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

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MAY 7 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: 627. 004005. Pynamin Forte. Review of Mouse Oncogenicity Study.

Tox. Chem. No. 025B
Project No. 1-2067

TO: Richard King, PM Team # 72
Special Review and Reregistration
Division (H7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 12/12/91
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C)
Roger Gardner 4/24/92
4-20-92

Record No(s). S397636

Background and Request:

Sumitomo Chemical Company has submitted a dietary mouse oncogenicity study on Pynamin Forte in response to the Registration Standard on Allethrins. The Toxicology Branch (TB-I) has been asked to review the submitted study.

Toxicology Branch Response:

TB-I has reviewed the mouse oncogenicity study on Pynamin Forte. It is classified as Core Supplementary and does not presently satisfy the regulatory requirement for a mouse oncogenicity study on Technical Pynamin Forte. TB-I believes that the mice could have tolerated significantly higher dose levels and is requesting that the Registrant submit the data from the 5-week range finding study on Pynamin Forte in mice along with a rationale on the selection of the dose levels for the chronic study from the range-finding study. The following paragraph is a short summary of the results of the study.

Pynamin Forte was tested in an oncogenicity study in mice at 0, 120, 600 and 3000 ppm in the diet. The systemic NOEL was 600 ppm and the LOEL was 3000 ppm (increased relative liver weights; increased incidence of moderate fat deposition in the

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centrilobular hepatocytes (males) associated with moderate enlargement and/or vacuolation of centrilobular hepatocytes; moderate generalised fat deposition (females) and increased incidence and degree of centrilobular hepatocyte enlargement (males)). The oncogenic NOEL was 3000 ppm (HDT). It appears that the animals could have tolerated much higher dose levels.

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Reviewed By: Pamela Hurley, Ph.D. Pamela M. Hurley 12/12/91
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner Roger Gardner 4-20-92
Section I, Tox. Branch (H7509C)

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DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity - Mouse (83-2)

TOX. CHEM. NO.: 025B

ACCESSION NUMBER/MRID NO.: 410996-02

TEST MATERIAL: Pynamin Forte

SYNONYMS: D-cis/trans allethrin

PROJECT NUMBER: SMO 247/881026

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

TESTING FACILITY: Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, England

TITLE OF REPORT: Pynamin Forte Potential Tumorigenic Effects in Prolonged Dietary Administration in Mice

AUTHOR(S): Mayfield, R.; Gopinathy, C.; Crook, D.; Offer, J; Anderson, A. and Gibson, WA.

REPORT ISSUED: March 20, 1989

CONCLUSION: Pynamin Forte was tested in a carcinogenicity study in mice at 0, 120, 600 and 3000 ppm in the diet. The systemic NOEL is 600 ppm and the LOEL is 3000 ppm (increased relative liver weights; increased incidence of moderate fat deposition in the centrilobular hepatocytes (males) associated with moderate enlargement and/or vacuolation of centrilobular hepatocytes; moderate generalised fat deposition (females) and increased incidence and degree of centrilobular hepatocyte enlargement (males)). The carcinogenic NOEL is 3000 ppm (HDT). It appears that the animals could have tolerated much higher dose levels.

Classification: Core Supplementary until the results from the 5-week range finding study are submitted along with the rationale for the selection of the dose levels for the mouse carcinogenicity study.

Testing Guideline Satisfied: None.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: 36.5% allyl homolog of cinerin I and 55.5% other allethrin stereoisomers

Description: orange/brown liquid
Batch #(s), Other #(s): Batch # 50310
Purity: 93.1%
Source: Sumitomo Chemical Co., Ltd.
Vehicle (if applicable): None.
Positive Control(s) (if applicable): N/A

2. Test Animals

Species and Strain (sexes): Male and female CD-1 mice
Age: 28 days upon receipt
Weight(s): Within a weight range of 4 grams upon arrival.
Source(s): Charles River breeding laboratories, Manston, Kent, U.K.
Housing: 4/cage, solid bottom polypropylene

3. Procedure:

a. Dietary Preparation: Pynamin Forte was mixed into the diet. A premix was first prepared by grinding the test material directly into the commercial animal diet (Labsure Laboratory Animal diet No. 2) and mixing for at least 3 minutes. The required concentrations were prepared by direct dilution of the pre-mix with untreated diet and mixed further for at least 7 minutes.

Frequency of preparation: The pre-mix was prepared weekly.

Storage conditions: The test material was stored at 4°C protected from light. Storage conditions of the prepared diets were not given in the report.

Stability Analyses: These analyses were conducted prior to initiation of the main study. Representative samples of freshly prepared diet at nominal concentrations of 25, 100 and 10000 ppm were analyzed for concentration of the test material at time 0, after storage at room temperature for 1, 2 and 3 weeks and after storage at 4°C for 1 and 2 weeks.

Homogeneity Analyses: These analyses were conducted prior to initiation of the main study. Representative samples at the same concentrations given for the stability analyses were sampled at discharge from the blender from the first kg discharged (the top), from the approximate center of the discharge (the middle) and from the final kg discharge (the bottom) and analyzed for concentration of the test material.

Concentration Analyses: Samples of diets prepared at weeks 1, 2, 4, 9, 10, 21, 24, 33, 45, 57, 58, 69, 80 and at termination were analyzed to check for accuracy of preparation.

b. Basis For Selection of Dose Levels: Dose levels were selected on the basis of the results from a 5-week range-finding study.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 81 weeks* male	female	Interim Sac. 52 weeks male	female	Interim Sac. 78 weeks** male	female
Contr.	0	52	52	12	12	24	24
1	120	52	52	12	12	24	24
2	600	52	52	12	12	24	24
3	3000	52	52	12	12	24	24

* Terminal sacrifice was started at 81 weeks. Since the terminal procedures took several weeks to complete, the treated animals continued to receive test compound in their diet until the day prior to being killed.

** Macroscopic and hematological examinations only - no microscopic examinations were done unless animal either died or was sacrificed *in extremis* during the treatment period.

d. Clinical Observations and Mortality: Animals were examined twice daily for mortality or moribundity. During the first 4 weeks of treatment, the animals were checked daily for clinical signs of toxicity. After that time, they were checked twice weekly. During these examinations the mice were palpated for masses.

e. Body Weight Determinations: Bodyweights were recorded at the time of allocation to the cage, one week prior to commencement of treatment, on day 1 of treatment, weekly for the first 13 weeks and monthly thereafter.

f. Food and/or Water Consumption: Food consumption was measured and reported per cage weekly for the first 13 weeks and monthly thereafter. Visual monitoring of water consumption was made but no accurate measurements were conducted. Efficiency of food utilization was calculated during the first 13 weeks, the period of fastest growth. Group mean intake of test material was calculated over the 81 weeks.

g. Clinical Pathology: (*) recommended by Guidelines

Hematology:

Collection times for blood (including # of animals): Venous blood smears were prepared where possible, but not examined, from all sporadic death animals during the study. During week 52, blood samples were collected from 12 males and 12 females per group. Similar samples were taken from all surviving satellite group animals at week 78 and from all surviving animals at week 82.

The following CHECKED (X) parameters were examined:

X	
x	Leukocyte count (WBC)*
x	Leukocyte differential count*
x	Cell morphology where applicable

h. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: All animals.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals.

i. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: interim group (52 weeks) - all

control and high dose and lungs, liver, kidneys and any macroscopically abnormal tissue in intermediate and low dose groups; all animals that died during the study.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: all animals in high dose and control groups and lungs, liver, kidneys and any macroscopically abnormal tissue in intermediate and low dose groups.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The weights of major organs of individual mice dying or killed preterminally during the study were recorded at the discretion of the pathologist. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
x	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	x	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder	x	Mammary gland*
x	Rectum*	xx	Testes*	x	Parathyroids*
xx	Liver*	x	Epididymides	x	Thyroids*
x	Gall bladder*	x	Prostate		Other
x	Pancreas*	x	Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*	x	Vagina		All gross lesions and masses
x	Larynx	x	Cervix	x	Harderian gland
x	Pharynx			x	Head*

* to preserve nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lachrymal gland and Zymbal's gland.

j. Statistical Analyses: The report stated that "the following sequency of statistical tests was used for food consumption, bodyweight, organ weight and clinical pathology data: If the data consisted predominantly of one particular value (relative

frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed by appropriate methods. Otherwise" the following tests were used when the circumstances warranted it: Bartlett's test to test for heterogeneity of variance between treatments; one-way ANOVA (if no significant heterogeneity detected); Kruskal-Wallis analysis of ranks (if heterogeneity of variance present); Student's t-test and Williams test for a dose-related response; and nonparametric equivalents of the t-test and Williams' test (Shirley's test) after the Kruskal-Wallis test.

B. RESULTS:

1. Dietary Preparation: The mean concentrations of Pynamin Forte in the test diet were within -6.7% and +9.2% of the nominal concentrations for all of the concentrations tested. It appears that the formulations were reasonable accurate. The homogeneity tests indicated that preparations using 3 premixes (25000, 2500 and 250 ppm) gave better accuracy of preparation (range of 92 - 98% of nominal) than using preparations of 2 premixes (4000 and 40000 ppm - consistently 35-48% above the nominal). Stability in the diet was confirmed for 3 weeks during storage in the animal feed hoppers (-3.9 - +49.2 % from nominal) and for 2 weeks during storage at -4°C (-5.0 - +51.2% from nominal). The greatest deviations were at the 25 ppm level and these were all in the positive range.

2. Clinical Observations and Mortality: The report stated that there were no treatment-related clinical signs of toxicity, although a summary table supporting this statement was not provided. In addition, there appeared to be no treatment-related mortalities. The following table taken directly from the report summarizes the mortality incidences throughout the study.

Mortalities Throughout the Study

Weeks of Treatment	Group and Treatment (ppm) Males				Females			
	0	120	600	3000	0	120	600	3000
0-52	3 (2)	2 (3)	5 (3)	3 (4)	6 (2)	3 (0)	1 (4)	1 (3)
53-81	12 (9)	6 (4)	5 (1)	9 (3)	6 (5)	5 (2)	15 (3)	5 (4)
81-85	0	3	0	0	1	1	4	0
Sacrif. Wk 52	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)
Sacrif. Wk 78	(13)	(17)	(20)	(17)	17)	(22)	(17)	(17)
Terminal Sacrif.	37	41	42	40	39	43	32	46
% Survival at Term.	71	79	81	77	75	83	62	88

() = Satellite group animals.

3. Body Weight Determinations: There were no treatment-related differences in mean bodyweight gains between the treated and control groups. The gains were analyzed by the following statistical procedures: Williams' test and Kruskal-Wallis analysis of mean ranks followed by Williams' test. The following table taken directly from the report summarizes body weight gains for selected time periods.

Bodyweight Gains for Selected Time Periods (g)

Treatment Period (weeks)	Group and Treatment (ppm) Males				Females			
	0	120	600	3000	0	120	600	3000
0-25	13.3	12.9	13.2	12.8	9.5	8.7	9.5	9.9
SD	3.1	3.7	4.4	2.6	3.2	3.1	2.6	2.8
25-53	3.9	5.0	3.9	3.9	5.3	6.1	4.9	4.5
SD	3.4	3.6	3.6	3.3	3.5	2.9	3.3	3.0
53-81	-0.3	0.3	-0.3	0.7	2.1	2.4	0.9	1.2
SD	3.7	2.5	3.6	2.4	4.6	2.7	4.0	2.5
0-81	18.4	19.2	17.7	17.6	17.3	17.8	15.6	15.4
SD	4.1	4.9	3.8	3.8	6.4	4.7	4.2	4.1

SD = Standard Deviation

4. Food and/or Water Consumption: No treatment-related differences in group mean food consumption were observed in any of the treated groups when compared to controls. The values in g/mouse for the first 13 weeks were as follows: 401, 394, 405 and 393 for males and 361, 363, 361 and 361 for females for controls, low dose, mid-dose and high dose groups, respectively.

   Calculated Achieved Intake of Pynamin Forte: The overall achieved intakes of the test material/dose level for the entire study were calculated using the mean overall food intakes and the overall mid-study mean bodyweights. The values were 0, 14.4, 71.6 and 349.8 mg/kg/day for males and 0, 15.0, 77.3, and 382.2 mg/kg/day for females for the controls, low dose, mid-dose and high dose groups, respectively.

5. Hematology: No treatment-related differences in white blood cell parameters were observed between the treated and control groups. Only slight differences were observed between values. Occasionally a value attained statistical significance when compared with control values, but these were not considered to be of any toxicological significance. For example, in week 82, significant decreases in lymphocytes and monocytes were observed in mid- and high dose males. The decreases were not dose-related and they were not present at week 78.

6. Gross Pathology: No treatment-related macroscopic lesions were observed in any of the treated groups.

7. Organ Weights: A statistically significant increase in mean relative liver weights were observed in high dose groups of both sexes at all sacrifice times. In males, marginally (but not significantly) higher mean liver weights were observed in the mid-dose group at weeks 52 and 78 but not at termination and in females, marginally (but not significantly) higher liver weights were observed in the mid-dose group at termination but not at 52 and 78 weeks. No other treatment-related differences in organ weights were observed in any of the treated groups. The following table summarizes relative liver weights for both sexes at weeks 52, 78 and 82.

Relative Liver Weights for Interim and Terminal Sacrifices

Sacrifice Time Weeks	Treatment (ppm) Males				Females			
	0	120	600	3000	0	120	600	3000
52	2.18	2.06	2.45	2.92*	1.82	1.74	1.85	2.39*
78	2.46	2.47	2.75	3.32*	1.86	1.83	1.92	2.35*
82	2.63	2.58	2.64	3.26*	1.83	1.87	1.95	2.46*

* Statistically significant from controls $p<0.01$ using Williams' test.

8. Histopathology:

a. Nonneoplastic lesions:

52-week interim sacrifice and those that died or were killed in extremis prior to 52 weeks: In high dose males there was an increased incidence of moderate fat deposition in the centrilobular hepatocytes when compared to controls (1/12, 0/12, 0/12 and 9/12 for controls, low, mid- and high dose groups, respectively). This change was associated with moderate enlargement (3/12 versus 0/12 in controls [0/12 and 1/12 in low and mid-dose groups]) and/or vacuolation of centrilobular hepatocytes (3/12 versus 0/12 in controls [0/12 and 1/12 in low and mid-dose groups]) in a small number of affected male mice. In high dose females there was moderate generalised fat deposition in hepatocytes in 3/12 animals (0/12 in controls and other groups). No other treatment-related effects were observed in any of the treated groups. In addition, no treatment-related

effects on the liver were observed in high dose animals which had either died or were killed prior to the 52-week sacrifice.

Terminal sacrifice and those that died or were killed in extremis after 52 weeks (including satellite animals that died): An increased incidence and degree of centrilobular hepatocyte enlargement was observed in high dose male mice when compared to controls. No other treatment-related effects were observed in any of the other treated groups, including those that died prior to termination. The following table summarizes the lesions of interest in the liver for animals that either died or were killed in extremis prior to termination (including satellite group) and animals that were sacrificed at termination.

Non-neoplastic Liver Lesions in Male and Female Mice

Lesion	Treatment (ppm)							
	Males				Females			
	0	120	600	3000	0	120	600	3000
Number Examined	63	59	55	59	59	54	59	59
Min. centrilobular hepatocyte enlarge.	17	20	16	27	1	2	2	2
Mod. centrilobular hepatocyte enlarge.	0	0	1	5	0	0	0	0
Min. generalized hepatocyte enlarge.	5	3	6	6	0	0	0	0
Mod. generalized hepatocyte enlarge.	4	6	0	0	0	0	0	0
Min. periportal hepatocyte enlarge.	1	0	0	0	1	0	0	0
Min. centrilobular hepatocyte vacuolat.	13	16	20	11	20	14	20	18
Mod. centrilobular hepatocyte vacuolat.	0	2	1	6	1	2	1	1
Min. generalized hepatocyte vacuolat.	0	1	0	1	2	1	0	1
Trace fat deposition centri. hepatocytes	9	8	10	11	5	7	7	7
Min. fat deposition centri. hepatocytes	3	5	14	6	5	2	2	1
Mod. fat deposition centri. hepatocytes	0	1	3	4	0	1	1	0
Trace gen. fat depos. hepatocytes	0	2	1	0	1	2	0	1
Min. gen. fat depos. hepatocytes	0	2	0	1	0	1	1	2
Mod. gen. fat depos. hepatocytes	0	1	0	0	2	0	1	0

b. Neoplastic lesions: There were no treatment-related increases in either the incidence or distribution of tumors recorded or in the number of tumor bearing mice in the treated animals when compared to controls. In addition, the authors stated that all tumors observed were considered to fall within the spontaneous tumor profile of the strain of mice employed in the study (however, historical control data were not provided to support this statement). The following table summarizes neoplastic lesions of interest in this study for animals that either died or were killed in extremis prior to termination (including satellite group) and animals that were sacrificed at termination.

Selected Neoplastic Lesions in Male and Female Mice

Lesion	Treatment (ppm) Males				Females			
	0	120	600	3000	0	120	600	3000
# mice examined	63	59	56	59	59	54	59	59
# tumor bearing mice	33	31	23	22	25	17	25	21
Lung - # examined	63	59	55	59	59	54	59	59
Pulmonary adenoma	8	11	4	7	6	7	4	6
Pulmonary adenocarcinoma	4	1	1	2	3	2	1	0
Liver - # examined	63	59	55	59	59	54	59	59
Benign liver cell tumors	9	7	6	8	0	1	0	1
Multiple benign liver cell tumors	3	1	4	2	0	0	0	0
Malignant liver cell tumors	4	5	2	2	0	0	0	0
Multiple malignant liver cell tumors	1	0	1	0	0	0	0	0
Lymphosarcoma	3	1	4	1	3	2	8	4
Pleomorphic lymphosarcoma	0	1	0	0	1	0	0	0
Lymphoid leukemia	0	1	0	0	1	1	1	0
Histiocytic Sarcoma	0	1	0	0	2	0	1	3
Myeloid leukemia	0	0	0	0	1	0	0	0

9. Quality Assurance Measures: Signed Good Laboratory Practice and Quality Assurance statements were provided for the study.

C. DISCUSSION: The systemic NOEL is 600 ppm and the LOEL is 3000 ppm (increased relative liver weights; increased incidence of moderate fat deposition in the centrilobular hepatocytes (males) associated with moderate enlargement and/or vacuolation of centrilobular hepatocytes; moderate generalised fat deposition (females) and increased incidence and degree of centrilobular hepatocyte enlargement (males)). The carcinogenic NOEL is 3000 ppm (HDT). The design and

conduct of the study is acceptable with the exception of the highest dose level tested. It appears that the animals could have tolerated much higher dose levels. The study is classified as Core Supplementary until the results from the 5-week range finding study are submitted along with the rationale for the selection of the dose levels for the mouse carcinogenicity study.

END