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SUBJECT: EPA Registration No. 432-487. 2-year rat chronic feeding/oncogenesis study with resmethrin. Study submitted as 6(a)2 data.
Tox. Chem. No. 83E

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Background:

The PENICK Corporation has submitted the final report of a 2-year chronic feeding/oncogenesis study with rats for review by TOXICOLOGY BRANCH. The conclusion of this study, according to the testing laboratory, was that resmethrin was associated with increased incidences of proliferative liver lesions and tumors in the high-dose test group.

Conclusions of Toxicology Branch review:

1. TOXICOLOGY BRANCH has concluded that resmethrin is associated with oncogenic effects at both the mid- and high-dose levels in the livers of female rats.

   TOXICOLOGY BRANCH has used the diagnosis of Drs. Becci and Cox (see Pathology Table 17a) to determine that there were both adenoma and nodular hyperplasia in female rats at the mid dose level (2,500 ppm) to justify the conclusion of oncogenic effects at this level. Nodular hyperplasia is considered a primary neoplasm in rats unless proven otherwise (see JNCI 64: 180-190 (1980).

2. The thyroid has also been indicated as being a possible target organ for oncogenic effects of resmethrin. Thyroid tissues for those animals in the low and mid dose groups not yet examined should be prepared and examined.

3. No clear NOEL was established for the effects of resmethrin on spleen weight.

4. The registrant and its consultants are invited to present their case that the thyroid tissue does not show oncogenic effects related to resmethrin and that the effects of resmethrin on spleen weight do not represent a toxic effect.

Review of Study

Title: A Lifetime Evaluation of the Dietary Administration of SBP-1382 to Wistar Albino Rats

Food and Drug Research Laboratories
May 2, 1980, Lab. No. 5271
EPA Accession No. 242782-3-4-5-6 (five volumes)

The one-year interim report of this study was reviewed by J. Doherty. June 26, 1980.

Groups of 60 male and 60 female weanling Wistar albino rats were dosed with 0, 500, 2500, or 5000 ppm of technical resmethrin in their diet. The test chemical was dissolved in corn oil and the controls were fed corn oil in their diet. The male rats were sacrificed during the 106th week, the female rats were sacrificed during the 113th week and this study is considered a lifetime feeding rather than a 2-year feeding study.

RESULTS

1. Mortality (See Pathology Table 1, Volume 2)

	Males	Females
Control	15/30% (23)*	19/38% (13)
Low Dose	18/36% (23)	27/54% (14)
Mid Dose	14/28% (29)	17/34% (14)
High Dose	20/40% (21)	25/50% (13)

* Number of survivors/percentage survival assuming 50 possible survivors; ( ) the number of rats dying spontaneously.

There was no dose-dependent decrease in survivors. Survival in this study is poor (expecially in the male group) because less than 50% of the possible animals survived.

2. Clinical signs - at the highest dose level, a transient increase in rats with tremors and hair loss was noted.

3. Body weight - a NOEL for depression of body weight is 500 ppm for both males and females.

4. Food Consumption - Statistically significant differences were noted but overall food consumption was within 3% of the controls for females and males.

5. On a mg/kg/day basis females consumed more of the test chemical than did the males across the dosing levels, (39.5, 193.7, 400.9, for males and 47, 232.7 and 450.3 for females).

6. Ocular effects. (Note: the interim report indicated a possible effect). The final report by R. C. Riis, D.V.M. states that ocular abnormalities were variable and not particularly related to systemic effects. The observations of a possible lesion as noted in the interim report were not further substantiated.

7. Hematology and urinalysis (analysis at 3, 12, 18 and 24 months for 6 rats of each sex). No consistent dose-related effects were noted in hematological analyses. At the mid and high dose levels, there were noted alterations in the content of albumin in the urine for females only. For example, at three months the albumin was higher than the control content; at 12, 18 and 24 months both the mid and high dose groups were lower (statistically significant) than the value reported for the control. The physiological and/or toxicological significance of the albumin in the urine and the resmethrin-induced decreases is not known.

8. Biochemistry - assays performed using six rats of each sex at 12 and 24 months. Glucose (mg %), SGPT and SAP were not affected by the presence of the test chemical in the mid- and high-dose groups. BUN was elevated at 12 months in females (see interim report) but no similar effect was noted at 24 months.

9. Organ weights - The interim report indicated differences in absolute and relative weights for liver and spleen for both males and females and possibly for adrenals and thyroids.

   The results of the terminal sacrifices showed that:

   a. Liver weight for both males and females in the high dose and for females in the mid dose test groups were higher.

   b. Thyroid weights for females in the high dose group were higher (32% absolute and 40% relative).

   c. Spleen weights. In males the mid and high dose groups were lower but dose dependence and statistical significance were not obtained. For females the following table has been prepared.

Spleen Weight Data

	12 Months		Terminal	
Dose Level	Abso. Wt.	Rel. Wt.	Abso. Wt.	Rel. Wt.
0	.76	.27	1.15	.36
500	.65	.24	.93	.27*
2500	.63*	.22*	.80*	.25*
5000	.58*	.21*	.81*	.28*

* statistically significant $P < .05$

A statistically significant decrease (25%) in relative spleen weight is noted in the low-dose test group. The consistency of appearance of decreases in spleen weight at both 12 months and at the terminal sacrifice allow the conclusion that this experiment does not demonstrate a NOEL at the lowest dose tested for adverse effects on the spleen.

Pathology

A. Gross Pathology

In particular, the liver exhibited clear indications of proliferative lesions in the female rats in the high dose test group.

B. Histopathology

Histopathology was performed on the rats sacrificed at 1 year and all of the control and high-dose group rats including survivors and spontaneous and moribund deaths. Histopathology was also performed on 10 rats/sex from the low and mid dose groups. For the remainder, only grossly abnormal organs were further examined microscopically. (Note that usually liver was examined histologically for all animals).

The initial reading of the slides was the responsibility of Dr. Mark K. Walter, a resident pathologist at FDRL during the summer of 1979. His report was reviewed by Dr. Peter Becci who noted that livers showed dose-dependent increases in proliferative lesions. Subsequently, Drs. Becci and George F. Cox, staff pathologists at FDRL, recut and reexamined (apparently extensively) slides of the lung and liver tissue. The pathology report (prepared by Drs. Becci and Cox) states that once they reread the slides, the slides were sent to Dr. Walter (who had moved to Iowa) for reexamination. Dr. Walter's diagnosis was different from that of Drs. Becci and Cox concerning the description of several liver lesions.

TOXICOLOGY BRANCH has accepted the diagnosis of Drs. Becci and Cox for their description of liver lesions. A summary of the tumors produced in the livers of female rats is shown in the following table.

Number of Animals Examined 1/		Hepatocellular Adenoma	Hepatocellular Carcinoma	Nodule* Hyperplastic	Total rats** affected
50	Control	0	0	1	1
46	500 ppm	0	0	0	0
49	2,500 ppm	5	0	5	7
49	5,000 ppm	13	8	13	24

* TOXICOLOGY BRANCH considers hyperplastic nodules as a primary neoplasia change in rats. Reference: "Histologic typing of liver tumors of the rat," JNCI 64: 180-190 (1980). See page 185 under primary neoplasms.

** Some rats have more than one lesion.

1/ under at the direction of [illegible] Toxicology Branch

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TOXICOLOGY BRANCH has concluded that on the basis of the data in this table, resmethrin, when administered in the diet at 2,500 and 5,000 ppm, resulted in a dose related increased number of tumors in female rats.

In male rats, there is only a possible oncogenic effect noted. For example, there were 0, 1, 1, and 3 occurrences of hyperplastic nodules in the control, low, mid and high dose groups respectively. There were 2 occurrences of hepatocellular carcinoma in the high-dose male group, whereas there were none reported in the other male test groups. Female rats, but not male rats, in the high-dose test group were also associated with increased incidences of anisokaryosis(

II. The following table shows the incidences of tumors (adenoma) found in the thyroid tissue.

Dose Group	Males	Females
Control	2/47 (4.2%)*	1/50 (2%)
500 ppm	4/26 (15.4%)	1/16 (6.3%)
2,500 ppm	3/27 (11.1%)	4/23 (17.4%)
5,000 ppm	8/47 (17.0%)	5/48 (10.4%)

*Number of adenomas/number animals examined ( ) percentage.

Based on both the percentage and the total number of adenomas obtained, an oncogenic effect is suggested, and can be shown to be statistically significant (personal communication B.Litt December 23, 1981).

The thyroid tissue did not show associated gross pathology other than that the high dose female test group had thyroid weights higher than controls (32% absolute and 40% relative). It is possible that the tumor type is small such that no associated gross observations are evident. The thyroid tissues from the low and mid dose groups that were not processed for histology should be prepared and examined. It is important to note that the thyroid tissue was examined by Dr. Walter only and there is no evidence that Drs. Becci and Cox further reexamined these tissues as they reexamined liver tissues.

III. Mammary and pituitary glands in females. The following table shows the frequency of occurrence of tumors in the mammary and pituitary glands in females.

	Mammary* Gland	Pituitary** Gland
Control	15/50 (30%)	15/45 (33.3%)
500 ppm	7/18 (39%)	17/31 (54.8%)
2,500 ppm	12/21 (57%)	22/34 (64.7%)
5,000 ppm	7/49 (14.3%)	12/46 (26.7%)

* benign fibro epithelial tumors: adenoma and fibroadenoma

** adenoma.

For both of these tissues, there is a disturbingly high frequency of occurrence of these commonly occurring tumors in the mid-dose group. However, the high-dose group is less than the control group. Thus, these observations do not support a dose-dependent response for an oncogenic effect. These observations are considered noteworthy but lend no toxicological interpretation at this time.

Conclusions:

1. This study demonstrates that resmethrin is oncogenic and produces liver tumors when fed in the diet at the level of 2,500 and 5,000 ppm for the lifetime of the Wistar female albino rats.

2. The thyroid tissue has also been identified as showing evidence of oncogenic effects.

3. As an oncogenesis evaluation, this study is classified as CORE MINIMUM. All of the tissues in the low- and mid-dose groups should have been prepared and examined histologically. Survival in the male group was poor.

4. As a chronic feeding study, this study is CORE MINIMUM. The laboratory tests were conducted on six and not eight rats of each sex/dose level.

5. This study does not demonstrate a NOEL for systemic effects. Spleen weight (both relative and absolute) were decreased and the spleen relative weight was statistically significantly reduced at the lowest test dose.

Addendum: This addendum is written at the request of Bill Burnam, Deputy Branch Chief, for the purpose of including the statistical methods used in the two year rat chronic feeding/oncogenesis study conducted with resmethrin. (Accession Nos. 242782 thru 242786 inclusive).

All the statistical methodology mentioned here was conducted by Food and Drug Research Labs, Inc.

All parameters from the clinical studies, organ weights, organ to body weight ratios, weekly body weights and food consumptions were evaluated statistically for differences among groups using analysis of variance. Differences among groups were deemed significant when the probability of rejecting the null hypothesis when true was less than 0.05. Any parameters where a difference was found among groups ($P < 0.05$) was then tested using the least significant difference test to determine which test group(s) differed from the control.

Mean body weights were analyzed using a randomized complete block design analysis of variance in which the weeks constituted the blocks.

The incidences of hyperplastic nodules, hepatocellular adenomas and hepatocellular carcinomas that were observed in female rats receiving the compound were found to be statistically significant ($P \leq 0.05$; Fishers Exact Test) when compared to their own controls. Furthermore, a highly significant dose response effect was observed with respect to the hyperplastic nodules and hepatocellular adenomas in female rats ($P \leq 0.01$; ordered chi-square test; reference Fed. Proc. 13:815, 1954).

The data for males showing one or more of the above mentioned responses were apparently analyzed in the same manner.

Note here also that the number of female rats examined with respect to liver lesions (see the data table on page 4 of John Doherty's review and page 10 of accession number 242782) were as follows and is based upon the findings of Drs. Becci and Cox.

Controls	50 animals
Low-dose	46 animals
Mid-dose	49 animals
High-dose	49 animals