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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No. 0052631

MEMORANDUM

DATE: June 22, 2004

SUBJECT: **Pyrethrins:** Report of the Cancer Assessment Review Committee (Third Evaluation)

PC Code: 069001

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Linda Taylor, Toxicologist
Reregistration Branch 1, Health Effects Division (7509C)

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Carmen J. Rodia, PM
Reregistration Branch 2, Special Review and Reregistration Division (7508C)

The Cancer Assessment Review Committee met on April 14, 2004 to re-evaluate the carcinogenic potential of Pyrethrins. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
PYRETHRINS (THIRD EVALUATION)
PC CODE 069001

FINAL

June 22, 2004

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

DATA PRESENTATION: _____
Linda L. Taylor, Ph.D., Toxicologist

DOCUMENT PREPARATION: _____
Jessica Kidwell, Executive Secretary

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

Karl Baetcke _____

William Burnam _____

Marion Copley _____

Vicki Dellarco _____

Kit Farwell _____

Jess Rowland _____

Linda Taylor _____

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

John Pletcher, Consulting Pathologist _____

Lori Brunsman, Statistical Analysis _____

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EXECUTIVE SUMMARY

On April 14, 2004, the Cancer Assessment Review Committee [CARC] of the Health Effects Division [HED] of the Office of Pesticide Programs [OPP] met to evaluate the available mode of action/mechanistic data on the thyroid and liver and determine whether the available data are sufficient to support a change in the carcinogenic classification of pyrethrins, based on the new guidelines.

Previously, the carcinogenic potential of pyrethrins was classified as **"likely to be a human carcinogen by the oral route"**, based on the occurrence of liver tumors in female Charles River CD rats and thyroid tumors in both sexes of Charles River CD rat [in accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (1996), CARC meeting February 3, 1999; TXR No. 013354]. The Committee recommended a linear low-dose approach for human risk characterization based on the most potent Q_1* value of the two tumor types [male thyroid]. This extrapolation was supported by the lack of data on the mode of action for tumor induction [TXR No. 013169].

Linda Taylor of Reregistration Branch 1 presented the mode of action/mechanistic data submitted for both tumor types by (1) describing the experimental design and findings of the studies; (2) presenting the weight of evidence for a determination of whether the available mechanistic data are sufficient to conclude that the thyroid tumors are due to thyroid-pituitary imbalance; and (3) whether pyrethrins cause liver and thyroid gland tumors through a dose-related proliferative response in the liver (replicative DNA synthesis and microsomal enzyme induction) and a secondary proliferative stimulation of thyroid follicular cells.

According to the EPA's *Draft Guidelines for Carcinogen Risk Assessment (July, 1999),* the Committee classified pyrethrins as **Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"** based on the following weight-of-the-evidence considerations:

(i) The occurrence of a benign and minimal liver tumor response only in female Crl:CD® (SD)IGS BR rats.

(ii) There was no treatment-related increase in liver tumors in male Crl:CD®(SD)IGS BR rats.

(iii) There was no treatment-related increase in tumors in either sex of Charles River CD mice.

(iv) There is no concern for mutagenicity.

The Committee further recommended that no quantification of human carcinogenic risk be determined for pyrethrins.

With regard to the thyroid tumors, there was a weak tumor response in a susceptible laboratory animal species that is not of concern for humans. The mode of action data for pyrethrins is consistent with the mode of carcinogenic action that has been established for a number of pesticides that induce thyroid follicular cell tumors in rats (Hurley et al., 1998). This mode of action involves a reduction of circulating thyroid hormone, which activates homeostatic processes that increase thyroid stimulating hormone (TSH) release from the pituitary. TSH release stimulates the thyroid gland to increase thyroid hormone synthesis and release. Persistently elevated TSH levels will lead to thyroid follicular cell hypertrophy and hyperplasia. While these effects are reversible on removal of the TSH stimulus, at least early in the process, continuous stimulation of the thyroid by TSH can lead to neoplasia.

Non-mutagenic [refers to chemicals that are not directly DNA reactive] chemicals that produce thyroid follicular cell tumors in rats by prolonged TSH stimulation are not likely to be carcinogenic to humans. As discussed in HED HOT SHEET #23 (dated 6/22/04), humans respond as do experimental animals to disturbances in thyroid function from various antithyroid stimuli; *e.g.,* iodide deficiency, partial thyroidectomy and goitrogenic chemicals; *i.e*., when circulating thyroid hormone levels go down, the TSH level rises, which in turn leads to thyroid hypertrophy and hyperplasia (goiter). Cellular and biochemical studies, however, provide compelling evidence that rats are substantially more sensitive than humans to the development of thyroid follicular cell tumors in response to thyroid hormone imbalance (IARC, 2001, Meek et al., 2003, EPA,1998; Dohler et al., 1979). There are a number of quantitative differences between rats and humans that explain this increased sensitivity of the rat. The rat has a much shorter thyroid hormone half-life than humans; for example, thyroxin (T4) half-life in the rat is ≈12 hours compared to 5-9 days in the human (Dohler et al., 1979). The longer half-life in humans is likely related to the presence of a high-affinity binding globulin for thyroxin that is absent in the rat. Binding of thyroid hormone to this globulin would account for slower metabolic degradation and clearance. Additionally, there is a larger thyroid hormone reserve in the human compared to the rat. The rat thyroid gland is more active than the human thyroid gland, as evidenced by increased turnover rate and increased hepatic clearance of thyroid hormones (T3, T4) in the rat compared to the human. Additionally, the constitutive TSH levels are approximately 25 times higher in rats than in humans, reflecting the increased activity of the thyroid-pituitary axis in rats (Dohler et al, 1979; McClain 1992). Lastly, rats appear to be very susceptible to thyroid neoplasia secondary to thyroid hormone imbalance. Modest changes in thyroid hormone homeostasis may promote tumor formation in rats. In contrast, data in humans suggest that prolonged TSH stimulation of the thyroid gland poses a negligible risk of thyroid carcinogenesis (Curran and DeGroot, 1991). Studies of individuals with hyperthyroidism (patients with Graves Disease, goiters) indicate the occurrence of thyroid cancer is rare (*e. g.*, Mazzaferri, 2000; Gabriele, *et al.*, 2003). Also, a study of environmental and heritable causes of cancer among 9.6 million individuals using the Nationwide Swedish Family-Cancer Database found that the environment did not appear to play a principal causative role in thyroid cancer (Czene, *et al.*, 2002). The only known human thyroid carcinogen is x-irradiation.

I. INTRODUCTION

On April 14, 2004, the Cancer Assessment Review Committee [CARC] of the Health Effects Division [HED] of the Office of Pesticide Programs [OPP] met to evaluate the available mode of action/mechanistic data on the thyroid and liver and determine whether the available data are sufficient to support a change in the carcinogenic classification of pyrethrins, based on the new guidelines.

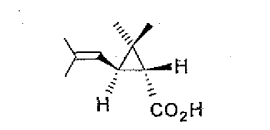
Pyrethrins was reviewed previously by the Cancer Peer Review Committee (CPRC) on February 22, **1995**, [TXR No. 0051384] and by the Cancer Assessment Review Committee (CARC) on February 3, **1999** [TXR No. 013354]. In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified pyrethrins as **"likely to be a human carcinogen by the oral route"** based on tumors at two organ sites in Charles River CD rats [liver tumors in females and thyroid tumors in males and females. The relevance of the observed tumors to human exposure could not be discounted. The Committee recommended a linear low-dose approach for human risk characterization. This extrapolation was supported by the lack of data on the mode of action for tumor induction [TXR No. 013169].

Pyrethrins Joint Venture [PJV] submitted mode of action [MOA]/mechanistic data [MRIDs 45889802 and 45669803] for both tumor types. The PJV requested that the Agency reconsider the carcinogenicity classification of pyrethrins based on the MOA data. The PJV asserts that the thyroid hormone data and liver enzyme induction data provide definitive evidence that the stimulation of the thyroid gland by Pyrethrins is similar to the well established mode of action by which Phenobarbital operates; namely, induction of liver mixed function oxidases leading to hepatocyte hypertrophy and cell proliferation; increased catabolism of thyroid hormones in liver serum [reduced T3/T4, resulting in a release of negative feedback control of the pituitary gland and increased serum TSH, thyroid follicular cell hypertrophy, cell proliferation, and ultimately, thyroid follicular cell adenomas. The PJV also asserts that the *"data provide definitive evidence that the liver tumors produced in the rat by Pyrethrins are induced only above a threshold dose by a rat-specific mode of action similar to that well described for Phenobarbital",* namely, induction of liver mixed function oxidase, replicative DNA synthesis, and ultimately liver cell adenomas. The PJV concludes that the *"data from the previous bioassay, taken together with the data in these mechanistic studies show that Pyrethrins, in common with other non-genotoxic oncogens, caused liver and thyroid gland tumors through a dose related proliferative response in the liver (replicative DNA synthesis and microsomal enzyme induction) and a secondary proliferative stimulation of thyroid follicular cells"*

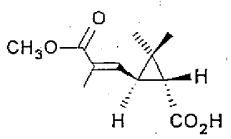
II. BACKGROUND INFORMATION

Pyrethrins are natural insecticides produced by certain species of the chrysanthemum plant. There are numerous agricultural, domestic home and garden, pet care, commercial/industrial/institutional/food and non-food/mosquito uses of pyrethrins. Pyrethrins are alkaloids. The concentrated extract from these flowers is called pyrethrum. Their relative instability to light limits their use outdoors and on food crops.

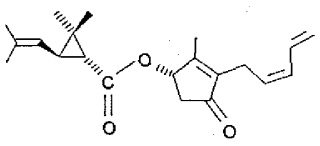
The structures of the components are shown below.



Chrysanthemic Acid



Pyrethric Acid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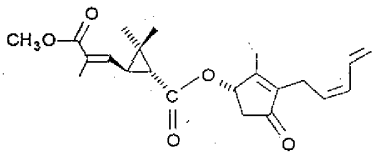
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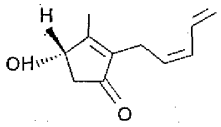
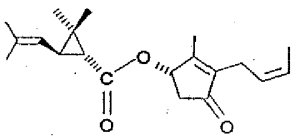


Pyrethrolone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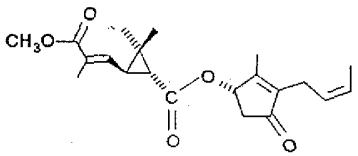
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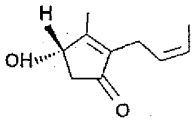

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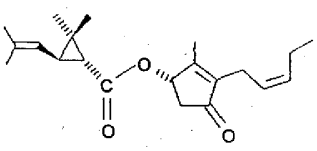
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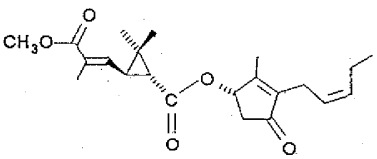
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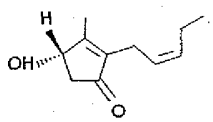

Jasmolone

III. EVALUATION OF MECHANISTIC DATA

The Pyrethrins Joint Venture [PJV] has submitted mechanistic data for both tumor types [thyroid effects (Summary Tables 1 and 2); liver effects (Summary Table 3)].

References: Finch, J. M., Martin, T., Travers, K. L., *et al.* (2002). Definitive Mechanistic Toxicity Study in Rats with Pyrethrins. Inveresk Research, Tranent, Scotland, Project No.455790; Report No. 21029. September 2, 2002. MRID 45889802. Unpublished.

Lake, B. G. (2002). An Investigation of Some Hepatic Enzyme Activities in Liver Samples Derived from Inversek Study 455790: Definitive Mechanistic Toxicity Study in Rats with Pyrethrins. TNO BIBRA International Ltd, Carshalton, Surrey, UK. TNO BIBRA Report No. 4024/2/2/2002; August 30, 2002. MRID 45889803. Unpublished.

Experimental Design: Female Sprague-Dawley [Crl:CD®(SD)IGS BR] rats [15/group/time point] were fed diets containing 0 [control, basal diet], 100 ppm, 3000 ppm, and 8000 ppm pyrethrins [PYR] and male Sprague-Dawley [Crl:CD®(SD)IGS BR] rats [15/group/time point] were fed a diet containing 0 [control, basal diet] and 8000 ppm pyrethrins for periods of 7 days, 14 days, 42 days, and 42 days followed by 42 days without treatment [recovery period]. As a positive reference compound, phenobarbital was fed *via* the diet to groups of Sprague-Dawley [Crl:CD®(SD)IGS BR] rats [15/sex/group/time point] of both sexes for periods of 7 days and 14 days. Liver samples from 8 rats/sex/group/time point/chemical were selected for hepatic enzyme analyses [MRID 45889803]. The results of the definitive mechanistic study are reported in MRID 45889802.

A. THYROID - Thyroid follicular cell tumors and follicular cell hyperplasia were observed in both sexes [males at the 1000 ppm and 3000 ppm dose levels and females at 3000 ppm only] in the bioassay. The mechanistic study provides data for male rats only at one dose level [8000 ppm], which is 2.7X-8X greater than the dose levels where tumors were observed in males. Dose levels of 100 ppm, 3000 ppm, and 8000 ppm were tested in females, which includes the dose level [3000 ppm] where thyroid [and liver] tumors were observed in the females.

BIOASSAY: Pyrethrins was administered at dose levels of 0, 100 ppm 1000 ppm and 3000 ppm in the bioassay. No thyroid hormone, thyroid weight, or hepatic enzyme activities data are available from this study. Follicular cell hyperplasia was observed in the 1000 ppm and 3000 ppm males [only slightly (not statistically-significantly) increased in the 3000 ppm females]. Follicular cell hypertrophy was not observed in either sex.

MECHANISTIC STUDY: Thyroid weight: 8000 ppm PYR males displayed increased thyroid weight after 7, 14, and 42 days; females displayed increased thyroid weight at 3000 ppm and 8000 ppm after 14, and 42 days. Thyroid Hormones: In 8000 ppm males, T3 and T4 were decreased and TSH was increased. Females at 3000 ppm and 8000 ppm displayed increased TSH only. After 7 and 14 days of exposure, the magnitude of the increase in TSH in females at 3000 ppm and 8000 ppm

exceeded that observed in the 8000 ppm males; after 42 days, the 8000 ppm female value exceeded the 8000 ppm male value. Microscopic Findings: 8000 ppm PYR males and 8000 ppm females displayed follicular cell hypertrophy at 7, 14, and 42 days; 3000 ppm females displayed this lesion only following 14 days. Phase II Marker of Hepatic Xenobiotic Metabolism Thyroxine UDP glycuronosyltransferase activity was increased in males and females at 8000 ppm at all three time points [days 7, 14, 42] and in the 3000 ppm females after 7 and 14 days. Increased BrdU staining: observed in the thyroid in a few males at 8000 ppm and in a few females at 3000 ppm and 8000 ppm following 7 days and 14 days only. % of cells labeling positive for BrdU: not affected by treatment for 7 days in either sex at 8000 ppm but was increased in the 3000 ppm females; following 14 days, an increase was displayed in females at 3000 ppm and in both sexes at 8000 ppm. No increase was observed after 42 days of exposure.

Following the 42-day recovery period, the thyroid hormones [TSH, T3, T4] values were comparable to those of the controls; thyroid follicular cell hypertrophy was not observed; and thyroxine UDPglycuronosyl transferase activity was no longer elevated. Thyroid weights were no longer elevated in the 8000 ppm males and 3000 ppm females, but they remained elevated at 8000 ppm in females.

- Given the nongenotoxic nature of the pyrethrins, the mode of action data are consistent with an enzyme-induced increased hepatic clearance of thyroid hormones. For the thyroid, data appear to support an indirect MOA for thyroid tumors, based on (1) non-genotoxicity; (2) increased thyroxine UDP glycuronosyltransferase activity (both sexes) [T3/T4 going down (males) *via* increased hepatic clearance]; (3) increased TSH levels in both sexes; and (4) hyperplasia observed at 1000 ppm and 3000 ppm in males. There was a weak tumor response, which was primarily benign; there was a flat dose response in males; the tumors were observed only at the high-dose level in females; and incidence of thyroid tumors in both sexes is outside historical control range.

- Data that detract from the proposed MOA include (1) hyperplasia not observed at any dose level in females; and (2) lack of dose response data in males. Data showing increased thyroxine UDP glycuronosyl transferase activity and alterations in thyroid hormones are available for males only at a dose level [8000 ppm] that is 2.7X/8X greater than the dose levels where tumors were increased.

Summary Table 1. THYROID EVIDENCE								
	thyroid weight	T3	T4	TSH	UDPGT	microscopic lesion	follicular cell tumor	
bioassay - males 100 ppm 1000 ppm 3000 ppm	[not weighed]	[not monitored]	[not monitored]	[not monitored]	not monitored]	follicular cell hyperplasia - ↑* ↑*	adenoma - ↑* ↑*	combined a & c - ↑* ↑*
mechanism - males 8000 ppm recovery	↑ days 7, 14, 42 -	↓ 7*** 14* -	↓7*** 14/42** -	↑7, 14/42*-	↑7**, 14/42*** -	follicular cell hypertrophy ↑7** 14/42*** -	adenoma N/A	combined a & c N/A
bioassay - females 100 ppm 1000 ppm 3000 ppm	[not weighed]	[not monitored]	[not monitored]	[not monitored]	not monitored]	follicular cell hyperplasia - - ↑	adenoma - - ↑*	combined a & c - - ↑
mechanism - females 100 ppm 3000 ppm 8000 ppm recovery	- ↑ days 14, 42 ↑ days 14, 42 ↑8000 ppm***	- - - -	- ↑ ↑* -	- ↑7/14*** 42 ↑7/14/42*** -	- ↑** 14/* 42 ↑7/14/42*** -	follicular cell hypertrophy - ↑7, 14*** ↑7* 14/42*** -	adenoma N/A	combined a & c N/A

* p<0.05; ** p<0.01; *** p<0.001; a adenoma; c carcinoma

Summary Table 2. [THYROID EFFECTS]						
Parameter	males		females			
	C	8000 ppm PYR	C	100 ppm PYR	3000 ppm PYR	8000 ppm PYR
7 days						
thyroid BrdU staining increased % cells labeling + for BrdU	0 2.96	3 3.33 [113]♪	0 2.65	0 2.85 [108]	4 7.82* [295]	5* 5.67 [214]
14 days						
thyroid BrdU staining increased % cells labeling + for BrdU	0 2.95	5* 11.54* [391]	0 4.96	0 6.70 [135]	5* 17.43* [351]	7** 33.48* [675]
42 days						
thyroid BrdU staining increased % cells labeling + for BrdU	0 3.78	0 4.86 [129]	0 4.47	0 3.56	0 3.74	0 3.62

* $p<0.05$; ** $p<0.01$; ♪[% of control]

- **DETERMINATION: WHETHER TUMORS DUE TO THYROID-PITUITARY IMBALANCE**

In accordance with the Agency's Policy Document entitled "Assessment of Thyroid Follicular Cell Tumors", March 1998 (EPA/630/R-97/002), the types of information necessary to characterize the mechanism of thyroid carcinogenesis are addressed as they apply to pyrethrins, as follows:

1. Consideration of whether the thyroid tumors associated with administration of pyrethrins can be attributed to disruption of the thyroid-pituitary hormonal balance (demonstration of antithyroid activity).

a.) **Increases in cellular growth *in vivo* (evidence required)**: Thyroid weight was increased in the mechanistic study in males at 8000 ppm after 7, 14, and 42 days of exposure and in females at 3000 ppm and 8000 ppm after 14 and 42 days of exposure. Thyroid weight remained elevated in females after the 42-day recovery period at 8000 ppm. Unfortunately, thyroid weights were not monitored in the rat bioassay. Thyroid follicular cell hyperplasia was observed in mid- dose males (1000 ppm) and in both sexes at the high-dose level (3000 ppm) in the bioassay. Thyroid follicular cell hypertrophy was observed in the mechanistic study after 7 days [8000 ppm, both sexes], 14 days and 42 days [both sexes at 8000 ppm, females at 3000 ppm], but not following a 42-day recovery period. In the mechanistic study, increased BrdU staining was observed in the thyroid in females at 3000 ppm [14 days] and 8000 ppm [7 and 14 days] and in males at 8000 ppm [14 days]. Also, the % of cell staining positive for BrdU was increased in females at 3000 ppm [7 and 14 days] and 8000 ppm

[7 and 14 days] and in males at 8000 ppm [14 days]. Following the recovery period, all monitored parameters had returned to control values, except the thyroid-weight increase in the 8000 ppm females.

b) **Hormonal changes [e.g., reduced thyroid hormones T3, T4 and increased TSH] (evidence required)**: Thyroid hormones were not monitored in the bioassay. In the mechanistic study, decreased T3 and T4 levels and increased TSH levels were observed in males at 8000 ppm [only dose tested in males] following 7 days and 14 days of exposure, and decreased T4 and increased TSH were observed in males following 42 days of exposure. Thyroid hormone values were comparable between the groups in males following the recovery period. In females, a dose-related increase was observed in TSH following all exposure durations.. Females displayed an increase in reverse triiodothyronine [rT3] following 7, 14, 42 days of exposure and following a 42-day recovery period, although the increase after 7 days was not dose-related. An effect on T3 and T4 was not demonstrated in females. TSH values were comparable among the groups of females following the recovery period.

c) **Site of action [intra-thyroidal, peripheral tissues, liver or other sites] (evidence required)**: The mechanistic study provided evidence that the liver is a site of action, as demonstrated by the increase in hepatic enzyme activities [cytochrome P450 enzymes and liver thyroxine UDPglycuronosyl transferase UDPGT] observed in both sexes at 8000 ppm and in females at 3000 ppm, with the magnitude of the change increasing with continued exposure for 7 days and 14 days and for 42 days [both sexes at 8000 ppm]. In males, the magnitude of the change was less following exposure for 42 days than after 14 days. Liver weights were increased slightly in the bioassay [111% (males); 112% (females) at 3000 ppm] *NOTE: In the bioassay, males at 3000 ppm displayed significantly increased **ALT** (561%-3318%) and **AST** (259%-650%) throughout the study.* Increased liver weights were observed in the mechanistic study following 7 days (males at 8000 ppm) and 7 and 42 days (females at 3000 ppm and 8000 ppm); comparable to control following recovery [42 days].

d) **Dose correlations (evidence required)**: The available data indicate that the effects on the liver weights, thyroid weights, liver UDPGT, and thyroid hormones occur at dose levels where thyroid follicular cell tumors have been observed in the **female** rat. Data for the male rat are limited to the occurrence of follicular cell hyperplasia at the same dose levels as the follicular cell tumors [in the bioassay]. The mechanistic data available for the male rat are at a dose level that is **2.7X and 8X greater** than the dose levels where the tumors were observed.

e) **Reversibility (evidence required):** The mechanistic study provides evidence of reversibility of the increase in liver P450 enzymes (females at 3000 ppm; both sexes at 8000 ppm), UDPGT (females at 3000 ppm; both sexes at 8000 ppm), T3 (males at 8000 ppm), T4 (males at 8000 ppm), TSH (females at 3000 ppm; both sexes at 8000 ppm), thyroid weight (females at 3000 ppm; males at 8000 ppm), liver weight (females at 3000 ppm; both sexes at 8000 ppm), follicular cell hypertrophy (females at 3000 ppm; both sexes at 8000 ppm). Thyroid weights remained elevated

in females at 8000 ppm following the recovery period [42 days] compared to controls [122% of control].

f) **Lesion progression (evidence desirable):** There was evidence of progression [hypertrophy/hyperplasia to neoplasia] in rats. In the bioassay, follicular cell hyperplasia was observed at the same dose levels as the follicular cell tumors. In the mechanistic study, follicular cell hypertrophy was observed following 7, 14, and 42 days of exposure at 8000 ppm, but only following 7 and 14 days of exposure in females at 3000 ppm [dose level where thyroid tumors observed]. Additionally, increased thyroid weight was observed in males following 7, 14, 42 days exposure [8000 ppm], and there was a dose-related increase in thyroid weight in females [3000 ppm and 8000 ppm] following 14 and 42 days of exposure. The thyroid was not weighed in the bioassay.

g): **Structure-activity analysis (evidence desirable)**: The structurally related pyrethroids, such as permethrin and cypermethrin, have been shown to cause lung and/or liver tumors in mice. Pyrethrins are usually formulated with Piperonyl butoxide and MGK-264, which are inhibitors of mixed function oxidases (MFO). Pyrethrins are metabolized by the MFO and thus, interact with the same physiological system in the liver. Piperonyl butoxide and MGK-264 both produce similar non-neoplastic lesions in the liver of rats and/or mice and have been indicated as producing at least some increases in hyperplasia and/or tumors of the follicular cells of the thyroid, as well as liver, in rats.

h) **Other studies (evidence desirable):** No additional data submitted.

2. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

There is no indication that genotoxicity or mutagenicity plays a role in the tumorigenic activity for pyrethrins.

3. Consideration of the occurrence of tumors in other tissues in addition to the thyroid follicular cell tumors (and relevant pituitary tumors).

There were no statistically-significant increases in any other tumor types in the male rats. However, high-dose female rats displayed a slight increase in **liver adenomas** in the bioassay, which was slightly outside the historical control range, and there is a statistically-significant positive trend and significant differences in pair-wise comparison of the high-dose group with the controls. There were no non-neoplastic lesions observed in the liver in either sex of rat.

4. Consideration of the dose-response.

Changes in thyroid hormone levels (T3, T5, TSH], thyroid weight, follicular cell hypertrophy, and UDPGT activity were observed in males at 8000 ppm [only dose tested] in the mechanistic study, while thyroid follicular cell hyperplasia and thyroid tumors were observed at 1000 ppm and 3000

ppm in the bioassay. Changes in TSH, thyroid weight, follicular cell hypertrophy, UDPGT levels were observed in a dose-related manner in females at the 3000 ppm and 8000 ppm dose levels in the mechanistic study, while thyroid follicular cell hyperplasia [not statistically significant] and thyroid tumors, as well as relative liver weight and liver tumors were observed in females at 3000 ppm in the bioassay.

B. LIVER - A causal relationship between enzyme induction and liver tumor formation following pyrethrins exposure has not been established. The proposed MOA is not inconsistent with the findings but is not conclusive. Although enzyme induction is consistent with liver hypertrophy and increased liver weights, which were both demonstrated in the mechanistic study, perturbation of these three parameters is not always associated with an increase in liver tumors. In the bioassay, neither liver hypertrophy nor liver hyperplasia was observed, and the liver tumors were observed only at the high-dose level, only in females, and they were benign only. The response was minimal but slightly outside the historical control range. The same enzymes induced in females were induced in males also, but no increase in liver tumors was observed in males in the bioassay. The fact that there was as much P450 induction in males as in females [8000 ppm] argues against a causal relationship between enzyme induction and liver tumor formation. NOTE: There are no data available on enzyme induction at 1000 ppm or 3000 ppm in males; from the female data available, it appears that enzyme induction would likely be observed at these bioassay dose levels in males.

Summary Table 3. LIVER EVIDENCE										
	liver weight	ALT	AST	7EROD	7PROD	test7αH	test16βH	test6βH	microscopic lesion	adenoma
bioassay - males				NM	NM	NM	NM	NM	hypertrophy	
100 ppm	rel111%C*	-	-						-	-
1000 ppm	-	-	-						-	-
3000 ppm	rel111%C*	↑[561-3318%C]	↑[259-650%C]						-	-
mechanism - males									hypertrophy	
8000 ppm	7*	-	-	7/14***	7/14***	7/14***	7**/14***	7/14***	↑*	N/A
recovery	-	-	-	-	-	-	-	-	-	
bioassay - females				NM	NM	NM	NM	NM	hypertrophy	
100 ppm	-	-	-						-	-
1000 ppm	-	-	-						-	-
3000 ppm	rel112%C*	-	-						-	↑**
mechanism - females									hypertrophy	
100 ppm	-	-	-	-	-	-	-	-	-	N/A
3000 ppm	7*42***	-	-	7**14*42**	7/14***42**	7***42***	7/14/42***	7/14/42***	7***14**42***	
8000 ppm	7*42***	-	-	7**14/42***	7/14***42**	7**14/43***	7/14/42***	7/14/42***	7*14***42**	
recovery	-	-	-	-	-	-	-	-		

* p<0.05; ** p<0.01; *** p<0.001; a adenoma; c carcinoma

Evidence of **liver cytotoxicity** was observed only in males in the rat bioassay at 3000 ppm. ALT [561%-3318% of control] and AST [259%-650% of control] were significantly elevated in males at 3000 ppm throughout the bioassay, but there was no increase demonstrated at 8000 ppm in the mechanistic study, although this might be due in part to the differences in the duration of the studies. Females did not displayed increases in ALT or AST in either the bioassay or the mechanistic study. The effect on liver weight in males in the bioassay is minimal and may be due to increased AST and ALT. Liver hypertrophy/hyperplasia was not observed in either sex in the bioassay, although liver hypertrophy was demonstrated in this mechanistic study in females at 3000 ppm and in both sexes at 8000 ppm. Thus, cytotoxicity is not associated with the female liver tumor response.

With respect to other modes of action for the liver tumors following pyrethrins exposure, there are adequate data to show that pyrethrins are not acting as a mutagen, and cytotoxicity is not associated with the female liver tumor response. While there is no evidence of peroxisome proliferation, no data have been submitted with respect to hepatic lipid peroxidation. Although a good association has been reported between the ability of chemicals to induce the cytochrome P-450 (CYP) 2B1/2 isozymes and their potential to promote hepatocarcinogenesis, when biochemical and tissue changes in rodents following exposure to 9 nongenotoxic NTP carcinogens were evaluated, it was concluded that **the only measurement that failed to correlate usefully with carcinogenicity was the induction of liver enzymes** [E HP, Vol. 110 (4): 363-375 (2002)]. Additionally, this article stated that there is "some controversy over the merit of determining transient increases in cell proliferation" (as determined in the study) "*versus* sustained increases, the latter of which have been suggested by some to be necessary to drive carcinogenesis." Pyrethrins appear to be a weak promotor/mitogen, although this is inconsistent with the results of the rat bioassay where there is evidence of cytotoxicity in males [significant elevations in ALT and AST] but there are no liver tumors in males. Other potential modes of action have not been investigated. It has been demonstrated that various types of liver tumor-promoting agents inhibit gap-junctional intercellular communication in the rat liver *in vivo*, and many hepatic tumor promoters and nongenotoxic carcinogens inhibit gap-junctional communication in rodent hepatocytes *in vitro*. Phenobarbital has been shown to reversibly decrease the abundance of the gap-junctional intercellular communication protein connexin 32 (x32) in both preneoplastic-altered hepatic foci and in centrilobular hepatocytes in which CYP 2B1/2 isozymes are induced (Cancer Commun. 2:21-31 (1990). Based on these findings, it has been suggested that agents that induce CYP 2Ba/2 isozymes and that reduce Cx32 in centrilobular hepatocytes are probably all liver tumor promoters (Cancer Res. 54: 3145-3152 (1994). Several studies have shown that PB-induced suppression of gap junction intercellular communication in hepatocytes is reversible upon withdrawal of PB treatment [Cancer Commun 2:21-31, 1990]. Gap junctions are transmembrane channels formed at the area of contact between cells that permit the transfer of small molecules and appear to be involved in the maintenance of homeostasis in multicellular organisms. Gap-junctional intercellular communication may mediate cell proliferation through regulating the passage of either growth stimulatory or inhibitory molecules between adjacent cells. Dysfunctional gap-junctional communication may result in the disruption of regulated cell division and may enhance preneoplastic cell growth [from Toxicol. Sci. 64(2): 192-199 (2001)]. Glutathione-S-transferase placental form focus, a putative preneoplastic lesion, is

induced following PB exposure and has been investigated as a measure of carcinogenic potential.

The available mode of action data for the **liver tumors** are not adequate; i.e., the MOA is unclear, however:

- the liver tumor response in the rat is weak;
- liver tumors occurred in the female rat only;
- the liver tumor response was observed only at the high-dose level [3000 ppm];
- the liver tumors were benign only; and
- the incidence [8%] is slightly outside the historical control range [0%-6%].

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

The CARC concluded:

(A) THYROID: The thyroid mode of action [MOA] is supported by the available data, based on the following:

1. Consideration of whether the thyroid tumors associated with administration of pyrethrins can be attributed to disruption of the thyroid-pituitary hormonal balance (demonstration of antithyroid activity).

- **evidence of goitrogenic activity *in vivo*:** (1) mechanistic study - both sexes displayed increased thyroid weights; thyroid follicular cell hypertrophy; increased BrdU staining in the thyroid, and increased % of cell staining positive for BrdU. (2) rat bioassay: both sexes displayed follicular cell hyperplasia.
- **clinical chemistry [hormonal] changes**: (1) mechanistic study - decreased T3 and T4 and increased TSH observed in males and increased TSH observed in females. (2) not monitored.
- **site of action**: (1) mechanistic study - LIVER, as evidenced by increased hepatic enzyme activities [cytochrome P450 enzymes and liver thyroxine UDPGT], increased liver weight; THYROID, as evidenced by increased thyroid weight and follicular cell hypertrophy. (2) rat bioassay - LIVER, as evidenced by liver toxicity in males [increased ALT and AST]. THYROID, as evidenced by increased thyroid weight and follicular cell hyperplasia.
- **dose correlation**: (1) mechanistic study - effects on liver weight, thyroid weight, liver thyroxine UDPGT, and thyroid hormones occurred at the dose level where thyroid follicular cell tumors were observed [females]. (2) rat bioassay - follicular cell hyperplasia occurred at the same dose levels as the follicular cell tumors (males).
- **reversibility**: (1) mechanistic study - with the exception of increased thyroid weight at the high-dose level in females [8000 ppm], all parameters [T3, T4, TSH, thyroid weight, follicular cell hypertrophy] returned to control values following removal of exposure. (2) rat bioassay - not assessed.

- **lesion progression**: (1) mechanistic study - follicular cell hypertrophy was observed at the dose level where follicular cell tumors were observed (female). (2) rat bioassay - follicular cell hyperplasia was observed at the same dose levels as the follicular cell tumors.
- **structure-activity analysis**. The structurally related pyrethroids, such as permethrin and cypermethrin, have been shown to cause lung and/or liver tumors in mice.

2. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

- There is no indication that genotoxicity or mutagenicity plays a role in the tumorigenic activity for pyrethrins.

3. Consideration of the occurrence of tumors in other tissues in addition to the thyroid follicular cell tumors (and relevant pituitary tumors).

- There were no statistically-significant increases in any other tumor types in the male rats. However, high-dose female rats displayed a slight increase in **liver adenomas** in the bioassay, which was slightly outside the historical control range, and there is a statistically-significant positive trend and significant differences in pair-wise comparison of the high-dose females with the controls. There were no non-neoplastic lesions observed in the liver in either sex of rat.

4. Consideration of the dose-response.

- Changes in thyroid hormone levels (T3, T5, TSH], thyroid weight, follicular cell hypertrophy, and UDPGT activity were observed in males at 8000 ppm [only dose tested] in the mechanistic study, while thyroid follicular cell hyperplasia and thyroid tumors were observed at 1000 ppm and 3000 ppm in the bioassay. Changes in TSH, thyroid weight, follicular cell hypertrophy, UDPGT levels were observed in a dose-related manner in females at the 3000 ppm and 8000 ppm dose levels in the mechanistic study, while thyroid follicular cell hyperplasia [not statistically significant] and thyroid tumors, as well as relative liver weight and liver tumors were observed in females at 3000 ppm in the bioassay.

(B) LIVER: The liver mode of action [MOA] is unclear. However, the data support pyrethrins as a weak, mitogenic, tumor promotor in the female rat.

V. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified pyrethrins as **"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"** based on the following weight-of-the-evidence considerations:

(i) The occurrence of a benign and minimal liver tumor response only in female Crl:CD®(SD)IGS BR rats.

(ii) There was no treatment-related increase in liver tumors in male Crl:CD®(SD)IGS BR rats.

(iii) There was no treatment-related increase in tumors in either sex of Charles River CD mice.

(iv) There is no concern for mutagenicity.

With regard to the thyroid tumors, the mode of action data for pyrethrins is consistent with the mode of carcinogenic action that has been established for a number of pesticides that induce thyroid follicular cell tumors in rats (Hurley et al., 1998). This mode of action involves a reduction of circulating thyroid hormone, which activates homeostatic processes that increase thyroid stimulating hormone (TSH) release from the pituitary. TSH release stimulates the thyroid gland to increase thyroid hormone synthesis and release. Persistently elevated TSH levels will lead to thyroid follicular cell hypertrophy and hyperplasia. Effects are reversible on removal of the TSH stimulus, at least early in the process. However, continuous stimulation of the thyroid by TSH can lead to neoplasia. Pyrethrins, like most antithyroid pesticides, operate at an extrathyroidal site by increasing hepatic metabolism and excretion of thyroid hormone.

Non-mutagenic chemicals that produce thyroid follicular cell tumors in rats by prolonged TSH stimulation are not likely to be carcinogenic to humans. Humans respond as do experimental animals to disturbances in thyroid function from various antithyroid stimuli, such as iodide deficiency, partial thyroidectomy and goitrogenic chemicals; *i.e.*, when circulating thyroid hormone levels go down, the TSH level rises, which in turn leads to thyroid hypertrophy and hyperplasia (goiter). Cellular and biochemical studies, however, provide compelling evidence that rats are substantially more sensitive than humans to the development of thyroid follicular cell tumors in response to thyroid hormone imbalance (IARC, 2001, Meek et al., 2003, EPA,1998; Dohler et al., 1979). There are a number of quantitative differences between rats and humans that explain this increased sensitivity of the rat. The rat has a much shorter thyroid hormone half-life than humans; for example, thyroxin (T4) half-life in the rat is ≈12 hours compared to 5-9 days in the human (Dohler et

al., 1979). The longer half-life in humans is likely related to the presence of a high-affinity binding globulin for thyroxin that is absent in the rat. Binding of thyroid hormone to this globulin would account for slower metabolic degradation and clearance. Additionally, there is a larger thyroid hormone reserve in the human compared to the rat. The rat thyroid gland is more active than the human thyroid gland, as evidenced by increased turnover rate and increased hepatic clearance of thyroid hormones (T3, T4) in the rat compared to the human. Additionally, the constitutive TSH levels are approximately 25 times higher in rats than in humans, reflecting the increased activity of the thyroid-pituitary axis in rats (Dohler et al, 1979; McClain 1992). Further, rats appear to be very susceptible to thyroid neoplasia secondary to hypothyroidism. In contrast, data in humans suggest that prolonged TSH stimulation of the thyroid gland poses a negligible risk of thyroid carcinogenesis (Curran and DeGroot, 1991). Studies of individuals with hyperthyroidism (patients with Graves Disease, goiters) indicate the occurrence of thyroid cancer is rare (*e. g.*, Mazzaferri, 2000; Gabriele, *et al.*, 2003). Also, a study of environmental and heritable causes of cancer among 9.6 million individuals using the Nationwide Swedish Family-Cancer Database found that the environment did not appear to play a principal causative role in thyroid cancer (Czene, *et al.*, 2002). The only known human thyroid carcinogen is x-irradiation.

It is noted that the CARC assessment of the thyroid mechanistic studies is not inconsistent with that of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, who concluded that the increased thyroid tumor incidence caused by pyrethrins is a threshold phenomena of negligible toxicological relevance to humans (JMPR report, 2003).

VI. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended that no quantification of human carcinogenic risk be determined for pyrethrins.

Although the rat thyroid follicular cell tumors with a known mechanism should not be used to evaluate human cancer hazard, they should be used as a biomarker that may lead to non-cancer outcomes; in particular, neurodevelopmental effects.

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