DATA EVALUATION RECORD

PERMETHRIN/109701

STUDY TYPE: CARCINOGENICITY - MOUSE (OPPTS 870.4200b/OECD 451)

MRID 45597105 (Main Study), 45597104

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
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Task Order No. 02-22

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DATA EVALUATION RECORD

TXR#: 0050465

STUDY TYPE: Carcinogenicity - mice, [feeding]

PC CODE: 109701 **DP BARCODE:** D280938
SUBMISSION NO.: S610304

TEST MATERIAL (PURITY): Permethrin (94.7%), brown liquid. Cis:trans 38.3:61.7

SYNONYMS: (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-carboxylate

CITATION: Barton, S.J., Robinson, S., and Martin, T. (2000) Permethrin technical: 100 week carcinogenicity/reversibility study in mice with administration by the diet. Inveresk Research, Tranent, EH33 2NE, Scotland. Inveresk Report No. 16839, Project No. 452695, FMC Study No. A95-4264, May 24, 2000. MRID 45597105. Unpublished.

Martin, T., Barton, S.J., and Weaver, R.J. (1996) Permethrin: 4-week preliminary study in mice with administration by the diet. Inveresk Research, Tranent, EH33 2NE, Scotland. Inveresk Report No. 11474A, Project No. 452585, FMC Study No. A95-4263, December 2, 1996. MRID 45597104. Unpublished.

SPONSOR: FMC Corporation, Chemical R and D Center, P.O. Box 8, Princeton, New Jersey 08543, USA.

EXECUTIVE SUMMARY: In a nonguideline mouse carcinogenicity study (MRID 45597105, 45597104) Permethrin technical (lot no. PL95-329, 94.7% a.i.) was administered to groups of 50 to 109 Crl:CD-1®(ICR)BR female mice in the diet at 0 or 5000 ppm (equivalent to 780 - 807 mg/kg bw/day) for 39, 52, 65, or 78 weeks. Groups of mice from all treatment groups were examined immediately after treatment and at weeks 79 and 101. Matching groups of untreated control mice were examined at each interval.

There were no compound-related effects on mortality or body weight. Body weight gain was slightly less in mice treated for 65 or 78 weeks and allowed to recover to week 101 (both 86% of the control weight). The overall food consumption was slightly decreased by 2-3% in some treated groups. The overall food efficiency in the pooled 52-week treatment groups was about 5% less than that of the controls.

At the end of each treatment period, the absolute liver weights were increased by about 44-53% compared to the control groups regardless of the treatment duration. Liver centrilobular hypertrophy and karyomegaly occurred in 87-100% and Kupffer cell hypertrophy was seen in 43-61% of treated animals compared to the controls (0-5%). Centrilobular hypertrophy and Kupffer cell hypertrophy at all dose durations was reversed to or near control levels during the recovery periods. Karyomegaly incidences were reduced by about 11-70% according to the length of the respective recovery periods, but were still present in 25-75% of the treated animals at the 101-week recovery. Inflammatory liver changes were seen in 75-95% of treated animals compared to 37-63% in the controls. The inflammatory liver changes increased in the control mice as a function of age; therefore, recovery was only seen in the treated groups allowed to recover to week 79. Amyloid deposits were increased in treated animals immediately after treatment, and continued to increase during the recovery period. Incidences of eosinophilic foci were significantly increased in the livers of treated groups only after the recovery periods and appeared to be related to the length of the treatment period. The activities of cytochrome P450 (CYP) mixed function oxidases in the livers of animals treated for 52 weeks were expressed both as specific activity (nmol/mg microsomal protein) and the total enzyme activity per liver. Specific activities of total CYP, CYP1A, CYP2B, CYP2E1, and CYP3A were unaffected by treatment, whereas, the specific activity of CYP4A was increased 3-fold. The total enzyme activities per liver of total CYP, CYP1A, CYP2B, CYP2E1, and CYP3A2 were increased in treated animals by 142-283%, and the activity of CYP4A was increased by 829% compared to the control values.

The incidences of Clara cell hyperplasia were increased in the lungs of all treated animals, and the incidences were significantly decreased during the recovery periods to weeks 79 and 101. The specific activities of CYP2B, CYP2E1, and CYP4A in animals sacrificed after 52 weeks of treatment were unaffected by treatment. The total enzyme activities of CYP2E1 and CYP4A expressed as activity/g lung were increased to only 133% and 125%, respectively, of controls.

Significant increases were seen in the incidences of basophilic and eosinophilic hepatocellular adenomas in female mice administered 5000 ppm in the diet for 39, 52, or 78 weeks followed by recovery to week 101 (7% to 10% compared to 1% in controls). The increased incidences were not treatment-duration (dose) related; treatment for 65 weeks resulted in no basophilic adenomas. Eosinophilic adenomas were increased after 78 weeks of treatment and after the recovery period (both 10% compared to 1-2% in controls). The incidences did not increase during the recovery period. No increases in hepatocellular carcinoma incidences were seen and the time to tumor onset for the adenomas was not different in treated animals compared to the controls. Lung bronchioloalveolar adenoma incidences increased immediately after treatment and continued to increase during the recovery periods compared to the controls. The incidences were 14%, 43%, 47%, 49%, and 49% for the control and 39, 52, 65, and 78 weeks exposure followed by recovery to week 101 ($p<0.01$). The lung adenomas did not occur any earlier in the treated animals than in the control groups, and there was no increase in lung carcinomas in treated animals.

This mouse carcinogenicity study is designed to test the progression and possible reversal of

toxic effects including benign liver and lung tumors and is classified as **Acceptable/Non-guideline**.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Permethrin technical

Description:	brown liquid, cis:trans 38.3:61.7
Lot/Batch #:	PL95-329
Purity:	94.7 % a.i.
Compound Stability:	Stable for 39 months at room temperature
CAS # of TGAI:	52645-53-1

2. Vehicle and/or positive control: The test substance was mixed with diet.

3. Test animals:

Species:	mice	
Strain:	Crl:CD-1®(ICR)BR	
Age/weight at study initiation:	~7 weeks/females: 24 g (mean)	
Source:	Charles River, Portage, Michigan, USA	
Housing:	Animals were housed individually in suspended polypropylene cages with stainless steel wire tops. Sterilized whitewood shavings were used as bedding. Tops with food hoppers were cleaned every 2 weeks, and the cages and water bottles were changed weekly.	
Diet:	Purina Rodent Chow (Mash) No. 5002, *ad libitum*	
Water:	local tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20 ± 2° C
	Humidity:	50 ± 15%
	Air changes:	15/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	Females: 19 days	

B. STUDY DESIGN:

1. In life dates: Start: May 1, 1996 End: Nov. 23, 1998

2. Animal Assignment/Dose Levels: Female mice were assigned to the test groups noted in Table 1 utilizing a computer assisted randomization procedure. Only one dose (5000 ppm in the diet) was used; the treatment and recovery times were varied. Initially, 50 animals were assigned to groups treated and sacrificed at weeks 39, 52, and 65; groups sacrificed at week 79 contained 75 mice; and groups scheduled for sacrifice at week 101 had 100 mice. To provide adequate samples at each sacrifice time, some mice were replaced or transferred among

groups, resulting in the numbers in Table 1.

TABLE 1: Study design[a]

Test Group	Permethrin dietary conc. (ppm)	Achieved dose[b] (mg/kg/day)	Duration of treatment (weeks)	Week of kill	No. mice[c]
Control, 1A	0	0	–	40	50
Control, 1B	0	0	–	53	52
Control, 1C	0	0	–	66	50
Control, 1D	0	0	–	79	67
Control, 1E	0	0	–	101	94
Treated, 2	5000	807	39	40	50
Treated, 3	5000	780	52	53	50
Treated, 4	5000	789	65	66	56
Treated, 5	5000	785	78	79	71
Treated, 6	5000	807	39	79	72
Treated, 7	5000	807	39	101	103
Treated, 8	5000	780	52	79	66
Treated, 9	5000	780	52	101	109
Treated, 10	5000	789	65	79	75
Treated, 11	5000	789	65	101	94
Treated, 12	5000	785	78	101	104
Control, 13A[d]	0	0	–	40	6
Control, 13B[d]	0	0	–	53	14
Treated, 14A[d]	5000	807	39	40	6
Treated, 14B[d]	5000	780	52	53	14

[a] Data obtained from pages 22, 23, and 72 of the study document, MRID 45597105.
[b] Average dose calculated by the reviewer from the data on page 72.
[c] Final no. animals after adjusting for mortality by shifting mice from identically treated groups.
[d] These groups were added to replace frozen liver and lung samples from the main study lost due to a freezer failure.

3. Dose selection: The dose level was selected based on the highest dietary concentration used in previous carcinogenicity studies and on a 4-week preliminary study in mice (Inveresk Project No. 452585, FMC Study A95-4263, MRID 45597104) where dietary-administration of 0, 5000, or 8000 ppm resulted in increased liver weights at 5000 and 8000 ppm and death of 2/10 animals at 8000 ppm. The dietary concentration of 5000 ppm was chosen because of the increased mortality seen at 8000 ppm. See the Appendix for more detailed information.

4. Diet preparation and analysis: The 5000 ppm dietary mixture was prepared every 41or 89 days by dissolving the appropriate

amount of test substance in acetone and preparing a premix by mixing the permethrin solution with Purina Rodent Chow (Mash) No. 5002 in a Hobart mixer. Evaporation of the acetone was assisted with a fan, and the final diet was prepared by mixing the proper aliquot of the premix with the untreated diet for at least 20 minutes on a Winkworth change drum mixer. Multiple batches were made at most preparatory intervals. The dietary mixtures were stored at room temperature in closed containers. Control diets were prepared in the same manner using the acetone premix minus the Permethrin and were used up to week 78 after which the commercial diet was used. The homogeneity and stability of 5000 and 8000 ppm dietary mixtures was tested prior to the study initiation. During the study, samples of each dietary mixture were analyzed for Permethrin content and homogeneity at all preparation intervals.

Results -
Homogeneity analysis: Three to 5 aliquots were taken from each batch and analyzed for Permethrin content. About 94 batches were prepared at 35 different preparation intervals during the study. The percent deviation from the nominal concentration (5000 ppm) and the coefficient of variation were calculated. The coefficient of variation was over 10% in only 5/94 batches tested and was never over 15%.

Stability analysis: Dietary samples containing 5000 and 8000 ppm Permethrin were determined to be stable stored for 89 days at room temperature; however, the experimental data were not supplied. The Permethrin technical was shown to be stable with essentially no loss after storage for up to 3 months.

Concentration analysis: Analysis of each batch of the dietary mixture for Permethrin content at all preparation intervals demonstrated concentrations that deviated from the nominal concentration by over ±10% in 7/94 batches and was never greater than ± 15%.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics: The F-max test was used to analyze for homogeneity of variance of the body weight and food consumption data. A parametric analysis of variance (ANOVA) was applied if the variances were homogeneous followed by individual group comparisons with Fisher's F-protected LSD method with the student's t-test. If the variances were not homogeneous, log or square root transformations were used to stabilize the variances. Non-parametric Kruskal-Wallis ANOVA was applied if the variances remained heterogeneous. Analysis of covariance was applied to the organ and terminal body weight data as covariants. The control groups were combined and the treatment groups of the same treatment duration were combined

for mortality analysis using Kaplan-Meier survival curves and comparisons by the Wilcoxon rank sum test modified for censored survival data.

The histological non-neoplastic and tumor findings were analyzed with Fisher's exact test. Logistic regression analyses were also applied to the pulmonary adenoma incidences.

Significant findings were flagged at $p < 0.05$ and $p < 0.01$. The reviewer considers the statistical procedures to be adequate.

C. METHODS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality and given detailed examinations and palpations for masses weekly.

2. Body weight: Animals were weighed weekly from weeks -1 to 13 and every 4 weeks thereafter. Animals that were in deteriorating condition or appeared to be rapidly losing weight were weighed more frequently.

3. Food consumption and compound intake: Food consumption for each animal was measured weekly during weeks 1-13 and during 1-week periods every 4 weeks thereafter. The mean food consumption per animal was calculated as g food/kg body weight/day. Food efficiency was not calculated. Compound intake (mg/kg bw/day) values were calculated each week the animals were weighed and food consumption was measured. The overall mean compound intake for each treated group was calculated by the reviewer (Table 1).

4. Water consumption: The water consumption was monitored weekly throughout the study by inspecting the water bottles.

5. Ophthalmoscopic examination: Eyes were not examined with an ophthalmoscope.

6. Hematology & clinical chemistry: Blood smears were prepared from animals that died at unscheduled times during the study, but apparently not from animals at the scheduled termination periods.

7. Urinalysis: Urinalysis is not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451.

8. Sacrifice and pathology: All animals that died during the study and those sacrificed on schedule were subjected to gross pathological examination. In order to evaluate cell proliferation, an intraperitoneal injection of bromodeoxyuracil (BrdU) at 100 mg/kg BW was given to selected animals in the 52, 65, 78, and 100 week treatment groups 1.5

to 2.5 hours before the animals were killed. The mice were killed by exsanguination after carbon dioxide asphyxiation, and the CHECKED (X) tissues were collected and fixed in 10% neutral buffered formalin or Davidson's fluid (eyes and optic nerves). Only the livers and lungs were weighed and examined histologically. These tissues were stained with H & E. The livers and lungs from animals in treatment group 14 were frozen in liquid nitrogen after the mice had been treated with Permethrin for 39 or 52 weeks and retained for ribosomal enzyme studies.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	X	Brain (multiple sections)*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		**GLANDULAR**
X	Ileum*			X	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	X	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
XX	Liver*+		Testes*+		**OTHER**
X	Gall bladder* (not rat)		Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct* (rat)		Prostate*	X	Skeletal muscle
X	Pancreas*		Seminal vesicle*	X	Skin*
	RESPIRATORY	X	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	X	Uterus*+		Coagulating glands
XX	Lung*++	X	Mammary gland*	X	Joint (tibio-femoral)
X	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200.
+Organ weight required in carcinogenicity studies.
++Organ weight required if inhalation route.

9. Cell proliferation measurements: Cell proliferation markers were used to monitor the presence, onset, and reversibility of increased cell division in the lung and liver. Two methods (PCNA and BrdU assays) were utilized to measure cell proliferation. In the BrdU assay, bromodeoxyuracil (BrdU) (100 mg/kg) was given by intraperitoneal injection to all mice 1.5 to 2.5 hours before scheduled kills. The BrdU is incorporated into newly synthesized DNA and can be visualized microscopically after staining the prepared slides using a bromodeoxyuracil antibody. Slides made of intestine sections from BrdU injected animals and non-injected animals served as positive and negative controls, respectively. The number of stained nuclei were counted and the results expressed as stained nuclei or counts/mm^2. The PCNA (proliferating cell

nuclear antigen) assay involves staining using an antibody against cyclin or proliferating cell nuclear antigen, which is synthesized during the G1 and S phases of the cell cycle. In this procedure, the cell sections were de-paraffinised in xylene and rehydrated through alcohol to water. The tissue sections were treated with 3% hydrogen peroxide in methanol, washed in water and in phosphate buffered saline. The sections were incubated with anti-PCNA/HRP conjugate. After washed in PBS, the section were treated with diaminobenzidine (DAB) chromogen. The sections were washed in water and counterstained with Haematoxylin, dehydrated through alcohol and xylene. The number of labeled nuclei in each lesion that was identified was counted and the labeling index was calculated and expressed as counts/mm^2. Slides of the cerebral cortex were used as negative controls and slides of the intestine were used for positive controls.

Originally, animals were selected from each of the 39-, 52-, 65-, and 78- week treatment groups for PCNA and BrdU assays. However, the report stated that "following the above selection, it was decided that assessment for PCNA should be conducted on liver sections of animals for which there was a diagnosis of hepatocullular carcinoma, basophilic cell focus or adenoma, or eosinophilic cell focus or adenoma. Attempts to stain for BrdU uptake were unsuccessful, and no data were available. It was also decided that lung sections should not be assessed for PCNA."

10. Measurement of cytochrome P450 (CYP) content and enzyme activities: Frozen liver and lungs from 14 control and 14 mice treated for 52 weeks (study groups 13B and 14B) were divided into 4 groups, each group contained liver and lung samples from 3 or 4 individuals. The organs were thawed, homogenized, and microsomes were isolated using standard centrifugal techniques. Microsomal protein was measured by the Lowry procedure.

The total CYP content was measured by treating the microsomal suspension with sodium dithionate then scanning (450-490 nm) with a Phillips single beam scanning spectrophotometer before and after bubbling the suspension with carbon monoxide. The CYP concentrations were calculated as nmol/mg protein using the extinction coefficient value of 91 mmol/cm.

The enzyme activities of CYP1A and CYP2B were determined by measuring ethoxyresorufin O-deethylation and pentoxyresorufin O-dependentylation activities, respectively, in the liver microsomal preparations using a continuous fluorimetric assay. The CYP3A activity was measured by incubating the microsome suspensions with [^{14}C]-testosterone and analyzing the metabolites using high pressure liquid chromatography (HPLC). CYP2E1 activity was measured by incubating the microsomes with[^{14} C]-chlorzoxazone and analyzing the metabolites with HPLC. 11-Hydroxylation and 12-hydroxylation activities were

used to determine CYP2E and CYP4A activities. These activities were measured by incubating [^{14}C]-lauric acid with the mouse liver or lung microsomes and measuring the resulting metabolites using HPLC.

II. RESULTS:

A. OBSERVATIONS:

1. Clinical signs of toxicity: There were no treatment-related significant increases in the incidences of clinical signs observed during the study comparing the treated groups to the pooled controls. When the treated groups were compared to pooled controls killed at various ages, possible treatment effects could not be distinguished from the signs seen in normal aging mice.

2. Mortality: There were no treatment-related effects on mortality.

B. BODY WEIGHT: Body weights and body weight gains at selected times during the study are presented in Table 2. The body weights of all treated groups were significantly less than the pooled control groups pretreatment (week 0) and were less for some groups, beginning with week 12 to the end of the study, but the differences were not treatment-duration related. The mean weight gains seen after 100 weeks in the groups that received 65 and 78 weeks of treatment at 5000 ppm were both 86% of the control weight gain. The mean weight gain amounts after 100 weeks in the 39- and 52-week treatment groups were 93% and 100% of the control group weight gain, respectively.

TABLE 2: Mean bodyweights (BW) and bodyweight gains (BWG) in mice treated with 5000 ppm Permethrin for various time periods[a]

BW and BWG/ treatment weeks	Control (g)	Treated for 39 wks. (g)	Treated for 52 wks. (g)	Treated for 65 wks. (g)	Treated for 78 wks. (g)
BW, Wk 0 (±SD)	26 (±2)	25 (±2)**	25 (±2)**	25 (±2)**	25 (±2)**
BW, Wk 39 (±SD)	38 (±5)	36 (±4)**	37 (±4)**	36 (±4)**	37 (±4)**
BW, Wk 52 (±SD)	39 (±5)	37 (±5)**	38 (±4)	37 (±4)**	37 (±4)*
BW, Wk 65 (±SD)	40 (±5)	39 (±5)*	39 (±5)	38 (±4)**	38 (±4)*
BW, Wk 78 (±SD)	40 (±5)	40 (±5)	39 (±5)	38 (±4)	38 (±4)**
BW, Wk 100 (±SD)	40 (±4)	39 (±4)	38 (±5)	37 (±3)**	37 (±4)**
BWG Wk 0-39	12	11	12	11	12
BWG Wk 0-78	14	15	14	13	13
BWG Wk 0-100 (% control)	14	14 (100)	13 (93)	12 (86)	12 (86)

[a] Data obtained or calculated from pages 66-68 in the study report, MRID 45597105.
* Significantly different (p<0.05) from the control.
** Significantly different (p<0.01) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: There was no treatment-related effect on food consumption. The average daily food consumption calculated weekly for the first 13 weeks was slightly higher in the treated groups compared to the pooled control groups, and, generally, was slightly lower thereafter. The increased food consumption in treated mice measured weekly was statistically significant in 4/13 weeks. After week 13, the food consumption in most of the treated groups was significantly less than the control group in 3/10 weekly measurement periods. The large numbers of animals in the pooled groups resulted in the small differences between the treated and control groups reaching statistical significance. The time-weighted average daily food consumption for the first 52 weeks of the study was 5.83 g/mouse/day for the controls and ranged from 5.70 to 5.80 g/mouse/day for the treated groups.

2. Compound consumption (time-weighted average): The average daily compound intake of the female mice for each of the treatment periods was calculated by the reviewer from the weekly compound consumption calculated by the study authors (based on the food consumption and body weight data) and is included in Table 1.

3. Food efficiency: The overall food efficiency at 13 weeks was 1.544 for the pooled control groups and 1.525 for the pooled treated groups. All groups gained an average of 8 grams over the 13-week period. The food efficiency over the first 52 weeks of the study was 0.611 for the pooled control and 0.582 for the pooled treated groups. The food efficiency was calculated by the reviewer using the food consumption and body weight data.

D. OPHTHALMOSCOPIC EXAMINATION: The eyes of the mice in this study were not examined with an ophthalmoscope.

E. BLOOD ANALYSES:

1. Hematology: Blood smears were prepared from only animals that died or were killed at unscheduled times during the study. Any results obtained from these preparations were not reported in the study document.

2. Clinical chemistry: Clinical chemistry parameters were not measured in the study

F. URINALYSIS: Urinalysis was not performed in the study.

G. SACRIFICE AND PATHOLOGY

1. Organ weight: The only organs weighed in the study were the liver and lungs. Absolute liver and lung weights and weights adjusted for body weights by covariant analysis after selected treatment and recovery periods are given in Table 3.

Liver: At the end of each treatment period, the group mean liver weights were increased in all treated animals by 44% to 53% ($p<0.01$) compared to the respective controls with no apparent relationship to treatment duration. The liver weights of mice that were treated for 65 weeks were comparable to the control liver weights by 79 weeks, which was the shortest recovery period tested (about 14 weeks). The mean absolute liver weights of animals treated for 39 and 52 weeks were also comparable to the control liver weights at 79 and 101 weeks. Liver weights of animals treated for 78 weeks and killed at week 101 were still slightly, but not significantly, elevated by about 6% compared to the controls. Prior to the recovery periods, the liver weights of treated animals adjusted to the body weights by covariant analysis were also significantly increased compared to the control groups. The liver weight changes can be correlated with a number of microscopic lesions seen during histopathology.

Lung: There were no treatment-related changes in lung weights.

TABLE 3: Selected group mean absolute (g) and adjusted (covariant analysis with body weight) liver and lung weights after treatment with Permethrin[a]

Organ, adjustment, weeks of treatment	Terminated at week 40	Terminated at week 53	Terminated at week 66	Terminated at week 79	Terminated at week 101
Liver, absolute wt., control	1.90 (±0.28)	2.00 (±0.28)	2.08 (±0.28)	2.14 (±0.39)	2.11 (±0.38)
Liver, absolute wt., 39-wks.	2.82 (±0.35)**	–	–	2.14 (±0.60)	2.16 (±0.41)
Liver, adj. for body wt., 39-wks.	2.82 (±0.04)**	–	–	2.17 (±0.07)	2.13 (±0.07)
Liver, absolute wt., 52-wks.	–	3.05 (±0.49)**	–	2.27 (±0.59)	2.23 (±0.46)
Liver, adj. for body wt. 52-wks.	–	3.05 (±0.05)**	–	2.23 (±0.07)	2.23 (±0.06)
Liver, absolute wt., 65-weeks	–	–	3.00 (±0.49)**	2.10 (±0.35)	2.20 (±0.39)
Liver, adj. for body wt. 65-wks.	–	–	3.01 (±0.06)**	2.15 (±0.07)	2.25 (±0.07)
Liver, absolute wt., 78-wks.	–	–	–	3.09 (±0.45)**	2.24 (±0.52)
Liver, adj. for body wt. 78-wks.	–	–	–	3.09 (±0.07)**	2.28 (±0.07)
Lung, absolute wt., control	–	0.43 (±0.38)	–	0.38 (±0.08)	0.32 (±0.08)
Lung, absolute wt., 52-wks.	–	0.36 (±0.09)	–	0.36 (±0.09)	0.33 (±0.09)
Lung, adj. for body wt. 52-wks.	–	0.36 (±0.04)	–	0.36 (±0.01)	0.33 (±0.02)
Lung, absolute wt. 78-wks.	–	–	–	0.36 (±0.09)	0.38 (±0.17)
Lung, adj. for body wt. 78-wks.	–	–	–	0.36 (±0.01)	0.38 (±0.02)

[a] Data obtained from pages 73-82 in the study report, MRID 45597105.
* Significantly different ($p<0.05$) from the control.
** Significantly different ($p<0.01$) from the control.

2. Gross pathology: Findings in treated animals that were consistent with treatment or appeared to increase with the duration of the treatment compared to the controls are listed in Table 4. The incidences of livers that appeared dark increased with the duration of treatment compared to the control groups and the differences became statistically significant at 65 and 79 weeks of treatment. An increase in the incidence of liver masses was seen only after 79 weeks of treatment. Increases in the incidences of lung masses were seen after all treatment periods, but the differences compared with the control groups were statistically significant only at 52 and 78 weeks. Consistent increases in the incidences of enlarged hepatic lymph nodes were seen in treated groups compared to the controls, but the differences were not statistically significant and did not show a clear dependency on the duration of treatment. The incidences of pale kidney were increased after 65 and 78 weeks of treatment compared to the control, but the differences were statistically significant only at 65 weeks. The incidences of enlarged spleen were also statistically significant after 39 and 65 weeks of treatment. However, the incidences of enlarged spleen also increased in the control groups with the age of the animals.

After the animals were exposed to 5000 ppm permethrin in the diet for 38, 52, 65, and 78 weeks, groups of these treated mice were given the control diet until study termination at 79 and 101 weeks allowing for various recovery periods. The effects of the recovery periods on the findings listed in Table 4 are shown in Table 5. The incidences of dark liver were still significantly increased at 101 weeks in animals treated for 78 weeks compared to the control, and slightly increased in animals treated for 65 weeks. The incidences of liver masses were significantly increased only in animals treated for 52 weeks and allowed to recover until week 101. The incidences of lung masses were significantly increased in all treated animals that were in the 101-week recovery group. While liver and lung masses remained elevated over the control values during the recovery periods, there was no clear dose duration-response relationship. The incidences of hepatic lymph node enlargement, pale kidneys, and enlarged spleens increased in all animals with age, and by week 101, the incidences in the control animals were as high or slightly higher than in the treated groups.

TABLE 4: Selected incidences of macroscopic findings after various weeks of treatment with 5000 ppm Permethrin in the diet [a]

Organ/Finding	Control 39 wks.	Treated 39 wks.	Control 52 wks.	Treated 52 wks	Control 65 wks.	Treated 65 wks.	Control 78 wks.	Treated 78 wks.

Liver/dark	0/50[b]	2/50	0/52	3/50	0/50	7/56**	0/67	6/71*
Liver/masses	0/50	1/50	0/52	0/50	0/50	1/56	0/67	6/71*
Lung/masses	1/50	4/50	0/52	6/50*	6/50	12/56	6/67	21/71**
Hepatic lymph node/ enlarged	4/50	8/50	3/52	7/50	1/50	5/56	8/67	14/71
Kidney/pale	0/50	0/50	2/52	4/50	2/50	12/56**	7/67	12/71
Spleen/enlarged	0/50	5/50*	6/52	9/50	2/50	10/56*	14/67	16/71

[a] Data obtained from pages 88-112 in the study report, MRID 45597105.
[b] Number of mice with lesion/number of mice examined.
* Significantly different ($p<0.05$) from the control, Fisher's exact test by the reviewer.
** Significantly different ($p<0.01$) from the control, Fisher's exact test by the reviewer.

TABLE 5: Selected incidences of macroscopic findings after various weeks of treatment with 5000 ppm Permethrin in the diet followed by various recovery periods [a]

Organ/ Finding	Control 78 wks.	Treated 39 wks., total 78 wks.	Treated 52 wks., total 78 wks.	Treated 65 wks., total 78 wks.	Control 101 wks.	Treated 39 wks., total 101 wks.	Treated 52 wks., total 101 wks.	Treated 65 wks., total 101 wks.	Treated 78 wks., total 101 wks.
Liver/dark	0/67[b]	1/72	0/66	3/75	1/106	2/103	3/109	4/94	7/104*
Liver/masses	0/67	2/72	4/66	2/75	6/106	9/103	15/109*	10/94	14/104
Lung/masses	6/67	21/72	17/66	34/75	13/106	44/103**	43/109**	38/94**	46/104**
Hepatic lymph node/enlarged	8/67	13/72	6/66	13/75	25/106	13/103	13/109	13/94	15/104
Kidney/pale	7/67	14/72	5/66	13/75	22/106	17/103	22/109	17/94	23/104
Spleen/enlarged	14/67	17/72	12/66	9/75	32/106	32/103	28/109	24/94	25/104

[a] Data obtained from pages 113-151 in the study report, MRID 45597105.
[b] Number of mice with lesion/number of mice examined.
* Significantly different ($p<0.05$) from the control, Fisher's exact test by the reviewer.
** Significantly different ($p<0.01$) from the control, Fisher's exact test by the reviewer.

3. Microscopic pathology

a) Non-neoplastic: Selected microscopic findings in female mice after 39, 52, 65, or 79 weeks of treatment with 5000 ppm permethrin in the diet are shown in Table 6. The possible reversal of these effects was tested by removal of the permethrin from the diet and feeding the animals the control diet to week 79 or 101 of the study. These findings are given in Table 7. The reviewer compared the recovery in Table 7 statistically (Fisher's exact test) to the findings immediately after treatment. The primary target for permethrin toxicity in female mice in this study is the liver. Incidences of inflammatory liver changes were increased in all treated mice compared to the control through treatment week 78 (Table 6). Thereafter, the inflammatory changes increased in the control animals as well, and the differences between treated and control animals were no longer significant (Table 7).

Incidences of centrilobular hypertrophy, Kupffer cell hypertrophy, karyomegaly, and pigmented liver were all significantly increased at all treatment periods without a recovery period compared to the controls (Table 6). None of these findings showed a clear dependence on the treatment duration. The incidences of amyloid deposits in the liver were also increased after 52, 65, and 78 weeks of treatment compared to the controls, and did increase slightly with increasing treatment duration.

The incidences of liver inflammatory changes, centrilobular hypertrophy, and Kupffer cell hypertrophy were significantly decreased to the control level in recovery animals fed the control diet following treatment (Table 7). Karyomegaly incidences in animals fed the control diet after treatment were significantly decreased compared to the animals examined immediately after treatment, but the recovery was at the control level only in the survivors of the group treated for the first 39 of 101 weeks. Since the karyomegaly incidences were high in animals that died prematurely during the recovery periods, the incidences in the total number of animals were also calculated. When the predecedents are included, the recovery never reaches the control level (values in Table 7). The incidences of liver pigment were only decreased in the group treated for the first 39 of 101 weeks. Amyloid deposit incidences in animals treated for 39 weeks increased during the recovery periods compared to animals examined immediately after the treatment period; no recovery was seen with amyloid deposit incidences at the other treatment durations.

In the lungs, incidences of Clara cell hyperplasia increased significantly in all treated animals immediately after the treatment periods compared to the control groups (Table 6). The Clara cell hyperplasia incidences were significantly decreased by about 46-69% and 39-75% in treated animals allowed to recover to 78 or 101 weeks, respectively (Table 7).

TABLE 6: Selected incidences of non-neoplastic microscopic findings after various weeks of treatment with 5000 ppm Permethrin in the diet [a]

Organ/Finding	Control 39 wks.	Treated 39 wks.	Control 52 wks.	Treated 52 wks	Control 65 wks.	Treated 65 wks.	Control 78 wks.	Treated 78 wks.
Liver/ inflammatory changes	16/43[b] (37%)	34/42** (81%)	20/40 (50%)	34/41** (83%)	26/41 (63%)	35/37** (95%)	22/40 (55%)	30/40* (75%)
Liver/centrilobular hypertrophy	0/43 (0%)	42/42** (100%)	0/40 (0%)	41/41** (100%)	1/41 (2%)	37/37** (100%)	2/40 (5%)	38/40** (95%)
Liver/Kupffer cell hypertrophy	0/43 (0%)	19/42** (45%)	0/40 (0%)	25/41** (61%)	0/41 (0%)	16/37** (43%)	0/40 (0%)	22/40** (55%)
Liver/karyomegaly	0/43[c] (0%)	42/44[c]** (95%)	0/40[c] (0%)	40/43[c]** (93%)	0/41[c] (0%)	49/56[c]** (88%)	1/40[c] (3%)	68/78[c]** (87%)
Liver/pigment	1/43 (2%)	12/42** (29%)	1/40 (3%)	15/41** (37%)	1/41 (2%)	16/37** (43%)	2/40 (5%)	16/40** (40%)

Liver/amyloid	0/44[c] (0%)	0/44[c] (0%)	0/49[c] (0%)	6/43[c] ** (14%)	0/49[c] (0%)	13/56[c] ** (23%)	1/67[c] (1%)	14/72[c] ** (19%)
Liver/eosinophilic foci	0/43	0/42	0/40	0/41	1/41	0/37	0/40	2/40
Lung/Clara cell hyperplasia	0/50[c] (0%)	36/50[c]** (72%)	0/49[c] (0%)	42/43[c]** (98%)	0/50[c] (0%)	50/56** (89%)	1/67 (1%)	61/71** (86%)

[a] Data obtained from pages 152-160 and pages 174-182 in the study report, MRID 45597105.

[b] Number of mice with lesion/number of surviving mice examined at the scheduled termination period.

[c] The total number of mice in this case included those that died prior to the scheduled study termination due to high lesion incidences in these animals.

*Significantly different ($p<0.05$) from the control, Fisher's exact test by the reviewer.

** Significantly different ($p<0.01$) from the control, Fisher's exact test by the reviewer.

TABLE 7: Selected incidences of non-neoplastic microscopic findings after various weeks of treatment with 5000 ppm Permethrin in the diet followed by various recovery periods [a]

Organ/ Finding	Control 78 wks.	Treated3 9 wks. total 78 wks.	Treated 52 wks. total 78 wks.	Treated 65 wks. total 78 wks.	Control 101 wks.	Treated 39 wks. total 101 wks.	Treated 52 wks. total 101 wks.	Treated 65 wks. total 101 wks.	Treated 78 wks. total 101 wks.
Liver/ inflammatory changes	22/40[a] (55%)	21/40** (53%)	24/40* (69%)	24/39** (62%)	31/32 (97%)	27/30 (90%)	34/41 (83%)	32/37 (86%)	32/39 (82%)
Liver/ centrilobular hypertrophy	2/40	5/40**	2/40**	8/39**	2/32	0/30**	1/41**	0/37**	2/39**
Liver/ Kupffer cell hypertrophy	0/40	5/40**	1/40**	2/39**	0/32	0/30**	0/41**	0/37**	2/39**
Liver/ karyomegaly	1/67[c] (1%)	42/72[c]** (58%)	39/66[c]** (59%)	57/74[c]** (77%)	1/106[c] (1%)	25/101[c]** (25%)	40/106[c]** (38%)	65/94[c]** (69%)	78/104[c]** (75%)
Liver/pigment	2/40 (5%)	16/40 (40%)	10/40 (25%)	20/39 (51%)	3/32 (9%)	2/30* (7%)	14/41 (34%)	23/37 (62%)	17/39 (44%)
Liver/amyloid	1/67[c] (1%)	7/72[c] (10%)	12/66[c] (18%)	15/74[c] (20%)	3/106[c] (3%)	18/101[c] (18%)	19/106[c] (18%)	22/94[c] (23%)	21/104[c] (20%)
Liver/ eosinophilic foci	0/67[c] (0%)	0/72[c] (0%)	0/66[c] (0%)	6/74[c+] (8%)	0/106[c] (0%)	1/101[c] (1%)	5/106[c+] (5%)	6/94[c++] (6%)	10/104[c++] (10%)
Lung/Clara cell hyperplasia	1/67[c] (1%)	19/72[c]** (27%)	19/66[c]** (29%)	25/74[c]** (34%)	1/107[c] (0.9%)	17/103[c]** (17%)	25/107[c]** (23%)	39/94[c]** (41%)	48/103[c]** (47%)

[a] Data obtained from pages 155-156, pages 161-173, pages 176-177, and pages 183 -189 in the study report, MRID 45597105.
[b] Number of mice with lesion/number of surviving mice examined, (ratio as %).
[c] The total number of mice examined included those that died prior to the scheduled sacrifice due to high lesion incidences in these animals.
* Significantly decreased ($p<0.05$) compared to the corresponding group in Table 6 examined immediately after treatment, Fisher's exact test by the reviewer.
** Significantly decreased ($p<0.01$) compared to the corresponding group in Table 6 examined immediately after treatment, Fisher's exact test by the reviewer.
[+] Significantly different ($p<0.05$) from the untreated control, 2x2 chi-square test by the reviewer.
[++] Significantly decreased ($p<0.01$) from the untreated control, 2x2 chi-square test by the reviewer.

b) Cell proliferation determinations: No significant differences in cell proliferation were reported in eosinophilic or basophilic liver lesions between treated and control mice with the proliferating cell nuclear antigen assays. Assays of tissues following bromodeoxyuracil incorporation were not successful.

c) CYP450 evaluations: The activities of various CYP450 enzymes in liver and lung microsomes from untreated mice and mice treated for 52 weeks with 5000 ppm permethrin are given in Table 8. The activities are expressed per mg ribosomal protein representing the enzyme specific activity, per g liver, and per liver to determine the amount of enzyme activity present. In the liver of treated animals the specific activities (activity/mg ribosomal protein) of total CYP, CYP2B, and CYP2E1 decreased and

CYP1A and CYP3A remained the same, while the specific activity of CYP4A increased approximately 3-fold (325% of the control value). When the liver CYP enzymes were expressed as activity/liver indicating the total activity available, total CYP, CYP1A, CYP2E1, and CYP3A were increased in treated animals by 200% to 283% of the control value. The total liver CYP4A activity in treated animals was increased by 829% compared to the control animals. The total CYP2B activity was only increased by about 142% and the CYP2B specific activity was decreased by about 40% compared to the control.

In the lungs, the CYP2E1 and CYP4A activities in treated mice expressed as per g lung were increased by 133% and 125%, respectively, over the control value, and the CYP2B activity was decreased by about 48% compared to the controls. The specific activities of all CYP isozymes tested in the lung were decreased in treated animals compared to the controls.

TABLE 8: Cytochrome P450 (CYP) enzyme activities after 52 weeks of treatment with 5000 ppm Permethrin in the diet [a]

Activity	Control	Treated
Liver, total CYP, nmol/mg microsomal protein	0.61 ±0.19[b]	0.48 ±0.16
Liver, total CYP, nmol/g liver	7.14 ±1.71	11.47 ±1.70 (161%)[c]
Liver, total CYP, nmol/liver	14.58 ±3.24	29.14 ±4.62 (200%)
Liver, CYP1A, pmol/mg microsomal protein	156 ±74	157 ±55
Liver, CYP1A, pmol/g liver	1806 ±679	3755 ±867 (208%)
Liver, CYP1A, pmol/liver	3648 ±1227	9515 ±2006 (261%)
Liver, CYP2B, pmol/mg microsomal protein	81 ±22	49 ±25
Liver, CYP2B, pmol/g liver	987 ±298	1139 ±343 (115%)
Liver, CYP2B, pmol/liver	2031 ±644	2885 ±848 (142%)
Liver, CYP2E1, pmol/mg microsomal protein	703 ±118	581 ±165
Liver, CYP2E1, pmol/g liver	8560 ±2197	14116 ±3072 (165%)
Liver, CYP2E1, pmol/liver	17772 ±5231	35576 ±6458 (200%)
Liver, CYP3A, pmol/mg microsomal protein	397 ±164	429 ±165
Liver, CYP3A, pmol/g liver	4594 ±1301	10379 ±3351 (226%)
Liver, CYP3A, pmol/liver	9370 ±2478	26472 ±8597 (283%)
Liver, CYP4A, pmol/mg microsomal protein	1136 ±694	3696 ±1135 (325%)
Liver, CYP4A, pmol/g liver	13913 ±8851	90039 ±28094 (647%)
Liver, CYP4A, pmol/liver	27614 ±15767	229033 ±74169 (829%)
Lung, CYP2B, pmol/mg microsomal protein	40.20 ±12.05	21.05 ±5.00
Lung, CYP2B, pmol/g lung	251.9 ±92.65	209.7 ±48.65 (83)
Lung, CYP2E1, pmol/mg microsomal protein	30.06 ±8.94	24.15 ±3.78
Lung, CYP2E1, pmol/g lung	182.3 ±46.66	242.1 ±44.59 (133%)

Lung, CYP4A, pmol/mg microsomal protein	45.98 ±13.51	36.13 ±12.10
Lung, CYP4A, pmol/g lung	282.9 ±81.34	352.5 ±72.89 (125%)

[a] Data obtained from page 87 in the study report, MRID 45597105.
[b] Enzyme activity ± standard deviation.
[c] (% of the control value).

d) Neoplastic: Selected incidences of neoplastic lesions seen in female mice immediately after dietary exposure to 5000 ppm Permethrin for 39, 52, 65, or 78 weeks are shown in Table 9. The effects after maintaining the exposed mice for up to 78 or 101 weeks on the control diet following the exposure periods are shown in Table 10. The incidence of basophilic hepatocellular adenomas was little affected by 78 weeks of treatment with Permethrin (control, 1%; treated, 4%) (Table 9). The incidence of eosinophilic hepatocellular adenoma was significantly increased in the livers of females after 78 weeks of treatment with 5000 ppm Permethrin (10%, $p<0.05$) compared to the corresponding control group (1%). Maintaining the animals on the control diet for up to week 78 or 101 following the exposure periods did not result in increased incidences of eosinophilic adenomas (incidences of eosinophilic adenomas were 10% at both the end of 78 weeks of treatment and at the 101-week recovery following 78 weeks of treatment), but did result in significantly increased incidences of basophilic adenomas at the 101-week recovery (4% following 78 weeks of treatment and 9% at the 101-week recovery time. The basophilic hepatocellular adenoma incidences were significantly increased after 39, 52, and 78 weeks of exposure followed by the control diet to week 101 (10%, $p<0.01$; 7%, $p<0.05$; and 9%, $p<0.01$; respectively) as compared to the control group (1%). The increases were not related to the length of the exposure period, and no basophilic adenomas were seen after 65 weeks of treatment, which tends to make the effect of Permethrin exposure questionable in this case. No increases in liver carcinoma incidences were seen in treated animals compared to the controls at any time period in the study.

Significantly increased incidences of bronchioloalveolar adenoma were seen in female mice treated for 52, 65, or 78 weeks and examined immediately after treatment compared to the corresponding control animals (Table 9). The incidences were 33%, 30%, and 42% at 52, 65, and 78 weeks of treatment, respectively, compared to 8%-10% in the control groups. Maintaining the animals on the control diet following the treatment period up to week 78 or 100 resulted in increased lung adenoma incidences of 31%, 42%, and 50% (all $p<0.01$) for 39, 52, and 65 weeks of treatment followed by control diet up to week 78; and incidences of 43%, 47%, 49%, and 49% (all $p<0.01$) for 39, 52, 65, and 78 weeks of treatment followed by the control diet up to week 100 (Table 10). The control group incidences were 10% and 14% for 78 and 100 weeks, respectively. The increases in lung adenoma incidences also showed a significant positive trend with length of exposure after the recovery periods at 78 weeks and 100 weeks ($p < 0.01$, Cochran-Armitage test by the reviewer). The earliest lung adenoma incidences were seen at week 40 in both treated and control animals. Lung carcinoma incidences were not increased by treatment with permethrin.

TABLE 9: Selected incidences of neoplastic microscopic findings after various weeks of treatment with 5000 ppm Permethrin in the diet [a]

Organ/Finding	Control 39 wks.	Treated 39 wks.	Control 52 wks.	Treated 52 wks	Control 65 wks.	Treated 65 wks.	Control 78 wks.	Treated 78 wks.
Liver/basophilic hepatocellular adenoma	1/44[b]	0/44	0/49	0/43	1/49	1/56	1/67 (1%)	3/72 (4%)
Liver/eosinophilic hepatocellular adenoma	0/44	0/44	0/49	2/43	0/49	2/56	1/67 (1%)	7/72* (10%)
Liver/hepatocellular carcinoma	0/44	0/42	0/49	0/43	0/49	0/56	1/67	2/72
Lung/bronchiolo-alveolar adenoma	4/50 (8%)	9/50 (18%)	5/49 (10%)	14/43** (33%)	4/50 (8%)	17/56** (30%)	7/67 (10%)	30/71** (42%)
Lung/bronchiolo-alveolar carcinoma	0/50	0/50	0/49	0/43	0/50	0/56	1/67	0/71

[a] Data obtained from pages 152-160 and pages 174-182 in the study report, MRID 45597105.
[b] Number of mice with lesion/total number of mice examined in the study group including those that died prior to the scheduled study termination.
* Significantly different ($p<0.05$) from the control, Fisher's exact test by the reviewer.
** Significantly different ($p<0.01$) from the control, Fisher's exact test by the reviewer.

TABLE 10: Selected incidences of neoplastic microscopic findings after various weeks of treatment with 5000 ppm Permethrin in the diet followed by various recovery periods [a]

Organ/ Finding	Control 78 wks.	Treated 39 wks. total 78 wks.	Treated 52 wks. total 78 wks.	Treated 65 wks. total 78 wks.	Control 101 wks.	Treated 39 wks. total 101 wks.	Treated 52 wks. total 101 wks.	Treated 65 wks. total 101 wks.	Treated 78 wks. total 101 wks.

Liver/ basophilic hepatocellular adenoma	1/67 (1%)	1/72 (1%)	3/66 (5%)	4/74 (5%)	1/106 (1%)	10/101** (10%)	7/106* (7%)	0/94 (0%)	9/104** (9%)
Liver/ eosinophilic hepatocellular adenoma	1/67	0/72	1/66	1/74	2/106 (2%)	3/101 (3%)	5/106 (5%)	4/94 (4%)	10/104* (10%)
Liver/ hepatocellular carcinoma	1/67	0/72	0/66	0/74	0/106	1/101	0/106	0/94	0/104
Lung/ bronchiolo-alveolar adenoma	7/67 (10%)	22/72** (31%)	28/66** (42%)	37/74** (50%)	15/107 (14%)	44/103** (43%)	50/107** (47%)	46/94** (49%)	50/103** (49%)
Lung/ carcinoma	1/67	1/72	1/66	3/74	1/107	4/103	2/107	2/94	2/103

[a] Data obtained from pages 155-156, pages 161-173, pages 176-177, and pages 183 -189 in the study report, MRID 45597105.
[b] Number of mice with lesion/total number of mice examined in the study group including those that died prior to the scheduled study termination.
* Significantly increased ($p<0.05$) compared to the corresponding control group at week 78 or 101, 2x2 chi square test by the reviewer.
** Significantly increased ($p<0.01$) compared to the corresponding control group at week 78 or 101, 2x2 chi square test by the reviewer.

II. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that treatment of female mice with 5000 ppm Permethrin in the diet resulted in reversible increases in liver weights, centrilobular hypertrophy, and karyomegaly. The changes are associated with an increased incidence of eosinophilic hepatocellular adenoma after 79 weeks of treatment. No increase was seen in eosinophilic adenoma incidence during any recovery period following the treatment periods. The relationship with permethrin treatment and the incidences of basophilic hepatocellular adenomas was considered to be equivocal. Reversibility was not demonstrated, but there was no progression from the adenomas to carcinomas in the study. The induction of cytochrome P450 mixed function oxidases in the liver was demonstrated after 52 weeks of treatment.

A reversible increase in Clara cell hyperplasia was seen in the lungs of treated animals compared to the controls that was associated with an increased incidence of bronchiolo-alveolar adenoma. The incidences of lung adenoma increased during the recovery periods following treatment for 39 and 52 weeks, but not following treatment for 65 or 78 weeks. Reversibility of the lung adenomas was not shown, but no progression to carcinoma was seen.

B. REVIEWER COMMENTS: There were no significant treatment-related effects on mortality. The body weight gain was decreased by about 14% in animals treated for 65 or 78 weeks followed by recovery through week 101 compared to the control animals. Food

consumption over the first 52 weeks of the study was about 5.83 g/mouse/day for the control group and ranged from 5.70 to 5.80 g/mouse/day for the treated groups. Food efficiency over the first 52 weeks was decreased in the pooled treated groups by about 5% compared to the pooled controls. A decrease in food efficiency is consistent with a toxic effect of treatment.

At the end of each treatment period, increased liver weights of about 44-53% were seen in all treated animals compared to the control group. There was no clear relationship between the increased liver weights and the length of the treatment period; however, liver weights of animals treated for 78 weeks did not quite recover to control levels by week 101. Liver weights of animals treated for 39 weeks recovered to control levels by week 79. The lung weights were not affected by treatment. Upon necropsy, the incidences of treated animals with dark livers were significantly increased after 65 and 78 weeks of treatment, and partial recovery was seen by week 101 in the 65-week treatment group but not in the 78-week treatment group. The incidence of liver masses was only slightly increased after 78 weeks of treatment and did not decrease during the recovery periods. The incidences of masses in the lung were significantly increased immediately after 78 weeks of treatment, and were increased at all time periods compared to the controls following the recovery to week 101. Significant increases in the incidences of pale kidneys and enlarged spleens seen after 65 weeks of treatment and slight, but consistent, increases in hepatic lymph node enlargement at all treatment durations were decreased to or near to the control levels during the recovery periods. There were no microscopic findings reported to confirm any treatment-related effects on the kidney or spleen.

Microscopic findings confirmed and expanded the gross observations in the liver and lungs. The liver is a primary target for permethrin toxicity in mice. Incidences of inflammatory changes, centrilobular hypertrophy, Kupffer cell hypertrophy and karyomegaly were significantly increased at all treatment durations; all these findings were significantly decreased during one or both recovery periods. Increased incidents of pigmented liver were seen at all treatment durations immediately after treatment, and were significantly decreased only after 39 weeks of treatment followed by recovery to 101 weeks. Incidents of amyloid deposits were increased immediately after 52, 65 and 78 weeks of treatment, and were also increased in the 39-week treatment group after the recovery periods. There were no decreases in amyloid deposit incidences during the recovery periods. Amyloid deposits were considered by the study authors to be a significant cause of the early deaths of animals in the study. The H & E dyes distinguished between basophilic and eosinophilic foci. The incidences of eosinophilic foci were not increased in treated animals immediately after the treatment periods. However, following the recovery periods, the incidents of eosinophilic foci were significantly increased compared to the controls in the 52-, 65-

, and 78- week treatment groups that were allowed to recover to week 101 and in the 65- week treatment group allowed to recover to week 78. Measurements of cellular proliferation did not show a treatment-related increase in cell proliferation in the livers of treated animals compared to the controls.

The total activity of cytochrome P450 (CYP) mixed function oxidase was shown to increase in the livers of animals treated for 52 weeks compared to the controls. However, for individual CYP isozymes, specific activity, expressed as pmol/mg microsomal protein, was increased for only CYP4A; the increase was 3-fold over the control value.

Increased incidences of Clara cell hyperplasia were seen in the lungs of treated animals at all treatment durations compared to the controls; the incidences were significantly decreased during the recovery periods, although still elevated over control values. The specific activities of CYP2B, CYP2E1, and CYP4A, expressed as pmol/mg microsomal protein, were not increased in the lung. Total activities, expressed as pmol/g lung, were either decreased (CYP2B) or only slightly increased (up to 133% of the control value for CYP2E1).

Treatment of Crl:CD-1®(ICR)BR female mice with a dietary level of 5000 ppm Permethrin for 39, 52, or 78 weeks followed by a control diet during a recovery period to week 101 resulted in significant increases (7% [$p<0.05$] to 10% [$p<0.01$]) in the incidences of basophilic hepatocellular adenoma compared to the control groups (1%). The increased incidences did not show a relationship to the treatment duration; mice treated for 65 weeks with recovery to week 101 did not develop any basophilic adenomas. The incidence of eosinophilic hepatocellular adenomas was increased in animals sacrificed after treatment for 78 weeks and also after being allowed to recover to week 101 (both 10%, $p<0.05$) compared to the control (1-2%). In contrast to the basophilic hepatocellular adenoma incidences, the eosinophilic hepatocellular adenoma incidences did not increase during the recovery period after the chemical agent was removed. However, reversibility of the adenoma incidences was not shown. There was no increase in the incidence of hepatocellular carcinoma seen in treated animals compared to the controls at any time period in the study, and the adenomas were not seen any earlier in the study in treated animals compared to the controls.

Significant increases in the incidences of lung bronchioloalveolar adenomas were seen in mice sacrificed after treatment with 5000 ppm Permethrin for 52, 65, or 78 weeks (33%, 30%, and 42% at 52, 65, and 78 weeks, respectively, $p<0.01$) compared to the control groups (8-10%). The lung adenoma incidences increased in all treated groups compared to the untreated controls (10-14%) during the recovery period after the Permethrin was removed from the diet and appeared to be related to the dose duration. The incidences were 31%, 42% and 50% ($p<0.01$) after 39, 52, and 65 weeks exposure, respectively,

followed by the control diet to week 79, and 43%, 47%, 49%, and 49% ($p<0.01$) after 39, 52, 65, and 78 weeks exposure, respectively, followed by recovery to week 101. There was no increase in the incidence of lung carcinoma in treated animals at any time point and the lung adenomas did not occur any earlier in the study in treated animals compared to the controls.

The observations of increased eosinophilic foci and adenomas as well as increased CYP4A are consistent with certain types of hepatic peroxisome proliferation.

C. STUDY DEFICIENCIES: There were no serious deficiencies in this complex study. The pooling of the control animals killed at 40 to 101 weeks of age should not have been used to compare the clinical observations of treated animals killed at specific times since some effects could be age-related. The statistics were included in some tables and not in others, and some statistical results were presented in the text, which was confusing.

DATA FOR ENTRY INTO ISIS

Carcinogenicity Study - mice (870.4200b)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
109701	45597105, 45597104	carcinogenicity, special study	mice-females	100 weeks	oral	diet	780-807 (5000 ppm in the diet)	Single dose; duration: 39, 52, 65, 78 weeks; recovery to 78 & 100 weeks	Not determined	Not determined; a single, known adverse effect dose was used	Liver, lungs	Liver inflammatory changes, centrilobular hypertrophy, Kupffer cell hypertrophy, karyomegaly, hepatocellular adenoma, pulmonary adenoma

APPENDIX: 4-Week Oral Toxicity [feeding] -[mouse]; Range-finding

TEST MATERIAL (PURITY): Permethrin (94.7% a.i.)

CITATION: T. Martin, S.J. Barton, and R.J. Weaver (1996) Permethrin 4 week preliminary study in mice with administration by the diet. Inveresk Research, Tranent, EH33 2NE, Scotland. Inveresk Report No. 11474A, Inveresk Project No. 452585, FMC Study No. A95-4263, Dec. 2, 1996. MRID 45597104. Unpublished

In a 4-week oral toxicity study (MRID 45597104) Permethrin (94.7% a.i., lot # PL95-329) was administered to groups of 10 Crl:CD-1®(ICR)BR female mice at dose levels of 0, 5000, or 8000 ppm (equivalent to 0, 1096-1390 or 1689-2152 mg/kg/day). Two additional groups of 5 each were added to serve as positive controls used to assess enzyme induction. One positive control group received 80 mg/kg 3-methylcholanthrene (in a 1% olive oil solution) by intraperitoneal injection 3 days before study termination; the second positive control group was given 0.1% phenobarbitone in the drinking water for 6 days. Treatment began 7 days prior to the day the mice were killed.

Two mice were found dead the morning after the first day of treatment with 8000 ppm. The only clinical sign noted was a slightly increased incidence of nervous behavior in both treated groups that persisted throughout the study. There were no treatment-related differences in body weight, food or water consumption, and no findings at terminal necropsy. The mean liver weights were increased by about 136% and 131% at 5000 and 8000 ppm, respectively, compared to the control group. The liver weights were also increased by 126% in the phenobarbitone control group.

The P450 enzyme activities assayed included ethoxyresorufin O-deethylation (EROD [CYP1A]), pentoxyresorufin O-dependentylation (PROD [CYP2B]), benzyloxyresorufin O-debenzylation (BROD [CYP3A]), and aminopyrine N-demethylation (APNDe [CYP1A]). Cytochrome P450 isoenzymes, CYP1A, CYP2B, CYP2E1, CYP3A, and CYP4A were assayed by western blotting against specific antibodies. The positive controls gave the expected response confirming the methodology. EROD activity was elevated 1.6 fold and APNDe was slightly increased at 8000 ppm compared to the untreated control group, but EROD, PROD, and BROD activities were not increased at 5000 ppm. The specific P450 content of treated animals was increased by 1.2 and 1.4 fold at 5000 and 8000 ppm, respectively. CYP4A was markedly induced at 5000 and 8000 ppm compared to the control group and CYP2B was marginally increased at 8000 ppm in 1 of 3 animals examined. No induction of the CYP1A, CYP2E1, or CYP3A was seen.

LOAEL and NOAEL values were not determined since the lowest dose (5000 ppm, 1096-1390 mg/kg/day) was known from previous studies to result in an adverse effect on the liver. The 5000 ppm dietary concentration was chosen for the recovery study because of the mortality seen at the 8000 ppm level.