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DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity Study, Mouse.

TOX. CHEM NO: New Chemical

ACCESSION NUMBER: D176679 MRID NO.: 421783-10

TEST MATERIAL: Sumilarv Technical; Chem No 1021-RAEG

SYNONYMS: (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine. S-31183.

STUDY NUMBER: GLN 83-2.

SPONSOR: Sumitomo Chemical Co., Osaka, Japan.

TESTING FACILITY: Hazelton Laboratories, America., Vienna, VA.

TITLE OF REPORT: Sumilarv -- Oncogenicity study in mice with S-31183.

AUTHOR(S): Merrill R. Osheroff, PhD, DABT

REPORT ISSUED: HLA 343-215, July 23, 1991.

CONCLUSION:

Technical Sumilarv was incorporated in the diets of mice at levels of 0, 120, 600 and 3000 ppm corresponding to experimental groups G1, G2, G3 and G4, respectively. These diet concentrations resulted in dietary consumptions of approximately 22, 90, and 410 mg/kg/day for G2, G3 and G4, respectively. The main study groups (50 animals/sex/group) of animals were maintained on these diets for 78 weeks. An interim sacrifice (10/sex/group) was carried out after week 52.

There were no neoplastic findings that were relatable to the administration of Sumilarv. There was a dose-response relationship between mortality and the administration of Sumilarv. There was also a pervasive distribution of amyloid throughout all experimental groups.

The NOEL for systemic effects was 120 ppm and the LEL for non-oncogenic effects was 120 ppm and was based on increased incidence of early deaths beyond week 56. Based on an observed dose-response relationship in early mortality and increasing dose of Sumilarv, it appears as though an MDT was reached.

Classification: Core minimum.

Special Review Criteria (40 CFR 154.7) There are not trigger considerations based on this study.

*A. MATERIALS:

*A.1. Test compound: . Description - Grayish white crystalline; Batch #PYG-87074; Purity - 95/3%; obtained from Sumitomo Chem.

*A.2. Test animals: Species: Mice; Strain: Crl::CD1(ICR)BR from Charles River Labs., Kingston, NY. Age: 29 days. Weight: Males 21.7 to 25.6 gm; Females 19.8 - 28.5 gm.

*A.3. Significant Dates: Start of study -- 5/90/88; Start of animal dosing -- 7/12/88; week 52 interim sacrifice; completion of necropsy -- 1/5/90; Submission of study report 7/23/91.

*B. STUDY DESIGN:

*B.1. ANIMAL ASSIGNMENTS. From 560 animals originally obtained from the supplier, 260 males and 261 females were available to be assigned by computer to four experimental groups (60/sex/group) as shown in Table 1:

	GROUP ASSIGNMENTS				
	Dose in	Main Study		Interim Sac.	
Test	diet	78 months		At week 52	
GROUP (ppm)		Male	Female	Male	Female
G1 Control	0	50	50	10	10
G2 Low (LDT)	120	50	50	10	10
G3 Mid (MDT)	600	50	50	10	10
G4 High (HDT)	3000	50	50	10	10

*B.2. DIET PREPARATION Fresh diets were offered to the animals on a weekly basis. The basal diet was Purina Certified Rodent Chow # 5002 which was fed directly to the control animals. Test agent diets were prepared using the following procedures: Crystalline Sumilarv was heated to a liquid state and divided into portions which were stored for future use in solid form under refrigeration. When ready for mixing in the diet, the solid Sumilarv was again liquified and mixed serially with the basal diet in a Hobart mixer. This premix was stored cool overnight and on the next day was further mixed with a mortar and pestle with basal diet to a concentration of 3000 ppm Sumilarv used for the G4 test animals. The 3000 ppm mix was further

diluted with basal diet to 120 and 600 ppm levels used for G2 and G3 test diets, respectively. The homogeneity of distribution and concentration of the test agent in the diets were verified using extraction procedures and HPLC methods to measure the test agent.

Results: Stability and Homogeneity. Agent stability studies in the diet were carried out for diets containing 50 ppm and 10,000 ppm Sumilarv. The studies were done in triplicate and indicated that Sumilarv was stable in the diet for at least 14 days, either at room temperature or when stored under refrigeration. The results showed recoveries of 98% to 103% of the target concentrations.

Homogeneity analyses of the diet were based on analyses of samples from the top, middle and bottom of one diet preparation. These samples ranged from 97% to 104% of the target dose. Adequate homogeneity was further reflected in the analyses of the different batches of diet over the 18 month study which indicated means of 97.6% to 98.7% of target concentrations with a relative standard deviations approximating 3%.

*B.3. HUSBANDRY. The animals were held for 2 weeks prior to assignment to the study. Animals on study were housed individually during the 18 month experimental study under normal laboratory conditions (12 hours of light and dark, 6 AM to 6 PM; temperature range of 65 to 78 degrees F.; relative humidity range of 26 to 78%) and received diets prepared weekly and water ad libitum from automatic water dispensers.

*B.4. STATISTICS Means and standard deviations were applied to many of the data using statistical significance based on 5% confidence limits. Other statistical procedures were used included a trend analysis (from NCI), Leven's test for homogeneity using several transformation procedures; Dunnett's test for significance; ANOVA, etc. It was not clear from the text of the report which statistical methods were used for which specific data.

*.B.5. QUALITY ASSURANCE. A quality assurance statement (page 5 of the report) was included in the report and was signed by Janet Milazzo on 7/23/91. A flagging statement by S. Yamane, Sumitomo, 8/1/91 (page 4 of the report) for criteria from 40 CFR 158.34 was reported as negative.

*C. METHODS AND RESULTS:

*C.1. OBSERVATIONS: The animals were inspected twice daily for signs of toxicity and mortality. Hands-on-physical examinations were performed once weekly.

Results: Cage-side observations. None of the clinical

signs were relatable to the test agent. A number of signs were seen in many of the animals including neurological signs (hunched back, tremors/convulsions, sensitivity to touch and reduced motor activity); animals coats discolored with urine stains, alopecia, rough hair coat and skin sores. Respiratory signs (primarily dyspnea) were seen in a number of animals (up to 5 animals in a group). Palpable tumor masses occurred rarely in both sexes. The incidence of hunched positions was greater in females while urine staining and the occurrences of swollen bodily areas were greater in males.

*C.2. MORTALITY/SURVIVAL. Results. Selected mortality/survival data are also shown in **Table 1.** Survival remained high in most test groups up to week 52 and dropped substantially from week 52 to week 78. From week 52, mortality in G2, G3 and G4 males increased at a greater rate than control males in proportion to the level of the test material in the diet. Dose-related increases in female mortality increased from about week 60. Males mortality rates were somewhat greater that females.

Prior to study termination, male mortalities were 28/50, 22/50, 14/50 and 9/50 for G1, G2, G3 and G4, respectively. Corresponding survival for females were 30/50, 27/50, 22/50 and 18/50, respectively.

*C.3. BODY WEIGHT Individual body weights were recorded prior to the initiation of the treatment diets, weekly for weeks 1-16 and every 4 weeks thereafter.

Results. Selected body weight data are shown in **Table 2.** Over the course of the 78 week exposure period, body weights were generally unaffected by the administration of the test material. The overall weights of G4 males deviated downward from the body weights of the other males and remained proportionally lower for the remainder of the study. Statistical evaluation of mean body weight data by the investigators revealed a significant depression in the mean absolute body weight of the G4 males (at week 13 an approx. 5% depression, at week 24, an approx 6% depression and at week 52, a 4% depression).

The female body weights were consistent between all of the 4 groups until about week 60 when the G4 female weights dropped away from the other three groups of animals. Body weights of G4 females were significantly depressed approximately 5.5% at week 76. Although body weights are significantly decreased for both sexes in the high dose groups, there is no biological significance attached to these findings. Other significant differences in mean absolute body weight values and mean body weight gain values (as well a food consumption) were considered incidental findings.

*C.4. FOOD CONSUMPTION AND COMPOUND INTAKE. Total food consumption and mean daily diet consumption was calculated weekly for weeks 1-16 and every 4 weeks thereafter.

Results. Selected food consumption data are shown in **Table 3.** Food consumption throughout the study was quite variable for all groups with no trends associated with treatment until late in the experimental period. In the last several weeks the males of G1, G2 and G3 tended to consume considerably more food than the G4 males. The same increase was seen in the corresponding females (G1-G3); however, the high dose females consumed considerably less food than the other three groups. There was no compound related trends for food consumption. Food consumption changes seemed to be reflected in body weight changes.

Overall mean chemical consumption data for the individual test groups were not presented in the report. Mean compound consumption rates were variable over the course of the study but were as might be expected. Mean compound consumption was presented as weekly means (mg/kg/day) which ranged as follows:

GROUP	PPM	MALES	FEMALES
Group 2	120	15 to 22 mg/kg/day	21 to 27 mg/kg/day
Group 3	600	76 to 105	103 to 142
Group 4	3000	391 to 522	476 to 680

*C.5. OPHTHALMOLOGICAL EXAMINATIONS Were carried out initially as a screening procedure only in order to remove animals which had obvious eye problems. There was no further in-life examinations and no histopathological observations which might relate any test substance induced changes.

*C.6. HEMATOLOGY. Planned hematological analyses were carried out for the 52 week interim sacrifice and for the terminal 78 week sacrifice, 10 animals/sex/group for each sacrifice. Blood was obtained by orbital sinus puncture using capillary tubes to collect the blood. The following CHECKED (X) parameters were examined.

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		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required of chronic studies.

Results: There were some group data which showed statistically significant changes from control animals however, none of the data from the interim sacrifice of terminal sacrifice

depicted changes that could be related to the exposures to Sumilarv. Hb analyses in the G4 females were significantly reduced at week 52 and reduced (without statistical significance) at week 78. There was not a corresponding effect in the Hb of the corresponding male treatment groups. The mean corpuscular volumes of G4 males were reduced at week 52 but not at week 78.

*C.7. NECROPSY. All animals that were found moribund or died spontaneously or were sacrificed on schedule (at weeks 53 and 79) were subjected to gross pathological examination. Sacrificed animals were euthanized using lethal sodium pentobarbital anesthesia. Necropsies included examination of the external condition of the body and gross examination of all organs and cavities of the body.

The following list of organs were weighed as marked (marked with a W) and/or examined histologically unless otherwise noted:

ORGANS TAKEN AT NECROPSY.

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

* Required for chronic studies.
+ Organ weight required in chronic studies.

*C.8. GROSS PATHOLOGY Gross pathological observations were presented in the report in three tables: 9A, 9B and 9C for the unscheduled deaths, interim sacrifices and terminal sacrifices, respectively. The results show very little gross pathology that was remarkable or relatable to the administration of the test agent other than the effects of amyloidosis discussed below. There was an increased incidence of granular/pitted/rough kidneys in the G4 males and females in unscheduled deaths and G4

females at week 53 interim kill and week 79 terminal kill.

*C.9. ORGAN WEIGHTS - Organ weights were obtained from animals at the 53 week interim sacrifice and at the end of the study (week 78) of 10 animals/sex/group/sacrifice. The organs weighed included the brain, liver/gall bladder, kidneys, spleen, testes, and ovaries and adrenal.

Results: There were no significant organ weight changes which could be ascribed to the test materials. Organ weight data were not presented for the unscheduled death necropsies which constituted a large percent of the animals used in the study. Organ weight data based on absolute and relative weights were somewhat variable and inconsistent between the different necropsy groups (interim or terminal) and between the sexes. Following 52 weeks of treatment, the mean absolute spleen and liver/gallbladder weights and mean liver to terminal body weight ratio values for the G4 females were significantly higher than respective control values. The increase in absolute weights of the spleen and liver/gall bladder was not seen in the corresponding G4 males, nor were these trends seen in either sex of corresponding terminal sacrifice males or female.

At the termination sacrifice, the mean absolute kidney weight value for G4 males was significantly lower than the control value. This effect was not seen in the terminal female organs or in the male or female organs of the interim necropsy groups.

*C.10. NON-NEOPLASTIC HISTOPATHOLOGY. The only non-neoplastic change which was relatable to the exposures of Sumilarv was due to amyloidosis. Other findings were seen which related to the general condition of the colony of animals and are discussed below.

Amyloidosis. Amyloidosis permeated a high percent of the animals in all test groups and was assumed by the pathologist to be the cause of death in most of the unscheduled deaths (see Table 4). The organs directly effected included the adrenal, thyroid, parathyroid, heart, spleen, kidney, liver, glandular stomach, duodenum, jejunum, ileum, testes and ovaries. Generally all of these organs were involved with the amyloid process on those animals effected. The gall bladder was affected by amyloidosis but not in an obvious dose response relationship. The lung, non-glandular stomach and pancreas were not significantly affected by amyloid.

Approximately 60 to 75% of the animals in all groups were affected by amyloid. It was present in 40 to 100% in the interim necropsy group (at 52 weeks) and was responsible for a high percentage of the deaths (40-84%) of the unscheduled deaths, leaving a correspondingly reduced number of animals for the

terminal sacrifice (Table 1).

Kidney. A second condition which had a high incidence was the mineralization of the kidney tubules and papilla, seen more frequently in the high dose males and females.

Infections. A review of the histopathology summaries by the pathologists for the different necropsy groups indicated that many of the animal deaths in all necropsy groups were associated with sepsis, infection and inflamations. There was no information on the preliminary health status of these animals regarding infection with bacteria, viruses or or the identification of parasites during necropsy.

Extramedullary Hematopoiesis was seen in several organs: liver, kidney, spleen, and mesenteric lymph nodes and noted in many of the necropsied animals and may be normal for this group of mice.

*C.11. NEOPLASTIC HISTOPATHOLOGY. None of the neoplastic findings in this study can be related to the treatment with Sumilarv. **Tables 5 and 6** show selected data for the liver and lung, respectively, and are presented as examples of the lack of tumorigenic responses in this CD-1 mouse study and the comparison of this study with data from historical controls for these organs. The comparison of the current study with the historical control data from the supplier (Charles River Laboratories) show the current control data are consistent with past historical controls.

*D. DISCUSSION:

*D.1. Design Objectives. The study met its design objectives relative to the incorporation of the test substance in the diet at the planned levels of 120, 600 and 3000 ppm. The number of unscheduled deaths increased with the exposure level correspondingly reducing the number of animals available for terminal necropsy.

*D.2. DOSE-RELATED FINDINGS. The mortality rates for all animals was comparable up to 52 weeks beyond which time, the mortality rates for G2, G3 and G4 animals increased in a dose response fashion. There was an obvious trend in mortality which was associated with the amyloid as a cause of death.

Mortalities were clearly affected by the administration of the test material as seen in the increase in the number of animals dying spontaneously (unscheduled deaths) and the decrease in the number of animals available for the terminal sacrifice shown in **Table 4.** Amyloidosis was the major cause of unscheduled deaths beyond week 52.

Amyloidosis is frequently reported as a finding in laboratory animals but is usually limited in its degree and primarily affects the kidney (Harkness and Wagner, 1983. On the other hand, some pathologists do not bother to report amyloid, considering it to be a background lesion (Lang 1993; and Lang 1980).

*D.3. POSITIVE FINDINGS NOT RELATED TO AGENT. Observations: There were a number of adverse signs observed throughout the experimental groups (discussed above). A number of these were related to the function of the nervous system (hunching, seizures, reduced activity) but it was not possible to relate this to the exposure of Sumilarv; nor to any organ pathology.

Body Weight Changes. Body weight changes (reduced in the G4 males early on, see Table 2) and in dose-response relationship in the last several weeks of the study seen in males and females. However, body weight changes, food consumption and agent consumption are not a significant finding in this study.

*E. REFERENCES.

-- Charles River Laboratories (1987). Spontaneous Neoplastic Lesions in the Crl:CD-1 (CICR)BR Mouse. Information compiled by Patricia L. Lang and published by CRL, Feb. 1, 1987.

-- Charles River Laboratories (1989). Survival of CrL:CD-1BR Mice During Chronic Toxicology Studies. Reference Paper, Fall 1989.

-- Lang, Patricia A. (1980). Letter to Martin L. de Vries, Penick Corporation, Orange NJ by P.A. Lang, consultant, Yardely, PA. Letter contains a review of amyloidosis in mice. Obtained from John Doherty, Toxicology Branch I.

-- Lang, Patricia A. (1993). Facsimile to John Doherty dated 4/23/93

*T1

TABLE 1. SURVIVAL FOR WEEKS 56 -78)

MALES

WEEK	Control	120 ppm	600 ppm	3000 ppm
56*	46/50	44/50	45/50	43/50
60	45/50	42/50	42/50	38/50
64	43/50	36/50	35/50	30/50
68	39/50	31/50	29/50	23/50
72	36/50	28/50	22/50	16/50
76	31/50	24/50	15/50	12/50
78	28/50	22/50	14/50	9/50
Total Unscheduled Deaths	23/50	28/50	37/50	42/50
	46%	56%	74%	84%

FEMALES

WEEK				
56*	47/50	45/49**	44/50	49/50
60	46/50	44/49	43/50	45/50
64	44/50	43/49	43/50	36/50
68	41/50	41/49	39/50	27/50
72	37/50	37/49	35/50	23/50
76	33/50	32/49	26/50	20/50
78	30/50	27/49	22/50	18/50
Total Unscheduled Deaths	20/50	23/49	29/50	32/50
	40%	47%	58%	64%

* Number of animals following interim sacrifice.
** Animal lost prior to interim sacrifice.

*T2 **TABLE 2. SELECTED MEAN BODY WEIGHT DATA (GM).#**

	MALES				FEMALES			
GROUP:	1	2	3	4	1	2	3	4
WEEK								
1	31.6	31.1	31.4	30.8	23.8	23.7	23.3	23.4
13	39.4	39.2	39.7	37.5*	29.3	29.7	28.9	29.3
52	42.7	41.7	43.4	40.9	34.3	35.3	35.0	34.8
76	42.3	42.6	44.9*	41.2	35.6	36.6	35.8	33.9*

Numbers of animals: 60/group for wk.1; 54-60/group for week 52; and 12 to 33/group for week 76.
* Significant at the 5% level.

*T3 TABLE 3. SELECTED MEAN FOOD CONSUMPTION DATA (GM/WK).

	MALES				FEMALES			
GROUP#	1	2	3	4	1	2	3	4
WEEK								
1	38.1	41.3	40.5	38.5	35.4	39.0	40.8	35.1
52	38.6	39.9	41.2*	39.5	38.2	40.8*	41.7*	40.0
76	42.6	41.7	46.0*	40.3	42.3	41.6	42.3	37.7*

Numbers of animals: 60/group for wk.1; 54-60/group for week 52; and 12 to 33/group for week 76.
* Increased significantly over control food consumption rates at the 5% level.

*T4

Table 4. SELECTED NON-NEOPLASTIC HISTOPATHOLOGY: AMYLOID.

	MALES				FEMALES			
GROUP	1	2	3	4	1	2	3	4
INTERIM NECROPSY								
Number	9	10	9	9	10	10	9	10
Amyloid*	6	8	7	5	4	4	5	10
UNSCHEDULED NECROPSY								
Number	23	28	37	42	20	23	29	32
Amyloid*	19	24	32	38	16	21	23	32
TERMINAL NECROPSY								
Number	28	22	14	9	30	27	22	18
Amyloid*	16	7	6	1	10	9	8	5
TOTALS								
Totals with Amyloid**	41	39	45	44	30	34	36	45
% with amyloid**	68	65	75	73	50	57	60	75

* Amyloid number is based on the tissues with the highest incidence of amyloid. The organs directly and uniformly effected included the adrenal, thyroid, parathyroid, heart, spleen, kidney, liver, glandular stomach, duodenum, jejunum, ileum, testes and ovaries.

** Based on 60 animals from all of the necropsy groups.

*T5

TABLE 5. SELECTED NEOPLASTIC HISTOPATHOLOGY LIVER

	MALES				FEMALES			
GROUP	1	2	3	4	1	2	3	4
INTERIM NECROPSY								
Hepat. Adenoma	0	0	2	1	0	0	0	0
Hepat. Carcinoma -	0	0	0	0	0	0	1	0
UNSCHEDULED NECROPSY								
Hepat. Adenoma	1	2	2	2	1	0	1	0
Hepat. Carcinoma	1	0	0	0	0	1	0	0
Hemangsarcoma	0	0	0	1	0	1	0	2
TERMINAL NECROPSY								
Hepat. Adenoma	2	1	4	2	0	0	1	0
Hepat. . Carcinoma	1	0	0	0	0	0	0	0
Hemangsarc.	0	1	0	0	0	0	0	1
TOTALS								
Liver Tumors	5	6	4	4	1	2	3	3
% Liver Tumors/of 60	8	10	7	7	2	3	5	5
By Sex Groups	19/240 (8%)				9/240 (4%)			
HISTORICAL CONTROLS for all tumors.***								
By Sex Groups	55/499 (11%)				16/497 (3%)			
Range***	(0- 16%)				(0- 6%)			

* includes all liver tumors ..
** Range includes the highest of any one type neoplastic lesion.
*** Charles River Laboratories (1987).

*T6

TABLE 6. SELECTED NEOPLASTIC HISTOPATHOLOGY LUNG**

	MALES				FEMALES			
GROUP	1	2	3	4	1	2	3	4
INTERIM NECROPSY								
Alveo./bronch. Adenoma	0	0	1	0	0	0	0	0
Alveolar/bronchogenic CA.-			1					
UNSCHEDULED NECROPSY								
Alv./Bronch. Adenoma	1	1	3	0	0	0	1	0
Alv. Bronch. Carcinom	1	2	2	1	0	0	0	0
TERMINAL NECROPSY								
Alveo./Bronch Adenoma	0	1	0	0	2	2	3	0
Alveo./Bronch. Carcinoma	3	2	0	0	3	3	1	0
TOTALS								
Lung Tumors	5	6	7	1	5	5	5	0
% Lung Tumors/of 60	10	10	17	2	8	10	8	0
By Sex Groups	23/240 (10%)				17/240 (7%)			
HISTORICAL CONTROLS (for all lung tumors).***								
By Sex Groups	45/496 (9%)				22/496 (4%)			
Range	(0- 14%)				(0- 9%)			

** Lists all lung tumors in this study.
*** Charles River Laboratories (1987).

*T7 **TABLE 7. MORTALITY AND AMYLOID IN UNSCHEDULED DEATHS.**

SEX	MALES				FEMALES			
Group	G1	G2	G3	G4	G1	G2	G3	G4
Unscheduled deaths	23	28	37	42	20	23	29	32
Death from amyloidosis	19	21	28	35	13	17	16	32
Percent Deaths from Amyloid	83	75	76	83	65	74	55	100

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