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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Tetramethrin (Neopynamin) - EPA Registration No. 10308-01 - Two-Generation Rat Reproduction Study with Neopynamin Forte and UDS Mutagenicity Assay with Neopynamin

Caswell No.: 844
Project No.: 9-1400
Record No.: 244,785
MRID Nos.: 407778-01;
407784-01

FROM: William Dykstra, Reviewer *William Dykstra 6/23/89*
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
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THRU: Robert Zendzian, Acting Section Head
Review Section I
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Requested Action

Review rat reproduction study with Neopynamin Forte and UDS mutagenicity assay with Neopynamin.

Conclusion and Recommendation

1. The two-generation rat reproduction study with Neopynamin Forte is acceptable as Core-Minimum data and fulfills the data requirement for a rat reproduction study with Neopynamin.
2. The UDS mutagenicity assay is acceptable.

Reviewed By: William Dykstra *William Dykstra 6/17/86*
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Robert Zendzian, *7/1/86*
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DATA EVALUATION REPORT

Study Type: 83-4 - Reproduction, Rat TOX Chem No.: 844
Accession No.: N/A MRID No.: 407778-01
Test Material: Neopynamin Forte
Synonyms: N/A
Study Number(s): HLA 343-~~174~~¹⁴⁷
Sponsor: Sumitomo Chemical Company, Ltd.
Testing Facility: Hazleton Labs, Vienna, VA
Title of Report: Two-Generation Reproduction Study in Rats with
Neopynamin Forte. *IT-61-0201.*
Authors: D.H. Pence, et al.
Report Issued: June 17, 1986

Conclusions:

The NOEL is 500 ppm, the mid-dose. At the LEL of 3000ppm, the high-dose, there were decreased body weights of males and females during the F₀ and F₁ growth phases, decreased food consumption of the F₀ females, decreased body weights of females during gestation and lactation of the F₀ and F₁ generation, decreased body weight of males and females during the 30-day postweaning period of the F₁ generation, decreased pup body weight in F₁ and F₂ litters, and increased incidence of bile duct hyperplasia of the liver in females of the F₁ parental animals.

Classification: Core-Minimum
Special Review Criteria (40 CFR 154.7): N/A

Review:

Two-Generation Reproduction Study in Rats with Neopynamin Forte (IT-61-0201) (Hazleton Project No. HLA 343-174; June 17, 1986). Quality Assurance was performed and the report was signed by the QA officer.

A. Materials:

1. Test Material - Neopynamin Forte, Lot No. 00402, purity 93.4%, a viscous brown liquid.
2. Test Animals - Species: Rat; Strain: Sprague-Dawley; Age: 4 weeks; Weight: Males 156 to 214 g, females 126 to 164 g; Source: Charles River, Kingston, NY.

B. Study Design:

1. Randomized groups of 4-week-old male and female Sprague-Dawley albino rats were used in the study as the F₀ parental animals. The rats were assigned to the following groups:

Group	No. of Animals		Dietary Level ppm
	Male	Female	
1 (Control)	13	26	0
2 (Low)	13	26	100
3 (Mid)	13	26	500
4 (High)	13	26	3000

Upon completion of weaning of the F₁ litters, 15 F₁ males and 30 F₁ females from each dietary group were randomly assigned to their respective groups to constitute the second generation parental animals.

2. Diet Preparation - Diet was prepared once each week and stored at room temperature. Samples of treated food were analyzed for stability and concentration at weeks 1, 2, 3, and 4 and once every 4 weeks thereafter.

Results - Results of diet analyses showed that the test material was stable for 7 days and was homogeneously distributed in the diet. Routine concentration analyses performed at specified intervals ranged from 32.8 to 116.4 percent of selected levels.

3. Animals received food (Purina Rodent Laboratory Chow®) and water ad libitum.
4. Statistics - Statistical evaluations of the data were performed and were considered significant at $p < 0.05$.

C. Methods and Results:

1. Observations - All animals were observed daily for toxic signs and mortality.

Results - Seven parental animals were found dead or sacrificed in extremis. There were one mid-dose male and one high-dose female F₀, and one control female, two low-dose male, and two high-dose female F₁ rats. Gross necropsy findings of these animals did not reveal any compound-related effects and the deaths were not attributed to treatment. Clinical observations which were observed more frequently in compound-treated groups in comparison to controls for parental F₀ and F₁ animals were alopecia, urine-stained fur, thinness, hunched appearance, rough hair coat, and rhinorrhea. These findings were not strictly dose-related and the toxicological significance, therefore, is uncertain.

2. Body Weight - Body weight was measured weekly for the F₀ and F₁ parental rats.

Results - Mean body weights of F₀ male and female animals of the mid- and high-dose groups were about 3 to 8 percent decreased during the growth period. At growth week 15, high-dose female F₀ body weight was 8 percent less than controls and was statistically significantly decreased.

In the F₁ growth period, mean body weight of the high-dose males and females was 7 to 10 percent decreased during growth. At week 18 of the F₁ growth period, body weight of high-dose females was 10 percent less than controls and was statistically significantly decreased.

Mean body weights of high-dose females during the F₀ and F₁ gestation periods were significantly decreased at days 0, 7, 14, and 20. Similarly, mean body weights of the high-dose females during the F₀ and F₁ lactation periods were significantly decreased at days 1, 4, 7, 14, and 21.

Mean body weights of mid- and high-dose males of the F₀ generation and high-dose males of the F₁ generation were decreased (4 to 6%) during the postmating phases in comparison to controls. The mean body weight of the low-dose males was significantly increased at week 25 in the F₁ postmating phase.

During the 30-day postweaning F₁ period, mean body weights of high-dose males and females were decreased (6% for males and 11% for females) in comparison to controls. Low-dose males during the F₁ postweaning period continued to exceed controls in body weight.

3. Food Consumption - Food consumption was determined weekly.

Results - Food consumption was decreased by 7 percent for high-dose females during weeks 6 to 15 of the F₀ growth phase. Food consumption of F₀ males and F₁ males and females was comparable between control and treated groups.

4. Evaluation of Mating and Reproductive Indices - Analysis of the mating and reproductive indices were performed for each generation.

Results - Gestation length in days was between 22.1 and 22.3 for the F₀ females and 22.3 and 22.4 for the F₁ females. There was no compound-related effect on gestation length.

With respect to reproduction indices and offspring survival data, there were no compound-related effects in the F₁ or the F₂ generations. There were no compound-related effects in female fertility rate, male fertility rate, or gestation index. Offspring survival indices were unaffected by treatment in the F₁ and F₂ litters.

In the F₁ litters, mean offspring body weight at the high-dose was significantly decreased in male and female pups at days 14 and 21 of weaning. These data are shown below.

<u>F₀ Generation (F₁ Litters)</u>					
<u>Mean Pup Body Weight</u> <u>(grams)</u>	<u>Group</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
	<u>Dose</u> <u>(ppm)</u>	<u>0</u>	<u>100</u>	<u>500</u>	<u>3000</u>
Males at Day 14		24.2	25.1	25.2	21.7*
Females at Day 14		22.9	24.3	23.6	20.6*
Males at Day 21		36.5	37.0	33.2	32.0*
Females at Day 21		34.8	36.6	35.9	30.3*

* p < 0.05

Similarly, in the F₂ litters, mean offspring body weight at the high-dose was significantly decreased in

males at day 7, males and females at day 14, and males at day 21. These data are shown below:

F₁ Generation (F₂ Litters)

Mean Pup Body Weight (grams)	Group Dose (ppm)	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
		0	100	500	3000
Males at Day 7		13.1	12.5	13.3	11.0*
Females at Day 7		12.3	11.9	12.5	10.7
Males at Day 14		25.7	25.1	26.7	21.0*
Females at Day 14		24.6	24.2	25.4	20.8*
Males at Day 21		39.4	38.3	41.5	32.3*
Females at Day 21		37.5	36.9	39.2	32.5

*p < 0.05

5. Sacrifice and Pathology - After the last F₁ litter was weaned, all surviving F₀ males and females were sacrificed, necropsied, and discarded. Gross observations were recorded.

After the last F₂ litter was weaned, F₁ animals continued to receive the appropriate diets for 30 additional days when 10 males and 25 females per group were randomly selected for gross and histopathological evaluation. All remaining F₁ animals were sacrificed, necropsied, and discarded. Gross observations were recorded.

In addition, five weanlings/sex/group from the F₁ and F₂ generations were selected randomly for gross necropsy and histopathologic evaluation. All remaining pups were sacrificed, necropsied, and discarded. Gross observations were recorded.

The following tissues from each animal selected for histopathological evaluation were preserved in 10% neutral buffered formalin, embedded in Paraplast, sectioned, stained with hematoxylin and eosin, and examined microscopically:

Brain	Duodenum, jejunum, ileum
Pituitary	Colon, cecum
Thoracic spinal cord	Mesenteric lymph node
Lumbar spinal cord	Urinary bladder
Eyes	Testes with epididymides

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Mandibular salivary glands	Prostate
Thyroid	Ovaries
Trachea	Uterus
Thymus	Femur
Esophagus	Femoral bone marrow smear
Heart	Lung ^a
Spleen	Liver ^b
Adrenals	Kidneys
Pancreas	Stomach
	Lesions

^aTwo sections examined microscopically.

^bTwo lobes examined microscopically.

Results - Gross pathology findings of F₀ males and females that were sacrificed after weaning of the F₁ offspring and F₁ parental animals sacrificed after the 30-day feeding period following weaning of the F₂ offspring did not show any compound-related effects.

Histopathological evaluation of the tissues showed a possible compound-related increase in bile duct hyperplasia in the liver of F₁ female high-dose rats, characterized by minimal to slight proliferation of bile duct and ductile cells.

The incidence of this lesion is shown below:

<u>Group</u>	<u>Female F₁ Parental Rats</u>			
	<u>Control</u>	<u>Low</u>	<u>Mid</u>	<u>High</u>
No. examined	25	25	25	25
Liver				
Bile Duct				
Hyperplasia	12	8	10	22

Clinical observation of offspring of the F₁ and F₂ litters showed an increased incidence of small and/or languid pups in high-dose F₂ litters in days 7, 14, and 21 in comparison to controls.

Evaluation of gross and microscopic observations of pups in the F₁ and F₂ litters did not reveal any compound-related lesions.

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DATA EVALUATION REPORT

Study Type: 84-2 - Mutagenicity

TOX Chem No.: 844

Accession No.: N/A

MRID No.: 407784-01

Test Material: Neopynamin

Synonyms: Tetramethrin

Study Number(s): 1280

Sponsor: Sumitomo Chemical Company, Ltd.

Testing Facility: Takarazuka Research Center, Osaka, Japan

Title of Report: In Vitro Unscheduled DNA Synthesis (UDS) Assay
of Neopynamin in Rat Hepatocytes. *IT-80-0213*

Author: S. Kogiso

Report Issued: June 30, 1988

Conclusions:

Hepatocytes were isolated from young male Sprague-Dawley rats and exposed for 20 hours to Neopynamin at six concentrations ranging from 0.2 to 100 $\mu\text{g}/\text{mL}$. The HDT was cytotoxic and formed a precipitate. Neopyramin was negative for mutagenic potential measured as induction of DNA-damage/repair. The positive control, 2-AAF, responded appropriately by inducing significant increases in both mean net grain counts (>10) and in the percentage of cells in repair ($>75\%$).

Classification: Acceptable

Special Review Criteria (40 CFR 154.7): N/A

Review:

In Vitro Unscheduled DNA Synthesis (UDS) Assay of Neopynamin in Rat Hepatocytes (Takarazuka Research Center Study No. 1280, June 30, 1988).

A. Materials:

1. The test compound was Neopynamin, Lot No. 60210, purity 94.0%; dissolved in DMSO.
2. Positive Control - 2-Acetylaminofluorene (2-AAF) dissolved in DMSO.

Animals - Five- and 6-week-old male rats of Sprague-Dawley strain were obtained from Charles River Japan, Inc. The diet (CE-2, Clea Japan, Inc.) and water were provided ad libitum. The rats were acclimatized and quarantined for a week. Seven- and 8-week-old male Sprague-Dawley rats weighing 282 to 328 g were used for the study.

Methods - Rat hepatocytes were isolated from 7- to 8-week-old male Sprague-Dawley rats following standard procedures by in situ perfusion with collagenase.

To determine dose levels of Neopynamin in the UDS assay, a preliminary cytotoxicity test was conducted at concentrations of 3, 10, 30, 100, and 300 $\mu\text{g}/\text{mL}$.

In the UDS assay, the isolated hepatocytes were exposed for 20 hours to Neopynamin at concentrations of 0.2, 1, 5, 25, 50, and 100 $\mu\text{g}/\text{mL}$. The test with the same cell population was conducted in duplicate for each dose and performed twice with different cell populations from different rats.

All slides were coded and analyzed in a blind manner. A net nuclear grain count was calculated by subtracting the highest count in background areas adjacent to the nucleus from a nuclear grain count. Fifty cells were analyzed for each test.

A two-way analysis of variance was used for net grain counts between Neopynamin-treated groups or positive control groups and the vehicle control group. Chi-square was used for the number of cells in repair in 100 cells observed.

Results - In the preliminary cytotoxicity test with Neopynamin, precipitates were observed at 30 $\mu\text{g}/\text{mL}$ and above and cytotoxicity was observed at 100 and 300 $\mu\text{g}/\text{mL}$.

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Therefore, the 100ug/mL dose was selected for the UDS assay as the highest dose. In the UDS assay, the mean net grain counts in the Neopynamin-treated groups ranged from -5.09 to -8.81 in Test I, and from -5.98 to -7.81 in Test II. These results were not different from the solvent controls which were -5.84 and -8.46 in Tests I and II, respectively.

Additionally, there was no significant difference between the vehicle control and Neopynamin-treated cells with respect to the number of cells having more than 5 net grain counts (cells in repair).

The positive control, 2-AAF, responded appropriately by inducing significant increases in both mean net grain counts (more than 10) and percentage of cells in repair (greater than 75%).