural similarity; however, carcinogenicity toxicity studies are not available for most of them. The chemical with the highest similarity to pyraclonil that also had carcinogenicity studies is cyantraniliprole (61.0% similarity), a ryanoid pesticide classified as "not likely to be carcinogenic to humans" based on the absence of increased tumor incidence in acceptable/guideline carcinogenicity studies in rats and mice, and there are no mutagenicity concerns. However, cyantraniliprole is not in the same chemical class as pyraclonil.

Pyraclonil has been classified as a pyrazole herbicide, however its structure is < 60% similar other pyrazole herbicides (per ChemID*plus*). Two other pyrazole pesticides are registered: halosulfuron-methyl (PC 128721) and pyroxasulfone (PC 090099). Halosulfuron-methyl was classified as Group E (no evidence of cancer in two species) in 1994. Pyroxasulfone was classified as "not likely to cause cancer at doses below those causing urinary calculi" (in male rat) in 2011. For pyroxasulfone, there were no tumors in mice and other tumors evaluated in rat by CARC were as follows: thyroid follicular cell and adrenal cortex tumors were not considered treatment related by CARC (statistically significant but within historical controls); additionally, mammary gland tumors did not occur in a dose-dependent manner and the high dose was within historical controls.

Pyraclonil has also been classified as a nitrile herbicide, however its structure is < 60% similar other nitrile herbicides (per ChemID*plus*). Two other nitrile pesticides are registered: bromoxynil (PC 035301) and dichlobenil (PC 027401). Bromoxynil is classified as Group C (possible human carcinogen) based on the increases in liver tumors in both sexes of the CD-1 mouse. Dichlobenil is classified as Group C (possible human carcinogen) based on hepatocellular tumors in both sexes of the rat, but only at an excessive dose in the male rat.

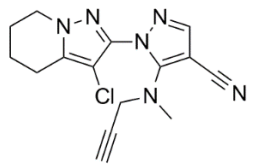
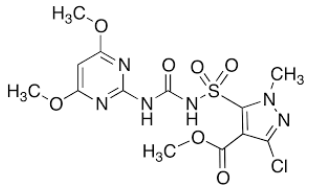
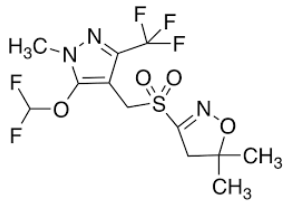
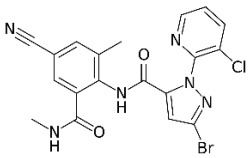
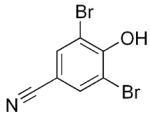
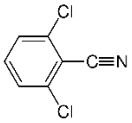
| Pyraclonil | Halosulfuron-methyl | Pyroxasulfone |
|---|---|---|
|  |  |  |
| **Cyantraniliprole** | **Bromoxynil** | **Dichlobenil** |
|  |  |  |

**Figure 2. Structures of pyraclonil and related chemicals.**

## 4. Subchronic and Chronic Toxicity

### A. Subchronic Toxicity

i. 90-Day oral toxicity study in mice

In a subchronic oral toxicity study (MRID 50971017), groups of ten Sprague Dawley CD-1 mice/sex/dose were administered SN 172391 (pyraclonil; 97.2% a.i.; lot # CR 21693 /01/960202) via the diet at nominal dose levels of 0, 20, 200, or 2000 ppm (equivalent to 0, 3.0, 28 or 302 mg/kg/day in males; 0, 4.1, 39 or 379 mg/kg/day in females) for up to 13 weeks. All significant findings occurred at the high dose. Male body weights were significantly decreased (5-14% compared to control), from week 4 to the end of the study, with the decrease becoming greater over time. Weekly food conversion (g of weight gained per g of food consumed) on weeks 3-13 tended to be lower in high dose males (range -0.1 to 3.0) than in control males (0.1-4.1). Total protein, albumin and cholesterol were increased in females only and correlate with liver pathological findings discussed below. Absolute and relative (to body) liver weights were significantly increased in females. In males, absolute liver weights were not increased; however, relative liver weights were statistically increased at a larger magnitude than the decrease in body weight, which indicates a toxicologically relevant increase in liver weight. In the liver, an increased incidence of slight to severe hepatocyte enlargement was noted in males, while an increased incidence of minimal to moderate centrilobular fat deposition was noted in the centrilobular hepatocytes of females. The changes in male liver are adaptive changes (not adverse).

This study is classified acceptable/guideline and satisfies the guideline requirements for a subchronic oral toxicity study (OCSPP 870.3100; OECD 408) in the mouse.

ii. 90-Day oral toxicity study in rat with neurological assessment

In a subchronic oral toxicity study (MRID 50971018), groups of ten Sprague-Dawley [Crj:CD(SD)] rats/sex/dose were administered SN 172391 (pyraclonil; 98.09% a.i.; lot # PP230081/2) via the diet at nominal dose levels of 0, 40, 2000, or 4000 ppm (equivalent to 2.2/2.4, 108/120, and 211/222 mg/kg/day in males/females) for up to 13 weeks.

There were no effects of treatment on mortality, clinical signs, grip strength, motor activity, sensory reactivity or functional observational battery (FOB) parameters, ophthalmoscopic examinations, urinalysis, or gross pathology.

Systemic toxicity was observed at 2000 ppm. In the females, significant treatment-related effects included increased total cholesterol (19%), increased absolute and relative thyroid (33-36%) and liver (17-20%) weights, and follicular cell hypertrophy in the thyroid (6/10 compared to 0/10 controls). In the males, treatment-related effects were limited to increased gamma glutamyl transferase (GGT) (2X control) during clinical chemistry observations.
At 4000 ppm, in the females, mean body weights were decreased by 8-11% compared to the control throughout treatment. There were no corresponding decreases in body weights in the males. Hematology findings in the males were limited to an increase in prothrombin time;

however, in isolation this finding is incidental. In the females, there were marginal (<10% change from control) decreases in hematocrit and hemoglobin concentration, and more substantial increases in platelet (29%) and reticulocyte (45%) counts coupled with increased relative spleen weight (17%), enhanced hematopoiesis in the bone marrow and spleen, as well as brown pigment deposition and congestion in spleen. Therefore, there is some evidence that treatment-related anemia was associated with hemolysis. However, since no obvious changes were observed in erythrocyte count, the overall slight hematological response is considered adaptive.

Clinical chemistry effects at 4000 ppm included increased GGT and decreased total bilirubin in the males and females. Additionally, in the females, increased total cholesterol and decreased triglyceride were observed. Together with the increase in male and female liver weights (21-41%) and liver hypertrophy in both sexes (7/10 males, 9/10 females), the clinical chemistry effects are adverse.

Thyroid effects at 4000 ppm in both sexes included increases in thyroid weight (39-48%) and thyroid follicular cell hypertrophy (7/10 males; 7/10 females). In 6/10 males (compared to 0/10 controls) the thyroid was grossly enlarged at necropsy. These co-occurring thyroid effects are considered adverse in the absence of hormone analyses. In females, significant increases in thyroid weight and incidence of thyroid follicular hypertrophy were also evident at 2000 ppm. Two out of 10 males examined at 2000 ppm also had thyroid follicular cell hypertrophy (compared to 0 controls).

This study is classified acceptable/guideline and satisfies the guideline requirements for a subchronic oral toxicity study (OCSPP 870.3100; OECD 408) in the rat.

iii. 90-Day oral toxicity study in rat with 4 weeks off to observe reversibility of effects

In a subchronic oral toxicity study (MRID 50971020), groups of ten Sprague-Dawley (CD CRL:CD BR) rats/sex/dose were administered SN 172391 (pyraclonil; 97.2% a.i.; lot # CR 21693/ 01/ 960202) via the diet at nominal dose levels of 0, 40, 2000, or 4000 ppm (equivalent to 0, 2.9, 154 or 324 mg/kg/day in males; 0, 3.9, 207 or 433 mg/kg/day in females) for up to 13 weeks. Additional groups of ten rats/sex were fed 0 or 4000 ppm for 13 weeks and then control diet for a further 4 weeks to examine possible reversibility of any observed effects.

Most significant findings occurred at the high dose. All males and 65% of females had soiled tails, and hair loss was noted in 60% of females and 20% of males. Both soiling and hair loss were noted for more than one third of the study days in females (soiled tail only for this duration in males), and occurred towards the end of the 13 week and 13 week + 4 week off-dose period.

Mean body weight in males was significantly decreased (15-18%) compared to controls during treatment (week 1-13) and remained lower than the control (14-16%) after treatment stopped. Male weekly body weight gain was decreased on weeks 1-5 and 13 of treatment; however, when treatment stopped, males on average gained more weight than controls. Females also showed significant reductions in mean body weight during treatment (11-17%); however, the difference was largest on week 1 and became less pronounced as the treatment progressed. After treatment

stopped, females had similar body weight to controls. Females lost weight on week 1; however, they had similar or higher body weight gain than controls for the remainder of the study.

Male food consumption was decreased sporadically (3-27%) during treatment. Female food consumption was decreased by 8-28% the first few weeks of treatment only and then returned to levels similar to those of the control; the only exception was a large decrease (18%) at week 13. In both sexes, the largest decrease was on week 1 (27-28%). After treatment ended, mean food consumption was similar to controls.

Food conversion (g body weight change per g food consumed) was decreased on week 1 for both sexes. Male food conversion was less than half that of controls, and females lost weight. Throughout the rest of the treatment period, food conversion was lower than controls in males only during weeks 4 to 8. Females had sporadic increases in food conversions during weeks 2-13, although the values were close to controls. After treatment ended (weeks 14-17), both sexes had higher food conversion than their corresponding controls.

At 13 weeks, cholesterol was increased at the mid and high doses, in a dose-dependent manner, in both sexes. This effect correlated with histopathology findings of the liver (increased incidence of minimal to slight liver hypertrophy) only in males at the high dose. However, the hepatocyte hypertrophy observed microscopically in males did not translate into significant liver weight increases in that sex (relative but not absolute liver weight was increased). On the other hand, absolute and relative liver weights were significantly increased in females at ≥2000 ppm without a significant increased incidence in hepatocyte hypertrophy.

At 13 weeks, thyroid follicular cell hypertrophy and colloid depletion were significantly increased in females at 2000 ppm (not statistically significant but accompanied by an increase in severity) and 4000 ppm (statistically significant). There was also a dose-dependent trend (ANOVA $p < 0.05$) in the mean severity of these findings in females. Additionally, males appear to show increased mean severity of the same findings at the high dose, but the trend is not statistically significant. In general, male rats were shown to have a high background of thyroid findings in other guideline studies.

This study is classified acceptable/guideline and satisfies the guideline requirements for a subchronic oral toxicity study (OCSPP 870.3100; OECD 408) in the rat.

iv. <u>Reproductive and Fertility Effects Study (parental effects only)</u>

In a two-generation reproduction and fertility study (MRID 50971032), groups of 24 Sprague-Dawley (Crl:CDBR) rats/sex/dose were administered AE B172391 (pyraclonil; 97.0-98.1% a.i.; batch #: AE B172391 00 1C99 0003) at dose levels of 0, 10, 100, or 1000 ppm for two successive generations (equivalent to premating levels of 0/0, 0.7/0.9, 7.3/9.2, or 74/88 mg/kg/day for P males/females; 0/0, 1.1/1.2, 11/13 or 108/122 mg/kg/day for F1 males/females; gestation levels of 0, 0.8, 8.8, or 100 mg/kg/day for P females and 0, 0.9, 8.9, or 96 mg/kg/day for F1 females; and lactation levels of 0, 1.5, 15, or 159 mg/kg/day for P females and 0, 1.5, 16, or 157 mg/kg/day for F1 females). The P generation rats were fed the test diets for at least 10 weeks prior to mating to produce the F1 litters. On post-natal day (PND) 21, one F1 pup/sex/litter was selected up to a total

of 30 F1 rats/sex/group and fed the same test diet as its dam (following weaning on PND 21) for at least 78 days prior to mating to produce the F2 litters.

There were no effects of treatment on mortality; clinical signs; premating, gestational or lactational body weights or body weight gains; food consumption; organ weights; or gross pathological findings. The incidence of slight follicular cell hypertrophy of the thyroid was increased in the 100 and 1000 ppm P males (6/24 and 10/24 treated, respectively, vs. 3/24 control), 1000 ppm P females (14/24 treated vs. 2/24 control), 1000 ppm F1 males (20/24 treated vs. 4/24 control), and 100 and 1000 ppm F1 females (7/24 and 12/24 treated, respectively, vs. 3/24 control).
The LOAEL for parental toxicity is 74 mg/kg/day (1000 ppm) based on increased incidence of thyroid follicular cell hypertrophy in P and F1 generations. The NOAEL for parental toxicity is 13 mg/kg/day (100 ppm).

This study is classified acceptable / guideline and satisfies the guideline requirements for a two-generation reproductive study (OCSPP 870.3800; OECD 416) in rats.

## B. Chronic Toxicity

### i. Carcinogenicity study in mice (low doses)

In a carcinogenicity study (MRID 50971036), groups of 52 CD-1 mice/sex/dose were administered AE B172391 Technical (pyraclonil; 98.0-98.1% a.i.; lot # AE B172391 00 1C99 0003) in the diet at dose levels of 0, 5, 50, or 500 ppm (equivalent to 0, 0.68, 6.7 or 68 mg/kg/day in males, and 0, 0.83, 8.6, and 87 mg/kg/day in females) for 18 months (83 weeks).

There were no treatment-related effects on mortality, clinical signs, palpable masses, body weight, food consumption or conversion, hematology parameters, organ weights, or macroscopic or microscopic pathology findings. Survival through week 78 was 56%, 62%, 63%, and 63% for males and 71%, 73%, 73%, and 81% for females in the 0, 5, 50, and 500 ppm groups, respectively.

This study is classified acceptable / guideline and satisfies the guideline requirements for a carcinogenicity study [OCSPP 870.4200b; OECD 451] in mice when considered together with MRID 50971035.

### ii. Carcinogenicity study in mice (high doses)

In a carcinogenicity study (MRID 50971035), groups of 16 (52-week treatment; satellite group) and 52 (78-week treatment; main group) ICR [Crl:CD1(ICR)]mice/sex/dose were administered pyraclonil (97.81% a.i.; lot # ANBP02Y01) in the diet at dose levels of 0, 1000, or 2000 ppm (equivalent to 0/0, 109/109, and 212/229 mg/kg/day in the 52-week group males/females and 0/0, 102/100, and 210/204 mg/kg/day in the 78-week group males/females).

There were no adverse effects on mortality, clinical signs, body weight or clinical chemistry. At week 52 (satellite group), absolute liver weights were increased by 30% at 1000 ppm and 40% at 2000 ppm in male mice but not in female mice. At week 78 (main group), absolute and relative

liver weights were increased in the 2000 ppm females, by 27% and 32%, respectively.

Treatment-related macroscopic findings included an increased ($p \leq 0.01$) incidence of dark colored livers in the 2000 ppm male mice (satellite and main groups). In the 2000 ppm main group females, treatment-related macroscopic findings in the liver included increased incidences of liver mass(es), spot(s), and enlarged livers. In some females these changes correlated with incidences of hepatic eosinophilic foci of cellular alteration at 1000 ppm and 2000 ppm (main group), which was also increased in incidence at the same doses ($p < 0.05$ and $p < 0.01$, respectively). Some hepatocellular hypertrophy was occasionally observed in males at ≥1000 ppm (satellite and main groups), but these sporadic findings were not considered adverse, as there were no changes in liver weights, clinical chemistry, or the incidence of neoplastic lesions for males.

This study is classified acceptable / guideline and satisfies the guideline requirements for a carcinogenicity study [OCSPP 870.4200b; OECD 451] in mice when considered together with MRID 50971036.

iii. Chronic/carcinogenicity study in rats (low doses)

In a combined chronic toxicity / carcinogenicity study (MRID 50971034), groups of 70 Sprague Dawley [Crl:CD(SD)BR] rats/sex/dose were administered AE B172391 (pyraclonil; 97.0-98.5% a.i.; batch # AE B172391 00 1C99 0003) in the diet at dose levels of 0, 10, 100, or 1000 ppm (equivalent to 0/0, 0.44/0.56, 4.4/5.8, or 44/58 mg/kg/day in males/females) for up to 104 weeks. After 52 weeks of treatment, 20 rats were euthanized for assessment of clinical pathology and microscopic findings. All other rats were treated for an additional 52 weeks (104 weeks total).

There were no effects of treatment on mortality, clinical signs, food consumption or food efficiency, body weight, ophthalmoscopic examinations, hematology, clinical chemistry, urinalysis, or gross pathology.

In the 1000 ppm main study males, absolute and relative liver weights were increased by 13% and 19%, respectively. These increases were corroborated by microscopic findings of minimal centrilobular hypertrophy in 1/19 decedent rats and 8/31 terminal euthanasia rats (9/50 total rats) compared to 1/50 control. However, in this case, the incidence of centrilobular hypertrophy is an adaptive (not adverse) response since no other liver lesions or corresponding clinical chemistry changes were detected.

In the 1000 ppm females, sertoliform tubular hyperplasia of the ovary was observed. Sertoli cell hyperplasia is thought to be derived from stromal cells in atrophic ovaries. This finding is associated with aging but may also be induced by chemicals. Because the lesion did not progress to tumors, in this case it is not adverse.

This study is classified acceptable/guideline and satisfies the guideline requirements for a chronic toxicity / carcinogenicity study (OCSPP 870.4300; OECD 453) in rats when considered together with MRID 50971037.

iv. Chronic/carcinogenicity study in rats (high doses)

In a combined chronic toxicity / carcinogenicity study (MRID 50971037), groups of 50 Sprague Dawley [Crl:CD(SD)] rats/sex/dose were administered pyraclonil (97.81% a.i.; lot # ANBP02Y01) in the diet at dose levels of 0, 2000, or 4000 ppm (equivalent to 0/0, 77/99 or 159/208 mg/kg/day in males/females) for up to 104 weeks (main study). A second group of 12 rats/sex/dose were administered pyraclonil at the same dose levels for up to 52 weeks.

There were no effects of treatment on mortality, clinical signs, palpable masses, or food consumption. There were no adverse decreases in body weight in males or females treated for up to 52 weeks, or in males treated up to 104 weeks. Females exposed to 4000 ppm in diet showed significant decreases in mean absolute body weight starting on week 52 (10% compared to controls) and through week 104 (17%).

GGT (γ-glutamyl transpeptidase) was increased at both doses of pyraclonil, in a dose-dependent manner, in both sexes at 52 weeks (78-211% males, 233-467% females), but only at the highest dose at 104 weeks (288% males, 400% females). Other changes in enzymes or cholesterol levels were not biologically significant or occurred only at 52 weeks (i.e., did not persist). The incidence of liver hypertrophy was significantly increased in males only at 52 weeks, but not at 104 weeks. At 104 weeks, females showed increased incidence of the following liver findings: lipofuscin deposition, foci of cellular alteration (eosinophilic) and bile duct hyperplasia at 4000 ppm. There were significant increases in absolute and relative liver weights of both sexes at 4000 ppm in the satellite and main study groups, and in both sexes at 2000 ppm in the main study group only. Periportal hepatocellular hypertrophy was observed in males at 4000 ppm in the satellite study, and females showed centrilobular hepatocellular hypertrophy.

At 52 weeks, the following thyroid-related effects were found in high dose males only: T3 and TSH were increased (40% and 68%, respectively); mean absolute and relative thyroid weights were significantly increased at 4000 ppm (54% and 44%, respectively, compared to controls). Follicular cell hypertrophy was also seen in high dose males at 52 weeks. On the other hand, females showed an increased incidence of follicular cell hypertrophy without any other thyroid effects at 52 weeks at both doses. By week 104, only TSH was increased (43%) in high dose males, and thyroid weights were not significantly increased compared to controls. Meanwhile in females, T4 was increased (25%), both absolute and relative thyroid weights were significantly increased (133% and 184%, respectively), and the incidence of follicular hypertrophy was still increased (but only at the highest dose), compared to controls, on week 104. Both sexes had a dose-dependent significant increase in incidence and severity of thyroid colloid degeneration at 2000 and 4000 ppm at 104 weeks, while males had this same increase at 52 weeks as well for both doses.

This study is classified acceptable/guideline and satisfies the guideline requirements for a chronic toxicity / carcinogenicity study (OCSPP 870.4300; OECD 453) in rats when considered together with MRID 50971034.

## C. Other Relevant Studies

### i. 14-Day oral toxicity in rat with thyroid measures

In a study to examine treatment-related changes on the liver and thyroids (MRID 50971015), groups of 16 Sprague-Dawley (Crj:CD SD) rats/sex/dose were administered pyraclonil (98.09% a.i.; lot # PP230081/2) via the diet at nominal dose levels of 0, 40, 2000, or 4000 ppm (equivalent to 0, 3.3, 161 or 298 mg/kg/day in males; 0, 3.2, 152 or 265 mg/kg/day in females) for 14 days. Body weight, food consumption, blood thyroid hormone concentrations, and liver and thyroid pathology were assessed after 1 or 2 weeks of treatment. A part of the liver was sampled and frozen at necropsy for measurement of hepatic enzymes.
There were no unscheduled deaths or clinical signs at any dose level. In the 4000 ppm group, body weights for both sexes were 5-8% lower than those in the control group at week 1. Females showed 7% lower body weights also at week 2. Food consumption for both sexes was significantly lower than that of the controls on week 1.

Effects on liver weight and enzyme activity indicate an adaptive response to pyraclonil. Liver weights were increased in a dose-dependent manner at 2000 and 4000 ppm, and in a time-dependent manner at weeks 1 and 2, in both sexes. At 4000 ppm, both sexes showed increased incidences of diffuse hepatocellular hypertrophy after one and two weeks of treatment. At 2000 ppm, males also showed increased incidence of hepatocellular hypertrophy on week 1, while females showed the same effect on week 2. CYP1A1 activity was significantly increased in both sexes at 4000 ppm after 1 and 2 weeks of treatment. In females, CYP1A1 activity was also significantly increased at 2000 ppm on week 1. CYP2B1 activity was significantly increased in males at 4000 ppm after 1 and 2 weeks of treatment, while in females it was increased only after 2 weeks of treatment at both 2000 and 4000 ppm. CYP3A2 activity was significantly increased in both sexes at 2000 and 4000 ppm, after 1 and 2 weeks of treatment, however the magnitude of the increase was much larger in females than in males. UDPGT activity against T3 was significantly different from the control only in females, and only on week 2 at the 2000 and 4000 mg/kg/day. Conversely, UDPGT activity against T4 was significantly increased in both sexes at 2000 and 4000 ppm, after 1 and 2 weeks of treatment.

Changes in thyroid hormones, thyroid weight and histopathology indicate thyroid toxicity. At 4000 ppm, males and females showed a significant decrease in blood T4 concentration (51% and 36%, respectively) after 1 week of treatment, with a concomitant increase in TSH (41%) in males only. At the same dose after 2 weeks of treatment, females had a significant decrease in T4 (38%) together with a significant increase in TSH (47%). Males also had a significant increase in TSH (109%), however their T4 levels were within the variability of the controls.

At 2000 ppm, males show a significant decrease in T4 (32%) and concomitant increase in TSH (54%) after one week of treatment, however females do not show changes in T4 or TSH at one week. At the same dose after 2 weeks of treatment, TSH was increased in both males and females (54% and 37%, respectively) while T4 was similar to controls in both sexes.

In males, thyroid weights increased in a dose- and time-dependent manner. At 2000 and 4000 ppm, relative (to body weight) thyroid weights show some increase (15-17%) after one

week of treatment. After 2 weeks both absolute and relative thyroid weights were significantly increased in males (32-40% and 37-46%, respectively).

Females also had dose-dependent increases in thyroid weight at 2000 and 4000 ppm; however, they showed the opposite temporal trend to males. The most significant increase in female thyroid weights occurred after one week of treatment (21% absolute, 24-33% relative), while after two weeks the increase manifested mainly at 4000 ppm (22% absolute, 32% relative).

At 4000 ppm, the incidence of thyroid follicular epithelial cell hypertrophy was increased (5/8 vs. 0/8 controls) in both sexes after 2 weeks of treatment. One male and 2 females (n= 8) also had the lesion at 2000 ppm. Thyroid follicular cell hypertrophy did not occur in any animals at doses below 2000 ppm. After one week of treatment, only 2 females at 4000 ppm showed the lesion.

The LOAEL was 2000 ppm (equivalent to 152 mg/kg/day) based on increased T4-specific UDPGT activity in the liver of both sexes; decreased blood levels of T4 and increased levels of TSH in males; and increased incidence of thyroid follicular hypertrophy and thyroid weights in both sexes. The NOAEL is 400 ppm (equivalent to 3.3 mg/kg/day). Liver activity of CYP1A1, CYP2B1 and CYP3A2 were increased in both sexes at 4000 ppm. CYP3A2 activity was increased in both sexes at 2000 ppm. In females only, CYP2B1, CYP1A1 (week 1 only) and T3-specific activities were increased at 2000 ppm. Also in females only, UDPGT at 2000 ppm on week 2 only, and both weeks at 4000 ppm.

This special study is classified acceptable/non-guideline and follows some of the guidelines for a repeated dose 28-day oral toxicity study (OCSPP 870.3050; OECD 407) in the rat.

### 5. Mode of Action Studies

There is no postulated mode of action for pyraclonil.

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

### 1. Carcinogenicity

Mice:

*Malignant Lymphomas*: The CARC identified observed increases in malignant lymphomas in female mice. However, since lymphomas are systemic tumors and only the liver and thyroid were evaluated in the second study, CARC concluded that the malignant lymphoma findings were inconclusive.

*Liver Tumors:* In the second study (MRID 50971035), there were statistically significant trends, and statistically significant pair-wise comparisons of the 2000 ppm group with the controls, for hepatocellular adenomas (19% vs. 2% controls), and hepatocellular adenomas and/or carcinomas combined (21% vs. 2% controls), all at $p < 0.01$. The incidence of adenomas at 2000 ppm exceeded both the concurrent control and the historical control range for the preforming

laboratory (0-3.9%; 2013-2015). Livers were fully evaluated in this study, and the tumor findings were supported by non-neoplastic findings of the liver. **The CARC concluded that the liver adenomas and adenomas/carcinomas combined are treatment related in females at the high dose (204 mg/kg/day). No treatment related tumors were seen in male mice.**

The doses were considered adequate when both studies are considered together. The first carcinogenicity study in mice (MRID 50971036) did not show any effects of treatment. A second mouse carcinogenicity study (MRID 50971035) was conducted at higher dose levels following the DART recommendations. The highest dose tested of 2000 ppm was considered adequate and not excessive in both sexes in the mouse. Liver weights and the incidence of dark colored livers were increased in males at 2000 ppm compared to controls. In females, treatment-related macroscopic findings in the liver included increased incidences of liver mass(es), spot(s), and enlarged livers. In some females these changes correlated with incidences of hepatic eosinophilic foci of cellular alteration at 1000 ppm and 2000 ppm, which was also increased in incidence and severity at the same doses. There was no increased mortality in treatment groups compared to controls. When considered together, the first and second mouse carcinogenicity studies are acceptable/guideline.

Rats:

*Mesenteric Lymph Node Tumors*: In the first study (MRID 50971034), hemangiomas of the mesenteric lymph node were evaluated in male rats, but there were no statistically significant trends or pair-wise comparisons in the data analyzed. Also, in the first study, there were no compound-related tumors observed in female rats. There was no dose-response and not much difference from low to high dose despite a 100X increase in dose. The doses used in the first study were not adequate (based upon DART conclusions). The DART determined that an additional study with limited evaluation of liver and thyroid should be generated similar to mouse (MRID 50971037). **The CARC determined the mesenteric lymph node hemangiomas are not treatment related at the high dose in males (1000 ppm or 44 mg/kg/day).**

*Histiocytic Sarcomas*: In the second study (MRID 50971037), male rats had a statistically significant trend for systemic histiocytic sarcomas at $p < 0.05$, but no pairwise significance, and no effect for mononuclear leukemia at 4000 ppm. There was low incidence and no statistical significance for these tumors in females. **The CARC determined the mesenteric systemic histiocytic sarcomas are not treatment related at the high dose in either sex (4000 ppm or 159 mg/kg/day in males, 208 mg/kg/day in females).**

*Liver Tumors*: In the second study (MRID 50971037), liver tumors were evaluated in female rats, but there were no statistically significant trends or pair-wise comparisons in the data analyzed. All adenomas and carcinomas in females were observed at final sacrifice. **The CARC determined the liver tumors are not treatment related in females at the high dose (4000 ppm or 208 mg/kg/day).**

*Thyroid Tumors*: In the second study (MRID 50971037), there was a statistically significant trend, and a statistically significant pair-wise comparison of the 4000 ppm group with the controls, for thyroid follicular cell adenomas in males, both at $p < 0.05$. There was also a

statistically significant trend at $p < 0.01$, and a statistically significant pair-wise comparison of the high dose group with the controls at $p < 0.05$, for thyroid follicular cell adenomas and/or carcinomas combined in males. The thyroids were fully evaluated in this study, and the tumor findings were supported by non-neoplastic findings of the thyroid. **The CARC determined the thyroid follicular cell adenomas are treatment related in males at the high dose (159 mg/kg/day).** Thyroid follicular cell tumors were evaluated in female rats, but there were no statistically significant trends or pair-wise comparisons in the data analyzed.

The doses were considered adequate. The first carcinogenicity study in rats (MRID 50971034) did not show any effects of treatment. A second rat carcinogenicity study (MRID 50971037) was conducted at higher dose levels based on the DART recommendations. The higher dose tested of 4000 ppm was considered adequate and not excessive in both sexes in the rat. Increased incidence of thyroid colloid degeneration was also observed at the lower dose of 2000 ppm in both sexes. In addition, females exposed to 4000 ppm in diet showed significant decreases in mean body weight, and both sexes showed increases in γ-glutamyl transpeptidase levels. When considered together, the first and second rat carcinogenicity studies are acceptable/guideline.

### 2. Mutagenicity

The was no evidence of increased gene mutations in bacteria or mammalian cells *in vitro*. Potential clastogenicity was seen in two in vitro chromosomal aberrations assays; however, pyraclonil was negative when tested in two *in vivo* micronucleus assays indicating a low potential for clastogenicity *in vivo*.

### 3. Structure Activity Relationship

Chemicals with available carcinogenicity studies had low structural similarity to pyraclonil (≤61%) and their cancer classifications ranged from "not likely to be carcinogenic to humans" to "possible human carcinogen" based on liver tumors in both sexes of mice (bromoxynil) or male rats (dichlobenil).

### 4. Mode of Action

There is no postulated mode of action for pyraclonil.

## VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005), the CARC classified pyraclonil as "Likely to be Carcinogenic to Humans". This classification is based on an increased incidence of hepatocellular adenomas and combined adenomas and carcinomas in female mice, and thyroid follicular cell adenomas and combined adenomas/carcinomas in male rats.

## VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

For the purpose of risk assessment, the CARC recommends a low dose extrapolation model

applied to the experimental animal tumor data be used for quantification of human cancer risk (Q1*).

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