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~~Pre-Registration~~ --Assessment of Chronic and Oncogenic Effects

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PERMETHRIN--ASSESSMENT OF CHRONIC AND ONCOGENIC EFFECTS

I. Availability and Usefulness of Studies:

Seven long-term chronic feeding/oncogenicity studies have been submitted to EPA in support of requests to register food and other uses of Permethrin. These studies are listed below.

Study Identification	Duration of Study (weeks)	Dosage Levels of Permethrin (mg/kg/day)
ICI Mouse	98	0, 37.5, 150, 375
FMC Mouse I	104	0, 3, 75, 600
FMC Mouse II	104	0, 4, 75, 300 (males) 0, 4, 375, 750 (females)
B-W Mouse	92	0, 10, 50, 250
ICI Rat	104	0, 25, 50, 125
FMC Rat	104	0, 1, 5, 25
B-W Rat	104	0, 10, 50, 250

The test material for all studies, with the exception of the B-W studies, was a 40:60 cis:trans isomer ratio of Permethrin. For the B-W studies, the cis:trans isomer ratio was 25:75. Although the cis isomer is known to be more toxic than the trans isomer (based on the results of acute oral and other toxicity studies), the difference in isomer ratios used in the chronic studies was not considered substantial enough to warrant separate consideration of the B-W studies.

One study, the FMC Mouse I study, has been judged to be of no usefulness due to a serious animal identification problem which arose during the study. This left an insufficient number of positively identified animals available at the end of the study to adequately evaluate the results.

One other study, the FMC Rat study, has been judged to be of only limited usefulness with regard to evaluation of lung tissues for tumors. This resulted from a failure to treat lungs from control and test animals in a comparable manner during preparation of these tissues for microscopic examination.

The remaining five chronic studies have been determined to be useful and valid for the purpose of evaluating the chronic and oncogenic effects of Permethrin.

The results of additional studies, particularly mutagenic, reproduction, teratology and metabolism studies, have also been considered in the overall assessment of the long-term effects of Permethrin.

II. Synopses of Individual Long-Term Studies:

ICI Mouse Study - Central Toxicology Laboratory, ICI
Report No. CTL/P/358 and CTL/P/359
January 27, 1978

Doses of 0 (control), 37.5, 150 and 375 mg/kg/day of Permethrin were administered in the diet to 70 male and 70 female Alderly Park strain mice/group for 98 weeks. Ten male and 10 female mice per group were sacrificed at 26 and 52 weeks and the remainder at 98 weeks. Relevant non-oncogenic effects observed during the study were increased mortality, increased liver enzyme (aminopyrine-N-demethylase) activity, increased liver weights, and eosinophilia of hepatocytes in both males and females at 375 mg/kg/day. Liver changes observed in this study were considered to be related in large measure to the induction of liver microsomal enzyme activity. Minimal liver changes were also observed at 150 mg/kg/day, but not at 37.5 mg/kg/day.

A slightly increased incidence of lung adenomas was observed in male mice in this study--10/70, 6/70, 12/70, and 17/70 for control, low, mid, and high dosage levels respectively. Statistical analysis of this finding revealed a positive trend for this lesion in Permethrin treated males (Armitage test); but after correction for departure from linearity, this trend was non-significant. Fisher's Exact test showed significance ($p < .01$) for increased risk among high dose vs. low dose mice; but when any single Permethrin treated male group was directly compared to the concurrent control group statistical significance was not observed ($p \geq 0.10$). Toxicology Branch considers the biological significance of this finding to be of only marginal concern when considered in isolation as an individual study, but notes that this finding does tend to support similar findings in the other 2 mouse studies.

FMC Mouse II Study - Bio/Dynamics Inc.
Project No. 76-1695
October 9, 1979

Doses of 0 (control), 4, 75 and 300 mg/kg/day of Permethrin were administered in the diet to groups of 75 male Charles River CD1 strain mice and doses of 0 (control), 4, 375 and 750 mg/kg/day to groups of 75 female mice for 104 weeks. Initial concerns regarding yellow staining of the ano-genital region, abdominal distention and amyloidosis of animals in this study have been dismissed as having no significant

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influence on the interpretation of results. Relevant non-oncogenic effects observed during the study were increased mortality in males at 300 mg/kg/day, increased liver weights in females at 375 and 750 mg/kg/day and increased lung weights in females at 750 mg/kg/day. Histopathologically, "focal areas of alveolar cell proliferation" (1) (an indicator of increased numbers of lung cells) was observed with a dose-related incidence in Permethrin treated females--3, 5, 11 and 13 for the control, low, mid and high dosage level females respectively. The proportion of female mice with "focal areas of alveolar cell proliferation" (1) as their most serious lung diagnosis was significantly higher ($P = 0.001$) among the high dose animals free of adenoma and/or carcinoma than among the comparable controls. Also, multifocal hepatocytomegaly (an indicator of increased liver cell volume) was observed with increased frequency in Permethrin treated females--0, 3, 6 and 9 for the control, low, mid and high dosage levels respectively. The nature of these lesions as precursors of neoplasms is disputed.

With regard to oncogenic effects, the initial pathology report (submitted with the study and dated February 7, 1980) indicated an increased incidence of bronchioalveolar adenomas in female mice--12, 14, 28 and 28 for control, low, mid and high dosage levels respectively. A second reading of the same lung slides (performed under contract to EPA; report dated February 23, 1981) also reported an increased incidence of alveolar cell neoplasms (adenomas and/or carcinomas) in female mice--15, 24, 35 and 44 for control, low, mid and high dosage level females respectively. The increased incidence reported in both readings was statistically significant. In addition, for the second reading, when this latter finding is adjusted for time of death (or time to tumor diagnosis), Peto's Prevalence method (2) shows statistical significance. The incidence of carcinoma, alone, when considered separately in the second report, was observed to increase in a dosage related manner (6, 7, 11 and 15) for control, mid and high dosage level females respectively. This trend is statistically significant ($P < 0.01$). In addition, direct comparison of the incidence of lung carcinoma in the high dose females (15/75) to the control females (6/74) revealed statistical significance.

The incidence of lung adenomas and/or carcinomas in female mice dying or sacrificed before the terminal sacrifice was 7/52, 6/38, 12/43 and 19/48 for the control, low, mid and high dosage level females respectively. Based on an analysis of time to diagnosis of tumor (at death) for these animals, a statistically significant decrease in latency, or time to tumor, was observed for combined tumor types, but not for carcinomas alone.

(1) EPL pathology report, dated February 23, 1981.

(2) Annex to Supplement II of I.A.R.C., 1981.

Toxicology Branch has determined the increased incidence of alveolar cell adenomas and carcinomas and the decreased latencies for these tumors in the lungs of female mice in this study to be statistically significant at both the mid and high dosage levels (375 and 750 mg/kg/day). Biologically, Toxicology Branch considers these tumors to be related to the ingestion of Permethrin.

A statistically significant increase in the incidence of hepatocellular neoplasms was also observed in female mice in this study at the mid and high dosage levels. The initial pathology report indicated 3, 2, 15 and 17 for hepatomas and the second report 6, 7, 25 and 30 for total hepatocellular neoplasms (adenomas and carcinomas) for control, low, mid and high dosage level females respectively. In the second report, hepatocellular adenomas were reported in 3, 4, 23 and 29 females and hepatocellular carcinomas in 4, 3, 3 and 2 females for the control, low, mid and high dosage level animals respectively. The incidence of hepatocellular adenomas was observed to increase in a dosage related manner. The number of carcinomas, however, did not increase with increasing dosage. Toxicology Branch has determined the increased incidence of hepatocellular adenomas in female mice at the mid and high dosage levels (375 and 750 mg/kg/day) to be statistically significant.

A joint FDA-EPA audit of this study conducted in late 1980 at Bio/Dynamics and FMC facilities did not reveal any inadequacies in the conduct or reporting of this study serious enough to compromise the usefulness or validity of these study results.

B-W Mouse Study - The Wellcome Foundation
Lab. No. HEFG 80-29
Received by EPA on December 17, 1980

Permethrin was administered in the diet to male and female CFLP strain mice for 92 weeks at dosage levels of 0 (control), 10, 50 and 250 mg/kg/day. One hundred mice/sex were used for the control group and 75 mice/sex for each of the test groups. Relevant non-oncogenic effects observed during the study were slightly decreased mortality in females at 50 and 250 mg/kg/day, increased liver weights in males and increased kidney weights in females at 250 mg/kg/day. Histopathologically, an increased incidence of cuboidal/columnar metaplasia of the alveolar epithelium was observed in the lungs of female mice at the high dosage level (250 mg/kg/day). The incidence for this lesion was 0, 0, 1 and 7% for the control, low, mid and high dosage levels respectively. Although controversial, this lesion is considered by some pathologists to be a precursor of lung neoplasms in mice.

For females in this study, but not for males, a dose-related trend in primary adenomatous tumors in the lungs was observed. The incidence of this neoplastic lesion in females was 3, 7, 9 and 20% for control, low, mid and high dosage level females respectively. The registrant (B-W) has submitted statistical data with the report of this study showing that by adjusting for time of diagnosis (or death), Peto's Prevalence method indicates a statistically significant ($p < .01$) relationship between dose of Permethrin and primary lung tumors in females. When these data are evaluated by Armitage's method for linear trend, the level of statistical significance for the dose-response trend is $p < .02$. In addition, comparison of the tumor rate in the high dose group (20%) to the rate in the controls (3%) shows high statistical significance by Fisher's Exact test. Also, for these same tumors, a dose-related decreased latency (or time to tumor) was observed. For females dying or sacrificed prior to terminal sacrifice, the incidence of this lesions was 0, 0, 6 and 26% for the control, low, mid and high dosage level females respectively. Further, with respect to malignant lung tumors in these females, 1 animal in the mid dosage level group and 2 animals in the high dosage level group were diagnosed as having metastasizing lung tumors whereas this diagnosis was not made for any tumors in the control or low dosage level animals. Toxicology Branch considers the increased incidence of lung tumors in female mice observed in this study, together with the other supportive evidence observed in this study, to be highly suggestive of a possible oncogenic effect in lungs -- particularly when considered in relation to the results of the other 2 oncogenic studies in mice.

ICI Rat Study - Central Toxicology Laboratory, ICI
Report No. CTL/P/357
Received by EPA on January 29, 1978

Doses of 0 (control), 25, 50 and 125 mg/kg/day of Permethrin were administered in the diet to 60 male and 60 female Wistar strain rats/group for 104 weeks. Twelve rats of each sex were sacrificed at 52 weeks and the remainder at 104 weeks. Relevant non-oncogenic effects observed during the study were slightly increased mortality in males and decreased mortality in females at 125 mg/kg/day, increased

liver weights in males and females at 125 and 50 mg/kg/day and in males only at 25 mg/kg/day, increased liver enzyme (aminopyrine-N-demethylase) activity in males and females at 125 mg/kg/day, and hepatocyte vacuolization or hypertrophy in males and females at 125 and 50 mg/kg/day. Additional effects observed were increased kidney weights in males at 125, 50 and 25 mg/kg/day, and increased pituitary weights in males at 125 and 50 mg/kg/day. Body tremors were also observed in males and females during the first 3 weeks of the study at 125 mg/kg/day.

No tumors considered by Toxicology Branch to be related to or attributable to the ingestion of Permethrin were observed in this study.

FMC Rat Study - Bio/Dynamics Inc.
Project No. 74R-1022
November, 1977

Permethrin was administered in the diet to 60 male and 60 female Long-Evans strain rats for 104 weeks at dosage levels of 0 (control), 1, 5 and 25 mg/kg/day. Ten males and 8 females from the 5 mg/kg/day group were sacrificed at 52 weeks to follow the hepatocyte hypertrophy observed in a previous 3 month study. The remaining animals were sacrificed at 104 weeks. Relevant non-oncogenic effects included increased liver weights for males at 25 and 5 mg/kg/day.

An increased incidence of adenomas and adenocarcinomas was initially reported in the lungs of male rats in this study. The reading of the original set of lung slides reported 1, 3, 6 and 5 lung neoplasms for control, low, mid and high dosage level males respectively. This increased incidence was not statistically significant ($p > .05$). These same lung slides were later reread by a second pathologist who reported 1, 3, 8 and 6 lung neoplasms for the respective groups. Based on the readings of the second pathologist, the increase in lung neoplasms was statistically significant ($p < .05$) for the mid and high dosage level males relative to the male control group. In a subsequent effort to perform a more critical and detailed evaluation of lung tumors in male rats, all available lung tissues from the male animals were step-sectioned at 250 micron intervals to exhaustion of tissue and read by the original pathologist who then reported revised figures of 8, 6, 10 and 10 for the respective groups (based on original and step-sectioned slides). This finding was not statistically significant ($p > .20$).

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Based on information supplied by FMC and on a joint FDA-EPA audit of the laboratories and personnel involved in the histological processing of the lung slides, Toxicology Branch has determined that inconsistencies in the technical methodologies used in the original sectioning and processing and also later in the stepsectioning and processing of these lung tissues introduced serious bias into all these results--largely due to inconsistent embedding techniques which resulted in unequal amounts of lung tissue being examined in the control and test groups. Readings of the original lung slides were biased in favor of finding relatively more tumors in the lungs of test animals whereas readings of the step-sectioned slides were biased in favor of finding relatively more tumors in the lungs of control animals.

In an effort to resolve the dilemma of which figures to use, Toxicology Branch has made additional theoretical calculations of lung tumor incidence based on equal amounts of lung tissue (adjusted) from all control and test groups. These "area adjusted" tumor incidences were calculated to be 1, 2, 5 and 4 for the original (non-step-sectioned slides) and 8, 8, 15 and 14 for all slides (original and step-sectioned slides). The statistical significance of these "area adjusted" findings was borderline (p approximately 0.10).

Evidence suggests the possibility of an oncogenic effect occurring in the lungs of treated male rats in this study. Sufficient uncertainty regarding the validity of the incidence figures, however, precludes making a scientifically supportable evaluation of the results.

B-W Rat Study - The Wellcome Foundation
Lab. No. HEFG 80-33
July 2, 1980

Permethrin was administered in the diet to 60 male and 60 female Wistar strain rats for 104 weeks at dosage levels of 0 (control), 10, 50 and 250 mg/kg/day. Relevant non-oncogenic effects observed during the study were increased mortality in males at 250 mg/kg/day, occasional body tremors in males and females at 250 mg/kg/day, increased liver weights in males at 250 mg/kg/day, hepatocyte hypertrophy in males and females at 250 mg/kg/day and focal disturbances in the growth pattern of thyroid follicular cells in males and females at 250 mg/kg/day. The microscopic liver and thyroid changes were also observed in males and females at 50 mg/kg/day.

With respect to tumors, none of the tumor types observed in this study were considered by Toxicology Branch to be related to or attributable to the ingestion of Permethrin.

III. Non-Oncogenic NOEL for Mouse Studies:

Relevant non-oncogenic effects observed in the 3 long-term mouse studies are tabulated below.

Study	Dosage Level of Permethrin (mg/kg/day)	Non-Oncogenic Effects
ICI Mouse	0	- None
	37.5	- None
	150	- Minimal changes in liver enzyme activity, liver weights and liver histopathology (eosinophilia of hepatocytes) (M&F)
	375	- Increased mortality (M&F), Increased liver enzyme activity (M&F), Increased liver weights (M&F), Eosinophilia of hepatocytes (M&F)
FMC Mouse II	0 (M&F)	- None
	4 (M&F)	- None
	75 (M only)	- None
	300 (M only)	- Increased mortality (M)
	375 (F only)	- Increased liver weights (F), "Focal areas of alveolar cell proliferation" (F), Multifocal hepatocytomegaly (F), Hepatocyte pigmentation (F)
	750 (F only)	- Increased liver weights (F), Increased lung weights (F), "Focal areas of alveolar cell proliferation" (F), Multifocal hepatocytomegaly (F), Hepatocyte pigmentation (F)
B-W Mouse	0	- None
	10	- None
	50	- None
	250	- Slightly increased liver weights (M), Slightly increased kidney weights (F), Cuboidal/columnar metaplasia of alveolar epithelium in lungs (F)

A consistent finding in all 3 mouse studies at high dosage levels was liver changes known to be associated with induction of the liver microsomal enzyme system. These changes included slightly increased liver weights and increased enzyme (aminopyrine-N-demethylase) activity. This induction phenomenon is a well-known and frequently occurring response to the administration of many exogenous compounds to experimental animals and man. It is widely accepted among knowledgeable scientists as being a normal and natural adaptive response of the organism to the presence of foreign chemicals and is not considered to be an adverse or toxicological effect of concern. Therefore, Toxicology Branch notes the presence of this phenomenon in mice treated with Permethrin, but will not use it to determine a NOEL. Of potentially more serious concern are other histopathological effects observed in liver cells that are not ordinarily associated with microsomal induction and could possibly be related to an adverse effect of Permethrin on the liver. These effects include excessive liver weight increases, multifocal hepatocytomegaly, hepatocyte pigmentation and possibly eosinophilia of hepatocytes. These and other toxicological manifestations were used to determine a non-oncogenic NOEL for the mouse studies.

Due to the considerably different dosage levels and spacing between levels employed in each of the 3 studies, Toxicology Branch believes the assignment of a specific NOEL to each mouse study would serve no useful purpose and might, in fact, be quite misleading. Based on a consideration of the totality of evidence in all 3 mouse studies, the NOEL for non-oncogenic effects in mice has been determined to be 50 mg/kg/day.

IV. Non-Oncogenic NOEL for Rat Studies:

Relevant non-oncogenic effects observed in the 3 long-term rat studies are tabulated below.

Study	Dosage Level of Permethrin (mg/kg/day)		Non-Oncogenic Effects
ICI Rat	0	-	None
	25	-	Increased liver weights (M), Increased kidney weights (M)
	50	-	Increased liver weights (M&F), Hepatocyte vacuolation (M&F), Hepatocyte hypertrophy (M&F), Increased kidney weights (M), Increased pituitary weights (M)

cont.

	125	-	Slightly increased mortality (M), Increased liver enzyme activity (M&F), Increased liver weights (M&F), Hepatocyte vacuolation (M&F), Hepatocyte hypertrophy (M&F), Increased kidney weights (M), Increased pituitary weights (M), Body tremors (1st 3 weeks) (M&F)
FMC Rat	0	-	None
	1	-	None
	5	-	Slightly increased liver weights
	25	-	(M), Increased liver weights (M)
B-W Rat	0	-	None
	10	-	None
	50	-	Hepatocyte hypertrophy (M&F) Changes in thyroid cells (M&F)
	250	-	Increased mortality (M), Increased liver weights (M), Increased liver weights (M), Hepatocyte hypertrophy (M&F), Changes in thyroid cells (M&F), Occasional body tremors (M&F)

As with mice, a consistent finding in all 3 rat studies was liver changes known to be associated with induction of the microsomal enzyme system. This induction phenomenon, for reasons already presented, was again not considered by Toxicology Branch to be an adverse or toxicological effect of concern. The other toxic effects listed above were used to determine a non-oncogenic NOEL for the rat studies.

For the FMC Rat Study, a NOEL of 5 mg/kg/day was determined. For the ICI Rat Study, a NOEL of < 25 mg/kg/day was determined. For the B-W Rat Study, a NOEL of 10 mg/kg/day was determined. Based on a consideration of the totality of evidence in all 3 rat studies, the NOEL for non-oncogenic effects in rats has been determined to be 5 mg/kg/day.

V. Overall Assessment of the Oncogenic Potential in Experimental Animals.

According to the International Agency for Research on Cancer (IARC), "the widely accepted meaning of the term 'chemical carcinogenesis' --- is the induction by chemicals of neoplasms that are not usually observed, the earlier induction by chemicals of neoplasms that are usually observed, and/or the induction by chemicals of more neoplasms than are usually found" (3). The Toxicology Branch considers this definition and the following seven criteria to evaluate oncogenicity in experimental animals when multiple oncogenic studies are available on a single chemical:

1. Oncogenicity in different a) species, b) strains, c) sexes and d) organs

Three strains of mice were used in the long-term mouse studies: a) ICI, Alderly Park, (SPF Swiss); b) FMC II, Charles River CD-1; and c) B-W, CFLP. In the ICI study, a slight (nonsignificant) increased incidence of lung adenomas was observed in male mice. Females in the FMC II study exhibited an increased incidence of lung and liver tumors. In the B-W study, female mice exhibited a trend in adenomatous tumors in the lungs. Only females of the Charles River CD-1 strain (FMC II study) showed a definite lung and liver oncogenicity due to Permethrin exposure.

Of the three long-term rat studies, the ICI (Wistar strain) and the B-W (Wistar strain) studies revealed no tumors, in either sex, which were considered to be related to or attributable to the ingestion of Permethrin. The FMC (Long Evans strain) study produced evidence suggestive of an oncogenic effect in the lungs of males only.

Among the six long term Permethrin studies, definite oncogenicity was observed in the lungs and liver of only one sex of one strain of mouse.

(3) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 22, p. 14, March 1980.

2. Presence of rare neoplasms and number of different types of neoplasms in one or more species

Lung and liver tumors were the predominant neoplasms of potential concern in the long-term mouse studies. In mice, lung and liver tumors are not rare neoplasms. Both tumor types occur spontaneously in control mice and have highly variable incidence rates (occasionally quite high) from study to study. With the exception of a marginal effect in males in the ICI study, increased incidences of lung tumors were observed only in females. An increase in the number of hepatomas was confined to females in the FMC II study. An increased incidence for this tumor type did not occur in either sex of the ICI or B-W studies. Other types of tumors observed in either sex in the three mouse studies were not attributed to ingestion of Permethrin.

No tumors considered to be related to ingestion of Permethrin were observed in the ICI or B-W Rat Studies. Evidence in the flawed FMC Rat Study suggests the possibility of an oncogenic effect in the lungs of male rats. Lung adenomas are not a common tumor type in this species. The uncertainty of incidence figures precludes making a scientifically supportable evaluation of this observation (see pp 6-7 for detailed discussion).

There was no increased incidence of rare or unusual tumors in any of the mouse or rat long-term studies.

3. Increased incidence of malignant neoplasms

The number of malignant lung tumors at termination of the ICI Mouse Study was small. For males, the incidence was zero for all groups. For females, the incidence was 0, 1, 1, 1 for the control and the three treated groups. This was also true at termination of the B-W Mouse Study. The incidence for males was 3, 1, 2, 1 and for females 0, 0, 1, 2 for the control group and each of the treated groups respectively. At termination of the FMC Mouse II Study, an increased incidence of alveolar cell neoplasms (adenomas/carcinomas) was observed in females only. The number of adenomas alone was 9, 17, 24, 29 for the female control and treated groups respectively. The number of lung carcinomas in these same groups was 6, 7, 11, 15 respectively. Only the 750 mg/kg/day level females exhibited a significantly increased lung carcinoma incidence relative to the control females. For males, the incidence was 7, 5, 13, and 4. In this same study, the incidence, at termination, of hepatocellular carcinoma among females was 4, 3, 3, 2 for the control and each of the treated groups and among males 16, 12, 19 and 8 respectively.

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The incidence of lung carcinoma in the FMC Rat Study was 1, 2, 5, 2 for the male control and treated groups respectively and zero for all female groups.

Histopathological evidence for increased malignancy of lung tumors was observed in only one sex of six mouse and rat studies. Evidence for increased malignancy of liver tumors was not observed.

4. Decrease in latency (time to tumor)

No decrease in latency for lung tumors was observed among the mice in the ICI study. A suggestive, not statistically significant, decreased latency was observed in the lung tumors among females in the B-W Mouse Study. The incidence in this study among females sacrificed or dying prior to termination of the study was 0, 0, 6 and 26% vs an overall termination incidence of 3, 7, 9 and 20% for the control and treated groups respectively. In the FMC Mouse II Study, an analysis of time to tumor (at death) for the females revealed a statistically significant decrease in latency for lung adenomas and carcinomas (combined tumors) but not for the carcinomas alone. The incidence for combined lung tumors prior to termination of the study was 7, 6, 12, and 19 vs 15, 24, 35 and 44 at termination for the female control and treated groups respectively.

No decreased latency for lung tumors was observed in the ICI or B-W long-term rat studies. The problem with histopathology data in the FMC Rat Study precluded any evaluation of latency period for lung neoplasms observed only in males.

In the FMC Mouse II Study, a decreased latency for liver tumors was not observed.

Of the six long-term studies involving Permethrin exposure, only the FMC Mouse II Study presented definite evidence of decreased latency for lung adenomas in female mice only.

5. Dose-response relationships

In the ICI Mouse Study, no substantive evidence was developed which could be interpreted as a dose-response relationship for lung adenomas in either sex. A dose-related, trend in lung adenomas was observed for females only in the B-W Mouse Study. The FMC II Mouse Study indicated a definite dose-related response for both lung and liver neoplasms for females only.

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No dose-response relationships for tumors were observed in either sex in any of the long-term rat studies.

A definite dose-related response for tumors was established only for female mice in the FMC Mouse II Study.

6. Mutagenicity in appropriate tests

A battery of mutagenicity tests has been performed on Permethrin to detect gene mutations, chromosomal aberrations and primary DNA damage. These tests included studies on S. typhimurium and E. coli (with and without activation), mouse lymphoma, dominant lethal, rat cytogenetics, mitotic recombination in yeast, DNA repair in E. coli and B. subtilis and unscheduled DNA synthesis in human fibroblasts. In none of these studies has Permethrin shown a mutagenic potential.

The mechanism of induction of oncogenicity observed only in female mice in the FMC Mouse II Study apparently does not operate by biological mechanisms which directly involve the genetic integrity of the cell.

7. Spontaneous tumor incidence in control groups

Historical control incidence data for the particular strains of mice and rats used in the six long term studies with Permethrin is limited. Data submitted by one registrant presented the historical control incidence of lung tumors in female mice of the CFLP strain used in the B-W study. The data for 807 female control mice from nine studies was abstracted from proprietary data unrelated to Permethrin. This fact precluded proper validation and analysis. Nevertheless, the female control incidence for lung tumors ranged from 7.5 to 30.0% with a mean of $165/807 = 20.4\%$. The incidence in the B-W Permethrin study, at termination, was 3, 7, 9 and 20% for the female control, low, mid and high level treated groups respectively.

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The following table gives pulmonary tumor incidences for various strains of inbred mice. (4)

Strain	Incidence (%)	Average Age (Mos.)	Comments
A	90	>18	
AB	36-54		Breeding female
	15-25		Virgin female
A/Jax	40		Male
	30		Female
BALB/c	29		Male
	26		Female
BL	26		
BN/b	25		
CC57W	24.5		
MAS/A	High		Both sexes
NGP/N	High		
PBA	77	12	
SWR	88	>18	

(4) Benirschke, K., Garner, F.M., and Jones, T.C., Ed. Pathology of Laboratory Animals. Springer-Verlag, NY, Vol. II, Table 12.1, p. 1055, 1978.

The incidence of lung adenomas in the ICI study males was 14, 9, 17 and 24% for the control and treated male groups respectively. In the FMC Mouse II Study, the incidence of combined lung neoplasms was 20, 32, 47 and 59% for the female control and treated groups respectively.

The female incidence of lung tumors observed in the B-W Permethrin study clearly falls within the range and is at or below the mean incidence observed in the historical female control data from various laboratories during the past 10-12 years. The incidence of lung adenomas in the ICI study males also falls below the range for this type of tumor in various strains of inbred mice (see table p. 15). In the FMC Mouse II Study, the combined lung neoplasm incidence for all female groups, except for the high level (750 mg/kg/day) group, is also near or within the range reported for various inbred strains.

The following table presents data for hepatocellular carcinoma in various strains of inbred mice (4).

Strain	Incidence (%)	Average Age (Mos.)	Comments
CBA	40.7	28.6	Male
	26.6	13.45	Female
CBAfbLwN	High	Older	Male
CeHeB	91	21.4	Breeding female
	58	24.1	Virgin female
C3H-Avy	99	12	Male
C3H-AvyfB	96	16	Foster-bred females
C3H/HeN	Common	Older	Male
C3H	85	14	Male
C3He	78	14	Male
C3Hf	72	14	Male

In the FMC Mouse II Study females, the only study and sex with an increased incidence of liver neoplasms, the incidence in the second pathology report was 8, 9, 33 and 40% for the combined tumors (adenoma/carcinoma) while the incidence of hepatocellular carcinomas alone was 5, 4, 4 and 3% for the female controls and the female treated groups respectively.

The incidence of combined liver neoplasms observed in the FMC Mouse II Study females falls within or below the incidence for various inbred strains of female mice and the incidence of hepatocellular carcinoma is well below the range.

The following table presents data from two sources on the incidence of spontaneous lung tumors in Osborne-Mendel and Fischer -344 rats.

Strain	Site	Tumor type	Number of rats (%)	
			of 975 males	of 970 females
Osborne-Mendel (5)				
Respiratory tract				
	Trachea	Undifferentiated sarcoma	1(0.1)	0
	Lung	Alveolar/bronchiolar adenoma	4(0.4)	2(0.2)
		Alveolar/bronchiolar carcinoma	3(0.3)	3(0.3)
		Mucoepidermoid carcinoma	1(0.1)	0
		Adenosquamous carcinoma	1(0.1)	0
Fischer-344 (6)				
	Lung/Trachea		<u>Of 846</u> 2.4%	<u>or 840</u> <1.0%

(5) Goodman, D.G., et. al. Neoplastic and Monneoplastic lesions in aging Osborne-Mendel rats. Tox. and Appl. Pharma. 55, 433-447, Table I (1980)

(6) Cueto, C.J., Env. Sc. and Health, V B15, 6, Table 1, p. 954 (1980)

The male incidence of lung neoplasms in the FMC Rat Study, from the second pathology report (step-sectioned slides), was 13, 10, 17 and 17% for the male control and each treated group respectively. The incidence of lung carcinomas, in the same report, was 2, 3, 8 and 3% for the male control and treated groups. No carcinomas were observed among any of the female groups. The flaws in the preparation of lung tissues in this study render the incidence figures questionable, however. The incidence of lung neoplasms in males in this study is much higher, even in the control group, than the incidence reported for two other strains of rats.

If the mouse historical control data are valid, they raise the possibility that these tumors may not be related to the ingestion of Permethrin but, rather, may simply be an expression of the variability of the spontaneous and naturally occurring incidence of this lesion in mice. Toxicology Branch recognizes some merit in this point of view. In the absence of historical incidence data for the specific strains used in the ICI and FMC Mouse II Studies, it is Toxicology Branch's opinion that relatively more weight should be placed on the observed incidence of lung tumors in concurrent control groups than on that in the other strain historical controls since concurrent controls more accurately reflect the particular conditions of any given study and reduce the number of unknown and uncontrolled variables to a minimum. In addition, Toxicology Branch considers the lung tumors observed in the three mice studies to be highly supportive of one another.

The incidence of lung neoplasms in the flawed FMC Rat Study, in the absence of such observations in the ICI and B-W rat studies and specific historical control incidence data for the Long-Evans rat strain, is very difficult to interpret. Their presence, however, cannot be ignored.

Absence of strain specific historical control data for the mice and rat strains used in the long-term studies severely limits the use of such data in the evaluation of the oncogenicity potential of Permethrin.

Summary and evaluation of criteria data

1. In the 3 long-term mouse studies, definite oncogenicity was observed in the lungs and liver of only one sex (female) of one strain (Charles River CD-1) of mice.
2. There was no increased incidence of rare or unusual tumors in the mouse or the rat studies. Suggestive evidence of increased incidence of lung adenomas was observed in male rats in a flawed long-term study.
3. An increase in malignant lung tumors was evident in only one of six long-term mouse and rat studies. No increase in malignant liver tumors was observed.
4. Of the six long-term studies, only the FMC Mouse II Study presented definite evidence of decreased latency for lung adenomas in female mice only.

5. Definite dose-related responses for tumors was established only for female mice in the FMC Mouse II Study.
6. In a battery of mutagenicity tests performed to detect gene mutations, chromosomal aberrations and primary DNA damage, no mutagenic potential was evident.
7. Historical control incidence data in the rodent strains tested is too limited to be of significance in evaluation of the oncogenic potential of Permethrin.

The evaluation of the weight of toxicological evidence leads the Toxicology Branch to conclude that, at dose levels above 250 mg/kg/day for a lifetime, Permethrin exhibits a weak oncogenicity potential in female mice. While the evidence in the FMC Rat Study is flawed to the extent that it precludes making a scientifically supportable evaluation, it is marginally suggestive that Permethrin may also have a very weak oncogenic potential in male rats. This potential is not supported by the other two long-term rat studies at dosage levels as high as 125 and 250 mg/kg/day. The mechanism of induction of oncogenicity observed in rodents apparently does not operate by biological mechanisms which directly involve the genetic integrity of the cell.

VI. Assessment of the Oncogenic Potential in Humans

Recently, several systems for ranking and classifying evidence from animal oncogenic studies have been developed or proposed. At present, only the International Agency for Research on Cancer (IARC) method has general acceptance. However, in evaluating the human oncogenicity potential for Permethrin, two additional systems were useful.

The IARC method classifies the evidence as either "sufficient" or "limited". Evidence which is sufficient requires the animal experiments to demonstrate "an increased incidence of malignant tumors: (a) in multiple species or strains, and/or (b) in multiple experiments (routes and/or doses); and/or (c) to an unusual degree (with regard to incidence, site, type, and/or precocity of onset)." (7) Data concerning dose-response, mutagenicity, and structure may provide additional evidence. Limited evidence, while not precisely defined by IARC, includes induction of "certain neoplasms, including lung tumors and hepatomas in mice, which have been considered of lesser significance than neoplasms occurring at other sites for the purpose of evaluating the carcinogenicity of chemicals" (7)

(7) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 22, pp 18-19, March 1980.

Using the IARC criteria, Permethrin animal data does not meet the requirements for the "sufficient" evidence category. Among six long-term mouse and rat studies, an increase in malignant lung tumors was not evident except in one sex (female) in the Charles River CD1 mouse strain used in the FMC Mouse II Study. This tumor type and site is not rare or unusual for mice. A decreased latency period for malignant tumors was not evident in this study. However, a dose-related trend was observed among the test groups. No evidence of mutagenic potential was observed in a battery of tests which included tests for DNA damage.

Using the IARC criteria and considering all available biological data, Toxicology Branch concludes that the evidence for Permethrin carcinogenicity must fall into the "limited" classification. The limited evidence strongly suggests that Permethrin is not a potent carcinogen in experimental animals but may possibly exhibit a weak oncogenic potential for female mice.

Weisburger and Williams (8) have proposed a mechanistic classification of carcinogens which is divided into two general categories: a) genotoxic and b) epigenetic. The genotoxic category contains those chemicals which function as electrophilic reactants or otherwise affect DNA. The epigenetic category contains carcinogenic substances for which no evidence of direct interaction with genetic material exists. The authors state.

"This classification, if ultimately validated, has major implications for risk extrapolation to humans of data on experimental carcinogenesis. Genotoxic carcinogens, because of their effects on genetic material, pose a clear qualitative hazard to humans. These carcinogens are occasionally effective after a single exposure, act in a cumulative manner, and act together with other genotoxic carcinogens having the same organotropism. Thus, the level of human exposure acceptable for "no risk" to ensue needs to be evaluated most stringently in the light of existing data and relevant mechanisms. Often, with powerful carcinogens, zero exposure is the goal.

On the other hand, with some classes of epigenetic carcinogens, it is known that their carcinogenic effects occur only with high and sustained levels of exposure that lead to prolonged physiologic abnormalities, hormonal imbalances, or tissue injury. Consequently, the risk from exposure may be of a quantitative nature.

(8) Weisburger, J.H. and Williams, G.M., Chemical Carcinogens, In Doull, J. (ed), Toxicology. The Basic Science of Poisons, 2nd ed., Macmillan Pub. Co., N.Y., 1980.

This is almost certainly the case with estrogens, which are carcinogenic at high, chronic exposure levels in animal studies, or otherwise every individual would develop cancer. Thus, with epigenetic carcinogens, it may be possible to establish a 'safe' threshold of exposure, once their mechanism of action is elucidated."

Because of the lack of knowledge concerning Permethrin's mechanism of action, the entire Weisburger and Williams classification system can not be fully utilized. However, the lack of positive evidence for mutagenic potential from a battery of tests, including DNA repair and unscheduled DNA synthesis, coupled with definite oncogenic evidence only at high dose levels for one sex in one mouse study among three studies, indicates that Permethrin falls into the epigenetic category. According to this system of classification, Permethrin falls into the group where the risk from exposure may be of a quantitative nature.

Squire (9) has also proposed a system for ranking animal carcinogens based on available data and the current state of knowledge. The system's "emphasis is on test animal data, since without further knowledge of mechanisms, this information is the most relevant to human risk. Whatever experimental data are to be included, however, the weight of scientific evidence should be considered in an appropriate system of carcinogen classification." The system includes the following factors: a) number of different species affected; b) number of histogenetically different types of neoplasms in one or more species; c) spontaneous incidence in appropriate control groups of neoplasms induced in treated groups; d) dose-response relationships; e) malignancy of induced neoplasms; and f) genotoxicity, measured in an appropriate battery of tests. Weighted numerical values were assigned to subparts of these factors by the author. Using these values the author ranks carcinogens into five classes. Classes I and II contain substances presenting the greatest potential hazard and have the highest priority for regulation. According to the author, chemicals in Classes III to V may permit many regulatory options including no action, approvals for limited uses, appropriate labeling, or public education programs.

Using this system of ranking potential carcinogens, Toxicology Branch determined that Permethrin clearly falls into Class V indicating the lowest potential risk of carcinogenicity.

(9) Squire, R.A., Ranking Animal Carcinogens: A Proposed Regulatory Approach. Science, Vol. 214, pp. 877-880, 20 Nov. 1981.

The weight of scientific biological evidence produced by six long-term mouse and rat studies plus the use of three oncogenicity ranking and classification systems lead the Toxicology Branch to conclude that Permethrin's potential for induction of oncogenicity in experimental animals is weak and that the risk of human oncogenic effects resulting from exposure to low levels of Permethrin is non existent or extremely low.