	(ug/ml)		(ug.hr/ml)			
	M	F	M	F	M	F
150	89.5	124	818	1064	4.08	4.08
200	122	153	1027	1688	4.11	4.12

In summary, SB-497115 attained a dose proportional plasma concentration at 150 and 200 mg/kg/day doses and the plasma concentration of males and females was not different. None of study animals died during the study.

13-Week Toxicity study in Mice

Key study findings: SB-497115 from 10 to 100 mg/kg/day doses for 13 weeks produced a non-dose dose proportional increase in systemic exposure of the compound. The treated animals showed no treatment related adverse effects. An MTD for the mouse carcinogenicity study were not identified.

Study no.: CD2004/00627/00 (protocol #G03324)/ — 7274-456 b(4)
Conducting laboratory and location: —
Date of study initiation & Completion: November 25, 2003 and September 10, 2004
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot # and % purity: TPO-E-01C with 99.9% pure as SB-497115-GR

Methods

Doses: 0 (vehicle control), 10, 60 and 100 mg/kg/day in 2% hydroxyl propyl methyl cellulose plus 0.2% sodium lauryl sulfate

Species/strain: — CD-1®(ICR)BR mice from — b(4)

Number/sex/group or time point (main study): 12/sex/group in 4 groups

Route, formulation, volume, and infusion rate: Oral gavage in 10 ml/kg

Satellite groups used for toxicokinetics: 21/sex/group of the study

Age: 6 weeks old

Weight: Mean body weight of 29.2 to 30.7 g (males) and of 22.7 to 23.1 g (females)

Sampling times: On week 28, 0.25-0.5 ml blood from jugular vein from non-fasted rats at 1, 2, 4, 8, 12 and 24 hr post dosing on week 28 was used for TK estimation

Unique study design or methodology (if any): a peer review of microscopic sections of selected organs was completed.

Observation and Times:

Clinical signs and mortality: Once daily during randomization and at the start and of treatment and end of the day, once weekly until necropsied.

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Body weights: Once during week –1, on the first day of treatment (prior to dosing) and once every week during the study
Food consumption: Once every week prior to treatment and weekly during the study
Ophthalmoscopy: Once prior to dosing, and on week 13 prior to dosing
Hematology: Blood samples by cardiac puncture on week 13
Clinical chemistry: Blood samples from jugular vein on week 14.
TK: Blood samples (0.25 to 0.5 ml) from 3/sex/group non-fasted animals in TK group during week 13 at 1, 2, 4, 8, 12 and 24 hr interval after dosing. The TK analysis was done by non-compartmental pharmacokinetic analysis using WinNonlin computerized program.
Urinalysis: Urine samples were not collected during the study.
Gross pathology: After 13 weeks of treatment, the animals were sacrificed and adrenal, aorta, brain, cecum, colon, clitoral gland, duodenum, epididymides, esophagus, eye/optic nerve, gallbladder, Harderian glands, heart, hind limb, ileum, jejunum, kidneys, liver, lung, lymph nodes, lesions, mammary glands, ovaries, pancreas, parathyroid, pituitary, rectum, salivary glands (mandibular, parotid), sciatic nerve, spinal cord (cervical, thoracic and lumber), skeletal muscle, skin, skull, spleen, sternum, stomach, testes, thymus, thyroid, trachea, tongue, urinary bladder, uterus, vagina and prostate were separated.
Organ weights: Adrenal, brain, heart, kidneys, liver, ovaries, testes, thymus and prostate.

Histopathology: The preserved tissues from control and high dose groups were processed to paraffin wax, sectioned stained (hematoxylin and eosin).

Results:

Mortality: Three animals, one each in 10, 60 and 100 mg/kg/day treatment groups died during the study. The cause of death of animals of 10 and 60 mg/kg/day treatment groups was gavage related. Moderately distended duodenum was reported at necropsy and the death was not treatment related.

Clinical signs: There were treatment related hunched appearance (0, 1, 0 and 1 male and, 0, 0, 2 and 1 female of the study groups) and rough hair coat was seen in 4, 7, 6 and 7 males and, 0, 1, 4 and 2 males of study groups.

Body weights and Food consumption: No treatment related changes.

Ophthalmoscopy: Bilateral focal opacity and unilateral left focal corneal opacity was seen in 1/male animal out of 12 animals of 10 mg/kg/day treatment group.

EKG: Not studied during the study

Hematology: On week 13, the hematology data of the study animals was similar to the data of control group animals.

Clinical chemistry: No significant effect of treatment was seen.

Urinalysis: The urine samples were not collected during the study.

Gross pathology: No treatment related effects were noted.

Organ weights: The testicular absolute and relative to brain weight of males included in 100 mg/kg/day treatment group was significantly increased ($p<0.05$). The relative to body and brain weights of thymus were significantly decreased ($p<0.05$).

Histopathology: Hepatocellular hypertrophy was present in 4 and 3 males and, 0 and 1 female of 0 and 100 mg/kg/day treatment groups. Focal necrosis was seen in 0 and 2 males and 4 and 0 females of 0 and 60 mg/kg/day treatment groups. Renal tubular necrosis was seen in 1 male and none in female of 40 mg/kg/day treatment group.

Toxicokinetics:

The orally administered compound achieved a non-dose proportional plasma concentration and, the concentrations in comparison to 10 mg/kg/day treatment group were 1.3 and 36.8 times in males and, 1.5 and 28.7 times in females included in 60 and 100 mg/kg/day treatment groups. The peak concentration was achieved in 4 hr excepting in females included in 10 mg/kg/day treatment group at 1 hr. The plasma concentrations in male and female animals were similar. The TK data is shown below in the table.

Toxicokinetic Parameters of 13-Week Toxicity Study in Male and Female Mice

Dose (mg/kg/day)	Males			Females		
	Cmax (ng/ml)	AUC $_{(0\text{-}24hr}$ (ng.hr/ml)	Tmax (hr)	Cmax(ng/ml)	AUC $_{(0\text{-}24hr)}$ (ng.hr/ml)	Tmax (hr)
10	2036.9	17303.2	4	3682.1	23218.0	1
60	72056.8	484565.5	4	48387.6	422847.4	4
100	94754.96	636932.3	4	113842.6	666477.7	4

For comparison, in humans the Cmax and AUC values at the maximum human dose (75 mg/day) were 11.4 mcg/ml and 146 mcg.hr/ml, respectively.

In summary, orally administered SB-497115 from 10 to 100 mg/kg/day (0.1-5 times MHD based on AUC) for 13 weeks in mice produced a non-dose dose proportional exposure of the compound. The treated animals showed no treatment related adverse effects. The target organs of toxicity were not identified.

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Study title: 14-Day Toxicity study in Rats
Key study findings: Similar plasma concentration was achieved in study males and females on day 1 and 28. Midzonal hepatocellular vacuolation of minimal nature was seen in 1 and 3 males and 1 and 5 females of 0 and 40 mg/kg/day (5 times MHD based on AUC) treatment groups and, renal cyst was present in 1/sex animals of 40 mg/kg/day treatment group. Sponsor though collected samples for stage dependent evaluation of spermatogenesis from the testes of 5 control and high dose males, the results was not submitted.

Study no.: CD2003/00252/00 (protocol #G02012)

Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.

Date of study initiation and completion: March 7, 2002 and February 19, 2003
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot #, and % purity: F033082 with 75.3% pure salt
Methods

Doses: 0, 3, 10 or 40 mg/kg/day in 2% hydroxyl propylmethyl cellulose plus 0.2% sodium lauryl sulfate

Species/strain: —CD®(SD) IGS BR rats from ———————— b(4)

Number/sex/group or time point (main study): 10/sex/group

Route, formulation, volume, and infusion rate: Oral gavage in a volume of 10 ml/kg.

Satellite groups used for toxicokinetics or recovery: 3/sex/group

Age: 12 weeks old

Weight: Mean weight of 404.9 to 406.5 g (males) and 250.9 to 252.9 g (females)

Sampling times: On day 1 and 14, 0.025-0.4 ml blood from 24 rats of TK groups (n=3/sex/group) were collected prior to dosing and 1, 2, 4, 8, 12 and 24 hr post dosing

Unique study design or methodology (if any): 1. Liver was used for the cytochrome P450 analysis and, (2) stage dependent evaluation of spermatogenesis from the testes of 5 control and high dose males.

Observation and Times:
Clinical signs: Once daily during randomization and treatment of group 1 to 4 animals.
Body weights: Once prior to treatment, once every day after treatment and terminally.
Food consumption: Once every week of treatment in main study group animals.
Ophthalmoscopy: Once prior to dosing, and on day 11 in main study group animals.
Hematology: Blood samples from main study groups animals collected from tail vein on day 14.
Clinical chemistry: Blood samples (from main study groups animals collected from tail vein on day 14 at 1, 2, 4, 8, 12 and 24 hr post dosing.
TK: Blood samples (0.25 to 0.4 ml) from TK group animals (3/sex/group)
Urinalysis: Overnight urine samples from main study group 16-18 hr fasted animals
Gross pathology: On day 14, livers from toxicokinetic group of animals (non-fasted) were used for hepatic cytochrome P450 system. Adrenal, aorta, brain, cecum, colon, duodenum, epididymides,

esophagus, eye/optic nerve, Harderian glands, heart, hind limb, ileum, jejunum, kidneys, liver, lung, lymph nodes, lesions, mammary glands, ovaries, pancreas, parathyroids, pituitary, rectum, rib, salivary glands, seminal vesicles, skin, skull, spinal cord, spleen, sternebra, stomach, testes, thymus, trachea, urinary bladder, uterus, vagina and prostate.
Organ weights (specify organs weighed if not in histopath table): Adrenal, brain, epididymides, kidneys, liver, ovaries, seminal vesicles, testes, thymus and prostate.

Histopathology: The rats of main study groups (1 to 4) were processed to paraffin wax, sectioned stained (hematoxylin and eosin). The microscopic examination of tissues belonging to control and high dose treatment groups was done. Study included the macroscopic observation of lesions detected in low and mid dose treatment group animals.

Results:

Mortality: No treatment related deaths were noted during the study.

Clinical signs: No treatment related effects were noted in the study animals.

Body weights and Food consumption: A retardation of 0, 1.03 and 16.9% in body weight gain was observed in males of 3, 10 and 40 mg/kg/day treatment groups. The body weights of females of high dose treatment group were not affected. The retardation was related to the 12.9% reduced food intake of these animals.

Ophthalmoscopy: Not performed on the study animals.
EKG: This parameter was not studied during the study

Hematology: No treatment related hematological effects were seen in the animals belonging to treatment groups.

Clinical chemistry: There were no statistically significant effects in study animals.

Urinalysis: The total urinary protein excretion was increased in statistically insignificant manner in 10 and 40 mg/kg/day treatment groups male rats only.

Gross pathology:

Organ weights: The testicular absolute and relative to body weight of males included in 40 mg/kg/day treatment group was significantly increased ($p=0.05$). The organ weights of females were not changed during the study. The absolute adrenal weight of 40 mg/kg/day treatment group was decreased insignificantly in males and significantly ($p<0.05$) in females.

Histopathology: Midzonal hepatocellular vacuolation of minimal nature was seen in 1 and 3 males and 1 and 5 females of 0 and 40 mg/kg/day treatment groups. Two and 3 females of 3 and 10 mg/kg/day treatment group had mild and moderate intensity vacuolation of liver. Focal necrosis was present in 1 out of 10 animals of 3 and 10 mg/kg/day treatment group. Renal cyst was present in 1/sex animals of 40 mg/kg/day treatment group. Sponsor collected samples for stage dependent evaluation of spermatogenesis from the testes of 5 control and high dose males but did not submit a report.

Toxicokinetics:

A linear increase in the plasma concentration was observed in animals included from 3, 10 and 40 mg/kg/day treatment groups as shown below in the table. The plasma concentrations on day 1 and 14 were similar as shown below in the table.

Dose (mg/kg/day)	Males				Females			
	Cmax (ug/ml)		AUC (0-24hr) (ug.hr/ml)		Cmax (ug/ml)		AUC (0-24hr) (ug.hr/ml)	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
3	2.49	5.01	13.5	25.2	3.56	8.01	14.0	28.6
10	22.6	37.1	107	198	19.7	30.5	81.4	130
40	76.2	71.6	613	672	79.1	88.2	611	628

In humans, the Cmax and AUC values at maximum recommended dose (75 mg/day) were 11.4 µg/ml and 146 µg.hr/ml, respectively.

In summary, a similar plasma concentration was reported in males and females on day 1 and 28 and, midzonal hepatocellular vacuolation of minimal nature was seen in 1 and 3 males and 1 and 5 females of 0 and 40 mg/kg/day treatment groups. Renal cyst was present in 1/sex animals of 40 mg/kg/day treatment group (5x MHD based on AUC). Sponsor though collected samples for stage dependent evaluation of spermatogenesis from the testes of 5 control and high dose males, the results was not submitted.

Study title: 14-Day Oral Toxicity study in Female Rats with a 4-week Reversibility Period

Key study findings: A dose proportional increase in plasma concentration and a dose related hepatocellular vacuolation was seen in 20 and 40 mg/kg/day treatment groups animals. The liver was identified as the target organ of toxicity and 20 mg/kg/day (2.4 times MHD based on AUC) was identified as the highest tolerable dose'. 'No effect dose' was not identified in the study.

Study no.: CD2003/00327/00 (protocol #G02321)

Reviewer: Chopra NDA No. 22-291

Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.
Date of study initiation: February 10, 2003 and November 10, 2003
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot #, and % purity: F033082 with 75.3% pure salt

Methods

Doses: 0, 20 or 40 mg/kg/day in a solution of 2% hydroxypropylmethylcellulose plus 0.2% sodium lauryl sulfate

Species/strain: CD®(SD) IGS BR female rats from [redacted] b(4)

Number/sex/group or time point (main study): 20/sex in control and 40 mg/kg/day groups and 10 animals in 20 mg/kg/day treatment group. On day 15, 10 females of group 1 to 3 were necropsied and on day 43, 10 females in group 1 and 3 were terminated.

Route, formulation, volume, and infusion rate: Oral gavage, (vol = 10 ml/kg)

Groups used for toxicokinetics or recovery: 3/sex/group, the animals were discarded on day 15.

Age: 12 weeks old

Weight: Mean weight of 281.0 to 284.1 g

Sampling times: On day 1 and 14, blood samples from inferior vena cava of rats included in TK groups (n=3/sex/group) were collected prior to dosing and 1, 2, 4, 8, 12 and 24 hr post dosing

Unique study design or methodology (if any): Urine samples were collected but were not analyzed.

Observation and Times:
Clinical signs and mortality: Once daily during randomization and treatment until animals in group 1 to 6 was necropsied. Detailed examination done on day 14 (main study group) and on day 42 (recovery group animals)
Body weights: Once prior to treatment, once every day after treatment and terminally. Recovery group animals were weighed weekly following day 15.
Food consumption: Once every week of treatment in main study group animals.
Hematology: Blood samples from main study groups animals collected from tail vein on day 14 and on day 43 at the termination.
Clinical chemistry: Blood samples (from main study groups animals collected from tail vein on day 14 and on day 43 at 1, 2, 4, 8, 12 and 24 hr post dosing.
TK: Blood samples (0.25 to 0.4 ml) from TK group animals (3/sex/group)
Urinalysis: Overnight urine samples from main study group 16-18 hr fasted animals
Gross pathology: On day 15, 10 animals/group were killed and 43 (remaining rats in group 1 and 3). At termination, (day 15 and 43), the organs separated were adrenal, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eye/optic nerve, Harderian glands, heart, hind limb, ileum, jejunum, kidneys, liver, lung, lymph nodes, lesions, mammary glands, ovaries, pancreas, parathyroids, pituitary, rectum, rib, salivary glands, seminal vesicles, skin, skull, spinal cord, spleen,

sternebrae, stomach, testes, thymus, trachea, urinary bladder, uterus, vagina and prostate. The liver, spleen and mesenteric lymph node were examined microscopically.

Organ weights (specify organs weighed if not in histopath table): Adrenal, brain, kidneys, liver, heart and thymus.

Histopathology: The rats of main study groups (1 to 4) were processed to paraffin wax, sectioned stained (hematoxylin and eosin). The microscopic examination of tissues belonging to control and high dose treatment groups was done. Study included the macroscopic observation of lesions detected in low and mid dose treatment group animals.

Results:

Mortality: No treatment related deaths were noted during the study.

Clinical signs: No treatment related effects were noted in the study animals.

Body weights and Food consumption: The body weight gain during the study of females of 20 and 40 mg/kg/day treatment groups was similar. There was no treatment related effect on the body weight of females. The food intake of these animals was not affected during the study.

Hematology: No treatment related hematological effects were seen.

Clinical chemistry: On day 14, a slight increase in total bilirubin (3.0 vs. 1.9 umol/l in control group animals) and decrease in bile acids (16.4, 11.3 and 6.4 umol/l) was seen in 20 and 40 mg/kg/day treated animals. During the recovery period these changes were similar.

Urinalysis: This was not done during the study.

Gross pathology: Pale liver was noted in 4 females of 40 mg/kg/day treatment group. No other adverse effects were reported.

Organ weights: The absolute and relative liver weights of females included in 40 mg/kg/day treatment group were significantly increased ($p=0.05$) and it was not different in recovery group animals. The organ weights of brain, heart, kidneys, adrenal, ovaries and thymus were not changed during the study.

Histopathology: Midzonal hepatocellular vacuolation was seen in 3 and 9 females of 20 and 40 mg/kg/day treatment groups. The intensity vacuolation of liver was minimal in 20 mg/kg/day treatment group. The intensity vacuolation of liver was of moderate and severe nature in 4 and 1 females of 20 and 40 mg/kg/day treatment group

Reviewer: Chopra NDA No. 22-291

Toxicokinetics:

A linear dose proportional increase in the plasma concentration was observed in animals included from 20 and 40 mg/kg/day treatment groups as shown below in the table. The plasma concentrations on day 1 and 14 were similar as tabulated below.

Dose (mg/kg/day)	Males		Females	
	Cmax (ug/ml)		AUC (0-24hr) (ug.hr/ml)	
	Day 1	Day 14	Day 1	Day 14
20	78.5	44.0	382	341
40	121	109	814	892

In summary, 14-day treatment in animals attained a dose proportional plasma concentration and the treatment caused dose related hepatocellular vacuolation in 20 and 40 mg/kg/day (2.4 and 6x MHD based on AUC) treatment groups animals. The liver was identified as the target organ of toxicity and this was identified as the highest tolerable dose'. 'No effect dose' was not identified in the study.

Study title: 28-Week Toxicity study in Rats

Key study findings: SB-497115 from 3 to 60 mg/kg/day for 28 weeks in rats produced a treatment related increase in systemic exposure of the compound. The exposure was similar in both sexes. Vacuolation of hepatocytes, hepatocytes hypertrophy and centrilobular degeneration/necrosis of liver, vacuolation/necrosis of pituitary, spleen, adrenal gland and lymphocytes necrosis and endosteal hyperostosis of femurs suggested that liver, pituitary, spleen, adrenal, lymphocytes and bone marrow as the target organs of toxicity. A dose of 30 mg/kg/day (5 times MHD based on AUC) was the highest tolerable dose in the study.

Study no.: CD2004/00332/00 (protocol #G03114). [redacted] 7274-374 b(4)
Conducting laboratory and location: [redacted]
Date of study initiation & Completion: June 16, 2003 and September 17, 2004
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot #, and % purity: F033082 with 75.3% pure salt

Methods

Doses: 0 (vehicle control), 3, 10, 30 or 60 mg/kg/day in 2% hydroxyl propylmethyl cellulose plus 0.2% sodium lauryl sulfate

Species/strain: [redacted]CD®(SD) IGS BR rats from [redacted] b(4)

Number/sex/group or time point (main study): 12/sex/group in 5 groups
Route, formulation, volume, and infusion rate: Oral gavage in 10 ml/kg volume.

Satellite groups used for toxicokinetics or recovery: No separate groups included
Age: 12 weeks old
Weight: Mean body weight of 367 to 369 g (males) and 233 to 235 g (females)
Sampling times: On week 28, 0.25-0.5 ml blood from jugular vein from non-fasted rats at 1, 2, 4, 8, 12 and 24 hr post dosing on week 28 was used for TK estimation
Unique study design or methodology (if any): a peer review of microscopic sections of selected organs was completed.

Observation and Times:
Clinical signs and mortality: Once during randomization, once at the start of treatment and daily during the treatment. Once weekly until necropsied.
Body weights: Twice before treatment, on the first day of treatment and once every week during the study
Food consumption: Once every week of treatment in main study group animals.
Ophthalmoscopy: Once prior to dosing, and on week 28 in main study group animals.
Hematology: Blood samples from jugular vein on week 4 and in week 28.
Clinical chemistry: Blood samples from jugular vein on week 4 and in week 28.
TK: Blood samples (0.25 to 0.5 ml) from animals during week 28 and 3 animals sacrificed at 1, 2, 4, 8, 12 and 24 hr interval after dosing (samples analyzed by GSK, PA. for the analysis.
Urinalysis: Overnight urine samples from main study group
Gross pathology: On week 28, the animals were fasted overnight and sacrificed and adrenal, animal ID, aorta, brain, cecum, colon, clitoral gland, duodenum, epididymides, esophagus, eye/optic nerve, Harderian glands, heart, hind limb, ileum, jejunum, kidneys, liver, lung, lymph nodes, lesions, mammary glands, nasal turbinates, nasopharanyx, ovaries, pancreas, parathyroids, pituitary, rectum, rib, salivary glands (mandibular, sublingual, parotid), seminal vesicles, skeletal muscle, skin, skull, spleen, sternebrae, stomach, testes, thymus, thyroid, trachea, tongue, urinary bladder, uterus, vagina and prostate were separated.
Organ weights (specify organs weighed if not in histopathology table): Adrenal, brain, epididymides, heart, kidneys, liver, lung, spleen, ovaries, testes, thymus and prostate.

Histopathology: The rats of main study groups (1, 4 and 5) were processed to paraffin wax, sectioned stained (hematoxylin and eosin). The suspected target organs of toxicity noted in high dose groups (liver, pituitary, adrenal, testis, femur/tibia (knee joint) embedded in paraffin for microscopic examination. Study included the macroscopic observation of lesions detected in low and mid dose treatment group animals.

Results:

Mortality: The treatment related deaths during the study were 11 males and 6 females of 60 mg/kg/day treatment group. Three, 2, 1 and 2 rats of 0, 3, 10 and 30 mg/kg/day treatment groups, respectively were also found dead during the study but these were not treatment related.
Clinical signs: The incidences of hunched appearance were 0, 1, 0, 0 and 4 in males and, 0, 0, 0, 1 and 3 in females of 0, 3, 10, 30 and 60 mg/kg/day treatment groups, respectively. Nasal bleeding was slightly more in animals of 60 mg/kg/day treatment group animals (5 males and 4 females out of 12/sex) and audible noisy and labored breathing was observed in 5 and 3 males, respectively. Two females of 60 mg/kg/day group were seen with noisy breathing.

Body weights and Food consumption: A retardation of 9.4 and 34.4% body weight gain was observed in males of 30 and 60 mg/kg/day treatment groups. The body weight gain among females in of high dose treatment group was similar to the control group females. The food intake of these animals was not affected.

Ophthalmoscopy: Bilateral diffuse cataract in 1/sex and unilateral left diffuse cataract was observed in 1 out of 5 males of 60 mg/kg/day treatment group.

EKG: Not studied during the study

Hematology: On week 4, a slight treatment related decrease in the number of RBCs (7.97 and 8.21 VS 9.17 and 8.64 million/ul in control males and female) and number of platelets, hemoglobin counts, absolute number of reticulocytes (533.7 and 250.0 th/ul VS 223.5 and 172.4 th/ul in control male and female) was seen in 60 mg/kg/day treatment group animals.

Clinical chemistry: An insignificant increase in the alkaline phosphatase were seen on week 4 and these were 165, 149, 169, 184 and 207 U/l in control, 3, 10, 30 and 60 mg/kg/day treatment groups. The total cholesterol amounts were slightly decreased at week 4 and week 28. The cholesterol levels of the study group animals were 96, 92, 88, 64 and 51 mg/dl and, on week 28 these were: 119, 107, and 107, 76 and not determined in control, 3, 10, 30 and 60 mg/kg /day treatment groups.

Urinalysis: On week 4, the total urinary protein excretion was increased (1.8 times) in statistically insignificant manner in 30 and 60 mg/kg/day treatment groups male rats only.

Gross pathology: No treatment related effects were noted.

Organ weights: The testicular absolute and relative to body weight of males included in 60 mg/kg/day treatment group was significantly increased ($p=0.05$). The absolute adrenal weight of 60 mg/kg/day treatment group males was decreased insignificantly and significantly ($p<0.05$) in females.

Histopathology: Vacuolation of hepatocytes of minimal nature was seen in 0 and 1 males and, 3 and 8 females 30 and 60 mg/kg/day treatment groups, respectively and, hepatocytes hypertrophy was

present 0 and 1 male and, 1 and 1 female of 30 and 60 mg/kg/day treatment groups. Centrilobular degeneration/necrosis was seen in 9 males and 1 female of 60 mg/kg/day treatment group. Vacuolation/necrosis of pituitary, spleen and lymphocytes was seen in animals of 60 mg/kg/day treatment groups. The incidences of necrosis in animals of 60 mg/kg/day treatment group were: adrenal cortex necrosis in 3 males and 1 female; pituitary pars distalis necrosis in 1 male and lymphocyte necrosis in 4 males. Adrenal cortical congestion was present in 1, 1 and 1 male and, 0, 0 and 2 female of 10, 30 and 60 mg/kg/day treatment group. Lymphoid depletion in mesenteric lymphoid node was observed in 7 males and 2 females of 60 mg/kg/day treatment group. Lymphoid depletion in mandibular lymphoid node was observed in 4 males and 3 females of 60 mg/kg/day treatment group. Endosteal hyperostosis of femur was present in 2 males of 60 mg/kg/day treatment group. Ultimobrachial cyst was present in 2 and 2 of 12 males of 0 and 60 mg/kg/day treatment group. Myocardial inflammation was present in 4 males and 2 females of high dose group and, myocardial degeneration was seen in 0, 0, 1, 1 and 9 males and 3, 1, 0, 0 and 6 females of 0, 3, 10, 30 and 60 mg/kg/day treatment group.

Toxicokinetics:

The plasma concentrations on week 28 increased in a linear fashion and the peak was seen from 1 to 4 hr in rats. There seems to be no significant difference in systemic exposure between male and female rats on week 28 of the study. The data is shown below in the table.

Toxicokinetic Data of 28-Week study in Male and Female Rats Following Oral Administration at 3, 10, 30 and 60 mg/kg/day SB-497115-GR

Dose (mg/kg/day)	Males			Females		
	Cmax (ug/ml)	AUC(0-24hr) (ug.hr/ml)	Tmax (hr)	Cmax (ug/ml)	AUC(0-24hr) (ug.hr/ml)	Tmax (hr)
3	4.4783	21.1300	1	6.2334	28.5231	1
10	10.4449	83.6834	1	21.5181	129.0403	2
30	66.4963	650.6330	2	83.4341	671.0909	2
60	64.0033	174.3667	4	148.9966	104.36331	4

In humans, the Cmax and AUC values at maximum recommended dose (75 mg/day) were 11.4 µg/ml and 146 µg.hr/ml, respectively.

The plasma concentration of the compound was 30.8 and 23.54 times respectively in male and female animals at 20 times the dose of the compound. Thus the exposure appears to be dose proportional up to 30 mg/kg/day group in both sexes. The systemic exposure of animals in the 60 mg/kg/day treatment group was not uniformly increased as the exposure was 8.3 and 36.6 times in males and females (based on AUC). A number of males of this treatment group died during the study.

In summary, SB-497115 from 3 to 60 mg/kg/day for 28 weeks in rats produced a treatment related increase in systemic exposure of the compound. The exposure was similar in both sexes. Vacuolation of the hepatocytes, hepatocytes hypertrophy and centrilobular degeneration/necrosis of liver, vacuolation/necrosis of pituitary, spleen, adrenal gland and lymphocytes necrosis and endosteal hyperostosis of femurs suggested that liver, pituitary, spleen, adrenal, lymphocytes and bone marrow as the target organs of toxicity. A dose of 30 mg/kg/day (5 times MHD based on AUC) was the highest tolerable dose in the study.

Juvenile Animals:

SB-497115-GR: Juvenile Oral (Gavage Once Daily) 28-day General Toxicity Study in the Male and Female Rats (study #CD2005/00661/00; Project No. 900710/GSK Reference No. G05081)

Key study findings: SB-497115 from 1 to 15 mg/kg/day for 2 weeks in juvenile rats produced a treatment related systemic exposure of the compound and plasma concentration were similar in both sexes. Lymph node hemorrhage and myeloid hypercellularity were reported in male and female of 15 mg/kg/day dose. In other juvenile study (CD2006/0065/100), SB-497115 at oral doses of 0, 5, 15 and 40 mg/kg/day from post partum day 5 to 30, i. e., 28 days duration in females produced only a slight reduction in RBCs, hemoglobin, hematocrit and, retinal atrophy (grade 1) in high dose male. Renal tubular hayline droplets and lymph node hemorrhage in 0, 2, 1 and 1 males and, 0, 1, 3 and 3 in females of 0, 5, 15 and 40 mg/kg/day treatment groups suggested lymphocytes and bone marrow as target organs of toxicity and a dose of 15 mg/kg/day was identified as the highest tolerable dose in both studies.

Study no.: 900710/GSK Reference No. G05081
Conducting laboratory and location: [redacted] b(4)
Date of study initiation & completion: June 19, 2005 and February 15, 2006
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot #, and % purity: F074359007-SB-497115 bismonoethanolamine with 97.4% pure salt

Methods

Doses: 0 (vehicle control), 1, 5 and 15 mg/kg/day suspensions at concentrations of 0, 2, 6 and 12 mg/ml (as active moiety) in 2% aq. (w/v) hydroxypropylemethylcellulose (HPMC), 0.2% aq. (w/v) sodium lauryl sulphate prepared weekly

b(4)

Species/strain: [redacted] CD®(SD) IGS BR mated female rats (day 4 postpartum. On Day 3 post partum (pp), after fostering, each litter was culled to 5/sex using a randomization procedure provided by a computer-based random number generator. The selected pups identified within litter

Number/sex/group or time point (main study): 4 groups of 10/sex/group in main study groups; additionally 18 animals/sex/treatment group for toxicokinetic evaluation on Day 13 pp.

Route, formulation, volume: Oral gavage in 5 ml/kg volume.

Sampling times: On pp day 13, 0.25-0.5 ml blood was collected from each pup and pooled to obtain 2 ml of blood in EDTA tube.

Unique study design or methodology (if any): a peer review of microscopic sections of selected organs was completed.

Group No Identification	Target Dose Level* (mg/kg/day)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Litters	Number of Pups	
					Main Study Phase	
					Male	Female
1 Control	0	0	5	6	10@+18**	10@+18**
2 Low dose	1	0.2	5	6	10@+18**	10@+18**
3 Mid dose	5	1	5	6	10@+18**	10@+18**
4 High dose	15	3	5	6	10@+18**	10@+18**

* The dose levels are expressed in terms of parent compound/base.

** Toxicokinetic animals were used for toxicokinetic evaluation on Day 13 pp.

@ Two additional pups/sex/litter for a total of 12 pups/sex/group were dosed commencing on Day 4 post partum. The additional pups were dosed until Day 27 post partum. On Day 28 post partum, 10 pups/sex/group were selected by a computer-generated randomization procedure to continue dosing until Day 31 post partum. All unselected juveniles were euthanized as outlined in Section 5.7.1. Unselected juveniles considered to be externally normal were discarded without further examination. No organ weights were taken on the unselected juveniles and no hematology, clinical biochemistry or urinalysis analysis was performed.

Observation and Times:

Clinical signs and mortality: Once daily at the time of randomization and twice daily for the mortality and clinical observations during pre-weaning and, once weekly post-weaning until necropsied.

Body weights: One daily during the treatment period (pp day 4 to pp day 31) and at the termination.

Food consumption: No observation was made.

Ophthalmoscopy: All animals were examined by indirect ophthalmoscopy and biomicroscopic (slit lamp) examinations during the last week of the treatment period by a board certified veterinary ophthalmologist.

Hematology & Clinical chemistry: Blood samples overnight fasted animals were collected at the time of termination.

Gross pathology: Animals that died during or at the termination of the study were necropsied and tissues preserved. The animals were euthanized and blood samples collected from aorta. The terminal body weight taken, fasted overnight and sacrificed and the tissues like adrenal, brain, heart, kidneys, liver, ovaries/testes, prostate, spleen, thymus were weighed.

The tissues were dissected out from each animal/group and preserved in neutral buffered 10% formalin: abnormalities; animal identification; adrenals; aorta (thoracic); brain; (cerebrum, cerebellum, midbrain and medulla oblongata); cecum; colon; duodenum; epididymides; esophagus; eyes with optic nerves[c]; femur[e], left (femoro-tibial joint); femur/tibia (right), Harderian glands[a]; heart; ileum; jejunum; kidneys; larynx[a]; liver (sample of 2 lobes); lungs (all lobes); lymph nodes (mandibular and mesenteric); mammary gland (inguinal) [b]; nasal cavities/skull (1 level); nasopharynx[a]; ovaries; pancreas; pituitary; preputial/clitoral gland[a]; prostate; rectum[a]; salivary gland (mandibular, sublingual, parotid); sciatic nerve; seminal vesicles; skeletal muscle (hind-limb); skin and fur (inguinal and dorsal thoracic) [a]; spinal cord (cervical a, lumbar and thoracic); spleen; sternum[e] (with bone marrow); stomach; testes[d]; thymus; thyroid lobes[f] (and parathyroids); tongue; trachea; urinary bladder; uterus (horns, body and cervix); vagina. (Key: [a] = Retained but not processed; [b] = Examined only if present in routine section of skin (mammary gland); [c] = Fixed in Davidson's fluid (all animals); [d] = Fixed in Bouin's fluid (all animals); [e] = Bone decalcified prior to sectioning; [f] = At least one parathyroid and one optic nerve should be examined; [g] = Infused with neutral buffered 10% formalin (all animals); [h] = Right hindlimb was retained in 10% neutral buffered formalin for future measurement of the femur). The femur/tibia was trimmed of skin and muscle and was retained intact and length of the right femur (greater trochanter to lateral condoyle) was measured and recorded.

Histopathology: Tissues from high dose and control groups were processed for histopathology changes embedding in paraffin wax, sectioned stained (hematoxylin and eosin).

Results:
Mortality: Two male pups, i.e., 1 of 1 and 15 mg/kg/day treatment groups were sacrificed. These were gavage related deaths.
Clinical signs: No treatment related clinical observations were reported.

Body weights and Food consumption: The initial and final body weights of males were 10.71 and 51.23 g (on pp day 19) and of females were 10.2 g and 54.73 g (on pp day 21). During post weaning period (pp day 29), the body weight gain in control and treated group males and females was similar, i.e., the body weight of males was 105.9, 107.0, 110.7 and 101.3 g in 4 study groups. The body weight of females (on pp partum day 29) was 90.3, 100.7, 99.1 and 96.5 g in 4 study groups.
Ophthalmoscopy: Bilateral diffuse cataract in 1/sex and unilateral left diffuse cataract was observed in 1 out of 5 males of 60 mg/kg/day treatment group.

EKG: Not studied during the study
Hematology: Statistically significant reduction in RBCs and hemoglobin and, an insignificant increase in absolute number of reticulocytes were seen in female of 15 mg/kg/day treatment group.

Clinical chemistry: No significant changes in clinical chemistry parameters.

Urinalysis: No significant or any change was reported in urine collected at the termination of the study.

Gross pathology: No treatment related effects were noted.

Organ weights: No treatment related changes.

Histopathology: Myeloid hypercellularity, renal fibrosis and subcutaneous abscess (grade 4) was reported in 1 male of 15 mg/kg/day group. Lymph node hemorrhage was reported in 0 and 1 males and, 1 and 2 females 1 and 15 mg/kg/day treatment groups, respectively.

Toxicokinetics:

The plasma concentrations of the compound were increased in a non-dose proportional manner from 1 to 3 hr of administration on day 13 postpartum (after 10^{th} dose) for 24 hr, i.e., till the next day. There seems to be no significant difference in systemic exposure between male and female juvenile rats. The data is shown below in the table.

Dose (mg/kg/day)	Males			Females		
	Mean C_{max} (ng/mL)	Mean $AUC_{(0-24)}$ (ng.h/mL)	Mean T_{max} (h)	Mean C_{max} (ng/mL)	Mean $AUC_{(0-24)}$ (ng.h/mL)	Mean T_{max} (h)
1	5079	81934	3.99	4975	96019	4.00
5	29118	468503	1.01	23995	432771	2.03
15	68068	1165188	3.99	63639	1238603	4.01

In summary, SB-497115 from 1 to 15 mg/kg/day for 2 weeks in juvenile rats produced a treatment related exposure of the compound and plasma concentration were similar in both sexes. Lymph node hemorrhage and myeloid hypercellularity were reported in male and female of 15 mg/kg/day dose. The identified target organs were lymphocytes and bone marrow. A dose of 15 mg/kg/day was the highest tolerable dose in the study.

In a subsequent juvenile study (CD2006/0065/100), SB-was administered at the dose at 0, 5, 15 and 40 mg/kg/day from post partum day 5 to 30, i.e., 28 days duration. One rat of 5 mg/kg/day group was found dead and cause of death was not determined. A slight reduction in RBCs, hemoglobin and hematocrit values were seen. The reticulocytes were increased in 40 mg/kg/day group. Serum cholesterol (46 and 29% in males and females) and triglycerides (52 and 32% in males and females)

were decreased in males of 40 mg/kg/day treatment group (data shown in sponsor's summary of the study and screened below):

Table 15c. Studies in Juvenile Animals (Continued) GSK Report No.: CD2006/00651/00 Study No.: G05264 Test Article: Eltrombopag (bis-monoethanolamine salt)

	Male				Female			
Daily Dose (mg/kg/day)	0	5	15	40	0	5	15	40
Numbers of Animals:								
Main	10	10	10	10	10	10	10	10
Toxicokinetic	9	9	9	9	9	9	9	9
AUC_{0-24} (µg.h/mL)								
Dose Day 5	-	23.4	135	552	-	21.9	143	475
Dose Day 30	-	48.3	237	904	-	44.1	217	795
C_{max} (µg/mL)								
Dose Day 5	-	3.4	16.5	76.9	-	4.5	21.5	64.6
Dose Day 30	-	8.4	34.1	103	-	10.8	44.5	113
Noteworthy Findings								
Unscheduled Deaths	0	1[a]	0	0	0	0	0	0
Clinical Observations	No treatment-related observations							
Body Weight Day 29 (%[b])	334.4 g	3.2	1.9	-2.8	206.6 g	-2.1	0.6	1.3
Body Weight Gains (%[b])								
Days 1 to 29	207.2 g	1.4	-0.3	-4.9	90.3 g	-4.5	-0.6	0.1
Hematology								
Red Blood Cells (RBC) (x 10^{12}/L)	7.9	7.9	7.9	7.5*	8.1	8.0	7.9	7.7
Hemoglobin (Hb) (g/L)	152	151	151	137***	152	151	149	144**
Hematocrit (HCT) (%)	46.7	47.1	46.8	43.8**	45.6	45.9	45.7	43.7*
Red blood cell distribution width (RDW) (%)	11.0	11.0	11.1	12.6***	10.3	10.4	10.5	11.5***
Reticulocytes (x 10^9/L)	213	241	252	254	162	173	186	216**
Clinical Biochemistry								
Cholesterol (Chol) (mmol/L)	1.4	1.2	1.1**	0.7***	1.6	1.6	1.6	1.1**
Triglycerides (Trig) (mmol/L)	0.8	0.7	0.6	0.4**	0.4	0.4	0.3	0.2
Urinalysis								
Protein (Prot-U) (mg/collection period)	2.5	2.4	3.1	5.2	0.9	0.8	1.1	1.2

Best Possible Copy

The histopathology changes of retinal atrophy (grade 1) in high dose male only (2 females of control group also had retinal atrophy), renal tubular hayline droplets, lymph node hemorrhage in 0, 2, 1 and 1 males and, 0, 1, 3 and 3 in females were noted in 0, 5, 15 and 40 mg/kg/day treatment groups. The identified target organ of toxicity was kidney and lymph nodes.

Rabbits

Study title: 7-Day Oral Toxicity study in Rabbits

Key study findings: SB-497115-GR from 80 to 200 mg/kg/day produced a linear increase in the plasma concentration of animals and liver histopathology of hepatocellular hypertrophy and erosion of stomach were observed in animals of 200 mg/kg/day treatment group. The target organ of toxicity was liver in both sexes and, stomach, thymus and kidneys were additional target organs of toxicity in males. A 'no effect dose (NOAEL)' was 150 mg/kg/day (0.4 times MHD based on AUC).

Study no.: CD2003/00854/00 (protocol #G03186)
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.
Date of study initiation: June 18, 2003 and April 4, 2004
GLP compliance: A statement was enclosed

Reviewer: Chopra NDA No. 22-291

QA report: yes (X) no ( )
Drug, lot #, and % purity: F033082 equivalent to 75.3% pure salt

Methods

Doses: 0, 80, 150 or 200 mg/kg/day in 2% hydroxy propylmethyl cellulose plus 0.2% sodium lauryl sulfate

Species/strain: New Zealand white rabbits from [redacted] b(4)

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume: Oral gavage in a volume of 5 ml/kg.

Satellite groups used for toxicokinetic or recovery: 3/sex/group

Age: 28-36 weeks old

Weight: Mean weight of 3.68 to 3.81 kg (males) and 3.50 to 3.68 kg (females)

Sampling times: On day 1 and 7, 0.5 ml blood from all rabbits (n=3/sex/group) were collected prior to dosing and 0.5, 1, 2, 4, 8 and 24 hr post dosing for TK estimation and, The 0.5 ml blood for hematology and blood chemistry were drawn on day 0 (prior to treatment) and on day 7 before treatment.

Unique study design or methodology (if any): The dose selection was based on 7-day oral dose ranging study performed at the doses of 80, 150, 200 and 300 mg/kg/day in 4/sex/group animals. The dose of 200 and 300 mg/kg/day was lethal in 1 and 2 out of 4 rabbits/group, The dose of 150 mg/kg/day was a tolerated dose and 200 mg/kg/day was selected as the high dose for the present study. In the present study, the brain, adrenal, duodenum, heart, ileo-cecal junction, ileum, jejunum, kidneys, lungs ovaries, stomach, testes and thymus from all the study animals were processed, fixed, stained and examined microscopically.

Observation and Times: (this information can be provided in a separate section OR evaluation times can be described for each parameter in the results section).
Clinical signs: Once daily during randomization and treatment.
Body weights: Daily once prior to treatment and, once every day after treatment and terminally.
Food consumption: Once 5 days before treatment and once daily during the treatment.
Ophthalmoscopy: Not done.
Hematology & Clinical chemistry: 0.5 ml blood via ear vein for hematology and blood chemistry were drawn on day 0 (prior to treatment) and on day 7 before treatment.
TK: Blood samples (5 ml) from all groups of animals were drawn and SB-497115 was estimated by protein precipitation followed by HPLC/MS/MS analysis.
Urinalysis: Not performed during the study
Gross pathology: On day 8, adrenal, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eye/optic nerve, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes, lesions, mammary glands, ovaries, pancreas, parathyroid, pituitary, rectum, rib, salivary glands, seminal vesicles, skin, skull, spinal cord, spleen, sternebrae, stomach, testes, thymus, trachea, urinary bladder, uterus, vagina and prostate were separated and preserved.

Organ weights: At the termination of the study, adrenals, brain, kidneys, liver, ovaries, heart, testes, thymus and prostate from each of the animals were separated and weighed.

Histopathology: The organs from each of the animals were separated in 10% formalin solution for histopathology changes were adrenal, cecum, duodenum, heart, ileocecal junction, ileum, jejunum, kidneys, liver, lungs, macroscopic lesions, ovaries, stomach, testes and thymus. These were processed, fixed, stained and examined microscopically

Toxicokinetics: About 0.5 ml blood was collected from ear vein of all the rabbits prior to dosing, 0.5, 1, 2, 4 and 24 hr after dosing on day 1 and 7 for the determination of systemic exposure (area under the plasma concentration-time curve (AUC) from the start of dosing to the last quantifiable timepoint ($AUC_{(0-t)}$) using the linear-logarithmic trapezoidal rule. Plasma samples were analyzed for SB-497115 using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis.
The maximum observed peak plasma concentration (C_{max}) and T_{max} were determined.

Results:

Mortality: No treatment related deaths were during the study.

Clinical signs: Blood stained feces and staining of the anogenital area was observed from day 8 in one male in the 200 mg/kg/day group.

Body weights and Food consumption: The body weight of control and treated groups of animals were similar during the study. The initial and final mean body weights of control group males were 3.7 and 3.7 kg and, of females were 3.7 and 3.7 kg, respectively. Among females, consumption was reduced from initial 125 (on day 1-2) to 114.7 (on day 7-8) g. One 200 mg/kg/day group female had a 25% reduction in food consumption relative to control on Day 8.

Hematology: No treatment related hematological effects were seen in the animals belonging to treatment groups. No significant increase in platelets and reticulocytes counts was reported.

Blood Chemistry: Serum triglycerides concentration increased by 2.5-fold (0.51 to 1.25 mmol/L) in females of 200 mg/kg/day by day 7. Mean serum cholesterol concentration was increased by 73% (to 0.69 mmol/L) on Day 7.

Organ Weight Changes: Mean liver absolute weights were increased by 13% and
13 to 14% in males and females. Absolute kidney weight was increased by 18 to 21% in males. An increase (32 to 33%) in Mean absolute adrenal weights was increased in males of 200 mg/kg/day group and, mean absolute adrenal weight was decreased by 15%

In females of 200 mg/kg/day group and no changes in relative weight were seen. Mean thymic weights (absolute and relative to body weight) were decreased by 27.8 and 30.2% in males belonging to 150 mg/kg/day and 200 mg/kg/day treatment groups.

Ophthalmoscopy: Not performed on the study animals.

EKG: This parameter was not studied during the study.

Clinical chemistry: Serum triglycerides were increased by 1.5 and 3.2 folds in male and females of 200 mg/kg/day treatment group. Serum cholesterol was increased by 73% (to 0.69 mm/l) in males treated with 200 mg/kg/day.

Gross pathology: There were no treatment related observations in the animals.

Organ weights: The absolute liver weight of study animals of 200 mg/kg/day group was increased insignificantly from 89.3 to 100.9 g (12.9%). Mean thymic weights (absolute and relative to body weight) were decreased in males given 150 mg/kg/day (27 to 28%) or 200 mg/kg/day (30 to 31%) compared with controls.

Histopathology: Hepatocellular hypertrophy was seen in 3/sex of 200 mg/kg/day treatment group. Periportal hepatic infiltrates was seen only in 1/3 males of 200 mg/kg/day. The additional histopathology findings of involution of thymus in 3 and 3 females of 150 and 200 mg/kg/day treatment groups were seen. This change was not seen in males included in the study. Two of 3 males of 200 mg/kg/day group showed minimal to mild vacuolization of renal cortical tubules (in the ascending portions of distal tubules) and a minimal to slight gastric erosion in 2 of 3 males of 200 mg/kg/day treatment group was an additional finding.

Toxicokinetics: A linear increase in the plasma concentration was observed in animals included in 80, 150 and 200 mg/kg/day treatment groups and the plasma concentrations in females was slightly more than in male of the study on day 7 as shown below in the table (scanned from sponsor's submission):

Increased exposure on Day 7 relative to Day 1 was observed for females at 150 mg/kg/day and for males and females at 200 mg/kg/day.

Mean Toxicokinetic Parameters						
Sex	Dose (mg/kg/day)	Day		AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max}[1] (h)
Male	80	1	Mean	16284	2489.3	2.03
			SD	1745	753.7	2.00-8.12
		7	Mean	18694	3227.2	1.02
			SD	3421	1363.1	1.02-1.08
	150	1	Mean	40534	4567.4	2.00
			SD	21461	1720.4	0.98-2.02
		7	Mean	48785	8118.4	1.03
			SD	9739	2119.3	0.52-1.98
	200	1	Mean	58396	6714.4	4.08
			SD	24844	427.9	2.07-8.00
		7	Mean	223282	15617.1	8.02
			SD	156531	8168.4	1.93-23.20
Female	80	1	Mean	18407	2936.2	1.02
			SD	2876	1900.6	0.47-8.05
		7	Mean	24008	4018.7	0.53
			SD	5257	1757.5	0.52-2.02
	150	1	Mean	30965	3927.8	1.03
			SD	9841	2225.2	0.97-2.02
		7	Mean	68756	8623.7	1.97
			SD	9227	1471.0	0.97-7.98
	200	1	Mean	65457	9430.8	1.97
			SD	40595	4163.0	0.95-2.00
		7	Mean	141593	19900.2	2.08
			SD	28444	6770.2	1.70-4.07

SD Standard Deviation
1. Median and range

In summary, SB-497115-GR at the doses 80, 150 or 200 mg/kg/day produced a linear increase of plasma concentration and the animals treated with high dose showed histopathological effect of hepatocellular hypertrophy, thymic involution, minimal to mild renal vacuolization and erosion of stomach in animals of 200 mg/kg/day treatment group. The identified target organ of toxicity was liver and stomach and, kidneys were additional target organ of toxicity in males of the study. The 'highest tolerable dose' was 150 mg/kg/day (0.4 times MHD based on AUC).

Dogs:

Study title: 14-Day Oral Capsules Dose Range Toxicity Study in Dogs

Key Study Findings: Repeat-dose administration of SB-497115 (60 mg/kg/day) for 14 days produced single cell hepatocellular necrosis, accumulation of pigment in Kupffer cells and hepatocellular

hypertrophy in the dog. A decrease in reticulocyte count and, increase in serum alanine aminotransferase, total bilirubin and alkaline phosphatase was observed in the dog indicating liver was affected in the treated animals.

Study Submission Date: December 12, 2003
Study No.: RD-2003/01089/00/1; D41290
GLP Compliance statement: Yes
Species: Beagle Dogs
Salt form: SB-497115 *bis*-monoethanolamine Gelatin capsule
Route: Oral by capsule

Methods: Two groups of dogs (1/sex) were treated with 0 and 60 mg/kg/day in capsules for 14 days as shown in the following dose regimen.

Study Design

Group	Dose	Number of Animals
1	0	1M/1F
2	60	1M/1F

Data collected: Toxicokinetics (Days 1 and 14), clinical signs, body weight, hematology, homeostasis, clinical chemistry, macroscopic and microscopic pathology were evaluated in dog treated with 60 mg/kg/day.

Results:

Reticulocyte count was decreased approximately 89% in the female in comparison to pretreatment value. Alanine aminotransferase and total bilirubin values were increased approximately 16- and 5-fold, respectively, in this female compared with the pretreatment values. Alkaline phosphatase activity was increased approximately 9- and 1.8-fold in the female and male, respectively. Both dogs had minimal-to-mild hepatocellular hypertrophy, and the female also had minimal hepatocellular single cell necrosis and accumulation of brown pigment in most Kupffer cells. A slight reduction in body weight (2%) was noted for the female. A sporadic decrease in food consumption was noted for both dogs.

Group No. (Dose: mg/kg/day)	**1 (0)**	**2(60)**	**1 (0)**	**2(100)**
Sex	**M**	**M**	**F**	**F**
Deaths (No.)	0	0	0	0
Body Weight gain (kg) – Day 1-13	0.24	-0.01	0.33	-012
Reticulocyte Count ($x10^9$/L) Week –1	56.8	38.3	77.5	55.4
Week 2	44.4	46.2	67.2	6.0
Serum alkaline phosphatase (U/L)				

Week –14	62	77	89	50
Week 2	53	136	82	447
Serum alanine aminotransferase (U/L)				
Week -1	41	35	29	35
Week 2	46	42	32	551
Total Bilirubin (umol/L)				
Week -1	1,2	3.5	1.0	1.2
Week 2	1.6	1.8	1.8	6.1
Incidence of Microscopic Findings				
Hepatocellular hypertrophy				
(Minimal)	NA	1	NA	0
(mild)	NA	1	NA	1/1
Multifocal Hepatocellular Single				
cell necrosis (mininmum)	NA	0	NA	1
Kupffer cell Brown Pigment				
(minimal)	NA	0	NA	1
Toxicokinetic Parameters (plasma):				
Day 1 AUC0-24 (ug.h/mL)	NA	169	NA	730
Day 14	NA	557	NA	2170
Cmax (ug/mL)				
Day 1	NA	20.6	NA	54.1
Day 14	NA	32.8	NA	134

In this study, SB-497115-GR formulation was administered orally to male and female dogs at a dose of 60 mg/kg/day for 14 days. Treatment produced single cell hepatocellular necrosis, accumulation of pigment in Kupffer cells and hepatocellular hypertrophy in treated dog. A decrease in reticulocyte count, increased serum alanine aminotransferase, total bilirubin and alkaline phosphatase were observed. The dose of 60 mg/kg/day exceeded the maximum tolerated dose.

Study title: 14-Day oral Gavage Toxicity Study in Dogs

Key study findings: A dose proportional plasma concentration of SB-497115 at 3 to 30 mg/kg/day SB-497115 was attained and the plasma concentrations in both sexes were similar. Based on the inflammatory cell infiltrate in liver and choroid plexus and, granuloma of skin in 1 female and sperm granuloma in 1 male of 30 mg/kg/day treatment group indicated that the low dose of 10 mg/kg/day (1.2x MHD based on AUC) was a 'no effect dose'.

Study no.: SB-497115/RSD-101V3C/1/G02015
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.
Date of study initiation & Completion: March 4, 2002 and October 10, 2002
GLP compliance: A statement was enclosed
QA report: yes (X) no ( )
Drug, lot #, and % purity: 20456-037 with a purity of 51.7% as free salt)

Methods

Doses: 3, 10 and 30 mg/kg/day (as base) in gelatin capsules (1 capsule/day in each of the treatment group)

Species/strain: ——— ı Beagle Dogs 9 to 10 months old b(4)

Number/sex/group or time point (main study): 3/sex/group

Route, formulation: Oral capsules

Groups used for toxicokinetics or recovery: --

Age: 9 to 10 months old

Weight: Mean weight of 10.5 to 10.9 kg (male) and 7.5 to 8.0 kg (female)

Sampling times: On day 1 and 14, 2 ml blood samples from the jugular vein of dogs (n=3/sex/group) were collected at 0.5, 1, 2, 4, 8 and 24 hr post dosing from the animals of each of the study treatment group

Observation and Times:
Clinical signs and mortality: Once daily for viability and detailed examination during randomization and on day 14
Body weights: Thrice prior to treatment, once every week and terminally.
Food consumption: Once prior to dosing and every week during study.
EKG: Once prior to dosing and 1-2 hr post dosing
Hematology: Once prior to dosing on day 1, 4 and 14.
Clinical chemistry: Blood samples were collected once prior to dosing on day 1, 4 and 14.
TK: Blood samples (2.0 ml/time point) from treated (3/sex/group) on day 1 and 14.
Urinalysis: The urine samples from main study group were collected
Gross pathology: After 14 days of treatment, the animals were sacrificed, adrenal*, aorta*, brain*, cecum, cervix, colon*, duodenum*, epididymides*, esophagus*, eye/optic nerve*, femur, gallbladder*, heart*, ileum*, jejunum*, kidneys*, larynx, liver*, lung*, lymph nodes* (mesenteric, popliteal), lesions*, mammary glands*, ovaries*, pancreas*, parathyroids*, pituitary*, prostate*, rectum, rib*, salivary glands* (mandibular, parotid), sciatic nerve*, skeletal muscle*, spinal cord (cervical and lumber) *, skin*, spleen*, sternum, stomach*, testes*, thymus*, thyroid*, trachea*, tongue, urinary bladder*, uterus*, vagina *and lumber vertebra were separated from all the dogs.
Organ Weights: On day 15/16 the liver of each of the animals was separated for cytochrome P450 analysis. Brain, liver, heart, kidneys (paired), adrenal glands (paired), thymus, testes (paired), epididymides (paired) and ovaries (paired) were separated and weighed.

Histopathology: The tissues marked asterisk (*) were examined histpathologically of all the dogs.

Results:

Mortality: None of the animals died during the study.

Clinical signs: The discolored (orange, dark orange or red/brown colored feces was reported in 2 and 5 dogs belonging to 10 and 30 mg/kg/day treatment groups.

Body weights and Food consumption: A slight body weight loss of 4.5 and 2.8% in males and, 0 and 1.35% in females of 10 and 30 mg/kg/day treatment groups was noted. On day 0, the initial body weights of males were 10.6, 10.7, 11.2 and 10.7 kg and, 7.8, 7.8, 8.1 and 7.4 kg of females of the study. The food intake of these animals on day 10 was 308, 356, 332 and 342 g/day in males and, 240, 298, 264 and 283 g/day in females.

Hematology & Clinical chemistry: No treatment related changes.

Urinalysis: No treatment related changes.

Gross pathology: No data was submitted.

Organ weights: In males, the mean thymus weight was increased by 4.0, 2.54 and 3.57 times and, kidney weights were increased by 1.2, 1.36 and 1.33 times in 3, 10 and 30 mg/kg/day treatment groups. The organ weights of female animals were not affected during the study.

Histopathology: The microscopic changes of inflammatory cell infiltrate in liver of 1 female, granuloma of skin in 1 female of 30 mg/kg/day treatment group were observed. The inflammatory cell infiltrate in choroid plexus was present in another female out of 3 females in 30 mg/kg/day treatment group. Sperm granuloma was seen in 1 male of 30 mg/kg/day treatment group.

Toxicokinetics:

A dose proportional increase in the plasma concentration within 1 to 2 hr of the administration of the compound was observed on day 1 and 14. The plasma concentrations of animals treated at 30 mg/kg/day was achieved at a delayed time of 4 hr. On day 1 and 14, the mean plasma concentrations, Cmax and AUC values in males and females were similar as shown below in the table (within +2SD).

	Cmax (ug/ml)		AUC (0-24hr) (ug.hr/ml)		Tmax (hr)	
	M	F	M	F	M	F
Day 1:						
3	2.89	4.64	27.9	40.7	2.01	2.01
10	9.89	18.4	90.0	147	1.02	2.02
30	47.8	43.1	618	590	2.03	2.03
Day 14						
3	5.14	5.45	60.8	55.4	2.0	2.0
10	11.5	11.6	152	205	1.0	2.0
30	53.4	49.2	695	869	4.0	4.0

In humans, the Cmax and AUC values at maximum recommended dose (75 mg/day) were 11.4 μg/ml and 146 μg.hr/ml, respectively.

In summary, orally administered 3 to 30 mg/kg/day SB-497115 (0.4-3 times MHD based on AUC) attained a dose proportional plasma concentrations and the plasma concentrations were similar in both sexes. The microscopic changes of inflammatory cell infiltrate in liver and choroid plexus and, granuloma of skin in 1 female and sperm granuloma in 1 male of 30 mg/kg/day treatment group indicated that the low dose of 10 mg/kg/day (1.2x MHD based on AUC) was a 'no effect dose'.

Study title: 52-Week Capsule Toxicity study in Dogs
(Study # CD2003/01/1148/00; #G03246)

Key study findings: SB-497115-GR treatment from a dose of 3 to 30 mg/kg/day (0.4-3 times MHD based on AUC) for 52 weeks in beagle dogs produced mild to moderate increases in serum alkaline phosphatase and bone-specific alkaline phosphatase. The increase was not associated with a significant alteration in histopathology of the treated animals, yet changes in the enzyme should be considered as an important toxicity.

b(4)

Study no.: CD2003/001148/00 (protocol #G03246) — /274-448
Conducting laboratory and location: '
Date of study initiation & Completion: October 17, 2003 and May 9, 2005.
GLP compliance: An unsigned statement was provided in the report
QA report: yes ( ) no (X)
Drug, lot #, and % purity: 202633-001 (purity not defined)
Methods

Doses: 0 (vehicle control), 3, 10 and 30 mg/kg/day in capsules for 52 weeks and interim groups (4/sex/group) were treated for 52 weeks. The doses were selected based on a 4-day oral dose-range toxicity study in dogs (#SB-497115/RSD-101T3T/1) at 10, 30, and 100 mg/kg/day). A dose of 100 mg/kg/day produced increased (up to 48-fold) liver enzymes (male only) and vacuolar hepatocellular hypertrophy and degeneration, necrosis and/or apoptosis (male and female) and reticulocyte counts were decreased at 100 mg/kg/day. In a 14-day oral toxicity study in dogs (study#SB-497115/RSD-101V3C/1 and RD2003/01089/00) SB-497115-GR was given at 3, 10, and 30 mg/kg/day and, 60 mg/kg/day. A dose of 60 mg/kg/day was not tolerated and produced a marked increase in serum alanine aminotransferase (16-fold), alkaline phosphatase (9-fold) and total bilirubin (5-fold) and a marked decrease (89%) in reticulocyte count.

b(4)

Species/strain: —
Number/sex/group or time point: 4
Route, formulation, volume, and infusion rate: Oral gavage in 10 ml/kg volume.
Satellite groups used for toxicokinetics or recovery: No separate groups were included
Age: 11 months
Body Weight: Mean body weight of 6.3 to 9.3 kg (males) and 5.7 to 8.9 kg (females)
Sampling times: The blood samples for TK analysis were collected on day 1, week 4, 13 and 26 from jugular vein from non fasted dogs at 1, 2, 4, 8, 12 and 24 hr post dosing
Unique study design or methodology (if any): --

Group Number	No. of Animals		Dose Level[1] (mg/kg/day)
	Males	Females	
1 (Control)[2]	4	4	0
2 (Low)	4	4	3
3 (Mid)	4	4	10
4 (High)	4	4	30

1. All doses are expressed in terms of free acid.
2. Animals in the control group received the vehicle control material (empty gelatin capsule) only.

Observation and Times:

Clinical signs and mortality: Clinical signs were observed twice daily during randomization, twice daily at the start and end of the each treatment day, twice during the 1st, 3 to 4 hr post dosing. The detailed observations were made once weekly through out the study.
Body weights: Once prior to treatment and once every week during the study
Food consumption: Once daily during the study.
Ophthalmoscopy: Once prior to dosing (week –2), and at the end of week 26 and 52.
Hematology: Blood samples from jugular vein on week 4, 13, 26 and week 52.
Clinical chemistry: Blood samples from jugular vein on week 4, 13, 26 and week 52. Serum bone specific alkaline phosphatase analysis was conducted on serum collected on week 52 using the [redacted] immunoassay manufactured by [redacted] b(4)

EKG: Ten-lead Electrocardiograms were recorded from each animal (nonanesthetized) once prior to the initiation of treatment and during Week 52 (pre and post dose). Heart rate; PR, QRS, QT, and RR intervals; and corrected QT interval (QTC was calculated using Van de Waters equation) were assessed.

TK: Blood samples (1 ml/animals) were collected during day 1, week 4, 13, 26 and 52. The plasma concentrations determined at GSK, King of Prussia.
Urinalysis: Overnight urine samples from main study group
Gross pathology: None of the animals were sacrificed for the gross pathology.
Organ weights (specify organs weighed if not in histopathology table): Organs were not dissected in the interim report.

Histopathology: The organs/tissues were not separated and no histopathology data included in the report.

Results:

Mortality: None of the animals died during the study.

Reviewer: Chopra NDA No. 22-291

Clinical signs: Discolored (orange, dark orange or red/brown colored) feces in 2 and 5 dogs belonging to 10 and 30 mg/kg/day treatment groups were reported.

Body weights and Food consumption: No treatment-related effects on food consumption were observed.

Blood Chemistry: On week 52, an increase of 16% in total protein for females given 30 mg/kg/day was seen. Serum albumin was not significantly affected in the animals and the effect linked to increased globulin. The bone-specific alkaline phosphatase was increased by 2.3- to 2.9-fold in males included in 10 and 30 mg/kg/day and 3-fold in females included 30 mg/kg/day group. The control values were 10.48 U/L (males) and 14.62 U/L (females). The increases in bone-specific alkaline phosphatase activity indicated the treatment might affect osteoblastic activity and bone formation.

Physical Examination/EKG Changes: There were no EKG changes in the treated dogs as seen in the sponsor's scanned table:

Source b(4) Species Canine
Study No. 7274-448 Restraint

						Duration / Intervals *						Morphology Assessment **					
Date	Exp ID	Group	Animal ID	Sex	HR	P	PR Int	QRS	QT Int	RR	QTcV	rhythm	P	QRS	ST	T	Note
21-Oct-04	Wk 52 PRE	1	H41241	M	120	35	85	50	190	475	295	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	1	H41242	M	84	40	90	55	230	650	250	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	1	H41243	M	108	30	80	55	185	550	224	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	1	H41244	M	132	30	85	40	185	440	234	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	1	H41255	F	84	40	95	50	220	655	250	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	1	H41257	F	128	30	100	40	195	495	239	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	1	H41259	F	128	40	90	45	185	495	229	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	1	H41260	F	102	30	105	50	210	620	243	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41245	M	126	40	95	40	190	470	296	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41246	M	64	40	120	50	225	980	226	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41247	M	84	30	95	50	210	715	235	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41248	M	96	35	105	40	225	625	256	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41261	F	108	40	100	40	210	540	280	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41262	F	90	40	110	45	225	630	257	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41263	F	132	30	90	45	185	470	231	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	2	H41264	F	84	40	125	50	230	725	254	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	3	H41249	M	78	25	95	50	220	700	248	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	3	H41250	M	78	40	90	40	210	790	228	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	3	H41251	M	78	30	90	50	195	795	213	✓	✓	✓	✓	✓	
21-Oct-04	Wk 52 PRE	3	H41252	M	78	30	110	40	230	860	258	✓	✓	✓	✓	✓	

* milliseconds units of measure ** numerical results are in ratified units ✓ denotes within normal limits

Note	Comment
general	10 lead ECGs recorded at 25 and 50 mm/sec; Heart rate estimated from 10 second segments
general	RR interval refers to interval preceding beat used for QT interval measurement; QTcV is Van deWater's corrected QT interval

Macroscopic Findings: No data was submitted.

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Organ weights: In males, the mean thymus weight was increased by 4.0, 2.54 and 3.57 times and, kidney weights were increased by 1.2, 1.36 and 1.33 times in 3, 10 and 30 mg/kg/day treatment groups. The organ weights of female animals were not affected during the study.

Histopathology: No treatment-related histomorphologic alterations were reported in the tissues examined.

Toxicokinetics: The oral administration of SB-497115-GR to male and female beagle dogs produced a dose related increase in plasma concentration in all animals at 24-hour. Tmax occurred between 1 and 12 hours after dosing. The plasma concentrations and systemic exposure (Cmax and AUC) between males and females were not significantly different on study day 1, weeks 4, 13, 26, 52. On day 1, non-dose proportional systemic exposures, i.e., 2- to 3-fold lower than the other sampling periods, were seen.

		Male				Female			
Daily Dose (mg/kg/day)		0	3	10	30	0	3	10	30
Number of Animals:	Toxicity	4	4	4	4	4	4	4	4
AUC_{0-24} (ng.h/mL):	Day 1	NA	12880	62370	182361	NA	23816	45361	270901
	Week 4	NA	22432	165571	486905	NA	29978	169771	452853
	Week 13	NA	25151	198331	381577	NA	29349	129145	476576
	Week 26	NA	33798	105598	461394	NA	38364	118351	488295
	Week 52	NA	49732	107957	368696	NA	66977	173730	467167
C_{max} (ng/mL):	Day 1	NA	1643.7	6468.2	17639	NA	2857.6	5516.1	21576
	Week 4	NA	2160.0	11883	31967	NA	2501.6	9663.6	30293
	Week 13	NA	2703.4	14371	28679	NA	3365.6	9890.2	35798
	Week 26	NA	4063.5	9106.3	39556	NA	4230.3	10792.3	41524
	Week 52	NA	5402.4	9895.2	31949	NA	6891.0	14392.2	37081
Noteworthy Findings: Clinical Chemistry:									

Note: In humans, the Cmax and AUC values at maximum recommended dose (75 mg/day) were 11.4 µg/ml and 146 µg.hr/ml, respectively.

In summary, SB-497115-GR treatment from 3 to 30 mg/kg/day (0.4-3 times MHD based on AUC) for 52 weeks to male and female beagles produced mild to moderate increases in serum alkaline phosphatase and bone-specific alkaline phosphatase. The increase was not associated with a significant alteration in histopathology of the treated animals. The important changes in the enzyme were considered drug related toxicity. There was a dose-dependent increase in the plasma concentration and accumulation was observed after repeated dosing since the systemic exposure was 2 to 3-fold higher after repeated dosing as compared to Day 1.

2.6.6.4 Genetic toxicology

1. Study title: SB-497119: Ames agar plate with Salmonella typhimurium and Escherichia coli

Key findings: The compound did not increase revertant colonies and was negative in the test.

Reviewer: Chopra NDA No. 22-291

Study no.: SB-497119/RSD-101KTS/1; G01565 b(4)

Conducting laboratory and location: [redacted]
Date of study initiation & completion: April 6, 2001 June 24, 2002
GLP compliance: UK GLP compliance statement was attached
QA reports: yes (X) no ( )
Drug, lot #, and % purity: KD 45207-166A1 and 100% pure

Methods

Strains/species/cell line: TA98, TA100, TA1535 and TA1537

Doses used in definitive study: 500, 1000 and 2500 ug/plate

Basis of dose selection: In dose-range toxicity study, 8 to 5000 ug/plate eltrombopag concentrations were used. In preliminary toxicity assay (1 plate/concentration), 62.5 to 2500 ug/plate concentrations were used. Precipitate was formed from 500 ug/plate but was claimed not to interfere with the detection and scoring of the plate colonies. Based on these observations, 62.5 to 2500 ug/plate was the concentration selected for main mutation test.

Negative controls: DMSO at 2.00 ug/plate for salmonella strains and, 10.0 ug/plate for WP2 uvrA and WP2 pKM101

Positive controls: 2.0 ug/plate for TA98, TA100, TA1535 and TA1537 strains
2-Nitrofuoquinoline –1- oxide 10 ug/plate for WP2 uvrA and WP2 pKM101 strains
Incubation and sampling times: 72 hr at 37°C

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The test was considered as positive if reproducible positive response was seen in 2 of the 3 tests and, negative if the compound was non-genotoxic in 2 of the 3 tests.

Study outcome: A severe toxicity or the precipitation was seen in culture at or above 250 ug/plate in TA98, TA100, TA1535, and TA1537, WPpKM101 WP2uvrA pKM101 in the presence or absence of S9. The precipitate was observed in plate containing 200, 500, 100 and 2500 ug/plate as shown below in the table but it did not affect the colonies counts, therefore the test was valid only up to 2500 ug/plate.

Table 2: Summary of Results for the Second Mutation Assay

Second Mutation Assay in the Presence of S9 (3 hour treatment)

Treatment / Concentration (ug/mL)		% RTG	Mutation frequency*	
			Treatment mean	Relative increa
DMSO	0.01 mL/mL	100	96	—
SB-497115	5	117	91	0.94
SB-497115	10	79	205	2.13#
SB-497115	15	74	213	2.21#
SB-497115	20	73	221	2.30#
SB-497115	40	57	190	1.98#
SB-497115	60	23	136	1.42
BP	2			

Second Mutation Assay in the Absence of S9 (24 hour treatment)

Treatment / Concentration (ug/mL)		% RTG	Mutation frequency*	
			Treatment mean	Relative increa
DMSO	0.01 mL/mL	100	84	—
SB-497115	0.5	110	75	0.90
SB-497115	1	101	95	1.14
SB-497115	2	90	78	0.93
SB-497115	4	56	94	1.13
SB-497115	8	14	133	1.59
	5			

Statistically significant increase
* mutant frequency per 10^6 viable cells
DMSO Dimethyl Sulphoxide
BP Benzo(a)pyrene
MMS Methyl Methanesulphonate

SB-497115 was not mutagenic up to 2500 ug/plate concentration.

2. **Study title: Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus**

Key findings: SB-497115 induced gene mutation and was mutagenic in the present in vitro test.

Study no.: SB-497115/RSD-101KFT/1; G01566
Conducting laboratory and location: [redacted] b(4)
Date of study initiation & completion: April 6, 2001 December 7, 2001
GLP compliance: UK GLP compliance statement was attached
QA reports: yes (X) no ( )
Drug, lot #, and % purity: KD 45207-166A1 and 100% pure

Methods
Positive controls – (i) Methyl methanesulfonate (MMS) at 10.0 ug/ml concentration in the presence of rat liver enzymes (S9 mix). The concentrations for 3 and 24 hr treatment were 10.0 and 5.0

ug/ml, respectively. (ii). Benzo (a) pyrene (BP) at 2.0 ug/ml concentration in the absence of metabolic activator, rat liver S-9 fraction.
Cell line Used: Stock of L5178Y, 3.7.2C, $TK^{+/-}$ mouse lymphoma cells stored in liquid nitrogen

Methods: In the preliminary dose ranging finding study in absence of S9, SB497115 was tested in a single culture tubes from 6.25 to 200 ug/ml concentrations for 3 hr and, 0.78 to 200 ug/ml for 24 hr treatment. Because of high toxicity at 100 ug/ml and above in the 3 hr treatment, the concentrations of 3.125, 6.25, 12.5, 25 and 50 ug/ml were selected for the mutation test in the presence of S9 mix. The mutation test in the absence S9 mix was conducted at 3.125, 6.25, 12.5, 25 and 37.5 ug/ml SB497115 concentrations in the single culture tubes for 3 hr. In the second mutation test (in the presence of S9), the concentrations used were 5, 10, 15, 20, 40 and 60 ug/ml for 3 hr treatment and, 0.5, 1, 2, 4 and 8 ug/ml concentrations were used for 24 hr treatment in the absence of S9.
In the third mutation test, the concentrations used were 5, 10, 20, 30, 40, 50, 60 and 70 ug/ml for 3 hr treatment.

The cultures with the compound were incubated for 2 days and TK mutations allowed to be expressed and maintained at $1X10^7$ cells/flask and the cells were adjusted to $1X10^4$ prior to plating for the determination of TFT resistance mutant frequency. The mutant frequency was determined after the addition of 5-trofluorothymidine (TFT). The mutant frequencies were counted as mutant frequency per 10^6 viable cells. The ratio of test article concentration to control frequency, mutant frequency colony ratio and ratio of total number of mutant colonies to the total cell colonies were calculated. The average absolute coloning efficiency of vehicle, small and large colony frequencies were calculated using relevant number of colonies. The test was conducted at the highest concentration of the compound achieved as the solution or the least toxicity (10-20%) of the negative control. If the compound showed an increase <1.5 times the negative control, the test was negative. If the compound induces 3 fold or greater in mutant frequencies over the concurrent background mutant frequency, the test was positive. The test should be positive in 2 of the 3 valid assays. The test within 1 and 2 times the negative controls, should be evaluated by the Dunnet's test of significance ($p<0.05$). If the results were questionable it was equivocal.

Results: SB-497115 was tested in 2 trials in the presence and in the absence of S-9 mix. There was 1.5 and 3 folds increase of mutation frequencies in the 3 assays and it was significantly greater than the control ($p<0.05$). SB-497115 at 10 ug/ml and above concentrations was genotoxic and as per the test criteria, it exerted moderate genotoxicity (>3.0 folds increase the negative control). Thus, SB-497115 induced gene mutation and chromosomal aberration in the present in vitro test.

The compound showed an increase in the number of the mutation frequencies in test 1, 2 and 3. The relative mutant frequencies increase in first mutation assay was 1.08, 1.97, 1.75, 1.69 and 1.56 times the control in cultures containing 3.125, 6.25, 12.5, 25 and 37.5 ug/ml SB497115 concentrations. The proportion of small to large colonies was 0.46, 0.55, 0.58, 0.54, 0.62 and 0.67 respectively with the above concentrations of the compound. The increase in the proportion of small to big colonies was not affected. The relative increase in the mutant frequencies was also seen in second and third

mutation assays. An increase of 12.96 to 18.83 times was seen with positive control, MMS. SB-497115 was mutagenic in the test. The mutant frequencies, the proportion of increase of small:big colonies are shown in the following tables of the sponsor:

Table 2 Summary Table of Mammalian Cell Mutation Data for 2-Aminoethanol (24 Hour Treatment)

GWRD Study No: V23485
GWRD Report No: WD2002/00089/00
Test Cells: Mouse Lymphoma L5178Y
Test for Induction of: Forward mutation in mammalian cells at the tk locus
Metabolising System: β-naphthoflavone and phenobarbitone-induced rat liver S9 fraction, with a glucose-6-phosphate-driven NADPH generating system
No. Replicate Cultures: 2

Tests in the Absence of S9-mix

Concentration of 2-Aminoethanol µg(base)/mL)	Test 1[b] (-)S9		Test 2[b] (-)S9	
	Relative Total Growth (%)	Mutant Freq. (x10^{-6})	Relative Total Growth (%)	Mutant Freq. (x10^{-6})
0[c]	99.92	149.87	100.01	230.45
20	94.88	253.78	83.61	296.07
30	96.89	271.21	73.86	424.14
40	82.67	336.39	88.51	384.04
43	89.54	337.55	70.56	502.57
Positive Control[d]	51.41	1701.60	40.66	2294.30

Footnote to Table 2

b 24 hour treatment
c Solvent control: 1% v/v Water for Injection
d Methyl Methanesulphonate at 5µg/mL (24 hour treatment in the absence of S9-mix)
- Denotes 'Not Tested' or 'Not Plated' for mutant frequency.
Relative total growth and mutant frequency values represent the mean of replicate cultures.
Statistically significant compared to solvent control $p<0.05$ **bold text**
There is evidence of an increasing trend in pooled mutation frequency (Linear Trend Test)
Positive controls fulfilled required criteria.

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SB-497115 induced gene mutation and chromosomal aberration in the present in vitro test.

3. **Study title: 2-Aminoethanolamine: Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus**
The test was conducted to observe the possible genotoxic effect of 2-aminoethanol in mammalian cell mutation test.
Key findings: Aminoethanolalamine was mutagenic in the test. b(4)

Study no.: WD2002/00089/00; V23485

Conducting laboratory and location: GlaxoSmithKline, Ware, Hertfordshire (UK)
Date of study initiation & completion: January 2, 2002 and September 11, 2002
GLP compliance: UK GLP compliance statement was attached

Reviewer: Chopra NDA No. 22-291

QA reports: yes (X) no ( )
Drug, lot #, and % purity: 10K 0191 and 99.9% pure

Methods
Positive controls – (i) Methyl methanesulfonate (MMS) at 1 mg/ml concentration in sterile water in 3 hr treatment and 0.5 mg/ml (for 24 hr treatment).
(ii). Dimethylbenzeneanthracene (DMBA) at 0.1 mg/ml in DMSO

Cell line Used: Stock of L5178Y, 3.7.2C, $TK^{+/-}$ mouse lymphoma cells stored in liquid nitrogen

Methods: The test was conducted at the maximum concentration of the bisethanolamine compound solution used for the previous mutation test study G01566 (SB-497119/RSD-101KFT/1) as reviewed above. The highest concentration of ethanolamine used in the study was 70 ug/ml bisethanolamine salt of the compound and that would contain 316 uM of 2-ethanolamine and this amounts to the maximum concentration of 43 ug/ml. In the present study, sponsor used the concentrations of 0, 20, 30, 40 and 43 ug/ml 2-aminothanolamine in the presence and absence of S9 mix. The study was performed in the presence and absence of S9 mix and at 3 hr and 24 hr treatment periods. The 4 tests in duplicate cultures were conducted, i.e., one in the absence and one in the presence of S9 mix for 3 hr treatment period and 2 in the absence of S9 mix only for 24 hr treatment period. The cultures with the compound were treated, incubated and evaluated as described in the above study (at S. #2) and the validity conditions.

Results: 2-Aminoethanolamine produced a slight increase of relative total growth of 107.4, 126.0, 132.0 and 101.2 % at 20, 30, 40 and 43 ug/ml ug/ml concentrations. The positive control MMS produced a decrease in the growth to 73.4% of the control group. The pooled mutant frequency were 242.6, 196.8, 176.7 or 245.5 $X10^{-6}$ in the cultures treated for 3hr with 20, 30, 40 and 43 ug/ml ug/ml 2-aminethanolamine. The pooled mutant frequency 24-hr data was 253.8, 271.2, 336.4 or 337.6 $X10^{-6}$ in the cultures treated with 20, 30, 40 and 43 ug/ml ug/ml 2-aminethanolamine. 2-Aminoethanolamine was mutagenic in the test.

4. **Study title: SB-497115-GR Ethanolamine Salt: Mutation Test at the TK Locus in Mouse Lymphoma L5178Y Cells**

Key findings: The compound induced an increase in the number of the mutation frequencies but the proportion of small to large colonies was not given in the study by sponsor. SB-497115 was mutagenic in the test.

Study no.: WD2003/00167/00; V23910

Conducting laboratory and location: GlaxoSmithKline, Ware, Hertfordshire (UK)
Date of study initiation: October 16, 2002 and August 3, 2003

Reviewer: Chopra NDA No. 22-291

GLP compliance: UK GLP compliance statement was attached
QA reports: yes (X) no ( )
Drug, lot #, and % purity: F033082 and 96.1% pure

Chemicals and positive control:

Positive controls – (i) Methyl methanesulfonate (MMS) stock solution (1.0 mg/ml) used at the 1.0 and 0.5 mg/ml concentration, respectively for 3 and 24 hr treatment. (ii). Dimethylbenzanthracene (DMBA) a stock solution of 0.1 mg/ml in the presence of metabolic activator, rat liver S-9 fraction. Cell line Used: Stock of L5178Y, 3.7.2C, $TK^{+/-}$ mouse lymphoma cells stored in liquid nitrogen

Methods: Three mutation assays were conducted (in duplicate cultures), one in the absence of S9 mix and cultures treated for 3 and 24 hr and, one in the presence of S9 mix, the cultures treated for 3 hr. In 3 hr treatment test, the mutation test in the absence of S9 mix was conducted by mixing the cells with 0.5, 8.0, 12 or 12 ug/ml of SB497115. The final concentrations of SB-497115 in the presence of S9 mix were 1, 6, 8 and 10 ug/ml. The mutation test for 24 hr treatment in the absence S9 mix was conducted at 2, 6, 8, 9 and 10 ug/ml SB497115 concentrations in duplicate culture tubes for 3 hr and, at 2.0, 6.0, 8.0, 9.0 and 10.0 ug/ml SB497115 concentrations in duplicate culture tubes for 24 hr incubation. The cultures tubes containing the compound were maintained for 2 days and allowed $TK^{+/-}$ mutations to be expressed and maintained at $1X10^6$ cells/ml and retaining $1X10^7$ cells/flask. The cloning was done in $1X10^5$ cells/ml prior to plating for the determination of TFT resistance mutant frequency). The mutant frequency was determined after the addition of 5-trofluorothymidine (TFT). The mutant frequencies were counted as mutant frequency per 10^6 viable cells as described in the above study at S.#2. The criteria of the positive test were, if the compound showed a significant increase in mutant frequencies in a dose related manner. The test was not significance if the variable increase was observed.

Results: SB-497115 was tested in 2 trials in the presence and in the absence of S-9 mix. There was no significant increase in the mutant frequencies at 0.5, 8.0, 12 or 12 ug/ml SB-497115. The mutant frequencies with MMS were 562.1. The % RTG values were 99.5, 84.4, 33.3, 20.4, 6.8 and 5.8% in cultures containing control (0), 4 compound treatment groups and DMBA. RGT was reduced in a dose related manner and it was 11.8% (vs. 100 and 37.1% in control and in MMS) cultures. At 24 hr treatment (-S9 mix), the pooled mutant frequencies were 302.3 and 270.0 at 9 and 10 ug/ml SB-497115 concentrations. A dose related decrease in mean RGT (%) from mean RGT of 100, 120.1, 77.7, 57.2, 21.0 and 12.3 in control, 2, 6, 8, 9, and 10 ug/ml concentration of SB-497115 and mean RGT were 83.9% in positive control (5 ug/ml MMS).

SB-497115 increased the mutant frequencies but was not concentration dependent. Thus the SB-497115 was clastogenic in the present in vitro test. The # mutant frequencies and their proportion of increase are shown in the following table from the sponsor submission:

Table 1 Summary Table

Genotoxicity: In Vitro — Report Title: WD2003/00157/00. SB-497115-GR: Mammalian cell mutation test at the thymidine kinase locus in mouse lymphoma L5178Y cells (GLP Study, V23910). — Test Article: SB-497115-GR

Test for Induction of: Forward mutation of the TK locus — No. of Independent Assays: 3 — Study No: V23910

Strain: L5178Y Mouse Lymphoma Cells — No. of Replicate Cultures: 2 (solvent); 2 (treatment and positive) — Location in CTD:

Metabolising System: βnaphthoflavone and phenobarbitone-induced rat liver S9-mix containing 108 µL S9-fraction per mL

Vehicles: Test Article: WFI Positive Controls: DMSO and WFI — GLP Compliance: Yes

Treatment: 3 hr treatment with and without S9-mix; Continuous treatment for 24 hr without S9-mix — Date of Treatment: November-December 2002

•Cytotoxic Effects: Concentrations were limited by solubility and cytotoxicity.

•Genotoxic Effects: SB-479115 was genotoxic following 24 hour incubation only.

Test Article	Dose Level µg/mL	3 hr Treatment -S9-mix Mean RTG (%)	3 hr Treatment -S9-mix Pooled Mutant Freq. (x10⁻⁶)	3 hr Treatment +S9-mix Mean RTG (%)	3 hr Treatment +S9-mix Pooled Mutant Freq. (x10⁻⁶)	24 hr Treatment -S9-mix Mean RTG (%)	24 hr Treatment -S9-mix Pooled Mutant Freq. (x10⁻⁶)
WFI	0	100.2	74.4 [1]	99.5	84.3	100	103.2
SB-497115-GR	0.5	22.3	116.2				
SB-497115-GR	1.0			54.4	x		
SB-497115-GR	2.0					120.1	103.3
SB-497115-GR	6.0			33.3	114.2	77.7	120.4
SB-497115-GR	8.0	25.9	116.2	20.4	93.2	57.2	147.4
SB-497115-GR	9.0					21.0	302.3 *
SB-497115-GR	10.0			6.8	97.2	12.3 [2]	270.0 *[2]
SB-497115-GR	12.0	18.5	x				
SB-497115-GR	14.0	11.8	90.4				
MMS	10.0	37.1	562.1				
MMS	5.0					83.9	250.0
DMBA	1			5.8	914.9		

1. Based on one vehicle control
2. Based on one replicate
x Not calculated statistically due to inconsistency between plates
* Statistically significant p<0.05

The compound showed an increase in the number of the mutation frequencies in test 1, 2 and 3. The proportion of small to large colonies was not given by the sponsor. SB-497115 was mutagenic in the test.

5. **Study title: SB-497115-GR: Micronucleus Assay in Sprague-Dawley rats after oral administration**

Key findings: SB-497115-GR was not clastogenic in vivo rat bone marrow micronuleus induction test up to11 times the human clinical exposure (based on C_{max}).

Study no.: WD2003/01250/00; R23395

Conducting laboratory and location: [redacted] b(4)
Date of study initiation& Completion: April 10, 2002 and May 7, 2004
GLP compliance: UK GLP compliance statement was attached
QA reports: yes (X) no ( )
Drug, lot #, and % purity: US0200061/F033082 and 96.1% pure

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Methods

Based on the results of the 4- and 10- day rat studies, eighty male Sprague-Dawley rats were administered oral gavage doses of 0, 120, 240 and 500 mg/kg (volume = 10 ml/kg) for 2 days. SB-497115 dose formulations were prepared as a suspension in 2% HPMC + 0.02% SLS in water. On day 2 (after 24 hr of the treatment with the compound), an additional group of 7 rats was added and animals administered a single dose of 20 mg/kg cyclophosphamide (the positive control). Four groups of satellite animals (3/group) were administered oral gavage doses of 0, 120, 240 and 500 mg/kg and included for the blood collection after 2 hr of the administration of the compound. After 24 hr of the dosing, the animals were killed and single femur per animal collected and processed for marrow pellet. The smears prepared, air dried and fixed and stained with acridine orange and erythrocytes (poly chromatic/normochromatic) and erythrocytes with mononuclear were calculated. The relative proportions of PCE and NCE in first 1000 cells/slide/animal determined. The 4000 PCE/animal were examined for micronuclei. The test was negative if it showed <2 times the response of the control value and it was positive if the response was 4 times the concurrent control animals. If the test give more than 1 to 2 above are further tested for statistical significance at $p<0.05$ using Dunnett's test.

Results:

No increase in MNPCE was seen in any of the bone marrow smears of the animals treated at the dose of 0, 120, 240 or 500 mg/kg. The increase in the number MNPCE was significantly higher in the animals treated with the positive control cyclophosphamide conforming validity of the assay. SB-497115-GR after 2 doses was not clastogenic in the test. Sponsor provided a summary table of the test and data of the individual animal was not given. The data given below is based on the summary result table of the sponsor (vol 3.14, pp 23 of the study).

Test article	Dose (mg/kg/day)	# Animals	Mean Proportion of PCE + SD	Mean % MPCE + SD
Vehicle	0	7M	59 + 7.8	0.04 + 0.05
SB-497115-GR	12	7M	65 + 9.7	0.04 + 0.02
SB-497115-GR	240	7M	54 + 5.9	0.05 + 0.04
SB-497115-GR	500	7M	44 + 9.8	0.03 + 0.02
Cyclophospham ide	20	7M	45 + 9.4	1.55 + 0.64

PCE = Polychromatic erythrocytes, MPCE = micronucleated polychromatic erythrocytes

SB-497115-GR (11 times the human clinical exposure based on C_{max}) was not clastogenic in vivo rat bone marrow micronuleus induction test.

6. Study title: Unscheduled DNA synthesis (UDS) in Rat Liver using in Vitro and in Vivo Procedure:

Reviewer: Chopra NDA No. 22-291

Key findings: SB-497115-GR showed no evidence of induction of unscheduled DNA synthesis (UDS) in the test.

Study no.: WD2003/01251/00; R23610/V23610
Conducting laboratory and location: [redacted] b(4)
Date of study initiation & completion: April 18, 2002 and May 7, 2004
GLP compliance: UK GLP compliance statement was attached
QA reports: yes (X) no ( )
Drug, lot #, and % purity: F033082 and 96.1% pure

Methods

SB-497115 dose formulations were prepared as a suspension in 2% HPMC + 0.02% SLS in water. The oral gavage doses of 0, 120, 240 and 500 mg/kg (volume = 10 ml/kg) in 4 groups (6 males/group) were administered twice in the time periods of 2 to 4 and 12 to 14 hr prior to the isolation of the hepatocytes. An additional group of 6 males was given a dose of 75 mg/kg acetamidofluorene (2-AAF) 12 to 14 hr prior to hepatocytes isolation. The bodyweight and the change in the clinical sign were measured. The hepatocytes from each of the treatment group animals and the positive control treatment group animals were separated and the solution diluted to 1.5 X 10^5 viable cells/ml. The adhered cells in the well plate radiolabel led with methyl-^{3}H thymidine (10 uCi/ml of WE-1) and fixed on a slide and developed with [redacted] The cell nuclei and cytoplasm with normal morphology were not scored. The net grain nucleus (NNG) and the number of cells corresponding to repairs and population average cytoplasm and nuclear grain count were estimated. The NNG values of 5 or greater and/or the nuclear grain count (N) is greater than 3 times the negative control values. If NNG value is less than 0, it is negative. The statistical significance at $p<0.05$ using Dunnett's test was estimated. b(4)

Results:

The net nuclear grain counts in 2-AAF was 27.2 and, these were -0.9, -0.3, -0.4 and -1.6 in the hepatocytes cultures of 0, 120, 240 or 500 mg/kg treatment groups male rats. The increase in the net nuclear grain counts in the positive control showed that the test validity. The mean% cells in repairs were 4.3, 6.5, 6.6 and 3.5 respectively in treatment group hepatocytes. Sponsor provided a summary table of the test and no data of the individual animal. The data is given below in the sponsor's table.

Test article	Dose (mg/kg/day)	# Animals	Mean NNG Counts	Mean % in Repairs
Vehicle	0	6M	-.0.9	4.3
SB-497115-GR	12	4M	-0.3	6.5
SB-497115-GR	240	4M	-0.4	6.6
SB-497115-GR	500	4M	-1.6	3.5
2Acetamidofluorene	75	74	27.2	93.5

SB-497115-GR showed no evidence of induction of unscheduled DNA synthesis (UDS) in the test.

In summary, Eltrombopag was not mutagenic or clastogenic in a bacterial mutation assay or in 2 in vivo assays in rats (micronucleus and unscheduled DNA synthesis, 11 times the human clinical exposure based on C_{max}). In the in vitro mouse lymphoma assay, eltrombopag was positive (<3-fold increase in mutation frequency).

2.6.6.5 CARCINOGENICITY STUDIES:

1. **MOUSE CARCINOGENICITY STUDY:**

STUDY DURATION: 2 Years (104 weeks male and female mice) b(4)

STUDY #: ——— /274-666/GSK#CD2006/00751/02

DATE OF INITIATION: November 15, 2004; **First Day of Dosing:** November 29, 2004

DATE OF COMPLETION: October 9, 2007; **Last day of Dosing:** November 29, 2006

MOUSE STRAIN: CD-1 mice ——— b(4)

ROUTE: Oral gavage

DOSING COMMENTS: All doses and concentrations (including analyte concentrations in plasma) were expressed in terms of SB 497115.

NUMBER OF ANIMALS: Control – 60/sex; Low Dose (25 mg/kg/day – 60/sex; Mid Dose (75 mg/kg/day) – 60/sex; High Dose 1 (150 mg/kg/day) – 60/sex; High Dose 2 (300 mg/kg/day) – 60/sex

BASIS OF DOSE SELECTION: Dose selection for this study was based on the recommendations of CAC-Ex (dated November 4, 2004). The dose of 300 mg/kg/day was lethal in 14-day toxicity study and based this, the Committee recommended 25, 75, and 150 mg/kg/day, respectively as low, mid and high doses.

DRUG LOT #, and % PURITY: Weeks 1-33 TPO-E-01C 99.9% - 07/31/2006;
Weeks 34-105 TPO-E-07 99.1% - 03/31/2008.

PRIOR FDA DOSE CONCURRENCE: Yes, CAC-Ex recommendations dated November 4, 2004. An additional 300 mg/kg/day treatment group was included by sponsor.

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The protocol for the mouse carcinogenicity study was reviewed earlier by Tamal Chakraborti (Pharmacologist). The Executive CAC Recommendations and Conclusions forwarded to sponsor were:

1. Based on the mortality at 300 mg/kg/day dose, the Committee recommended the low-, mid- and high-doses of 25, 75, and 150 mg/kg/day, respectively for the proposed 2-year oral carcinogenicity study in CD-1 mice.

2. The Committee also recommended that if the number of animals in a dosed group drops to 20/group, the sponsor should not terminate that group or the study but should contact the Division about how to proceed or how to modify the dosing.

MOUSE CARCINOGENICITY: Negative

1. **Study Title SB-497115-GR: 2-Year Oral Gavage Carcinogenicity Study in CD-1® Mice**

Key study findings: In a 2-year carcinogenicity study, CD-1 mice (60/sex/group) were administered 0, 25, 75, 150, or 300 mg/kg/day SB-497-115. Dosing for the 300 mg/kg/day groups was terminated on Week 2 due to mortality. The dose for the 150 mg/kg/day groups was reduced to 115 mg/kg/day on week 21 and dosing for the 150/115 mg/kg/day groups was terminated on week 44 due to mortality. Tubular vacuolar degeneration/necrosis was observed in 14 males of 150/115 mg/kg/day and 22 females of 150/115 mg/kg/day groups (sacrificed on Week 64). A statistically significant treatment-related decrease in survival was observed in the remaining female groups. However, survival in these groups was adequate for carcinogenicity assessment. There were no statistically significant positive trends or positive pairwise increases in tumor incidence observed in this study. Eltrombopag was not carcinogenic in mice at doses up to 75 mg/kg/day (exposures up to 4 times the human clinical exposure based on AUC, respectively).

Adequacy of the carcinogenicity study and appropriateness of the test model: A 2-year carcinogenicity bioassay in 2 species is required by the Agency for the assessment of carcinogenic potential of a compound. The sponsor submitted mouse carcinogenicity study with SB-497115 in the present submission. The study was done according to standard procedures with adequate number of animals. The dose selection of the study was based on Ex-CAC recommendation dated November 4, 2004. An additional 300 mg/kg/day treatment group was included by sponsor.

Evaluation of tumor findings: Statistical evaluation for the incidences of tumors was conducted by a statistical reviewer. There were no treatment related tumors or increase in tumor incidence reported.

Study no.: [redacted] #7274-666/GSK#CD2006/00751/02

Conducting laboratory and location: [redacted] b(4)

Date of study initiation & completion: November 15, 2004 & October 9, 2007
GLP compliance: A statement of compliance was submitted
QA report: yes (X) no ( )
Drug, lot #, and % purity: Weeks 1-33 TPO-E-01C 99.9% 77.8% 07/31/2006
Weeks 34-105 TPO-E-07 99.1% 77.7% 03/31/2008.
CAC concurrence: November 4, 2004

Methods:

Doses:

Basis of dose selection: Dose selection for this study was based on the Ex-CAC recommendation (dated November 4, 2004). Dose selection for this study was based on the recommendations of CAC-Ex (dated November 4, 2004). A dose of 300 mg/kg/day was lethal and based this, the Committee recommended 25, 75, and 150 mg/kg/day, respectively as low, mid and high doses. Sponsor included an additional 300 mg/kg/day treatment group.

Species/strain: CD-1 mice / b(4)

Number/sex/group (main study): Five groups of mice (60/sex) approximately 6 weeks old weighing about 21.6 to 34.4 g (males), and 20.0 to 28.2 g (females).

Route, formulation, volume: Oral gavage

Frequency of dosing: Daily

The design and dosage regimen are shown in the following table.

Group Number	Dose Level[1] (mg/kg/day)	Dose Concentration (mg/mL)	Number/Sex
Toxicology Animals			
1	0	0	60
2	25	2.5	60
3	75	7.5	60
4[3,4]	150/115	15/11.5	60
5[2]	300	30	60
Toxicokinetic Animals			
6	0	0	48
7	25	2.5	48
8	75	7.5	48
9[3,4]	150/115	15/11.5	48
10[2]	300	30	48

1. Doses are expressed in terms of the parent compound.
2. Groups 5 and 10 were last dosed on Day 15 for males and Day 14 for females and terminated on Day 17 for males and Day 16 for females.
3. Dose Levels for Groups 4 and 9 Females were decreased on Day 143 (Week 21)
4. Groups 4 and 9 mice were not dosed after 22 September 2005 (study Day 298 for males and study Day 297 for females).

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Observations:

Drug stability/homogeneity: The test article suspensions tested on Weeks 1, 4, 13, 26, 39, 52, 65, 67, 68 remix, 71, 78, 91, and 104. The homogeneity results of the samples were within ±5% of the average of the sample values.

Dual controls employed: No
Interim sacrifices: No
Deviations from original study protocol: The treatment of the animals of 300 mg/kg/day treatment group was stopped in week 2 due to mortality, and the remaining animals in the group were euthanized on week 8. The dose for the 150 mg/kg/day groups was reduced to 115 mg/kg/day on week 21 due to mortality. The survival rate for the 150/115 mg/kg/day treatment group was 40% on week 59. Treatment of this group was stopped on day 297 (males) and 298 (females) and the surviving animals were euthanized on week 64-65.

Observation times

Mortality: The animals were observed for mortality at the start and end of each working day. Those animals that died or were euthanized during the treatment period were necropsied. Only the thoracic and abdominal cavities of toxicokinetic animals that died or were euthanized during the treatment period were macroscopically examined.

Clinical Signs: Each animal was observed twice daily (a.m. and p.m.) throughout the study and findings recorded. Cage side observations were made for each carcinogenicity animal after dose administration. Detailed clinical examinations were performed at least once prior to treatment, once weekly, and on the day of scheduled euthanasia; abnormal findings were recorded. Each grossly visible or palpable mass was recorded weekly.

Body Weight Changes: The animals were weighed once prior to initiation of dosing week -1, on day 1 (prior to dosing), weekly for weeks 1 through 26, and every 4 weeks during the study duration. The animals of TK groups were also weighed in week 67 for blood collection.

Food Consumption: Individual food consumption was recorded weekly beginning week -1, on day 1 (prior to dosing), weekly for weeks 1 through 26, and every 4 weeks during the study duration.

Ophthalmoscopy: On study weeks 28, 42, 55, 68, 82, 94, and 104, all surviving mice (groups 1-4) were examined by an ophthalmologist by using a slit lamp and indirect ophthalmoscope. A mydriatic ([redacted] , was used into each eye on the first day week 42 for the males. b(4)

Clinical Pathology: The blood samples were collected for hematology parameters evaluation except from group 4 and 5 animals.

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Toxicokinetics: Blood samples collected from animals of groups 6 through 9 (groups 6 through 8 for week 67) at predose (group 8 male during week 67) and at 1, 2, 4, 8, and 24 hours postdose. The samples from control animals were collected and processed for plasma at about the same time. SB-497115 concentration was estimated using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis.

Histopathology:
Peer review: yes ( ), no (x)
At week 105, the surviving animals of main groups were anesthetized, sacrificed, weighed and necropsied. A necropsy was performed and all tissues were discarded without further examination. The animals were examined for external and internal abnormalities and the tissues listed below were taken from each animal and preserved in 10% neutral-buffered formalin. Eyes, optic nerves, Testes and epididymides were fixed in Davidson's, trimmed and processed.

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Covance 7274-666 GSK Reference No. G04280

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities (lesions)	X	Pancreas	X
Adrenals	X	Parathyroids	X
Animal identification site		Pituitary gland	X
Aorta (thoracic)	X	Preputial gland	X
Brain	X	Prostate	X
Cecum	X	Rectum [a]	
Cervix	X	Salivary gland	
Clitoral Gland	X	mandibular	X
Colon	X	sublingual	X
Duodenum	X	parotid	X
Epididymides [b]	X	Sciatic nerve	X
Esophagus	X	Seminal vesicles	X
Eyes [b]	X	Skeletal muscle (hind-limb)	X
Femur (Femoro-tibial joint)	X	Skin	X
Gallbladder	X	Spinal cord	
Harderian glands [b]	X	cervical	X
Heart	X	thoracic	X
Ileum	X	lumbar	X
Jejunum	X	Spleen	X
Kidneys	X	Sternum with bone marrow	X
Larynx	X	Stomach	X
Liver (2 lobes)	X	Testes [b]	X
Lung	X	Thymus	X
Lymph node -		Thyroids	X
mandibular	X	Tongue	X
mesenteric	X	Trachea	X
Mammary gland (inguinal)	X	Urinary bladder	X
Nasal cavities and Nasopharynx [a] with skull		Uterus	X
Optic Nerve [b]	X	Vagina	X
Ovaries	X		

a Collected and held for possible microscopic examination.
b Preserved in Davidson's fixative for all euthanized animals.
Note: The animal identification device was collected but not examined.

The tissues listed above from all animals in groups 1, 2, and 3 were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. The lesions were sectioned, stained with hematoxylin and eosin for microscopic examination. Unscheduled sacrifices and early deaths in the treated animals through day 219, week 32 had protocol specified tissues processed and examined for microscopic changes. The ophthalmoscopy was performed.

Analysis of Data: The Path/Tox System (supplied by [redacted]) was used for the direct online capture of in life toxicology and anatomic pathology data. Levene's test (for variance homogeneity) transformations were used to stabilize the variance. Comparison tests, one-way analysis of variance (ANOVA) was used (if applicable) to analyze clinical pathology, food consumption, and body weight data and if significant, Dunnett's t-test was used for pairwise

b(4)

comparisons between treated and control groups. Group comparisons were evaluated at the 5.0%, two-tailed probability level. Only data collected on or after the first day of treatment were analyzed statistically.

Results:

Clinical Observations: Reduced activity was observed in 9, 11, 11 and 14 males and 7, 10, 9 and 17 females, fewer excreta was in 0, 1, 2 and 6 males and, 1, 1, 3 and 5 females administered 0, 25, 75, and 150/115 mg/kg/day, respectively. Opacity in eyes was noted on week 8 in 3, 5, 5 and 29 males and 10, 7, 21 and 40 females administered 0, 25, 75 and 150/115 mg/kg/day, respectively. This was the primary test article-related clinical sign. The opaque eyes were seen on week 34 in control mice. Periorbital squinting was reported in 7, 7, 8 and 18 males and, 2, 7, 9 and 12 females administered 0, 25, 75 150/115 mg/kg/day, respectively.

Mortality: Five females and 5 males treated at 300 mg/kg/day dose died in early phase and sponsor euthanized the remaining males (on day 14) and females (day 17) of this group. The survival rate of animals of 150/115 mg/kg/day treatment group was 40% on week 63 and week 61 in males and females, respectively. The 150/115 mg/kg/day males and females were not treated from day 297 and 298, respectively and surviving mice were euthanized on week 64. The summary of survival rate is shown in the table below (data extracted from sponsor's table 2).

Table 2 Summary of Survival Data

GROUP AND DOSE LEVEL (MG/KG/DAY)	(MALE) WEEK:	0	2	3	4	11	14	18	20	26	34	48	63	82	104
1	#	60	60	60	60	60	60	60	60	60	60	54	44	40	22
0	%	100	100	100	100	100	100	100	100	100	92	75	68	37	
2	#	60	60	60	60	60	59	57	56	56	55	51	39	33	26
25	%	100	100	100	100	100	98	95	93	93	92	85	65	55	43
3	#	60	60	60	60	59	58	58	56	56	54	49	41	36	23
75	%	100	100	100	100	98	97	97	93	93	88	82	68	60	38
4	#	60	60	60	60	58	55	49	48	40	31	25	23	0	0
150	%	100	100	100	100	97	92	83	81	69	53	43	40	0	0
5	#	60	55	0	0	0	0	0	0	0	0	0	0	0	0
300b	%	100	100	0	0	0	0	0	0	0	0	0	0	0	0

Animals/sex/group = 60
a Group 4 mice were not dosed after 22 September 2005 (study Day 298 for males and study Day 297 for females);
b Groups 5 and 10 were last dosed on Day 15 for males and Day 14 for females and terminated

Table 2 Summary of Survival Data

Reviewer: Chopra NDA No. 22-291

GROUP AND DOSE LEVEL (MG/KG/DAY)		(FEMALE) WEEK: 0	2	3	4	11	14	18	20	26	34	48	63	82	104
1	#	60	60	60	60	60	60	60	59	58	58	57	55	50	35
0	%	100	100	100	100	100	100	100	98	97	97	95	92	83	58
2	#	60	60	59	59	57	57	56	56	56	55	53	51	42	22
25	%	100	100	98	98	95	95	93	93	93	92	88	85	70	37
3	#	60	60	60	60	60	60	60	60	60	60	56	45	33	49
75	%	100	100	100	100	100	100	100	100	100	100	93	75	60	38
4	#	60	59	56	55	49	45	44	41	40	35	26	24	0	0
150	%	100	98	93	92	82	75	73	68	67	58	43	40	0	0
5	#	60	55	0	0	0	0	0	0	0	0	0	0	0	0
300b	%	100	100	0	0	0	0	0	0	0	0	0	0	0	0

Animals/sex/group = 60
a Group 4 mice were not dosed after 22 September 2005 (study Day 298 for males and study Day 297 for females);
b Groups 5 and 10 were last dosed on Day 15 for males and Day 14 for females and terminated

The statistical reviewers of the studies concluded that there was no significant positive trend in mortality in males. There was a positive trend for mortality in the 25 and 75 mg/kg/day female treatment groups as show in the above table.

Body weights: The body weight gain in treated animals was similar to those of control group animals and w not affected during the study treatment period. The final body weights on study week 102 were 38.4, 37.9 and 38.4 g for males and, 33.5, 33.1 and 32.8 g of females treated with 0, 25 and 75 mg/kg/day, respectively. The changes in body weight of animals are shown in following graphs (for males and females) provided by the sponsor.

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Figure 3 **Mean Body Weights - Males**

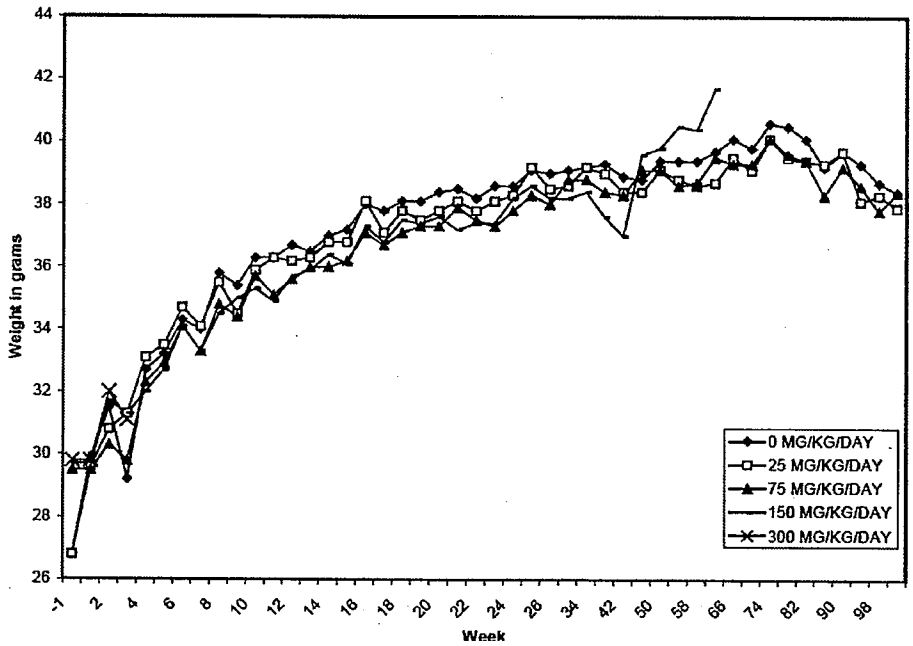
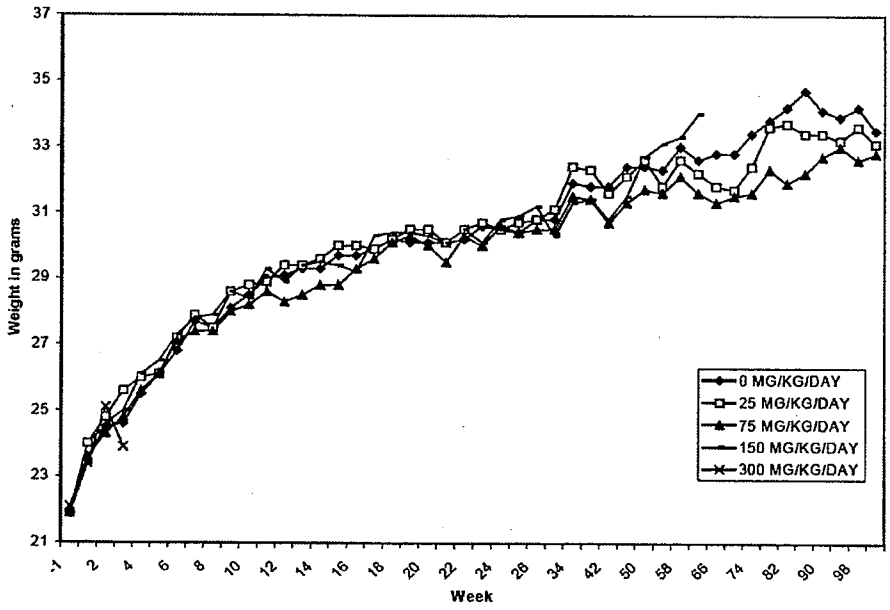


Figure 4 **Mean Body Weights - Females**



Food consumption: Food consumption was not affected during the study. On study week 1 and 102, the food consumption was 6.07 and 6.09 g/day, respectively in males and 5.43 and 5.8 g/day, respectively, in females.

Hematology: The hematology parameters were not affected during the study.

Ophthalmic examinations: Opaque eyes were noted in females given 75 mg/kg/day and in males of the 150 mg/kg/day treatment group by study week 8. Total bilateral diffuse cataract was detected in 14 males and 34 females of 150 mg/kg/day treatment group by indirect ophthalmoscopy. Additional incidences of cataract were also detected in 75 mg/kg/day groups by the slit lamp method. The incidences were 1 and 15 males and, 7 and 24 females administered 75 and 150/115 mg/kg/day, respectively.

Histopathology:

Non-neoplastic: An increased incidence of lenticular degeneration was observed in 75 mg/kg/day females and 150 mg/kg/day males and females. A dose related increase in renal degeneration/ necrosis was reported in animals that died during the study. The incidences were 0, 7 and 14 in males and 3, 4 and 13 in females administered 25, 75 and 150 mg/kg/day, respectively. Tubular vacuolar degeneration/necrosis was seen in 1, 1, and 14 males and 2, 3 and 22 females administered 25, 75 and 150 mg/kg/day, respectively. Among males, unilateral degeneration of seminiferous tubules was seen in 9, 8 and 16 males administered 0, 25 and 75 mg/kg/day, respectively.

Neoplastic: There were no treatment related tumors or an increase in tumor incidence in animals administered 0, 25, 75 and 150/115 mg/kg/day SK-497115, respectively.

Toxicokinetics:

On week 4, the peak plasma concentration of SB-497115-GR from a dose of 25 to 300 mg/kg/day was achieved 1 to 4 hr after dosing in both males and females. On week 67, systemic exposure (AUC) was similar to week 4 indicating that the drug was uniformly absorbed throughout the study and there was no accumulation after repeated dosing. The TK data is shown below (sponsor's table scanned below).

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Composite Toxicokinetic Parameters for SB-497115 from Male and Female Mice Following Oral Administration of SB-497115

Week	Sex	Dose-level mg/kg/day	$AUC_{0\text{-}24}$ (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)
4	Male	25	170316	18446	1.00
		75	685294	58586	4.00
		150	901337	99089	2.00
	Female	25	216832	26863	1.00
		75	641587	87511	1.00
		150	1264843	111500	4.00
67	Male	25	160682	16808	2.00
		75	578270	65546	2.00
	Female	25	241726	42913	1.00
		75	603163	75350	1.00

Note: Group 9 animals were suspended from dosing during Week 42 and sacrificed in Week 64. Therefore, no Week 67 samples were collected for this group.

MOUSE STUDY COMMENTS: Administration of SB-497115 up to a dose of 75 mg/kg/day (4x MHD based on AUC) was not associated with the induction of tumors. The mouse carcinogenicity study with eltromobag is adequate.

RAT CARCINOGENICITY STUDY:

STUDY DURATION: 2 Years (104 weeks male and female rats)
STUDY #: — #7274-638/GSKG04281/#CD2007/00923/00
DATE OF INITIATION: December 2, 2004; **First Day of Dosing**: December 9, 2004
DATE OF COMPLETION: September 28, 2007; **Last day of Dosing**: December 27, 2006
RAT STRAIN: SD rats — CD™(SD)IGS BR b(4)
ROUTE: Oral gavage
DOSING COMMENTS: All doses and concentrations (including analyte concentrations in plasma) were expressed in terms of the parent compound, SB-497115.

NUMBER OF ANIMALS: a. Main Study: Control – 60/sex; Low Dose (10 mg/kg/day) – 60/sex; Mid Dose (20 mg/kg/day) – 60/sex; High Dose (40 mg/kg/day) – 60/sex
b. TK Study groups: a. Main Study: Control – 6/sex; Low Dose (10 mg/kg/day) – 6/sex; Mid Dose (20 mg/kg/day) – 6/sex; High Dose (40 mg/kg/day) – 6/sex

BASIS OF DOSE SELECTION: Dose selection was based on a 14-day oral toxicity study and a 28-week oral toxicity study with SB-497115-GR in rats. SB-497115-GR was administered orally by gavage to male and female rats —CD™(SD)IGS BR)] at doses of 3, 10, or 40 mg/kg/day for 14 days. Reversible minimal midzonal hepatocellular vacuolation was observed at the 40 mg/kg/day dose level. In the 28-week chronic toxicity study, oral doses of 3, 10, 30 and 60 mg/kg/day SB-497115 were administered. The 60 mg/kg/day dose produced mortality and the sponsors proposed dose of 40 mg/kg/day was close to lethal dose of 60 mg/kg/day dose. Based on this, CAC-ex recommended a b(4)

dose of 30 mg/kg/day as the high dose for the carcinogenicity study and recommended low and mid doses were 5 and 10 mg/kg/day, respectively. Sponsor used the oral doses of 0, 10, 20 and 40 mg/kg/day SB-497-115 in rat carcinogenicity study.

PRIOR FDA DOSE CONCURRENCE: Yes. CAC-Ex recommendations dated November 4, 2004:

1. The Committee considers that the sponsors proposed high dose of 40 mg/kg/day for the 2-year rat carcinogenicity study is too high as this dose is close to the lethal dose of 60 mg/kg/day. Based on the mortality at 60 mg/kg/day, the Committee recommended 5, 15, and 30 mg/kg/day as low, mid and the high doses, respectively.

2. The Committee also recommended that the sponsor should contact the Division about how to proceed if the number of animals in a group drops to 20.

2. **Study Title SB-497115-GR: 2-Year Oral Gavage Carcinogenicity Study in SD-Rats**

Key study findings: In 2-year carcinogenicity study, SD rats (60/group) were administered orally 0, 10, 20 and 40 mg/kg/day SB-497-115. A dose-related increase in the incidence of non-neoplastic lenticular degeneration was seen in females. There were no drug-related tumors or increase in tumor incidence in study animals. Eltrombopag was not carcinogenic in mice at doses up to 40 mg/kg/day (exposures up to 5 times the human clinical exposure based on AUC).

Adequacy of the carcinogenicity study and appropriateness of the test model: A 2-year carcinogenicity bioassay in 2 species is required by the Agency for the assessment of carcinogenic potential of SB-497115. The number of animals/treatment group in the study was adequate and sponsor conducted histopathology evaluation of animals of the all of the study groups.

Study no.: —— #7274-638/GSKG04281/#CD2007/00923/00

Conducting laboratory and location: [redacted] b(4)

Date of study initiation & completion: December 4, 2004 & September 28, 2007
GLP compliance: A statement of compliance was submitted
QA report: yes (X) no ( )
Drug, lot #, and % purity: #TPO-E-02C (99.3%) —— , F076633 (99.0%); and F083255 (99.8%) b(4)
CAC concurrence: Yes
Methods:

Doses:

Basis of dose selection:

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In 14-day oral toxicity study (GlaxoSmithKline # CD2003/00327/00), oral gavage doses of 0, 3, 10, or 40 mg/kg/day SB-497115-GR were administered to 4 groups of male and female rats. Reversible hepatocellular vacuolation was seen in 40 mg/kg/day treatment group. In a 28-week oral toxicity study, (GlaxoSmithKline #CD2004/00332/01), the high dose of 60 mg/kg/day SB-497115 was lethal in 11 of 12 males and 4 of 12 females of the group. Regenerative anemia, increased liver weights (females only) and microscopic changes consisting of hepatocellular vacuolation, hypertrophy, degeneration and necrosis, adrenal vacuolation/ degeneration and pituitary vacuolation were observed in high dose group animals. CAC-Ex recommendations dated November 4, 2004 - The Committee considers that the sponsors proposed high dose of 40 mg/kg/day for the 2-year rat carcinogenicity study is too high as this dose is close to the lethal dose of 60 mg/kg/day. Based on the mortality at 60 mg/kg/day, the Committee recommended 5, 15, and 30 mg/kg/day as low, mid and the high doses, respectively. Sponsor employed 10, 20 and 40 mg/kg/day as high, mid and low doses in the study.

Species/strain: — CD™(SD)IGS BR)] rats b(4)
Number/sex/group (main study): Four groups of rats (60/sex/group) approximately 6 weeks old with mean weight of 194 to 197 g (males), and 169 to 172 g (females).
Route, formulation, volume: Oral gavage, 10 ml/kg
Frequency of dosing: Daily

The design and dosage regimen are shown in the following table.

Group Number	Dose[a,b] (mg/kg/day)	Dose Concentration[a,b] (mg/mL)	Number/Sex
Toxicology Animals			
1[c]	0	0	60
2	10	1	60
3	20	2	60
4	40	4	60
Toxicokinetic Animals			
5[c]	0	0	6
6	10	1	6
7	20	2	6
8	40	4	6

a. The dose volume was 10 mL/kg.
b. Dose concentrations were adjusted for salt form and purity using a correction factor specific to each batch of test article.
c. Animals in the control groups received the control article (2% aqueous HPMC with 0.2% SLS in RO water) only.

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Observations:
Drug stability/homogeneity: The concentration and homogeneity analysis of the test article formulations and vehicle formulation were verified prior to the initiation of dosing for the preparations used during Weeks 1, 4, 11, 12, 26, 39, 44, 51, 61 (Groups 2 and 4 only), 66, 78, 91 and 104. The analysis results were within the acceptable range of ±10% of nominal and homogeneity results were within ±5% of the average values.

Dual controls employed: No

Interim sacrifices: No
Deviations from original study protocol: None

Observation times

Mortality: Each of the study animals was observed twice daily (a.m. and p.m.) throughout the study period for mortality and morbid signs. Cage side observations for abnormal findings were made for each animal. Detailed clinical examinations for each carcinogenicity animal were performed at least once prior to treatment, once weekly, and on the day of scheduled euthanasia; abnormal findings or an indication of normal was recorded. Gross, visible or palpable mass was recorded weekly for the location, time of onset, size, appearance and progression. The thoracic and abdominal cavities of animals in the toxicokinetic groups that died or were euthanized during the treatment period were macroscopically examined.

Clinical Signs: Each of the study animals was observed twice daily (a.m. and p.m.) throughout the study. Cage side observations were made for each carcinogenicity animal after dose administration. Detailed clinical examinations were performed at least once prior to treatment, once weekly, and on the day of scheduled euthanasia; abnormal findings were recorded.

Body Weight Changes: All animals were weighed at least once prior to treatment for randomization, on Day -1 (prior to dosing), weekly from weeks 1 through 16, and every 4 weeks thereafter. In addition, body weights were also collected at week 46.

Food Consumption:
The daily food consumption of carcinogenicity animals was assessed for 4 days during the week prior to initiation of dosing, for weeks 1 through 16, and daily for one week out of each month thereafter. If no pellets remained, food consumption was considered normal.

Ophthalmoscopy: The eyes of carcinogenicity animals were examined by a board-certified veterinary ophthalmologist using an indirect ophthalmoscope and a slit lamp on Days 186, 267, 361, 453, 550, 634, and 701 (weeks 27, 39, 52, 65, 79, 91, and 101, respectively).

Hematology: Blood samples from the animals of main study group were collected for hematology parameters into the tubes containing potassium EDTA and, total red blood (erythrocyte) and white blood cell count, platelet count, hemoglobin concentration differential leukocyte count, hematocrit, total leukocyte count, mean corpuscular volume red blood cell distribution width, mean corpuscular hemoglobin, reticulocyte count, mean corpuscular hemoglobin concentration, blood smear for cell morphology (examined in the study if suggestive of any change).

Clinical Pathology & Clinical Chemistry: The blood samples from main study groups were collected from jugular vein at euthanasia.

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Toxicokinetics: In week 50, blood samples collected from overnight fasted animals of groups 5 through 9. The samples from control animals were collected and processed for plasma collection at about the same time.

Histopathology:
Peer review: yes ( ), no (x)
At week 104 or 105, the surviving animals of main groups (group 1 to 4) were anesthetized, sacrificed, and weighed. A necropsy was performed in week 50 for TK animals and the animals were discarded without further examination. The main study group animals were examined for external and internal abnormalities and the tissues listed below were taken from each animal and preserved in 10% neutral-buffered formalin.

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Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities (lesions)	X	Pituitary	X
Adrenals	X	Preputial/clitoral gland	X
Animal identification device		Prostate	X
Aorta (thoracic)	X	Rectum	
Brain	X	Salivary gland	
Cecum	X	mandibular	X
Colon	X	sublingual	X
Duodenum	X	parotid	X
Epididymides	X	Sciatic nerve	X
Esophagus	X	Seminal vesicles	X
Eyes/Optic Nerves	X	Skeletal muscle (hind-limb)	X
Femur (Femoro-tibial joint)	X	Skin	X
Harderian glands	X	Spinal cord	
Heart	X	cervical	X
Ileum	X	thoracic	X
Jejunum	X	lumbar	X
Kidneys	X	Spleen	X
Larynx	X	Sternum with bone marrow	X
Liver (2 lobes)	X	Stomach	X
Lung	X	Testes	X
Lymph node -		Thymus	X
mandibular	X	Thyroid	X
mesenteric	X	Tongue	X
Mammary gland (inguinal)	X	Trachea	X
Nasopharynx and nasal turbinates		Urinary bladder	X
Ovaries	X	Uterus with cervix	X
Pancreas	X	Vagina	X
Parathyroids	X		

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In addition, eyes, optic nerve, testis and epididymides were fixed in Modified Davidson's solution. The following tissues of the dead animals mentioned below were also examined: Group 2 Males B87393 and B87405, Group 3 Male B87475, and Group 4 Male B87484, Eyes and optic nerve: Group

2 Female B87677, Group 3 Female B87729, and Group 4 Female B87752, testis, epididymides, eyes, optic nerves, and Harderian glands: Group 2 Male B87363 and Group 4 Female B87787 - Eyes, optic nerves, and Harderian glands: Group 3 Female B87745, Group 4 Females B87772, B87787, and B87806, Testis, epididymides, eyes, optic nerves, and Harderian glands: Group 1 Males B87304 and B87314, Group 2 Males B87378 and B87387, Group 3 Males B87462 and B87471 were also examined.

Analysis of Data: The Path/Tox System (supplied by ——————— was used for the direct online capture of in life toxicology and anatomic pathology data. Levene's test (for variance homogeneity), transformations were used to stabilize the variance. Comparison tests, one-way analysis of variance (ANOVA) was used (if applicable) to analyze clinical pathology, food consumption, and body weight data and if significant, Dunnett's t-test was used for pairwise comparisons between treated and control groups. Statistical Analysis Software (SAS) was used to perform statistical analysis group comparisons were evaluated at the 5.0%, two-tailed probability level. b(4)

Results:

Clinical Observations: Convulsions were reported in 2, 5, 4 and 9 out of 60 males and, 2, 2, 1 and 5 females out of 60 females administered 0, 10, 20 and 40 mg/kg/day, respectively. At week 36, opaque eye(s) were seen in 0, 1, 0 and 9 males administered 0, 10, 20 and 40 mg/kg/day, respectively.

Mortality: Mortality during the study consisted of 174 rats (71 males and 103 females), and survival to terminal euthanasia was comparable in control and SB-497115-treated groups at week 104 (78, 68, 78, and 73% for males 59, 56, 58 and 64% for females given 0, 10, 20 and 40 mg/kg/day, respectively). Thirteen of the early deaths were attributed to gavage error. There were no test article-related deaths identified during the study.

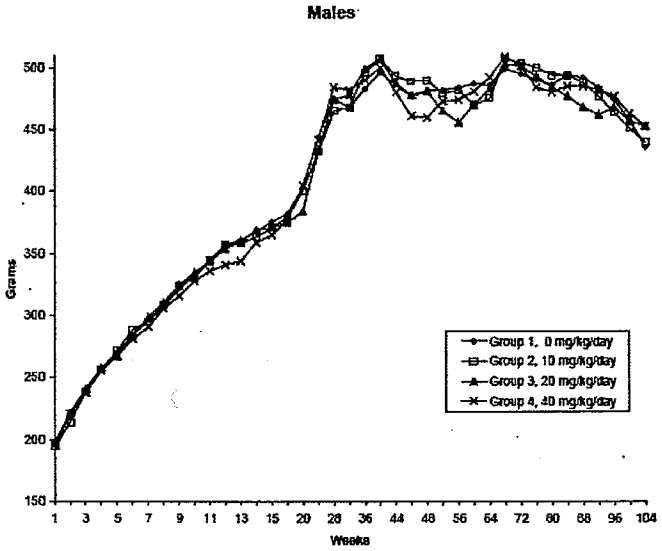
Text Table 1
Survival Data

	Males				Female			
Dose Level (mg/kg/day)	0	10	20	40	0	10	20	40
Unscheduled Deaths								
Weeks 1-52	0	6	3	3	0	1	5	3
Weeks 53-78	3	10	2	3	5	7	7	6
Weeks 79-106	10	9	11	11	21	19	15	14
Total Unscheduled Deaths	13	25	16	17	26	27	27	23
Terminal Necropsy	47	35	44	43	34	33	33	37
Total	60	60	60	60	60	60	60	60

Body weights: At week 104, the mean final body weight of rats administered 10, 20 and 40 mg/kg/day was increased in a dose related manner by 102.6, 106.7 and 107.1% of control, respectively, in males and by 121.6, 144.6 and 135.1% of control, respectively, in females. The body weight of study group

animals was significantly ($p>0.5 - 0.05$) higher in all study groups than the mean body weight of control group animals. The initial (study week 1) and final (study week 104) body weights were 196 and 436 g, respectively, for control group males and 172 and 246 g, respectively, for control group females.

Figure 2 Mean Body Weight Data



Food Consumption: The daily food consumption was increased slightly by 4, 11 and 11% in males administered 10, 20 and 40 mg/kg/day, respectively. Food consumption was not affected in study females.

Hematology: In males, the % reticulocyte was increased from 2.4 to 3.9% in 40 mg/kg/day treatment group. The absolute reticulocytes counts were increased but not of statistical significance (189.5 $\pm$110.6 in control to 254.3$\pm$177.6 in 40 mg/kg/day group). The absolute platelets counts were increased slightly in a dose-related manner (894, 1016, 1067 and 1171X10^3/ul for 0, 10, 20 and 40 mg/kg/day SB497115, respectively, $P<0.05$). These parameters were not affected in study females. All other hematology parameters changes during the study are shown in the following sponsor's table 7 (p 126 and 127).

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TABLE 7
SUMMARY OF CLINICAL HEMATOLOGY DATA

		TEST ARTICLE	CONTROL	SB-497115-GR		
		GROUP	1	2	3	4
		LEVEL (mg/kg/day)	0	10	20	40
GROUP		MCHC G/DL Week 105	PLATELET TH/UL Week 105	RDW % Week 105	WBC TH/UL Week 105	NEUT AB TH/UL Week 105	LYMPHO AB TH/UL Week 105	LDC AB TH/UL Week 105
1M	Mean	36.4	894	17.4	7.4	3.8	3.1	0.0
	SD	1.30	243.5	1.11	2.29	1.75	0.94	0.03
	N	46	46	46	46	46	46	46
2M	Mean	36.5	1016	17.2	6.6	3.4	2.8	0.0
	SD	1.03	270.5	1.05	2.31	1.77	0.65	0.04
	N	35	35	35	35	35	35	35
3M	Mean	36.6	1067*	17.8	7.9	4.2	3.2	0.0
	SD	1.14	283.5	1.06	2.44	2.07	0.92	0.03
	N	44	44	44	44	44	44	44
4M	Mean	36.3	1171*	18.9*	8.4	4.7	3.3	0.0
	SD	1.16	321.7	1.98	2.92	2.71	0.72	0.03
	N	42	42	42	42	42	42	42
Statistics		P	P	P	P	P	P	X

* = P < or =0.05
P = ANOVA (and Dunnett's, if applicable)
X = not analyzed

Ophthalmic examinations: A drug-related increased incidence of cataract formation was reported. The incidences of cataract by indirect and slit-lamp method are shown below in the sponsor's table (Table 4; pp 80 Document CD2007/00923/00):

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Table 4 Summary of Ophthalmic Observations

INDIRECT

WEEKS 27-101

NUMBER OF ANIMALS AFFECTED

CATEGORY / KEYWORD / QUALIFIER	SEX:	MALE				FEMALE			
	GROUP:	1	2	3	4	1	2	3	4
	DOSE:	0	10	20	40	0	10	20	40
	NUMBER:	60	54	58	59	60	59	56	59
*** TOP OF LIST ***									
LENS									
POSTERIOR SUBCAPSULAR CATA									
BILATERAL-DIFFUSE		0	0	1	3	0	0	0	0
UNILATERAL LEFT-DIFFUSE		0	0	0	0	0	0	0	2
TOTAL CATARACT									
BILATERAL-DIFFUSE		0	0	0	7	0	0	0	0
UNILATERAL LEFT-DIFFUSE		0	0	0	2	0	0	0	0
POSTERIOR CAPSULAR CATARACT									
UNILATERAL LEFT-FOCAL		0	0	1	0	0	0	0	0
BILATERAL-DIFFUSE		0	1	1	7	0	1	0	2
UNILATERAL RIGHT-DIFFUSE		0	0	0	3	0	0	0	0
UNILATERAL LEFT-DIFFUSE		0	0	0	3	0	0	0	1
PALPEBRAE/CONJUNCTIVA									
CHROMODACRYORRHEA									
BILATERAL-DIFFUSE		0	0	0	0	0	1	0	1
UNILATERAL RIGHT-DIFFUSE		0	2	1	0	0	0	1	1
UNILATERAL LEFT-DIFFUSE		0	1	1	1	0	0	0	2
DISCHARGE									
UNILATERAL LEFT-DIFFUSE		0	1	0	0	0	0	0	0
LACRIMATION/EPIPHORA									
UNILATERAL RIGHT-DIFFUSE		0	0	1	0	0	0	0	0
UNILATERAL LEFT-DIFFUSE		1	0	1	1	0	1	0	0
FUNDUS									
ATROPHY									
UNILATERAL RIGHT-DIFFUSE		0	0	0	0	0	1	0	0
HEMORRHAGE(S)									
BILATERAL-FOCAL		0	0	0	1	0	0	0	0
UNILATERAL LEFT-DIFFUSE		0	1	0	0	0	0	0	0
FUNDUS									
RETINOCHOROIDITIS									
UNILATERAL RIGHT-DIFFUSE		0	0	0	1	0	0	0	0
GLOBE									
PHTHISIS BULBI									
UNILATERAL LEFT-FOCAL		1	0	0	0	0	0	0	0
PANOPHTHALMITIS									
UNILATERAL LEFT-FOCAL		0	1	0	0	0	0	0	0
EYE EXAM									
NO VISIBLE LESIONS		60	54	58	59	60	59	56	59
*** END OF LIST ***									

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TABLE 4
SUMMARY OF OPHTHALMIC OBSERVATIONS

SLITLAMP

WEEKS 27-101	NUMBER OF ANIMALS AFFECTED							
SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
CATEGORY / KEYWORD / QUALIFIER — DOSE:	0	10	20	40	0	10	20	40
NUMBER:	60	54	58	59	60	59	56	59
*** TOP OF LIST ***								
LENS								
POSTERIOR SUBCAPSULAR CATA								
UNILATERAL LEFT-FOCAL	0	1	0	0	1	0	0	0
BILATERAL-DIFFUSE	1	0	2	10	0	0	0	4
UNILATERAL RIGHT-DIFFUSE	0	0	0	1	0	0	0	0
UNILATERAL LEFT-DIFFUSE	0	0	0	0	0	0	0	3
BILATERAL-CENTRAL	1	0	0	0	0	0	0	0
TOTAL CATARACT								
BILATERAL-DIFFUSE	0	0	0	7	0	0	0	0
UNILATERAL LEFT-DIFFUSE	0	0	0	1	0	0	0	1
POSTERIOR CAPSULAR CATARACT								
UNILATERAL LEFT-FOCAL	0	0	1	0	0	0	0	0
BILATERAL-DIFFUSE	0	1	3	10	0	1	0	4
UNILATERAL RIGHT-DIFFUSE	1	0	0	2	0	0	0	1
UNILATERAL LEFT-DIFFUSE	0	1	0	2	0	1	0	1
PALPEBRAE/CONJUNCTIVA								
CHROMODACRYORRHEA								
BILATERAL-DIFFUSE	0	0	0	0	0	1	0	1
UNILATERAL RIGHT-DIFFUSE	0	2	1	0	0	0	1	1
UNILATERAL LEFT-DIFFUSE	0	1	1	1	0	0	0	1
DISCHARGE								
UNILATERAL LEFT-DIFFUSE	0	1	0	0	0	0	0	0
LACRIMATION/EPIPHORA								
UNILATERAL RIGHT-DIFFUSE	0	0	1	0	0	0	0	0
UNILATERAL LEFT-DIFFUSE	1	0	1	1	0	1	0	0
GLOBE								
PHTHISIS BULBI								
UNILATERAL LEFT-FOCAL	1	0	0	0	0	0	0	0

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Gross pathology: The incidences of opaque eyes in the treated males were 6 of 42 males and none in females of 40 mg/kg/day group. The number of neoplastic and non-neoplastic masses produced in treatment groups was similar to controls. The incidence of enlargement of the pituitary gland was decreased in treatment groups and were 4, 2, 2 and 1 in males and, 18, 8, 10 and 7 in females administered 0, 10, 20, and 40 mg/kg/day SB497115, respectively. There were no other dose/treatment related effects observed. There was an indication that the masses produced during the study were lesser and of smaller in size in treated animals compared to the control group animals. The incidence of swollen skin was increased in high dose males.

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Text Table 2
Frequent Causes of Death

	Males				Female			
Dose Level (mg/kg/day)	**0**	**10**	**20**	**40**	**0**	**10**	**20**	**40**
Neoplasms								
Pituitary	4	3	3	2	15	7	6	4
Mammary	0	0	0	0	8	4	6	5
Histiocytic Sarcoma	1	0	2	2	1	1	0	2
Lymphoma/Leukemia	0	1	0	1	0	1	2	0
Fibroma/Fibrosarcoma	0	3	1	1	0	0	1	1
Non-Neoplastic Processes								
Pododermatitis	2	2	0	1	0	1	1	0
Gavage-Related Injury	0	4	2	1	1	1	3	1

Histopathology:

Non-neoplastic: The increased incidences of bilateral lens degeneration were in 1/47, 1/35, 3/42 and 16/43 males and, 0/33, 0/33, 1/33 and 3/37 females belonging to 0, 10, 20 and 40 mg/kg/day groups. The changes in males of 40 mg/kg/day treatment group were increased in comparison to control. An equivocal increase in severity of lens degeneration was noted in females treated at 40 mg/kg/day.

Neoplastic: There were no drug-related tumors or increase in tumor incidence observed in this study. A significant ($p<0.5$ and not at 0.05) reduction in pancreatic islet cell adenoma was observed in males only at 20 & 40 mg/kg/day doses. The incidences were 5/47, 3/35, 0/44 and 1/43 among males at 0, 10, 20 & 40 mg/kg/day, respectively. This effect was not considered treatment-related.

Toxicokinetics:
The sensitivity of assay was limited to 10.0 ng/ml SB-497115. Peak plasma drug levels were achieved between 1.00 and 4.00 hours of the dosing and the systemic exposure (AUC) in both sexes was similar. The plasma peak concentration was dose proportional as a 4-fold increase concentration was seen in animals of 10 and 40 mg/kg/day treatment groups. On week 26, the mean steady state systemic exposure (AUC values) in animals treated with 40 mg/kg/day were 6.1-, 6.2-, and 3.6-fold in males (mean Cmax values were 5.6-, 4.8- and 1.3-fold) and 4.8, 5.6 and 7.0 fold in females (mean Cmax values were 3.4-, 4.2- and 3.9-fold) during weeks 4, 11, and 26, respectively.

A summary table of toxicokinetic values is provided below:

Dose (mg/kg/day)	Male (n=6)					
	Mean C_{max} (µg/mL)			Mean $AUC_{(0-t)}$ [b] (µg.h/mL)		
	Week 4	Week 11	Week 26	Week 4	Week 11	Week 26
10	11.0	16.4	25.2[a]	90.8	90.1	161[a]
20	39.4	48.1	44.6	251	302	307
40	61.3	78.1	63.4	555	560	581
Dose (mg/kg/day)	Female (n=6)					
	Mean C_{max} (µg/mL)			Mean $AUC_{(0-t)}$ [b] (µg.h/mL)		
	Week 4	Week 11	Week 26	Week 4	Week 11	Week 26
10	15.2	21.8	22.9[a]	84.7	102	111[a]
20	26.0	46.8	52.7	179	274	335
40	52.0	91.0	90.2	405	571	773

a N is 4 for males and 5 for females in Week 26.
b AUC calculations for Week 4 include blood samples collected at 0(predose), 1, 2, 4, 8, and 24 hours after dosing whereas AUC calculations for Weeks 11 and 26 include blood samples collected at 0 (predose), 1, 2, 4, 8, 12 and 24 hours after dosing.

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A dose proportional plasma concentration of SB-497115 was achieved in carcinogenicity study of rats treated at oral gavage doses of 0, 10, 20 and 40 mg/kg/day.

The survival among treatment and control group animals was similar. The statistical reviewers (Steven F. Thomson and Karl K. Lin, Ph.D., Division of Biometrics 6 dated June 3, 2008) in their study data analysis concluded that there was no statistically significant positive trend or positive pairwise increase in lethality among all treatment groups of the study. SB-497115 did not induce tumor of any class, benign or malignant in study rats.

RAT STUDY COMMENTS: There were no drug-related tumors or increase in tumor incidences during the study at doses up to 40 mg/kg/day (5x MHD based on AUC). The rat carcinogenicity study with eltrombopag was adequate.

2.6.6. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

A. Study title: SB-497115-GR: Oral female fertility and early embryonic development study in rats.

Key findings: Eltrombopag did not affect female fertility or early embryonic development in rats at doses up to 20 mg/kg/day (2 times the human clinical exposure based on AUC). However, at a maternally toxic dose of 60 mg/kg/day (7 times the human clinical exposure based on AUC) in rats, eltrombopag treatment was associated with increased pre- and post-implantation loss (leading to a 27% decrease in live litter size) and reduced fetal body weight.

Reviewer: Chopra NDA No. 22-291

Study no.: CD2003/00612/00
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA
Date of study initiation: March 18, 2003
GLP compliance: A statement of compliance was attached.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: F033082 and 99.3% pure

Methods

Doses: 10, 20 and 60 mg/kg/day b(4)
Species/strain: Sprague-Dawley rats – CD(SD)IGSBR
Number/sex/group: 25 female/group
Route, formulation, volume, and infusion rate: Oral gavage in 2% hydroxypropyl methylcellulose containing 0.2% sodium lauryl sulfate.
Satellite groups used for toxicokinetics: Not done
Study design: The 4 groups of female rat were treated at the doses of 0, 10, 20 and 60 mg/kg/day (vol = 10 ml/kg) 2 weeks before mating. On day 15, these were cohabited with untreated males for up to 7 days and tested for the presence of sperm in vaginal lavage and it was considered as day 0 postcoitum. The treatment continued through the cohabition period and up to day 6 postcoitum. The females that did not mate after 7 days were paired with another male for additional 7 days. Males were discarded after mating. The animals sacrificed on pc day 20.
Parameters and endpoints evaluated: Clinical observations were performed on pretreatment day 1 (females) and males during cohabitation period.
Body weight: on each pretreatment day 6 and 17, each dosing day and on day 7, 10, 14, 17 and 20 post corium. Food consumption for females at the interval of day 1 to 8 and 8 to 15 of the treatment period and on day 0 to 7, 7 to 14 and 14 to 20 postmortem
Estrus cycle: Vaginal cytology during day 6 through 19 of pretreatment period, during days 1 to 15 of the treatment period and daily until mated.
Mortality and dead deliveries: Two control females (R03F7704 – delivered 1 litter and R03F7775) died because of moribund conditions.
Fetal Observations: Embryo-fetal survival, fetal weight and gravid uterine weight recorded at the time of necropsy on day 20, # of corpora lutea, # live and dead fetuses, fetal weight, # implantation sites, # resorption and their relative position recorded. Gross examination of placenta from live and dead fetuses performed. Each live and dead fetus was examined externally. The uterus and ovaries of each of the F0 females and malformed fetuses were retained in phosphate buffer.

Results

Mortality: None of the females included in the treatment groups died.

Clinical signs: One female of 20 mg/kg/day treatment group showed low posture labored breathing.

Body weight: The retardation in the percent body weight gain during treatment and gestation time was 17.1% and during gestation period the retardation in percent body weight gain was 15.8%. The initial and final body weight of control females was 259 and 423 g.
Food consumption: The food consumption of the 60 mg/kg/day treatment group females was significantly less than the control and other groups of females, 116 g/day Vs 127 g/day in control.

Toxicokinetics: Not done

Necropsy: No notable necropsy observations were noted in dams.

Fetal Data:

The number of matings, fertility index and number of corpora lutea were similar in treated and control group females. The number of implantations and live litter size in the animals of 60 mg/kg/day treatment group were statistical significant lower than control and other treatment groups. There were increased number of early resorptions and reduced average number of fetuses in 60 mg/kg/day treatment group than the control group. At this dose, the compound was maternal and fetal toxic in rats.

Table for the Summary of Reproductive Indices in Female Rats Examined at Mid Gestation (Reproduced from Sponsor's Submission)

Group	Vehicle	SB-97115 Dose (mg/kg) 10	20	60
Variable				
# Animals	25	25	25	25
Mean Estrous Cycle/15 days	3.5 + 0.1	3.4 + 0.1	3.5 + 0.1	3.6 + 0.1
Days of Mating	2.4 + 0.3	1.9 + 0.1	1.8 + 0.2	2.0 + 0.3
% Mating Index (#)	96 (24)	100.0 (25)	100.0 (25)	100.0 (25)
# Fertility Index	91.7 (22/24)	100.0 (25/25)	100.0 (25)	88.0 (22)
# Corpora lutea	15.5 + 0.5	15.9 + 0.3	16.1 + 0.6	15.0 + 0.6
# Implantations	14.4 + 0.5	15.2 + 0.3	14.7 + 0.5	12.1 + 0.5*
Pre-Implatation Loss	7.2 + 2.4	4.6 + 1.2	7.9 + 2.7	16.4 + 3.9
Early Resorp.	0.7 + 0.2	0.6 + 0.1	1.5 + 0.3	2.1 + 0.5*
Late Resorption	0.1 + 0.1	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0
Live litter size	13.7 + 0.6	14.6 + 0.4	13.2 + 0.4	10.0 + 0.5*
# Total resorptions	15.2 + 1.7	13.9 + 3.4	14.8 + 1.1	12.1 + 4.1
Live Males % index	56.8 + 2.9	53.0 + 2.7	50.5 + 2.6	51.3 + 3.0
Gravid Uterine Weight (g)	77.6 + 3.0	82.0 + 2.1	76.2 + 2.4	53.7 + 3.0
Fetal Data:				
# (Fetuses/Litter)	287/21	364/25	331/25	219/22
Male	3.74 + 0.05	3.68 + 0.04	3.73 + 0.06	3.00 + 0.07
Female	3.54 + 0.04	3.50 + 0.04	3.53 + 0.06	2.78 + 0.07
Fetal Observations:				
Enlarged Placenta	0	0	0	1/1
External Mouth/Jaw (Cleft	0	0	1/1	0

lip)				
External eyelid not evident	0	0	1/1	0
Fused Placenta	0	0	0	2/1
Enlarged Placenta	0	0	0	1/1

* Significantly different from control values $P < 0.05$
IMPLANTATION INDEX = (IMPLANTATIONS/CORPORA LUTEA) X 100; RESORPTION INDEX = (TOTAL RESORPTION/IMPLANTATIONS) X 100

In summary, Eltrombopag did not affect female fertility or early embryonic development in rats at doses up to 20 mg/kg/day (2 times the human clinical exposure based on AUC). However, at a maternally toxic dose of 60 mg/kg/day (7 times the human clinical exposure based on AUC) in rats, eltrombopag treatment was associated with embryolethality (increased pre- and post-implantation loss resulting in a 27% decrease in live litter size) and reduced fetal body weight in the female fertility study.

B. Study title: SB-497115-GR: Oral male fertility and early embryonic development study in rats.

Key findings: SB-497115 (10, 20 & 40 mg/kg/day) when administered in male rats for 14 days before mating with untreated females until mating confirmed (42 to 46 doses) produced decrease in body weight gain and slightly reduced food consumption in 40 mg/kg/day group during the first 2 weeks of the treatment period. Testicular weights in 40 mg/kg/day group was increased but there were no effects on mating and fertility of treated males, nor any effects on survival, growth or external morphology of the fetuses sired by the treated males. . The NOAEL for male fertility study was 40 mg/kg/day (5 times MHD based on AUC).

Study no.: CD2005/00236/00

Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.

Date of study initiation and completion: August 29, 2005 and October 3, 2005
GLP compliance: A statement of compliance was attached.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: F074359007 and 76.8% base

Methods

Doses: 10, 20 and 40 mg/kg/day (vol = 10 ml/kg) once daily beginning on Day 1 of the study and continuing until termination (42 to 46 doses).

Species/strain: Sprague-Dawley rats — CD™(SD)IGS BR [redacted] b(4)

Number/sex/group: 25 male/group

Route, formulation, volume, and infusion rate: Oral gavage in 2% hydroxypropyl methylcellulose containing 0.2% sodium lauryl sulfate.

Satellite groups used for toxicokinetics: Not done

Study design: Selected males and females were assigned to 4 treatment groups.

Group	Dose[a] (mg/kg/day)	Number of Males
1	0 (vehicle)	25
2	10	25
3	20	25
4	40	25

a. Dose is expressed as the SB-497115 active moiety.

Starting on treatment day 15, males were cohabited 1:1 with untreated females for up to 7 days. The mating was confirmed in a vaginal lavage. Mated females were separated from the males (post coitum day 0) and males that had not mated after 7 days of cohabitation were paired with a second female until mating was confirmed. Treatment of the males was continued through the cohabitation period (day 43 to 47). Mated females and their litters were euthanized on day 20 pc.

Viability checks and clinical observations: performed daily during the treatment period (males) and during the cohabitation and postcoitum period (females). Detailed of weights and the intrauterine position recorded. Gross examination of live and dead fetuses was done. The placenta of each of the live and dead fetus was examined for gross changes.

Parameters and endpoints evaluated: Clinical observations were performed on postcoitum day 6, 7, 18 and 21.

No unscheduled deaths occurred during the study. Females reaching the end of the cohabitation period without evidence of mating were continued on study until they delivered a litter or until 20 days after the end of their cohabitation when they were euthanized by carbon dioxide asphyxiation and had a gross examination of the cervix and vagina. Those females reaching 20 days after the end of their cohabitation were examined to the extent possible according to procedures described below for cesarean sections.

Embryo-Fetal Survival, Fetal Weight and Uterine Weight

Mated females were examined, euthanized on Day 20 pc and their cervix and vagina examined. The ovaries were removed and the corpora lutea were counted. Each gravid uterus with at least 1 live fetus was weighed and its contents examined. Implantation sites (stained with ammonium sulfide if apparently not pregnant), resorptions and live and dead fetuses were counted and their relative positions were recorded. Placenta from each of live and dead fetuses examined and each live fetus was weighed individually and euthanized by an overdose of sodium pentobarbital given orally.

External Fetal Examinations

Each live and dead fetus was examined externally. Fetal sex was determined by external examination. Malformations are those fetal observations judged to potentially affect survival, growth, development, functional competence or external appearance.

Male Termination and Necropsy: On Day 43 to 47, males were euthanized by carbon dioxide asphyxiation and were necropsied.
Organ Weights Testis (left intact, right parenchyma), epididymides and corpus together (separate for right cauda), ventral prostate, and 1 seminal vesicle from each male were weighed. The left testis and epididymides (except for right cauda) were fixed in Bouin's for approximately 24 hours and then transferred to 70% ethanol. The right testis parenchyma and right cauda epididymis were placed immediately on ice, and were then frozen at approximately -20°C.

Results

Mortality: None of the males included in the treatment groups died.

Clinical signs: No treatment related effects were seen.

Body Weight
A decrease of 12% in mean body weight gain was noted from Days 1 to 15 of the treatment in 40 mg/kg/day group males as compared to the control group males. It was associated with a slight decrease in food consumption at this dose (see below). The body weight gain in males of the 10 and 20 mg/kg/day treatment groups from days 1 to 8 and days 1 to 15 of treatment were comparable to control and 10 mg/kg/day group after cohabitation.
There were no effects on body weight of the untreated females that were mated with the treated males.

Reproductive Performance of Males: There was no effect of SB-497115-GR on reproductive performance at any dose level. Mating index, fertility index and number of days needed for mating in the SB-497115-GR treated groups were comparable to control values.

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Male (M0) Mating Trial - Group Means

Protocol No. G04428

Endpoints:		No. of days needed for mating	Mating Index (%)	Fertility Index (%)
Test Used:		KW	CA	CA
Dose (mg/kg/day)	SB-497115-GR			
0 Control	Mean	1.8	100.0 (25/25)	100.0 (25/25)
	SEM	0.2		
	N	25		
10	Mean	2.0	100.0 (25/25)	92.0 (23/25)
	SEM	0.2		
	N	25		
20	Mean	1.7	100.0 (25/25)	100.0 (25/25)
	SEM	0.2		
	N	25		
40	Mean	2.1	100.0 (25/25)	92.0 (23/25)
	SEM	0.4		
	N	25		

N = Number of animals during treatment period.
Mating Index = (Number of males mated/Total number treated) * 100
Fertility Index = (Number of males impregnating at least one female/Number of males mated) * 100

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Embryo-Fetal Survival, Fetal Weight and Gravid Uterine Weight: Administration of 10, 20 or 40 mg/kg/day to the male rats did not affect The embryo-fetal survival, fetal weight or gravid uterine weight of females that the males impregnated in 10, 20 and 40 mg/kg/day groups were similar to control group.

Pre- and postimplantation loss, mean number of live fetuses, percent live males, gravid uterine weights and fetal weights were also similar in the compound-treated and control groups as shown below in table.

APPEARS THIS WAY
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Female (F0) Cesarean Section Deliveries - Group Means

Protocol No. G04428

Endpoints: / Test Used:		Corpora Lutea KW	Implants KW	% Pre-Implant Loss KW	Postimplantation Loss: Early Resorption	Late Resorption	Dead Fetuses	Total KW	%	Live Fetuses KW	% Live Males KW	Gravid Uterus Weight (g) KW
Dose (mg/kg/day) SB-497115-GR												
0 Control	Mean	15.3	14.9	2.3	0.6	0.0	0.0	0.6	3.7	14.4	52.2	82.9
	SEM	0.3	0.3	0.7	0.1	0.0	0.0	0.1	0.9	0.3	2.3	1.8
	N	25	25	25	25	25	25	25	25	25	25	25
10	Mean	15.0	14.7	2.3	1.0	0.0	0.0	1.0	7.3	13.6	55.6	79.4
	SEM	0.3	0.3	0.9	0.2	0.0	0.0	0.2	1.5	0.4	2.6	2.4
	N	23	23	23	23	23	23	23	23	23	23	23
20	Mean	15.7	14.7	4.8	1.1	0.0	0.0	1.1	10.0	13.6	55.9	81.8
	SEM	0.8	0.5	1.8	0.3	0.0	0.0	0.3	4.1	0.8	2.8	1.8
	N	25	25	25	25	25	25	25	25	25	24	24
40	Mean	15.0	14.3	4.8	0.6	0.0	0.0	0.6	4.1	13.7	54.1	79.9
	SEM	0.4	0.5	2.5	0.2	0.0	0.0	0.2	1.1	0.5	2.7	2.1
	N	23	23	23	23	23	23	23	23	23	23	23

N = Number evaluated
Group Means are means of litter values
% Preimplantation Loss = 100 x (Corpora Lutea - Implants) / Corpora Lutea
Total Postimplantation Loss = No. Early Resorption + No. Late Resorption + No. Dead Fetuses
% Postimplantation Loss = 100 x (Total Postimplantation Loss/ Implants)
% Live Males = 100 x (Total No. of Live Males / (Total No. of Live Males + Total No. of Live Females))

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Necropsy Observations
There were no treatment-related findings in males at necropsy.

External Fetal Examinations
There were no external fetal findings in this study excepting fused placenta in 20 mg/kg/day group was seen and it was not considered to be related to treatment.
Organ Weights:
A 15.7% increases in mean testes weights ($p>0.05$) in the 40 mg/kg/day group was reported. The testes to body weight ratios was also increased by the same intensity.
An insignificant slight 8.4% ($p \leq 0.05$) increase in mean caput/corpus epididymis weights in the 40 mg/kg/day group was noted. Organ weights in the 10 and 20 mg/kg/day groups were comparable to control values.

The treatment in males up to 40 mg/kg/day (5 times MHD based on AUC) did not affect the reproductive performance, number of matings, fertility index and any functional or histopathology change in testes. The number of corpora lutea in untreated females were similar in treated and control group females.

C. Study title: SB-497115-GR: Oral Embryo-Fetal Development study in SD rats.

Key findings: SB-497115-GR was maternally toxic (decreases in body weight gain and food consumption) at the dose of 60 mg/kg/day (7 times MHD based on AUC) when administered during organogenesis. Fetal survival was not adversely affected by test article treatment. However, at the maternally toxic dose of 60 mg/kg/day, fetal weights were significantly reduced (7%), and there was

a slight increase in the incidence of the fetal variation, cervical rib. The NOAEL for embryonic and fetal development was 20 mg/kg/day (2 times MHD based on AUC).

Study no.: CD2003/00938/00; G03034
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.

Date of study initiation & Completion: May 16, 2003 and March 26, 2004
GLP compliance: A statement of compliance was attached.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: F033082 and 99.3% pure

Methods

Doses: 10, 20 and 60 mg/kg/day
Species/strain: Sprague-Dawley rats —.CD™(SD)IGS BR b(4)
Number/sex/group: 22 pregnant female/group
Route, formulation, volume, and infusion rate: Oral gavage in 2% hydroxypropyl methylcellulose containing 0.2% sodium lauryl sulfate.
Satellite groups used for toxicokinetics: Not done
Study design: The 4 groups of pregnant rats were administered oral gavage doses of 0, 10, 20 and 60 mg/kg/day (vol = 10 ml/kg) from postcoitum day 6 to 17 (gestation day 7 to 18). On post coitum day 21, these animals were killed and fetuses were evaluated. The corpora lutea, implantation sites, resorption, live and dead fetuses counted, weighed and the intrauterine position recorded. Gross examination of live and dead fetuses was done. The placenta of each of the live and dead fetus was examined for gross changes.
Parameters and endpoints evaluated: Clinical observations were performed on postcoitum day 6, 7, 18 and 21.
Body weight: taken daily on day 6 to 18 and on day 21 post coitum. Food consumption for females taken on day 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 21 postcoitum. Mated females were sacrificed on day 21
Fetal Observations: Each live and dead fetus was examined externally and about one-half of the fetuses were used for abdominal and thoracic viscera examination. These fetuses were decapitated and head preserved in Bouin's solution and eviscerated and fixed in absolute alcohol. The remaining fetuses were processed for skeletal abnormalities after fixing with Alizarin Red S stain. The uterus, ovaries and gross lesions and malformed fetal viscera and the fetuses not processed for skeletal examination of the treatment groups and the corresponding tissues of the control group animals were stored in phosphate buffer formalin solution.

Results

Mortality: None of the females included in the treatment groups died.

Reviewer: Chopra NDA No. 22-291

Clinical signs: One female of 20 mg/kg/day treatment group showed low posture labored breathing and it was not considered as treatment related.

Body weight: In the high dose group, the decrease in percent body weight gain during treatment and gestation period was 17.1% and, 15.8%, respectively as compared to control.
Food consumption: The food consumption of the 60 mg/kg/day treatment group females was significantly less than the control and other groups of females, 116 g/day Vs 127 g/day in control.
Toxicokinetics: Not done

Necropsy: No notable necropsy observations were noted in dams. The number of fetuses, and corpora lutea and the sites of implantations in the treated animals and control groups were similar. A dose related mean 6.0, 8.9, 9.1 and 9.3 preimplantation losses was seen in 0, 10, 20 and 60 mg/g/day treatment groups dams. It was a slight but dose related change. The fetal growth in the 60 mg/kg/day treatment group was lesser than in control indicating that the compound was feto-toxic. The fetal viability was not changed. The maternal data is shown below:

Table Maternal and Fetal Observations in Rats at Cesarean Section

	Vehicle Control	SB-497115-GR (mg/kg) 10	20	60
# Litters	22	22	22	21
# Fetuses	251	322	284	299
# Implantations[1] (mean +SD)	12.7 + 0.4	13.3 + 0.3	12.5 + 0.5	13.6 + 0.3
Pre-Implantation Losses (mean+SD)	6.0 +1.5	8.9 + 1.9	9.1 + 2.2	9.3 + 1.8
Mean Viable (mean +SD) Non Viable[1](mean +SD)	12.5 + 0.4 0	12.8 + 0.3 0	11.5 + 0.5 0	13.3+ 0.3 0
Resorptions Total Early[1] (mean +SD) Late[1] (mean +SD)	 0 0.2 + 0.1 0.0 + 0.0	 0 0.5 + 0.2 0.0 + 0.0	 0 1.0 + 0.2 0.0 + 0.0	 1 0.2 + 0.1 0.0 + 0.0
Corpora Lutea[1] (mean +SD)	13.6 + 0.5	14.7 + 0.4	13.9 + 0.5	15.1 + 0.5
Sex Ratio (M/F)	1.132	1.041	1.088	1.029
Fetal Body Wt. Males(g) (mean +SD) Females(g) (mean +SD)	 5.84 + 0.08 5.49 + 0.06	 5.70 + 0.07 5.44 + 0.08	 5.67 + 0.07 5.35 + 0.08	 5.41* + 0.06 5.15 + 0.07

* Significantly less than vehicle control ($p < 0.01$)

Fetal Evaluations:

Table 3. Summary of the Incidence of Fetal Skeletal Variations

SB-497115-GR	Dose groups (mg/kg)				0
10 20 60					
No. of Fetuses/No. of litters					
No. Litters	22	22	22	21	
No. Fetuses/Litter Examined:	138	143	128	138	
Skeletal Variations:					
Cervical Vertebral					
-Arch Reduced Ossification	0/0	2/2	0/0	1/1	
-Cervical Rib Reduced Ossification	1/1	2/2	0/0	6/5	
Hind paw Phalanges:					
-Incomplete Ossification	0/0	2/2	0/0	1/1	
Rib					
--Incomplete Ossification	0/0	1/1	0/0	3/1	
Sternebrae					
-Split	1/1	1/1	0/0	0/0	
-Incomplete Unossified	1/1	0/0	0/0	0/0	
Brain					
-Third Ventricle Absent	0/0	1/1	0/0	0/0	
Nares					
-Small	0/0	0/0	1/1	0/0	

Table 3 Shows that SB-497115 produced an increase in the incidences of reduced or incomplete ossification of ribs. These were of no significant or clinical importance as these incidences were within the historical control incidences. There was an increase in the incidence of the fetal variation, cervical rib at high dose. There were no dose or treatment related external and visceral malformations (see table below).

Table 2. Summary of the Incidence of Fetal External and Visceral Malformations and Variations

	SB-497115-GR			
Dose groups (mg/kg)	0	10	20	60
No. of Fetuses (No. of litters)				
No. Litters	22	22	22	21
No. Fetuses/Litter given a:				
External Examination:	275	269	252	279
Visceral Examination:	137	138	124	141
Variations/Malformations:				
External				
-Generalized Edema	0(0)	1(1)	0(0)	0(0)
-Exencephaly	0(0)	1(1)	0(0)	0(0)

-Micrognathia	0(0)	0(0)	1(1)	0(02)
-Tongue Misshapen	0(0)	0(0)	1(1)	0(0)
Visceral				
-Innominate Absent	1(1)	1(1)	0(0)	0(0)
-Carotid Origin from Innominate	0(0)	0(0)	1(1)	0(0)

* Seen in the same animal (Male fetus # 07 litter # 400)
H Seen in the same animal (Male fetus # 07 litter # 402)

D. Study title: SB-497115-GR: Oral Embryo-Fetal Development study in Rabbits.

Key findings: Administration of SB-497115-GR during the period of organogenesis resulted in deaths at 150 mg/kg/day (0.6 times MHD based on AUC) dose and a minimal adverse effect of reduced stools and retardation in body weight gain was seen in rabbits included in 80 mg/kg/day. It was non-teratogenic in rabbits.

Study no.: CD2003/01136/00; G03322
Volume #, and page #: 3.16, pp 1
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA.

Date of study initiation and Completion: November 23, 2003 and July 26, 2004
GLP compliance: A statement of compliance was attached.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: F033082 and 99.3% pure

Methods

Doses: 0, 30, 80 and 150 mg/kg/day were administered from day 7 to day 19 post coitum. b(4)
Species/strain: New Zealand White rabbits
Number/sex/group: 22 pregnant female/group (the actual number of pregnant rats used were 21, 19, 20 and 22 in 3 control and treatment groups).
Route, formulation and, volume: Oral gavage in 2% hydroxypropyl methylcellulose containing 0.2% sodium laurly sulfate, volume 5 ml/kg.
Study design: Animals sacrificed on post coitum day 29
Clinical Observations: all animals examined twice daily for mortality and changes in the physical signs once before treatment and daily throughout the study and full examination was done on days 4, 7 to 20, 25 to 29 pc.
Body weight: Once before treatment and daily throughout the study and full examination was done on days 4, 7 to 20, 25 to 29 pc. Food consumption was measured daily for all the animals during the study.

Fetal Observations: Embryo-fetal survival, fetal weight and gravid uterine weight recorded at the time of necropsy on day 20, # of corpora lutea, # live and dead fetuses, fetal weight, # implantation sites, # resorption and their relative position recorded. Gross examination of placenta from live and dead fetuses performed. Each live and dead fetus was examined externally for abnormalities. Each of the fetuses was eviscerated and placed in 85% ethanol/15% methanol for staining with alizarin red S and skelatal examination. The uterus and ovaries of each of the F0 female and malformed fetuses retained in phosphate buffer for further test.

Toxicokinetic: 0.5 ml blood obtained on day 11 (day 5 of dosing) from 3/group of the study at 0 (prior dosing), 0.5, 1, 2, 4, 8 and 24 hr post dosing.

Necropsy: On day 29, all animals were sacrificed, reproductive tract separated, ovaries removed and corpora lutea counted. The uterus weighed, placentas counted, live and dead fetuses number and their position determined. Early, late and empty implantation sites recorded. The abnormal tissues, ovaries and uterus preserved in 10% formalin and retained. The observed abnormalities were classified as major malformation, minor external, visceral skeletal variations.

Results

A. Maternal Dam Data:

Mortality: Two females included in 150 mg/kg/day treatment group and 1 animal of control group died during the study.

Clinical signs: Decreased fecal excretion 2 and 3 females and, left paw brown stains in 1 and 3 females belonging to 80 and 150 mg/kg/day treatment groups were observed. Loss of hairs on inguinal area in 1, 2, 3 and 3 animals and, thin hair cover in urogenital areas in 2, 3, 4 and 6 females were noted.

Body weight: The retardation in percent body weight gain during treatment period from day 7 to 19 was 4.8 and 19.0% in females included in 80 and 150 mg/kg/day treatment groups. The animals after the treatment period attained similar body weights, i.e., 0.20 and 0.20 kg from day 20 to 29. The initial and final body weight of control females was 2.9 and 3.31 kg respectively.

Food consumption: The food consumption of the 150 mg/kg/day treatment group females was similar to the control groups of females, the initial and final food consumption of control female were 127 g/day Vs 124 g/day.

Pathology: Dark colored foci in stomach of 1, 2, 0 and 3 females of 150 mg/kg/day treatment group and, dark colored raised area was seen in liver and gall bladder was observed in 1 female of 150 mg/kg/day treatment group.

Toxicokinetics: A non-dose proportional plasma increase in compound concentrations was seen in females within 2 to 4.01 hr of the administration of the compound. The TK data is shown below in the sponsor's table.

Summary Table for TK for
SB-497115 in Pregnant Rabbits

SB-497115-GR	T max (hr)	Cmax (ng/ml)	$AUC_{(0-24h)}$
30	2.0	1206	7560
80	4.0	5685	42636
150	4.02	12502	89573

Fetal Observations & Evaluation:

The number of fetuses and corpora lutea and the sites of implantations in the treated animals and control groups were similar. An increase of pre-implantation losses was noted in females included 150 mg/g/day treatment groups. The fetal growth was similar in the animals of all study groups and was similar to the control group indicating that the compound was not feto-toxic.

Table Maternal and Fetal Observations in Rabbits

	Vehicle Control	SB-497115-GR (mg/kg) 30	80	150
# Pregnant Animals	20	19	20	20
# Fetuses	157	147	148	151
#Corpora Lutea[1] (mean +SD)	8.6 + 0.27	8.8 + 0.38	8.5 + 0.49	8.9 + 0.24
# Implantations[1] (mean +SD)	8.3 + 0.31	8.2 + 0.38	7.9 + 0.5	7.8 + 0.5
Pre-Implantation Losses (%mean+SD)	4.3 +1.72	7.9 + 1.77	7.5 + 2.59	12.7+ 4.88
Mean Viable (mean +SD) Non Viable[1](mean +SD)	7.9 + 0.38 0	7.7 + 0.31 0	7.4 + 0.47 0	7.6+ 0.48 0
Resorptions Total Early[1] (mean +SD) Late[1] (mean +SD)	 0 0.4 + 0.2 0.0 + 0.0	 0 0.4 + 0.14 0.1 + 0.05	 0 0.4 + 0.15 0.1 + 0.07	 1 0.2 + 0.09 0.0 + 0.05
Sex Ratio (M/F) % Male	55.9+ 4.2	48.2+ 5.4	43.7+ 2.59	43.4+ 4.03
Fetal Body Wt. Males(g) (mean +SD) Females(g) (mean +SD)	 39.7 + 1.17 39.2 + 0.92	 40.6 + 1.07 40.2 + 0.97	 40.4 + 1.06 40.1 + 1.13	 43.4 + 1.05 42.1 + 1.22

* Significantly less than vehicle control ($p < 0.01$)

Table Summary of the Incidence of Fetal External and Visceral Malformations and Variations in Rabbits

	SB-497115			
Dose groups (mg/kg)	0	30	80	150
No. Litters	20	19	20	20
No. Fetuses given a:				
External Examination:	157	147	148	151
Visceral Examination:	157	147	148	151
Skeletal Examination	79	74	73	74
No. of Fetuses				
Variations/Malformations:				
Major Malformation (total)	0(0)	0(0)	0(0)	0(0)
Minor External/Visceral Anomalies5(5)	7(8)	2(2)	6(9)	
External				
-Arthrogryposis	0(0)	0(0)	1(1)	0(0)
-Domed Head	0(0)	0(0)	0(0)	1(1)
Visceral				
-Lung and Thymus Agenesis	1(1)	1(1)	0(0)	0(0)
-Liver Vestigeal lobe	1(1)	1(1)	0(0)	2(2)
-Gall Bladder Agenesis	0(0)	0(0)	0(0)	1(1)
-Gall Bladder Hypoplasia	(0)	0(0)	0(0)	0(0)

The incidence of external and visceral variations/malformations was not dose or treatment related and there was only 1 variation/malformation which occurred in more than one litter and this was considered as not treatment related. Thus, the compound did not produce any treatment related fetal abnormality or malformation.

Table 6. Summary of the Incidence of Fetal Skeletal Variations

	SB-497115-GR			
Dose groups (mg/kg)	0	30	80	150/90
No. Litters	20	19	20	20
No. Fetuses Examined	157	147	148	151
Skeletal Variations:	No. of Fetuses (No. of litters)			
Sternebrae				
-Fused Branched	0/0	1/1	1/1	3/3
-Extra	0/0	1/1	0/0	1/1
-Misaligned	0/0	0/0	1/1	0/0
Pubis				
-Reduced Ossification	0/0	1/1	1/1	1/1

Thoracic Vertebral centrum				
-Bipartite	5/8	1/1	4/9	4/9
-Semi-bipartite	3/4	1/1	4/6	2/2
-Absent	0/0	0/0	0/0	1/1

Note: Variations which occurred at a greater incidence in control groups or which occurred only at the low or mid dose were not included.

There was no clinically significant effect of the compound on the fetal development in pregnant rabbits including in 30, 80 and 150/90 mg/kg/day treatment groups. No increase in the incidence of external, visceral or skeletal malformations or the variations was seen in rabbits treated during the period of organogenesis. The incidences were similar to vehicle treated control rabbits.

In conclusion, administration of SB-497115-GR during the period of organogenesis resulted in deaths at 150 mg/kg/day (0.6 times MHD based on AUC) dose and the dose was reduced to 90 mg/kg/day. A minimal adverse effect of reduced stools and body weight retardation was seen in 80 mg/kg/day (0.3 times MHD based on AUC) treatment group animals. No treatment related increased incidence of external, visceral or skeletal malformations or the variations were observed. SB-497115-GR was non-teratogenic in the rabbits included in the study.

E. SB-497115-GR: Oral Pre- and Postnatal Development Study in Rats Study # GSK No. 605014

Key study findings: Oral gavage doses of 10, 20 or 60 mg/kg/day (1-7 times MHD based on AUC) SB-497115 when administered from day 6 postcoitum (pc) through day 20 postpartum (pp) in pregnant rats, caused vaginal bleeding, decreased activity, red serous fluid around the vagina, anemia, rough hair coat, diarrhea, brown watery feces and ptosis at high dose. The high dose was lethal and sponsor terminated this group during the study. No adverse effects on pregnancy, parturition or lactation were reported in animals of 10 and 20 mg/kg/day (2 times MHD based on AUC) treatment groups. The pups of these treatment group dams showed no treatment related adverse effect on growth, development, neurobehavioral parameters and reproductive functions of offspring (F1).

Study no.: CD2005/00331/02; G05014
Conducting laboratory and location: GlaxoSmithKline, King of Prussia, PA
Date of study initiation: September 14, 2006
GLP compliance: A statement that the study was in compliance with GLP was enclosed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Batch Number F074359007 (Sub lot of TPO-E-03.Mil), (assigned purity 97.4%)

Methods

Doses: 0, 10, 20 and 60 mg/kg/day by oral gavage once daily on Day 6 pc through Day 20 pp

Species/strain: Ninety-six time-mated female – CD™(SD) rats from b(4)

Number/sex/group: 24/group

Route, formulation, volume: Oral gavage

Satellite groups used for toxicokinetics: No; plasma concentration of the compound was determined in 10 days old pups at 3, 8 and 22 hr post dose to dams.

Study design: The dams treated by oral gavage once daily on Day 6 pc through Day 20 pp with the aim to determine the effects of SB-497115 on pregnancy, parturition, lactation and on pre- and postnatal survival and also on neurobehavioral growth and, the development of reproductive parameters of F1 and F2 generations.

Parameters and endpoints evaluated: F0 Females were examined for the changes in clinical observations (day 6 and 21 pc and on Day 1 and 21 pp), body weight (day 6 and 21 pc) and food consumption on day 6 to 10, 10 to 14, 14 to 19 and 19 to 21 pc and, on postpartum day 1 to 4, 4 to 8, 8 to 12 and, 12 to 16.

Postweaning Evaluations

One/sex F0 rats were mated and F1 offspring (1M and 1F) were delivered. These rats were mated to deliver F2 (2M and 2F). F1 Pups were tested for auditory startle reflex (postnatal day 27 to 31), passive avoidance test for learning/retention once between postnatal Day 40 and 50 using an automated system and, motor activity (postnatal day 54 to 61). F2 Pups were tested for passive avoidance (postnatal Day 40 to 50) and auditory startle reflex (postnatal Day 70 to 77).

F1 offspring spontaneous locomotor activity was tested in animals challenged with single subcutaneous injection of 0.5 mg/kg d-amphetamine sulfate. The challenge dose was administered between postnatal Day 54 and 61 and each of the rats was monitored for an additional 1.2 hr.

F1 mating and parturition observations:

The vaginal smears prepared and estrous cycle of 1F females were determined from postnatal day 64 to 73 (beginning on postnatal day 73 to 80) until mated. Mated females (F1) were weighed on day 0, 7, 14 and 21 pc and day 1 and 7 pp and were allowed to deliver their F2 litters. The dams were checked frequently between 0800 and 1800 hours to record the date and time parturition, number of pups and the day parturition was completed (recorded as pp day 0). The dead F2 offsprings were counted and the test done to determine if offspring was 'stillborn'/'born alive' and all of these were necropsied. F1 females were euthanized on post natal 23 and uterine contents examined for implantations (stained with ammonium sulfide if apparently not pregnant), resorptions and live and dead fetuses were counted.

F2 pup evaluations and termination: Pups were examined externally, their number, weights and fetal sex determined. Live offspring were individually weighed on postnatal days 1 and post-partum day 7. The cause of death (still born/born dead) determined. Surviving F2 offspring were euthanized by carbon dioxide asphyxiation on Day 7 pp and discarded.

Results

F_0 Dams:

Observations: In F0 females of 60 mg/kg/day treatment group, decreased activity, red/brown staining of the bedding, red serous fluid around the vagina, pale appearance, unkempt hair coat, fecal staining, light brown watery feces and ptosis were observed.

Mortality: The dams died during the study or sacrificed during gestation or immediately postpartum were recorded. Dams in the high dose group were euthanized during late gestation or immediately postpartum due to excessive toxicity. Two of 4 dams of high dose group that delivered prior to termination, cannibalized their offspring. A retardation of 23% in body weight gain and 18% decrease in food consumption was noted. On pc day 19 to 21, a mean body weight loss (26 g) from and severely decreased (85%) food consumption was seen.

No deaths or drug-related observations were seen in the F0 females from the 10 or 20 mg/kg/day groups.

There were no effects on gestation length or parturition, no significant differences among the groups for numbers of live born offspring (F1) or their viability, and no drug-related offspring malformations.

F_0 necropsy: There were no treatment-related external or visceral findings. (Visceral exam was done only on dead or externally malformed pups.) No dose or treatment related effects were noted in litters of 10 or 20 mg/kg/day treatment groups. Two litters in the 20 mg/kg/day group had thin hair coat on the body or head. Two pups from 1 litter in the 20 mg/kg/day group had kinked tails. Only a limited number of pups were available for examination prior to the decision to terminate (and discard the dams and offspring) from the 60 mg/kg/day group, and examination of some of these pups was precluded by cannibalization.

F_1 physical development: There were no effects on offspring (F1) body weight or attainment of physical landmarks of sexual maturation (balano-preputial skinfold separation in males and vaginal opening in females). There were no drug-related effects on offspring (F1) startle reflex, passive avoidance behavior, motor activity. The growth of F1 generation rats was similar in compound treated and control groups of pups as shown in the table below).

F_1 behavioral evaluation: The behavioral parameters in rats born to 10 and 20 mg/kg/day treatment groups and control group animals were similar and the learning (neurophamacological parameters of memory and learning) and retention were not affected in F1 generation.

F_1 reproduction: The fertility or reproductive performance (estrous cyclicity, mating and pregnancy incidence, parturition, uterine implantations) among the F1 rats treated with the compound was similar (see table below). F2 pups number, survival rate, viability, body weight and external morphology born to F1 dams were similar to the control group animals.

F_2 findings: The developmental parameters of F2 generation of rats were similar to the control group animals. The passive avoidance test for learning/retention evaluated in F2 offspring once between postnatal Day 68 and 70 using an automated system was similar indicating the compound did not exert an adverse effect up to 20 mg/kg/day.

The peri- and post- natal data of the animals included in the study are shown in the following table (condensed and modified from sponsor's table on page 14 of report #cd2005-00331-02):

Study Type: Reproductive and Developmental Toxicology **Study No. G05014**

COMPOSITE TABLE FOR THE PERINATAL & POST NATAL PARAMETERS IN RATS

Daily Dose (mg/kg)	0 (Control)	10	20	60
F0 Females:				
No. Pregnant	24	24	23	n/a
No. Pregnant Survivors to Term	24	24	23	n/a
Gestation Body Weight Change				
– Day 6 to 19 pc (%a)	113.7	2	3	2
Day 6 to 21 pc (%a)	144.4	0.2	-23*	-57*
Lactation Body Weight Change (%a)	23.1	16	13	n/a
Gestation Food Consumption (%a)				
Day 6 to 10 pc	97.6	-1	2	5
Day 10 to 14 pc	101.3	-1	3	-3
Day 14 to 19 pc	138.5	-4	0.4	-18*
Day 19 to 21 pc	51.8	0	0.3	-85
Lactation Food Consumption (%a)				
Day 1 to 4 pp	107.9	0	0.3	N/A
Day 4 to 8 pp	208.9	-2	-3	N/A
Day 8 to 12 pp	246.9	3	-1	N/A
Day 12 to 16 pp	272.3	4	0.5	N/A
Mean Duration of Gestation (days)	21.3	21.4	21.4	21.5
Mean No. of Implantations	13.0	13.5	12.8	14.5b

Reviewer: Chopra NDA No. 22-291

Average Pup Delivery Time (min)	9.1	9.9	10.7	24.2b
Necropsy Observations	-	-	-	-
F1 Litters: No. Viable Litters at Birth (Day 1)	24	24	23	n/a
(Preweaning) No. Viable Litters at Weaning (Day 21)	24	24	23	n/a
Mean No. Live Pups Born/Litter	12.5	12.8	12.0	5.5*b
Mean No. Live Male Pups Born/Litter	6.5	6.3	6.5	2.5b
Mean No. Live Female Pups Born/Litter	6.0	6.5	5.5	3.0b
Mean No. Stillborn/Litter	0.1	0.2	0.3	0b
Postnatal Survival				
Day 4 pp (% Survival Day 1 to 4 pp)	99.7	99.7	100	n/a
Survival to Weaning (% Survival Day 4 to 7) (Day 7 to 14, Day 14 to 21 pp)	99.7	99.7	97.9	n/a
Weight at Day 1 pp (g) M	7.42	7.25	7.27	n/a
Weight at Day 1 pp (g) F	7.04	6.91	6.89	n/a
Weight at Weaning (g) M	47.7	48.6	48.9	n/a
Weight at Weaning (g) F	46.4	46.2	47.1	n/a
F1 Males: No. Evaluated	24	24	23	0
(Postweaning) Mean Body Weight Day 98 pp (g)	536	543	543	n/a
Behavioral & Growth Parameters Evaluation: Mean Age of Balano-Preputial Separation (days) Sensory Function (Startle reflex)	43.5 -	43.7 No effect	43.9 No effect	n/a n/a
Learning and Memory (Passive Avoidance) -	No effect	No effect	n/a	
Mean No. Days Prior to Mating	3.4	3.3	3.9	n/a
No. of Males that Mated	23/24	20/24	21/23	n/a
No. of Fertile Males	21/23	20/20	20/21	n/a
F1 Females: No. Evaluated	24	24	23	0
(Postweaning) Mean Body Weight Day 98 ppa (g)	307	304	308	n/a
Mean Age of Vaginal Patency (days)	32.3	32.2	32.6	n/a
Sensory Function (Startle Reflex)	-	No effect	No effect	n/a
Motor Activity	-	No effect	No effect	n/a
Learning and Memory (Passive Avoidance)	-	No effect	No effect	n/a
Mean No. of Estrous Cycles Premating	2.5	2.2	2.1	n/a
Mean No. Days Prior to Mating	3.4	3.3	3.9	n/a
No. of Females Sperm-Positive	23	20	21	n/a
Mean Gestation Body Weights Day 21 pc (g)	427	437	432	n/a
No. of Pregnant Females	21	20	20	n/a
Mean Duration of Gestation (days)	21.2	21.5	21.3	n/a
Average Pup Delivery Time (min)	10.1	9.1	10.2	n/a
Mean No. of Implantations	14.6	15.3	13.7	n/a
F2 Litters: No. Evaluated	21	20	21	n/a
Mean No. Live Pups Born/Litter	14.4	14.4	13.5	n/a

Mean No. Live Male Pups Born /Litter	7.5	7.0	7.0	n/a
Mean No. Live Female Pups Born /Litter	6.9	7.4	6.5	n/a
Mean No. Dead Pups Born/Litter	0	0.1	0	n/a
Postnatal Survival to Day 7 pp (% Survival Day 1-7 pp)	99.7	99.7	100	n/a
Weight at Day 1 pp (g) M	6.65	6.89	6.80	n/a
Weight at Day 1 pp (g) F	6.34	6.56	4.42	n/a
Weight at Day 7 pp (g) M	14.17	14.00	14.52	n/a
Weight at Day 7 pp (g) F	13.64	13.34	13.56	n/a

- No noteworthy findings. pp postpartum pc postcoitum n/a not applicable (group terminated)
a. For controls, group means are shown. For treated groups, percent differences from controls are shown. *= Statistical significance ($p \leq 0.05$) is based on raw data (not on the percent differences). b. Value is based on only 2 litters because group was terminated due to excessive toxicity

SB-497115 at oral doses of 0, 10, 20 and 60 mg/kg/day (1-7 times MHD based on AUC) in pregnant rats from day 6 postcoitum (pc) through day 20 postpartum (pp) produced deaths among the high dose treatment group. The dead animals showed overt toxicity of reduced activity, body weight loss, vaginal and rectal bleeding and red serous fluid around the vagina. Two of 4 dams of the group aborted and cannibalized their offspring, showing marked toxicity. No adverse effects on the development of neurobehavioral or reproductive functions of the F1 and F2 pups were noted.

TK analysis: SB-497115 was detected in the plasma of all 10 day old rat pups (F1) for the entire 22 hour sampling period following administration of drug to the F0 dams. Plasma concentrations increased with dose. The composite plasma concentrations were similar across the measured time points (3, 8 and 22 hours). Since 60 mg/kg/day treatment group was terminated early, no TK data was available for this group. The table below summarizes the TK data for postpartum rat pups (F1).

Dose	Plasma Conc. 3 hr (ng/mL)	Plasma Conc. 8 hr (ng/mL)	Plasma Conc 22 hr (ng/mL)	AUC_{0-22} (ng.h/ml)
10 mg/kg/day				
Male	3264	3408	3402	69895
Female	3287	3796	3813	77070
20 mg/kg/day				
Male	10029	11654	11315	231977
Female	10729	10570	10489	219123

SPECIAL TOXICOLOGY:

1. Study Title SB-497115-GR: **Investigative Phototoxicity Study to Evaluate Effects on Eyes in Female Albino CD-1 and Pigmented B6C3F1 Mice** (Study No. AFA00546