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

006755

JUN 17 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 10308-1 - Tetramethrin (Neopynamin) - Combined Chronic Toxicity and Oncogenicity Study in Mice

Caswell No.: 844
Project No.: 7-0962
Record No.: 200981
Accession No.: 402763-01
(2 Vols.)

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THRU: Edwin Budd, Section Head
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Requested Action

Review combined chronic toxicity and oncogenicity study in mice.

Conclusions and Recommendations

1. The Dynamac review covers all information in Accession Nos. 262778, -88 (the original study report, 11 volumes), Accession No. 402763-01 (revised pathology report, 2 volumes), and Accession Nos. 402804-00, -01 (historical control data).

2. The chronic toxicity/oncogenicity mouse feeding study with tetramethin is acceptable as Core-Guideline data. The oncogenic potential in the mouse study is negative. The systemic NOEL is 12 ppm. At the LEL of 60 ppm there were decreased absolute and relative weights of the thyroid and pituitary in male mice.

3. Based on the results (DER of April 11, 1983 by J. Doherty) of the 13-week pilot study in mice, the highest dose level in the chronic study, which was 1500 ppm, is considered the MTD.

   In the pilot study, there were decreased relative weights in the thyroid, adrenal, and pituitary in both sexes at 1500 and 5000 ppm. In the chronic study, the absolute and relative weight of the thyroid and pituitary in male mice was also significantly decreased at 60, 300, and 1500 ppm. In both the pilot study and the chronic study, there were no histopathological lesions associated with the organ weight decreases. Additionally, in the pilot study, there were body weight decreases in males (-9%) and females (-7.5%) associated with higher food consumption at the high dose of 5000 ppm. These body weight decreases and organ weight decreases are clearly indicators of significant toxicity in the pilot study and support an MTD of 1500 ppm in the chronic study.

   The actual intake of tetramethrin for the 1500 ppm male and female mice in the chronic study as determined by actual measurements of food consumption, body weight, etc., was 289 and 392 mg/kg/day, respectively (see page 10 in the Dynamac review).

Tetramethrin can be regarded as having adverse effects on the endocrine organs in mice.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-02-4225
DYNAMAC No. 330-A
January 5, 1988

006755

DATA EVALUATION RECORD

TETRAMETHRIN (NEO-PYNAMIN)

Chronic Toxicity/Oncogenicity Feeding Study--Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
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Signature: [illegible]

Date: 1-5-88

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EPA: 68 02-4225 6755
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DATA EVALUATION RECORD

TETRAMETHRIN (NEO-PYNAMIN)

Chronic Toxicity/Oncogenicity Feeding Study--Mice

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DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Combined chronic feeding/oncogenicity--mice.

ACCESSION NUMBERS: 262778-262788 and 402763-01.

TEST MATERIAL: Neo-Pynamin--3,4,5,6-tetrahydrophthallimidomethyl -1(R)cis, trans-chrysanthymate.

SYNONYMS: Tetramethrin.

STUDY NUMBER(S): 343-136.

SPONSOR: Sumitomo Chemical Company Ltd., Osaka, Japan.

TESTING FACILITY: Hazleton Laboratories America Inc., Vienna, VA.

TITLE OF REPORT: Combined Chronic Toxicity and Oncogenicity Study.

AUTHOR(S): Cox, R. H., Dudeck, L. E., Alsaker, R. D., et al.

REPORT ISSUED: April 17, 1986; Amendment May 29, 1987.

CONCLUSIONS:

Under the conditions of the study, there was no clear evidence of an oncogenic response when Neo-Pynamin was fed to $B6C3F_1$ mice at dietary levels of 12, 60, 300, and 1500 ppm for 2 years. There was a significant increase ($p \leq 0.05$) in the incidence of hemangiosarcoma of the spleen in mid-dose males (300 ppm), but when hemangiosarcomas at all sites were analyzed there was no significant increase. Adenomas of the Harderian gland were significantly increased in males receiving 1500 ppm when compared to controls but there was no dose-related trend. There were no important effects of dosing on body weights, food consumption, or clinical laboratory findings. The absolute and relative weights of the thyroid and pituitary (at termination) were significantly ($p \leq 0.05$) decreased in males receiving 60, 300, and 1500 ppm Neo-Pynamin when compared to controls, but there were no correlating histologic changes in the endocrine organs. Based on these organ weight changes the LOEL for systemic toxicity is 60 ppm and the NOEL is 12 ppm Neo-Pynamin.

Classification: Core Guideline.

A. MATERIALS:

1. Test Compound: Neo-Pynamin; description: light yellow chunks, batch No. 00811; purity: 93.3%.

2. Test Animals: Species: mice; strain: $B6C3F_1$; age: 7 weeks; weight: males--14.3-26.4 g, females--12.9-22.2 g; source: Harlan Sprague Dawley, Indianapolis, IN.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test groups (after elimination of ones with extreme body weight values):

Test Group	Dose in diet (ppm)	Main Study (24 months) Males	Main Study (24 months) Females	Satellite Groups[a] A Males	Satellite Groups[a] B Males	Satellite Groups[a] A Females	Satellite Groups[a] B Females
1 Control	0	50	50	20	20	20	20
2 Low (LDT)	12	50	50	20	20	20	20
3 Mid (MDT)	60	50	50	20	20	20	20
4 Mid (MDT)	300	50	50	20	20	20	20
5 High (HDT)	1500	50	50	20	20	20	20

[a]Ten/sex/group A at 12 and 24 months for blood; tissues saved for histopathology. Ten/sex/group B at 6 and 18 months for blood; animals were then discarded.

Rationale for Dose Selection: Selection of the dose levels was based on a 13-week feeding study at levels of 500, 1500, and 5000 ppm. There was a 4 to 13 percent decrease in body weight gain in males and females receiving 1500 ppm and decreased mean weight of thyroid, adrenals, pituitary, and ovaries. These organ weight changes occurred in both sexes in a dose-related manner. Mean liver weights were elevated 21% in high-dose males and 14% in high-dose females.

2. Diet Preparation: The concentration of the test compound was adjusted to 100 percent active ingredient for calculation of dietary levels. Diet was prepared weekly. Storage conditions were not specified. Samples of treated food were analyzed for concentration at week 1 and every 4 weeks thereafter. Stability and homogeneity were determined prior to study initiation.

Results: It was reported that Neo-Pynamin/diet mixtures were stable for at least 7 days at room temperature. Samples were found to be homogeneous. Table 1 shows the mean values for dietary concentrations and the percent of target for each dietary level over the 104 weeks of the study. There was some indication of large variations in the 12- and 60-ppm diets at weeks 60 and 76; the samples were, therefore, reanalyzed. The reanalysis indicated nonhomogeneity within the diets. Thereafter, diets were prepared with test material that was ground more thoroughly and sieved to provide uniform particle size. The accuracy of the samples was improved.

TABLE 1. Analyzed Mean Dietary Levels of Neo-Pynamin Fed to Mice (Weeks 1-104)[a]

	Nominal Dietary Levels (ppm)			
	12	60	300	1500
Mean (ppm) ± S.D.	11.3±1.4	58.9±6.8	297±15.9	1504±79.9
CV[b]	12.0	11.5	5.4	5.3
Percent of target	94.0	98.0	99.0	100.3

[a]Test material was not detected in the control diet; the detection limit was 0.2 ppm.

[b]CV = coefficient of variation in percent; calculated by reviewers.

3. Food and Water Consumption: Animals received food (Purina Rodent Laboratory Chow No. 5001) and water ad libitum.

4. Statistics: Homogeneity of variances was analyzed by Levene's test and heterogeneous data transformed using the following (in order): $\log_{10}$, square, square root, reciprocal, angular, or rank transformation. Homogeneous data (untransformed or transformed) were analyzed by analysis of variance (ANOVA). Group comparisons were then performed with Dunnett's test or a modified Tukey-Kramer multiple comparison (equal or unequal variances). Cumulative survival data were analyzed with the computer program of Thomas, Breslow, and Gart (1977).

5. A quality assurance statement was signed and dated April 11, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of toxicity and mortality and detailed observations were recorded weekly.

   Results: There were no significant dose-related trends in survival; mortality was significantly lower in males receiving 300 ppm than in controls. At study termination, survival ranged from 80 to 92 percent in male groups and 80 to 88 percent in female groups (Table 2).

   There were no compound-related signs of toxicity. Females receiving 1500 ppm appeared thin from weeks 101 to 103 weeks. Commonly observed signs such as hair loss, urine stains, and rough coats were noted in all groups. The incidence of tissue masses was normal for the strain and age of mice and was similar in control and dosed groups.

2. Body Weight: Mice were weighed weekly for 14 weeks and every other week thereafter.

   Results: Table 3 presents body weight data at selected intervals. Mean body weights were similar in dosed and control groups.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated. Food and water consumption were recorded weekly for the first 14 weeks and every other week thereafter.

   Results: It was reported that there was a significant decrease in food consumption for females receiving 12 ppm during the first year of the study but that overall food consumption for 104 weeks was similar in dosed and control groups. Mean food consumption for females receiving 12 ppm was 46.4 g/mouse/week through week 52, compared to 48.1 g/week/mouse for control females. Over 104 weeks, mean weekly food consumption values were 44.7 g/mouse/week in the group receiving 12 ppm, compared to 46.2 g/mouse/week

TABLE 2. Cumulative Mortality and Percent Survival[a] in Mice Fed Neo-Pynamin for 2 Years

Dose Group (ppm)	Mortality (Percent Survival) at Selected Weeks				
	13	26	52	78	105
			Males		
0	1 (98)	0 (98)	5 (92)	0 (92)	14 (77)
12	0(100)	0(100)	0(100)	1 (98)	13 (78)
60	3 (95)	4 (93)	0 (93)	9 (85)	14 (77)
300	0(100)	0(100)	0(100)	1 (98)	6 (90)
1500	2 (97)	0 (97)	3 (95)	5 (92)	11 (82)
			Females		
0	2 (97)	3 (95)	4 (93)	6 (90)	17 (72)
12	1 (98)	2 (97)	5 (92)	7 (88)	11 (82)
60	2 (97)	3 (95)	6 (90)	8 (87)	16 (73)
300	2 (97)	2 (97)	4 (93)	8 (87)	12 (80)
1500	2 (97)	4 (93)	0 (93)	6 (90)	17 (72)

[a]Survival was based on 60 mice/sex/group; calculated by reviewers.

TABLE 3. Mean Body Weights (g) of Mice Fed Neo-Pynamin for 2 Years

Weeks	Dietary Level (ppm)				
	0	12	60	300	1500
			Males		
0	21.6	21.8	21.9	21.4	21.3
14	27.9	27.9	27.8	27.1	26.9
26	29.9	29.9	30.1	29.3	29.4
52	34.3	33.9	34.6	33.3	33.0
104	33.2	33.4	32.8	32.0	32.9
			Females		
0	18.0	17.6	17.6	17.6	17.8
14	25.1	24.8	24.4	24.6	24.3
26	27.2	26.5	26.2	27.0	26.4
52	31.5	30.7	30.9	31.4	30.0
104	30.6	31.0	29.8	30.2	29.3

for controls; these latter values did not differ significantly. Mean compound intake is presented in Table 4.

4. Ophthalmology: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood was collected by orbital sinus puncture before treatment and at 27, 53, 79, and 105 weeks for hematology and from the abdominal aorta or orbital sinus for clinical chemistry analysis from satellite groups of 10 animals (10/sex/group/interval). Bone marrow smears were obtained at the same intervals. The CHECKED (X) parameters were examined.

a. Hematology

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†

X Total plasma protein (TP)
X Leukocyte differential count
Mean corpuscular HGB (MCH)
Mean corpuscular HGB concentration (MCHC)
Mean corpuscular volume (MCV)
X Reticulocyte count
X Erythrocyte morphology
X Myeloid/erythrocyte cell ratio
X Heinz bodies

Results: There were no changes in hematology parameters that were consistent between intervals or between sexes nor were there any changes that were clearly dose related. At week 27, leukocyte counts were increased ($p < 0.05$) in males receiving 300 ppm and females receiving 300 or 1500 ppm when compared to controls and platelet counts were slightly increased ($p < 0.05$) in males receiving 300 or 1500 ppm. At week 79, reticulocyte counts were increased (1.1 percent) in males receiving 1500 ppm. No other significant changes or trends were apparent.

b. Clinical Chemistry

Electrolytes
X Calcium†
X Chloride†
Magnesium†
Phosphorus†
X Potassium†
Sodium†

Enzymes
X Alkaline phosphatase (ALP)
Cholinesterase
Creatinine phosphokinase†
X Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)†
X Serum aspartate aminotransferase (also SGOT)†

Other
X Albumin†
Blood creatinine†
X Blood urea nitrogen†
X Cholesterol†
X Globulins
X Glucose†
X Total bilirubin†
X Total protein†
Triglycerides

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 4. Mean Compound Intake in Mice Fed Neo-Pynamin for 2 Years

	Dietary Level (ppm)				
	0	12	60	300	1500
Males	0.0	2.27±0.54	11.90±4.47	57.82±14.76	289.4±73.3
Females	0.0	2.64±0.64	13.81±3.47	68.20±15.87	391.9±78.5

[a]Calculated by our reviewers based on data in CBI Table 5, pp. 110-120.

Results: No compound-related effects on clinical chemistry parameters were seen. There were several values in dosed groups that differed significantly ($p < 0.05$) from the appropriate control; however, the changes were not consistent between intervals or dose related and both increases and decreases were seen.

6. Urinalysis: Urine was collected from fasted animals at 0, 6, 12, 18, and 24 months. The CHECKED (X) parameters were examined.

	Appearance†	X	Glucose†
	Volume†	X	Ketones†
X	Specific gravity†		Bilirubin†
X	pH	X	Blood†
	Sediment (microscopic)†		Nitrate
X	Protein†		Urobilinogen

Results: Urinalysis values were similar in dosed and control groups.

7. Sacrifice and Pathology: All animals that died and that were sacrificed at 12 or 24 months were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were, in addition, weighed.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta†	XX	Brain† (fore-, mid-, and hind-)
X	Salivary glands†	XX	Heart†	X	Peripheral nerve†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (two levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†,a
X	Duodenum†	XX	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	X	Thymus†		Glandular
X	Ileum†		Urogenital	XX	Adrenals†,a
X	Cecum†	XX	Kidneys†		Lacrimal gland
X	Colon†		Urinary bladder†	X	Mammary gland†
	Rectum†	XX	Testes†	XX	Parathyroids†,a
XX	Liver†	X	Epididymides	XX	Thyroids†,a
X	Gall bladder†	X	Prostate		Other
X	Pancreas†	X	Seminal vesicle	X	Bone†
	Respiratory	X	Ovaries[a]	X	Skeletal muscle†
X	Trachea†	X	Uterus†	X	Skin
X	Lung			X	All gross lesions and masses
				X	Head, three coronal sections

†Recommended by Subdivision F (October 1982) Guidelines.

[a]Weighed postfixation.

Results:

a. Organ Weight: At the 12-month sacrifice, the liver-to-body weight ratio in males receiving 1500 ppm Neo-Pynamin (4.65±0.34%) was slightly but significantly greater than controls (4.16±0.30%), but the absolute mean weights did not differ from controls and mean body weight at sacrifice was about 10% lower than controls. The absolute and relative adrenal weights were increased in both sexes receiving 12 ppm when compared to controls, but there was no effect at the three higher doses. Ovary weight in females receiving 1500 ppm was decreased when compared to controls, but this was not considered related to dosing and the decrease was not statistically significant.

At terminal sacrifice, the mean weights of the thyroid and pituitary were significantly ($p \leq 0.05$) lower than controls in males receiving 60, 300, and 1500 ppm; the weights relative to body weight were also reported to be significantly decreased in the same groups of males. There was a significant ($p \leq 0.05$) increase in adrenal weights in males receiving 12 ppm Neo-Pynamin but there was no dose-related trend. The absolute and relative adrenal weights in females receiving 1500 ppm were 13 percent lower than in controls ($p \leq 0.05$). No histologic findings were noted in any of these endocrine organs. Table 5 summarizes data on mean weights of thyroid, adrenal, and pituitary at terminal sacrifice. The authors also reported that there was a compound-related decrease in spleen weights in males receiving 300 and 1500 ppm Neo-Pynamin (see Section E, Reviewers' Discussion and Interpretation of Results).

b. Gross Pathology: No increases in the incidence of any gross findings were observed in dosed mice. The findings were those normally found in mice of this strain and age.

c. Microscopic Pathology:

1. Nonneoplastic: There were no compound-related nonneoplastic histologic findings at either the interim sacrifice or in animals that died or were sacrificed at termination. All findings were considered incidental and were common to mice of this strain and age. Table 6 summarizes the incidence of the more frequently occurring lesions.

2. Neoplastic: Neoplastic lesions are summarized in Table 7. The study authors concluded that there was no evidence of increased neoplasia in dosed mice. They noted the "substantial" number of splenic hemangiosarcomas in males receiving 300 ppm and suggested that this was not compound related since there was an absence of a dose-related trend and an absence of hemangiosarcomas in the spleen of high-dose males. No comment was made on

TABLE 5. Selected Organ Weights (g ± S.D.) at Week 105 in Mice Fed Neo-Pynamin[a]

Dietary Level (ppm)	Thyroid		Adrenal		Pituitary	
	Absolute (mg)	Relative (% x 10^2)	Absolute (mg)	Relative (% x 10^2)	Absolute (mg)	Relative (% x 10^2)
			Males			
0	8.0±2.8	2.7±1.0	6.1±1.6	2.1±0.7	2.0±0.8	0.68±0.27
12	8.3±3.8	2.9±1.5	8.3±2.3*	2.7±0.9*	2.3±0.9*	0.81±0.32*
60	5.2±1.1*	1.8±0.4*	6.7±1.7[b]	2.3±0.7[b]	1.7±0.7*	0.57±0.23*
300	4.9±2.3*	1.7±0.9*	6.0±1.2	2.1±0.5	1.4±0.5*	0.51±0.20*
1500	5.6±1.8*	2.0±0.7*	5.7±1.2	2.0±0.5	1.4±0.5*	0.51±0.18*
			Females			
0	6.2±1.8	2.3±0.66	8.3±2.0	3.1±0.71	2.4±0.8	0.90±0.30
12	6.3±2.1	2.3±0.76	8.6±1.8	3.2±0.73	2.7±1.8	0.99±0.71
60	6.3±2.0	2.4±0.78	8.4±2.0	3.1±0.72	2.5±0.8	0.93±0.31
300	7.7±1.4	2.9±4.2	7.6±1.9	2.8±0.74	2.0±0.7[b]	0.74±0.24[b]
1500	6.8±1.8	2.6±0.7	7.2±1.4*	2.7±0.55*	2.3±0.8	0.88±0.35

[a]These values were recalculated by our reviewers using the individual animal data in the CBI Report Amendment of May 29, 1987.

[b]Found to differ significantly from control by the report authors.

*Significantly different from controls by analysis of variance followed by Duncan's multiple range test ($p < 0.05$); analysis by reviewers.

TABLE 6. Selected Nonneoplastic Lesions in Mice Fed Neo-Pynamin for 2 Years[a]

Organ/Lesion	Males					Females				
	Dietary Level (ppm)					Dietary Level (ppm)				
	0	12	60	300	1500	0	12	60	300	1500
Lung	(70)[b]	(70)	(70)	(70)	(69)	(70)	(70)	(70)	(70)	(70)
Squamous metaplasia	6	9	2	5	13	3	5	6	6	4
Epithelial hyperplasia, focal	9	8	7	13	17	6	7	6	6	5
Harderian gland	(69)	(69)	(69)	(68)	(69)	(67)	(68)	(69)	(69)	(70)
Inflammation	3	0	3	7	4	1	0	0	0	0
Necrosis	5	2	4	2	3	1	6	4	5	8
Adrenal cortex	(68)	(69)	(69)	(70)	(69)	(67)	(68)	(69)	(69)	(70)
Hyperplasia, focal	8	2	5	8	9	0	0	0	0	0
Degeneration	6	1	0	6	2	2	3	2	4	2
Testes	(68)	(70)	(70)	(70)	(69)					
Degeneration	1	5	5	7	3					
Ovaries						(67)	(68)	(68)	(66)	(66)
Ovarian cysts						9	10	11	8	7

[a]Includes animals sacrificed at 1 year and at termination and those found dead or sacrificed moribund.

[b]The numbers of tissues examined are in parentheses.

TABLE 7. Neoplastic Lesions in Mice Fed Neo-Pynamin for 2 Years[a]

Organ/Neoplasm	Males Dose Level (ppm) 0	12	60	300	1500	Females Dose Level (ppm) 0	12	60	300	1500
Liver	(69)[b]	(69)	(69)	(69)	(69)	(70)	(70)	(69)	(70)	(70)
Hepatocellular carcinoma	15	10	11	9	11	0	5	1	3	3
Hepatocellular adenoma	14	15	18	19	17	6	8	7	4	12
Adenoma/carcinoma	28	24	27	26	26	6	13	8	7	14
Hemangiosarcoma	4	5	4	2	0	1	1	0	0	0
Lung	(70)	(70)	(70)	(70)	(69)	(70)	(70)	(70)	(70)	(70)
Alveolar/bronchiolar adenoma	10	6	8	6	9	5	2	1	4	5
Alveolor/bronchiolar carcinoma	0	3	1	1	4	1	0	0	0	0
Spleen	(68)	(67)	(68)	(70)	(68)	(70)	(68)	(67)	(70)	(70)
Hemangiosarcoma	0	1	1	7	0	1	1	1	2	0
Hemangioma	0	0	1	0	0	0	0	0	0	[illegible]
Pituitary	(61)	(51)	(65)	(65)	(66)	(64)	(57)	(63)	(56)	(56)
Adenoma	0	0	0	0	0	0	3	1	2	0
Thyroid	(68)	(67)	(69)	(70)	(69)	(69)	(68)	(67)	(69)	(69)
Follicular cell adenoma	0	1	2	2	0	3	3	1	3	[illegible]
Mammary gland	(35)	(11)	(27)	(35)	(24)	(64)	(67)	(67)	(70)	(69)
Adenocarcinoma	0	0	0	0	0	2	0	0	1	3
Uterus						(69)	(69)	(68)	(70)	[illegible]
Adenocarcinoma						2	0	0	0	[illegible]
Endometrial stromal polyp						0	2	3	2	0
Harderian gland	(68)	(69)	(69)	(68)	(69)	(67)	(68)	(69)	(69)	[illegible]
Adenoma	1	5	5	3	7	2	5	4	4	2
Multiple organs	(70)	(70)	(68)	(70)	(70)	(70)	(70)	(70)	(70)	[illegible]
Lymphoma, lymphoblastic	7	2	3	5	5	13	6	14	3	[illegible]
mixed cell	0	2	1	1	1	1	2	3	2	[illegible]
stem cell	0	2	4	1	2	1	8	5	4	3
histiocytic	0	0	0	0	0	2	1	0	0	[illegible]
lymphocytic	0	0	2	4	0	5	2	0	1	3
not specified[c]	0	0	0	0	0	2	1	1	0	[illegible]
Total lymphomas	7	6	10	11	8	24	20	23	10	[illegible]
Leukemia, mononuclear	0	1	0	0	0	0	3	1	0	0

(continued)

TABLE 7. Neoplastic Lesions in Mice Fed Neo-Pynamin for 2 Years[a] (concluded)

Organ/Neoplasm	Males					Females				
	Dose Level (ppm)					Dose Level (ppm)				
	0	12	60	300	1500	0	12	60	300	1500
All sites										
Hemangioma	1	1	1	2	0	0	3	1	3	1
Hemangiosarcoma	4	6	5	9	0	2	3	2	3	1

[a]Includes animals that died or were sacrificed moribund as well as those sacrificed at 12 months and at termination. If incidence was less than 3% in all groups, the neoplasm was not tabulated.

[b]Number of tissues examined.

[c]Cell type not specified because of autolytic changes.

[d]Spleen, liver, subcutaneous, peritoneal, muscle, bladder, ovaries, and uterus (primary tumors only).

the incidence (7/69) of adenomas of the Harderian gland in males receiving 1500 ppm (see the discussion in Section E).

D. STUDY AUTHORS' CONCLUSIONS:

There were no effects of dosing on survival. Clinical signs were those commonly observed for mice. Mean body weights and growth rates were comparable among groups and there was no effect of dosing on food and water consumption. There were no effects in hematology or clinical chemistry parameters related to dosing and results of urinalyses were not remarkable. Gross findings were those commonly observed for mice and none were considered compound related. Decreased mean organ weights and organ-to-body weight ratios were found for pituitary and thyroid/parathyroid of males receiving 60, 300, or 1500 ppm but there were no corresponding histomorphologic changes. Spleen weights were decreased for the 300- and 1500-ppm groups of males. No evidence of increased neoplasia was detected at levels of 12, 60, 300, or 1500 ppm. There were no nonneoplastic lesions related to dosing. The NOEL was considered to be 12 ppm Neo-Pynamin.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

Statistical analysis (by our reviewers) of the incidence of adenoma of the Harderian gland of males (but not females) receiving 1500 ppm indicated a significant increase when compared to controls (Fisher's exact test, $p < 0.05$). The laboratory historical incidence of adenoma of the Harderian gland based on two studies (120 males, 119 females) was 7.2% (range 0-16%) and 4.6% (range 0-10%) in untreated males and females, respectively. In the NTP data base the range for males (36 studies) was 0-12% and the mean 2.7%. Since the incidence of Harderian adenomas in high-dose males in the tetramethrin study (10%) is within historical range, the increase over concurrent control is not considered of biological importance.

The incidence of hemangiomas of the spleen in males (but not females) receiving 300 ppm Neo-Pynamin was also found to be significantly increased ($p \leq 0.05$) when compared to controls. It was noted from review of individual animal data that all the hemangiosarcomas in the spleens of males were in animals that survived to final sacrifice.

Nonmetastatic hemangiosarcomas at all sites (liver, spleen, muscle, subcutaneous, and peritoneum) were 4/69 in control males and 5/69, 6/69, 9/69, and 0/69 in males receiving 12, 60, 300, and 1500 ppm Neo-Pynamin (Table 7). It is appropriate to combine these circulatory system tumors. The increase is not significant in 300-ppm males when hemangiosarcomas at all sites are combined. The NTP historical incidence for hemangiosarcomas of the circulatory system is 2.7% in 2343 male $B6C3F_1$ controls (range 0-12%);[2] the concurrent control incidence is 5.8%.

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[1] Haseman, T. K., Huff, J., and Boorman, G. A. 1984. Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12: 126-135.

[2] Ibid.

The incidence of liver, lung, and hematopoietic tumors found in all groups of males or females are within the normal historical range found for $B6C3F_1$ mice. It is our assessment that there is no clear oncogenic response in this study.

It is possible that the mice could have tolerated a higher dose since there was no effect on survival, body weights, hematology, clinical chemistry, or urinalysis. However, the rationale for choice of the high dose (as reported) appears to be adequate--a decreased body weight gain at 1500 ppm in a 13-week study as well as decreased weight of thyroid, adrenals, and ovaries.

The LOEL (60 ppm) and NOEL (12 ppm) for the study were based on decreased pituitary and thyroid weights. Although the weights of these endocrine organs were reported to be significantly decreased (Table 5), there were no gross or histopathologic findings. It was noted that these organs were weighed after fixation (as per protocol).

The absolute and relative organ weights for thyroid, adrenals, and pituitary were reanalyzed statistically by our reviewers using ANOVA followed by Duncan's multiple-range test (Table 5). This analysis essentially supported the results of the study authors who used different methods. The study authors used reciprocal transformation of data for thyroids, pituitary, and adrenals of males. In agreement with the authors, there was a significant decrease in the absolute and relative weights of thyroid and pituitary of males receiving 60, 300, and 1500 ppm and of adrenals of females receiving 1500 ppm Neo-Pynamin. The significant increase in adrenal weight in both sexes receiving the lowest dose (12 ppm) at 12 months and in males receiving 12 ppm at 24 months cannot be assessed to be of any biological importance.

The study authors noted that spleen weights were decreased in males receiving 300 and 1500 ppm and stated that there were no corresponding "histomorphologic" changes. Examination of the mean data showed that the standard deviations were extremely high in some of the groups (Table 8). Examination of the individual organ weights for spleen indicated several values between 1 and 3 g for spleen weights, compared to an expected value of less than 0.1 g. The excessive spleen weights correlated with malignant lymphoma. Control male No. 11 with a spleen weight of 1.20 g had malignant lymphoma and male No. 224 receiving 12 ppm with and a spleen weight of 1.99 g had leukemia. The abnormally high values in all groups of females also correlated with malignant lymphoma; for example, in the group of females receiving 300 ppm, spleen weights of 3.41, 1.14, 1.28, and 1.37 g were seen in four mice (Nos. 657, 664, 682, and 685) that had malignant lymphoma. It is our assessment, therefore, that the decreased spleen weight in males receiving 300 and 1500 ppm were not compound related. The incidence of malignant lymphoma in the spleen of control males at terminal sacrifice was 4/47, compared to 2/49 in the 300-ppm group and 1/49 in the 1500-ppm group.

TABLE 8. Meam Spleen Weights in Mice Fed Neo-Pynamin for 2 Years

Dietary Level (ppm)	Weight at Study Termination (g ± S.D.)	
	Males	Females
0	0.10±0.17	0.28±0.48
12	0.13±0.28	0.17±0.11
60	0.09±0.10	0.16±0.14
300	0.08±0.04	0.30±0.55
1500	0.07±0.04	0.22±0.25

Based on a decrease in absolute and relative weights of thyroid and pituitary in males receiving 60, 300, or 1500 ppm Neo-Pynamin, we assess that for systemic toxicity the NOEL is 12 ppm and the LOEL is 60 ppm.