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Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity- dog

GUIDELINE #: 83-1

P.C. Code: 129032

MRID #: 421783-09

TEST MATERIAL: S31183 (94.9%)

SYNONYMS: Sumilarv, Nylar

STUDY NUMBERS: 91/0776

SPONSOR: Sumitomo Chemical Company, Ltd.
Osaka, Japan

TESTING FACILITY: Life Sciences Research, Ltd.
Suffolk, England

TITLE OF REPORT: S31183: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 52 Weeks.

AUTHORS: E.A. Chapman

REPORT ISSUED: August 1, 1991

CONCLUSIONS:

When the S-31183 was administered to male and female beagle dogs for approximately one year, the NOEL was 100 mg/kg/day and the LOEL was 300 mg/kg/day based on statistically and biologically significant decreases in body weight gain and increases in relative liver weights in both sexes.

In males receiving 300 mg/kg/day there was also significant increases in cholesterol levels throughout the study and increases in triglyceride levels at week 50. Mild anemia was also present in males at this dose level and was characterized by significant decreases in hemoglogin and red cell count when compared to controls.

At 1000 mg/kg, death was reported in two of the four males and was attributed to hepatic failure. In the remaining males, there

was a significant increase in prothrombin time, and in both sexes there were increases in hepatic enzyme levels and gross and microscopic hepatic lesions. Decreases in body weight gain were also reported for both sexes and there was an increase in relative and absolute liver weights at the high dose level.

CLASSIFICATION: Guideline

MATERIALS:

The test material was S-31183, lot number 87074, 94.9% active ingredients. The material was a thick brown liquid and was administered to the test animals in gelatin capsules. The test animals were male and female beagle dogs, obtained from Consort Ltd., Herefordshire, England, weighing from 7.0 to 9.2 kg. At the start of the study, the animals were 16 to 20 weeks of age (23 to 27 weeks at start of treatment).

METHODS:

Prior to the initiation of the study, the identity, strength and purity of the test material and the stability of the material in gelatin capsules was determined. The maximum time between the preparation of the gelatine capsules and their use was 4 days.

After under going an acclimation period of approximately 7 weeks, during which animals were vaccinated, dewormed and dipped for ectoparasites, animals were randomly assigned to treatment groups. During the acclimation period, animals also had blood samples collected for health screening. All dogs were individually housed in an environment that allowed for a 12 hour light/dark cycle. The temperature and relative humidity in the environment ranged from 18 to 25°C, and 70%, respectively.

During the study, water was provided ad libitum. Each animal was offered 400 grams of food daily and food was withheld from the animals shortly before dosing. On days when blood and urine were collected, uneaten food was withdrawn in the afternoon prior to sample collection.

Dogs were assigned to the following treatment groups after being ranked according to body weights:

GROUP #	DOSAGE (mg/kg)	# ANIMALS	
		M	F
1	0	4	4
2	30	4	4
3	100	4	4
4	300	4	4
5	1000	4	4

Control animals received empty 1/2 ounce gelatin capsules. Based on the body weights of the test animals, the test material was administered in either one capsule or two. Animals received test material daily for 52 weeks.

Daily observations were conducted to assess the toxicity of the compound and the general health of the animals. All abnormalities were recorded. Weekly detailed physical examinations were conducted and abnormalities were also recorded.

Food consumption was recorded daily, body weights were recorded weekly and food efficiency ratios were calculated monthly. Ophthalmoscopic examinations were conducted prior to the beginning of the study and at weeks 26 and 51. Blood was collected at weeks 12, 24, 37 and 50 for hematology and serum chemistry evaluations. Urinalysis was conducted prior to treament and on weeks 11, 23, 35 and 49.

The following parameters were evaluated following sample collection:

x Hematocrit (HCT)
x Hemaglobin (HGB)
x Leucocyte count (WBC)
x Erythrocyte count (RBC)
x Platelet count
x Leucocyte differential
x Mean corpuscular hemaglobin
x Mean corpuscular hemaglobin concentration
x Mean corpuscular volume
x Reticulocytes

Blood clotting measurements:
Thromboplastin time
Clotting time
x Prothrombin time

Electrolytes:
x Calcium
x Chloride
Magnesium
x Phosphorus
x Potassium
x Sodium

Enzymes:
x Creatinine phophokinase
x Alkaline phosphatase
x Lactic dehydrogenase
x SGPT (ALT)
x SGOT (AST)
Gamma glutamyl transferase
Glutamate dehydrogenase
Cholinesterase

Other Serum Chemistry Values:
x Albumen
x Blood creatinine
x BUN
x Cholesterol
x Globulin
x Glucose
x Total Bilirubin
x Total protein
x Triglycerides
Serum protein electrophoresis

Urinalysis
x Appearance
x Volume
x pH
x Spec gravity
x Protein
x Glucose
x Ketones
x Blood
x Urobilinogen
x Nitrites
x Bilirubin
x Sediment

All animals were sacrificed by exsanguination while under sodium pentobarbitol anesthesia. A full gross necropsy was performed on all animals. Animals sacrificed in extremis were also subjected to a full necropsy. Tissues were preserved in 4% formaldehyde. Eyes and optic nerves were fixed in Davidson's fluid and were stored in 70% methylated spirits.

MCV and MCH values were calculated. A costal bone marrow smear was obtained from each of the dogs that was killed prior to the termination of the study and for those sacrificed after 52 weeks on the stuidy. Samples were examined for cellularity and myeloid: erythroid ratios were determined.

The following CHECKED (x) tissues were collected for histological examination, embedded in parrafin and stained with hematoxylin and eosin. Weighed organs are designated by (xx).

Digestive system	Cardiovasc./Hemat.	Neurologic
x Tongue	x Aorta	xx Brain
x Salivary glands	xx Heart	x Periph. nerves
x Esophagus	x Bone marrow	x Spinal cord
x Stomach	x Lymph nodes	x optic n.
x Duodenum	xx Spleen	
x Jejunum	xx Thymus	Glandular
x Ileum		x Parathyroids
x Cecum		xx Adrenals
x Colon	Urogenital	xx Thyroid
x Rectum	xx Kidneys	xx Pituitary
	x Urinary bladder	x Mammary
xx Liver	xx Testes	
x Gall bladder	x Epididymides	Other
x Pancreas	xx Prostate	x Bone
	x Urethra	x Skin
Respiratory	xx Ovaries	x Skel. muscle
x Trachea	xx Uterus	x All gross lesions
xx Lung	x Vagina	
x Larynx		

STATISTICAL ANALYSIS:

Group means and standard deviations were determined for food consumption, body weights and organ weights. Incidences of clinical signs were determined based on the number of days in which signs were observed. Incidence values were expressed as a percent of the maximum possible occurence and rounded to the nearest 5%. Student's T Test was used to determine intergroup differences in body weight changes and in blood, serum chemistry and urinary parameters. Intergroup differences in organ weights were analyzed using Dunnett's Test and differences in histopathology lesions between control and treated groups were assessed using Fisher's Exact Test. Significance was at $p < 0.05$.

QUALITY ASSURANCE:

A statement of Quality Assurance, dated August 1, 1991 was included in the submission. A GLP compliance statement dated December 13, 1991 was also included.

RESULTS:

Test Substance Analysis

The content of S-31183 in the gelatin capsules used in the low dose and high dose groups was determined by HPLC. Based on the analysis, when capsules were stored at 50 degrees centigrade for 5 days, the content of S-31183 ranged from 98 to 103% of the initial content at the lowest dose and 103% of the initial concentraion at the highest dose. After two days of storage, the capsules contained between 103 and 105% of the initial concentration at the lowest dose and between 102 and 103% at the highest dose. Based on this, it was concluded that after 4 days, the estimated content of the test material in the capsules was 100% of the initial content and, on average, 97 and 103% of the intended content for the high and low dose groups, respectively.

Clinical Signs

The most commonly observed clinical signs were salivation, emesis and diarrhea. Thin appearance was reported for 2 males in the high dose group and 2 males at 300 mg/kg. Salivation was increased in incidence at 1000 mg/kg, and was occasionally reported at 300 and 30 mg/kg. Diarrhea was reported on occasion in all groups but was increased in frequency in the high dose male and female groups. Emesis occured with a greater frequency in the high dose animals and isolated incidences were reported at 100 mg/kg in both sexes. (See Table I).

Mortality

Two high dose males were sacrificed <u>in extremis</u>. One animal had a thin appearance and inappetance was observed throughout the time that this animal was on the study (17 weeks). This animal also lost 3.3 kg in body weight during this time period. Liver failure was diagnosed after sacrifice. The other animal was sacrificed on week 31 after liver failure was diagnosed and confirmed.

Body Weight, Weight Gain and Food Consumption

Lower mean body weight gains were reported in males receiving 300 and 1000 mg/kg of the test material. These lower weight gains were apparent during the first 13 weeks of the study. At 300 mg/kg, the body weight gains were 46.5 % lower than controls and at 1000 mg/kg, body weight gains were 89.3 % lower. The compound did not appear to have an effect on males receiving 30 and 100 mg/kg and after the initial 13 weeks body weight gains were similar for these two groups of males.

At the end of the study, the body weight gains were comparable to controls, with the exception of a significantly ($p < 0.01$) lower weight gain reported for males receiving 300 mg/kg. However after recalculating the weight gains from the data provided for the highest dose group in males, there also appeared to be a significant difference in the body weight gains when animals in the 1000 mg/kg group were compared to controls. The differences in weight gains reported in male dogs receiving the test compound at doses greater than 100 mg/kg suggest that there may be a relationship between the administration of the test material and the observed effects on body weight gain. (See Table II).

Females receiving 100, 300 and 1000 mg/kg of the test material gained weight but weight gain was less than that recorded for controls, especially from weeks 26 to 39 of the study. Differences of 71.5% were reported for 300 mg/kg dogs and differences of approximately 86% were reported for females receiving 1000 mg/kg/day. The totals for body weight change at the end of the study suggest that there may be an association between the dose level of S31183 that was administered and body weight gains especially at the two highest dose levels in females.

Food consumption was decreased for high dose males., overall but was variable throughout the study. No effect on food consumption was reported for high dose females or for the other dose groups. There did not appear to be an effect on food efficiency.

Hematology, Serum Chemistry and Urinalysis

Red cell parameters were affected in males at 12 and 24 weeks and in females at 12 and 37 weeks. In males receiving 300 mg/kg and in females receiving 100, 300 and 1000 mg/kg, low erythrocyte counts and hemoglobin concentrations were reported when compared to controls. The decreases in these parameters were sporadic in occurence in females, and in males in the high dose group, these findings were not as apparent. In males, however, the significance of the findings at 300 mg/kg should not be discounted because they are not present to the same degree at 1000 mg/kg, since two males were available in the high dose group for evaluation at the last two intervals.

High values for mean cell volume (MCV) were also reported in males and females receiving the highest doses of S31183, but these values are within the normal range for dogs. (See Table III).

At the highest dose tested, platelet counts were high and prothrombin times were prolonged throughout the study. In males receiving 300 mg/kg, platelet counts were high at 12, 24, 37 and 50 weeks. At 100 mg/kg, high platelet counts were recorded at weeks 37 and 50.

7

The serum chemistry parameters that appeared to be most affected by the administration of the test material in animals receiving 1000 mg/kg were alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol and triglycerides. In the highest dose group, there were reported increases in AP, ALT and AST in both sexes. Triglyceride levels were also elevated, but the elevation was primarily at week 50. Cholesterol levels were increased in both sexes, but in males the elevation was only recorded after week 24 and returned to normal by week 50. Decreases were reported for both calcium and chloride concentrations; however, these did not persist and were considered only marginal decrements, not significantly outside of the normal range.

At 300 mg/kg and 100 mg/kg, AP and triglyceride levels were elevated in both males and females. Hypercholesterolemia was reported in males at both of these dose levels and persisted through week 50.

At the lowest dose tested, a slight increase in cholesterol was reported; however, the reported increase appeared to be caused by one animal with high levels of cholesterol that persisted throughout the study. The reported increase was not outside of the acceptable normal range for dogs and was not considered to be related the administration of the test material.

With regard to urinalysis, high urine volume and low urine pH were reported for 1000 mg/kg males throughout the study. In high dose females, decreased urine pH was reported at week 35. In the absence of renal lesions, the biological significance of these findings is unknown.

The compound did not ap[pear to have an effect on bone marrow function based on the results of the marrow smears.

Gross and Microscopic Pathology

Large livers and livers with irregular surfaces were found on gross examination of males receiving 1000 mg/kg. (See Table V). One male had numerous masses in the liver and a mass which replaced the renal lymph node.

Microscopically, hepatic damage characterized by centriacinar fibrosis and bile duct hyperplasia was observed in high dose males and females. Chronic inflammatory infiltrates, cystic degeneration, cellular vacuolation were also present. The number of animals with these pathological findings was significantly higher when the high dose was compared to the control group for both sexes. ($p < 0.05$)

Increased liver weights were also present at the lower dose groups; however, at 30 mg/kg and 100 mg/kg, the weight increase was not associated with morphological changes and was probably an adaptive change and of little biological significance. Females receiving doses below 300 mg/kg did not have statistically significant increases in absolute liver weights.

Degeneration of the seminiferous epithelium and hyperplasia of the transitional epithelium of the bladder was observed in males. The effect on the seminiferous epithelium was not believed to be related to treatment because it affected only a small number of tubules and is commonly observed in male dogs of this age. The changes in the bladder were also not considered to be related to the compound since there was no dose related increase in this lesion and it was found in one female receiving 300 mg/kg, one male receiving 1000 mg/kg and one male and one female receiving 100 mg/kg.

There were no other gross or microscopic lesions attributable to the administration of the test material.

DISCUSSION:

The results of this study indicate that following chronic administration of the compound S31183, the liver was the target organ with hepatic toxicity being reported at dose levels of 300 mg/kg and above. Gross and microscopic lesions and clinical pathology could be correlated to hepatotoxicity. In addition, the observed increase in prothrombin time could also be associated with liver because of the role of that organ in the production of clotting factors 2, 7, 9 and 10. Prothrombin times are statistically elevated at the high dose level starting at week 12 and continuing through week 50. If considered alone, prothrombin time would have little biological significance; however, when this finding is analyzed in light of the gross and microscopic pathology it provides further support to the conclusion that the chemical is hepatoxic.

Although the findings at 1000 mg/kg suggest that the clinical pathology at the next lower dose is incidental, it should be kept in mind that the evaluations made after week 17 were not made on an entire group of animals due to early deaths of two high dose males. Hematological findings in males suggest a mild anemia at 300 mg/kg. This is based on reported decreases in red blood cell counts, hemoglobin and packed cell volume (hematocrit).

Although the hematology values appear to be in a biologically normal range and represent only mild changes, when these are coupled with serum chemistry data, the observed effects on red cell parameters may be secondary to hepatic toxicity. Abnormal lipid metabolism as evidenced by increases in both serum

cholesterol and triglycerides has also been associated with a decrease in the life span of red blood cells and an increase in fragility may result in a mild or slow hemolysis that may barely be perceptible and may be further compromised by reduced hepatic synthesis of clotting factors.

The hepatotoxicity observed in this chronic dog study appears to be associated with the administration of the test material and is supported by secondary effects on other parameters such as hematology and clotting times. The study satisfies the requirements for a chronic non-rodent toxicity study pursuant to Guideline 83-1.

The NOEL is 100 mg/kg and the LOEL is 300 mg/kg. These values differ from those provided in the report summary; however it is the opinion of this reviewer that the effects observed at 100 mg/kg (increased liver weight, sporadic changes in red blood cell counts, hemoglobin and cholesterol) were not biologically significant. The increased liver weights ~~were~~ appeared to be adaptive in nature and were not accompanied by gross or microscopic pathology. Although the MCV values were statistically elevated at 100 mg/kg, these values were within the normal range of 66 to 77 u^3 for dogs. In their report, the author states that elevations in hepatic enzymes also occured in animals receiving 100 mg/kg; however, data analysis show that neither ALT or AST enzyme levels were elevated and, AST levels were, in fact, significantly lower than controls. In conclusion, the effects reported at 100 mg/kg were non-specific and did not differ in magnitude from the effects observed at 30 mg/kg.

TABLE I
CLINICAL OBSERVATIONS and FREQUENCY

MALES

Clinical Obs.	Number of Observations Dose Group (mg/kg) 0	30	100	300	1000
Diarrhea	51	15	56	75	110
Salivation	0	1	0	7	125
Emesis	0	1	0	0	37
Thin Appearance	0	1	0	287	176
FEMALES					
Diarrhea	59	3	22	81	83
Salivation	2	3	0	4	247
Emesis	4	2	0	0	15
Thin Appearance	0	0	0	0	0

The possible number of observations for all females and for males in Groups 1 thru 4 was 1456. The possible number of observations for males in Group 5 (1000 mg/kg) was 1061. This takes into account the deaths that occured prior to the termination of the study.

TABLE II

MEAN BODY WEIGHT CHANGE (kg)

Males Interval (days)	Dose Group (mg/kg) 0	30	100	300	1000
0- 91	2.8	2.5	2.0	1.5*	0.3#
91- 182	0.4	0.5	0.1	0.1	0.3
182- 273	0.6	0.7	0.5	0.5	1.1
273- 364	0.1	0.2	0.1	0.0	0.6*
Total 0 - 364	3.9	3.9	2.7	2.0**	2.3**a
Females					
0- 91	1.8	1.9	1.9	1.5	0.9*
91- 182	0.8	0.6	0.2	-0.1*	0.2
182- 273	0.3	0.5	0.7	0.6	0.9*
273- 364	0.7	0.5	0.2*	0.2*	0.1*
Total 0 - 364	3.5	3.4	3.0	2.2*	2.0*

a = a corrected value from data provided in Table 3 of the submission).

* = $p < 0.05$
** = $p < 0.01$
\# = $p < 0.001$

TABLE III
MEAN HEMATOLOGY VALUES

Females

Parameter: PCV (%)		DOSE (mg/kg) 0	30	100	300	1000
Week	-1	42	39	42	41	41
	12	44	42	41	39*	42
	24	43	43	45	41	43
	37	47	45	40*	38**	41*
	50	42	42	44	41	43

Parameter: Hg (g%)						
Week	-1	13.7	12.6	13.9	13.1	12.9
	12	15.5	14.1*	13.3**	13.2#	14.5
	24	14.9	14.4	15.2	13.9	14.5
	37	16.8	15.4	13.8**	13.2**	14.1**
	50	14.5	13.6	14.4	13.6	14.3

Parameter: RBC ($10^6/m^3$)						
Week	-1	6.30	5.96	6.40	6.00	5.92
	12	6.80	6.35	5.85**	5.66**	6.11*
	24	6.41	6.29	6.43	5.88	6.05
	37	7.03	6.56	5.73**	5.48**	5.79**
	50	6.51	6.25	6.49	6.25	6.30

Parameter: MCV (u^3)						
Week	-1	66	66	66	68	69
	12	65	66	69#	69#	69#
	24	67	69	70*	70*	71#
	37	67	68	70**	69*	72#
	50	65	67*	67*	66	69#

Parameter: PT (sec)						
Week	-1	8.3	8.8	8.4	8.6	8.7
	12	8.2	8.4	8.0	8.1	8.9*
	24	8.1	8.4	8.2	8.4	8.6
	37	8.3	8.2	8.1	7.9	8.6
	50	7.4	7.6	7.4	7.4	8.6*

MEAN HEMATOLOGY VALUES

Males

Parameter: PCV (%)		DOSE (mg/kg) 0	30	100	300	1000
Week	-1	42	41	41	40	39
	12	42	41	42	39	40
	24	44	43	42	39	42
	37	46	44	45	42	46
	50	45	43	44	42	47

Parameter: Hg (g%)						
Week	-1	13.1	13.5	13.6	12.5	12.6
	12	14.4	13.7	14.0	12.6*	12.9
	24	15.0	14.5	14.4	13.1*	13.8
	37	16.4	15.2	15.5	14.4*	16.2
	50	15.1	14.6	14.4	13.7	15.5

Parameter: RBC ($10^6/m^3$)						
Week	-1	6.29	6.11	6.19	5.90	5.88
	12	6.47	6.02	6.21	5.63**	5.75*
	24	6.59	6.20	6.15	5.61**	5.97
	37	6.82	6.35	6.52	5.96*	6.59
	50	6.83	6.52	6.59	6.18	6.99

Parameter: MCV (u^3)						
Week	-1	66	67	67	67	67
	12	65	67	68*	68*	69**
	24	66	69	68	70*	70*
	37	67	68	69	70	70
	50	65	66	66	68	68

Parameter: PT (sec)						
Week	-1	8.4	8.3	8.6	8.4	8.6
	12	7.7	7.9	7.6	8.5	9.7*
	24	8.1	7.8	8.1	8.6**	9.2#
	37	7.9	7.7	8.0	8.5**	10.6#
	50	7.4	7.2	7.3	7.6	12.1#

Data taken from Table 5 of report.
*** = p < 0.05; ** = p < 0.01; # = p < 0.001**

14

TABLE IV
MEAN SERUM CHEMISTRY VALUES

Females

Parameter: AP (IU/L)		0	30	100	300	1000
Week	-1	62	91	85	93	76
	12	53	72	89	130*	168**
	24	48	47	49	106	146*
	37	41	66	84	146**	127*
	50	72	75	68	94	109
Parameter: ALT						
Week	-1	27	34	32	27	30
	12	40	43	27	36	178*
	24	32	42	28	35	258*
	37	36	43	29	41	299*
	50	28	49	27	38	121*
Parameter: AST						
Week	-1	24	28	24	24	26
	12	31	36	26	26	45
	24	20	24	20	25	43
	37	28	31	22	21	45
	50	16	49*	23	23	30*
Parameter: Triglycerides						
Week	-1	35	42	38	31	25
	12	17	24	21	52#	57#
	24	34	41	35	53*	57**
	37	37	47	57*	65**	64**
	50	47	54	48	59	58
Parameter: Cholesterol (mg%)						
Week	-1	154	148	142	156	202*
	12	155	157	244**	283#	207
	24	199	156	197	213	181
	37	162	189	298**	306**	211
	50	222	168*	172*	179	173*

MEAN SERUM CHEMISTRY VALUES

Males

Parameter: AP (IU/L)		0	30	100	300	1000
Week	-1	109	123	86	104	95
	12	89	80	98	155	321#
	24	54	56	63	112*	221#
	37	61	50	99	139**	227#
	50	55	59	114	151*	273#

Parameter: ALT (iu/L)						
Week	-1	33	30	24	20*	24
	12	44	39	29	47	385#
	24	40	39	28	40	393**
	37	57	39	29	57	287#
	50	44	45	38	76	255**

Parameter: AST (IU/L)						
Week	-1	29	26	18**	25	17**
	12	43	42	26**	34	77#
	24	30	29	17	18	74*
	37	34	32	18**	29	46*
	50	33	30	17*	38	45

Parameter: Triglycerides						
Week	-1	31	38	31	32	36
	12	18	20	27	52	177#
	24	25	36	44	64	143#
	37	33	43	45	73#	58**
	50	33	33	59**	62**	49

Parameter: Cholesterol (mg %)						
Week	-1	130	158	136	176*	145
	12	128	172	210**	262#	225**
	24	109	172*	223#	265#	221**
	37	129	195*	247#	294#	142
	50	103	154	265#	251**	103

= $p < 0.001$ * = $p < 0.05$ ** = $p < 0.01$

TABLE V
MEAN ABSOLUTE ORGAN WEIGHTS (g)

Males

	Dose Groups				
	0	30	100	300	1000
LIVER	365	476	538*	613**	697**
LUNGS	119	112	109	110	102*

Females

LIVER	389	416	486	561*	542**
THYROIDS	0.57	0.76	0.85	1.04**	0.93*

None of the other organs that were weighed showed statistical differences between control and treated groups for either sex.