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

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361
JAN 31 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

January 22, 1996

SUBJECT: 004005. d-Allethrin (Pynamin Forte). Review of Range Finding Study for Mouse Oncogenicity Study (83-2)

PC Code 004005
DP Barcode D218869
Case No. 818518
MRID No. 43760401

Tox. Chem. No. 025B
Reregistration Case No. 0437
ID No. 004005

TO: Dana Lateulere, CRM Team # 72
Reregistration Branch
Special Review and
Reregistration Division (7508W)

FROM: Pamela M. Hurley, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)
Pamela M. Hurley 1/22/96

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C)
Roger Gardner 1/22/96
KB 1/23/96

Background and Request:

Sumitomo had submitted a dietary mouse oncogenicity study (MRID 41099602) conducted with Pynamin Forte in response to the Registration Standard on allethrins. The Toxicology Branch (TB-I) reviewed the study and classified it as Core Supplementary pending receipt of the range-finding study along with a rationale on the selection of the dose levels for the chronic study. Sumitomo submitted the range-finding study (MRID 43760401) with the rationale for dose selection and TB-I has been asked to review the submission and upgrade the study if appropriate.

Toxicology Branch Response:

TB-I has reviewed the range-finding study and the rationale for dose selection and agrees with the Registrant that the highest dose tested in the mouse oncogenicity study was adequate. Therefore, the mouse oncogenicity study conducted with Pynamin Forte is upgraded to acceptable and is acceptable for regulatory

purposes. The guideline requirement for a mouse oncogenicity study to be conducted with technical pynamin forte has been fulfilled (83-2). The following paragraphs summarize the results of the range-finding study and the main oncogenicity study.

Technical pynamin forte (93.1% a.i.) was tested in a carcinogenicity study in groups of 52 CD-1 mice/sex/dose at 0, 120, 600 or 3000 ppm in the diet (MRID 41099602). The calculated intakes were 0, 14.4, 71.6 or 349.8 mg/kg/day for males and 0, 15.0, 77.3 or 382.2 mg/kg/day for females. **The systemic NOEL is 600 ppm and the LOEL is 3000 ppm** (increased relative liver weights (up to 135% of controls in males and up to 134% of controls in females); increased incidence of moderate fat deposition in the centrilobular hepatocytes in males (9/12 for the high dose group versus 1/12 in controls) associated with moderate enlargement and/or vacuolation of centrilobular hepatocytes (3/12 in high dose males versus 0/12 in controls for both parameters) at 52 weeks; moderate generalised fat deposition (3/12 in high dose females versus 0/12 in controls) at 52 weeks and increased incidence and degree of centrilobular hepatocyte enlargement in high dose males at termination. **There was no indication of any increase in any type of neoplasia.**

In a subchronic toxicity range-finding study (MRID 43760401), pynamin forte (93.4% a.i.) was administered to 10 CD-1 mice/sex/dose in the diet at dose levels of 0, 100, 300, 1000, 3000 or 10000 ppm (0, 14, 43, 144, 423 or 1550 mg/kg/day for males and 0, 16, 45, 157, 438 or 1522 mg/kg/day for females) for 5 weeks. There were changes in clinical chemistry at all dose levels (decreases in triglycerides in both sexes (28 - 77% of controls; more significant in females) and increases in albumin in females (106 - 119%). At 300 ppm and above, there was slight to minimal enlargement of hepatocytes and at 3000 ppm and above, there were increases in liver weights. At 10,000 ppm, there were decreases in bodyweight (83%) and bodyweight gain (34%) in males, inferior food utilization efficiency: males (267%) and females (158%), and changes in both hematological and other clinical chemistry parameters in both sexes. **Technically, the NOEL is 100 ppm, the lowest dose tested and the LEL is 300 ppm, based on enlargement of hepatocytes, increases in liver weights, supporting clinical chemistry, decreases in body weight and body weight gain, inferior food efficiency and hematological effects. However, most of the significant effects were observed at 10000 ppm. It appears that the NOAEL is closer to 3000 ppm and the LOAEL is 10000 ppm.**

The submitted rationale for dose selection stated that the doses were selected on the following data from the range finding study: decreased body weight gains in males at 1000 ppm and higher and in females at 10000 ppm, increased liver weights in both sexes at 3000 ppm and higher and centrilobular enlargement in males at 1000 ppm and in females at 3000 ppm and higher. The

Registrant also submitted to the Agency that "there are no food uses for Pynamin Forte and that the principal basis for determining the MTD is therefore absent. Further, Pynamin Forte is not structurally related to any known carcinogens; it is not a mutagen; and the subchronic data have failed to show morphologic effects in organs which may result in neoplasia. Finally, Pynamin Forte is not used in any products which present a significant exposure in terms of the human life span (e.g. treated fabrics, insect repellents or constant-use indoor aerosols). Thus neither Pynamin Forte's chemistry, nor its toxicology nor its use patterns call for an oncogenic MTD."

For decision and information purposes on RED production for the allethrins, TB-I has been continually updating the toxicology data base for this group of pesticides as the data reviews are completed. The following is a discussion of the background on these chemicals and a list of completed toxicology study requirements for registration for each of the technical products involved. These only include studies which TB-I has in our files. They do not include anything that has been submitted to the Agency and has not yet been sent to TB-I.

The Toxicology Chapter of the Registration Standard for Allethrins was written in 1987. This chapter included the following 5 technical/manufacturing use allethrin products registered as pesticides:

1. allethrin (pynamin, PC Code 004001, Caswell # 025)

2. d-trans allethrin (bioallethrin, PC Code 004003, Caswell # 025A)

3. S-bioallethrin (esbiol, PC Code 004004, Caswell # 025C)

4. d-cis/trans allethrin (pynamin-forte, PC Code 004005, Caswell # 025B)

5. esbiothrin (PC Codes 004003 and 004004, Caswell #'s 025A and 025C)

The major components of **allethrin** comprise a mixture of 8 different isomers. This product is the least insecticidally active and is the least pure. It may have already been dropped from the re-registration process. The major components of **pynamin-forte** comprise a mixture of 4 of the 8 isomers. The major components of bioallethrin, esbiol and esbiothrin comprise mixtures in various proportions of 2 of the 8 isomers: d-trans chrysanthemic acid of d- and l-allethrolone. **Bioallethrin** is approximately a 50/50 mixture, **esbiol** is approximately a 90/5 mixture and **esbiothrin** is approximately a 72/21 mixture.

In the Registration Standard, it was agreed that the required chronic studies for bioallethrin, esbiol and esbiothrin would be satisfied by chronic studies conducted on esbiothrin. Allethrin and pynamin forte were to have totally separate testing requirements each. On the basis of these decisions, at this time, the following data requirements have been satisfied for each of the products:

Allethrin

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	No
81-2	Acute Dermal Toxicity	Yes	No
81-3	Acute Inhalation Toxicity	Yes	No
81-4	Primary Eye Irritation	Yes	No
81-5	Primary Dermal Irritation	Yes	No
81-6	Dermal Sensitization	Yes	No
82-1(a)	Subchronic Oral (rodent)	Yes	No
82-1(b)	Subchronic Oral (non-rodent)	Yes	No
82-2	21-Day Dermal	Yes	No
83-1(a)	Chronic Toxicity (rodent)	Yes	No
83-1(b)	Chronic Toxicity (nonrodent)	Yes	No
83-2	Oncogenicity (mouse)	Yes	No
83-5	Oncogenicity (rat)	Yes	No
83-3(a)	Teratology (first species)	Yes	No
83-3(b)	Teratology (second species)	Yes	No
83-4	Multigeneration Reproduction	Yes	No
84-2(a)	Mutagenicity - Gene Mutation	Yes	No
84-2(b)	Mutagenicity - Structural Chromosomal Aberrations	Yes	No
84-2(c)	Mutagenicity - Other Genotoxic Effects	Yes	No
85-1	Metabolism	Yes	No

Pynamin Forte

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Yes
81-3	Acute Inhalation Toxicity	Yes	Yes
81-4	Primary Eye Irritation	Yes	Yes
81-5	Primary Dermal Irritation	Yes	Yes
81-6	Dermal Sensitization	Yes	Yes
82-1(a)	Subchronic Oral (rodent)	Yes	Yes[1]
82-1(b)	Subchronic Oral (non-rodent)	Yes	Yes[2]
82-2	21-Day Dermal	Yes	No
83-1(a)	Chronic Toxicity (rodent)	Yes	Yes
83-1(b)	Chronic Toxicity (nonrodent)	Yes	Yes
83-2	Oncogenicity (mouse)	Yes	Yes
83-5	Oncogenicity (rat)	Yes	Yes
83-3(a)	Teratology (rat)	Yes	Yes
83-3(b)	Teratology (rabbit)	Yes	Yes
83-4	Multigeneration Reproduction	Yes	No
84-2(a)	Mutagenicity - Gene Mutation	Yes	No[4]
84-2(b)	Mutagenicity - Structural Chromosomal Aberrations	Yes	No[4]
84-2(c)	Mutagenicity - Other Genotoxic Effects	Yes	No[4]
85-1	Metabolism	Yes	Possibly[3]

[1]An acceptable chronic feeding study in the rat is available. The subchronic study is not required.
[2]An acceptable chronic feeding study in the dog is available. The subchronic study is not required.
[3]Metabolism studies were submitted on bioallethrin to cover all the allethrins. The decision on whether or not the studies can cover all the allethrins needs to be considered by HED (possibly the metabolism committee).
[4]The requirement for mutagenicity testing may be satisfied by either the old guidelines (listed above) or the new guidelines, which are a Salmonella assay (Ames), a mammalian cell forward mutation assay and an in vivo cytogenetics assay.

Bioallethrin

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Possibly[1]
81-3	Acute Inhalation Toxicity	Yes	Yes
81-4	Primary Eye Irritation	Yes	No
81-5	Primary Dermal Irritation	Yes	No
81-6	Dermal Sensitization	Yes	No
82-1(a)	Subchronic Oral (rodent)	Yes	No[7]
82-1(b)	Subchronic Oral (non-rodent)	Yes	Yes
82-2	21-Day Dermal	Yes	No
83-1(a)	Chronic Toxicity (rodent)	Yes	Yes[5]
83-1(b)	Chronic Toxicity (nonrodent)	Yes	Yes[2]
83-2	Oncogenicity (mouse)	Yes	No[3]
83-5	Oncogenicity (rat)	Yes	Yes[5]
83-3(a)	Teratology (rat)	Yes	Yes
83-3(b)	Teratology (second species)	Yes	No
83-4	Multigeneration Reproduction	Yes	Yes[4]
84-2(a)	Mutagenicity - Gene Mutation	Yes	Yes
84-2(b)	Mutagenicity - Structural Chromosomal Aberrations	Yes	No[6]
84-2(c)	Mutagenicity - Other Genotoxic Effects	Yes	Yes
85-1	Metabolism	Yes	Yes

[1]Possibility that study may be acceptable under current standards for acutes - only 1 sex tested, can still obtain Tox. Category.
[2]A 6 month study is available on bioallethrin and is graded Core-minimum. This study was acceptable in the past for a chronic study. The 1-year study on esbiothrin is supplementary, upgradable to guideline upon submission of data from preliminary study. The chronic dog study on esbiothrin would also satisfy this requirement if it is upgraded.
[3]The oncogenicity study in the mouse was conducted on esbiothrin and is graded supplementary pending submission of preliminary range-finding study. The mice could have tolerated higher dose levels. This study will satisfy requirements for bioallethrin if upgraded.
[4]This requirement has been satisfied by an acceptable reproduction study conducted on esbiothrin.
[5]This requirement has been satisfied by an acceptable chronic/oncogenicity feeding study conducted on esbiothrin.
[6]This study may possibly be upgradable pending submission of additional data.
[7]This study may be upgraded pending submission of histopathology tables.

Esbiothrin

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	Yes
81-3	Acute Inhalation Toxicity	Yes	Yes
81-4	Primary Eye Irritation	Yes	Yes
81-5	Primary Dermal Irritation	Yes	Yes
81-6	Dermal Sensitization	Yes	Yes
82-1(a)	Subchronic Oral (rodent)	Yes	Yes[3]
82-1(b)	Subchronic Oral (non-rodent)	Yes	No[1]
82-2	21-Day Dermal	Yes	Yes
83-1(a)	Chronic Toxicity (rodent)	Yes	Yes
83-1(b)	Chronic Toxicity (nonrodent)	Yes	No[1]
83-2	Oncogenicity (mouse)	Yes	No[2]
83-5	Oncogenicity (rat)	Yes	Yes
83-3(a)	Teratology (rat)	Yes	Yes
83-3(b)	Teratology (rabbit)	Yes	Yes
83-4	Multigeneration Reproduction	Yes	Yes
84-2(a)	Mutagenicity - Gene Mutation	Yes	Yes
84-2(b)	Mutagenicity - Structural Chromosomal Aberrations	Yes	No[5]
84-2(c)	Mutagenicity - Other Genotoxic Effects	Yes	No[5]
85-1	Metabolism	Yes	Possibly[4]

[1]The 1-year study on esbiothrin is supplementary, upgradable to guideline upon submission of data from preliminary study. The requirement for a subchronic study in non-rodents will be satisfied by this study if it is upgraded.
[2]The oncogenicity study in the mouse is graded supplementary pending submission of preliminary range-finding study. The mice could have tolerated higher dose levels.
[3]This requirement has been satisfied by an acceptable chronic feeding study in the rat.
[4]Metabolism studies were submitted on bioallethrin to cover all the allethrins. The decision on whether or not the studies can cover all the allethrins needs to be considered by HED (possibly the metabolism committee).
[5]The requirement for mutagenicity testing may be satisfied by either the old guidelines (listed above) or the new guidelines, which are a Salmonella assay (Ames), a mammalian cell forward mutation assay and an in vivo cytogenetics assay.

Esbiol

		Required	Satisfied
81-1	Acute Oral Toxicity	Yes	Yes
81-2	Acute Dermal Toxicity	Yes	No
81-3	Acute Inhalation Toxicity	Yes	No
81-4	Primary Eye Irritation	Yes	No[1]
81-5	Primary Dermal Irritation	Yes	No[2]
81-6	Dermal Sensitization	Yes	No
82-1(a)	Subchronic Oral (rodent)	Yes	No
82-1(b)	Subchronic Oral (non-rodent)	Yes	No
82-2	21-Day Dermal	Yes	No
83-1(a)	Chronic Toxicity (rodent)	Yes	Yes[6]
83-1(b)	Chronic Toxicity (nonrodent)	Yes	No[3]
83-2	Oncogenicity (mouse)	Yes	No[4]
83-5	Oncogenicity (rat)	Yes	Yes[6]
83-3(a)	Teratology (first species)	Yes	No
83-3(b)	Teratology (second species)	Yes	No
83-4	Multigeneration Reproduction	Yes	Yes[5]
84-2(a)	Mutagenicity - Gene Mutation	Yes	Yes
84-2(b)	Mutagenicity - Structural Chromosomal Aberrations	Yes	Pending[8]
84-2(c)	Mutagenicity - Other Genotoxic Effects	Yes	Pending[8]
85-1	Metabolism	Yes	Possibly[7]

[1]Study may possibly be upgraded if scoring method submitted.
[2]This study had an unusual test design. Needs additional information.
[3]The 1-year study on esbiothrin is supplementary, upgradable to guideline upon submission of data from preliminary study. The chronic dog study on esbiothrin will satisfy this requirement if upgraded.
[4]The oncogenicity study in the mouse was conducted on esbiothrin and is graded supplementary pending submission of preliminary range-finding study. The mice could have tolerated higher dose levels. This study will satisfy requirements for esbiol if upgraded.
[5]This requirement has been satisfied by an acceptable reproduction study conducted on esbiothrin.
[6]This requirement has been satisfied by an acceptable chronic/oncogenicity feeding study conducted on esbiothrin.
[7]Metabolism studies were submitted on bioallethrin to cover all the allethrins. The decision on whether or not the studies can cover all the allethrins needs to be considered by HED (possibly the metabolism committee).
[8]TB-I has received these studies but has not reviewed them as yet.

EPA Toxicologist: Pamela Hurley Pamela M Hurley, Date 1/22/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 1/22/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [feeding]-[mouse]; 5 Week Range-Finding Study

DP BARCODE: D218869 SUBMISSION CODE: S493044
P.C. CODE: 004005 TOX. CHEM. NO.: 025B

TEST MATERIAL (PURITY): Pynamin forte (93.4%)

SYNONYMS: d-Allethrin, d-cis/trans allethrin

CITATION: Edwards, C.A., K. Connaughton, D. Crook, et al. (1986) Pynamin forte® toxicity to mice by repeated dietary administration for 5 weeks. Huntingdon Research Centre Limited, Huntingdon, Cambridgeshire, England. Laboratory Project Number SMO 237/8625, October 9, 1986. MRID 43760401. Unpublished.

SPONSOR: Sumitomo Chemical Company, Ltd. Osaka, Japan

EXECUTIVE SUMMARY:

In a subchronic toxicity range-finding study (MRID 43760401), pynamin forte (93.4% a.i.) was administered to 10 CD-1 mice/sex/dose in the diet at dose levels of 0, 100, 300, 1000, 3000 or 10000 ppm (0, 14, 43, 144, 423 or 1550 mg/kg/day for males and 0, 16, 45, 157, 438 or 1522 mg/kg/day for females) for 5 weeks.

There were changes in clinical chemistry at all dose levels (decreases in triglycerides in both sexes (28 - 77% of controls; more significant in females) and increases in albumin in females (106 - 119%). At 300 ppm and above, there was slight to minimal enlargement of hepatocytes and at 3000 ppm and above, there were increases in liver weights. At 10,000 ppm, there were decreases in bodyweight (83%) and bodyweight gain (34%) in males, inferior food utilization efficiency: males (267%) and females (158%), and changes in both hematological and other clinical chemistry parameters in both sexes.

Technically, the NOEL is 100 ppm, the lowest dose tested and the LEL is 300 ppm, based on enlargement of hepatocytes, increases in liver weights, supporting clinical chemistry, decreases in body weight and body weight gain, inferior food efficiency and hematological effects. However, most of the significant effects were observed at 10000 ppm. It appears that the NOAEL is closer to 3000 ppm and the LOAEL is 10000 ppm.

This range-finding toxicity study is classified Supplementary. It was conducted to determine the dose levels for the mouse oncogenicity study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: pynamin forte
   Description: orange/brown liquid
   Lot/Batch #: batch # 50310
   Purity: 93.4% ai.
   Stability of compound: "sufficient for duration of study"
   CAS #: 42534-61-2
   The following is a generalized structure of one of the memebers of the allethrin family.

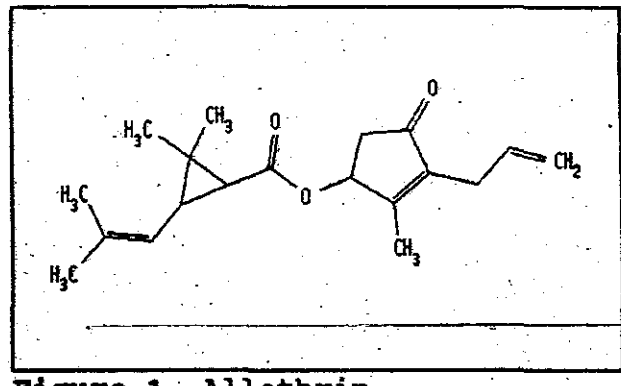

Figure 1 Allethrin

2. Vehicle and/or positive control: N/A

3. Test animals: Species: mice
   Strain: CD-1
   Age and weight at study initiation: 28 days old, within a weight range of 2 g for males and 1 g for females.
   Source: Charles River UK Ltd., Manston, Kent, England.
   Housing: 5/sex/cage in solid bottom polypropylene cages (33x15x13 cm high) with autoclaved sifted sawdust as bedding.
   Diet: Labsure Laboratory Animal Diet No. 2 ad libitum
   Water: tap water ad libitum
   Environmental conditions: Temperature: 21°C
   Humidity: 50%
   Air changes: Not stated
   Photoperiod: 12 hours light, 12 hours dark
   Acclimation period: 15 days

B. STUDY DESIGN:

1. In life dates - start: 10/24/85 end: 2-4/12/85

2. Animal assignment

Animals were assigned randomly, stratified by body weight to the test groups in table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet ppm	Mean Dose to Animal mg/kg	# Males per Group	# Females per Group
Control	0	0	10	10
Low	100	14 (♂) 16 (♀)	10	10
Mid 1	300	43 (♂) 45 (♀)	10	10
Mid 2	1000	144 (♂) 157 (♀)	10	10
Mid 3	3000	423 (♂) 438 (♀)	10	10
High	10000	1550 (♂) 1522 (♀)	10	10

3. Diet preparation and analysis

Diet was prepared weekly by grinding the test substance directly into the diet as a premix and diluting the premixes with additional quantities of the untreated diet. It wasn't specifically stated, but it appears that the treated diets were probably stored at room temperature., Homogeneity and stability were tested prior to commencement of the study. Both homogeneity and stability studies were conducted at dose levels of 25, 100 and 10000 ppm. Stability studies were conducted at either room temperature for up to 3 weeks or at 4°C for 1 and 2 weeks. Samples of treated food at all dose levels were taken during the first week of the study and during the last week for concentration analyses.

Results - Homogeneity Analysis: Mixing appeared to be homogeneous. In the cases where two pre-mix preparations were done, the actual concentrations were somewhat higher than the nominal concentrations (33.8-37.0 versus 25 ppm

nominal), however, they were consistently homogeneous. Where 3 premix preparations were done, the concentrations were closer to the nominal (22.8-24.9 versus 25 ppm nominal).
Stability Analysis: Pynamin forte was stable for 3 weeks during storage in the animal feed hoppers and for 2 weeks during storage at 4°C.
Concentration Analysis: With the exception of two measurements (group 2, weeks 1 and 5), mean results were within ± 5% of the nominal values. The 2 measurements were 23 and 22% above the nominal value.

The analytical data indicated that the mixing procedure was adequate and that the varaince between nominal and actual dosage to the animals was acceptable.

4. Statistics - The following paragraphs are taken directly from the report.

"If the data consist predominantly of one particular value (relative frequency of the mode exceeds 75%), the proportion of animals with values different from the mode were analysed by appropriate methods. Otherwise:

Bartlett's test (1) was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (2) was used.

Except for pre-dose data, analyses of variance was followed by Student's 't' test and Williams' test (4) for a dose-related response, although only the one thought most appropriate for the response pattern observed was reported. The Kruskal-Wallis analysis was followed by the non-parametric equivalents of the 't' test and Williams' test (Shirley's test, (3))."

Where appropriate, analysis of covariance may be used in place of analysis of variance in the above sequence. For most parameters, the appropriate covariant is the same parameter at pre-dose. For organ weight data, the final bodyweight may be used as a covariate in an attempt to allow for differences in bodyweight which might influence the organ weights."

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality.

2. Body weight

Animals were weighed weeks -2 and -1, on the day of commencement of dosing (day 1) and on days 4, 8, 15, 22, 29 and 36.

3. Food consumption and compound intake

Food consumption for each cage was determined on a weekly basis and mean weekly diet consumption was calculated as g food/mouse/week. Food efficiency values were calculated as weight of food consumed per unit gain in bodyweight. The quantity of compound intake in terms of mg/kg/day was calculated from the mean weekly food consumption and the group mean bodyweight data.

4. Ophthalmoscopic examination

Eyes were examined before treatment commenced and during week 5 by means of a Keeler indirect ophthalmoscope. Prior to examination, the pupils of all animals were dilated using a Tropicamide ophthalmic solution.

5. Blood was collected during week 5 from the orbital sinus of all mice under a light ether anaesthesia. Blood from 5/group/sex was used for hematological investigations and blood from the remaining animals was used for the biochemical measurements. Free access to food and water was permitted for animals prior to blood sampling. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood clotting measurements*	x	Packed cell volume (PCV)
	(Thromboplastin time)	x	Cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
	Calcium*	x	Albumin*
	Chloride*		Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
	Phosphorus*	x	Total Cholesterol
	Potassium*	x	Globulins
	Sodium*	x	Glucose*
			Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)	x	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
x	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
x	Leucine aminopeptidase (LAP)		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from all animals at weeks 4/5. Food but not water was removed overnight from animals sampled for urinalysis. The CHECKED (X) parameters were examined.

X		X	
	Appearance	x	Glucose
x	Volume	x	Ketones
x	Specific gravity		Bilirubin
x	pH		Blood
x	Sediment (microscopic)		Nitrate
x	Protein	x	Urobilinogen
		x	Total reducing substances
		x	Bile pigments
			Haem. pigments

* Not required for subchronic studies

7. Sacrifice and Pathology

At termination, all surviving animals were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (Y) organs were weighed. The report stated that "as the terminal procedures took several days to complete, the treatment of individual treated animals continued until

the day they were killed. The duration of the treatment period, however, was reported as 5 weeks." An extensive list of tissues were preserved for future microscopic examination. However, only a few were actually microscopically examined. These are checked below.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*	y	Brain*Periph.
	Salivary glands*	y	Heart*		nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)[T]
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*	y	Spleen*		Eyes (optic n.)[T]
	Jejunum*		Thymus*		
	Ileum*		UROGENITAL		GLANDULAR
	Cecum*		Kidneys*+		Adrenal gland*
	Colon*	xy	Urinary bladder*	y	Lacrimal gland[T]
	Rectum*		Testes*[+]		Mammary gland[T]
xy	Liver*[+]	y	Epididymides		Parathyroids*[++]
	Gall bladder*		Prostate		Thyroids*[++]
	Pancreas*		Seminal vesicle		OTHER
	RESPIRATORY	y	Ovaries		Bone
	Trachea*		Uterus*		Skeletal muscle
y	Lung*				Skin
	Nose			x	All gross lesions and masses*
	Pharynx				
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
[+] Organ weight required in subchronic and chronic studies.
[++] Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations :

1. Toxicity - No treatment-related clinical signs of toxicity were observed.

2. Mortality - There were no mortalities during the study. However, one 300 ppm females mouse disappeared during week 4 and could not be found. This animal was excluded from the study.

B. Body weight and weight gain: The report stated that "overall, a lower mean bodyweight gain was observed at termination for male mice receiving 10000 ppm, when compared with the controls. Bodyweight change of other male treated groups and all female groups showed no clear effect of treatment with Pynamin Forte, although slightly lower mean

bodyweight gains were noted for males receiving 1000 or 3000 ppm. The mean bodyweight losses, observed for all groups of males at week 4 were considered to be the result of overnight deprivation of food during urine collection which occurred prior to weighing." The Toxicology Branch (TB-I agrees with the authors' conclusions. The following table summarizes the results.

Group Mean Bodyweights and Bodyweight Gain (g)			
Dose (ppm)	Week 0	Week 5	Gain Weeks 0-5
Males			
0	24.6	34.3	8.5
100	28.7	34.3	7.4
300	25.4	33.5	7.5
1000	27.8	32.6	5.9**
3000	27.7	32.4	5.8**
10000	23.4	28.4**	2.9**
Females			
0	22.0	25.9	3.9
100	21.3	24.7	3.4
300	21.2	24.2	3.0
1000	20.8	24.2	3.4
3000	21.2	24.0	2.8
10000	21.3	23.7	2.4

C. Food consumption and compound intake:

1. Food consumption - There was a marginal decrease in mean food consumption in the high dose male mice (143 g/mouse versus 157 in the controls (91% of control value)). This was not statistically significant.

2. Compound consumption (time-weighted average) - The following average comsumption values for pynamin forte was as follows:

Average Compound Intake Over 5 Weeks		
Concentrations (ppm)	Males (mg/kg/day)	Females (mg/kg/day)
100	14	16
300	43	45
1000	144	157
3000	423	438
10000	1550	1522

3. Food efficiency - Food efficiency was calculated as follows: mean food consumption/mean bodyweight gain. The report stated that "the food utilization efficiency of male mice receiving 10000 ppm was markedly inferior to that of the controls. A similar but less pronounced finding was observed among females of this group." No effects were observed in any of the other groups. TB-I agrees that there appears to be an effect at the highest dose level, especially in males. The values are summarized as follows:

Food Efficiency in Male and Female Mice (Weeks 1-5)	
Dose (ppm)	Food Efficiency
Males	
0	18.5
100	21.8
300	20.1
1000	26.4
3000	26.1
10000	49.4
Females	
0	34.6
100	40.6
300	41.9
1000	38.5
3000	45.6
10000	54.5

D. Ophthalmoscopic examination - No abnormalities were observed at the pre-treatment examination. After 5 weeks, a corneal opacity was observed in the eye of one male mouse receiving 3000 ppm. Since there was no indication of a dose-response, this is not considered to be related to treatment.

E. Blood work:

1. Hematology - In the high dose group, the report stated that decreased mean hemoglobin, red blood cell count and PCV values were observed in both sexes when compared to the control group at 5 weeks. However, the differences were not large and in males, only PCV was statistically significantly different from the control value. No other differences were considered to be related to treatment. The following table summarizes the results.

Selected Mean Hematological Values for Males and Females at 5 Weeks			
Dose (ppm)	Hemoglobin (g/dl)	RBC ($X10^6/mm^3$)	PCV (%)
Males			
0	14.2	8.8	48
100	14.2	9.0	48
300	15.4	8.9	49
1000	13.7	8.6	46
3000	13.7	8.8	46
10000	12.8	8.0	43**
Females			
0	14.7	9.3	48
100	14.4	8.8	48
300	14.0	9.0	46
1000	14.8	9.5	49
3000	14.7	9.5	49
10000	13.3**	8.2**	44**

**Statistically significant $p < 0.001$ (Williams' test)

2. Clinical Chemistry - The report stated that there were decreased mean triglyceride values for all treated groups in both sexes. The individual values in females were consistent with this observation but the individual male values showed no clear trends with dose although the majority of the individual values were below those recorded for control males. There were also increases in group mean albumin values in high dose males and in all treated female groups, increases in mean protein in the high dose group of both sexes and increases in cholesterol values in high dose females. The authors believed that the slightly higher mean GPT values may be collated with increased liver weight although dosage-related trends in the enzyme data were not distinct. They also stated that there was no clear dosage-related pattern in glucose values although females showed lower mean values than the control counterparts. The following table summarizes the results.

Selected Mean Clinical Chemistry Values for Males and Females						
Dose (ppm)	Triglyc.	Albumin	Protein	Cholest.	GPT	Glucose
Males						
0	94	2.8	5.2	111	31	194
100	72*	2.9	5.7	129	27	177
300	68**	2.9	5.5	114	28	144
1000	68**	3.0	5.7	104	28	124
3000	72**	2.9	5.5	122	24	179
10000	53**	3.3**	5.8*	130	45*	124**
Females						
0	127	3.1	5.5	90	24	187
100	85**	3.3**	5.8	85	25	158
300	54**	3.3**	5.5	78	33	156
1000	52**	3.4**	5.7	93	29	154
3000	50**	3.3**	5.7	111	39**	147
10000	36**	3.7**	6.4**	133**	34**	139

* $p < 0.05$; ** $p < 0.01$ (Williams' test)

F. Urinalysis - No treatment-related differences were found between treated groups and the control group.

G. Sacrifice and Pathology:

1. Organ weight - Statistically significant increases in liver weights were observed in both sexes at 3000 and 10000 ppm when the organ weights were adjusted for final bodyweight as covariate. None of the other changes were considered to be toxicologically significant. The following table summarizes the liver weight data.

Group Mean Liver Weights for Both Males and Females		
Dose (ppm)	Males	Females
0	1.86 (1.97)	1.47 (1.56)
100	1.83 (2.17)	1.51 (1.47)
300	1.85 (1.92)	1.47 (1.45)

Group Mean Liver Weights for Both Males and Females		
Dose (ppm)	Males	Females
1000	2.02 (2.18)	1.57 (1.48)
3000	2.25** (2.17)	2.00** (2.11)
10000	3.13** (2.55)	2.73** (2.67)

**p < 0.01 (Williams' test)

2. Gross pathology - The liver was enlarged in both the 3000 (7/10) and 10000 (10/10) ppm groups in females when compared to the control group. Accentuation of the lobular markings of the liver was also seen in 4/10 male and 3/10 female mice at 10000 ppm versus 0/10 in the control groups. Other observed changes were considered to be incidental.

3. Microscopic pathology

a) Non-neoplastic - Slight to minimal enlargement of hepatocytes was observed in male and female mice receiving 300, 1000, 3000 or 10000 ppm in the diet. No other treatment-related changes were observed. The following table summarizes the microscopic changes observed in the liver.

Microscopic Pathology in the Liver												
Effect	Males						Females					
Slight enlargement of centrilobular hepatocytes	0/10	0/10	2/10	8/10	7/10	5/10	0/10	0/10	1/9	2/10	8/10	2/10
Minimal enlargement of centrilobular hepatocytes	0/10	0/10	0/10	0/10	2/10	5/10	0/10	0/10	0/9	0/10	0/10	8/10

b) Neoplastic - N/A

III. DISCUSSION

This study was a range-finding study for the mouse oncogenicity study. As such, it is classified as Core Supplementary, mainly because of the length of time the animals were exposed to the test chemical (5 weeks versus 13 weeks) and because of the lack of a complete microscopic examination.

Technically, the NOEL is 100 ppm, the lowest dose tested and the LEL is 300 ppm, based on the enlargement of the hepatocytes and supporting clinical chemistry, but most of the significant effects were observed at 10000 ppm. Based on the results of this study, the highest dose selected for the oncogenicity study was 3000 ppm. The Toxicology Branch agrees with the selection of 3000 ppm as the high dose for the oncogenicity study, mainly because of the significant decreases in bodyweight and bodyweight gain and the inferior food efficiency observed at 10000 ppm in this range-finding study.

C11775

Tox. Chem No. 025B File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mouse oncogenicity/ Huntingdon Res. Cntr./ #SMO 247/881026; 3/20/89 Update	Pynamin Forte; 93.1% pure	41099602	Technical pynamin forte (93.1% a.i.) was tested in a carcinogenicity study in groups of 52 CD-1 mice/sex/dose at 0, 120, 600 or 3000 ppm in the diet (MRID 41099602). The calculated intakes were 0, 14.4, 71.6 or 349.8 mg/kg/day for males and 0, 15.0, 77.3 or 382.2 mg/kg/day for females. The systemic NOEL is 600 ppm and the LOEL is 3000 ppm (increased relative liver weights (up to 135% of controls in males and up to 134% of controls in females); increased incidence of moderate fat deposition in the centrilobular hepatocytes in males (9/12 for the high dose group versus 1/12 in controls) associated with moderate enlargement and/or vacuolation of centrilobular hepatocytes (3/12 in high dose males versus 0/12 in controls for both parameters) at 52 weeks; moderate generalised fat deposition (3/12 in high dose females versus 0/12 in controls) at 52 weeks and increased incidence and degree of centrilobular hepatocyte enlargement in high dose males at termination. There was no indication of any increase in any type of neoplasia.	N/A	Acceptable

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001922

Chemical:	**d-Allethrin**
PC Code:	**004005**
HED File Code	**13000 Tox Reviews**
Memo Date:	**01/31/96**
File ID:	**TX011775**
Accession Number:	**412-01-0073**

HED Records Reference Center
12/18/2000