CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Benzobicyclon

Chemical Code #6076 , **Tolerance** #53164
SB 950 # NA

4/4/13, 1/31/14

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible 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, possible adverse effect (non-reproductive)
Teratology, rat:	No data gap, possible adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, possible 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 #268660 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T140131
Revised by Name, T. Moore, 1/31/14

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

53154-0028 268644, "SAN1315H: 24-month Chronic Oral Toxicity and Carcinogenicity study in Rats"; 835; rat; The Institute of Environmental Toxicology, Tokyo, Japan, Study # IET 95-0168, 7/12/99; Kitazawa, T.; SAN1315 H, Lot No.6F0502, 99% pure, a pale yellow powder, was administered to 50/sex rats by feed at 0, 10, 20, 50 or 100 ppm for males [mean daily test substance intake: 0, 0.334, 0.667, 1.696 and 3.43 mg/kg/day] and 0, 100, 1000 or 10000 ppm for females [mean daily test substance intake: 0, 4.19, 42.2 and 427 mg/kg/day], respectively, for 24 months. Additional 35/sex rats were allotted to each of the dosing group as the satellite group. After weeks 26, 52 and 78, 10 males and 10 females from each group of satellite group were selected and served as interim kills for evaluation of chronic toxicity with the test substance. Mortality: 16, 9, 9, 12 and 6 for 0, 10, 20, 50 or 100 ppm male main study groups, respectively; 12, 6, 15 and 12 for 0, 100, 1000 and 10000 ppm female main study groups, respectively. No test substance treatment related changes in body weight, bodyweight gains, food consumption and food efficiency in main study group males and females were observed. High dose females showed clinical signs including red adhesive substance on integument as well as red sebum at macroscopic observation at final necropsy. Decreased urine pH, increased values of hematology parameters including MCV, MCH and MCHC in high dose group females, increased organ weights including liver, kidney, and spleen at interim kills at 25, 52 weeks or final sacrifice at 104 weeks, enlarged spleen at macroscopic observations were observed. Increased hyaline droplet deposition in kidney proximal tubular cells of the high dose males at interim kills at 52 and 78 weeks as well as in all was observed. **NOEL (No Observed Effect Level)**: for male rats: 50 ppm (1.696 mg/kg/day) due to histopathological findings in kidney; for female rats: 1000 ppm (42.2 mg/kg/day) due to clinical signs, urinalysis, organ weights and macroscopic findings. **Acceptable** (Pan& Leung, 2/14/13).

CHRONIC TOXICITY, RAT

See Combined, Rat above.

CHRONIC TOXICITY, DOG

53154-0026 268642, "SAN 1315H: Chronic toxicity study by oral administration to Beagle Dogs for 52 weeks"; 831; Beagle Dog; Biosafety Research Center, Shizuoka 437-1213, Japan, Kitajima, S., Laboratory Study No.: 3307, 6/2/99; SAN 1315H, Batch No.6F0502, 99% pure, a pale yellow powder, was administered to 4/sex Beagle Dogs orally by gelatin capsule at 0, 10, 100, or 1000 mg/kg/day for 52 weeks. Animals were observed for general condition, hematology, blood chemistry, ophthalmology and urinalysis. No mortality. No test substance treatment related changes in body weight, bodyweight gains, food consumption, hematology, blood chemistry, ophthalmology and urinalysis organ weights and histopathology in both males and females were observed. **NOEL (No Observed Effect Level)**: for male and female Beagle Dogs: 1000 mg/kg/day. **Acceptable** (Pan& Leung, 2/5/13).

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

53154-0027 268643, "SAN 1315H: Carcinogenicity study by dietary administration to CD-1 Mice for 78 weeks"; 832; CD-1 Mice; Huntingdon Life Sciences Ltd., Cambridgeshire, PE17 5HS, England, Powell, L., Laboratory Study No.: SBT 007/983709, 8/16/99; SAN 1315H, Batch No.6F0502, 99% pure, a pale yellow powder, was administered to 50/sex CD-1 Mice orally by diet at 0, 300, 3000, or 30000 ppm [mean test substance intake: 37, 373 and 3817 mg/kg/day for males, 45, 473 and 4807

mg/kg/day for females, respectively] for 78 weeks. Animals were observed for general condition, hematology, during necropsy and histopathology. Mortality: 12, 21, 15 and 12 in males; 15, 13, 10 and 9 (one additional mouse was found dead during termination) in females of Groups 1, 2, 3, and 4, respectively. No test substance treatment related changes in body weight, bodyweight gains, food consumption, and hematology. Increased absolute and relative liver weight was observed in high dose females. Histopathology examination showed increased centrilobular hepatocyte hypertrophy in high dose males and females. No test substance treatment related changes in neoplastic findings. **NOEL (No Observed Effect Level)**: 3000 ppm (373 and 473 mg/kg/day for male and female CD-1 Mice, respectively) based on changes in liver histopathology. **Acceptable** (Pan& Leung, 2/8/13).

REPRODUCTION, RAT

** **53164-0031; 268647**; "A Two-Generation Reproduction Study in Rats with SAN 1315H"; (N. Shimizu; The Institute of Environmental Toxicology, Tokyo, 105-0014, Japan; Study No. IET 95-0169; 5/6/99); Twenty-four Crj:CD (SD) rats/sex/group were treated in the diet with 0, 100, 1000, or 20000 ppm of SAN 1315H (benzobicyclon technical) (lot no. 6F0502; purity: 99%) for 2 generations. The treatment periods for the P generation included 10 weeks prior to mating, mating, 3 weeks of gestation and 3 weeks of lactation. At that time 24 F1 animals/sex/group were selected as parents and treated for an additional 10 weeks, followed by mating and 3 weeks each for gestation and lactation of the F2 generation ((M) 0, 6.38 to 7.46, 63.6 to 73.3, 1320 to 1531 mg/kg/day; (F) 0, 5.97 to 13.0, 60.1 to 130.5, 1233 to 2664 mg/kg/day). There were no apparent treatment-related deaths among the parents nor treatment-related effects upon mean body weights. The mean food consumption of both sexes in the 20000 ppm groups of both generations was actually greater than that of the control group throughout much of the study. The mean absolute and relative liver weights for the females and the mean relative liver weight of the males in the 20000 ppm treatment group of both generations were greater than those of the controls ($p<0.05$, 0.01 or 0.001). However, there were no related histological findings. The mean absolute and relative adrenal weights of the 20000 ppm females in the P generation and the mean absolute adrenal weight of the 20000 ppm females in the F1 generation were greater than the control values ($p<0.001$). No histological lesions were noted in the adrenals of the F1 generation females. The mean absolute and relative weights of the testes and the epididymides of the 20000 ppm males in the F1 generation were greater than the control values ($p<0.05$, 0.01 or 0.001). However, no related histological lesions were evident. The mean absolute and relative kidney weights of both sexes in the 20000 ppm group and the mean relative kidney weight of the 1000 ppm males were greater than the control values for both generations (NS, $p<0.05$, 0.01 or 0.001). In the histopathological examination, increased hyaline droplet degeneration and tubular basophilic changes were noted in the proximal tubules of the kidneys of the 1000 and 20000 ppm males of both generations ($p<0.001$). There was an increased incidence of granular casts in cystically dilated renal tubules of the 20000 ppm males in both generations ($p<0.01$ or 0.001). An increased incidence of hydropic degeneration in the basophilic cells of the pituitary gland was also noted for the 20000 ppm males in the F1 generation ($p<0.01$). There was no apparent treatment-related effect on sperm count, motility, and morphology. There was no treatment-related effect upon the time to preputial separation in the males and the time to vaginal opening for the females of the F1 generation. There were no treatment-related effects upon the reproductive parameters or development of the pups. **Adverse effect indicated (non-reproductive endpoint):** increased hyaline droplet degeneration in the proximal tubules of the male rats. **Parental Systemic NOEL: (M)** 100 ppm (based on the renal lesions noted for the males in the 1000 ppm group of both generations, (M) 6.38 to 7.46 mg/kg/day), **(F)** 1000 ppm (based upon increased liver, kidney and adrenal weights noted for females of both generations in the 20000 ppm group, (F) 60.1 to 130.5 mg/kg/day); **Reproductive NOEL:** 20000 ppm (based upon the lack of treatment-related effects for the 20000 ppm group, (M) 1320 to 1531 mg/kg/day, (F) 1233 to 2664 mg/kg/day); **Developmental**

NOEL: 20000 ppm (based upon the lack of treatment-related effects on the development of the offspring in the 20000 ppm group); (F) 1233 to 2664 mg/kg/day); **Study acceptable**. (Moore, 1/28/13)

TERATOLOGY, RAT

**** 53164-0029; 268645**; "SAN 1315H: Oral (Gavage) Teratogenicty Study in the Rat"; (A. Fuchs; Covance Laboratories GmbH, 48163 Munster, Germany; Study No. 612-011; 7/7/97); Twenty five mated Sprague Dawley female rats/group were dosed orally, by gavage with 0, 40, 200 or 1000 mg/kg/day of SAN 1315H (benzobicyclon technical) (batch no. 960108N; purity: 99%) from gestation day 6 through gestation day 15. No deaths resulted from the treatment. There was no treatment-related effect on mean body weight gain or food consumption. There was no treatment-related effect on litter parameters or fetal body weight. The incidence of malformed fetuses was not treatment-related. The number of litters with fetuses having renal pelvic cavitation (unilateral and bilateral) was greater for the 1000 mg/kg treatment group (0: 6/22 vs. 11/24). **Possible adverse effect:** increased incidence of renal pelvic cavitation in the fetal kidney. **Maternal NOEL:** 1000 mg/kg/day (based upon the lack of treatment-related effects at the highest treatment level); **Developmental NOEL:** 200 mg/kg/day (based upon the increased number of litters having fetuses with renal pelvic cavitation in the kidneys of the 1000 mg/kg fetuses); **Study acceptable.** (Moore, 1/22/13)

TERATOLOGY, RABBIT

** 53164-0030; 268646; "SAN 1315H: Oral (Gavage) Teratogenicty Study in the Rabbit"; (A. Fuchs; Covance Laboratories GmbH, 48163 Munster, Germany; Study No. 612-010; 1/26/98); Eighteen mated New Zealand White female rabbits/group were dosed orally by gavage with 0, 40, 200 or 1000 mg/kg/day of SAN 1315H (benzobicyclon technical) (batch no. 960108N; purity: 99%) from gestation day 6 through gestation day 18. Some difficulties were encountered during the study due to the premature deaths of study animals as a consequence of gavaging errors. Therefore, 3, 1 and 2 additional animals, respectively, were assigned to the control, 40 and 200 mg/kg groups. However, in addition, three other animals in the control group were found dead on gestation days 12, 18 and 28, respectively. In the 40 mg/kg group, another animal was found dead on gestation day 17 (death possibly due to pneumonia). In the 200 mg/kg group, one animal was found dead on gestation day 19 (death possibly due to diarrhea). In the 1000 mg/kg group, two animals were found dead on gestation days 13 (possible intubation error) and 17, respectively. At the conclusion of the treatment period, there were 12, 13, 17 and 12 does in the 0, 40, 200 and 1000 mg/kg groups, respectively, which had litters of live fetuses. There was no treatment-related effect on body weight gain until the 15th to 19th day of gestation during which the 1000 mg/kg does lost weight. The mean food consumption for these animals was also less than that of the control group during this period. There was no apparent treatment-related effect upon the development of the fetuses. The number of litters with malformed fetuses increased from 0/12 for the control to 7/17 for the 200 mg/kg group. However, this effect was not apparent for the 1000 mg/kg group in which there were 2/12 litters with malformed fetuses. No adverse effect was apparent. Maternal NOEL: 200 mg/kg/day (based upon reduced body weight gain for the 1000 mg/kg does); Developmental NOEL: 1000 mg/kg/day (based upon the lack of treatment-related effects on the 1000 mg/kg fetuses); Study acceptable. (Moore, 1/23/13)

GENE MUTATION

** 53164-0032; 268648; "SAN 1315H Technical: Reverse Mutation Test"; (M. Akanuma; Kodaira Laboratories, The Institute of Environmental Toxicology, Kodaira-shi, Tokyo 187, Japan; Study No. IET 94-0051; 10/3/94); *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with SAN 1315H Technical (lot no. KS-2-146; purity was not reported) at concentrations ranging from 200 to 5000 ug/plate in the 1st trial and from 156 to 5000 ug/plate in

the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 1/29/13)

CHROMOSOME EFFECTS

**** 53164-0040; 268656**; "A Chromosomal Aberration Test of SAN 1315H in Cultured Chinese Hamster Cells"; (K. Saigo; Skin Nippon Biomedical Laboratories, Ltd., Kagoshima 891-13, Japan; Study No. SBL 52-12; 4/30/96); Chinese Hamster Lung cells (CHL/IU) were incubated with SAN 1315H (benzobicyclon technical) (lot no. 941208; purity: 99%) at concentrations ranging from 5 to 40 ug/ml, nonactivated, 24 hours of treatment, 2.5 to 20 ug/ml, non-activated, 48 hours of treatment and 5.0 to 40 ug/ml, activated, 6 hours of treatment, 18 hours of incubation, at 37° C. In all of the assays, the cells were incubated the last 2 hours with Colcemid prior to fixation. All of the incubations were performed with duplicate cultures. A phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was a treatment-related increase in the percentage of cells with chromosomal aberrations under conditions of both activation and non-activation. **Adverse effect indicated:** increased incidence of chromosomal aberrations. The positive controls were functional. **Study acceptable.** (Moore, 1/29/13)

DNA DAMAGE

** 53164-0041; 268657; "A Micronucleus Test of SAN 1315H in Mice"; (K. Saigo; Skin Nippon Biomedical Laboratories, Ltd., Kagoshima 891-13, Japan; Study No. SBL 52-13; 7/10/96); Six male CD-1 mice/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% sodium carboxymethylcellulose), 500, 1000 or 2000 mg/kg of SAN 1315H (benzobicyclon technical) (lot no. 941208; purity: 99%). A positive control group of 6 animals was injected intraperitoneally with 2.0 mg/kg of mitomycin C. At 48 hours post-dose, the bone marrow in the femurs of each animal was recovered and the percentages of micronucleated immature erythrocytes (PCEs)/PCEs/animal and percentage of PCEs per the total population of erythrocytes (immature and mature)/animal were determined. There was no treatment-related increase in the percentage of micronucleated PCEs. There was a treatment-related reduction in the percentage of immature erythrocytes at the 2000 mg/kg treatment level ($p<0.05$). **No adverse effect indicated.** The positive control was functional. **Study acceptable.** (Moore, 1/31/13)

** 53164-0042; 268658; "SAN 1315H Technical: DNA Repair Test (Rec-Assay)"; (M. Akanuma; Kodaira Laboratories, The institute of Environmental Toxicology, Kodaira-shi, Tokyo 187, Japan; Study No. IET 94-0050; 10/3/94); *Bacillus subtilis* H17 (rec^+) and M45 ($recE^-$) strains were exposed to concentrations of SAN 1315H Technical (lot no. KS-2-146; purity was not reported) ranging from 20 to 1000 ug/disk for 24 hours at 37° C under conditions of non-activation and activation in duplicate samples for a single trial. A phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. The differences in the zones of inhibition between the repair deficient strain and the repair competent strain were not sufficient to deem a positive response with or w/o activation. Positive controls were functional. **No adverse effect indicated. Study acceptable.** Moore, 2/4/13)

NEUROTOXICITY

Rat Acute Neurotoxicity Study

No study submitted nor required at this time

Rat Subchronic Neurotoxicity Study

53154-0025 268641, "Oral (Diet) Subchronic Neurotoxicity Study of Benzobicyclon in Rats"; 862; Rat; Charles River Laboratories, Horsham, PA, Barnett, J., Laboratory Project ID: 20018181, 11/16/12; Benzobicyclon, Batch No.1A0110, 99.9% pure, a slight yellow powder, was administered to 10/sex Rats by diet at 0, 1000, 5000, or 20000 ppm [mean test substance intake: 0, 61.8, 306.5, and 1290.0 mg/kg/day for males; 0, 72.3, 373.6, and 1498.8 mg/kg/day for females, respectively] for at least 91 consecutive days. Animals were observed for general condition, detailed clinical examinations, functional observational battery (FOB), motor activity tests, neurohistology and neurohistopathology. No mortality. No test substance treatment related changes in body weight, bodyweight gains, food consumption, functional observational battery (FOB) and motor activity tests, ophthalmology, organ weights and neurohistopathology in both males and females were observed. Positive control studies conducted by the testing facility demonstrated the ability of the observational methods to detect major neurotoxic endpoints and the sensitivity and reliability of the activity-measuring device and testing procedures. **NOEL (No Observed Effect Level)**: for male and female Rats: 20000 ppm (1290.0 and 1498.8 mg/kg/day for male rats and female rats, respectively). **Acceptable** (Pan& Leung, 2/1/13).

SUBCHRONIC TOXICITY STUDIES

Rat Subchronic Dietary Toxicity Study

53154-0022 268638, "SAN1315H: 90-Day Subchronic Oral Toxicity Study Followed by a 4-Week Recovery in Rats"; 821; rat; The Institute of Environmental Toxicology, Tokyo, Japan, Study # IET 95-0167, 6/21/99; Kitazawa, T.; SAN1315 H, Lot No. 960108N, 99% pure, a pale yellow powder, was administered to 12/sex rats by feed at 0, 20, 100, or 400 ppm for males [mean daily test substance intake: 0, 1.13, 5.73 and 22.74 mg/kg/day] and 0, 100, 400, 2000 or 10000 ppm for females[mean daily test substance intake: 0, 6.29, 25.17, 125.9 and 630 mg/kg/day], respectively, for 13 weeks. Additional group of 6/sex rats were allotted to the satellite group (recovery group) and received the test substance in diet at 0, 20, and 400 ppm for males [mean test substance intake: 0, 1.134 and 22.48 mg/kg/day] and 0, 100, and 10000 ppm for females [mean test substance intake: 0, 6.28 and 644 mg/kg/day], respectively, for 13 weeks followed with a 4-week recovery period when animals were kept on a basal diet. No mortality. No test substance treatment related changes in body weight, bodyweight gains, food consumption and food efficiency in both recovery and non-recovery group males and females were observed. Statistically significant drop of mean specific gravity, decreased yellow appearance or increased pale yellow appearance and increased urine volume in 400 ppm group males of non-recovery and recovery groups at 13 week was observed. The statistically significant changes in specific gravity and appearance continued in the recovery groups at 17 week. Statistically significant shift of urine pH to acidity in 400 ppm group females of non-recovery and recovery groups at 13 week was observed. This effect was reversed at 17 weeks in the recovery group. Statistically significant drops of hematocrit, hemoglobin, and red blood cell, and statistically significant increase in mean corpuscular hemoglobin in 400 ppm group males of non-recovery groups at 13 week was observed, these effects were reversed at 17 week. Statistically significant increase in mean corpuscular volume in 2000 and 10000 ppm group females of non-recovery groups at 13 week was observed. The effects were reversed at 17 week. In blood chemistry examination, decreased alkaline phosphatase, increased blood urea nitrogen and creatinine in 400 ppm males were observed at 13 weeks and were reversed at 17 weeks. Increased relative weights of liver and kidneys in 400 ppm non-recovery group males, increased absolute and relative weights of liver and kidneys in 10000 ppm non-recovery group females at 13 weeks were observed and both effects were reversed at 17 weeks. Increased absolute and relative weights of ovaries were observed at 17 weeks recovery group females. In terminal kill at 13 week, increased pale colored kidney was observed in 400 ppm group non-recovery males, with reversal at 17 weeks. Microscopically, increased deposition of hyaline droplets in proximal tubular cells, increased granular casts in cystically dilated tubules and papillary mineralization was observed in 400 ppm group males at 13 weeks. The effects of increased granular casts in cystically dilated tubules and

papillary mineralization in 400 ppm group males persisted at 17 weeks. **NOEL (No Observed Effect Level)**: for male rats: 20 ppm (1.134 mg/kg/day); for female rats: < 100 ppm (6.29 mg/kg/day) due to urinalysis results. **Acceptable** (Pan& Leung, 1/29/13).

Rat 21-Day Repeated Dosing Dermal Toxicity Study

53154-0024 268640, "A 21-Day Dermal Toxicity Study of Benzobicyclon in New England White Rabbits"; 822; New England White Rabbit; WIL Research, Ashland, OH, and Experimental Pathology Laboratories, Inc., Sterling, VA. Study # WIL-331008, 11/15/12; Morris, T.; Benzobicyclon, Batch No. 1L0108, 98% pure, a light yellow powder, was moistened by deionized water, administered to 10/sex New England White Rabbits by dermal application at 0, 100, 300, or 1000 mg/kg/day for 21 consecutive days, 6 hours per day. Animals were observed for general condition, clinical and detailed physical examinations, and the application sites were scored. No mortality or dermal irritation was reported in control or test animals. However, slightly higher incidence of muscle degeneration in treated skin of 100 and 1000 mg/kg/day males and 100, 300 and 1000 mg/kg/day females were observed. Male rabbits did not exhibit dose-response relationship, but all female groups revealed dose related response. No statistical significance was evident in either sex. No test substance treatment related changes in body weight, bodyweight gains, food consumption, hematology, blood chemistry, ophthalmology, and pathology in both males and females were observed. **Systemic and Dermal NOEL (No Observed Effect Level)**: for male and female New England White Rabbits: 1000 mg/kg/day. **Acceptable** (Pan& Leung, 1/31/13).

Dog Subchronic Oral Toxicity Study

53154-0023 268639, "SAN1315H: Subchronic Toxicity Study by Oral Administration to Beagle Dogs for 13 Weeks"; 821; Beagle Dog; Biosafety Research Center, Shizuoka 437-1213, Japan. Study # 3306, 5/6/98; Kitajima, S.; SAN1315 H, Lot No. 6F0502, 99% pure, a pale yellow powder, was administered to 4/sex Beagle dogs by gelatin capsules at 0, 20, 200, or 2000 mg/kg/day for 13 weeks. No mortality. No test substance treatment related changes in body weight, bodyweight gains, food consumption, hematology, blood chemistry, ophthalmology, urinalysis and pathology in both males and females were observed. **NOEL (No Observed Effect Level)**: for male and female Beagle dogs: 2000 mg/kg/day. **Acceptable** (Pan& Leung, 1/29/13).

Mouse 28-Day Immunotoxicity Study

53154-0044 268660, "A 28-day Immunotoxicity study of Benzobicyclon by diet in Rats"; 857; Rat; Charles River Laboratories, Horsham, PA, Hoberman, A., Laboratory Protocol No. 20026473: 10/8/12; Benzobicyclon, Batch No.1A0110, 99.2% (w/w) pure, a slight yellow powder, was administered to 10 male rats by diet at 0, or 15000 ppm [mean test substance intake: 0 and 1181.8 mg/kg/day, respectively] for 28 days. An additional group of 10 male rats were given control diet for 24 days followed with 50 mg/kg of cyclophosphamide via intraperitoneal injection for 4 consecutive days before euthanasia. All rats were administered 0.5 mL sRBC (sheep Red Blood Cells) via intravenous administration once 4 days before euthanasia (on day 25 of study). The rats were observed for vitality, general appearance, body weight and food consumption. At the end of the 28-day treatment period, all rats were euthanized by carbon dioxide asphyxiation, and organ weights were measured. The splenocyte suspension was prepared and its vitality was tested. A primary IgM response to sRBC was enumerated using a modified hemolytic plaque assay, permitting the number of antibody-forming cells (AFC) present in the whole spleen to be calculated. No mortality. No test substance treatment related changes in body weight, bodyweight gains, food consumption, organ weights and AFC were observed. Sparse hair coat on limbs was observed in 1 treated rat. Mild dehydration in 1 positive control animal at clinical observation and slight dilation of kidney right pelvis in 1 positive control animal at necropsy was observed. Positive control group animals demonstrated decreased body weight gain, food consumption, relative food consumption,

and food efficiency during the last week of the dosing period as well as decreased spleen and thymus weights and decreased splenic antibody forming cells. **NOEL (No Observed Effect Level)** for immunotoxicity: 15000ppm (1181.8 mg/kg/day) in male rats after 28 day treatment. **Acceptable** (Pan& Leung, 2/15/13).

RAT METABOLISM

** 53164-0043; 268659; "SAN 1315H: Metabolism in the Rat"; (D. Shaw; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE17 5HS, England; Study No. DSK 170/963807; 7/15/99); Sprague-Dawley rats of both sexes were dosed with either [2,4-Bicyclooctene-^{14}C]-SAN 1315H (label A) (batch no. 209-010-042, specific activity: 42 mCi/mmol; batch no. 109-299-046, specific activity: 46 mCi/mmol) or [Ring-U-^{14}C-benzoyl]-SAN 1315H (label B) (batch no. 109-291-055, specific activity: 55 mCi/mmol). The specific activity of the dosing preparations was adjusted with the addition of non-radiolabeled SAN 1315H (batch no. 960108N, purity: 99%). Four studies were performed; excretion balance, bilie duct cannulation, pharmacokinetic, and tissue distribution. In the excretion balance study, 5 animals/sex/group were dosed orally by gavage with 10 or 500 mg/kg of either radiolabeled test material. An additional cohort of 5 animals/sex/group was injected iv with 10 mg/kg of either radiolabeled test material. Urine and fecal samples were collected at various time points up to 4 days post-dose. In the bile duct cannulation study, 3 bile duct-cannulated rats/sex/group were dosed with 10 mg/kg of either radiolabeled test material or 500 mg/kg of Label A and bile, urine and fecal samples were collected for up to 48 hours post-dose. In the pharmacokinetic study, 15 animals/sex/group received a single dose of 10 or 500 mg/kg of Label A or 7 daily doses of 10 mg/kg of Label A. Blood samples were collected up to 7 days post-dose. Blood samples were collected at specified time intervals up to 7 days post-dose. In the tissue distribution study, 6 animals/sex/group were dosed orally by gavage with 10 or 500 mg/kg of Label A., 3 animals/sex were dosed orally with 10 mg/kg of Label B and 9 animals/sex were dosed orally with 10 mg/kg/day for 7 days with Label A. For the 10 mg/kg group (Label A), 3 animal/sex/time point were euthanized at 6 and 24 hours post-dose. For the 10 mg/kg group (Label B), 3 animals/sex were euthanized at 6 hours post-dose. For the 500 mg/kg group (Label A), 3 animals/sex/time point were euthanized at 6 and 48 hours post-dose. For the 10 mg/kg/day group (Label A), 3 males/time point were euthanized at 3 and 24 hours post dose and 3 females/time point were euthanized at 4 and 48 hours post dose. Distribution of radiolabel was also assessed in the tissues of the animals in the excretion balance study at 96 hours post-dose. Excretion of radiolabel via the urine represented 2 to 3% of the administered dose for the 10 mg/kg treatment level and less than 1% for the 500 mg/kg group. Ninety one to 96% of the radiolabel was recovered in the feces at 10 mg/kg, increasing to 95 to 99% at 500 mg/kg. In the bile duct-cannulation study, the radiolabel in the bile ranged from 6 to 14% of the administered dose at 10 mg/kg and 1.5 to 2% of the dose at 500 mg/kg. For the 10 mg/kg treatment group, the total absorbed dose ranged from 11% (Label A, males and females) to 28% (Label B, females). For the 500 mg/kg group, absorption was limited to 3 to 4% of the administered dose (Label A). The author of the report speculated as to the reason for the discrepancy in the absorbed dose for the two radiolabeled test materials, but could not offer a definitive rationale. In the pharmacokinetic study, the plasma Cmax level for the 10 mg/kg group was 0.37 ug equiv./g, increasing to 0.49 to 0.56 ug equiv./g after treatment at 10 mg/kg/day for 7 days. Measurement of radiolabel in the plasma at 24 hours after the 2nd , 4th , 6th and 7th treatments revealed a plateauing effect with concentrations increasing from 0.17 to 0.26 ug equiv./g for the males and from 0.20 to 0.40 ug equiv./g for the females. The time at which the maximal plasma levels (Tmax) were achieved was 6 hours for the single dose treatment and 3 to 4 hours for the multiple dose treatment. For the 500 mg/kg group, Cmax was 2 ug equiv./g and Tmax ranged from 3 to 6 hours. The T1/2 times of radiolabel in the plasma ranged from 32 to 57 hours irrespective of the dosing regimen. In the tissue distribution study, the liver and kidneys were the primary sites of recovery. The T1/2 times of the radiolabel in the liver ranged from 88 to 108 hours after treatment at 10 mg/kg and 67 to 69 hours after treatment at 500 mg/kg. For the kidneys, the T1/2 times for the radiolabel varied according to the treatment regimen; 76 to 85 hours for 10 mg/kg, 131 to 150 hours for 10 mg/kg/day, and 46 to 67 hours for 500 mg/kg. Urine, feces and bile which were collected in the excretion balance and bile cannulation

studies were analyzed for metabolites. Up to 11 urinary and 18 bile metabolites were isolated. Due to the limited absorption of the test material, any one metabolite represented only a fraction of a percent of the administered dose. Four metabolites were identified in the urine and 3 were identified in the bile. Cleavage of the keto linkage and loss of the thiopentyl moiety were the two sites of metabolism. **Study acceptable.** (Moore, 2/21/13)

METABOLITES

1315-070

** 53164-0033; 268649; "Mutagenicity Study of 1315P-070 with the Bacterial Reverse Mutation Assay"; (Y. Oguma; *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with 1315P-070 (benzobicyclon metabolite) (lot no. KS-4-3234; purity: > 95%) at concentrations ranging from 1.2 to 5000 ug/plate in the 1st trial and from 313 to 5000 ug/plate in the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 2/5/13)

1316P-076

** 53164-0034; 268650; "Mutagenicity Study of 1315P-076 with the Bacterial Reverse Mutation Assay"; (Y. Oguma; BML, Inc., Safety Studies Section, Safety Evaluation & Reagent Division, Kawagoe-shi, Saitama 350-1101, Japan; Study No. 6674; 10/13/13); *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with 1315P-076, (benzobicyclon metabolite) (lot no. TT-4-196; purity: 99.47%) at concentrations ranging from 1.2 to 5000 ug/plate in the 1st trial and from 313 to 5000 ug/plate in the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 2/5/13)

1315P-570

** 53164-0035; 268651; "Mutagenicity Study of 1315P-570 with the Bacterial Reverse Mutation Assay"; (Y. Oguma; BML, Inc., Safety Studies Section, Bicellology Division, Kawagoe-shi, Saitama 350-1101, Japan; Study No. 6298; 1/21/99); *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with 1315P-570 (benzobicyclon metabolite) (lot no. JI-5-112; purity: >98%) at concentrations ranging from 1.2 to 5000 ug/plate in the 1st trial and from 156 to 5000 ug/plate in the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 2/5/13)

1315P-683

** 53164-0036; 268652; "Mutagenicity Study of 1315P-683 with the Bacterial Reverse Mutation Assay"; (Y. Oguma; BML, Inc., Safety Studies Section, Bicellology Division, Kawagoe-shi, Saitama 350-1101, Japan; Study No. 6301; 1/21/99); *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with 1315P-683 (benzobicyclon metabolite) (lot no.

JI-5-111; purity ≥ 98%) at concentrations ranging from 1.2 to 5000 ug/plate in the 1st trial and from 156 to 5000 ug/plate in the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 2/6/13)

1315P-960

** 53164-0037; 268653; "Mutagenicity Study of 1315P-960 with the Bacterial Reverse Mutation Assay"; (Y. Oguma; BML, Inc., Safety Studies Section, Bicellology Division, Kawagoe-shi, Saitama 350-1101, Japan; Study No. 6300; 1/21/99); *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with 1315P-960 (benzobicyclon metabolite) (lot no. TT-4-121; purity: ≥ 98%) at concentrations ranging from 1.2 to 5000 ug/plate in the 1st trial and from 313 to 5000 ug/plate in the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 2/7/13)

1315P-966

** 53164-0038; 268654; "Mutagenicity Study of 1315P-966 with the Bacterial Reverse Mutation Assay"; (Y. Oguma; BML, Inc., Safety Studies Section, Bicellology Division, Kawagoe-shi, Saitama 350-1101, Japan; Study No. 6299; 1/21/99); *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with 1315P-966 (benzobicyclon metabolite) (lot no. 405-95; purity: 99.40%) at concentrations ranging from 1.2 to 5000 ug/plate in the 1st trial and from 313 to 5000 ug/plate in the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 2/12/13)

1315P-DAC

** 53164-0039; 268655; "Mutagenicity Study of 1315P-DAC with the Bacterial Reverse Mutation Assay"; (Y. Oguma; BML, Inc., Safety Studies Section, Kawagoe-shi, Saitama 350-1101, Japan; Study No. 4480; 1/8/96); *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were treated with 1315P-DAC (benzobicyclon metabolite) (lot no. KS-4-2; purity: > 95%) at concentrations ranging from 1.2 to 5000 ug/plate in the 1st trial and from 156 to 5000 ug/plate in the 2nd trial with and w/o activation. The treatment included preincubation for 20 minutes followed by plate incorporation for 48 hours at 37° C. Each treatment level was plated in duplicate. 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. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 2/8/13)