Reviewed by: Sheryl K. Reilly, Ph.D. Sheryl K Reilly 2/18/93
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Melba S. Morrow, D.V.M. MSM 2/18/93
Section II, Tox Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic 90-day Oral Toxicity

GUIDELINE #: 82-1

TOX. CHEM. #: 129032

MRID #: 413217-16

TEST MATERIAL: S-31183, Lot No. PYG-87074

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

STUDY NUMBERS: 343-208

SPONSOR: Sumitomo Chemical Co., Ltd.
Kitahama, 4-Chome 5-33
Chuo-Ku, Osaka 541 Japan

TESTING FACILITY: Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22180

TITLE OF REPORT: Subchronic Toxicity Study with S-31183 in Rats

AUTHOR: Raymond A. Cox, Ph.D.

REPORT ISSUED: March 8, 1989

CONCLUSIONS: Under the conditions of the study, when S-31183 was administered to Crl:CDBR rats at doses of 0, 400 ppm (equivalent to a mean of 23.49 mg/kg/day for males (M), and 27.68 mg/kg/day, females (F)), 2,000 ppm (117.79 mg/kg/d (M), and 141.28 mg/kg/d (F)), 5,000 ppm (309.05 mg/kg/d (M), 356.30 mg/kg/d (F)), and 10,000 ppm (641.81 mg/kg/d (M), 783.96 mg/kg/d (F)), for 90 days, the NOEL for systemic toxicity in rats of either sex was 400 ppm, and the LOEL was 2,000 ppm, based on higher mean total cholesterol and phospholipids, decreased mean red blood cell, hematocrit and hemoglobin counts, and significantly higher liver-to-body weight ratios at that concentration, in male rats compared with males on the control diet. Female rats did not demonstrate these effects until the 5,000 ppm level of the test substance, however, a

negative trend in mean red blood cell volume at the 2,000 ppm concentration of the test substance was observed in the female rat. In addition, both sexes also demonstrated slightly increased hepatocyte:cytoplasm and cytoplasm:nucleus ratios, and decreased sinusoidal spaces at the 2,000 ppm concentration of the test substance, but the significance of these observations is unclear. This study satisfies the criteria set forth in the Subdivision F Guidelines (82-1) for a subchronic oral study.

CLASSIFICATION: Guideline
TOX. CATEGORY: N/A

MATERIALS: Technical grade S-31183, lot number PYG-87074, 95.3% pure, a light grey crystalline solid which turns pale yellow in the liquid state, was the test material. Crl:CDBR (Sprague-Dawley) rats were the test species. The animals were obtained from Charles River Laboratories, and were 6 weeks old at the initiation of dosing. The males weighed from 189.8-241.1 g, and females weighed from 151.2-197.2 g.

METHODS: Upon arrival, the animals were acclimated to the laboratory for 14 days. The rats were caged individually after 11 days. Following the 14-day acclimation period, the animals were kept in an environment with a 12-hour light:dark cycle, temperature range between 66-77 °F, and relative humidity 21-67% for the duration of the study.

The animals were assigned to the following treatment groups[1] (10 males and 10 females per group):

Group #		Dietary Levels (ppm)	Mean* Compound Consumption-Males (mg/kg/day)	Mean Compound Consumption-Females (mg/kg/day)
1	(Controls)	0	0	0
2	(Low)	400	23.49	27.68
3	(Mid-1)	2,000	117.79	141.28
4	(Mid-2)	5,000	309.05	356.30
5	(High)	10,000	641.81	783.96

[1]*Data taken from pages 2 and 27 of the submission.
Mean compound consumption over the 13-week study

The test mixture was given to the animals in their food, since the most likely route of human exposure is oral. Dietary mixtures of the test material were prepared weekly in the following manner. The test material was heated in a 50-60 °C water bath for 3-4 hours

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until liquified, weighed, then thoroughly pestled into 5 kg rodent feed (Purina Certified Rodent Chow #5002) to achieve a 10,000 ppm premix. The premix was further blended for 1 hour with a Hobart mixer, then stored overnight at room temperature. The different test feeds were made up the following day by serial dilution of the premix. After 8 weeks, the test feed preparation procedure was modified, and the test feeds prepared by initially mixing the test material into the control feed for 2 minutes with a Waring blender (instead of being pestled), followed by 1 hour mixing in Hobart mixer.

Homogeneity of the control, premix and test feeds was determined with High Peformance Liquid Chromatography (HPLC) on 50 g samples of feed collected from the top, middle and bottom layers of the feed, prior to initiation of the study, and again at 8 and 9 weeks, when modifications in the mixing occurred. Duplicate 50 g samples were stored in the freezer.

Concentration of the control, premix and test feeds was verified in 50 g sample aliquots via HPLC, prior to initiation of the study and weekly when the feeds were prepared.

The stability of the test material was tested by comparing samples from 50 ppm and 10,000 ppm test diets prepared on day 0 with samples of those diets which were stored at room temperature and under refrigeration for 7 and 14 days. Stability analyses were performed with HPLC.

The animals were observed twice daily for mortality and moribundity, and a careful examination for toxicity was performed once a day. Body weights were recorded prior to initiation, then weekly throughout the 13 week study, and at sacrifice or the time of death. Food consumption was measured weekly, and water consumption twice weekly, throughout the study.

Indirect ophthalmoscopic examinations were conducted prior to treatment and at week 13, using 1% Mydriacyl as a mydriatic.

After 13 weeks, all animals were housed in individual urine collection racks and fasted overnight for clinical sampling. After the fasting period, blood was collected from the orbital sinus of ketamine-anesthetized animals, for hematologic and blood chemistry analysis. The following parameters were assessed:

<u>Hematology</u>*

leukocyte count *
erythrocyte count *
hemoglobin *
hematocrit *
platelet count *
corrected leukocyte count
leucocyte differential count
cell morphology
mean cell hemoglobin
mean cell volume
mean cell hemoglobin concentration

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Blood Chemistry

sodium *
potassium
chloride
total protein *
albumin *
globulin
calcium *
phosphorus *
total bilirubin *
blood urea nitrogen *
creatinine
glucose *
aspartate aminotransferase *
alanine aminotransferase *
alkaline phosphatase
total cholesterol
phospholipid
triglyceride
gamma glutamyltransferase

Urinalysis[a]

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

* Values Required by Subdivision F Guidelines
[a] specific gravity and volume were not reported

Full gross necropsies were performed on all animals in this study, and at the end of 13 weeks, all animals that were still alive were weighed, anesthetized with sodium pentobarbital, and sacrificed by exsanguination. Liver, kidneys, testes and adrenals (postfixation) were weighed, and several other tissues and organs (see table below) were preserved in 10% neutral buffered formalin. The following tissues were collected for histopathological examination from control and high-dose test groups, and from animals dying unexpectedly during the course of the study. In addition, gross lesions, kidneys, lungs, and livers from all animals were prepared for histopathology by embedding the tissues/organs in paraffin, sectioning, and staining with hematoxylin and eosin.

The following tissues/organs were collected as required by the Subdivision F Guidelines[2]:

brain with brainstem (medulla/pons, cerebellar cortex, cortex)
pituitary
thyroid/parathyroids
thymus
lung
trachea
heart
testes
epididymides
ovaries
uterus
liver
spleen
aorta
esophagus
stomach
duodenum

sternum (with bone marrow)	jejunum
salivary glands (mandibular)	ileum
mammary gland	colon
thigh musculature	cecum
eyes	rectum
femur including articular surface	urinary bladder
kidneys	mesenteric lymph node
adrenals	sciatic nerve
pancreas	skin
	spinal cord (cervical, mid-thoracic, lumbar)

[2]From pages 20-21 of the submission

QUALITY ASSURANCE: A statement of quality assurance dated 3/9/89 was included in the submission, along with a statement of compliance with good laboratory practices dated 3/8/89.

STATISTICAL ANALYSIS: Body weights, food and water consumption, clinical pathology data (except cell morphology and urinalysis), and organ weights of the control animals were statistically compared with the data from the same sex of the test animals, using Levene's test of homogeneity of variances, which included one-way ANOVA followed by Dunnet's. Terpstra-Jonckheere's test for monotonic trend was also used, which included a simple linear regression of untransformed data and regression ANOVA, or a simple linear regression of rank-transformed data and regression ANOVA. The comparisons were at the 5%, two-tailed probability level, unless a trend in the data indicated a one-tailed test would be more appropriate.

RESULTS:

Homogeneity, Concentration, and Stability of Control, Premix, and Test Feeds

Analysis of the feed by high performance liquid chromatography (HPLC) demonstrated homogeneity in all samples. (See table 1, below; homogeneity, concentration, and stability data are from pages 45 and 46 of the submission).

The mean concentration values were acceptable, but a fair amount of sample variation during weeks 4-7 resulted in a change in feed preparation (mixed in a Waring blender 2 minutes, followed by 1 hour mixing in Hobart mixer). Concentration analysis revealed no unacceptable sample variation after the change in mixing procedure was made. (See table 2, below).

The test material was found to be stable at 50 ppm and 10,000 ppm levels for 14 days, when stored under refrigeration and at room

temperature conditions. (See table 3, below).

Table 1: Mean Values for Homogeneity

Percent of Target Concentration (in ppm:)

	400 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Pretest:				
Top	101	99	99.78	101
Middle	101	99.1	98.3	100
Bottom	99.8	98.4	98.9	102
8 weeks:				
Top	95.3	96.5	99.2	97.6
Middle	107	98	98.8	98.7
Bottom	99	97	98.4	104
9 weeks:				
Top	96	94	100	100
Middle	101	96.5	98	99
Bottom	102	96	101	101

Table 2: Concentration Analysis - Representative Intervals*

Percent of Target Concentration (duplicate 50 g samples)

Week #	400 ppm	2,000 ppm	5,000 ppm	10,000 ppm
1	91.3, 92.5	97, 104	106, 101	99, 99
3	95.3, 90.3	96, 100	102, 100	102, 104
5	93.8, 89.3	108, 107	89.8, 88.2	87.1, 96.4
7	102, 104	96, 83	92.8, 103	121, 99.5
8**	100	97	98.8	100
9**	99.8	95.5	99.6	100
11	97.5, 94.5	99, 98	98, 98	100, 98.9
13	97, 102	100, 99	101, 100	99, 98.8

* performed prior to study & weekly

** Dose verification is provided for one sample only, when mixing procedure changed.

Table 3: Stability Analysis

	Percent of Target Concentration (values = duplicate 50 g samples)	
Stability of Test Material in Feed stored for:	50 ppm	10,000 ppm
14 days, refrigerated	102, 101	101, 102
14 days, room temperature	100, 101	102, 103

Mortality/Clinical Observations

There were no treatment-related effects reported for clinical signs of toxicity, and treatment had no effect on mortality in either sex. One female rat from Group 3 was sacrificed during week 11, because the animal suffered severe injury to the hard palate as the result of catching its nose or teeth in the cage.

Body Weight

Mean body weights decreased with increasing dose of the test material during the study, indicating a compound-related effect. At 13 weeks, Group 4 (5,000 ppm) and Group 5 (10,000 ppm) animals exhibited significantly lower mean body weight values than control animals in both sexes ($p \leq 0.05$): male rats in Group 4 = 8.4% (decrease in mean body weight); Group 5 males = 12.2%; Group 4 females = 9%; and Group 5 females = 11.7%.

Food Consumption and Compound Consumption

The mean food consumption values were similar between groups, although in females, there was a significant negative monotone trend (weeks 1-13) in total consumption data.

The consumption of the test compound, decreased over the 13 weeks study, as body weight increased. This would be expected when animals are fed a fixed concentration (in ppm) of the test substance in the feed, instead of adjusting the dose to the weight of the animals. The mean compound consumption for the study (weeks 1-13), plus the high and low values, are presented below (mg/kg/day)[4].

Mean Compound Consumption, Weeks 1-13
(mg/kg/day)

	Males			Females		
Group	Mean	Low	High	Mean	Low	High
2	23.49	18.04	36.02	27.68	21.95	38.45
3	117.79	87.15	178.64	141.28	118.55	189.96
4	309.05	237.28	477.82	356.30	294.57	478.34
5	641.81	499.41	945.95	783.96	629.32	1137.08

[4]From page 27 of the submission

Clinical Chemistry, Hematology

a. Urinalysis: No treatment related effects were observed in the urine data.

b. Serum Chemistry: Mean total cholesterol values for Groups 3, 4 and 5 males, and Groups 4 and 5 females, were significantly higher than the control animals of the same sex, and phospholipids were significantly higher than controls in the Groups 3, 4 and 5 males and Group 5 females. These observations appear to be related to the test material, and may be associated with the increase in absolute liver weights and liver-body-weight ratios observed in these same groups. Mean total protein and albumin were slightly but significantly higher ($p \leq 0.05$) in both sexes of Group 5 animals, compared with control animals. A significant positive trend for males and females ($p \leq 0.05$) was observed in aspartate aminotransferase and calcium, and significant positive trends ($p \leq 0.05$) in blood urea nitrogen, glucose, and globulin was observed in females. These findings appear to have been affected by the test material, and may be indicative of an alteration in hepatic function when effects are considered collectively (enzyme changes, BUN, and lipids/cholesterol).

MEAN CLINICAL CHEMISTRY VALUES (± Std. Dev.)

Group #	Dosage Level (ppm)	Total Cholesterol (mg/dl)	Phospholipid (mg/dl)	Total Proteins (g/dl)	Albumin (g/dl)
	Males				
1	0	62±9.6	95±10.9	6.9±0.2	4.9±0.21
2	400	72±9.6	102±15.1	6.7±0.38	4.8±0.39
3	2,000	92±13.2*	126±13*	6.8±0.42	4.9±0.24
4	5,000	121±47.3*	167±47.9*	7.1±0.42	5.1±0.55
5	10,000	128±24.9*	169±29.2*	7.5±0.4*	5.6±0.34*
	Females				
1	0	77±15	129±25.6	7.1±0.42	5.3±0.49
2	400	81±20.3	133±29.8	7.1±0.37	5.4±0.46
3	2,000	81±10.8	124±15.5	6.9±0.4	5.3±0.41
4	5,000	100±14.0*	145±21.7	7.2±0.51	5.7±0.49
5	10,000	136±19.5*	200±24.9*	7.9±0.53*	6.3±0.53*

*Significantly different from controls, $p \leq 0.05$.

c. Hematology: Decreased mean red blood cell (RBC), hemoglobin and hematocrit values were exhibited in Groups 3, 4 and 5 males, and in Groups 4 and 5 females, which appears to be due to the test material. A negative trend in mean red cell volume was observed in Groups 3 and 5 females, which was slightly but significantly less than the control values ($p \leq 0.05$). The mean cell hemoglobin values in Groups 4 and 5 males were significantly higher ($p \leq 0.05$) than controls, and there was a positive trend ($p \leq 0.05$) in mean cell hemoglobin concentration in females. The biological significance of these observations are unclear.

MEAN CLINICAL HEMATOLOGY VALUES (± STD. DEV.)

Group #	Dosage Level (ppm)	RBC (HI/ul)	Hemoglobin (g/dl)	Hematocrit (%)	Mean Cell Volume (FL)
	Males				
1	0	9.5±0.396	17.2±0.68	48±1.56	50.6±1.83
2	400	9.32±0.281	17.2±0.52	48.5±1.4	52.1±1.35
3	2,000	8.79±0.334*	16.1±0.86*	45±2.43*	51.2±1.77
4	5,000	8.69±0.575*	16.4±0.93*	45.3±2.79*	52.2±1.16
5	10,000	8.92±0.297*	16.7±0.63*	45.9±1.33*	51.5±0.7
	Females				
1	0	8.71±0.319	17.0±0.56	47.7±1.58	54.8±1.45
2	400	8.52±0.299	16.6±0.41	46.5±1.14	54.6±1.06
3	2,000	8.93±0.274	17.1±0.58	47.4±1.76	53.1±1.28*
4	5,000	8.18±1.004*	15.9±2.01*	44±5.29*	53.9±1.46
5	10,000	8.30±0.264*	15.8±0.42*	43.9±1.08*	52.9±1.17*

*Significantly different from controls, $p \leq 0.05$.

Organ Weights, Gross Pathology

Mean absolute liver weights and liver-to-body weight values (at the end of the study) for males of Groups 3, 4 and 5, and females of Groups 4 and 5 were significantly higher than controls. Kidney-to-body weight ratios were significantly higher in the Groups 4 and 5 males than control animals. The mean adrenal-to-body weight ratio was significantly higher in Group 5 males than control animals. Positive trends in mean absolute adrenal weights in males, and adrenal-to-body weight ratios in males and females, were observed.

Gross pathological findings of the liver, including enlargement, pale, dark or red areas, and/or mottled appearance, were observed in 0/10, 1/10, 0/10 and 5/10 males, and 0/10, 2/10, 1/10, 0/10 and 2/10 females, in Groups 1-5, respectively. There does not appear to be an association to treatment in females (no trends). In males, it is possible that observations at the highest dose tested are related to the test material, but the biological significance of this observation is unclear.

Histopathology

Treatment-related, microscopic findings consisted of slight increases in cytoplasmic content, decreased nucleus/cytoplasm ratio, and decrease in sinusoidal spaces in all Groups 3-5 rats. Increased cytoplasmic content parallels the increased cholesterol observed in male rats in Groups 3-5 and females of Groups 4 and 5, and may be due to an increased cholesterol production in these treatment groups.

DISCUSSION: Under the conditions of the study, when S-31183 was administered to Crl:CDBR rats at doses of 0, 400 ppm (equivalent to a mean of 23.49 mg/kg/day for males (M), and 27.68 mg/kg/day, females (F)), 2,000 ppm (117.79 mg/kg/d (M), and 141.28 mg/kg/d (F)), 5,000 ppm (309.05 mg/kg/d (M), 356.30 mg/kg/d (F)), and 10,000 ppm (641.81 mg/kg/d (M), 783.96 mg/kg/d (F)), for 90 days, the NOEL for systemic toxicity in rats of either sex was 400 ppm, and the LOEL was 2,000 ppm, based on higher mean total cholesterol and phospholipids, decreased mean red blood cell, hematocrit and hemoglobin counts, and significantly higher liver-to-body weight ratios at that concentration, in male rats compared with males on the control diet. Female rats did not demonstrate these effects until the 5,000 ppm level of the test substance, however, a negative trend in mean red blood cell volume at the 2,000 ppm concentration of the test substance was observed in the female rat. In addition, both sexes also demonstrated slightly increased hepatocyte cytoplasm and cytoplasm:nucleus ratios, and decreased sinusoidal spaces at the 2,000 ppm concentration of the test substance, but the significance of these observations is unclear. This study satisfies the criteria set forth in the Subdivision F Guidelines (82-1) for a subchronic oral study.

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