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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, DC 20460

JUL 18 1995

OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Fipronil

FROM: Virginia Dobozy, V.M.D., M.P.H. *Virginia A Dobozy*  
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Toxicology Branch II  
Health Effects Division (7509C)

and

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Manager, Carcinogenicity Peer Review Committee  
Science Analysis Branch  
Health Effects Division (7509C)

TO: Richard Keigwin  
Product Manager #10  
Insecticide/Rodenticide Branch  
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THROUGH: Stephanie D. Irene Ph.D. *Stephanie D Irene*  
Acting Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on Feb. 01, 1995 and June 07, 1995 to discuss and evaluate the weight-of-the-evidence on fipronil with particular reference to its carcinogenic potential. The CPRC concluded that fipronil should be classified as a Group C - possible human carcinogen - and recommended that the RfD methodology be used for the estimation of human risk. The classification of Group C was based on increases in thyroid follicular cell tumors in both sexes of the rat, which were statistically significant by both pair-wise and trend analyses.

The RfD methodology was selected because the thyroid tumors appeared to be related to a disruption in the thyroid-pituitary status and there was no apparent concern for mutagenicity or available information from structurally related analogs.

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## SUMMARY

Administration of fipronil in the diet to CD-1 mice resulted in a statistically significant increase in hepatocellular carcinomas at the highest dose tested (HDT) only in male mice, accompanied with a statistically significant positive trend. The incidence of hepatocellular carcinomas at the HDT was within the range of historical controls, but slightly above the mean. The consensus of the CPRC was that the increased tumor incidence was due to variability and was not compound related. There were no increases in tumor incidences reported for female mice. The CPRC agreed that dosing in the mouse study was adequate in both sexes.

Administration of fipronil in the diet to Charles River CD rats resulted in statistically significant increases in thyroid tumors in both sexes. In males there were statistically significant increases in thyroid follicular cell adenomas and combined adenomas/carcinomas at three dose levels, and follicular cell carcinomas at the HDT. There were also statistically significant positive trends for the adenomas, carcinomas and combined adenomas/carcinomas. In female rats there were statistically significant increases in thyroid follicular cell adenomas and combined adenomas/carcinomas at the HDT only, accompanied with a statistically significant positive trend for the adenomas and combined adenomas/carcinomas. The incidences of thyroid follicular cell adenomas, carcinomas and combined adenomas/carcinomas in male rats and thyroid adenomas and combined adenomas/carcinomas in female rats exceeded that of historical controls. The CPRC agreed that dosing in the rat study was adequate in both sexes.

Fipronil is a member of a new class of chemicals known as phenylpyrazoles, for which no structure-activity correlations can be proposed. Fipronil does not appear to have mutagenic activity.

The classification of Group C was based on the increases in thyroid follicular cell tumors in both sexes of the rat, statistically significant by both pair-wise and trend analyses. The RfD approach was selected because there appeared to be sufficient evidence for relating the thyroid tumors in the rat to a disruption of the thyroid-pituitary status (a full discussion of this analysis is found in the body of this document - Section F, number 8) and there was no apparent concern for mutagenicity or available information from structurally related analogs.

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**A. Individuals in Attendance at one or both meetings:**

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Stephanie Irene	<u>Stephanie Irene</u>
William Burnam	<u>William Burnam</u>
Karl Baetcke	<u>Karl Baetcke</u>
Marcia Van Gemert	<u>Marcia Van Gemert</u>
Kerry Dearfield	<u>Kerry Dearfield</u>
Elizabeth Doyle	<u>Elizabeth A Doyle</u>
Marion Copley	<u>Marion Copley</u>
Hugh Pettigrew	<u>Hugh Pettigrew</u>
Esther Rinde	<u>Esther Rinde</u>

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Virginia Dobozy <sup>1</sup>	<u>Virginia Dobozy</u>
Mike Ioannou	<u>J.M. Ioannou</u>
Lori Brunzman	<u>Lori Brunzman</u>
Lucas Brennecke <sup>2</sup> (PAI/ORNL)	<u>Lucas Brennecke</u>

3. Other Attendees:

Bernice Fisher, David Anderson, John Whalan, Linnea Hansen, Kit Farwell, Raymond Locke, and Amber Aranda

<sup>1</sup>Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

<sup>2</sup>Signature indicates concurrence with pathology report.

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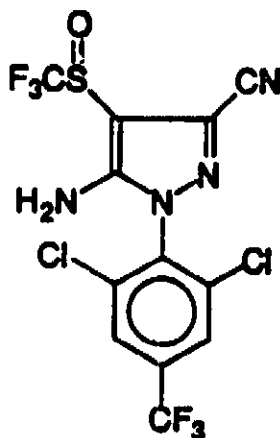
Carcinogenicity Peer Review of Fipronil  
Feb. 01 and June 07, 1995**B. Material Reviewed**

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Virginia Dobozy, and tables and statistical analysis by Lori Brunzman. The material reviewed is attached to the file copy of this report.

**C. Background Information**

Fipronil, or 5-amino-1-(2,6-dichloro-4-trifluoromethyl phenyl)-3-cyano-4-trifluoromethylsulphonylpyrazole (also referred to as M&B 46030), is proposed as a soil applied granular formulation for use in field corn for control of Northern and Western corn rootworm larvae and wireworms. Application was made to the Agency for an Experimental Use Permit (EUP) and Temporary Tolerance of fipronil as a 1.5% formulation with proposed temporary tolerances of 0.02 ppm in field corn (grain), 0.05 ppm in corn forage and 0.07 ppm in corn fodder. Toxicology Branch II recommended that the temporary tolerance for fipronil not be granted based on the potential of the chemical for carcinogenicity and neurotoxicity. Furthermore, Toxicology Branch II recommended that an EUP with crop destruct be granted when the data gaps for the acute toxicity studies on the technical and formulated chemical are fulfilled (June 7, 1994 memo from Virginia Dobozy to Robert Brenni./Daphne Waldo/PM 10). Fipronil has been reviewed by the Health Effects Division RfD/Peer Review Committee for evaluation of the existing toxicology data base and for determination of the Reference Dose for this chemical. The chemical has not been previously reviewed by the Health Effects Division's Cancer Peer Review Committee.

The Chemical Abstracts Registry Number (CAS No.) for fipronil is 120068-37-3. The structure of the chemical is shown below:

**FIPRONIL**

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Carcinogenicity Peer Review of Fipronil  
Feb. 01 and June 07, 1995**D. Evaluation of Carcinogenicity Evidence****1. CD-1 Mouse Carcinogenicity Study**

Reference: A. Broadmeadow, 1993. M&B 46030: Oncogenicity Study by Dietary Administration to CD-1 Mice for 78 Weeks. Study # LSR 92/RHA313/0971. Life Science Research Limited. MRID # 429186-49.

Material: M&B 46030 (Fipronil); purity: 95.4%

**a. Experimental Design**

Six groups of 20 male and 20 female CD-1 mice per group were treated with M&B 46030 in the diet at dosages of either 0, 0.1, 0.5, 10, 30 or 60 ppm for 52 weeks to measure the chronic toxicity of the chemical. (Group mean measured dosages in mg/kg/day were 0.011, 0.055, 1.181 and 3.430 for males and 0.012, 0.063, 1.230 and 3.616 for females, in the 0.1, 0.5, 10 and 30 ppm dose groups, respectively<sup>3</sup>). An additional six groups of 52 male and female mice were treated at the same dosages for 78 weeks to test the carcinogenic potential of the chemical. The standard measures of ante-and post-mortem toxicity were evaluated.

Due to excessive mortality, males and females in the 60 ppm groups were sacrificed during Week 10 of the study. The study report indicated that survival in the other groups was comparable or exceeded the control group. The Statistical Review from the Science Analysis Branch (SAB) also found that male and female mice showed no significant changes in mortality with increasing doses of fipronil.

**b. Discussion of Tumor Data**

There was an increased incidence of malignant hepatocellular tumors (carcinomas) in males in the 30 ppm group as compared to the controls at the carcinogenicity phase necropsy. The study report indicated that the difference in incidence was not statistically significant and when benign and malignant tumors were considered together, the incidences were similar between the treated and control groups. However, the Statistical Review found that male mice had a significant dose-related increasing trend in hepatocellular carcinomas at  $p < 0.01$  when animals that died before observation of the first tumor were excluded. There was also a significant difference in the pair-wise comparison of the 30 ppm

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<sup>3</sup>Dosages for the 60 ppm dose groups were not measured in mg/kg/day, due to excessive early mortality in this group. (The approximate equivalent of 60 ppm for the mouse would be 8.6 mg/kg/day.)

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dose group with the controls for hepatocellular carcinomas at  $p < 0.05$ . The statistical findings for hepatocellular tumors in males are presented in Table 1. No increase in tumor incidence was reported for female mice.

Table 1. Fipronil - Charles River CD-1 Mouse Study

Male Hepatocellular Tumor Rates<sup>†</sup> and  
Peto's Prevalence Test Results (p values)

	<u>Dose (ppm)</u>				
	0	0.1	0.5	10	30
Adenomas (%)	10/65 (15)	3 <sup>a</sup> /68 (4)	2/63 (3)	6/58 (10)	6/65 (9)
p =	0.341	0.987 <sup>n</sup>	0.972 <sup>n</sup>	0.791 <sup>n</sup>	0.851 <sup>n</sup>
Carcinomas (%)	1/61 (2)	1/58 (2)	2/60 (3)	1/51 (2)	6 <sup>b</sup> /62 (10)
p =	0.009 <sup>**</sup>	0.440	0.218	0.343	0.029 <sup>*</sup>
Combined (%)	11/65 (17)	4/68 (6)	4/63 (6)	7/58 (12)	11 <sup>c</sup> /65 (17)
p =	0.068	0.980 <sup>n</sup>	0.907 <sup>n</sup>	0.754 <sup>n</sup>	0.509

<sup>†</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

<sup>n</sup>Negative change from control.

<sup>a</sup>First adenoma observed at week 46, dose 0.1 ppm.

<sup>b</sup>First carcinoma observed at week 54, dose 30 ppm.

<sup>c</sup>One animal in the 30 ppm dose group had both an adenoma and a carcinoma.

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

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

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The incidence in the control group was lower than the mean incidence for this tumor type in control animals from this laboratory. In seven studies conducted from July 1988 to January 1989 involving 379 animals, the mean incidence rate of hepatocellular carcinoma in male CD-1 mice was 8.4% (range: 1.7-17.3%).

The consensus of the CPRC was that the increased tumor incidence in male mice was due to variability and was not compound related.

c. Non-neoplastic Lesions

Systemic signs of toxicity included: 1) decreased body weight gain in the 30 ppm group males and females at most of the evaluation periods; values for the 10 ppm group were also decreased but less consistently; 2) decreased food consumption in the 30 ppm group females; 3) decreased food conversion efficiency in the 10 and 30 ppm group males; 4) altered white blood cell differential counts in the 30 ppm group females; 5) increased incidence of liver pathology on gross examination in the 30 ppm group males in the carcinogenicity phase; 6) increased absolute and/or relative liver weights in the 10 and 30 ppm group males and females in both the toxicity and carcinogenicity phases; 7) increased incidence of periacinal microvesicular vacuolation in the liver of the 10 and 30 ppm group males at the toxicity and carcinogenicity phase necropsies; 8) increased incidence of hepatocellular hyperplasia and chronic degenerative changes in the liver of the 30 ppm group males which died or were sacrificed during the treatment period of the carcinogenicity phase. Based on these findings, the Lowest Observed Effect Level (LOEL) was 10 ppm (1.181 mg/kg/day for males and 1.230 mg/kg/day for females); the No Observed Effect Level (NOEL) was 0.5 ppm (0.055 mg/kg/day for males and 0.063 mg/kg/day for females).

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

At the 30 ppm dose level, mean body weight gain in both sexes was decreased (at 13 weeks, there was a 26% decrease in males and a 17% in females; at 78 weeks, there was a 14% decrease in males and a 19% decrease in females). Mean food consumption was decreased in the 30 ppm group females (more than 10% decrease). Decreased food efficiency values were reported in the 30 ppm group males. Differential white blood cell counts showed that the 30 ppm group females had a slightly lower percentage of neutrophils and slightly higher percentage of lymphocytes after 76 weeks of treatment. The following evidence of liver pathology was observed in the 30 ppm group: 1) increased incidence of abnormal findings on gross examination in the males in the carcinogenicity phase; 2) increased absolute and/or relative



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liver weights in the males and females in both the toxicity and carcinogenicity phases; 3) increased incidence of periacinal microvesicular vacuolation in the liver of the males at the toxicity and carcinogenicity phase necropsies; 4, increased incidence of hepatocellular hyperplasia and chronic degenerative changes in the liver of the males which died or were sacrificed during the treatment period of the carcinogenicity phase. In light of these systemic effects, the 30 ppm dose level is considered to be an adequate dose for assessing the carcinogenic potential of fipronil in mice. The adequacy of this dose for carcinogenicity testing has been reviewed by the Health Effects Division's RfD/Peer Review Committee and is supported by their recommendation.

## 2. CD Rat Combined Chronic Toxicity/Carcinogenicity Study

Reference: P. Aughton, 1993. M&B 46030: Combined Oncogenicity and Toxicity Study by Dietary Administration to CD rats for 104 Weeks Including a 13 Week Reversibility Period on Completion of 52 Weeks of Treatment. Study # LSR 93/RHA432/0166; Pharmaco-LSP, Limited. MRID # 429186-48.

Material: M&B 46030 (Fipronil); purity: 95.4%

### a. Experimental Design

Fifteen CD rats/sex/group were administered technical M&B 46030 in the diet for 52 weeks to assess the chronic toxicity of the chemical. An additional 15 rats/sex/group were fed the chemical for 52 weeks and then were untreated for an additional 13 weeks to test the reversibility of treatment-related changes. Fifty (50) rats/sex/group were supposed to be treated for 104 weeks to assess the carcinogenic potential of the chemical. The doses administered in all the phases were 0, 0.5, 1.5, 30, and 300 ppm (males: 0, 0.019, 0.059, 1.27 and 12.68 mg/kg/day; females: 0, 0.025, 0.078, 1.61 and 16.75 mg/kg/day). Standard pre- and post-mortem evaluations of toxicity were included in the study along with measures of thyroid function.

The carcinogenicity phase of the study was terminated early (after 89 and 91 weeks of treatment in males and females, respectively) due to excessive mortality and to ensure that a sufficient number of animals were available for the terminal sacrifices. The study report indicated that no treatment-related differences in mortality between the groups were observed. However, the SAB Statistical Review found that there was a significant increasing trend in mortality with increasing doses of fipronil in male rats. Female rats showed no significant incremental changes in mortality with increasing doses.

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The protocol violation does not affect the validity of the study for several reasons. First, the premature termination occurred near the end of the study. Second, the registrant has cited literature references indicating that in general, the longevity of the CD rat has been decreasing and therefore, a shortened life span was not unique to this study. Third, the study was long enough to have tumors develop in the treated groups.

b. Discussion of Tumor Data

In males, there were increased incidences of thyroid follicular cell adenomas in all dose groups, and increased incidences of thyroid follicular cell carcinomas in the 30 and 300 ppm groups. In females there were increased incidences of thyroid follicular cell adenomas only at the HDT, and thyroid follicular cell carcinomas at 0.5, 30 and 300 ppm. The SAB Statistical Review found that male rats had significant increasing trends in thyroid follicular cell adenomas, carcinomas, and combined adenomas and/or carcinomas, all at  $p < 0.01$ . There were significant differences in the pair-wise comparisons of the 1.5 and 30 ppm dose groups with the controls for thyroid follicular cell adenomas and combined adenomas and/or carcinomas, all at  $p < 0.05$ . There were also significant differences in the pair-wise comparisons of the 300 ppm dose group with the controls for thyroid follicular cell adenomas, carcinomas and combined adenomas and/or carcinomas, all at  $p < 0.01$ .

Female rats had a significant increasing trend and a significant difference in the pair-wise comparison of the 300 ppm dose group with the controls for thyroid follicular cell adenomas and combined adenomas and/or carcinomas, all at  $p < 0.01$ .

Statistical analyses are presented in Tables 2 and 3. The study report indicated that only the 300 ppm group males and females exceeded the historical incidence of the thyroid follicular cell tumors, either alone or in combination, for this strain of rat in the performing laboratory (Table 4).

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Table 2. Fipronil - Charles River CD Rat Study

Male Thyroid Follicular Cell Tumor Rates<sup>†</sup> and  
Peto's Prevalence Test Results (p values)

	<u>Dose (ppm)</u>				
	0	0.5	1.5	30	300
Adenomas (%)	0/63 (0)	1/61 (2)	5/63 (8)	3/62 (5)	12 <sup>a</sup> /61 (20)
p =	0.000 <sup>**</sup>	0.116	0.014 <sup>*</sup>	0.038 <sup>*</sup>	0.000 <sup>**</sup>
Carcinomas (%)	0/59 (0)	0/57 (0)	0/62 (0)	1 <sup>b</sup> /60 (2)	5/57 (9)
p =	0.000 <sup>**</sup>	-	-	0.186	0.007 <sup>**</sup>
Combined (%)	0/63 (0)	1/61 (2)	5/63 (8)	4/62 (6)	16 <sup>c</sup> /61 (26)
p =	0.000 <sup>**</sup>	0.116	0.014 <sup>*</sup>	0.024 <sup>*</sup>	0.000 <sup>**</sup>

<sup>†</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

<sup>a</sup>First adenoma observed at week 42, dose 300 ppm.

<sup>b</sup>First carcinoma observed at week 53, dose 30 ppm.

<sup>c</sup>One animal in the 300 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.

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

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

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Table 3. Fipronil - Charles River CD Rat Study

Female Thyroid Follicular Cell Tumor Rates<sup>†</sup> and Exact Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (ppm)</u>				
	0	0.5	1.5	30	300
Adenomas (%)	0/48 (0)	0/49 (0)	0/50 (0)	0/45 (0)	8 <sup>a</sup> /46 (17)
p =	0.000 <sup>**</sup>	1.000	1.000	1.000	0.002 <sup>**</sup>
Carcinomas (%)	0/48 (0)	1/49 (2)	0/50 (0)	1/45 (2)	2 <sup>b</sup> /46 (4)
p =	0.084	0.505	1.000	0.484	0.237
Combined (%)	0/48 (0)	1/49 (2)	0/50 (0)	1/45 (2)	10/46 (22)
p =	0.000 <sup>**</sup>	0.505	1.000	0.484	0.001 <sup>**</sup>

<sup>†</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.<sup>a</sup>First adenoma observed at week 62, dose 300 ppm.<sup>b</sup>First carcinoma observed at week 79, dose 300 ppm.Note: Significance of trend denoted at control.Significance of pair-wise comparison with control denoted at dose level.If \*, then  $p < 0.05$ . If \*\*, then  $p < 0.01$ .

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Historical control data were submitted from 359 male and 365 female rats from studies which commenced between June 1987 and September 1989. The incidence of benign and malignant follicular cell tumors in these animals is presented in Table 4.

Table 4  
Historical Control Data for Thyroid  
Tumors Submitted by Testing Facility

	Male	Female
Number Examined	359	365
Follicular Cell Carcinoma	4 (1.1%)	6 (1.6%)
Follicular Cell Adenoma	22 (6.1%)	5 (1.4%)
Total Follicular Cell Tumors	26 (7.2%)	10 (2.7%)

c. Non-neoplastic Lesions

Evidence of systemic toxicity included: 1) neurotoxicity (including seizures which resulted in death) in the 1.5, 30 and 300 ppm group males and females; 2) decreased body weight gain in the 300 ppm group males and females and the 30 ppm group females; 3) decreased food consumption and food conversion efficiency in the 300 ppm group males and females at the beginning of the study; 4) decreased hematology parameters in the 300 ppm group males and females in comparison to the control groups (values were comparable to pretreatment measures); 5) alterations in clinical chemistry (increased cholesterol and calcium values; protein alterations with increased total protein, decreased albumin and increased globulins) mostly in the 30 and 300 ppm group males and females; protein alterations were seen in the 1.5 ppm group males after 76 and 81 weeks of treatment; 6) alterations in thyroid hormones (increased TSH and decreased T<sub>4</sub> levels) in all treated groups at some time points with the 30 and 300 ppm group males and females consistently affected; 7) alterations in urinalysis parameters (lower pH, higher protein, elevated urine volume with decreased specific gravity) in the 30 and 300 ppm groups (predominantly males); 8) changes on gross necropsy (large and/or pale kidneys and large livers, adrenals and thyroids) in the 30 and 300 ppm group males and females; 9) increased absolute and relative

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weights of the liver and thyroids in the 30 and 300 ppm group males and females; 10) increased incidence and severity of progressive senile nephropathy in the 30 and 300 ppm group males and females. Based on these findings, the LOEL was 1.5 ppm (0.059 mg/kg/day for males and 0.078 mg/kg/day for females); the NOEL was 0.5 ppm (0.019 mg/kg/day for males and 0.025 mg/kg/day for females).

Effect of Fipronil on Thyroid Hormones

Thyroid hormones (TSH,  $T_4$  and  $T_3$ ) were measured after 1, 4, 12, 24 and 50 weeks of treatment and after 2, 4, 7 and 11 weeks of the reversibility period. The TSH and  $T_4$  levels were the most consistently affected parameters. The TSH levels were significantly elevated in the 30 ppm group males and the 300 ppm group males and females at most of the time points during the study. The  $T_4$  levels were significantly decreased in the 1.5, 30 and 300 ppm group males and females at most of the time points. After one week of treatment, the value was zero for both the males and females in the 300 ppm groups. There were only occasional significant differences in the  $T_3$  values of the treated animals. Table 5 summarizes the TSH and  $T_4$  values during treatment.

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Table 5  
Thyroid Hormone Changes in Rats Treated  
with M&B 46030 in the Diet for up to 91 Weeks<sup>a</sup>

	Dose Levels (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
<b>After 1 Week of Treatment</b>										
TSH (ng/ml)	4.7	7.1	6.2	11.8***	20.3***	3.5	3.5	3.2	3.6	7.6***
T <sub>4</sub> (µg/dl)	2.99	3.02	2.23*	1.16***	0.00***	2.32	1.86	2.58	1.26**	0.00***
<b>After 4 Weeks of Treatment</b>										
TSH	5.2	8.0	6.5	11.2**	22.9***	3.8	3.9	3.3	3.9	7.5***
T <sub>4</sub>	3.14	2.70*	2.56**	1.84***	0.37***	3.03	2.48*	2.36*	1.46***	0.79***
<b>After 12 Weeks of Treatment</b>										
TSH	5.7	7.2 (6.0) <sup>b</sup>	5.8	6.1	18.4***	3.4	3.4	2.9	3.5	8.7***
T <sub>4</sub>	5.18	4.74 (4.38*)	3.96**	3.50***	1.22***	3.62	2.85**	2.87*	2.05***	1.10***
<b>After 24 Weeks of Treatment</b>										
TSH	7.2	10.0	6.9	8.6	21.0***	3.2	3.7	3.2	3.9	6.6***
T <sub>4</sub>	4.58	3.81*	3.35***	2.43***	0.76***	2.85	3.09	3.49**	2.98	1.46***
<b>After 80 Weeks of Treatment</b>										
TSH	13.0	17.1	12.4	26.6*	57.3***	6.2	8.0	5.5	6.1	13.5***
T <sub>4</sub>	5.93	5.51	4.83**	3.90***	2.07***	3.31	3.45	3.00	2.06***	1.38***

<sup>a</sup> Extracted from Tables 1,2A-E (pages 156-160) of the study report.

<sup>b</sup> Values in parenthesis calculated after exclusion of one outlier.

\* Significantly different from controls,  $p < 0.05$

\*\* Significantly different from controls,  $p < 0.01$

\*\*\* Significantly different from controls,  $p < 0.001$

During the reversibility period, the TSH and T<sub>4</sub> levels in the females were comparable to the controls; the T<sub>3</sub> levels were significantly elevated in the 30 and 300 ppm groups at some of the time points. The TSH levels in the 300 ppm group males remained significantly elevated through the reversibility period, although the values decreased at each subsequent time point. The T<sub>4</sub> levels in the treated males were not comparable to the control group until after 11 weeks of reversibility period. The T<sub>3</sub> levels in the treated males were essentially comparable to the controls.

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Feb. 01 and June 07, 1995**d. Adequacy of Dosing for Assessment of Carcinogenic Potential**

At the 300 ppm dose, the following deviations were observed in males and females: 1) clinical signs of neurotoxicity (including seizures which resulted in death); 2) decreased overall mean body weight gain (by 18% in males and 25% in females); 3) decreased food consumption and food conversion efficiency at the beginning of the study; 4) decreased hematology parameters in comparison to the control groups; 5) alterations in clinical chemistry (increased cholesterol and calcium values; protein alterations with increased total protein, decreased albumin and increased globulins); 6) alterations in thyroid hormones (increased TSH and decreased T<sub>4</sub> levels); 7) alterations in urinalysis parameters (lower pH, higher protein, elevated urine volume with decreased specific gravity; predominantly in males); 8) changes on gross necropsy (large and/or pale kidneys and large livers, adrenals and thyroids); 9) increased absolute and relative weights of the liver and thyroids; 10) increased incidence and severity of progressive senile nephropathy.

The dose levels used in this study are considered adequate for testing the carcinogenic potential of fipronil. This conclusion is supported by the recent evaluation of this study by the Health Effects Division's RfD/Peer Review Committee which determined the dose levels of this study to be adequate for testing the carcinogenic potential of fipronil.

**E. Additional Toxicology Data on Fipronil****1. Metabolism**

Reference: P. Powles, C. Biol, M.I. Biol, 1992. (14-C)-M&B 46,030: Absorption, Distribution, Metabolism, and Excretion in the Rat (2 Volumes). Hazleton UK, Study # HUK Report No. 7040-68/117. MRID # 429186-55

Material: [U-<sup>14</sup>C] - M&B 46,030, radiochemical purity: > 98.0%  
Unlabeled M&B 46,030, chemical purity: > 99.3%

<sup>14</sup>C-Fipronil was administered orally in carboxymethylcellulose to groups (5 sex/dose) of male and female Sprague-Dawley rats at a low oral dose (4 mg/kg), repeated low oral dose (4 mg/kg x 14 days), and a single high dose (150 mg/kg).

The rate and extent of absorption appeared similar among all dose groups, but may have been decreased at the high dose. Distribution

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data showed significant amounts of residual radioactivity in carcass, g.i. tract, liver, adrenals, and abdominal fat at 168 hours post-dose for all rats in all dose groups. Repeated low oral dosing or a single high oral dose resulted in an overall decrease in the amount of residual radioactivity found, but an increase in the amount in abdominal fat, carcass, and adrenals.

Feces appeared to be the major route of excretion for fipronil derived radioactivity, where between 45-75% of an administered dose was excreted. Excretion in urine was between 5-25%. Increases in the percentages excreted in urine and feces were observed with repeated low oral dosing or a single high dose, while the percentage found in all tissues combined decreased. There were no significant sex-related differences in excretion.

Several metabolites were identified in urine and feces of fipronil dosed rats. Major metabolites in urine included two ring-opened products of the metabolite M&B 45,897, two oxidation products (M&B 46,136 and RPA200766), and parent chemical (M&B 46,030). In feces, parent M&B 46,030 was detected as a significant fraction of the sample radioactivity as well as the oxidation products M&B 46,136 and M&B 45,950.

Pharmacokinetic investigations showed that at the single low oral dose, whole blood half-life ranged from 149.4-200.2 hr in male and female rats, with 0-168 hr AUCs approximately equal between sexes. At the single high oral dose, whole blood half-life was noticeably decreased to 54.4 hr in male rats and 51.2 hr in female rats. Blood AUCs at this dose were approximately proportional to the increase in dose.

## 2. Mutagenicity

Fipronil has been tested in several mutagenicity studies. Fipronil has not provided evidence for mutagenic activity in any of the submitted acceptable tests. However, based on revised current mutagenicity requirements, a micronucleus test has been identified as a data gap.

a) Reference: C.B. Clare, 1988. Study to Determine the Ability of M&B 46030 to Induce Mutation in Four Histidine-Requiring Strains of *Salmonella typhimurium*. Microtest Research Limited; Study # MAB 20/S. MRID # 429186-52.

In two independently performed *Salmonella typhimurium*/mammalian

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microsome reverse gene mutation assays, strains TA1535, TA1537, TA98, or TA100 were exposed to 0.8, 4, 20, 100, or 500  $\mu\text{g}/\text{plate}$  M&B 46030 (initial trial) or 25, 50, 100, 200, or 400  $\mu\text{g}/\text{plate}$  (confirmatory trial) both in the presence and absence of S-9 activation. Cytotoxicity was seen in the majority of strains at 500  $\mu\text{g}/\text{plate}$  +/- S9 in the initial assay; therefore, the high dose was lowered in the confirmatory assay to 400  $\mu\text{g}/\text{plate}$  +/- S9. M&B was assayed up to a sufficiently high level and there was no evidence of mutagenicity in any strain. This study was classified as acceptable.

b) Reference: R.R. Marshall, 1988. Study to Evaluate the Chromosome Damaging Potential of M&B 46030 by its Effects on Cultured Human Lymphocytes Using an *in vitro* Cytogenetics Assay. Microtest Research Limited; Study # MAB 20/HLC. MRID # 429186-53.

Human lymphocytes derived from one male and one female healthy human donors were exposed to M&B 46030 doses of 75, 150, or 300  $\mu\text{g}/\text{ml}$  with or without S9 activation. Reduced mitotic indices were seen in cultures treated with 300  $\mu\text{g}/\text{ml}$  +/- S9; this level was also reported to be near the solubility limit of the test material in this assay system. There was, however, no indication of a clastogenic effect at any dose with or without S9 activation. Findings with the positive controls confirmed the sensitivity of the test system to detect clastogenesis. This study was classified as acceptable.

c) Reference: J.M. Lloyd, 1993. M&B 46030: Investigation of Mutagenic Activity at the HGPRT Locus in a Chinese Hamster V79 Cell Mutation System. Pharmaco-LSR Limited; Study # 93/RHA304/0566. MRID # 429186-51.

In two independent Chinese hamster V79 cell HGPRT forward gene mutation assays, M&B 46030 was assayed at intended concentrations of 0.8, 4, 20, 100 or 500  $\mu\text{g}/\text{ml}$  +/- S9. M&B 46030 was neither cytotoxic nor mutagenic in the presence or absence of S9 up to insoluble levels ( $\geq 100$   $\mu\text{g}/\text{ml}$  +/- S9). Findings with the positive controls confirmed the sensitivity of the test system to detect mutagenesis. This study was classified as acceptable.

d) Reference: C.N. Edwards, 1993. M&B 46030: Assessment of Clastogenic Action on Bone Marrow Erythrocytes in the Micronucleus Test. Pharmaco-LSR; Study # 90/RHA305/1377. MRID # 429186-50

Groups of five male and five female CD-1 mice receiving single oral gavage administrations of 25 mg/kg M&B 46030 were sacrificed 24, 48 or 72 hours posttreatment. Similar groups were administered either 1 or 5 mg/kg, and bone marrow cells were harvested 24 hours

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postexposure. No evidence of overt toxicity, cytotoxicity to the target organ, or increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) was observed in males or females from the high-dose group. Based on the lack of an effect, slides prepared from two males and two females administered 50 mg/kg in a preliminary test were re-evaluated; these findings provided no convincing evidence of cytotoxicity and no appreciable increase in the MPE frequency. While there was no indication of a genotoxic effect, observations from only two males and two females administered 50 mg/kg and sacrificed at 72 hours are insufficient to confirm that a genotoxic effect was not observed. Additional animals should have been tested at this dose level (or a slightly higher dose level). This study was classified as unacceptable.

Several metabolites (numbered 46136, 45950, 200766, 46513, 104615 and 105048) all tested negative in the Salmonella assay.

### 3. Structure-Activity Correlations

Fipronil is a member of a new class of chemicals known as phenylpyrazoles. Therefore, no structure-activity correlations can be proposed.

Fipronil acts by reversing the effect of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in insects. Inhibition interferes with the transport of chloride ions across the cell membrane causing uncontrolled CNS activity and subsequent death of the insect. The closest class of compounds to fipronil, as defined by their mechanism of action, is the cyclodienes. These chemicals also act as antagonists of the GABA regulated chloride channel but in insects it is not at the same site within the channel as fipronil<sup>4</sup>. The cyclodienes include chemicals such as chlordane, aldrin, dieldrin, heptachlor and endrin.

### 4. Reproduction Study

#### Reproductive Toxicity in Rats

Reference: V.C. King, 1992. M&B 46030: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations. Life Science Research Limited; Study # LSR 92/RHA425/0309. MRID # 429186-47.

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<sup>4</sup>Mode of Action of Fipronil, prepared by D.F. Bushey, dated August 20, 1994; submitted December 14, 1994 (DP Barcode: D210219).

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Material: M&B 46030 (fipronil); purity: 95.4%

M&B 46030 was administered to two generations of male and female CD rats continuously in the diet at concentrations of 0, 3, 30 and 300 ppm (equivalent to 0, 0.25, 2.54, and 26.03 mg/kg/day, respectively).

Parental (systemic) toxicity was noted in the form of the following: 1) increased mortality in the 300 ppm group males and females in the  $F_0$  and  $F_1$  generations; 2) decreased body weight gain pre-mating in the 300 ppm group males and females in the  $F_0$  and  $F_1$  generations and in the 300 ppm group females during gestation and lactation in the  $F_0$  generation; 3) food consumption in the 300 ppm group males and females during pre-mating in the  $F_0$  generation; 4) the absolute and relative weights of the thyroid glands and the liver were increased in the 30 and 300 ppm group males and females of the  $F_0$  and  $F_1$  generations; the absolute and relative weights of the ovaries were decreased in the 300 ppm group females in the  $F_0$  generation; the

absolute weight of the pituitary gland was decreased in the 30 and 300 ppm group females and the relative weight was decreased in all the treated female groups in the  $F_1$  parental animals; the absolute and relative weights of the testes in the 300 ppm group males were decreased in the  $F_1$  parental animals; 5) increased incidence of centriacinar fatty vacuolation in the livers of the 300 ppm group females in both the  $F_0$  and  $F_1$  generations; and 6) increased incidence of follicular epithelial hypertrophy of the thyroid glands in the 300 ppm group males and females in the  $F_0$  generation and in the 30 and 300 ppm group females in the  $F_1$  generation.

Reproductive toxicity was noted in the form of the following findings in the 300 ppm group: 1) clinical signs of toxicity in the  $F_1$  and  $F_2$  offspring; 2) decreased litter size in the  $F_1$  and  $F_2$  litters; 3) decreased body weights in the  $F_1$  and  $F_2$  litters; 4) decrease in the percentage of  $F_1$  parental animals mating; 5) reduction in fertility index in  $F_1$  parental animals; 6) reduced post-implantation survival and offspring postnatal survivability in the  $F_2$  litters; and 7) delay in physical development in the 300 ppm group of the  $F_1$  and  $F_2$  litters.

Parental (systemic) Toxicity NOEL = 3 ppm (0.25 mg/kg/day for males and 0.27 mg/kg/day for females)

Parental (systemic) Toxicity LOEL = 30 ppm (2.54 mg/kg/day for males and 2.74 mg/kg/day for females) based on increased weight of the thyroid glands and liver in males and females; decreased weight of

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the pituitary gland in females; and an increased incidence of follicular epithelial hypertrophy of the thyroid gland in the females.

Reproductive Toxicity NOEL = 30 ppm (2.54 mg/kg/day for males and 2.74 mg/kg/day for females).

Reproductive Toxicity LOEL = 300 ppm (26.03 mg/kg/day for males and 28.40 mg/kg/day for females) based on clinical signs of toxicity in the F<sub>1</sub> and F<sub>2</sub> offspring; decreased litter size in the F<sub>1</sub> and F<sub>2</sub> litters; decreased body weights in the F<sub>1</sub> and F<sub>2</sub> litters; decrease in the percentage of F<sub>1</sub> parental animals mating; reduction in fertility index in F<sub>1</sub> parental animals; reduced post-implantation survival and offspring postnatal survivability in the F<sub>2</sub> litters; and delay in physical development in the F<sub>1</sub> and F<sub>2</sub> offspring.

#### 5. Additional Studies

a) Reference: D.H. Peters et al, 1991. M&B 46030 - An Investigation into the Potential Effects on Thyroid Function in Male Rats Using the Perchlorate Discharge Test. Huntingdon Research Centre Limited; Study # M&B 353/90920. MRID # 429779-04.

The effect of M&B 46030 on thyroid function was compared to propylthiouracil (PTU), a known inhibitor of thyroid organification, and Noxyflex, a thiourea compound known to lower thyroxine levels in rats. There was no evidence of an inhibition of iodide incorporation by either M&B 46030 or Noxyflex, whereas treatment with PTU produced decreases in the amount of <sup>125</sup>I incorporated in the thyroid and in the blood:thyroid ratios along with elevated levels of <sup>125</sup>I in the blood.

b) Reference: D.H. Peters et al, 1991. M&B 46030 - An Investigation into the Potential Effects on Thyroid Function in Male Rats by Studying Thyroxine Clearance. Huntingdon Research Centre Limited; Study # M&B 352/90958. MRID # 429186-54.

The effect of M&B 46030 on thyroxine pharmacokinetics was compared to phenobarbital. There was no effect of M&B 46030 on clearance after one day of treatment, however after 14 days, there was a decrease in the thyroxine terminal half life (52% of control level) and increases in clearance and volume of distribution (261% and 137% of control level, respectively). The effects seen with phenobarbital treatment were similar, although quantitatively not as severe, but were evident on Day 1 of treatment.

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Feb. 01 and June 07, 1995**F. Weight of Evidence Considerations**

The Committee considered the following regarding toxicology data on fipronil in a weight-of-evidence determination of carcinogenic potential.

1. In the mouse carcinogenicity study, male mice had a significant dose-related increasing trend and a significant difference in the pair-wise comparison of the 30 ppm dose group with the controls for hepatocellular carcinomas. However, the male control animals in this study had an incidence of hepatocellular carcinomas that was much lower than the mean incidence of male historical control animals and comparable to the lower limit of the range for these tumors. The consensus of the CPRC was that the increased tumor incidence in male mice was due to variability and was not compound related. No increase in tumor incidence was reported for female mice.

2. Male rats had significant increasing trends in thyroid follicular cell adenomas, carcinomas, and combined adenomas and/or carcinomas, all at  $p < 0.01$ . There were significant differences in the pair-wise comparisons of the 1.5 and 30 ppm dose groups with the controls for thyroid follicular cell adenomas and combined adenomas and/or carcinomas, all at  $p < 0.05$ . There were also significant differences in the pair-wise comparisons of the 300 ppm dose group with the controls for thyroid follicular cell adenomas, carcinomas and combined adenomas and/or carcinomas, all at  $p < 0.01$ .

Female rats had a significant increasing trend and a significant difference in the pair-wise comparison of the 300 ppm dose group with the controls for thyroid follicular cell adenomas and combined adenomas and/or carcinomas, all at  $p < 0.01$ .

Fipronil was demonstrated to have a potent effect on thyroid hormone regulation. At 300 ppm (12.68 and 16.75 mg/kg/day in males and females, respectively), the  $T_4$  levels were zero after one week of treatment. During the reversibility period (13 weeks of no treatment), these values in the treated males did not return to levels comparable to the control animals until 11 weeks post-treatment.

3. At doses of 0.8-500  $\mu\text{g}/\text{plate}$ , fipronil was not mutagenic to four Ames bacterial strains in the presence or absence of S9 activation.

4. At doses of 75-300  $\mu\text{g}/\text{ml}$ , fipronil did not produce evidence of a clastogenic effect on human lymphocytes in the presence or absence of S9 activation.

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5. At doses of 0.8-500  $\mu\text{g}/\text{ml}$ , fipronil was not mutagenic at the HGPRT locus of Chinese hamster V79 cells in the presence or absence of S9 activation.

6. In the micronucleus test, there was no evidence of clastogenic action on bone marrow erythrocytes of mice treated with a single dose of 50 mg/kg of fipronil. However, the study was classified as not acceptable because only two male and two female mice were treated at this dose. This remains as a data gap and a repeat micronucleus test is required.

7. Fipronil is a member of a new class of chemicals known as phenylpyrazoles. Therefore, no structure-activity correlations can be proposed.

**8. Consideration of the Use of the Threshold Model for Fipronil**

In the evaluation of fipronil, the Committee was asked to consider the possibility of using the threshold model for thyroid neoplasms. The following discussion has been taken from the Amitrole Peer Review Document (dated Nov. 30, 1992) and revised for Fipronil.

The following guidance is given in the Agency's DRAFT Policy Document [Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988]:

"Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (eg., iodine deficiency) have demonstrated the significance of long-term thyroid-pituitary hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia.. Hyperplasia and sometimes neoplasia of the pituitary may also be seen. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations..... the Agency concludes that:

a. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels:

b. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when

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thyroid-pituitary homeostasis exists; and

c. models that assume thresholds may be used to assess the risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance."

Two basic questions must be addressed before this policy is applied.

"The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second question concerns the procedures to be employed in estimating the risks of these agents."

"The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assignment is based on knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response."

Determination of whether neoplasms are due to thyroid-pituitary imbalance

The document goes on to describe the 3 factors that should be considered in making this determination (answering the first question, or "qualitative issue"). These are addressed as they apply to Fipronil as follows:

FACTOR I. Consideration of whether the thyroid tumors associated with administration of Fipronil can be attributed to disruption of the thyroid-pituitary hormonal balance. [In addressing this factor, the Policy states that 6 indicators should be considered.]

a. Goitrogenic activity in vivo: In the combined chronic toxicity/carcinogenicity study, thyroid follicular cell hyperplasia was infrequently reported in the animals sacrificed at the scheduled necropsies (interim, following the reversibility period and terminal) and in those which died or were sacrificed during the treatment period. There was no increased incidence in treated animals. In the subchronic toxicity study in the rat (MRID # 429186-43), the incidence of hypertrophy of the follicular epithelium was increased in males and females in the 300 ppm groups.



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b. **Clinical chemistry changes** [e.g., reduced thyroid hormone and increased TSH serum concentrations]: In the combined chronic toxicity/carcinogenicity study in rats, thyroid hormones (TSH,  $T_4$  and  $T_3$ ) were measured after 1, 4, 12, 24 and 50 weeks of treatment and after 2, 4, 7 and 11 weeks of the reversibility period. The TSH and  $T_4$  were the most consistently affected parameters. The TSH levels were significantly elevated in the 30 ppm group males and the 300 ppm group males and females at most of the time points during the study. The  $T_4$  levels were significantly decreased in the 1.5, 30 and 300 ppm group males and females at most of the time points. After one week of treatment, the value was zero for both the males and females in the 300 ppm groups. There were only occasional significant differences in the  $T_3$  values of the treated animals.

c. **Specific evidence of reduced hormone synthesis** [e.g., inhibited iodine uptake] or **increased thyroid hormone clearance** [e.g., enhanced biliary excretion]: In a special study (MRID # 429186-54), there was no effect of fipronil on thyroxine clearance after one day of treatment. However, after 14 days, there was a decrease in the thyroxine half-life (52% of control level) and increases in clearance and volume of distribution (261% and 137% of control level, respectively).

d. **Evidence of progression** [e.g., hypertrophy/hyperplasia, nodular hyperplasia - neoplasia]: There was no evidence of progression [hypertrophy/hyperplasia to neoplasia] in rats.

e. **Reversibility of effects after exposure is terminated**: During the reversibility period, the TSH and  $T_4$  levels in the females were comparable to the controls; the  $T_3$  levels were significantly elevated in the 30 and 300 ppm groups at some of the time points. The TSH levels in the 300 ppm group males remained significantly elevated through the reversibility period, although the values decreased at each subsequent time point. The  $T_4$  levels in the treated males were not comparable to the control group until after 11 weeks of reversibility period. The  $T_3$  levels in the treated males were essentially comparable to the controls.

**SAR to other thyroid tumorigens**: No structure-activity comparisons can be made.

Based on the overall judgment of the six indicators in Factor I, it may be concluded that there are sufficient data to determine whether there is suggestive evidence that the thyroid tumors in the rat associated with the administration of fipronil may be due to a

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disruption in the thyroid-pituitary status.

**FACTOR II:** Consideration of the extent to which genotoxicity may account for the observed tumor effects.

The genotoxicity data on fipronil itself are negative. (The micronucleus study has been classified as unacceptable.) There is no indication that genotoxicity plays a role in the tumorigenic activity for fipronil.

**FACTOR III:** Evaluation of neoplasms other than thyroid follicular cell tumors (and relevant pituitary tumors).

There was an increased incidence of malignant hepatocellular tumors (carcinomas) in males in the 30 ppm group as compared to the controls at the carcinogenicity phase necropsy. Male mice had a significant dose-related increasing trend in hepatocellular carcinomas at  $p < 0.01$  when animals that died before observation of the first tumor were excluded. There was also a significant difference in the pair-wise comparison of the 30 ppm dose group with the controls for hepatocellular carcinomas at  $p < 0.05$ . However, the consensus of the CPRC was that the increased tumor incidence in male mice was due to variability and was not compound related.

**Factors to be Considered in Determining the Method to be Used in Estimating the Risks of Fipronil**

Again, this guidance was taken from the Amitrole Peer Review Document and revised for fipronil. The Committee was requested to consider these points when determining the method to be used for estimating the carcinogenic risk for fipronil.

Guidance given in the EPA DRAFT policy for proceeding with the quantitation of risk is as follows:

a. "Threshold considerations should be applied in dose-response assessments for those chemical substances where (1) only thyroid tumors (and relevant pituitary tumors) have been produced; (2) the tumors can be attributed to a disruption in thyroid-pituitary hormonal homeostasis; and (3) potential mechanisms other than thyroid-pituitary imbalance (eg., genotoxicity) can be discarded.

b. Special attention should be given to chemicals (1) that have induced thyroid tumors (and relevant pituitary tumors) that may be due to thyroid-pituitary imbalance, and (2) where there is also evidence of either a genotoxic potential or the induction of

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neoplasms at sites other than the thyroid (or pituitary). Generally, those cases will be approached using various principles laid out in the EPA Guidelines for Carcinogen Risk Assessment. A strong rationale must be articulated for handling these agents otherwise.

c. For those chemicals producing thyroid tumors that do not seem to be acting via thyroid-pituitary hormonal inhibition, dose-response assessments will be performed in accordance with the EPA Guidelines for Carcinogen Risk Assessment<sup>5</sup>

Based on the overall judgment of the 6 indicators in FACTOR I and adding in FACTORS II and III, sufficient data exist with which to conclude that there is suggestive evidence that the thyroid tumors in the rat associated with the administration of fipronil may be due to a disruption in the thyroid-pituitary status. All of the criteria for a threshold effect have been met except the data do not show a progression of the lesions and there is no information on SAR. Nevertheless, other mechanisms of tumor induction by fipronil cannot be excluded.

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<sup>5</sup>A new policy document is in process, which currently states these phrases differently: 1. "Threshold considerations will be incorporated into thyroid (and relevant pituitary) cancer dose-response assessments for chemicals that (a) cause disruption of thyroid-pituitary homeostasis and (b) are judged not to have genotoxic activity relevant to carcinogenicity. Dose-response relationships for neoplasms other than the thyroid (or pituitary) should be evaluated using mechanistic information bearing on their induction and various principles laid out in the Agency's cancer risk assessment guidelines. 2. Threshold considerations may be applied in thyroid cancer dose-response assessments on a case-by-case basis for chemicals that (a) produce thyroid-pituitary imbalance and (b) are judged to have genotoxic activity related to carcinogenicity. The implications of the genotoxic events to the thyroid carcinogenic responses need to be carefully evaluated. In some cases thyroid cancer dose-response relationships may be characterized in more than one way. 3. Threshold considerations will not be applied in thyroid cancer dose-response assessments for substances operating through mechanisms not involving thyroid-pituitary imbalance. However, case-by-case exceptions may arise, based on mode of action data."

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9. The following endpoints were considered by the CPRC in considering a threshold approach for fipronil:

From the Combined chronic toxicity/carcinogenicity study in rat:

- Thyroid tumors
- Increase in thyroid weights
- Increase in liver weights
- Alterations in thyroid hormones (decrease in T<sub>4</sub> and increase in TSH)

Although not used for selecting a NOEL, the thyroid special study in the rat did confirm that there was an increased metabolism of thyroid hormones. In the study, the fipronil-treated animals had a decrease in thyroxine terminal half-life and an increase in clearance and volume of distribution as compared to control values.

All of the endpoints above that were observed either in the thyroid or liver were considered to be directly related to the thyroid neoplastic response in rats.

In addition, the same endpoints were examined in other species (with the exception of the thyroid tumors which were observed only in the rat). The following endpoints were observed in the carcinogenicity study in mice, reproduction study in rats and subchronic toxicity study in rats.

From the carcinogenicity study in mice:

- increased liver weights
- increased incidence of hepatocellular hyperplasia and chronic degenerative changes in the liver

From the reproduction study in rats:

- increased liver and thyroid weights in the F<sub>0</sub> and F<sub>1</sub> generations
- increased incidence of follicular epithelial hypertrophy of the thyroid glands in the F<sub>0</sub> and F<sub>1</sub> generations

In the mouse carcinogenicity study, male mice had a statistically significant increase in hepatocellular carcinomas. However, the male control animals in this study had an incidence of hepatocellular carcinomas that was much lower than the mean incidence of male historical control animals and comparable to the lower limit of the range for these tumors. Therefore, this finding was not considered in the weight-of-the-evidence for classification of carcinogenic potential.

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Presented below are the endpoints, NOEL's and LOEL's in mg/kg/day for fipronil:

Factors Considered for Determining NOEL for Fipronil			
Study	Endpoint	NOEL (mg/kg/day)	LOEL (mg/kg/day)
Combined Chronic Toxicity/ Carcinogenicity - Rat	thyroid tumors	0.019 (♂), 1.61 (♀)	0.059 (♂), 16.75 (♀)
	↑ thyroid weight	0.059 (♂), 0.078 (♀)	1.27 (♂), 1.61 (♀)
	↑ liver weights	0.059 (♂), 0.078 (♀)	1.27 (♂), 1.61 (♀)
	↓ T <sub>4</sub> ↑ TSH	0.019 (♂), 0.025 (♀) 0.059 (♂), 0.078 (♀)	0.059 (♂), 0.078 (♀) 1.27 (♂), 1.61 (♀)
Carcinogenicity - Mouse	↑ liver weights ↑ liver hyperplasia & degenerative changes	0.055 (♂), 0.063 (♀) 1.181 (♂), 3.616 (♀)	1.181 (♂), 1.230 (♀) 3.430 (♂), >3.616 <sup>6</sup> (♀)
Reproduction - Rat	↑ liver weights in parental animals of F <sub>0</sub> and F <sub>1</sub> generations	0.25 (♂), 0.27 (♀)	2.54 (♂), 2.74 (♀)
	↑ thyroid weights in parental animals of F <sub>0</sub> and F <sub>1</sub> generations	0.25 (♂), 0.27 (♀)	2.54 (♂), 2.74 (♀)
	↑ hypertrophy thyroid follicular epithelium in parental F <sub>0</sub> generation	2.54 (♂), 2.74 (♀)	26.03 (♂), 28.40 (♀)
	↑ hypertrophy thyroid follicular epithelium in parental F <sub>1</sub> generation	0.25 (♂), 0.27 (♀)	2.54 (♂), 2.74 (♀)

The NOEL and LOEL which represented the majority of the observations were 0.06 mg/kg/day (rounded from 0.059 mg/kg/day) for the NOEL and 1.3 mg/kg/day (rounded from 1.27 mg/kg/day) for the LOEL. Only two endpoints had NOEL's lower than 0.06 mg/kg/day. These were for thyroid tumors in males and decreased T<sub>4</sub> values in males and females<sup>7</sup>.

For comparison purposes, the NOEL selected for calculation of the reference dose (RfD) for fipronil is 0.02 mg/kg/day, based on neurotoxicity and alterations in thyroid hormones.

<sup>6</sup>There was no hyperplasia observed in female mice.

<sup>7</sup>That is, the thyroid tumors occurred at a lower dose level than all but one of the other non-neoplastic and/or hormonal endpoints.

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**G. Classification of Carcinogenic Potential:**

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that fipronil should be classified as a Group C - possible human carcinogen and that for the purpose of risk characterization the RfD approach should be used for quantification of human risk.

This decision to classify fipronil as a Group C carcinogen was based on evidence of increased incidences of thyroid tumors in both sexes of the Charles River CD rat. Statistically significant increases were found for thyroid follicular cell adenomas, carcinomas and combined adenoma/carcinomas in male rats, for thyroid follicular cell adenomas and combined adenomas/carcinomas in female rats. The incidences of thyroid tumors exceeded that of historical controls in both sexes of the rat.

Fipronil is a member of a new class of chemicals known as phenylpyrazoles, for which no structure-activity correlations can be proposed. Fipronil does not appear to have mutagenic activity.

The CPMC also considered the possibility of using the threshold model for thyroid neoplasms based on the Agency's DRAFT Policy Document, "Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988." The CPMC concluded that there appeared to be sufficient evidence for relating the thyroid tumors in the rat to a disruption of the thyroid-pituitary status (a full discussion of this analysis is found in the body of the document - Section F, number 8).

The RfD approach was selected because the thyroid tumors in the rat appeared to be related to a disruption of the thyroid-pituitary status and there was no apparent concern for mutagenicity or available information from structurally related analogs.

011616

Carcinogenicity Peer Review of Fipronil  
Feb. 01 and June 07, 1995

**H. Induces Cancer Call --Fipronil**

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to fipronil resulted in statistically significant increased incidences of thyroid follicular cell tumors in both sexes of Charles River CD rats (adenomas, carcinomas and combined adenomas/carcinomas in males and adenomas and combined adenomas/carcinomas in females). The incidences of these tumors exceeded that of historical controls.

The Committee agrees that fipronil induces cancer in animals.