2.4. REPRODUCTIVE TOXICOLOGY

2.4.1. FERTILITY STUDIES

2.4.1.1. Study Of Fertility And Early Embryonic Development To Implantation With SC-58635 By Oral Administration In The Rat, Document No.: PSA95C-30-SA4294; Date: 15-May-1995 (Vol. 1.55, p. 1-339 & Vol. 1.56, 1-230)

Included as an appendix to this report was:
Evaluation Of The SC-58635 Plasma Concentration Data From The Study Of Fertility And Early Embryonic Development To Implantation With SC-58635 By Oral Administration In The Rat, SA4294, Document No.: MRC-95S-0086; Date: 27-Apr-1995 (Vol. 1.56, p. 179-193)

Study Nº: SA4294/B 95723
Report Nº: PSA95C-30-SA4294
Study Aim: To evaluate the effects of SC-58635 on fertility and early embryonic development in rats
Compound: SC-58553 (Lot Nº 94K014-A1B, -A3B) suspension in 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O
Dose & Route:
♂: 0, 60, 300, and 600 mg/kg/day po, at least 28 days prior to mating, throughout the study.
♀: 0, 60, 300, and 600 mg/kg/day po, 14 days prior to placement for mating, during the mating period and in gestation from Day 0 to 7.
Control Vehicle: 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O
Animals: 115♂ & 115♀ Sprague-Dawley rats, strain Crl:CD®(SD)BR; Age: 12 & 10 wk old for ♂ and ♀, respectively; weighing 367 - 444 g for ♂ and 191 - 267 g for ♀ rats.
Study Location:

Study Date: 10/4/1994 - 1/23/1995
Compliance with GLP/QAU: Yes
Study Design:

Group	Dose (mg/kg/day)	Nº of Rats		
		Treated		Untreated
		♂	♀	♀
Vehicle Control	0	25	25	25
SC-58635	60	25	25	24*
SC-58635	300	25	25	25
SC-58635	600	25	25	25

*Assigned male died prior to mating with untreated females.

The following parameters were investigated: clinical signs; body weight (measured on Gestation Days 0, 3, 7, 10 and 13); food consumption recorded for the mated females (treated and untreated) during gestation intervals 0-3, 3-7, 7-10 and 10-13; estrous cycles; uterine examination on Gestation Day 13; PK; and terminal gross pathological examinations; sperm mortality; spermatozoa counts and morphology of spermatozoa.

Results: One ♂ in both the control and the 60 mg/kg/day groups died of unknown causes during the study. One ♂ in 600 mg/kg/day was killed on Day 102 in a moribund condition as a result of esophageal perforation. No treatment related clinical findings were noted for the male or treated females. There were no effects on body weights. Food consumption was increased for males receiving 300 mg/kg/day from weeks 2 to 3 and for females from weeks 1 to 2 of the premating

period. No gross pathological findings were treatment related. The estrous cycles of the SC-58635 treated females were not affected. The mating and fertility indices, conception rates and mean day of mating were unaffected in all animals. Sperm motility, spermatozoa counts and morphology were not changed by the treatment. The number of live fetuses and implantation sites were significantly lower in all SC-58635 treated groups. Significantly higher preimplantation losses were seen for the all treated females and this SC-58635 induced preimplantation loss was dose-dependent.
In conclusion, male fertility was not affected by SC-58635 treatment at the dose levels up to 600 mg/kg/day. For the treated females, the number of live fetuses was significantly lower and significantly higher preimplantation losses at dose levels ≥60 mg/kg/day.

2.4.1.2. Study Of Fertility And Early Embryonic Development To Implantation With SC-58635 By Oral Administration In The Female Rat, (SA 4345), Document No.: P30S4345; Date: 04-Nov-1996 (Vol. 1.57, 1-272)

Study Nº: SA4345/95813
Report Nº: P30S4345
Study Aims: To investigate the effects of SC-58635 on fertility and early embryonic development in female rats after oral administration.
Compound: SC-58635 (Lot Nº: 94K014-A3B, 99.8% purity)
Vehicle: 0.5% Methylcellulose

Group	Dose (mg/kg/day)	Nº of ♀
1 (Vehicle Control)	0	25
2	15	25
3	30	25
4	50	25
5	300	25

Dose and Route: 15, 30, 50, and 300 mg/10 ml/kg/day po by gavage
Animals: 125♀ rats, Crl:CD®(SD)BR, 10 weeks of age, weighing 211-258 g, 25/group.
Study Date: 2/7/95 (1st day of treatment) - 3/26/95 (necropsy)
Study Site:

GLP/AUC: Yes
Study Design: The female rats were given SC-58635 or vehicle control daily from 14 days prior to mating, throughout the mating and through Gestation Day 7. The following observations were conducted:

- Clinical Signs and Mortality - 2x/day.
- Body Weight and - 1x/week during premating treatment period and Gestation Days 0, 3, 7, 10, and 13.
- Food consumption - 1x/week during premating treatment period and Gestation Days 0-3, 3-7, 7-10, and 10-13.
- Necropsy - on Gestation Day 13. The reproductive tract was removed and the corpora lutea were counted. The uterine contents were examined. The following tissues were preserved: uterus, mammary glands (cervical and inguinal), vagina, ovaries, and any abnormalities.
- Mating and fertility indices, and the conception rates were calculated as follows:
  Mating Index (%) = (Nº of females mating)/(Nº of females placed for mating) x 100
  Fertility Index (%) = (Nº of females pregnant)/(Nº of females placed for mating) x 100
  Conception Rate (%) = (Nº of pregnant females)/(Nº of mated females) x 100
- Reproductive Indices were calculated as follows:
  Preimplantation loss (%) = (Nº of corpora lutea - Nº of implants)/(Nº of corpora lutea) x 100

Post implantation loss (%) = (Nº of implants - Nº of live embryos)/(Nº of implants) x 100

Results:

- Mortality and Clinical Findings- One ♀ @ 50 mg/kg/day died on study Day 29 prior to mating due to the handling error with macroscopic findings of pulmonary edema and fluid in the tracheal lumen and a clot in the cranial cavity. Because mating was not observed, a ♀ @ 50 mg/kg/day was sacrificed at the end of the mating period and was found to be pregnant. No remarkable clinical signs were noted attributable to the treatment.
- Body Weight and Food Consumption - No treatment related effects were observed. Food consumption values for the 15 mg/kg/day treated females were significantly lower during the prestudy period.
- Gross Pathological Findings - Adhesion between the liver and adjacent structures, such as the diaphragm or intra-abdominal fat was seen in 3 females @ 300 mg/kg/day. This change was associated with intrahepatic abnormalities, such as depressed and/or dark and/or pale area(s). The sponsor stated that these findings were incidental. Dark mucoid material in the vaginal lumen was the most frequent observation in all groups.
- Estrous Cycles - Not affected.
- Reproductive Performance and Parameters - The mating and fertility indices, conception rates and mean day of mating were unaffected. The numbers of corpora lutea were significantly ↓ for the 300 mg/kg/day group. Significantly decreased numbers of implantation sites and live embryos were seen in the females @ 50 and 300 mg/kg/day. These reductions resulted in significantly ↑ pre- and post implantation losses (%) in these groups. The following table shows reproductive indices for each group.

Reproductive Indices	Dose (mg/kg/day)				
	Vehicle Control	15	30	50	300
Nº of Corpora Lutea	17.5±2.04	17.3±3.15	17.6±2.33	17.2±2.80	15.7±2.42**
Nº of Implantation Sites	16.5±1.90	16.3±2.65	16.9±2.09	14.0±3.76*	11.4±4.56***
Nº of Live Embryos	15.4±1.74	14.4±3.33	14.9±2.61	11.6±4.41***	9.4±4.18***
Nº of Dead Embryo	0.0±0.21	0.0±0.00	0.0±0.00	0.1±0.23	0.0±0.00
Nº of Early Resorption	1.0±1.17	1.9±1.94	2.0±1.61	2.4±3.55	2.0±1.93
% Preimplantation Loss	5.8±6.52	5.7±7.37	4.0±5.12	18.0±19.21*	27.7±25.40***
% Post Implantation Loss	6.4±6.67	11.9±12.81	12.0±9.34	16.7±21.02*	20.3±21.90**

Significantly different from control value: *p<0.05; **p<0.01; ***p<0.001 (Mann-Whitney).

In conclusion, no evidence of SC-58635 induced toxicity was observed in the females at any dose level. There was no affect on mating and fertility indices, conception rates or the mean day of mating when female rats were treated with SC-58635 at dose levels up to 300 mg/kg/day. For the treated females, the numbers of implantation sites and live embryos were significantly ↓ at dose levels of ≥50 mg/kg/day that resulted in significantly ↑ pre- and post-implantation losses. In addition, significant reductions in the numbers of corpora lutea were seen in the ♀ @ 300 mg/kg/day. Therefore, the NOAEL was 30 mg/kg/day for female rats in this study.

2.4.1.3. Study Of Fertility And Early Embryonic Development Through Implantation With SC-58635 In The Female Rat (2 Week Oral Administration Followed By 2 Week Reversal Prior To Mating)(SA 4402), Document No.: P30S4402; Date: 26-Aug-1996 (Vol. 1.57, p. 273-464)

Report Nº:	P30S4402
Study Nº:	SA4402
Study Aim:	To evaluate the reversibility of SC-58635 induced effect on fertility and early embryonic development in female rats
Compound:	SC-58635 (Lot Nº 94K014-A3B)

Vehicle Control: 0.5% (w/v) methylcellulose and 0.1% Polysorbate in distilled and deionized H_2O

Dose & Route: 0, 60 and 300 mg/kg/day po by gavage

Animals: 75 ♀ rats, Crl:CD®(SD)BR, weighing 177-245 g, 8-9 weeks of age, 25/group

Group	Dose (mg/kg/day)	Nº of ♀
1	0	25
2	60	25
3	300	25

Study Location:

Compliance with GLP/QAU: Yes

Study Date: 6/13/95 - 8/6/95

Study Design: The animals were orally dosed with SC-58635 daily for 14 days followed by a 14-day reversal period before mating. The following parameters were monitored.

- Mortality and Clinical Signs - 2x/day.
- Physical Examination - 1x/week.
- Body Weight & Food Consumption - Gestation Days 0, 3, 7, 10, and 13.
- Necropsy - Gestation Day 13.
- Estrous Cycles - The estrous cycles were determined for 10 days prior to mating.
- Mortality and Clinical Signs - No death occurred. No treatment-related clinical signs were noted.
- Body Weight & Food Consumption - No SC-58635 treatment related effects were noted.
- Estrous Cycles - The estrous cycles were not altered.
- Maternal Reproductive Performance - Neither mating nor fertility indices were affected. Conception rates in the treated ♀ were comparable to the control. There were no significant changes in the numbers of corpora lutea, implantation sites, live and dead fetuses, early absorption or pre and post implantation losses.
- Necropsy - No remarkable findings were obtained during gross pathological examination.

Results: In the previous study (Study Nº SA4294)(2.3.1.1.), results showed that SC-58635 at doses ≥60 mg/kg caused a significant ↓ in the numbers of live fetuses and ↑ in the preimplantation losses when ♀ rats were treated with SC-58635 14 days before mating, and through Gestation Day 7. These effects were not observed in the present study when treated females were allowed to have a 14-day recovering period before mating. The length of treatment with SC-58635 in the current study was shorter than that indicated in the Study Nº SA4294. Therefore, under the current study condition, the effects of SC-58635 on female reproductive performance might be reversible.

2.4.2. TERATOLOGY STUDIES

2.4.2.1. An Embryo-Fetal Developmental Toxicity Study Of SC-58635 In Rats, SA 4362, Document No.: PSA95S-30-SA4362; Date: 06-Dec-1995 (Vol. 1.58, p. 1-172)

Included as an appendix to this report was:
Evaluation Of Plasma SC-58635 Concentrations In An Embryo-Fetal Developmental Toxicity Study In Rats, SA 4362, Document No.: MRC95S-30-950168; Date: 22-Sep-1995 (Vol. 1.58, p. 146-168)

Study Nº: SA4632

Report Nº: PSA95S-30-SA4632

Study Aim: To determine the possible adverse effects on the pregnant female rats and on the development of the embryo and fetus following multiple oral administration of SC-58635 on Gestation Days 7-18.

Compound: SC-58635 (Lot Nº 94K014-A3B) suspension in 0.5% methylcellulose (w/v), 0.1% polysorbate 80 (v/v) in dist. H_2O

Dosage & Route: 0, 10, 30, and 100 mg/kg/day, 10 ml/kg po from Gestation Days 6-17 for 12 days

Animals: ♀ (VAF) CD strain rats, weighing 182-288g, ~4 months of age, 20/group for the Study Nº SA4632, and 6/group and 2 in the control group for the companion PK study

Study Location: G.D. Searle, Skokie, IL

Compliance with QAU: Yes

Study Design: Pregnant female rats were dosed with SC-58635 at 0, 10, 30, or 100 mg/kg/day for 12 days (Gestation Days 6-17). All animals were observed for clinical signs at least once daily. All animals were sacrificed on Gestation Day 20. All maternal and fetal data were collected at necropsy. Blood samples were collected on Gestation Days 6 & 16 at 2, 3, 4, and 24 hr post dosing. Plasma SC-58635 concentrations were determined by a validated method.

Results:

- Clinical Observations & Mortality - One at 100 mg/kg in the companion PK study died due to dosing error. Two animals in the control group were excluded from the study due to inadvertent deprivation of H_2O intakes. No remarkable treatment-related clinical signs were noted.
- Food Consumption and Body Weight - No treatment-related changes were seen.
- Maternal Reproductive Performance - The data from any reproductive indices (Nº of corpora lutea, implantations, resorptions, dead fetuses, preimplantation loss, and postimplantation loss) were comparable across all dose groups. There was a slight but not statistical significant decrease in the number of live fetuses observed in the 100 mg/kg group. The mean (±SD) live fetuses for control, 10, 30, and 100 mg/kg Groups were 13.7±2.1, 13.8±2.0, 13.5±2.0, and 12.1 ±3.0, respectively.
- Toxicokinetics - Dose-dependent but not dose-proportional increases in C_{max} and AUC were noted on Gestation Days 6 & 16. The summarized PK parameters obtained on Gestation Days 6 & 16 are presented in the following table. C_{max} and AUC values were higher on Gestation Day 16 than those values obtained on Gestation Day 6 for the animals receiving 10 and 30 mg/kg/day indicating that accumulation of SC-58635 had occurred after repeated dosing.

Parameter	10 mg/kg		30 mg/kg		100 mg/kg	
	Gestation Day 6	Gestation Day 16	Gestation Day 6	Gestation Day 16	Gestation Day 6	Gestation Day 16
AUC_{0-24} (μg•hr/ml)	20.3	37.1	43.9	67.0	134	115
AUC/Dose	2.03	3.71	1.46	2.23	1.34	1.15
C_{max} (μg/ml)	1.79	2.81	3.01	5.03	6.37	7.45
C_{max}/Dose	0.179	0..81	0.100	0.168	0.0637	0.0745
T_{max} (hr)	3.00	3.00	3.00	3.00	4.00	4.00

- Fetal Parameters - Live fetal body weights were similar among treated and control groups. Fetal external and visceral examination revealed that one fetus in the 10 mg/kg group with major malformation (elongated nose, right anophthalmia, displaced left eye, displaced ears, no mouth opening, a papillated left flap of tissue located next to the left eye, and missing the left kidney and ureter). Skeletal examination of this particular fetus also showed some alterations (malformations) with the characteristics of split, misshaped, and unossified skull bones. **Based on the data from skeletal examination, the incidence in the wavy ribs appeared to increase in the fetuses at 30 and 100 mg/kg groups with values of 7 in 4 litters and 23 in 7 litters respectively as compared with 5 in 2 litters in the control group.** It should be noted that data

from the historical controls should be employed to compare the incidence for alterations (malformations) and variations in the external, visceral, and skeletal examinations.

Therefore, the no-observable-adverse-effect-level (NOAEL) for the maternal, reproductive, and fetal development in the present study was 100, 30, 10 mg/kg/day, respectively.

2.4.2.2. An Oral Study Of Embryo-Fetal Development In The Rat Administered SC-58635 (SA 4599), Document No.: P20S4599; Date: 03-Dec-1997 (Vol. 1.59, p. 1-375)

Included as an appendix to this report was:
Pharmacokinetics Of SC-58635 in An Oral Study Of Embryo-Fetal Development in The Rat Administered SC-58635 (SA4599), Document No.: M3097210; Date: 04-Sep-1997 (Vol. 1.59, p. 353-369)

Study Nº: SA 4599/COV6127-353
Report Nº: P20S4599
Study Aim: To evaluate the maternal and embryo-fetal toxicity and teratogenic potential of SC-58635 when administered once daily via oral gavage to pregnant rats during the period of organogenesis.
Compound: SC-58553 (Lot Nº 95K010-A1A) suspension in 0.5% methylcellulose (w/v) (400 cps) & 0.1% polysorbate 80 (v/v) in H_2O
Dose & Route: 0, 10, 30 and 100 mg/kg/day, 10 ml/kg for 12 days (Gestation Days 6-17) by gavage
Control vehicle: 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O, 10 ml/kg
Animals: ♀ Crl:CD®BR rats ~ 10-11 weeks of age, weighing 208 - 298 g at time of mating, 30/group for Toxicology study and 2-6/group for PK study.
Study Location:
Study Date: 6/3/97 - 6/20/97
Compliance with GLP/QAU: Yes
Study Design: Groups of 32-36 pregnant rats were dosed with SC-58635 or vehicle once daily for 12 days (Gestation Days 6-17) by oral gavage. Group assignments and dose levels are as follows:

Group	Dose	Nº Rats/Group	
	(mg/kg/day)	Toxicology Study	PK Study
1 (Control)	0	30	2
2 (Low)	10	30	6
3 (Mid)	30	30	6
4 (High)	100	30	6

The following parameters were monitored.

- Mortality and Clinical Signs - 2x/day.
- Body Weight & Food Consumption - Gestation Days 0, 6, 8, 10, 12, 14, 16, 18 and 20.
- PK - on Gestation Days 6 and 17 at 0, 2, 3, 4 and 24 hr post dose.
- Cesarean Section and Necropsy - Gestation Day 20. Each animal were examined for cervical, thoracic, or abdominal visceral abnormalities. Abnormal viscera were preserved in 10% neutral-buffered formalin. The uterus from each gravid female was excised, weighed, and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions, and any abnormalities. The uteri of apparently non-pregnant females were stained with ammonium sulfide for verification of pregnancy status. The ovaries were examined for the number of corpora lutea.

- Fetal Examination - Each fetus was sexed, weighed, examined for external abnormalities and sacrificed. Visceral examination was performed on ½ of the fetuses from each litter for assessing soft tissue development. The remaining fetuses were processed for skeletal examination using the Alizarin Red S staining method. This evaluation included examination of the skull, long bones, vertebral column, rib cage, extremities, and pectoral and pelvic girdles. Bone alignment and degree of ossification were assessed. All fetuses were kept in Bouin's fixative (fetuses examined for visceral abnormalities) or glycerin (fetuses examined for skeletal abnormalities).

Results:

- Clinical Observations and Mortality - One ♀ @ 10 mg/kg was found dead on Gestation Day 20 as a result of dosing error (perforated esophagus) with clinical signs of swollen dorsal cervical area, swollen right shoulder, and few or no feces.
- Body Weights and Food Consumption - Significantly lower mean body weight (↓ 4% relative to control) and body weight change (↓ 28.5%) values were noted for the animals @ 30 mg/kg during the pretreatment interval (Gestation Days 0-6). A significant reduction (↓13.6 %) in food consumption was observed for the high dose group during Gestation Days 6-8.
- Gross Pathology - The Group 2 ♀ that died on Gestation Day 20 had a perforated esophagus, a large amount of food in the thoracic cavity and enlarged adrenals. Another Group 2 ♀ had a diaphragmatic hernia. There were no treatment related changes in gravid uterine weights, corrected terminal weights, and net body weight gains. The pregnancy rates were 97, 100, 100, and 100% for the main study females in Groups 1-4, respectively. The mean numbers of corpora lutea and implantation sites and percent preimplantation loss values of the SC-58635-treated animals were comparable to those of the control group. Values for the mean percent of early, late, and total resorptions and viable fetuses were comparable among all groups. There were no dead fetuses and the sex ratios and mean covariate fetal weights were similar among all groups.
- Fetal Evaluations - **A dose dependent increase in diaphragmatic hernia (soft tissue malformation) was noted.** The fetal and litter incidences for diaphragmatic hernia in each group are presented in the following table.

		Dose (mg/kg)			
		0	10	30	100
VISCERAL EVALUATION					
Litters Evaluated		29	29	30	30
Fetuses Evaluated		209	215	216	221
Soft tissue Malformations					
Diaphragmatic Hernia	Fetal Incidence	0	0	8 (3.7%)	31 (14%)
	Litter Incidence	0	0	6 (20%)	13 (43%)
SKELETAL EVALUATION					
Litters Evaluated		29	29	30	30
Fetuses Evaluated		207	218	211	222
Skeletal Variations					
Unossified Vertebral Centrum	Fetal Incidence	5 (2.4%)	0	3 (1.4%)	11 (5.0%)
	Litter Incidence	4 (14%)	0	3 (10%)	7 (23%)
Bipartite Vertebral Centrum	Fetal Incidence	5 (2.4%)	4 (1.8%)	1 (0.5%)	8 (3.6%)
	Litter Incidence	4 (14%)	3 (10%)	1 (3.3%)	7 (23%)
5th Sternebrae Incomplete Ossification	Fetal Incidence	64 (31%)	71 (33%)	88 (42%)	109 (49%)
	Litter Incidence	23 (79%)	22 (76%)	25 (83%)	29 (97%)
Sternebrae Asymmetrically Ossified	Fetal Incidence	3 (1.4%)	1 (0.5%)	12 (5.7%)	9 (4.1%)
	Litter Incidence	2 (6.9%)	1 (3.4%)	9 (30%)	8(27%)
Skeletal Malformations					
Absent Bone in Skull	Fetal Incidence	0	0	0	1 (0.5%)
	Litter Incidence	0	0	0	1 (3.3%)
Vertebral Anomaly with/without Rib Anomaly	Fetal Incidence	0	0	0	1 (0.5%)
	Litter Incidence	0	0	0	1 (3.3%)

- PK - SC-58635 was absorbed systemically and plasma levels of SC-58635 increased non-proportionally with dose. The mean PK parameters are presented in the following table.

PK Parameter	10 mg/kg		30 mg/kg		100 mg/kg	
	Gestation Day 6	Gestation Day 17	Gestation Day 6	Gestation Day 17	Gestation Day 6	Gestation Day 17
AUC_{0-24} (μg•hr/ml)	45.7	47.6	54.3	104	140	115
AUC/Dose	4.57	4.76	1.81	3.47	1.4	1.15
C_{max} (μg/ml)	3.79	3.20	4.91	5.43	7.66	7.41
C_{max}/Dose	0.379	0.320	0.164	0.181	0.0766	0.0741
T_{max} (hr)	3.00	3.00	3.00	4.00	4.00	3.00

2.4.2.3. A Range-Finding Study of SC-58635 In Pregnant Rabbits, Document No.: PSA95S-30-EX4310; Date: 27-Mar-1995 (Vol. 1.60, p. 1-69)

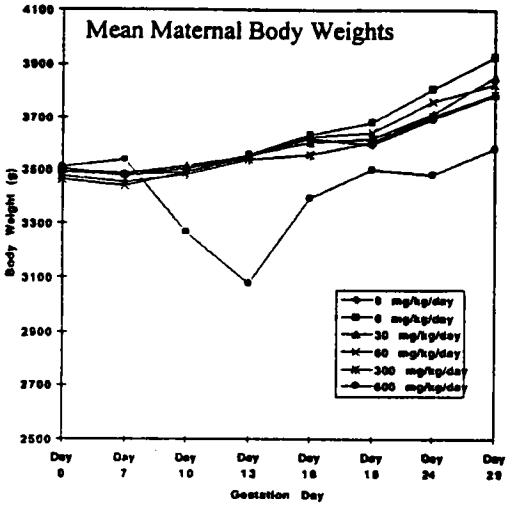
Included as an appendix to this report was:
Searle Memo Report Of SC-58635 Plasma Concentrations In A Range-Finding Study Of SC-58635 In Pregnant Rabbits, EX4310, Document No.: MRC-95S-0032; Date: 26-Jan-1995 (Vol. 1.60, p. 58-64)

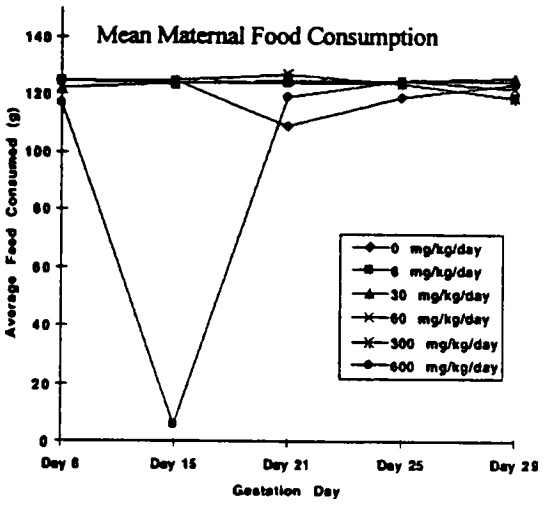
Study Nº: EX4310
Report Nº: PSA-95S-30-EX4310
Study Aim: To evaluate the potential toxic effects of SC-58635 on fetal viability in rabbits
Compound: SC-58553 (Lot Nº 94K014-A3B) suspension in 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O
Dose & Route: 6, 30, 60, 300, and 600 mg/kg/day, 10 ml/kg for 12 days by gavage
Control vehicle: 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O, 10 ml/kg
Animals: 36 nulliparous ♀ New Zealand White rabbits, approximately 5 mon of age; Strain Hra:(NZW)SPF; Weight: 3045 - 3929 g; 6/group
Study Location: G.D. Searle, Skokie, IL
Study Date: 11/28/94 - 12/20/94
Compliance with GLP/QAU: No
Study Design: Pregnant rabbits, 6 groups of 6, were orally administered with SC-58635 (6, 30, 60, 300, or, 600 mg/kg) or vehicle via gavage for 12 days from Gestation Days 7-18. Animals were examined daily for mortality and clinical signs starting on Gestation Day 7. Body weights were measured on Gestation Days 0, 7, 10, 13, 16, 19, 24, and 29. Food consumption was measured for the 24 hr interval on Gestation Days 7-8, 14- 20-21 24-25, and 28-29. Blood samples was taken on Gestation Days 7 and 17 at selected time points for the determination of plasma SC-58635 levels. All surviving animals were sacrificed on Gestation Day 29. Gross pathological examine was performed and the reproductive tracts were evaluated to acquire the numbers of corpora lutea, implantations, resorptions, and live and dead fetuses. All fetuses were individually weighed and examined.

Results:

- Clinical Signs and Mortality - No treatment-related mortality occurred at any dosage level. One rabbit from each of 6 and 300 mg/kg/day group died from gavage error on Gestation Day 11. Post mortem examinations showed foamy, fluid-filled lungs. One female in 600 mg/kg/day group aborted on Gestation Day 24. Two rabbits in the same group showed clinical signs of red materials.

- Body Weight and Food Consumption - Decreased body weights during treatment period, Gestation Days 7-18 and a marked decrease in food consumption between Gestation Days 14-15 were noted in rabbits receiving SC-58635 600 mg/kg/day group as depicted in the following figure.





- Female Reproductive Parameters - It appeared that no drug related effects on the mean numbers of corpora lutea, implantations, resorptions, fetal weights and live or dead fetuses were noted in groups of rabbits receiving ≤300 mg/kg/day. In the 600 mg/kg/day group, significantly increased post-implantation losses ($p \leq 0.003$) and decreased live fetuses ($p \leq 0.014$) were noted. External examination of fetuses revealed no SC-58635 treatment associated changes. In conclusion, SC-58635 had significant maternal toxicity and embryo-fetal toxicity at the level of 600 mg/kg/day by the evidence of weight losses, reduced food consumption, clinical signs, significantly higher post-implantation loses, and significantly reduced live fetuses.
- PK- SC-58635 could be detected in all plasma samples indicating that it was systemically available at all dosage levels. Mean plasma SC-58635 concentrations on treatment Days 1 and 11 (Gestation Days 7 and 17) are listed in the following table.

Dose (mg/kg)	Plasma SC-58635 Concentration (μg/ml)							
	2 hr		3 hr		4 hr		24 hr	
	Day 1	Day 11	Day 1	Day 11	Day 1	Day 11	Day 1	Day 11
6	0.108	0.167	0.132	0.155	0.125	0.173	-	0.0132
30	0.49	0.454	0.535	0.462	0.559	0.673	0.0591	0.0663
60	0.838	0.655	1.17	1.02	0.837	0.737	0.227	0.212
300	1.64	2.65	1.94	3.45	1.89	2.97	1.69	1.95
600	2.1	10.1	2.83	8.49	2.54	10.2	2.64	6.25

2.4.2.4. A Pilot Study Of SC-58635 In Rabbits, Document No.: PSA95S-30-EX4309; Date: 20-Feb-1995 (Vol. 1.60, p. 70-85)

Included as an appendix to this report was:
Searle Memo Report Of SC-58635 Plasma Concentrations In The Pilot Study Of SC-58635 In Pregnant Rabbits, EX4309, Document No.: MRC-95S-0031; Date: 23-Jan-1995 (Vol. 1.60, p. 83-85)

Study Nº	EX4309
Report Nº	PSA95S-30-EX4309
Study Aim:	To evaluate the potential toxic effects of SC-58635 and to establish PK data for dosage selection in a range-finding study in rabbits

Compound: SC-58553 (Lot Nº 94K014-A1B) suspension in 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O

Dose & Route: 200, 400, and 600 mg/kg/day, 10 ml/kg for 4 days by gavage

Animals: 6 mated ♀ New Zealand White rabbits (Gestation Days 19-21), 6 to 7 months of age; Strain Hra:(NZW)SPF; Weight: 3494 - 4267 g; 2/group

Study Location: G.D. Searle, Skokie, IL

Study Date: 11/7-11/1994

Compliance with GLP/QAU: No

Study Design: Female rabbits, 2/group, were given SC-58635 suspension in 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O at levels of 200, 400, or 600 mg/kg/days for 4 days by gavage. Clinical signs and mortality were monitored daily. Blood sampling was taken for plasma SC-58635 determination at approximately 2, 3, 4, and 24 hr following the first dosing and 24 hr following the last dosing. Animals were subjected to post-mortem examinations on day 5.

Results: Animals receiving 600 mg/kg/day had decreased body weights (↓ 5% on Day 5) with signs of few, soft, and small or no feces. No significant changes were attributable to the treatment at post-mortem. Plasma SC-58635 concentrations were as followings:

Dose	Mean Plasma SC-58635 Levels (μg/ml)				
(mg/kg/day)	Day 1				Day 4
	2 hr	3 hr	4 hr	24 hr	24 hr
200	3.44 ± 0.51	3.52 ± 0.97	2.77 ±0.61	1.97 ± 0.41	1.75 ± 0.82
400	3.86 ± 0.86	3.46 ± 0.42	3.78[a]	3.61 ± 0.69	3.70 ± 0.78
600	3.55 ± 0.17	4.93 ± 0.98	3.73 ± 0.15	5.72 ± 0.56	5.99 ± 2.76

[a] Data was obtained from a single animal.

Based on data presented in the current study, SC-58635 was considered to be toxic at 600 mg/kg/day level.

2.4.2.5. A Segment II Developmental Toxicity Study Of SC-58635 In Rabbits, Document No.: PSA95S-30-SA4342; Date: 25-Oct-1995 (Vol. 1.60, p. 86-255)

Included as an appendix to this report were:

1. Evaluation Of Plasma SC-58635 Concentrations In A Segment II Developmental Toxicity Study Of SC-58635 In Rabbits (SA4342), Document No.: MRC95S-30-950134; Date: 24-Jul-1995 (Vol. 1.60, p. 223-245)
2. Final Report Amendment No. 1: A Segment II Developmental Toxicity Study Of SC-58635 In Rabbits (SA4342), Document No.: P31S4342; Date: 16-Oct-1997 (Vol. 1.60, p. 249-255)

Study Nº: SA4342

Report Nº: PSA95S-30-SA4342

Study Aim: To determine the possible adverse effects on the pregnant female rabbits and on the development of the embryo and fetus following multiple oral administration of SC-58635 on Gestation Days 7-18.

Compound: SC-58635 (Lot Nº 94K014-A3B) suspension in 0.5% methylcellulose (w/v), 0.1% polysorbate 80 (v/v) in dist. H_2O

Dosage & Route: 0, 60, 150, and 300 mg/kg/day, 10 ml/kg po from Gestation Day 7-18 for 12 days

Animals: New Zealand White ♀ rabbits (Hra:SPF), weighing 2821-4821 g, 4-6 months of age, 20/group

Study Location: G.D. Searle, Skokie, IL

Study Date (In-Life): 2/5/95 - 3/3/1995.

Compliance with QAU: Yes

Study Design: Pregnant female rabbits were dosed with SC-58635 at 0, 60, 150, or 300 mg/kg/day for 12 days (from Gestation Days 7-18). All animals were observed for clinical signs at least once daily. All rabbits were sacrificed on Gestation Day 29 and all maternal and fetal data were collected. Blood samples were collected on Gestation Days 7 & 19 at 1, 2, 3, 4, 8, and 24 hr post dosing. Plasma SC-58635 concentrations were determined by a validated method.

Results:

- Clinical Observations & Mortality - Two in each of 60 and 300 mg/kg groups were found dead as results of dosing errors. Reduced feces, soft stool and fecal tinted fur were seen scattered across all groups.
- Food Consumption and Body Weight - Food intake and body weight gains were comparable among treated and control animals.
- Toxicokinetics - Dose-dependent but not dose-proportional increases in C_{max} and AUC were noted on Gestation Days 7 & 19. The following table showed summarized PK parameters obtained on Gestation Days 7 & 19. C_{max} and AUC values were higher on Gestation Day 19 than those values obtained on Gestation Day 7 indicating that accumulation of SC-58635 had occurred after repeated dosing.

Parameter	60 mg/kg		150 mg/kg		300 mg/kg	
	Gestation Day 7	Gestation Day 19	Gestation Day 7	Gestation Day 19	Gestation Day 7	Gestation Day 19
AUC (μg•hr/ml)	14.9	22.5	24.5	41.5	37.4	89.0
AUC/Dose	0.249	0.375	0.164	0.227	0.125	0.297
Cmax (μg/ml)	0.951	1.49	1.41	2.37	1.76	5.14
C_{max}/Dose	0.0158	0.0248	0.00942	0.0158	0.00585	0.0171
T_{max} (hr)	8.00	8.00	8.00	8.00	4.00	8.00

- Fetal Parameters - External and visceral fetal examination showed a slight increase in sternebrae fused of the fetuses at 150 mg/kg group. There was a slight and dose-dependent increase in the incidence of misshapen sternebrae in the fetuses at 150 and 300 mg/kg groups during skeletal examination.

	Dose (mg/kg/day)			
	Control	60	150	300
Nº. Live Fetuses Examined	177	121	153	111
Nº Litter Examined	20	17	20	16
TYPE AND NUMBER OF FETAL ALTERATIONS: (Nº of Fetuses/Nº of Litters)				
Ribs Fused (M)	1 (0.6%)/1	2 (1.7%)/1	2 (1.3%)/2	4 (3.6%)/4
Sternebrae fused (M)	1 (0.6%)/1	2 (1.7%)/2	13 (8.5%)/10	5 (4.5%)/4
Sternebrae Misshapen (V)	2 (1.2%)/2	3 (2.5%)/3	6 (3.9%)/4	5 (4.5%)/2

- Maternal Reproductive Performance - Oral administration of SC-58635, at dosages of 150 mg/kg/day, to pregnant rabbits did not have adverse effects on the average of corpora lutea, implantations, resorption and live or dead fetuses. In contrast, at the dose level of 300 mg/kg, SC-58635 caused significant decreases in the numbers of live fetuses and significant increases in the post-implantation losses (resorption and dead fetuses) as shown in the following table.

REPRODUCTIVE STATUS OF PREGNANT FEMALES AT SACRIFICE

DOSE GROUP		FETAL OBSERVATIONS: CORPORA LUTEA	IMPLANTATIONS	RESORPTIONS	LIVE FETUSES	DEAD FETUSES	PREIMP. LOSS(a)	POSTIMP LOSS(b)
CONTROL	MEAN	11.2	9.6	0.8	8.9	0.0	1.6	0.8
	IMN(c)	10.9	9.6		8.9		1.1	0.6
	STD	2.1	2.4	1.1	2.2	0.0	1.6	1.1
	N	20	20	20	20	20	20	20
60 MG/KG SC-58635	MEAN	10.2	8.1	1.0	7.1	0.0	2.1	1.0
	IMN(c)	10.0	8.3		7.4		1.4	0.7
	STD	1.8	2.2	1.5	2.3	0.0	2.6	1.5
	N	17	17	17	17	17	17	17
	P-VALUE (TREND)				.025*			
150 MG/KG SC-58635	MEAN	9.9	8.9	1.3	7.7	0.0	1.0	1.3
	IMN(c)	9.6	9.0		7.9		0.8	0.9
	STD	1.7	2.1	1.4	2.6	0.0	1.0	1.4
	N	20	20	20	20	20	20	20
	P-VALUE (TREND)				.025*			.17
300 MG/KG SC-58635	MEAN	10.9	9.5	2.6	6.9	0.1	1.4	2.6
	IMN(c)	10.4	9.4		7.2		0.9	2.1
	STD	2.7	3.0	2.0	3.2	0.3	1.8	2.0
	N	16	16	16	16	16	16	16
	P-VALUE (TREND)	(d)	.21	(e)	.018*	(e)	1.0	.001*

(a) Calculated as Corpora Lutea - Implantations
(b) Calculated as Resorptions + Dead
(c) IMN computed only for those parameters statistically analyzed
(d) Tested only for global homogeneity, p-value = .19
(e) Parameter not statistically analyzed

Therefore, the lowest no-observable-effect level (NOEL) for maternal, reproductive, and developmental toxicity in the rabbits were 300, 150 and 60 mg/kg, respectively.

2.4.3. PERINATAL/POSTNATAL STUDY

2.4.3.1. A Study Of Pre And Postnatal Development With SC-58635 By Oral Administration In The Rat, (SA 4404), Document No.: P30S4404; Date: 21-Mar-1997 (Vol. 1.61-1.64)

Included as an appendix to this report was:
Evaluation Of Plasma Concentration Data In A Study Of Pre And Postnatal Development With SC-58635 By Oral Administration In The Rat, SA4404, Document No.: M3096141; Date: 28-Oct-1996 (Vol. 1.64, p. 202-218)

Study Nº:	SA4404/95903
Report Nº:	P30S4404
Study Aims:	To examine the effects of SC-58635 on gestation, parturition and lactation in the dams and the development, survival, physical development, behavior and reproductive performance of the pups.
Compound:	SC-58635 (Lot Nº 95K010-A1A)
Vehicle:	0.5% methylcellulose (w/v) + 0.1% polysorbate 80 (v/v) in dist. H_2O
Dosage & Route:	0, 10, 30, or 100 mg/10 ml/kg po by gavage from Gestation Day 6 to Days 21-23 post partum
Animals:	Sprague-Dawley rats, Crl:CD®(SD)BR, 12 weeks of age, weighing 212-292 g, 25 ♀/group

Study Date (In-Life): 10/2/95 - 2/20/96
Study Site:

GLP/AUC: Yes

Study Design: Groups of 25 pregnant ♀ (F_0) were given SC-58635 at doses of 10, 30, and 100 mg/10 ml/kg/day from Gestation Day 6 through Days 21-23 post partum by oral gavage. F_1 generation (1/sex from each litter), weaned on Day 21 post partum, was examined for physical, reflex/sensory development, behavior and reproductive performance. All non-selected pups were subjected to a gross examination. The following observations were performed.

Group	Compound	Dose (mg/kg/day)	Nº of mated ♀
1	Vehicle Control	0	25
2	SC-58635	10	25
3	SC-58635	30	25
4	SC-58635	100	25

F_0 Generation:

- Clinical Signs and Mortality - 2x/day
- Body Weight - Gestation Days 0, 6, 9, 12, 15, 18, and 20 and Post Partum Days 0, 4, 7, 10, 14, 17 and 21.
- Food Consumption - Gestation Days 0-6, 6-9, 9-12, 12-15, 15-18, and 18-20.

F_1 Litter Observation:

- Clinical Condition - 1x/day during the lactation period.
- Body Weight - Days 4, 7, 10, 14, 17, and 21 post partum.
- Culling - On Day 4 post partum, the litter was culled to 8 pups (4♂ & 4♀)
- Physical Development - Day 1 post partum and onward: pinna unfolding; Day 7 post partum and onward: tooth eruption; Day 12 post partum and onward : eye opening.
- Reflexological Development - Day 2 post partum until all pups in the litter had a positive response or until culling on Day 4 post partum; the negative geotaxis test was evaluated from Day 8 post partum until all pups in the litter had a positive response; the auricular startle response was assessed from Day 12 post partum until all pups in the litter have a positive response.
- Weaning and Selection for F_1 Adult Generation - Day 21 post partum, 1♂ and 1♀ were selected from each litter to form F_1 adult generation.

F_1 Adult Observation:

- Clinical Condition - 2x/day
- Body Weight - Gestation Days 0, 6, 9, 12, 15, 18, and 20.
- Physical Development - ♀: Day 26 post partum and onward, assessment of vaginal opening; ♂: Day 35 and onward, assessment of preputial separation.
- Visual Function (pupillary closure and visual placing) - Day 21 post partum
- Behavior Performance -
  Motor Activity: Figure 8 mazes assessment on Days 35 (±1) and 60 (±2) post partum.
  Auditory Startle Habituation: startle habitation assessment on Day 55 (±2) post partum.
  'E' Water Maze: Days 60 and 70 post partum.
- Mating Procedure - On Day 85, 1♂ and 1♀ from the same dose group were placed together for a maximum of 14 days. Vaginal lavage was examined for spermatozoa and to identify pregnancy.
- Observation at parturition - 3x/day from Gestation Day 20 for signs of parturition and any sign of dystocia.

F_2 Generation:

- Pups - On Day 0 post partum, the pups were weighed and examined for malformations, sexed and the number of alive and dead recorded.
- Clinical Condition and Mortality - 1x/day.
- Body Weight - Days 0 and 4 post partum.

Terminal Sacrifice:

F_0 and F_1 Adult Generation - Necropsy and gross pathological examination were performed. F_1 males were sacrificed immediately after the end of mating period. F_1 females that failed to mate were sacrificed 26-28 days after the end of mating period. F_0 dams were sacrificed on Days 21-23 post partum and the number of implantation sites were recorded. F_1 dams were sacrificed on Days 4 or 5 post partum and the number of implantation sites were recorded. The following were retained in 10% neutral buffered formalin for fixation and possible future histopathological examination: animal identification, seminal vesicles, epididymides[10], testes[10], mammary glands (thoracic and inguinal), uterus, vagina, ovaries, abnormal tissues, and prostate. All digestive tracts retained as abnormal tissues of all F_0 females who died preterminally or were sacrificed in a moribund condition, and selected tissues retained as abnormal for the F_1 females in the 30 and 100 mg/kg/day treated groups were prepared for histological examination.

F_1 and F_2 Pups - Pups dying or sacrificed as malformed on or before Day 7 post partum for the F_1 generation and Day 4 post partum for the F_2 generation were placed in Bouin's fluid for subsequent examination using a modified Barrow and Taylor[11] technique. A complete necropsy was performed on pups of the F_1 generation dying or sacrificed between Days 8 and 21 post partum and postweaning F_1 generation not selected for breeding or for the determination of plasma concentrations of SC-58635.

On Day 4 or 5 post partum, any externally abnormal F_2 generation pups were examined as described above for pups dying or malformed. Externally normal F_2 generation pups were euthanized and discarded without further examination.

PK/TK:

F_0 Generation: Plasma samples were obtained from 5 dams/group for the vehicle control, 10 and 30 mg/kg/day treated groups and 4 dams/group for the 100 mg/kg/day treated group.

F_1 Generation - Plasma samples were obtained from pups that were not selected for breeding for the determination of SC-58635 plasma concentrations at terminal sacrifice.

Results:

F_0 Generation:

- Clinical Signs and Mortality - Deaths or moribund were found in 1 ♀ @ 30 mg/kg/day and 8 ♀ @ 100 mg/kg/day group with clinical findings of fur staining of the muzzle and urogenital regions, thin body condition and prominent backbone, body condition dehydrated/weak, cold to touch, decreased muscle tone, decreased activity, pale skin, shallow respiration, discharges from eyes/vagina, and firm abdominal structure. Deaths were the result of peritonitis and/or gastrointestinal lesions.
- Body Weights and Food Consumption - Similar body weights and body weight gains during gestation and lactation were seen in the control and treated groups. A dose-related, transitory, decrease in food consumption was noted for all treated groups from Gestation Days 6 to 9 (78.3, 76.3, 75.0, and 71.4 grams/animal for the control 10, 30, and 100 mg/kg/day groups, respectively).
- F_0 Reproductive Performance - A slight ↓ in the gestation index was seen in ♀ at the 30 and 100 mg/kg/day groups (95.8 and 92.0 %, respectively vs. 100% in the control group) as a result of the deaths of one pregnant animal in the 30 mg/kg/day group and 2 pregnant animals in the 100 mg/kg/day group during gestation. A significant ↓ in the mean number of live pups was observed in mid- and high-dose ♀ (15.6, 14.5 and 14.1 live pups/litter in the control, 30 and 100 mg/kg/day groups, respectively). A significant ↑ in the incidence of litters with dead pups was

[10] Fixed with Zenker's fluid for sacrificed rats only.

[11] Barrow, M.V. and Taylor, W.J., 1969. A rapid method for detecting malformations in rat fetuses. J. Morph. 127: 291-306.

also observed in mid dose (5 dead pups from 23 litters) and high dose (8 dead pups from 23 litters) groups.

F_1 Generation:

- F_1 Pups - Pup viability, body weights, survival and lactation indices were comparable across all groups and there were no treatment-related clinical observations for the F_1 generation pups. Dilation of various gastrointestinal segments, digesta changes and/or urinary bladder changes were noted in pups born to dams that were found dead or at moribund in the 100 mg/kg/day group. These observations might be secondary to the deteriorating condition of the dams.
- Visual Function - Comparable results were seen in all groups for the visual placing and pupillary closure.
- Physical Development - Pups born to dams in mid and high dose groups showed significant delayed in the mean days of preputial separation. The mean day of development of tooth eruption and the values for righting reflex, negative geotaxis and auricular startle were similar between groups.
- Behavior Assessment - No remarkable findings were attributable to the treatment.
- Reproductive Performance - There were no significant differences in the parental and maternal performance parameters (mating and fertility index, conception rate, gestation index, length of gestation, implantation sites and live birth index).

F_2 Generation:

- Viability, Clinical Signs, Body Weights and Gross Pathological Findings - No differences were found.

PK/TK: SC-58635 was absorbed and systemically available to the F_0 dams and their offspring The following table shows the range of plasma concentrations of SC-58635 seen in the dams and pups.

DOSE (mg/kg/day)	Range of Plasma Concentrations of SC-58635 (μg/ml)	
	DAMS	PUPS
10	0.175 - 0.660	<0.0250 - 0.0484
30	0.422-1.20	<0.0250 - 0.435
100	<0.0250 - 2.44	<0.0250 - 7.15

Based on the results of this study the NOAEL for the survival, physical development, behavior and reproductive performance of the F_1 ♂ and ♀ was 100 mg/kg/day as only minor changes were seen in development. The NOAEL for F_0 toxicity was 10 mg/kg/day due to mortality at 30 and 100 mg/kg/day and an increase in dead pups at 30 and 100 mg/kg/day.

2.5. GENETIC TOXICOLOGY

2.5.1. IN VITRO TESTS

2.5.1.1. An Evaluation Of The Mutagenic Potential Of SC-58635 In The Ames Salmonella/Microsome Assay, Document No.: PSA-94S-4242; Date: 18-Jul-1994 (Vol. 1.65, p. 1-23)

Study Nº: SA4242
Report Nº: PSA-94S-4242
Study Aim: To evaluate mutagenic potential of SC-58635 using Ames Salmonella/microsome assay
Compound: SC-58553 (Lot Nº C00025) dissolved in DMSO, 100 mg/ml
Dose: 10, 50, 100, 500, 1000, and 5000 μg/plate.
Vehicle Control: DMSO, 50 μl/plate
Indicator Cells: *Salmonella typhimurim* strains (histidine auxotrophs) TA97a, TA98, TA100, TA1535 and TA1538
S9 Mix: Aroclor 1254-induced rat-liver S9 homogenate
Positive Control:

Chemical	S9 Mix	Tester Strains	Conc. (μg/plate)
NaN_3 (sodium azide)	-	TA1535, TA100	1
2-Nitrofluorene	-	TA 1538, TA98	2.5
ICR-191 acridine	-	TA97a	0.5
2-Aminoanthracene	+	TA97a, TA98, TA100, TA1535, TA1538	1.0

Test Article Exposure Time: 48 hr at 37ºC
Study Location: Searle Research and Development, Skokie, IL
Study Date: 5/3/94 - 5/5/94
Compliance with GLP/QAU: Yes

Results: A precipitate was observed when *Salmonella typhimurim* (all tested strains) incubated with SC-58635 at concentrations 1000 and 5000 μg/plate and colony counts were not determined at these concentrations. SC-58635 was toxic at concentrations of ≥500 μg/plate as a reduction in the number of revertants and the presence of microcolonies. Therefore, SC-58635, at concentrations up to 500 μg/plate, was not mutagenic at any concentrations under current testing system.

2.5.1.2. An Evaluation Of The Mutagenic Potential Of SC-58635 In The CHO/HGPRT Mutation Assay, Document No.: PSA-94S-4299; Date: 05-Dec-1994 (Vol. 1.65, p. 24-52)

Study Nº: SA4299
Report Nº: PSA-94S-4299
Study Aim: To evaluate mutagenic potential of SC-58635 using CHO/HGRT mutation assay
Compound: SC-58553 (Lot Nº 94K014-A1B) dissolved in DMSO
Positive Controls: ICR-191 acridine, 1 μg/ml; 3-methylcholanthrene (MCA), 1 μg/ml
Dose:
Range-Finding: 0.08, 0.27, 0.80, 2.67, 8.0, 2.67, 8.0, 26.67, 80.0, 266.67, and 800.0 μg/ml
-S9: 4, 8, 12, and 16 μg/ml
+S9: 15, 30, 45, and 60 μg/ml
Indicator Cells: CHO cells (subline K1-BH4)

S9 Mix: The 9000 x g supernatant fraction of the liver homogenate from Aroclor 1254-treated rats
Exposure Time: -S9: 20-24 hr at 37°C; +S9: 4 hr at 37°C
Study Location: G.D. Searle, Skokie, IL
Study Date: 10/5/94 - 11/4/1994
Compliance with GLP/QAU: Yes
Study Design: Cells were treated with various concentrations of SC-58635 or positive control compounds, either for 20-24 hr without metabolic activation or approximately 4 hr with metabolic activation.

Results: Results of the dose range finding cytotoxicity test in the presence or absence of metabolic activation are shown in the following table.

Compounds	Concentration (μg/ml)	Relative Cell Survival (%)	
		-S9	+S9
DMSO	1% (v/v)	100	100
SC-58635	0.08	68	94
	0.27	88	81
	0.80	82	83
	2.67	65	78
	8.00	49	81
	26.67	NC	60
	80.00	NC	NC
	266.67	NC	NC
	800	NC	NC

NC = Not cloned due to insufficient cell numbers.

Data from the mutation experiment with or without S9 mix are presented in the following table. Apparently, under the test condition without S9 mix, the concentrations of SC-58635 used did not reach maximum condition as 47% of cell survival were observed at 16 μg/ml, the highest concentration tested[12]. Therefore, celecoxib was not mutagenic at doses up to 16 μg/ml and 45 μg/ml in the absence and presence of S9 activation, respectively.

Compounds	-S9			+S9		
	Concentration (μg/ml)	Cell Survival on Day 1 (%)	Mutant Colonies/1x10^6 Clonable Cells	Concentration (μg/ml)	Cell Survival on Day 1 (%)	Mutant Colonies/1x10^6 Clonable Cells
DMSO	1%(v/v)	100	1.5	1% (v/v)	100	1.1
ICR-191 acridine	1	27	362.8**	-	-	-
MCA	-	-	-	1	53	166.1**
SC-58635	4	78	1.7	15	80	0.6
	8	68	1.0	30	69	1.7
	12	58	3.8	45	7	0.0
	16	47	0.6	60	NC	-

** significant at p≤0.01.

2.5.1.3. An Evaluation Of The Potential Of SC-58635 To Induce Chromosome Aberrations In Vitro In Chinese Hamster Ovary (CHO) Cells, Document No.: PSA-94S-4302; Date: 17-Nov-1994 (Vol. 1.65, p. 53-92)

Study Nº: SA4302
Report Nº: PSA94S-SA4302
Study Aim: To evaluate mutagenic ability of SC-58635 to induce chromosomal aberrations in CHO-WBL cells
Compound: SC-58553 (Lot Nº 94K014-A1B) dissolved in DMSO

[12] ICH S2A Document: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, 19 July 1995.

Dose: 0.08, 0.27, 0.80, 2.67, 8.0, 26.67, 80.0, 266.67, and 800.0 μg/ml for range-finding study; 10, 20, and 40 μg/ml for assay condition -/+ S9 activation mixtures
Vehicle Control: DMSO, 200 μl
Positive Controls: Mitomycin C (MMC), 0.5 μg/ml; Cyclophosphamide (CP), 5 μg/ml
Indicator Cells: CHO cells (subclone WBL)
Exposure Time: -S9, 4 and 24 hr; +S9, 4 hr
Study Location: G.D. Searle, Skokie, IL
Study Date: 10/4/95 - 11/2/94
Compliance with GLP/QAU: Yes
Study Design: Cells with or without metabolic activation system (liver S9 homogenate) were treated with various concentrations of SC-58635 for 4 hr or 24 hr. Cells were washed and fresh complete culture medium was added. Twenty one to 22 hr from the beginning of dosing, colcemid (0.1 μg/ml) was added to cells for 1.5-2 hr. Cells were then collected and metaphase analysis was performed. The following parameters were calculated:

% Aberrant Cells = (Total number of cells with at least one aberration)/(Total number of cells examined per dose)x 100

Cell with >1 Aberration = (Total number of cells with two or more aberrations)/(Total number of cells examined per dose) x 100

Aberrations/cell = (Total number of aberrations)/(Total number of cells) x 100

Results: In the range finding cytotoxicity experiment, results showed that no viable cells could be found at the doses ≥80 μg/ml and precipitations occurred at doses ≥266.67 μg/ml in the presence or absence of S9. **An increase in cell endoreduplication was observed in cells treated with SC-58635 in the presence of activation mix. Higher frequency of endoreduplicated cells was noted at 30 and 40 μg/ml as shown in the following table.** The biological significance of this increasing incidence of cell abnormality is unknown.

Treatment	Dose	Cell Viability (%)			Endoreduplication (%)		
	(μg/ml)	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
DMSO	20 μl	100	100	100	0	1	0.5
SC-58635	20	100	100	116	4	-	0
	30	-	70	97	-	10	3
	40	41	46	45	14	9	17

No viable cells were noted in all experiments when cells were treated with 80 μg/ml of SC-58635. Data from the 4 hr aberration assays are shown in the following table.

Treatment	Dose	Nº Cells Scored		Abs/Cells		%Cells w/Abs		% Cells w/>1 Abs		% Cell Survival	
	μg/ml	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
DMSO	20 μl	200	200	0.010	0.000	1.0	0.0	0.0	0.0	100	100
MMC	0.5	66		≥0.667		45.5		13.6		72	
CP	5.0		71		≥0.704		43.7		18.3		55
SC-58635	10	200	200	0.000	0.010	0.0	1.0	0.0	0.0	101	107
	20	200	200	0.000	0.010	0.0	0.5	0.0	0.5	94	100
	40	200	200	0.020	0.030	2.0	1.0	0.0	0.5	49	41

2.5.2. IN VIVO TEST

2.5.2.1. An Evaluation Of The Potential Of SC-58635 To Induce Micronucleated Polychromatic Erythrocytes In The Bone Marrow Of Rats (Micronucleus Test), Document No.: PSA95S-30-SA4326; Date: 10-Mar-1995 (Vol. 1.65, p. 93-139)

Included as an appendix to this report were:

1. Final Report Amendment No. 1: An Evaluation Of The Potential Of SC-58635 To Induce Micronucleated Polychromatic Erythrocytes In The Bone Marrow Of Rats (Micronucleus Test), Document No.: PSA96S-31-SA4326; Date: 25-Mar-1996 (Vol. 1.65, p. 133-135)
2. Final Report Amendment No. 2: An Evaluation Of The Potential Of SC-58635 To Induce Micronucleated Polychromatic Erythrocytes In The Bone Marrow Of Rats (Micronucleus Test), Document No.: P31S4326; Date: 26-Feb-1997 (Vol. 1.65, p. 136-137)
3. Final Report Amendment No. 3: An Evaluation Of The Potential Of SC-58635 To Induce Micronucleated Polychromatic Erythrocytes In The Bone Marrow Of Rats (Micronucleus Test), Document No.: P33S4326; Date: 05-Mar-1997 (Vol. 1.65, p. 138-139)

Study Nº: SA4326
Report Nº: PSA95S-30-SA4326
Study Aim: To evaluate the potential of SC-58635 to induce micronuclei in the bone marrow polychromatic erythrocytes of 8 week old Sprague-Dawley rats
Compound: SC-58553 (Lot Nº 94K014-A1B) suspension in 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O; Cyclophosphamide (CP), 60 mg, served as positive control
Dose & Route: 150, 300, and 600 mg/kg/day for 3 days, 10 ml/kg, oral gavage
Control Vehicle: 0.5% methylcellulose (w/v) & 0.1% polysorbate 80 (v/v) in H_2O
Animals: 30♂ & 30♀ Sprague-Dawley rats, strain CD(SD)BR, ~8 weeks of age, weighing 239.6 - 262.5 g for ♂ and 183.8 - 199.6 ♀ for & rats
Study Location: G.D. Searle, Skokie, IL
Study Date: 12/6/1994 - 1/5/1995
Compliance with GLP/QAU: Yes
Study Design:

Group	Dose	Nº Animals
Vehicle Control	10 ml	5/Sex
Cyclophosphamide	60 mg	5/Sex
SC-58635	150 mg	5/Sex
SC-58635	300 mg	5/Sex
SC-58635	600 mg	5/Sex

Animals were randomly assigned into 5 groups of 10 (5♂ & 5♀) and orally (by gavage) received either vehicle, cyclophosphamide (60 mg/kg) or SC-58635 (150, 300, or 600 mg/kg, 10 ml/kg) once daily for 3 days. Clinical signs and mortality were monitored. Animals were sacrificed on Day 4, approximately 24 hr post last dosing. Bone marrow from tibia of each animal was extracted; four smears were prepared and stained with acridine orange. Slides were evaluated for micronuclei in polychromatic (PCE) and erythrocytes.

Results: No overt clinical signs or mortality were observed. No SC-58635 induced micronucleus formation in any treatment group. In contrast, cyclophosphamide caused a significantly higher incidence ($p \leq 0.01$) of micronucleus formation compared to the vehicle control. **SC-58635 did not cause micronucleated polychromatic erythrocytes in the bone marrow of rat. Therefore, SC-58635 was not a clastogen.**