Reviewed by: Sheryl K. Reilly, Ph.D. Sheryl Reilly 3-17-93
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Melba S. Morrow, D.V.M. Melba 3-17-93
Section II, Tox Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day Feeding Studies - Non-rodent

GUIDELINE #: 82-1

TOX. CHEM. #: 129032

MRID #: 421783-07

TEST MATERIAL: S-31183, Lot No. PTG-86011

SYNONYMS: 2-[1-Methyl-2-(4-phenoxyphenoxy)ethoxy]-pyridine, SUMILARV, NYLAR

STUDY NUMBERS: NNT-80-0037

SPONSOR: Sumitomo Chemical Company, Limited
5-33 Kitahama, 4-Chome
Chuo-Ku, Osaka 541 Japan

TESTING FACILITY: Sumitomo Chemical Company, Limited

TITLE OF REPORT: SUMILARV -- Three Month Oral Toxicity Study of S-31183 in Dogs

AUTHOR: Minoru Nakano

REPORT ISSUED: May 6, 1988

CONCLUSIONS: Under the conditions of the study, when S-31183 was administered to male and female beagle dogs at doses of 0, 100, 300 and 1000 mg/kg for 90 days, the NOEL for systemic toxicity in dogs of either sex was 100 mg/kg/d, and the LOEL was 300 mg/kg/d, based on significantly higher absolute liver weights and liver-to-body weight ratios in the males, and enlargement of hepatocytes observed in females at that concentration, compared with dogs on the control diet. The changes in liver weights, organ-to-body weight ratios, and increase in size observed in the hepatocytes at 300 mg/kg/d are probably not due to hepatotoxicity, however; rather, these changes appear to be adaptations of the liver to detoxifying the test substance, since these changes are observed in both groups at the highest dose tested. This study satisfies the criteria set forth in the Subdivision F Guidelines (82-1) for a subchronic oral study in non-rodent animals.

CLASSIFICATION: Guideline
TOX. CATEGORY: N/A

MATERIALS: Technical grade S-31183, a water-insoluble crystalline substance (melting point 45°C) lot number PTG-86011, 97.2% pure, was the test material. According to the registrants, the compound has a known stability when temporarily melted below 100 °C, and becomes yellow, viscous fluid when melted at 60 °C (page 8 of the submission).

Beagle dogs (16 males, 16 females) were the test species. The animals were obtained from White Eagle Laboratories Inc. (USA), and were 6 months old at the initiation of the study. The males weighed from 7.7 to 11.1 kg, and females weighed from 7.1 to 11.1 kg.

METHODS: Dosage of the test compound was determined in a preliminary study, wherein S-31183 was given to dogs for 4 weeks at dose levels of 100, 300 and 1000 mg/kg/d. The only observation reported was that the dogs receiving the highest dose had enlarged hepatocytes.

In the present study, the animals were randomly assigned to 4 treatment groups, based on the results of the preliminary study: 0 (controls), 100, 300 and 1000 mg/kg/d of test substance. Four male and 4 female dogs were assigned to each group. On the day preceding the study, the dogs were individually housed in aluminum cages and given 300 g of solid dog food (Lab Diet Purina-Taiyo Pet-Food, Lab Chows) and tap water ad libitum until the next morning. The dogs were maintained in an environment with a 12-hour light:dark cycle, temperature 23±2 °C, and relative humidity 55±15%, and ventilated at least 8 times per hour for the duration of the study.

The test mixture was melted at about 60 °C, weighed, then packed into gelatin capsules. The capsules each held about 1200 mg of the test substance. The dosage (# capsules) to be given to each dog was determined on the days body weight was measured. On day 1 of administration, the dogs were given a dose based on their weight that day; subsequent dosages were recalculated after weighing, then the actual dose amount adjusted for the following day. The control group received the same number of capsules as the high dose group, except the capsules were empty. The dogs were given the capsules once a day (9:30 a.m.), 7 days a week, for approximately 91 days.

During the dosing period, the dogs were observed prior to dosing and every 2-3 h daily until 5 p.m. on weekdays and at least 3 times a day after dosing on holidays for clinical signs. Body weights were recorded once a week from 2 weeks preceding initiation and throughout the 13-week study, then at sacrifice. Food consumption was measured daily.

2

Indirect ophthalmoscopic examinations were conducted prior to treatment and at weeks 6 and 12, first macroscopically or with a fundus camera (RC-II, Kowa Co., Ltd.), then using Midrin P (Santen Pharmaceutical Co., Ltd.) as a mydriatic.

Electrocardiography was performed on each dog on weeks 0, 5 and 12 of the study, and heart rate, amplitude of P and R waves, PR interval, and QRS and QT intervals recorded.

Blood was drawn from the cephalic vein just prior to administration of the test substance, and at weeks 0, 4, 8 and 12 during the study, for hematologic and blood chemistry analysis. Urinalysis was performed on weeks 0, 6 and 11. The following parameters were assessed:

Hematology*

erythrocyte count *
platelet count *
leukocyte count *
leucocyte differential count
mean corpuscular volume
erythroblast count
activated partial thromboplastin time
mean corpuscular hemoglobin concentration
hematocrit *
hemoglobin *
reticulocyte count
erythrocyte sedimentation
prothrombin time
erythrocyte sedimentation rate

Blood Chemistry

sodium *
potassium
total protein *
protein fraction
albumin *
globulins
total cholesterol
phospholipids
triglycerides
direct bilirubin
total bilirubin *
creatinine
glucose *
GOT
ALP *
CPK
GPT *
gamma-GTP
LDH
calcium *
urea nitrogen *
uric acid

Urinalysis

pH	occult blood *
appearance *	bilirubin *
glucose *	urobilinogen
ketones *	microscopic examination of sediment *
protein *	specific gravity *
volume	

* Values Required by Subdivision F Guidelines; inorganic phosphate was not reported for blood chemistry

The registrants also submitted fecal data (occult blood), tests of liver function (BSP retention), and renal function (PAH retention).

At the end of the study, full gross necropsies were performed on all animals. It took 3 days to complete all necropsies, and animals of each sex from each group were equally distributed for necropsy over the 3 days. The animals that were not necropsied on day 91 of the study remained on their dosing program until sacrifice. Liver, kidneys, testes and adrenals (postfixation) were weighed, and several other tissues and organs (see table below) were preserved in 10% neutral buffered formalin and prepared for histopathology by embedding the tissues/organs in paraffin, sectioning, and staining with hematoxylin and eosin (also PAS staining of the liver and kidneys). Part of the liver was fixed with formalin-alcohol solution; eyes were fixed in Davidson's fixative. Liver samples from the control and high-dose groups were examined with an electron microscope.

The following tissues/organs were collected as required by the Subdivision F Guidelines:

brain	tongue	rectum
pituitary	larynx	urinary bladder
thyroid/parathyroids		lymph nodes
thymus	vagina	sciatic nerve
lung	gall bladder	skin
trachea	testes	spinal cord
heart	epididymides	
bone marrow	ovaries	* oviduct data missing
salivary glands	uterus *	
mammary gland	liver	
thigh musculature	spleen	
eyes, including optic nerve	aorta	
kidneys	esophagus	
adrenals	stomach	
pancreas	duodenum	
prostate	jejunum	
	ileum	
	colon	
	cecum	

QUALITY ASSURANCE: A statement of compliance with good laboratory practices dated 12/31/91 was included in the submission.

STATISTICAL ANALYSIS: Data were analyzed for significant differences between the control and test groups by the ASSIT method[1].

RESULTS:

Mortality

Treatment with the test compound had no effect on mortality in either sex.

Clinical Observations

Clinical abnormalities included vomiting and soft stools in all groups, including the control animals, but there was no significant difference in occurrence of these symptoms between the groups. In the 100 and 300 mg/kg/d groups, the incidence of soft stool/diarrhea was comparable to control animals. Diarrhea was observed only once in 1 female of the 100 mg/kg/d group, and once in 1 male and 2 females in the 300 mg/kg/d group, during the 3-month study. Mucous stool was observed once in 1 female in the control group, and once in 1 male and 1 female of the 100 mg/kg/d group. Bloody stool was seen once in 1 female in the control group, but the significance of this finding is unclear. In the 1000 mg/kg/d group, 1 male and 3 females had soft stools relatively frequently and occasional diarrhea during the first month of administration; this subsided in these animals by day 30. Additionally, a yellow, viscous substance, believed to be the test material, was observed in the stool in this group. It is possible that the viscous nature of the test substance caused the diarrhea/soft stools in this group, rather than its toxicity.

Food Consumption

The mean food consumption values were similar between groups, and no changes attributable to the test material were observed during the study. In the 100 mg/kg/d males, there was a statistically significant decrease in food consumption during weeks 10 (4%) and 12 (10%) compared with the controls; however, these differences were small and no other abnormalities were observed.

Body Weight

No significant changes or differences in body weight were observed in any of the groups during the study.

Opthalmoscopy

No changes attributable to the test compound were observed.

One female in the 1000 mg/kg group exhibited small, star-shaped tapetal cells on the border of the tapetum in both eyes, but this anomaly was observed prior to the start of and throughout the study. These cells, while rare, are apparently found in normal beagles, and were not caused by the test substance.

One male dog in the high dose group developed a mass on the back of its left eye (day 81 of the study). The mass did not change and its cause was undetermined.

Electrocardiography

S-31183 had no significant effect on electrocardiogram readings.

Urinalysis

No treatment-related effects were observed.

Blood Chemistry

Total proteins and phospholipids were significantly greater than controls in females at the 1000 mg/kg/d dose at week 4 ($p<0.05$); these values remained greater than controls throughout the rest of the study, but the differences were not statistically significant. Phospholipid values were slightly but not significantly higher in the 100 and 300 mg/kg/d females during the administration of the test compound. At week 4, α_1 globulin in 1 female in the 1000 mg/kg/d group was significantly lower than controls ($p<0.01$), and at week 8, this dog and another female dog in this group exhibited significantly lower ($p<0.05$) levels of α_1 globulin. By the 12th week, the levels of α_1 globulin remained less than controls, but the difference was not significant. These changes appear to be caused by the test compound; however, it is not clear if it is due to the toxicity of the compound or detoxification of the compound by the liver or alteration of liver functions (see liver histopathology results, below).

GPT (aspartate aminotransferase) in 1 male in the 1000 mg/kg/d group was significantly lower than control on week 4 ($p< 0.01$), but this finding was probably not clinically significant, since subsequent values did not differ significantly from controls.

These findings correspond to an increase in total proteins and aspartate aminotransferase observed in rat subchronic toxicity studies of S-31183, thought to be due to alterations in liver function caused by the test compound.

No other abnormalities in blood chemistry were observed.

Dosage Level (mg/kg/d)	Blood Chemistry Data			
	Phospholipid (mg/dl) - females			
	Week 0	**Week 4**	**Week 8**	**Week 12**
Control	298	285	323	338
100	301	283	328	343
300	294	313	354	358
1000	307	346 *	384	388
	Total Protein (g/dl) - females			
	Week 0	**Week 4**	**Week 8**	**Week 12**
Control	5.0	5.1	5.3	5.2
100	5.3	5.2	5.3	5.5
300	4.9	5.2	5.3	5.3
1000	5.2	5.6 *	5.6	5.5
	α_1 globulin (%) - females			
	Week 0	**Week 4**	**Week 8**	**Week 12**
Controls	3.9	4.4	4.0	3.9
100	3.8	3.6	3.2	3.0
300	3.9	3.4	3.0 *	2.9
1000	4.1	2.8 **	2.9 *	2.5
	GPT (U/l) - males			
	Week 0	**Week 4**	**Week 8**	**Week 12**
Controls	47	51	50	55
100	44	44	51	51
300	44	42	43	51
1000	39	32 **	38	43

* significantly different from the control group ($p<0.05$)
** significantly different from the control group ($p<0.05$)

Hematology

No changes attributed to the test compound were observed.

Organ Weights

Males in the 300 and 1000 mg/kg/d groups had slightly but significantly greater absolute liver weights than controls ($p<0.05$). Males in the 300 mg/kg/d group had significantly greater relative liver weight:body weight ratios than controls ($p<0.05$). Males in the 300 mg/kg/d group also had significantly greater submandibular salivary gland weights ($p<0.01$) than controls, and females in the 100 mg/kg/d group had significantly greater uterine weights ($p<0.01$) than controls. There were no dose-related tendencies in these differences, however, and thus they are not likely attributable to test compound, but more likely due to normal variation in organ weights (from tables 38-41 and appendixes 34-37 of the submission).

Gross Pathology

Gross pathological observations (from table 42 of the submission) in the 100 mg/kg/d group included a concavity on the surface and a white focus on the capsule of the liver in one male, retraction of bilateral anterior and middle lobes and adhesion of accessory lobe and right posterior lobe of the lungs in 1 female. The reason for the liver observation is unclear, but the lung anomalies in the female were probably congenital.

Aberrations in the 300 mg/kg/d group included a dark red region on the left middle lobe of the lungs in 1 male and a grayish yellow region on the left middle lobe of the lungs in another male; an adhesion of left anterior and middle lobes, yellowish-grey discoloration on the tip of right middle lobe, and white spots scattered on tips of the bilateral posterior lobes of 1 female. These findings in the lung could be related to findings of interstitial pneumonia in the male with the dark red focus on the lung, and bronchopneumonia in the other male (grayish-yellow focus on the lungs). The liver of the male with the grayish-yellow focus on the lungs was also slightly enlarged, relative to body size. The female dog with lung anomalies was found to have interstitial pneumonia. The lung abnormalities are therefore likely due to an infection, rather than the test compound.

An enlarged liver relative to body size was observed in 2 males and 1 female in the 1000 mg/kg/d group. Other anomalies in this group included: black nodes on the right atrioventricular valve in 1 male and 1 female of this group; a grayish-yellow retraction on right middle lobe of the lungs in 1 female; scattered red spots on the thymus of 1 male; and white granules on the mucous membrane of the gall bladder in 1 female. The lung anomalies appear to be related to a pulmonary infection. The reasons for the

heart nodes are not clear. The enlarged liver corresponds to enlarged hepatocytes and increased smooth endoplasmic reticulum observed histopathologically (see below), and may be due to an adaptation of the liver to detoxification of the test compound.

External findings included a mass on abdominal skin of 1 female 100 mg/kg/d, and the back of 1 male 1000 mg/kg/d. These were areas of dermatitis, and likely not caused by the test compound.

Histopathology

Enlargement of hepatocytes was observed in females of the 300 mg/kg/d group, and both sexes of the 1000 mg/kg/d group. All male animals of the high dose group exhibited minimal to moderate enlargement of the hepatocytes in the lobular central zone; one also had vacuolation in the lobular central zone. In the 300 mg/kg/d group, minimal enlargement from central to intermediate lobular zone in 3/4 females. The 1000 mg/kg/d females exhibited slight enlargement from the lobular central to peripheral zones (3/4 dogs); 1/4 had minimal enlargement in lobular central zone. Eosinophilic cytoplasmic bodies were observed in 2 males and 2 females in the 1000 mg/kg/d group. Fibrosis, hepatocellular vacuolization or ballooning degeneration of hepatocytes was observed in a few animals of both sexes, including controls. Electron micrography showed increased smooth endoplasmic reticulum in all dogs of the 1000 mg/kg/d group. All females in the 1000 mg/kg/d group exhibited minimal to slight dilatation of the smooth endoplasmic reticulum. The histopathological findings in the high dose group could be indicative of an adaptive response of the liver in the process of detoxification of the test compound.

Other histopathological findings did not appear to be caused by the test compound, because of their randomness or lack of dose-responsiveness, and/or occurrence in all test groups, including controls. These results were reported as follows:

Histopathological Observations*:	Controls	100 mg/kg/d	300 mg/kg/d	1000 mg/kg/d
Mesenteric lymph nodes - Minimal to slight dilatation of the lymph sinus.		2♂		1♂
Submandibular lymph nodes - Minimal brown staining in medullary region.	2♂/1♀	3♂	3♂/1♀	2♂
Thymus - Minimal to slight atrophy.	1♂/1♀	2♂/3♀	1♂/1♀	
Minimal lymphocytic hyperplasia.	1♂			
Spleen - Minimal to moderate local congestion.	3♂/2♀	2♂/1♀	4♂/2♀	1♂/3♀
Minimal/slight Gamna-Gandy node.	1♀	2♀	2♀	
inimal/slight hemorrhage in trabecular artery wall.	2♀	1♂/1♀	1♂	1♂/2♀
Lungs - Minimal to moderate interstitial pneumonia.	1♂/2♀	2♂/4♀	3♂/2♀	
Moderate bronchopneumonia.			1♂	1♀
Conglobulation of foamy cells.	1♀		1♂/1♀	1♀
Interstitial fibrosis.			1♂/1♀	
Moderate change in thickness of capsule.			1♂/1♀	
Dilatation of bronchi.			1♂	1♀
osinophilic infiltration.				1♂
Trachea - Minimal ↑ in goblet cells and cell infiltrate in lamina propria mucosae.	1♀	1♂/1♀	1♂/1♀	1♀
Heart - Minimal hematoma in right atrioventricular valve.				1♂/1♀
Stomach - Minimal hyperplasia of lymphatic follicle in glandular region.		2♂		1♂
Small intestine - Minimal dilatation of lumen, congestion of ciliated epithelium.		3♂/3♀	1♂/3♀	3♂/1♀

Histopathological Observations*, continued:	Controls	100 mg/kg/d	300 mg/kg/d	1000 mg/kg/d
Large intestine - Minimal mucosal congestion.		1♀		
Pancreas - Slight focal degeneration of acinar cells.				1♂
Cellular infiltration to pancreatic duct wall	1♀			
Adrenals - Slight enhancement of vacuolation of cortical cells in zona fasciculata or zona glomerulosa.		1♂	1♀	
Skeletal muscle - minimal focal cell infiltration.		1♀	1♂	
Skin - Minimal dermatitis.		1♀		1♂
Minimal focal cell infiltration in corium.		1♀		
Salivary glands (parotid, submandibular) - Minimal lymphocytic infiltration.	2♂/2♀	2♂/1♀	1♂/1♀	
Thyroid - Slight hyperplasia of perifollicular cells.	2♀	1♂/2♀	1♀	
Moderate localized cellular infiltrate.		1♀		
Parathyroid - Unclassifiable Cyst.	2♂/1♀		1♀	1♂
Kidneys - Minimal to slight calcification in uriniferous tubules at renal papilla.	2♂/2♀	2♂/3♀	4♂/3♀	4♂/3♀
Minimal to slight vacuolization of tubular epithelial cells.	1♂/3♀	3♂/1♀	1♀	1♂/3♀
Regeneration of tubular epithelial cells.	2♂/1♀	2♀	1♀	2♂/2♀
Hyaline cylinders in tubules.	1♂/2♀			1♂
Localized cellular infiltration of cortical interstice.	2♂/2♀	1♂/3♀	1♂/3♀	1♂/3♀
Prostate - Minimal cellular infiltration.	1♂	1♂		

Histopathological Observations*, continued:	**Controls**	**100 mg/kg/d**	**300 mg/kg/d**	**1000 mg/kg/d**
Uterus - Minimal localized hemorrhage.	1♀	1♀		
Vagina - Minimal lymphatic infiltration.	1♀			1♀
Pituitary - Unclassifiable anterior lobe cyst.	3♂	1♀	1♂/2♀	1♂/1♀

* Data from appendixes 38-45 of the submission

Miscellaneous Tests:

No changes attributable to the test compound were observed in the fecal examination, liver or renal function tests.

DISCUSSION:

When S-31183 was administered to male and female beagle dogs at doses of 0, 100, 300 and 1000 mg/kg for 90 days, the changes attributable to the test compound included soft stool and diarrhea, and hepatocellular effects, e.g., changes in absolute liver weight, increased relative liver:body weight ratios, and increased hepatocellular size and smooth endoplasmic reticulum. The NOEL for systemic toxicity in dogs of either sex was 100 mg/kg/d, and the LOEL was 300 mg/kg/d, based on significantly higher absolute liver weights and liver-to-body weight ratios in the males, and enlargement of hepatocytes observed in females at that concentration, compared with dogs on the control diet. The changes in liver weights, organ-to-body weight ratios, and increase in size observed in the hepatocytes at 300 mg/kg/d are probably not due to hepatotoxicity, however; rather, these changes appear to be adaptations of the liver to detoxifying the test substance, since these changes are observed in both groups at the highest dose tested.

1.Yamazaki, M. et al., Statistical methods appropriate for general toxicological studies in rats. Algorithms for multiple comparisons of treatment groups with control. **J Takeda Res. Lab., 40** (3/4): 163-187 (1981).