SUMMARY OF TOXICOLOGY DATA

Fenpyrazamine

Chemical Code # 6077, Tolerance # 53165
SB 950 # New A.I.

Original: 9/21/12, revised: 1/14/13

I. DATA GAP STATUS

Chronic toxicity, rat: No data gap, no adverse effect
Chronic toxicity, dog: No data gap, no adverse effect
Oncogenicity, rat: No data gap, no adverse effect
Oncogenicity, mouse: No data gap, no adverse effect
Reproduction, rat: No data gap, no adverse effect
Teratology, rat: No data gap, no adverse effect
Teratology, rabbit: No data gap, no adverse effect
Gene mutation: No data gap, no adverse effect
Chromosome effects: No data gap, no adverse effect
DNA damage: No data gap, no adverse effect
Neurotoxicity: No data gap, no adverse effect

Toxicology one-liners are attached.

All record numbers through #268397 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
## indicates a study on file but not yet reviewed.
File name: T120921A
Revised by T. Moore, 9/21/12, R. Pan, 1/14/13.
II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 0046; 262230; “S-2188 Technical: Combined Chronic Toxicity/Oncogenicity (Feeding) Study in the Wistar Rat” (Sommer, E.W., Harlan Laboratories Ltd. (former RCC Ltd.), Itingen, Switzerland, Harlan Laboratories Study No. A08897, 05/26/2009). 870.4300. S-2188 Technical (Batch number 030-050914-1G, purity = 94.7%) was admixed to the diet and fed to 50 Han:Rcc:WIST (SPF) rats per sex per dose at dose levels of 0 (diet only), 100, 300, 1200, or 2400 ppm (0, 4.25, 12.72, 51.90, and 106.76 mg/kg/day, respectively for males, and 0, 5.29, 15.64, 63.58, and 130.25 mg/kg/day, respectively for females) for at least 104 weeks with 20 additional animals per sex per dose (satellite group animals) for at least 52 weeks. Mortality totals (main group animals) were as follows- males: 10 (4 spontaneous, 6 sacrificed in extremis)/50, 13 (6 spontaneous, 7 sacrificed in extremis)/50, 13 (4 spontaneous, 9 sacrificed in extremis)/50, 8 (4 spontaneous, 4 sacrificed in extremis)/50, 16 (6 spontaneous, 10 sacrificed in extremis)/50, respectively; females: 11 (1 spontaneous, 10 sacrificed in extremis)/50, 13 (2 spontaneous, 11 sacrificed in extremis)/50, 14 (2 spontaneous, 12 sacrificed in extremis)/50, 11 (3 spontaneous, 8 sacrificed in extremis)/50, 8 (4 spontaneous, 4 sacrificed in extremis)/50, respectively. Mortality totals (satellite group animals) were as follows- males: no mortalities; females: 0/20, 1/20 (spontaneous), 0/20, 0/20, 0/20, respectively. No treatment-related clinical signs were observed. No treatment-related palpable nodules/masses were observed during the detailed weekly examinations. Functional Observational Battery (FOB), conducted on each satellite group animal at week 49 of treatment, revealed no treatment-related effects. Results of the locomotor assessments on the satellite group animals indicated no treatment-related effects. A treatment-related decrease in mean body weights was observed in both sexes of main group animals at 2400 ppm. No consistent effects on mean food consumption were observed. Treatment-related decreases in the mean red blood cell, mean corpuscular, and mean corpuscular hemoglobin levels in males at week 52 at 2400 ppm and treatment-related decreases in mean hemoglobin and in mean hematocrit levels and a treatment-related increase in the mean prothrombin time in females at week 52 at 2400 ppm were observed. A decrease in the mean creatinine level at weeks 26 and 52 in both sexes at 2400 ppm, treatment-related increases in mean alkaline phosphatase and mean gamma glutamyl transpeptidase levels in males at week 52 at 2400 ppm, an increase in the mean total protein level in both sexes at week 52 at 2400 ppm, and an increase in the mean albumin level in females at week 52 and in males at weeks 26 and 52 at 2400 ppm were observed. Urinalysis revealed no treatment-related effects. A treatment-related increase in mean relative liver weight was observed in satellite group males at 1200 and 2400 ppm and in satellite group females at 2400 ppm and in main group males and females at 2400 ppm. Macroscopic examination revealed treatment-related thickened liver in main group males at 2400 ppm. Microscopic examination revealed treatment-related hepatocellular hypertrophy in main and satellite group males and females at 1200 and 2400 ppm, diffuse follicular hyperplasia in the thyroid in main group males at 2400 ppm, and diffuse follicular hypertrophy in the thyroid in main group females at 2400 ppm. Micropathological examination revealed no dose-related neoplastic tumors in the main and satellite group animals. No adverse effects. NOEL (M) = 12.72 mg/kg/day (300 ppm) and NOEL (F) = 15.64 mg/kg/day (300 ppm) (based on an increase in hepatocellular hypertrophy). Acceptable. (Corlett and Leung, 6/14/2012)

CHRONIC TOXICITY, RAT

See Combined, Rat above.
CHRONIC TOXICITY, DOG

** 0044; 262228; “A 1-Year Oral Dose Toxicity Study of S-2188 Active Ingredient in Dogs” (Sato, S., Safety Research Center of Ina Research Co., Ltd., Nagano, Japan, Study no. ST06263, 02/18/2009). 870.4100. S-2188 active ingredient (Lot no. 030-050914-1G, purity = 94.7%) was administered via gelatin capsules to 4 beagle dogs per sex per dose at dose levels of 0 (empty capsules), 5, 25, or 100 mg/kg/day for 52 weeks. No mortalities occurred. Clinical observations (conducted daily) and detailed clinical observations (conducted weekly) revealed dose-related normal stools containing white substances in both sexes at 100 mg/kg/day. No treatment-related effects on mean body weight and mean food consumption were observed. Ophthalmology revealed no treatment-related ocular lesions. A statistically significant decrease in mean corpuscular hemoglobin concentration and a statistically increase in mean corpuscular volume were observed at week 52 in males at 100 mg/kg/day; a statistically significant increase in mean platelet count was observed at weeks 13, 26, and 52 in females at 100 mg/kg/day. A statistically significant increase in the mean alkaline phosphatase level at week 39 in males at 100 mg/kg/day was observed. Urinalysis revealed no treatment-related effects. A treatment-related increase in mean absolute liver weight was observed in males at 100 mg/kg/day. Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination revealed treatment-related centrilobular hepatocellular hypertrophy in both sexes at 100 mg/kg/day. No adverse effects.

NOEL (M/F) = 25 mg/kg/day (based on centrilobular hepatocellular hypertrophy). Acceptable.

(Corlett and Leung, 05/08/2012)

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

** 0045; 262229; “S-2188 Technical: 78-Week Oncogenicity (Feeding) Study in the CD-1 Mouse” (Sommer, E.W., Harlan Laboratories Ltd. (formerly RCC Ltd.), Itingen, Switzerland, Harlan Laboratories Study No. A08875, 06/24/2009). 870.4200. S-2188 Technical (Batch number 030-050914-1G, purity = 94.7%) was admixed to the diet and fed to 52 CD-1 mice per sex per dose at dose levels of 0 (diet only), 100, 1500 (males)/2000 (females), or 3000 (males)/4000 (females) ppm (0, 11.13, 176.15, and 349.03 mg/kg/day, respectively for males, and 0, 13.85, 282.82, and 551.78 mg/kg/day, respectively for females) for at least 78 weeks with 12 additional animals per sex per dose (satellite group animals) for 52 weeks. Mortality totals (main group animals) were as follows- males: 23 (17 spontaneous, 6 sacrificed in extremis)/52, 14 (6 spontaneous, 8 sacrificed in extremis)/52, 15 (10 spontaneous, 5 sacrificed in extremis)/52, 15 (9 spontaneous, 6 sacrificed in extremis)/52, respectively; females: 15 (8 spontaneous, 7 sacrificed in extremis)/52, 24 (13 spontaneous, 11 sacrificed in extremis)/52, 16 (10 spontaneous, 6 sacrificed in extremis)/52, 18 (12 spontaneous, 6 sacrificed in extremis)/52, respectively. Mortality totals (satellite group animals) were as follows- males: 1 (spontaneous)/12, 0/12, 0/12, 0/12, respectively; females: 0/12, 2/12 (1 spontaneous, 1 sacrificed in extremis)/12, 1 (spontaneous)/12, 5 (3 spontaneous, 2 sacrificed in extremis)/12, respectively. No treatment-related clinical signs were observed. No treatment-related palpable nodules/masses were observed during the detailed weekly examinations. A treatment-related decrease in mean body weights during the middle of the dosing interval was observed in males at 3000 ppm. No treatment-related effects on mean food consumption were observed. A treatment-related decrease in the mean red blood cell level and treatment-related increases in mean corpuscular volume and mean corpuscular hemoglobin levels were observed in males at 3000 ppm after 78 weeks; treatment-related decreases in mean red blood cell, hemoglobin, and hematocrit levels were observed in females at 4000 ppm after 78 weeks. A treatment-related increase in mean relative liver weight was observed in satellite group males at 1500 and 3000 ppm and in satellite group females at 2000 and 4000 ppm and in main group males at 3000 ppm and in main group females at 4000 ppm. Micropathological examination revealed no treatment-related neoplastic tumors in the satellite group animals or in the main group animals. Microscopic examination revealed treatment-related hepatocellular hypertrophy in main group males at 3000 ppm and in main group and satellite group females at 4000 ppm. No adverse
**REPRODUCTION, RAT**

NOEL (M) = 11.13 mg/kg/day (100 ppm) and NOEL (F) = 13.85 mg/kg/day (100 ppm) (based on an increase in mean relative liver weights in the satellite group animals). Acceptable. (Corlett and Leung, 05/22/2012)

53165-0043, 262227; "S-2188: Two-generation reproduction toxicity study in the Han Wistar rat"; (Harlan Laboratories, LTD (formerly RCC, LTD), Switzerland, Laboratory Project ID: Harlan Laboratories Study no. A08954, Gerspach, R., Weber, K. and Flade, D., 4/29/09); S-2188 Technical grade, Lot no. 030-050914-1G, 94.7% pure; Groups of 24 male and 24 female Han Wistar rats were administered by diet admixture to 0, 400, 1000 or 3000 ppm test substance S-2188 for at least 70-day pre-pairing period, during pairing period and, for females, during gestation and lactation periods. The P generation dams were allowed to rear the post-culling F1 pups to day 21 post partum before being sacrificed. On or soon after day 21 post partum, 24 male and 24 female F1 pups per group were selected randomly from as many different litters as possible to provide the F1 generation. The selected F1 animals were reared on their respective test diets for at least 70 days prior to pairing, during pairing, and for females, during gestation and lactation until day 21 post partum. F1 dams were allowed to rear their post-culling young in the lactation periods until day 21 post partum. On or soon after day 21 post partum all F2 pups and F1 parent animals were sacrificed. There were no test substance related mortality or clinical signs. P generation: Lower food consumption and body weights throughout the pre-pairing, gestation and lactation periods, and lower body weight gains throughout the pre-pairing and gestation periods were observed in group 4 females. Thickened (enlarged) thyroid was recorded in 13/24 males in group 4. Increased incidents of enlarged liver and enlarged thyroid were observed in group 4 females at terminal examination. Increased absolute and relative liver weights in group 3 and 4, increased absolute and relative thyroid weights in group 4 males were observed. In females, absolute liver weight was increased in group 4, relative liver weight, absolute and relative thyroid weight were increased in group 3 and 4. Increased postnatal loss, lower body weights and body weight gains were noted in group 4 F1 pups. Enlarged thyroid was recorded in F1 pups. F1 generation parent animals: Statistically significantly lower food consumption, higher relative food consumption was noted in pre- and after pairing period in group 4 males. Mean body weight was statistically significantly lower in groups 3 and 4 males in pre-pairing period and in group 4 males after pairing. In after pairing period, lower body weight in group 3 males was observed without statistical significance. Statistically significantly lower food consumption and body weights were noted for group 4 female throughout the pre-pairing, gestation and lactation periods. Implantation rate was statistically significantly decreased and post-implantation loss was statistically significantly increased in group 4, as a result, the mean litter size was statistically significantly lower in group 4. In increased postnatal loss occurred in group 2, 3 and 4. Enlarged thyroid was found in group 3 and 4 females at terminal examination. Statistically significantly increased absolute liver weight in group 4, relative liver weight in group 3 and 4, and relative thyroid weight in group 4 was observed in F1 males. Statistically significantly higher mean absolute thyroid weight in group 3 and 4, relative weights of liver and thyroid weights in group 4 occurred in F1 females. F2 pups: Increased number of pups was found dead in group 3 at first litter check after birth. Mean weights of both male and female group 4 pups were statistically significantly lower from day 1 post partum until weaning. Pathology data: Increased incidences of enlarged livers and thyroid glands occurred in groups 3 and 4 at macroscopic examinations. Microscopic examinations revealed increased hepatocellular hypertrophy, mainly centrilobular, in the liver of P and F1 parent animals at 1000 and 3000ppm groups, except for the male P generation animals at 1000 ppm group. Follicular hypertrophy was noted in the thyroid gland of P and F1 parent animals in both sexes at 1000 and 3000ppm groups, except for the male F1 animals at 1000 ppm. Parental NOEL: 400 ppm due to changes in liver and thyroid weight in P and F1 generation parent animals, lower body weight and microscopic findings in P and F1 parent animals. Reproductive NOEL: 1000 ppm due to reduced implantation and
increased post-implantation loss in F1 generation group 4 offspring. **Offspring NOEL:** < 400 ppm
due to increased post-natal loss in F1 generation offspring. **Study acceptable.** (Pan and Leung,
4/9/2012)

**  53165-0042, 262226; "S-2188: Preliminary reproduction toxicity study in the Han Wistar rat ";
(RCC, LTD, Switzerland, Laboratory Project ID: RCC Study no. A58948, Pössnecker, A., and
Flade, D., 1/2/08); S-2188 Technical grade, Lot no. 030-050914-1G, 94.7% pure; Groups of 5 male
and 5 female Han Wistar rats were exposed by feed admix to 0 (only diet), 400, 1000 or 3000 ppm
test item once daily on 14 days prior to pairing, during the pairing, gestational and lactational
(only dams) periods until day 21 post partum. Eight pups of each litter were necropsied on day 28 post
partum, a week (on respective diets) after weaning. The male animals were sacrificed after the
gestation period of the dams. There was no mortality or treatment related clinical signs. Reduction
of body weight was observed in treated males during the after pairing period and in high dose group
females during gestation period. During the first week of the pre-pairing period (days 1-8) and first
week after pairing, mean relative food consumption (grams of food consumed/kg of body
weight/day) was statistically significantly higher for the high dose males. Reproduction data
indicates decreased mean number of live pups and increased post-implantation loss in the high
dose group. The mean food consumption between day 21 and day 28 post partum for the F1 pups
was decreased in high dose group. **Study supplemental.** (Pan and Leung, 3/27/2012)

**  TERATOLOGY, RAT

**  53165-0040, 262222; "S-2188: Prenatal developmental toxicity study in Han Wistar rat ";
(Harlan Laboratories, LTD (formerly RCC, LTD), Switzerland, Laboratory Project ID: Harlan
Laboratories Study no. B77466, Gerspach, R., and Flade, D., 3/23/09); S-2188 Technical grade,
Lot no. 030-050914-1G, 94.7% pure; Groups of 22 mated female Han Wistar rats were exposed by
gavage to 0, 30, 125 or 500 mg/kg test item in 1% CMC (carboxymethyl cellulose) once daily from
day 6 to 20 post coitum. There was no mortality or treatment related clinical signs. Statistically
significant decrease of food consumption, body weight and body weight gain was observed in 500
mg/kg group female toward the end of the treatment period. There was a statistically significant
decrease in body weight gain in the 125 mg/kg group females at days 19 and 20. Reproduction data
indicates decreased mean fetal body weight and increased placental weight in the high dose group.
**Maternal NOEL:** 30 mg/kg for decreased body weight gain in the 125 and 500 mg/kg group dams;
**Developmental NOEL:** 125 mg/kg for decreased fetal body weight and increased placenta weight
in the high dose group. **Study acceptable.** (Pan and Leung, 3/23/2012)

**  53165-0041, 262224; "S-2188: Dose range-finding developmental toxicity study in Han Wistar
rat"; (Pössnecker, A., RCC, LTD, Toxicology, Switzerland, Laboratory Project ID: RCC Study no.
A08908, 11/3/06); S-2188 Technical grade, batch no. 030-050914-1G, 94.7% pure; Groups of 5
mated Han Wistar rats were exposed to test substance at 150, 300 or 500 mg/kg by gavage from
day 6 to 20 post coitum. Additional 5 rats were exposed to the control vehicle, 1% CMC by gavage
from day 6 to 20 post coitum. One female from the 500 mg/kg group was found dead on day 11 post
coitum. No clinical signs were observed for any females in the study. Decreased body weight gain
was observed in the females of the 300 and 500 mg/kg groups from around day 16 to 21
post-coitum. Decreased total food consumption was noted in 500 mg/kg group females. Necropsy
findings of the female found dead in the 500 mg/kg group included stomach filled with gas, contents
of stomach showed little reddish particles and the fore stomach revealed a reddish surface. The
lungs were partly emphesematous and with reddish colorations. No treatment related changes
were found in necropsy for surviving females, and reproduction data. Mean fetal body weights were
decreased for the test substance treated animals, with statistically significant reduction of fetal
weights observed in the 500 mg/kg group. Mean weights of placenta were statistically significantly
increased with treatment of test substance. External examinations and skeletal examinations
revealed no abnormal findings. **Study supplemental.** (Pan and Leung, 3/21/2012)

**TERATOLOGY, RABBIT**

** 53165-0041, 262225; "Teratology study in rabbits with S-2188 technical grade "; (Sumitomo Chemical Company, LTD, Environmental Health Science Laboratory, Konohana-ku, Osaka, Japan, Laboratory Project ID: Study no.4073, Inawaka, K., 7/23/08); S-2188 Technical grade, Lot no. 030-050914-1G, 94.7% pure; Groups of 24 artificially inseminated Kbl:NZW SPF rabbits were exposed once daily to test material at 0, 30, 50 or 90 mg/kg by oral gavage from gestation day 6 through 27. No mortality. The numbers of pregnant animals were 20, 19, 21 and 22 in control, 30, 50 and 90 mg/kg, respectively. Abortions and premature deliveries were observed in 1 and 7 animals of 50 and 90 mg/kg groups, respectively. Clinical signs including orange urine and red fluid under grid floor of cage were observed in animals that aborted or premature delivered. Severe body weight loss was observed in animals that aborted or prematurely delivered. Reduced food consumption was noted in 50 and 90 mg/kg group females. Necropsy results of the animals that aborted and premature delivered found muddy and dark brown content in the cecum, pale heart, pale liver, white focus in the liver and distension of the gallbladder. Pale liver was also observed in one animal of the 50 mg/kg group. There were no treatment-related findings in any parameters for caesarean section findings. **Maternal NOEL:** 30 mg/kg due to reduced maternal food consumption and body weight changes at 50 and 90 mg/kg dose levels; **Reproductive NOEL:** 50 mg/kg due to increased abortion and premature deliveries in the 90 mg/kg group females. **Developmental NOEL:** 90 mg/kg due to no evidence of developmental and fetal toxicity at dose levels tested. **Study acceptable.** (Pan and Leung, 3/26/2012)

53165-0039, 262221; "Dose range-finding teratology study in rabbits with S-2188"; (Inawaka, K., Sumitomo Chemical Company, LTD, Environmental Health Science Laboratory, Konohana-ku, Osaka, Japan, Laboratory Project ID: Study no. D0279, 6/23/08); S-2188 Technical grade, Lot no. 030-050914-1G, 94.7% pure; Groups of 5 inseminated rabbits were exposed to test substance at 60, 90, 120 or 150 mg/kg by gavage from gestation day 6 to 27. Six more were exposed to the control vehicle, 1% CMC (aqueous solution of sodium carboxymethyl cellulose in water), one of which was excluded from the study on gestation day 6 due to red fluid under grid floor of cage. There were 2 non pregnant animals, one from 90 mg/kg group and one from 150 mg/kg group. Maternal death was observed in 1 animal from the 120 mg/kg group and 1 animal from the 150 mg/kg group. Abortions or premature deliveries were observed in 1, 2, and 3 animals in 90, 120, and 150 mg/kg groups, respectively. Clinical signs including orange urine, red fluid under grid floor of cages and red fluid around vagina were observed in the 120 and 150 mg/kg groups. The animal that died in the 150 mg/kg group was not pregnant and was found dead after showing orange urine, red fluid under grid floor of cage and pale eyes. Decreased (not statistically significant) body weight change and food consumption was observed in the females of the 120 and 150 mg/kg groups during most of the treatment period. No treatment related changes were found in necropsy for surviving females, at maternal caesarean section and external findings for live fetuses. **Study supplemental.** (Pan and Leung, 3/19/2012)

**GENE MUTATION**

** 53165-0047; 262231; “Reverse Mutation Test of S-2188-DC in Bacterial Systems” ; (S. Kitamoto; Sumitomo Chemical Company, Ltd, Environmental Health Science Laboratory, Konohana-ku, Osaka, Japan; Study No. 4082; 1/30/08); S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA were treated with S-2188-DC (Fenpyrazamine Technical) (lot no. 07SC4171306; purity: 100%) at concentrations ranging from 156 to 5000 ug/plate with a preincubation of 20 minutes, followed by plate incorporation and incubation for 48 hours at 37°C under conditions of activation and non-activation. Two trials were performed with triplicate samples for each treatment level. A phenobarbital and 5,6-benzoflavone-induced rat liver S9
fraction was used to metabolize the test material. There was no treatment-related increase in the incidence of reverse mutation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/20/12)

** 53165-0047; 262232; “Reverse Mutation Test of S-2188-Technical Grade in Bacterial Systems”; (S. Kitamoto; Sumitomo Chemical Company, Ltd, Environmental Health Science Laboratory, Konohana-ku, Osaka, Japan; Study No. 4032; 9/27/06); S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2uvrA were treated with S-2188 Technical Grade (Fenpyrazamine Technical) (lot no. 030-050914-1G; purity: 94.7%) at concentrations ranging from 156 to 5000 ug/plate with a preincubation of 20 minutes, followed by plate incorporation and incubation for 48 hours at 37°C under conditions of activation and non-activation. Two trials were performed with triplicate samples for each treatment level. A phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the incidence of reverse mutation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/21/12)

** 53165-0047; 262233; “Gene Mutation Assay in Chinese Hamster V79 Cells In Vitro (V79/HPRT) with S-2188 Technical Grade”; (H. E. Wollny; RCC Cytotest Cell Research GmbH, D-64380 Rossdorf, Germany; Study No. 1043100; 6/18/07); Chinese hamster V79 cells were exposed for 4 hours at 37°C to S-2188 Technical Grade (Fenpyrazamine Technical) (lot no. 030-050914-1G; purity: 94.7%) at concentrations ranging from 2.5 to 60 μg/ml (non-activation) and 12.5 to 200 ug/ml (activation) in the first trial. In the second trial, the cells were exposed for 24 hours under conditions of non-activation at concentrations ranging from 25 to 130 μg/ml and for 4 hours under conditions of activation at concentrations ranging from 20 to 160 μg/ml. A phenobarbital and beta-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the mutation frequency in either of the trials. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/23/12)

** 53165-0047; 262234; “In Vitro (V79/HPRT) Chromosomal Aberration Test on S-2188 Technical Grade in Chinese Hamster Lung Cells (CHL/IU)”; (S. Kitamoto; Sumitomo Chemical Co. Ltd., Konohana-ku, Osaka 554-8558, Japan; Study No. 4029; 12/20/06); Chinese hamster V79 were exposed to concentrations of S-2188 Technical Grade (Fenpyrazamine Technical) (lot no. 030-050914-1G; purity: 94.7%) ranging from 60 to 150 ug/ml w/o activation and from 20 to 160 ug/ml with activation for 6 hours, followed by an additional 18 hours of incubation in the first experiment. In the second experiment, cells were exposed for 24 hours under conditions of non-activation at concentrations ranging from 25 to 130 μg/ml and for 4 hours under conditions of activation at concentrations ranging from 20 to 160 μg/ml. A phenobarbital and beta-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. The increased incidence of chromosomal aberrations at the highest treatment levels (+activation) was not sufficient to identify it as a positive effect. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/26/12)

** 53165-0047; 262235; “Micronucleus Test on S-2188 Technical Grade in CD-1 Mice”; (S. Kitamoto; Sumitomo Chemical Co. Ltd., Konohana-ku, Osaka 554-8558, Japan; Study No. 4030; 5/30/07); Five male CD1 mice/group/time point were treated orally by gavage with 0 (aqueous 0.5% methyl cellulose) or 2000 mg/kg of S-2188 Technical Grade (Fenpyrazamine Technical) (lot no.
030-050914-1G; purity: 94.7%) and euthanized at 24 and 48 hours post-dose. An additional 5 animals/group were dosed with 500 or 1000 mg/kg and euthanized at 24 hours post-dose. As a positive control, another group of 5 male mice was treated by oral gavage with 60 mg/kg of cyclophosphamide and euthanized at 24 hours post-dose. The number of micronucleated polychromatic erythrocytes (PCE) in 2000 PCEs/animal and the ratio of PCEs to the total number of erythrocytes were reported. There was no treatment-related increase in the percentage of micronucleated PCEs. **No adverse effect indicated.** Positive control was functional. **Study acceptable.** (Moore, 3/26/12)

**NEUROTOXICITY**

**Rat Acute Neurotoxicity Study**

**S-2188 Technical: Acute Oral Neurotoxicity (Gavage) Study in Rats**;
(E.W. Sommer; RCC, LTD, Zeliweg 1, CH-4452 Itingen, Switzerland; Study No. B36336; 6/13/08);
Ten Hanlbm:WIST (SPF) rats/sex/group were dosed orally by gavage with 0, 80, 400 or 2000 mg/kg of S-2188 Technical (batch no. 030-050914-1G; purity: 94.7%). No deaths resulted from the treatment. There was no treatment-related effect upon the mean body weights or food consumption. No treatment-related effects were exhibited in any of the functional domains or functional measurements of the functional observational battery (FOB). The session totals for total distance and number of rearings in the motor activity assessment were significantly lower for 400 and 2000 mg/kg males on the 1st day at 2 hours post-dose (p<0.01 or 0.05). The total number of rearings for the 2000 mg/kg females was less than that of the control group as well (p<0.01). There was no treatment related effect upon brain weight or treatment-related lesions in the nervous tissues of the study animals. Due to the lack of treatment-related effects in other domains in the FOB, the apparent treatment-related effect upon two of the motor activity parameters was not deemed to be neurotoxicologically significant. **No adverse effect evident.** Reported Rat Acute Neurotoxicity NOEL: (M/F) 2000 mg/kg (based upon the lack of treatment-related effects at the 2000 mg/kg treatment level) **Study unacceptable**, possibly upgradeable to acceptable with the submission of concurrent positive control study data. (Moore, 3/29/12)

The study was upgraded to acceptable upon submitting of an acceptable positive control study: “Acrylamide: 28-Day Oral Neurotoxicity (Gavage) Validation Study in Rats (Document #: 53165-0056, Record #: 268397)”. The positive control study demonstrated the evidence of the ability of personnel to observe major neurotoxic endpoints including motor activity and neuropathology parameters, and of the sensitivity and reliability of the activity-measuring devise and procedures. **Acceptable.** (Moore, 3/29/12, upgraded, Pan & Leung, 1/11/13)

30-050914-1G; purity: 94.7%). No deaths resulted from the treatment. No treatment-related lesions noted in the neuropathy parameters. **Study supplemental.** (Moore, 4/17/12)

**Rat Subchronic Neurotoxicity Study**

**S-2188 Technical: 90-Day Oral Neurotoxicity Peak-Effect Study in Rats**;
(E.W. Sommer; RCC, LTD, Zeliweg 1, CH-4452 Itingen, Switzerland; Study No. B37721; 8/23/07);
Three Wistar rats/sex/group were dosed orally by gavage with 0 (vehicle: aqueous 1% carboxymethylcellulose), 100, 1000, or 2000 mg/kg/day of S-2188 Technical (batch no. 030-050914-1G; purity: 94.7%). No deaths resulted from the treatment. No treatment-related clinical signs were evident during the 7-day observation period. There were no treatment-related lesions noted in the necropsy. **Study supplemental.** (Moore, 4/17/12)
group were less than the control values over the course of the study (p<0.01 or 0.05). The mean food consumption of both sexes in the 3000 ppm group was less than that of the controls (p<0.01 or 0.05). No apparent treatment-related effects were evident in the FOB or motor activity assessments. The mean relative brain weight of the 3000 ppm females was greater than the control value (p<0.05). However, no lesions were noted in the histopathological examination of the nervous tissue. No neurotoxic adverse effect was noted. Reported Rat Subchronic Neurotoxicity NOEL: (M/F) 3000 ppm ((M) 223.6 mg/kg/day, (F) 248.4 mg/kg/day) (based upon the lack of neurotoxicity-related effects noted in the 3000 ppm treatment group). Study unacceptable, possibly upgradeable to acceptable with the submission of concurrent positive control study data. (Moore, 4/17/12)

The study was upgraded to acceptable upon submitting of an acceptable positive control study: “Acrylamide: 28-Day Oral Neurotoxicity (Gavage) Validation Study in Rats (Document #: 53165-0056, Record #: 268397)”. The positive control study demonstrated the evidence of the ability of personnel to observe major neurotoxic endpoints including motor activity and neuropathology parameters, and of the sensitivity and reliability of the activity-measuring devise and procedures. Acceptable. (Moore, 3/29/12, upgraded, Pan & Leung, 1/11/13)

Rat Positive Control Study
53165-0056, 268397 “Acrylamide: 28-Day Oral Neurotoxicity (Gavage) Validation Study in Rats”, rat, (Sommer, E.W., Flad, D., and Krinke, G. J., 1/5/2005, RCC, LTD, Zeliweg 1, CH-4452 Itingen, Switzerland. Laboratory Project ID: RCC Study No. 852323). Acrylamide, white crystalline powder, batch no. 433301/1 34203452, >99.5% pure. Groups of 10 HanBrl:WIST (SPF) rats/sex were exposed to acrylamide in 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80, at 0, 3, 10 or 30 mg/kg/day by oral gavage for 28 days. The high dose group animals received 30 mg/kg/day for 17 days only due to the progressive manifestation of severe gait abnormalities. Mortality: One male and 2 female rats in the high dose group were killed in extremis or found dead near the end of the study. Clinical signs were observed in high dose group rats starting week 3 till the end of week 4: uncoordinated movements, paresis in males and females of high dose group, decreased spontaneous activity and ruffled fur in all females, breathing noise in one female. Weekly outside cage observations in high dose group rats in weeks 3 and 4 included piloerection, hunched posture, uncoordinated movements, hindleg paresis, decreased spontaneous activity, dyspnea, no push-off kind legs, pain response and red. muscle tone. Iridic light reflex and prolonged rearing were observed only in females. Statistically significantly decreased forepaw and hindpaw grip and body temperature, statistically significantly increased landing foot was observed in high dose rats in week 4 at Functional observational battery. Statistically significantly decreased body temperature was also observed in 3 and 10 mg/kg/day male rats in week 4. Statistically significantly decreased motor activity including distance traveled, rearing and center time was observed in high dose group males at week 3 and 4, in high dose group females at weeks 2, 3 and 4. Decreased (not statistically significantly) food consumption in high dose group males and females in week 2 to 4 was observed. Statistically significantly decreased body weight from day 15 to 28, and statistically significantly decreased body weight gain from day 8 to 28 was observed in high dose males and females. Statistically significantly decreased body weight gain was also observed in 10 mg/kg/day females at days 15 and 28. Pathology report indicated that the high dose male killed in extremis and one of the females found dead during the study had prominent peripheral neuropathy, especially affecting their dorsal root ganglia. The other high dose female found dead during the study could not be evaluated due to autolysis. Treatment related microscopic findings included presence of necrotic Purkinje cells in the cerebellum of high dose rats, neuronal chromatolysis of Gasserian ganglion and dorsal root ganglion in 30 and 10 mg/kg/day group rats, neuronal cytoplasmic vacuolation of Gasserian ganglion in 10 mg/kg/day group, neuronal cytoplasmic vacuolation of dorsal root ganglion in 30 mg/kg/day group rats, degeneration of nerve fibers of Gasserian ganglion, dorsal root ganglion, dorsal and ventral spinal nerve root, sciatic
nerve, tibial nerve, plantar nerve and gastrocnemius muscle in 30 mg/kg/day group rats. Degeneration of nerve fibers of ventral spinal nerve root and gastrocnemius muscle in 10 mg/kg/day group rats was also observed. NOEL (No Observed Effect Level): 3 mg/kg/day based on pathological findings. Acceptable. (Pan & Leung, 1/11/13)

**RAT METABOLISM**

53165-0051, 262239; “The metabolism and excretion of [14C] S-2188 in the rat upon administration of single oral high and low doses “; (Analytical Phase: PTRL West, Inc., Hercules, CA 94547; In-Life Phase: Northview Pacific Laboratories, Hercules, CA, 94547), Laboratory Project ID: PTRL West 1440W (Sumitomo Chemical Company No.: QNM-0027), Dohn, D., Kovatchev, A. and Estigoy, L. 9/30/07; [14C]S-2188, specific activity: 55 mCi/mmol (2.04 GBq/mmol), Manufacturer Lot no. CFQ14368 Batch 1; Groups of four male and 4 female rats were dosed by oral gavage with 3 mg/kg (low dose group) or 300 mg/kg (high dose 1 group) [14C] S-2188. Additional 6 rats (4M/2F) were dosed with 300 mg/kg [14C] S-2188 (high dose 2 group) due to urine collection failure of the 6 (4M/2F, high dose 1 group) rats previously dosed. Urine and cage wash, expired air, and feces were collected continuously for up to 7 days after dosing. The test compound was metabolized and excreted quickly within the first two days after dosing, with majority of the excretion occurring in urine (80%-85%, 48hrs) and feces (8.5%-11.7%, 48hrs). Negligible radioactivity was detected in the CO2 traps. Residual radioactivity was measured in selected tissues at 7 days after dosing. The residual amounts of radioactivity in tissues were insignificant at 7 days after dosing. Significant metabolites were identified from pooled urine and feces samples. Study acceptable. (Pan and Leung, 4/17/2012)

53165-0051, 262240; “The metabolism, excretion and tissue distribution of [14C] S-2188 in the rat upon administration of repeated doses”; (Analytical Phase: PTRL West, Inc., Hercules, CA 94547; In-Life Phase: Northview Pacific Laboratories, Hercules, CA, Project ID: PTRL West 1555W (Sumitomo Chemical Company No.: QNM-0026), Quistad, G. and Kovatchev, A., L. 9/24/07; [14C]S-2188, specific activity: 55 mCi/mmol (2.04 GBq/mmol), Manufacturer Lot no. RIS2006-008; Thirty six rats (18M/18F) were dosed by oral gavage with 3 mg/kg [14C] S-2188 daily for up to 14 consecutive days. Groups (A to F) of 3 males and 3 females were sacrificed on day 2, 7, 11, 15, 19 or 24. The concentrations of S-2188 derived 14C were measured in selected tissues/organs and in the carcass at each time interval. The radioactive residues in tissues and carcasses were low throughout the study period. Measurement of 14C in excreta was performed in Group F rats during entire time on test. Analysis of metabolites was carried out in selected tissues and selected excreta samples of Group D and F rats, respectively. Major metabolites in tissues measured were a mixture of S-2188-CH2OH-DC and MPPZ, those in excreta were a mixture of S-2188-CH2OH-DC and MPPZ, MPPZ sulfate and S-2188-DC. Study supplemental. (Pan and Leung, 4/23/2012)

53165-0052, 262241; “The pharmacokinetics of [14C] S-2188 in the rat upon administration of single oral low and high doses” ; (Analytical Phase: PTRL West, Inc., Hercules, CA 94547; In-Life Phase: Northview Pacific Laboratories, Hercules, CA, Project ID: PTRL West 1434W (Sumitomo Chemical Company No.: QNM-0022), Dohn, D., L. 9/10/07; [14C]S-2188, specific activity: 55 mCi/mmol (2.04 GBq/mmol), Manufacturer (Amersham Biosciences, UK) code: CFQ14368 Batch 1; S-2188 (Lot No. 4CM03-R2G, purity of 99.4%) was used to dilute [14C] S-2188 prior to application and reference standard; Thirty three rats (16M/17F) fitted with jugular vein cannulae received a single dose of 3 mg/kg or 300 mg/kg [14C] S-2188 by oral gavage. Blood was drawn at specific intervals into heparinized tubes up to 5 days after dose administration. Study supplemental. (Pan and Leung, 4/23/2012)

53165-0053; 262242; “The Tissue Distribution of [14C]S-2188 Technical in the Rat upon Administration of Single Oral High and Low Doses”; (G.B. Quistad, A. Kovatchev; Northview Pacific
Laboratories, Hercules, CA and PTRL West, Inc., Hercules, CA; Project ID No. PTRL West 1441W; 9/6/07); Twelve Wistar rats/sex/group were dosed orally by gavage with 3 or 300 mg/kg of \([^{14}C]\)S-2188 Technical (lot no. CFQ14368 Batch 1, radiochemical purity: 99.4%; chemical purity: 99.9%; specific activity: 55 mCi/mmol). The specific activity of the dosing preparation was adjusted with unlabeled S-2188 Technical (lot no. 4CM03-R2G, purity: 99.4%). For the 3 mg/kg group, 3 animals/sex/time point were euthanized at 0.25, 1, 4, and 12 hours post-dose. For the 300 mg/kg group, 3 animals/sex/group were euthanized at 0.5, 6, 24 and 72 hours post-dose. Tissue distribution of the radiolabel was ascertained for each time point for both cohorts. The radiolabel in the plasma, liver and kidneys was further assayed for metabolites. The times for the euthanizations were selected based on the respective pharmacokinetic parameters; \(1/2\) Cmax (absorption phase), Cmax, \(1/2\) Cmax (elimination phase), and 1/10 Cmax (see project no. 1434W). Over the time course, the highest concentration of radiolabel was recovered from the stomach and stomach contents at 0.25, 1, and 4 hours post-dose for both sexes in the 3 mg/kg treatment group. At 12 hours, the highest level was found in the caecum and remainder of the large intestine and the contents of these two regions. The liver, kidneys and small intestine with its contents also demonstrated levels which were more than twice as great as that of plasma over the course of the sampling period. For both sexes in the 300 mg/kg group, the stomach with its contents was the site of the highest concentration of radiolabel throughout the 72-hour sampling period. In contrast to the 3 mg/kg group, levels which were achieved in the caecum and remainder of the large intestine percentagewise were much less than that recovered from the stomach over the sampling time course. By 72 hours, only the stomach had a concentration of radiolabel which was greater than 2 times that of the plasma. For the 3 mg/kg group, radiolabel in the stomach and its contents represented 77 and 71, 61 and 49, and 60 and 35% of the recovered radiolabel at 0.25, 1, and 4 hours post-dose for the males and females, respectively. The radiolabel in the caecum and the remainder of the large intestine and the contents of these regions at 12 hours post-dose represented 49% of the recovered radiolabel for both the males and females. For the 300 mg/kg group, the radiolabel in the stomach and its contents represented 83 and 68, 57 and 53, and 88 and 77% of the recovered radioactivity at 0.5, 6 and 24 hours post-dose for the males and females, respectively. The percentage of administered dose recovered at 72 hours was too minimal to assess the percent recovery in respective tissues. Among the moieties which were identified in the plasma, liver and kidneys, S-2188-DC and the combined total of S-2188-DC and MPPZ were the predominate metabolites for both sexes at both treatment levels. The parent compound represented a minor portion of the identified radiolabel. Over time, the percentage of the recovered radiolabel which was assigned to an identifiable metabolite decreased. An increasing percentage of the radiolabel in the liver was unextractable. **Study supplemental.** (Moore, 4/25/12)

**SUBCHRONIC TOXICITY**

**Rat Subchronic Dietary Toxicity Study**

** 0038; 262218; “S-2188 Technical: 13-Week Repeated Dose Oral Toxicity (Feeding) Study in the Wistar Rat” (Sommer, E.W. et al., RCC Ltd, Toxicology, Itingen, Switzerland, RCC Study No. A08886 (Sumitomo Chemical Company no. QNT-0009), 12/21/2006). 870.3100. S-2188 Technical (Batch number 030-050914-1G, purity = 94.7%) was admixed to the diet and fed to 12 Wistar rats per sex per dose at dose levels of 0 (diet only), 300, 600, 1000, or 3000 ppm (0, 19.12, 37.69, 64.00, and 196.11 mg/kg/day, respectively for males, and 0, 20.54, 42.04, 68.56, and 207.32 mg/kg/day, respectively for females) for 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. Occasional decreases in mean body weight and mean food consumption were observed in both sexes at 3000 ppm during the treatment period. Hematology, clinical chemistry, and urinalysis revealed no treatment-related effects. A treatment-related increase in mean relative liver weight was observed in both sexes at 3000 ppm. Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination
revealed treatment-related centrilobular hepatocellular hypertrophy in both sexes at 3000 ppm and treatment-related follicular thyroid cell hypertrophy in males at 3000 ppm. **No adverse effects.** NOEL (M) = 64.00 mg/kg/day (1000 ppm) and NOEL (F) = 68.56 mg/kg/day (1000 ppm) (based on centrilobular hepatocellular hypertrophy and an increase in mean relative liver weights). **Acceptable.** (Corlett and Leung, 04/17/2012)

**Rat 28-Day Repeated Dosing Dermal Toxicity Study**

No adverse effects. NOEL (M/F, systemic and skin) = 1000 mg/kg/day (based on no effects at the highest dose tested). **Acceptable.** (Corlett and Leung, 05/04/2012)

**Rat 4-Week Immunotoxicity Study**

No adverse effect indicated. The positive control was functional. **Study acceptable.** (Moore, 4/26/12)

**Dog Subchronic Oral Toxicity Study**

Ophthalmology revealed no treatment-related ocular lesions. Statistically significant decreases in mean red blood cell and mean hemoglobin levels at weeks 4 and 8 and in mean corpuscular hemoglobin concentration at week 8 in males and statistically increases in mean corpuscular volume and in mean platelet count at weeks 8 and 13 in males were observed at 150 mg/kg/day; a statistically significant increase in mean corpuscular volume at weeks 8 and 13 and a statistically
A significant decrease in mean corpuscular hemoglobin concentration at week 8 in females at 150 mg/kg/day were observed. Statistically significant increases in the alanine aminotransferase level in males at 150 mg/kg/day and in the gamma-glutamyl transpeptidase level in females at week 13 at 50 and 150 mg/kg/day and in the mean total bilirubin level at weeks 4 and 8 and in the mean urea nitrogen level at week 4 in males at 150 mg/kg/day were observed; statistically significant decreases in the mean albumin level in males at weeks 4 and 8 and in the mean total cholesterol and mean phospholipids levels in females at weeks 4 and 13 at 150 mg/kg/day were observed. Urinalysis revealed no treatment-related effects. A treatment-related increase in mean relative liver weight was observed in males at 150 mg/kg/day. Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination revealed treatment-related centrilobular hepatocellular hypertrophy in both sexes at 50 and 150 mg/kg/day. No adverse effects. NOEL (M/F) = 25 mg/kg/day (based on centrilobular hepatocellular hypertrophy). Acceptable. (Corlett and Leung, 04/26/2012)

**Mouse Subchronic Dietary Toxicity Study**

"0037; 262217; “S-2188 Technical: 13-Week Repeated Dose Oral Toxicity (Feeding) Study in the CD-1 Mouse" (Sommer, E.W. et al., RCC Ltd, Toxicology, Itingen, Switzerland, RCC Study No. A08864 (Sumitomo Chemical Company no. QNT-0011), 03/12/2007). 870.3100. S-2188 Technical (Batch number 030-050914-1G, purity = 94.7%) was admixed to the diet and fed to 12 CD-1 mice per sex per dose at dose levels of 0 (diet only), 200, 2000, 4000, or 6000 ppm (0, 27.96, 296.21, 639.54, and 1022.57 mg/kg/day, respectively for males, and 0, 33.48, 363.31, 719.60, and 1098.26 mg/kg/day, respectively for females) for 13 weeks. No mortalities occurred. No effects on mean body weights were observed. No toxicologically relevant effect on food consumption was observed. A treatment-related decrease in mean hematocrit levels at 4000 and 6000 ppm in males and at 6000 ppm in females was observed. A treatment-related increase in mean relative liver weight was observed in both sexes at 2000, 4000, and 6000 ppm. Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination revealed treatment-related centrilobular hepatocellular hypertrophy in both sexes at 2000, 4000, and 6000 ppm. No adverse effects. NOEL (M) = 27.96 mg/kg/day (200 ppm) and NOEL (F) = 33.48 mg/kg/day (200 ppm) (based on centrilobular hepatocellular hypertrophy and an increase in mean relative liver weights). Acceptable. (Corlett and Leung, 04/11/2012)