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DATA EVALUATION REPORT

STUDY TYPE:	Teratogenicity - Developmental Toxicity, Rabbit
GUIDELINE #:	83-3
TOX. CHEM. #:	129032
MRID #:	421783-11 ~~413217-20 H~~ (Amended MRID No. 413217-20)
TEST MATERIAL:	SUMILARV (S-31183)
SYNONYMS:	NYLAR, 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]-pyridine
STUDY NUMBERS:	NNT-80-0003
SPONSOR:	Sumitomo Chemical Company, Limited 5-33, Kitahama 4-chome, Chuo-Ku Osaka 541, Japan
TESTING FACILITY:	Sumitomo Chemical Company, Limited 5-33, Kitahama 4-chome, Chuo-Ku Osaka 541, Japan
TITLE OF REPORT:	Sumilarv--Study of S-31183 by Oral Administration During the Period of Fetal Organogenesis in Rabbits
AUTHOR:	Atsuko Hirohashi
REPORT ISSUED:	August 30, 1989

CONCLUSIONS: Under the conditions of the study, when SUMILARV was administered to pregnant JW-NIBS rabbits on days 6-18 of gestation, no developmental effects were reported. Dams received doses of 0, 100, 300 and 1000 mg/kg/d.

The maternal NOEL was 100 mg/kg/d; the maternal LOEL was 300 mg/kg/d, based on the occurrence of premature delivery/abortions, soft stools, emaciation, lusterless fur, decreased activity and bradypnea/deep breathing. At 1000 mg/kg/d, the number of premature births/abortions increased as did the frequency and number of animals with the clinical symptoms that were reported at the 300 mg/kg dose. Gross pathological findings were also present in the high dose dams (see discussion).

The developmental NOEL was > 1000 mg/kg/day; however, there were only 4 litters available for evaluation at this dose level.

CORE CLASSIFICATION: Supplementary, but upgradeable, pending receipt of summary data which provides gravid uterine weights, appropriate information on totally resorbed litters, data which separates early/late resorptions from stillbirths, and summary data for litter incidence of malformations/variations.

MATERIALS:

1. Test Compound: Sumilarv (S-31183), Lot # PTG-86011, 97.2% pure) a pale yellow solid, soluble in acetone or methanol, water insoluble.

2. Test Animals: Male and female JW-NIBS rabbits were the test species. The animals were obtained from Nihon Seibutsu Kagaku Research Institute. Males were 6 months old, and weighed between 2.43 - 3.00 kg upon arrival; females were 5 months old, and weighed between 2.39 - 2.96 kg.

METHODS:

1. Dose Selection: In a preliminary study, to determine which test dosages would be used in the present study, nonpregnant female rabbits were given 100, 300, or 1000 mg/kg/d of the test substance by gavage for 14 consecutive days, to determine general toxic effects of the test substance. In that study, the animals in the 300 mg/kg dose group exhibited a decrease in body weight gain by day 3 of dosing, while the animals in the 1000 mg/kg dose group exhibited a decrease in body weight throughout the entire dosing period. One of the rabbits in the high dose group stopped eating from day 7 until the end of the study. Upon necropsy, traces of hemorrhage in the stomach, and a thinning in the wall of the cecum and changes in the properties of its contents were observed in this rabbit. Data was not provided in the submission for the preliminary study. These three dosage levels were chosen for use throughout the present study.

2. Animal Husbandry/Mating: The animals were acclimated for two weeks prior to the study, then housed individually in wire mesh floor cages. The animal room air was replaced at least 10 times per hour; lighting was on a 12 hour:12 hour light:dark cycle (7:00 am-7:00 pm). Room temperature was maintained at 22 ± 2 °C, and humidity at 55 ± 10%. Animals were fed a solid rabbit chow (NRT-1, lot Nos. 200, 231 and 280, Nihon Seibutsu Kagaku Research Institute), and provided potable well water ad libitum.

Female rabbits were placed in the males' cages in the morning every day, until copulation was observed twice, or once if vaginal smears confirmed the presence of spermatozoa. This was counted as day 0 of gestation. Dams were weighed on days 0, 6, 9, 12, 15, 18,

22, 25 and 28 of gestation; food consumption was measured on these same days, excluding day 0.

3. Administration of the Test Compound: Dosing was initiated on day 6 of gestation. The test substance was melted at 50 °C, cooled to room temperature, then administered orally to the test animals by gavage, using a rubber catheter fitted with a silicon tube, once a day from day 6 to day 18 of gestation. The volume/kg body weight was based on the specific gravity of the melted compound (1.156 @ 24°C), and each animal's body weight on day 6 of gestation. Control animals were given sterile distilled water by gavage. The animals were observed daily throughout the study for signs of toxicity.

3. Group Arrangement: The animals were assigned to the following treatment and control groups based on comparable body weights and mating partners:

Test Substance	Dose Level (mg/kg)	Volume Administered by gavage (ml/kg)	Number of animals
Controls	0	1.00	15
S-31183:	100	0.09	17
"	300	0.26	15
"	1000	0.87	18

4. Maternal and Fetal Examination: On day 28 of gestation, the animals were euthanized with sodium pentobarbital, and cesarean section and necropsy performed. The organs in the thoracic and abdominal cavities were examined, and the number of corpora lutea, implantations, live fetuses, and dead embryos and fetuses recorded. The sex of all fetuses was determined, and live fetuses were examined for external abnormalities and body weight. All fetuses that did not exhibit external abnormalities were subjected to visceral examination and eyes, heart, and kidneys removed and fixed in Bouin's solution for later examination. Fetuses with abnormalities were fixed in 10% formalin and not examined for skeletal or internal anomalies. Aborted or premature offspring were fixed in 10% formalin and examined for external and visceral abnormalities, if observation was possible.

5. Statistical Analysis: Student's t Test was used to compare the following tests between the S-31183 treated groups and controls, at the 5% level of significance:

Body weight of dams
Body weight gain of dams
Food consumption
Food consumption/kg body weight

Number of corpora lutea
Number of implantations
Number of live fetuses
Body weight of live fetuses
Number of ossified sacral and caudal vertebrae
Number of ossified proximal and middle phalanges of forelegs

The Rank Sum Test was used to compare the following in the control and treated groups at the 5% significance level:

Implantation rate
Percentage of post-implantation loss
Sex ratio
Incidences of anomalies and variations in fetuses
Incidence of unossified 5th and 6th sternebrae
Incidence of unossified 1st metacarpal bone
Incidence of unossified 5th middle phalanx
Incidence of unossified talus

Nonpregnant animals, and animals that died due to improper administration of the test material, or which were sacrificed prior to initiation of the study, were not included in the statistical analysis. Therefore, the number of animals per group used in the statistical analysis were 14, 12, 14 and 13, in the control, 100, 300 and 1000 mg/kg groups, respectively.

QUALITY ASSURANCE: A statement of quality assurance dated 7/28/89 was included in the submission, along with a statement of compliance with good laboratory practices dated 12/13/91.

RESULTS:

Maternal Toxicity

1. Mortality: Seven animals died or were sacrificed during the study. One control animal was sacrificed on day 13 of gestation, one week after fracturing the right front foreleg during the initial dosing procedure. The fracture kept the animal from eating properly, and it was losing weight. Two animals from the 100 mg/kg group were sacrificed on day 6 of gestation, prior to initial dosing of the other animals, because they had been losing weight due to decreased food consumption. Necropsy revealed that one of the animals was not pregnant. One animal of the 300 mg/kg group died after gavage on day 8 of gestation. The animal initially had symptoms of dyspnea, followed by bleeding from the mouth, and death within minutes. Necropsy revealed blood in the trachea, esophagus, stomach, and lungs, and on the gavage tube. It was concluded that death resulted from improper administration of the test material. The animal was not pregnant. One animal died during the administration period in the 1000 mg/kg group, and 2 in this group were sacrificed due to moribundity; all 3 of these animals were pregnant.

2. Clinical Observations: One animal in the control group and 1 in the 100 mg/kg group exhibited soft stools, and 1 control animal had episodes of sneezing following gavage on days 15-20 of gestation. Two rabbits in the control group lost hair in the neck region (1 during days 20-25 and the other on days 23-28 of pregnancy). No signs of toxicity were observed in the 100 mg/kg group. Soft stools, emaciation, lusterless fur, decreased activity and bradypnea/deep breathing were observed in 3 animals which prematurely delivered or died during the study in the 300 mg/kg group. In the 1000 mg/kg group, ananastasia, diarrhea, decreased activity, lusterless fur, bradypnea, and emaciation, was observed in 9 animals which delivered prematurely or died during the study; 1 animal from this group exhibited soft stools (days 12-13, 17) and emaciation (days 20-22), but was not pregnant.

3. Body Weight and Food Consumption: There was no statistically significant difference in mean body weight gain and food consumption between control, 100 mg/kg and 300 mg/kg groups, although food consumption in the 300 mg/kg group was approximately 15-18% less than controls from days 15 to 25. In the 1000 mg/kg group, mean body weight decreased 10.9% between days 6-22, and was significantly less than controls on days 9 ($p<0.05$), 12, 15, 18, 22 ($p<0.01$), and 25 ($p<0.05$). The mean food consumption decreased in all groups during the dosing period (between days 6-18); this difference was most pronounced in the 1000 mg/kg dosage group (↓ 74%). Food consumption differences between the 100 and 300 mg/kg groups and the controls were not significant during the study; however, the 1000 mg/kg animals consumed significantly less ($p<0.01$) food than control animals on days 9, 12, 15, 18 and 22. The difference in food consumption was the most pronounced on day 18 (72% less than controls), which was the last day of dosing. Food consumption and body weights increased to the same degree as control animals by day 28 of gestation. Thus, the body weight loss and food consumption decrease observed in the high-dose animals exhibited during the dosing period appeared to be dependent upon the test material. See Table I for data.

4. Gross Pathological Observations: In the control group, one animal aborted or prematurely delivered, and upon necropsy, secondary placenta and retention of blood in vagina and left uterine horn were observed. Two of the control animals had traces of petechiae scattered throughout the lungs, 1 exhibited a slight discoloration of the heart, and 1 had miliary-sized white dots or spots scattered on the placenta. The significance of these findings is not clear, but the findings in the lung may be due to gavage.

Two of the 100 mg/kg group had scattered petechiae throughout the lungs, and the medial side of the left lobe of the liver of one of the dams was pale brown in color. One of the animals in this group had an accessory spleen. The significance of these findings is unclear, but the lung petechiae are possibly due to damage

caused by gavage.

In the 300 mg/kg group, scattered petechial hemorrhages were observed in the lungs of 2 animals. Three dams exhibited discoloration of the heart, 2 of which had discoloration of the kidneys, and 1 of these had discolored liver and spleen. These same 2 animals exhibited distention of the gallbladder, and several findings in the digestive tract, such as traces of hemorrhage in the stomach, cecum, and colon, gastric ulcer, distention of the cecum, viscous contents of the cecum; both aborted or delivered prematurely. A 4th animal exhibited distention of the gallbladder, traces of hemorrhage in the stomach, gas retention in the stomach, and viscous contents in the cecum. This dam had a solid substance in the stomach, probably the test substance and delivered prematurely. It appears that the test substance may have attributed to the premature delivery and some of the other symptoms exhibited in this treatment group; however, the trace hemorrhages found in the GI tract and gastric ulcer may have been caused by administration of the compound by gavage.

The 1000 mg/kg group exhibited many of these same symptoms, in more animals. Seven animals in this group exhibited some or all of the following symptoms of toxicity: discoloration of kidneys, liver, and heart; traces of hemorrhage in the stomach and cecum; retention of a hardened substance in the stomach, possibly the test substance; viscous, oily or slightly hardened contents in the cecum; distention of the gallbladder; dark black-brown or dark green watery bile; thinned gallbladder wall; ulcerated lesion in the gallbladder; and adhesion of gallbladder and liver. It appears that the test substance, which is not water soluble, had an effect on the gallbladder and lower intestine. It is possible that digestion of the test material is very low, due to its poor solubility.

Reproductive Effects

Premature delivery was observed in 1/14, 0/14, 3/14, and 6/13 animals in the control, 100, 300, and 1000 mg/kg/d groups, respectively. No toxicity was observed in the 100 mg/kg/d group. There were no significant differences in the numbers of corpora lutea, implantations, post-implantation losses, numbers of live fetuses, sex ratios, or body weights of live fetuses between the control and treated groups. White, scattered spots were observed on the placenta in 1 of each of the control, 300 and 1000 mg/kg groups; the 1000 mg/kg rabbit had prematurely delivered. The significance of the white spots is unclear. Implantation occurred at the rate of 89.7%, 91.5%, 88.8%, 87.5% in the control, 100, 300 and 1000 mg/kg groups, respectively; there was no significant difference in implantation rates amongst the groups. See Table II for cesarean section observations.

Fetal Effects

1. Litter Data: Living fetuses were found in 13/14, 12/12, 11/14, and 4/13 pregnant females in the control, 100, 300, and 1000 mg/kg treated groups, respectively.

2. External Examination: One control fetus had multiple malformations consisting of cranioschisis, cleft palate, manus valga and umbilical hernia. One of the 100 mg/kg group exhibited articular flexion contracture in the foreleg. None of the fetuses exhibited external anomalies in the 300 and 1000 mg/kg groups.

3. Skeletal Examination: A defect in the 3rd distal phalanx of the hindleg was observed in 1 fetus (1.1%) of the 300 mg/kg group. Fusion of the cervical vertebrae was observed in 12 fetuses (12.9%) of the control group; 9 fetuses (10%) of the 100 mg/kg group; and 2 fetuses (7.7%) of the 1000 mg/kg group. Other anomalies observed included asymmetrical sternebrae, hypoplasia of the 3rd distal phalanx of the foreleg, and hypoplasia of the 2nd distal phalanx of the hindleg in approximately 1.1% of the fetuses in the 300 mg/kg group. The 100 mg/kg group had significantly greater numbers of ossified middle phalanges of the forelegs than the control group, but the other treatment groups were comparable with the controls. There were no other statistically significant differences in skeletal variations between the control and treated groups.

4. Visceral Examination: Two fetuses in the 300 mg/kg group exhibited visceral malformations, which included a cystic lung, hypoplasia of the left atrial auricle, persistent truncus arteriosus, and ventricular septal defect in one of the fetuses, and a gallbladder defect in the other. One fetus in this group had a slight vascular anomaly, which consisted of a persistent left azygos vein. No significant differences in the incidences of visceral abnormalities between drug and control groups were observed.

DISCUSSION: The test compound appeared to have a dose-related effect on food consumption, with concomitantly diminished weight gain. With regard to weight gain during the dosing period, the high dose group demonstrated significantly less weight gain than controls ($p<0.01$). This corresponded to a significantly less ($p<0.01$) food consumption in this group, when compared with the controls. By the end of the gestational period, there was no significant difference in food consumption and body weight gain between the groups.

Clinical signs of toxicity were present in the high dose dams, including ananastasia, diarrhea, decreased activity, lusterless fur, bradypnea, and emaciation. These signs were observed in 9 animals which also delivered prematurely or died during the study.

Upon gross examination of those dams, it appeared that the compound had dose-related effects on digestion, including some hemorrhaging in the stomach, cecum, and colon, retention of gas and a viscous material in the cecum. There were several incidences of distended gallbladders and changes in bile appearance in the high-dose group and appearance of the liver, which indicated the compound had an effect on the liver, perhaps due to its indigestibility.

There were no compound-related developmental abnormalities.

STUDY DEFICIENCIES: The study does not meet the Subdivision F Guideline criteria for core guideline or minimum classification, based on the following deficiencies in data reporting. The primary deficiency in this study was the low number (4) of litters available for analysis in the high dose group. In this study, the low numbers of available litters can be attributable to the maternal toxicity, which is considerable in the high dose group.

The study also does not separate the number of early and late resorptions from stillbirths, nor does it report totally resorbed litters, or distinguish between premature deliveries and abortions. Gravid uterine weights were not reported, so corrected weight gains could not be ascertained. Litter incidence data for malformations/ variations was not presented in a summary format.

TABLE I

Group	Number of Animals[4]	MEAN BODY WEIGHT GAIN DURING GESTATION (kg)[1] day of gestation: 6	9	12	15	18	22	25	28
Controls	14	0.00	0.02	0.04	0.10	0.11	0.15	0.18	0.19
100*	12	0.00	0.00	0.02	0.10	0.10	0.15	0.18	0.22
300*	14	0.00	-0.01	0.01	0.05	0.02	0.05	0.06	0.17
1000*	13	0.00	-0.05[2]	-0.12[3]	-0.19[3]	-0.29[3] (12)	-0.30[3] (10)	-0.12[2] (6)	0.16 (4)

Mean Food Consumption (g/day)[5]

Group	Day 6 (day 1 of dosing period)	Days 9-18 (dosing period)	Days 22-25 (post-dosing period)	Day 28 (last day of Gestation)
Control	168.0	151.25	132.5	112.0
100*	159.0	139.75	130.5	118.0
300*	167.0	131.50	110.5	114.0
1000*	158.0	68.50[6]	65.0[7]	116.0

* mg/kg/day of S-31183

[1] Data from Table 2 of the submission
[2] Significant difference from control group ($p<0.05$)
[3] Significant difference from control group ($p<0.01$)
[4] The numbers in parentheses represent the changing number of animals due to death or premature delivery
[5] Data calculated from Table 4 of the submission
[6] Mean food consumption for days 9, 12, 15 & 18; food consumption for each of those days was significantly different from the control group ($p < 0.01$)
[7] Value on day 22 was significantly different from controls ($p < 0.01$), but not significantly different on day 25

TABLE II: Cesarean Section Observations[1]

Dose:	Control	100 mg/kg/d	300 mg/kg/d	1000 mg/kg/d
#Animals mated	15	17	15	18
#Pregnant	15	13	14	13
Pregnancy rate (%)	100	76.5	93.3	72.2
Maternal wastage:				
#Died (total)	1	2	1	3
#Died/pregnant	1	1	0	3
#Nonpregnant	0	4	1	5
#Aborted/premature delivery	1	0	3	6
Total # litters	13	12	11	4
# Corpora lutea	116	106	107	32
Corpora lutea/dam	8.9	8.8	9.7	8.0
Total implantations	104	97	95	28
Preimplantation loss (%)	10.3	8.5	11.2	12.5
Implantations/dam	8.0	8.1	8.6	7.0
Implantation rate (%)	89.7	91.5	88.8	87.5
Postimplantation loss*	10	6	6	2
Early	7	2	4	2
Late	3	4	2	0
Postimplantation losses/dam	0.76	0.50	0.55	0.50
Total # live fetuses	94	91	89	26
#Live fetuses/dam	7.2	7.6	8.1	6.5
Mean fetal weight (g)	36.12	36.04	37.43	37.54
Sex ratio (% males)	50.00	57.14	47.19	53.85

* The study does not separate the number of prematurely delivered from aborted fetuses, nor dead fetuses from the number of resorptions

[1] Data extracted from Tables 1 and 6 of the submission

TABLE III

FETAL EXAMINATIONS

	Concentration of S-31183 (mg/kg)			
	0 (Controls)	**100**	**300**	**1000**
A. External Examination[1]				
# pups (litters) examined	94 (13)	90 (12)	89 (11)	26 (4)
# pups (litters) affected	1 (1)	1 (1)	0 (0)	0 (0)
Individual Observations:				
Cranioschisis	1	0	0	0
Cleft palate	1	0	0	0
Articular flexion contracture of the left front foreleg	0	1	0	0
Manus Valga	1	0	0	0
Umbilical hernia	1	0	0	0
B. Skeletal Examination[2]				
# pups (litters) examined	93 (13)	90 (12)	89 (11)	26 (4)
# pups (litters) affected with malformations:	0 (0)	0 (0)	1 (1)	0 (0)
# pups (litters) affected with anomalies:	12 (7)	9 (7)	2 (2)	2 (1)
# pups (litters) affected with variations:	26 (11)	14 (10)	12 (8)	7 (4)
Individual Observations:				
1. Malformations:				
Defect of distal phalanx of hindleg	0	0	1	0
2. Anomalies:				
Fusion of cervical vertebrae	12	9	0	2

Table III, continued

	Concentration of S-31183 (mg/kg)			
	0 (Controls)	100	300	1000
Asymmetrical sternebrae	0	0	1	0
Hypoplasia of distal phalanx of foreleg	0	0	1	0
Hypoplasia of distal phalanx of hindleg	0	0	1	0
3. Variations:				
Bipartite cervical vertebrae	0	0	1	0
Deformed cervical vertebrae	5	0	0	1
Deviated caudal vertebrae	0	1	3	0
13 ribs	7	3	5	2
Bipartite sternebrae	0	0	0	1
Bipartite hyoid bone	12	5	3	0
Shortened hyoid bone arch	1	1	0	1
Curved hyoid bone arch	1	2	1	3
Lumbar rib	1	2	0	0
C. Visceral Examination[3]				
# pups (litters) examined	93 (13)	90 (12)	89 (11)	26 (4)
# pups (litters) with visceral malformation:	0 (0)	0 (0)	2 (2)	0 (0)
# pups (litters) with visceral anomaly:	0 (0)	0 (0)	1 (1)	0 (0)
# pups (litters) with visceral variation:	19 (10)	16 (8)	18 (8)	10 (3)

Table III, continued

	Concentration of S-31183 (mg/kg)			
	0 (Controls)	100	300	1000
1. Malformations:				
Cystic lung	0	0	1	0
Hypoplasia of left atrial auricle	0	0	1	0
Persistent truncus arteriosus	0	0	1	0
Gallbladder defect	0	0	1	0
2. Anomalies:				
Persistent left azygos vein	0	0	1	0
3. Variations:				
Abnormally located posterior vena cava	17	14	14	9
Abnormally located right subclavian artery	5	0	5	0
Bifurcation of vermiform appendix	0	2	2	1

[1]Data extracted from Table 7 and Appendices 8-1 to 8-4 of the submission
[2]Data extracted from Tables 8 to 11 and Appendices 9-1 to 9-12 of the submission
[3]Data extracted from Tables 14 to 16 and Appendices 12-1 to 12-12 of the submission