UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE 5-16-80

SUBJECT: *EPA Reg. Nos. 279-3013 and 279-3014, Evaluation of 24-month Mouse Oncogenesis Study with Permethrin.*

TOX Chemical 652 BB

FROM: *John Doherty, 5/15/80*
Toxicology Branch/HED (TS-769)

TO: *F. D. R. Gee, PM #17*
Registration Division (TS-767)

Action Requested:

Review two year mouse oncogenesis/oral toxicity study submitted as one of the requirements for conditional registration of Pounce 3.2E and Pounce technical insecticides.

Conclusion:

1. *Toxicology Branch (TB) has reviewed the final report of the subject mouse oncogenesis study and has concluded that the results indicate a dose dependent oncogenic effect in female mice. Specifically, continuous feeding of mice with permethrin for 24 months resulted in a dose dependent increase in hepatomas. For the reasons presented in this review, TB does not accept the registrants assertion that the increased occurrences of hepatomas were incidental.*

   Statistically significant increases in bronchioalveolar adenoma in female mice were also noted and were considered by TB to be related to ingestion of permethrin.

2. *The increased incidences of hepatocellular carcinomas in male mice were considered by TB to be possibly related to permethrin in the diet.*

3. *Future conditional registrations of permethrin products should await a risk analysis for the oncogenic effects of the active ingredient.*

4. *TB was unable to critically evaluate the gross necropsy results because the original report was transcribed and an edited version was included on the histopathology report. It was also not possible to tell who trimmed and selected tissues for histopathologic examination. Therefore, the records of necropsy, slide preparation and other transit information should be audited and, if warranted, a confirmatory histopathologic evaluation of the microscopic slides should be performed. An audit would not likely affect the conclusion of TB that permethrin induces the oncogenic effects mentioned above, but may indicate that the same effects occur at lower doses or at higher incidences than reported. This information would be essential for a proper risk analysis.*

DETAILED REVIEW OF STUDY

Identification of Study:

"A Twenty-four Month Oral Carcinogenicity Study of FMC 33297 in Mice". Final Report.

Bio/dynamics Inc., Project No. 76-1695, Report dated October 9, 1979 (Submitted to FMC Corporation).EPA Accession Nos. 242174 and 242175.

Study Design:

This study was designed to assess the carcinogenic potential of permethrin (FMC 33297) in Charles River CD-1 mice. Groups of 75 mice of each sex were fed technical permethrin in their diet for 24 months. The males were dosed with 0, 20, 500, and 2000 ppm and the females were dosed with 0, 20, 2500, and 5000 ppm. The cis/trans ratio of the technical permethrin used was not stated.

Results:

1. Survival:

All groups except the high dose males had essentially equivalant survival rates during the study. Except for this group there were at least 20 survivors at 2 years for histopathological examination.

		Mice Surviving 24 Months	
Group	Dosage Level	Male	Female
I	0 ppm	20	22
II	20 ppm	27	33
III	500 (males) or 2500 (females) ppm	26	23
IV	2000 (males) or 5000 (females) ppm	12*	22

*Significantly different from controls.

2. Physical Observations (During Study):

Two anomalies were noted: 1) higher incidence of yellow staining in the ano-genital region (males only) and 2) abdominal distention (males and females). The toxicological significance of these anomalies is uncertain.

3. Body Weight and Food Consumption:

No consistant differences between test and control groups were noted.

4. Differential Leukocyte Counts:

Two deviations between test and control groups were noted:

i. lymphocyte counts (%) of the mid and high dose females and of the high dose males were lower than controls,

ii. neutrophil counts (%) for the high dose males were higher than controls.

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5. *Organ Weights and Organ to Body Weight Ratios (determined on 2-year survivors).*

   i. *Testes - High dose males were 38% lower in absolute weight and 35% lower in relative weight. This was statistically significant ($p \leq 0.01$).*

      The low dose group was 7% and 7% lower and the mid dose group was 15% and 12% lower in absolute and relative weights respectively. The differences at the low and mid doses were reported as not being statistically significant, but the progressive depression in weight is noted.

   ii. *Liver - In test females, there was a progressive increase in absolute weight compared to the controls (6%, 26%, and 42%) and relative weight (3%, 16%, 36%). The differences were significant at the mid and high dose levels for absolute weight, and at the high dose for relative weight. No increase in liver weights was noted in test males.*

   iii. *Brain - In the high dose females, the relative brain/body weight ratio was statistically lower than in controls (10%).*

   iv. *Lung - In the high dose females, the absolute lung weight was higher than controls (24%).*

   The differences noted above support a NOEL of 20 ppm.

6. *Pathology*

   NOTE: Following terminal sacrifice at 2 years, the organs to be weighed were removed. The remaining entire carcass was placed in fixative and returned to the sponsor (FMC Corporation) for gross necropsy and histology. Dr. William R. Rapp, D.V.M., an independent pathologist, evaluated the microscopic slides for abnormalities.

   The pathology report is dated Feb. 7, 1980. Necropsy and histopathology was conducted on essentially all animals in the study. This included animals sacrificed at termination and those dying or sacrificed during the course of the experiment.

Neoplastic Changes:

i. Number of animals in each test group with one or more neoplasms.

Sex	*Group I*	*Group II*	*Group III*	*Group IV*
Males	*43*	*44*	*53*	*43*
Females	*35*	*42*	*53*	*52*

An increased incidence of females with neoplasms is noted.

ii. The observed neoplasms of particular concern are listed in the following table.

Neoplasm	*Sex*	*Group I*	*Group II*	*Group III*	*Group IV*
Bronchioalveolar adenoma	*M*	*18*	*19*	*20*	*17*
	F	*12*	*14*	*28**	*28**
Hepatoma	*M*	*16*	*21*	*18*	*17*
	F (1)	*3*	*2*	*15**	*17**
Hepatocellular carcinoma	*M*	*4*	*6*	*13(2)*	*5*
	F	*0*	*2*	*3*	*0*

(1) Supports a dose dependent effect.

(2) Not statistically significant by the log rank test but unusually high.

** Statistically significant by the log rank test ($p < 0.05$).*

iii. The pathologist's report states that bronchioalveolar adenomas observed in control and test mice were morphologically similar and that the increased incidences in the higher dose females were not, in his opinion, sufficiently large for this common neoplasm to clearly define an oncogenic effect.

The pathologist's report also states that, in his opinion, increased incidences of hepatoma and hepatocellular carcinoma in mice are not always a good indicator of oncogenic potential unless the experimental data is "remarkable" or ancillary data supports the conclusion. In this study, the sponsor asserts that the incidence is not "remarkable" and that there were no ancillary data to warrant a conclusion that permethrin was oncogenic.

iv. *Toxicology Branch does not concur with the pathologist's interpretation of the data because of the reasons presented below:*

a. *The data on absolute and relative liver weight as presented in Appendix H clearly indicate an ancillary effect that accompanies the increased incidences of hepatoma in females. For example, livers from many of the female mice were not included in subsequent weighting calculations because a "mass" was observed in the liver and the weights were considered invalid for the purposes of such calculations. In spite of these "throw outs" there was still noted a dose dependent increase in both absolute and relative liver weights. (See results in part 5 of this review).*

The frequency of occurrence of female mice with liver "masses" is as follows:

(See Appendix H of Section B)

Group	Dose (ppm)	Frequency	% of Female Survivors with Liver "Masses"
I	0	1 of 22	4.5%
II	20	1 of 34	3%
III	2500	8 of 24	33%
IV	5000	13 of 22	59%

The above is interpreted by TB as indicative of a dose response for "masses" in the liver of female mice and represents an ancillary effect.

b. *A pronounced dose relationship was also obtained when the incidence of hepatoma among female survivors only is considered.*

Group	Dose (ppm)	Frequency	% of Female Survivors with Hepatoma
I	0	0 of 22	0%
II	20	1 of 34	3%
III	2500	6 of 24	25%
IV	5000	13 of 22	59%

c. A dramatic increase in incidences is also noted among surviving female mice with the lesion bronchioalveolar adenoma. Moreover, there is also a dramatic dose dependent relationship between female mice having both of the lesions described as bronchioalveolar adenoma and hepatoma.

		Female Mice with Bronchio-alveolar Adenoma		Female Mice with Both Bronchioalvelar Adenoma and Hepatoma	
Group	Dose (ppm)	Frequency	%	Frequency	%
I	0	4 of 22	18	0 of 22	0
II	20	8 of 34	24	0 of 34	0
III	2500	15 of 24	63	4 of 24	17
IV	5000	13 of 22	59	8 of 22	36

d. The pathologist's report considers the hepatomas to be benign. However, it must be kept in mind that according to many investigators, benign liver neoplasms have the potential to progress to carcinomas. The above table showing a dose response for mice having both lung and liver lesions is suggestive of metastasis.

e. The pathologist's report asserts that male mice do not develop increased incidences of hepatoma relative to the controls.

The following table indicates, however, that the lesion of hepatocellular carcinoma (HC), which can be considered as a type of hepatoma, may be related to ingestion of permethrin.

Group	Dose (ppm)	Average Survival of all Males in Group (in months)	Number of Survivors at 2 years	Incidences of HC	Average Survival of All Males with HC (in Months)
I	0	19.8 (4.9)*	22	4	21.0 (2.6)*
II	20	20.0 (5.7)	26	6	22.0 (5.35)
III	500	20.0 (5.8)	26	13	22.0 (1.85)
IV	2000	17.7 (5.8)	12**	5	20.2 (2.8)

*(Standard deviation)

** Statistically significantly lower.

This table indicates that mid dose male mice lived longer than the high dose males and developed more incidences of hepatocellular carcinoma than either the controls, low dose or the high dose groups. These data can be interpreted as permethrin causing an oncogenic effect that develops in the later months of feeding (for example, the last 4 of the 24 months). The failure of the high dose group to show the oncogenic effect may be related to poor survival in this group.

Of the 13 male mice with hepatocellular carcinoma in the 500 ppm group, the lesion apparently metastasized to the lung and kidney in some cases because 4 mice were reported as having a lesion in their lungs and one mouse was reported as having a lesion in its kidney described as "hepatocellular carcinoma".

Non-neoplastic Changes:

i. A variety of non-neoplastic changes were noted in the pathologist's report. These were considered as being incidental in occurrence.

Discussion and Conclusion:

1. The "in-life" phase of this study (part performed by Bio/dynamics Inc.) was audited by Theodore Ellison, Ph.D., consultant in toxicology. Dr. Ellison's conclusion was that the raw data in the form of data reports, log books and correspondence supports the information presented in the final report.

2. This study is classified as Core-Guidelines.

3. This study demonstrates that female mice fed doses of permethrin at 2500 and 5000 ppm developed significantly higher incidences of hepatoma. The five to eight fold increase in this particular lesion relative to the control and low dose groups is considered by TB to be in excess of an incidental occurrence.

4. The increased incidences of lesions described as "bronchioalveolar adenoma" (occurring in mid and high dose females) is considered by TB as being related to exposure to permethrin.

5. Hepatocellular carcinoma that was noted in mid dose males is considered by TB to be possibly related to exposure to permethrin.

6. The NOEL for this study, based on non-oncogenic effects, is 20 ppm.

May 1, 1980

Status of Permethrin Review

Acting Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

Associate Deputy Assistant Administrator
for Pesticide Programs (TS-766)

The complete oncogenic study on permethrin has recently been received in this Branch. It is under review by Dr. John Doherty on a high priority basis. Preliminary results indicate the pesticide causes an increase in hepatomas in female mice. Final evaluation is due in three weeks.

William L. Burnam

cc: Dr. McGrath

TS-769:WLBURNAM:fmm:CM#2X7731O:Rm816:5/1/80.

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