CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
HUMAN HEALTH ASSESSMENT BRANCH

SUMMARY OF TOXICOLOGY DATA
Kasugamycin Hydrochloride

Chemical Code # 6197, Document Processing Number (DPN) # 53293

20 January 2015

DATA GAP STATUS

Combined toxicity, rat: No data gap, no adverse effect
Chronic toxicity, dog: No data gap, no adverse effect
Oncogenicity, mouse: No data gap, no adverse effect
Reproduction, rat: No data gap, no adverse effect
Developmental toxicity, rat: No data gap, no adverse effect
Developmental toxicity, 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: Not required at this time

Toxicology one-liners are attached.

All record numbers through 280663 (Document No.53293-0053) were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T150120 prepared by H. Green.
These pages contain summaries of studies. Individual worksheets may contain additional effects.

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**METABOLISM AND PHARMACOKINETICS**

**53293-0050**  280660, “Metabolism of 14C-Kasugamycin in Rats,” (T. Cheng, Covance Laboratories Inc., Madison, WI., Laboratory Project ID. Covance 6434-110, November 20, 1998). Four, 5, or 9 Fischer 344 F344-NHLa CFV rats per sex per group received a single oral gavage dose of 14C(U)-hexopyranosyl-kasugamycin hydrochloride hydrate at 100 or 1000 mg/kg. Two rats per sex received a single oral gavage dose of the vehicle (sterile deionized water). Group mean total recoveries of administered radioactivity for Groups A and B (100 mg/kg) and C, and D (1000 mg/kg) ranged from 90.6% to 96.7% with 87.7% to 94.5% eliminated in feces and 1.35% to 3.26% excreted in urine, respectively. Most of the radioactivity was eliminated during 48 hours post dosing. Radioactivity remaining in the carcass and all tissues combined was ≤ 0.13% of the total dose at 168 hours postdose (terminal sacrifice). Results for the pharmacokinetic groups included group mean maximum blood radioactivity concentrations (C\(_{\text{max}}\)) of 1.47 ug/g (males) and 2.17 ug/g (females) for group E (low dose, 100 mg/kg) and 6.40 ug/g (males) and 5.23 ug/g (females) for group F (high dose, 1000 mg/kg) reached at 1 hour (T\(_{\text{max}}\)) for both sexes in both groups. Elimination half lives (T\(_{1/2}\)) were 1.41 hours (males) and 1.17 hours (females) at 100 mg/kg and 1.40 hours (males) and 1.55 hours (females) at 1000 mg/kg. For the tissue distribution groups, tissues were collected at 1, 2, and 6 hours postdose for Group G (100 mg/kg) and at 1, 3, and 4 hours postdose for Group H (1000 mg/kg). Among all the examined samples (excluding the GI tract and contents), kidneys and lymph nodes had higher residue concentrations than plasma at all 3 time points. As a reference, values from the 168 hour postdose sacrifice for Group A (100 mg/kg) and C (1000 mg/kg) animals indicated residues were not detectable in lymph nodes and plasma but still detectable in kidneys. For bile cannulated animals, Groups I (100 mg/kg) and J (1000 mg/kg), bile was collected at 24 hours predose, 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours postdose. No radioactivity was detected in bile, indicating the absence of biliary elimination and enterohepatic recirculation of radioactivity. At 100 mg/kg, the mean recovery of administered radioactivity was 3.31% (males) and 1.75% (females) in urine, 87.2% (males) and 80.4% (females) in feces, and 1.70% (males) and 12.7% (females) in carcass through 48 hours postdose. At 1000 mg/kg, mean recovery was 6.07% (males) and 6.7% (females) in urine, 88.2% (males) and 52.4% (females) in feces, and 3.29% (males) and 37.2% (females) in carcass. Kasugamycin was not extensively metabolized in the rat. More than 90% of administered radioactivity was eliminated in the feces as unchanged parent compound. Less than 5% of administered radioactivity was absorbed from the gastrointestinal tract. A portion of the absorbed kasugamycin was eliminated in urine as unchanged kasugamycin. Less than 0.02% of kasugamycin was metabolized to the metabolites kasugamycinic acid and kasuganobiosamine through deamination, oxidation, decarboxylation, and hydrolysis. Acceptable. (Green, 12/31/14).
GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat

**53293-0021, 0022  280616, 280267, “Acute Oral Toxicity Study of Kasugamycin Hydrochloride Technical in Rats,” (S.M. Glaza, Hazleton Wisconsin, Inc., Madison, WI. Laboratory Project ID. 20504630, September 29, 1992). Five fasted Crl:CD®BR albino rats per sex received a single oral gavage dose of Kasugamycin hydrochloride technical (80.6%) at 5000 mg/kg followed by a 14-day observation period. All animals survived to the end of the 14-day observation period, showed no treatment-related clinical signs, and gained bodyweight. Necropsy results were unremarkable. Record 280616 provides the purity of the test article used. LD50 (M & F) > 5000 mg/kg. Toxicity category IV. Acceptable. (Green, 11/14/14).

Acute dermal toxicity

**53293-0021, 0022  280616, 280618, “Acute Dermal Toxicity Study of Kasugamycin Hydrochloride Technical in Rabbits (EPA Guidelines),” (S.M. Glaza, Hazleton Wisconsin, Inc., Madison, WI. Laboratory Project ID. 20504631, September 29, 1992). Five New Zealand White rabbits per sex received a single 24-hour dermal exposure (clipped, unabraded, occluded skin) to kasugamycin hydrochloride technical (80.6%) at 2000 mg/kg followed by a 14-day observation period. Treated sites were washed with water at the end of the 24 hour exposure period. All animals survived to the end of the 14-day observation period with no treatment-related clinical signs. Slight erythema (score of 1) was noted in 1 female on day 1 and was resolved (score of 0) on day 3. No dermal irritation was indicated for any of the other animals at any time during the study. All animals gained bodyweight during the study. No treatment-related changes were noted at necropsy. Record 280616 provides the purity of the test article used. LD50 (M & F) > 2000 mg/kg. Toxicity category III. Acceptable. (Green, 11/14/14).

Acute inhalation toxicity, rat

**53293-0022  280619, “Acute Inhalation Toxicity Study in Rats – Limit Test,” (J. Durando, Eurofins | Product Safety Laboratories, Dayton, NJ, Study No. 25917, July 20, 2009). Five Sprague-Dawley rats per sex were exposed to Kasugamycin technical (84.4% kasugamycin hydrochloride hydrate) for 4 hours by whole body inhalation at 2.07 mg/l gravimetric (with a mean mass median aerodynamic diameter (MMAD) of 3.8 µm and a mean geometric standard deviation (GSD) of 2.05) followed by a 14 day observation period. All animals survived to the end of the 14-day observation period and gained bodyweight. In chamber animal observations included hunched posture and hypoactivity. After removal from the chamber, all animals recovered and appeared active and healthy over the 14 day observation period. No treatment-related changes were noted at necropsy. LC50 (M & F) > 2.07 mg/l. Toxicity category IV. Acceptable. (Green, 11/17/14).
Primary eye irritation, rabbit

**53293-0022 280620, “Primary Eye Irritation Study in Rabbits,” (J. Durando, Eurofins | Product Safety Laboratories, Dayton, NJ, Study No. 25918, July 20, 2009).** The right eyes of 3 female New Zealand albino rabbits were treated with 0.1 ml (0.07 g) of Kasugamycin technical (84.4% kasugamycin hydrochloride hydrate) followed by a 72-hour observation period (eyes remained unrinsed). At 24 hours after treatment, corneal opacity was noted in one treated eye (scores of 1 for opacity and area) and all 3 treated eyes showed minimal conjunctivitis. All animals were free of ocular irritation at 72 hours after treatment. All animals appeared active and healthy during the study. Toxicity category III. Acceptable. (Green, 11/17/14).

Primary dermal irritation

**53293-0022 280621, “Primary Skin Irritation Study in Rabbits,” (J. Durando, Eurofins | Product Safety Laboratories, Dayton, NJ, Study No. 25919, July 20, 2009).** Three female New Zealand albino rabbits received a single 4-hour exposure (clipped, unabraded, semi-occluded skin) to Kasugamycin technical (84.4% kasugamycin hydrochloride hydrate) at 0.5 g. At the end of the exposure period, pads were removed, and the test sites were cleansed of any residual test article. During the first 48 hours after patch removal, all 3 treated sites showed well-defined erythema (scores of 2) and slight edema (scores of 2). The irritation decreased by 72 hours to very slight erythema and edema (scores of 1), and all animals were free of erythema and edema by day 7 (although desquamation was noted for 1 animal on day 7 that was resolved by day 10). All animals appeared active and healthy during the study. Toxicity category III. Acceptable. (Green, 11/18/14).

Dermal sensitization

**53293-0022 280622, “Dermal Sensitization Study in Guinea Pigs (Buehler Method),” (J. Durando, Eurofins | Product Safety Laboratories, Dayton, NJ, Study No. 25920, July 20, 2009).** Twenty female Hartley albino guinea pigs were induced (one 6-hour topical application per week for 3 weeks) with 0.4 g of Kasugamycin technical (84.4% kasugamycin hydrochloride hydrate) at 65% w/w in distilled water followed by one 6-hour topical challenge application at 33% twenty-seven days after the first induction application. An additional group of 10 naïve control females also received a single, topical 6-hour challenge application at 33%. Very faint erythema (score of 0.5) was noted for 2/20 test group animals and for 1/10 naïve control animals 24 hours after challenge patch removal. All sites were clear (scores of 0) at 48 hours. Positive controls were functional. Dermal sensitization was not indicated using the Buehler method. Acceptable. (Green, 11/18/14).

SUBCHRONIC STUDIES

Rat Subchronic Dietary Toxicity Study:

**53293-0026 280636, : “Kasugamycin: 13-Week Oral Subchronic Toxicity Study in Rats,”** (N. Nakashima, The Institute of Environmental Toxicology, Tokyo, Japan, Study No. IET 89-0083, April 1991). Twelve Wistar rats (Jcl:Wistar) per sex per group received kasugamycin
(monohydrochloride) (64.5%) in the diet at 0 (basal diet), 300, 1000, 3000, and 6000 ppm for 13 weeks. Group mean test article intake during the study was 17.5, 58.2, 176.7, and 354.8 mg/kg/day for males and 20.3, 69.2, 201.0, and 395.5 mg/kg/day for females at 300, 1000, 3000, and 6000 ppm, respectively. All animals survived to scheduled necropsy with no treatment-related clinical signs. Group mean bodyweights at 6000 ppm were reduced for males (4% to 9%) from treatment week 2 and for females (3% to 6%) from week 3 compared to controls. Values were statistically significant from week 2 to week 13 for males and at week 6 for females. Group mean food consumption for males and females at 1000, 3000, and 6000 ppm was significantly decreased during study week 1 compared to controls. Group mean water consumption was significantly increased at 6000 ppm during weeks 1 to 4, 6, 9, 12, and 13 for males and during weeks 2 and 13 for females and, at 3000 ppm during weeks 1 to 4 for males vs controls. Group mean urine pH values at week 13 were significantly decreased for males and females at 6000 ppm and for females at 3000 ppm compared to controls. Additionally, a significant increase in the epithelial cell count in the urine sediment of females was noted at 6000 ppm. Significant decreases were noted in group mean values for hematocrit, hemoglobin, and erythrocyte counts for males and females at 6000 ppm, and for males at 3000 and 1000 ppm vs controls. Serum chemistry changes included significant decreases in alanine aminotransferase activity, total protein, albumin, and globulin, and increases in chlorine in both sexes at 6000 ppm vs controls. At 6000 ppm, significant increases were noted for absolute and relative cecum weights and relative kidney weights in both sexes, for relative brain, salivary gland, and testes weights in males, while decreases were noted for absolute and relative liver weights in males vs controls. At 3000 ppm, significant increases were noted for absolute and relative cecum weights in both sexes and for relative kidney weights in females, and decreases were noted for absolute and relative ovary weights in females vs controls. Relative ovary weights were also significantly decreased at 1000 ppm vs controls. Necropsy results were unremarkable. Treatment-related histology results at 1000, 3000, and 6000 ppm included significant increases in the incidence of eosinophilic bodies in the proximal tubular cells of the kidney in males and in foam cell aggregation in the lung of females vs controls. NOEL = 300 ppm (17.6 mg/kg/day for males and 20.3 mg/kg/day for females) based on kidney and lung histology. No adverse effect. Acceptable. (Green, 11/21/14).

**Dog Subchronic Dietary Toxicity Study**

**53293-0030  280640, “13-Week Dietary Toxicity Study with Kasugamycin in Dogs,” (P. J. Thomford, Hazleton Wisconsin, Inc., Madison, WI., Laboratory Project ID. HWI 6434-101, September 30, 1993). Four beagle dogs per sex per group received Kasugamycin hydrochloride technical (80.6%) in the diet at 0 (basal diet), 300, 3000, and 6000 ppm for 13 weeks. High dose animals received treated diet at 6000 ppm from test days 1 through 41 and an untreated soft-food diet (Alpo®) (because of low food consumption, bodyweight loss, and tongue erosions/ulcerations) until test day 50, when they began receiving treated diet at 4500 ppm. Group mean test article intake during the treatment period was 10.59, 105.96, 157.14, and 212.10 mg/kg/day for males and 11.44, 107.89, 172.93, and 185.33 mg/kg/day for females at 300, 3000, 4500, and 6000 ppm, respectively. Treatment-related clinical signs included 2 dogs per sex with swollen mouths, 3 males and 4 females with tongue lesions, and 4 per sex with excessive salivation at 3000 ppm, and 4 per sex with excessive salivation and tongue lesions and 2 males and 1 female with swollen mouths at 6000 ppm. At 6000 ppm, group mean bodyweight was significantly reduced for males at weeks 6 through 9 and for females at weeks 4 through 9.
Group mean food consumption was reduced for males and females at 6000 ppm for weeks 1 through 5 compared to controls (values for females were statistically different). Treatment-related findings at necropsy included increased erosions/ulcerations of the tongue at 3000 ppm (3/4 males and 4/4 females) and 6000 ppm (all animals), diffuse reddening of the tongue in 1/4 females at 6000 ppm, and a depressed area of the tongue in 1/4 males at 3000 ppm vs controls (0 for both sexes). Additionally, diffuse reddening of the oral cavity was noted for 1/4 males at 300 ppm, 1/4 males and 2/4 females at 3000 ppm, and 1/4 females at 6000 ppm vs controls (0 for both sexes). Treatment-related histology was limited to changes in the tongues of animals coincident with the gross pathology findings for males and females at 3000 and 6000 ppm. Tongue erosions/ulcerations followed a consistent progression. Lesions began with loss of the papillae of the dorsal epithelium progressing from the side of the tongue to the center. After loss of the papillae, the epithelium atrophied resulting in complete ulceration. Ulcerated areas frequently contained serous fluid along with minimal to slight chronic-active inflammation. NOEL (M & F) = 300 ppm (10.59 mg/kg/day for males and 11.44 mg/kg/day for females) based on clinical signs and gross pathology and histology of the tongue. No adverse effect. Acceptable. (Green, 12/2/14).

**Rat Repeated Dosing 21-day Dermal Toxicity Study**

**53293-0033, 0034, 0035 280643, 280644, 280645, “A 21-Day Dermal Toxicity Study of Kasugamycin Technical in Sprague Dawley Rats,” (S.D. Seidel, WIL Research Laboratories, LLC, Ashland, OH., Study No. WIL-476005, November 16, 2009). Ten Crl:CD(SD) rats per sex per group were dermally treated (clipped, intact, occluded skin) with Kasugamycin technical (71.5%) at 0 (deionized water), 50, 250, and 500 mg/kg/day 6 hours per day, 7 days per week for 3 weeks. On study day 8, treatment levels for females were changed from 250 and 500 mg/kg/day to 100 and 200 mg/kg/day, respectively, due to the severity of dermal observations. Clinical observations at the test sites 1 hour after unwrap included dose site reddened in 2/10 males and 4/10 females at 250/100 mg/kg/day, and in 8/10 males and 9/10 females at 500/200 mg/kg/day; and scabbing within dose site in 2/10 males and 4/10 females at 250/100 mg/kg/day, and in 8/10 males and 9/10 females at 500/200 mg/kg/day beginning on study days 7 and 5 for males and females, respectively. Dermal observations at the treatment sites included erythema, edema, eschar formation, coriaceousness, pinpoint scabbing, and encrustation in males and females at 250/100 and 500/200 mg/kg/day that progressed in severity and number of animals affected during the study. There were no treatment-related effects on bodyweight, food consumption, hematology, serum chemistry, urinalysis, ophthalmology, or organ weights. Treatment-related gross pathology included scabbing on the skin of the application site of 4/10 males and 2/10 females at 500/200 mg/kg/day. The two females also had open sores on the treatment site. Treatment-related histology was noted at the dermal treatment sites of high dose animals. Nine of 10 males and 7/10 females at 500/200 mg/kg/day had acanthosis, acute inflammation, and/or ulceration effecting the treatment site skin. Two females also had minimal granulomatous inflammation. Record 280644 is a rat 7-day dose range-finding dermal toxicity study. Record 280645 is a test substance stability analysis waiver request/discussion. Systemic NOEL = 500 mg/kg/day for males and 200 mg/kg/day for females. Dermal NOEL (M & F) = 50 mg/kg/day based on dermal application site observations. No adverse effect. Acceptable. (Green, 12/10/14).
**Rat 7-Day Dose Range-Finding Dermal Toxicity Study**

53293-0034  280644, “A 7-Day Dose Range-Finding Dermal Toxicity Study of Kasugamycin Technical in Sprague Dawley Rats,” (S.D. Seidel, WIL Research Laboratories, LLC., Ashland, OH., Study No. WIL-476004, September 18, 2009). Five Crl:CD(SD) rats per sex per group were dermally treated (clipped, intact, occluded skin) with Kasugamycin technical (71.5%) at 0 (deionized water), 50, 250, 500, and 1000 mg/kg/day for 6 hours per day for 7 days. Dermal treatment with kasugamycin technical was well tolerated systemically. There were no treatment-related effects on survival, clinical observations, bodyweights, food consumption, or hematology findings at any treatment level. Treatment-related application site dermal effects, which were more severe in females, were noted for both sexes at 250, 500, and 1000 mg/kg/day during study days 0 to 7. At 250 mg/kg/day; 1/5 females had very slight erythema (Draize score of 1), and 3/5 females and 1/5 males had pinpoint scabbing. At 500 mg/kg/day; 1/5 males and 1/5 females had very slight erythema, 1/5 females had very slight edema (Draize score of 1), and 4/5 males and 2/5 females had pinpoint scabbing. At 1000 mg/kg/day; 2/5 males and 2/5 females had very slight erythema, 1/5 males and 1/5 females had slight erythema (Draize score of 2), 1/5 females had moderate erythema (Draize score of 3), 1/5 males and 3/5 females had very slight edema, 1/5 males and 1/5 females had slight edema (Draize score of 2), 3/5 males and 2/5 females had pinpoint scabbing, and 1/5 males and 2/5 females showed coriaceousness. 500 mg/kg/day was considered a maximum tolerated dose. Supplemental data. (Green, 12/9/14). No worksheet.

**Mouse Subchronic Dietary Toxicity Study**

**53293-0025 280635, “Kasugamycin: Toxicity study by dietary administration to CD-1 mice for 13 weeks”; 821; mice; Life Science Research Limited, Suffolk, IP23 7PX, England. Lab Project ID: TMN-0156, LSR 90/0345, 7/3/1990; Holmes, P.; Kasugamycin technical, Lot No. KP-821, 64.8% pure, a pale-brown powder. Groups (1-5) of 12/sex CD-1 mice were treated with the test substance at daily oral doses of 0, 300, 1000, 3000 or 10000 ppm for 13 weeks (mean average test substance intake: 41.15, 135.4, 408.5 and 1559 g/kg/day for males in 300, 1000, 3000 or 10000 ppm group, respectively; 57.98, 170.9, 565.6 and 1834 mg/kg/day for females in 300, 1000, 3000 or 10000 ppm group, respectively). Mortality: 1M, 1M&2F, 2M&1F in groups 1, 4 and 5 respectively. One of group 1 males died at bleed on week 13. One of group 4 males was found dead on week 14. Two of group 5 males were humanely killed on week 12. Perianal abrasion, base of tail abrasion, perigenital abrasion, scrotal ulceration was observed among other similar signs before the kill. One female in group 4 was humanely killed on week 12. Parianal and dorsal abrasion, perianal, limb and dorsal ulceration was observed before kill. Another female of this group was found dead on week 7. One female in group 5 was found dead on week 6. Perianal reddening was observed before death. Reduced total food consumption in 10000 ppm group female mice, reduced body weight gain in 10000 ppm group males and females was observed. Perianal reddening was observed in other mice from groups 4 and 5 starting from week 4 until the end of the 14 week study period. Increased neutrophile count, decreased platelet count in 3000 and 10000 ppm group males, increased total white blood cells in 10000ppm females were observed at hematology measurement. Reduced total cholesterol in 3000 and 10000 group females, and in 10000 ppm group males was observed at blood chemistry measurement. Histopathology findings included increased numbers of animals with anus active chronic inflammation and ulceration in 3000 and 10000 ppm group males and females, increased number of female mice with kidney basophilia/hyperplasia at pars recta in 3000 and 10000 ppm group. NOEL (No Observed Effect Level): 1000 ppm (135.4 mg/kg/day for males and 170.9 mg/kg/day for females) due to hematology and histopathology observations.
CHRONIC STUDIES

Combined Toxicity, rat

**53293-0036 280646, “Kasugamycin: 24-month oral chronic toxicity and oncogenicity study in rats”; 831; rat; The Institute of Environmental Toxicology, Tokyo 187, Japan. Lab Project ID: TMN-0120, ID-0901987, 9/16/1987; Kitazawa, T.; Kasugamycin (hydrochloric salt), Lot No. KP-570, 67.1% pure, a pale brown powder. Groups (1-4) of 70/sex SPF Wistar rats were treated with the test substance at daily oral doses of 0, 30, 300, or 3000 ppm for up to 104 weeks (interim examinations at weeks 26 and 52: maximum 10/sex/group) (mean average test substance intake: 1.146, 11.31 and 115.9 mg/kg/day for males in 30, 300, and 3000 ppm groups, respectively; 1.371, 13.42 and 139.8 mg/kg/day for females in 30, 300, and 3000 ppm groups, respectively). Cumulative mortality: 9, 14, 11 and 6 for groups 1, 2, 3 and 4 males, respectively; 19, 14, 12 and 11 for groups 1, 2, 3 and 4 females, respectively. Slightly decreased bodyweight, fluctuation of the food and water consumption in group 4 males in the beginning of the treatment period was observed. The overall bodyweights and food and water consumptions were not different from control groups. Increased urine protein in all treated male animals observed in urinalysis conducted at 104 weeks, No treatment related effects on hematology were observed except for slight reduction of hematocrit, hemoglobin and RBC in the group 4 females after 26 weeks of treatment. Reduced alkaline phosphatase in group 4 males and females throughout the study, decreased glutamic pyruvic transaminase in group 4 females after 26 and 52 weeks treatment, decreased total cholesterol in group 4 females after 52, 78 and 104 weeks treatment was reported at blood biochemical examinations. Histopathological examinations revealed increased brown pigment in kidney proximal tubule cell, increased foam cell aggregation in lung in group 4 males and females, increased nasal cavity rhinitis in group 4 males, and increased hepatocellular atrophy in group 4 females. NOEL (No Observed Effect Level): 300 ppm (11.31 mg/kg/day for males and 13.42 mg/kg/day for females) due to blood biochemistry, organ weights, and histopathology findings in the group 4 animals. Study Acceptable. (Pan& Leung, 12/19/2014).

Chronic Toxicity, dog

**53293-0049 280659, “52-Week Dietary Toxicity Study with Kasugamycin in Dogs,” (J. C. Albretsen, Covance Laboratories Inc., Madison, WI, Covance Study No. 6434-117, April 28, 2003). Four beagle dogs per sex per group received Kasugamycin Hydrochloride Technical (72.8%) in the diet at 0 (basal diet), 300, 1000, and 3000 ppm for 52 weeks. Group mean test article consumption was 10.5, 30.5, and 99.6 mg/kg/day for males and 9.4, 33.4, and 103.6 mg/kg/day for females at 300, 1000, and 3000 ppm, respectively, during the 52 week treatment period. Treatment-related effects were noted in serum chemistry and urinalysis results at 3000 ppm at week 52. Minimally higher urea nitrogen (statistically significant for males) and creatinine (statistically significant for females) values were noted in both sexes at 3000 ppm. Also at 3000 ppm, group mean urine volume was lower for both sexes (statistically significant for males) and urine specific gravity was minimally higher in both sexes. There were no treatment-related effects on physical examination data, clinical signs, ophthalmology,
bodyweights, bodyweight change, food consumption, organ weights, gross pathology, or histology. Chronic NOEL (M & F) = 1000 ppm (30.5 mg/kg/day for males and 33.4 mg/kg/day for females) based on serum chemistry and urinalysis changes. No adverse effect. Acceptable. (Green and Leung, 12/4/14).

**Oncogenicity, rat**

See Combined Toxicity, rat above.

**Oncogenicity, mouse**

**53293-0038 280648, “Oncogenicity study by dietary administration to CD-1 mice for 78 weeks”; 832; mice; Life Science Research Limited, Suffolk, IP23 7PX, England. Lab Project ID: TMN-0122, LSR 91/HKC006/1010, 6/19/1992; Holmes, P.; Kasugamycin technical, Lot No. KP-821, 64.8% pure, a pale-brown orange powder. Groups (1-4) of 72/sex CD-1 mice were treated with the test substance at daily oral doses of 0, 50, 300, or 1500 ppm for 52 weeks (interim examination: maximum 20/sex/group) to 78 (terminal sacrifice: the remaining surviving animals) weeks (mean average test substance intake: 5.93, 34.94, and 186.3 mg/kg/day for males in 50, 300, and 1500 ppm groups, respectively; 7.25, 42.49, and 215.2 mg/kg/day for females in 50, 300, and 1500 ppm group, respectively). Mortality: 1M, 4M/2F, 2F and 9M/1F in groups 1, 2, 3 and 4, respectively, at interim phase; 22M/8F, 20M/10F, 20M/4F and 21M/9F, for groups 1, 2, 3 and 4, respectively, at terminal phase. No treatment related effects on mortality, clinical signs, and hematology were observed. The following observations were made: increased absolute and relative spleen weights in high dose males killed after 78 weeks with increased number of high dose group male mice killed or dying during the interim phase having enlarged spleen. Other observations reported including decreased number of high dose group male mice killed after 78 weeks having enlarged spleen, increased incidence of extramedullary hemopoiesis in high dose group male mice killed or dying during the interim phase, and in 300 ppm group male mice killed after 78 weeks did not demonstrate any dose response relationship, and therefore were not determined to be treatment-related. No carcinogenicity indicated for the test substance treated mice. NOEL (No Observed Effect Level): 300 ppm (34.94 mg/kg/day) for males due to increased absolute and relative spleen weights; 1500 ppm (215.2 mg/kg/day, no effect at highest dose tested) for females. Study Acceptable. (Pan& Leung, 12/10/2014).

**REPRODUCTIVE TOXICITY, RAT**

**53293-029 280639, “Two-Generation Reproduction Study with Kasugamycin in Rats,” (S.M. Henwood, Hazleton Wisconsin, Inc., Madison, WI., Laboratory Project ID. HWI 6434-102, November 12, 1993). Twenty-five F0 and F1 Crl:CD® BR VAF/Plus® rats per sex per group received Kasugamycin Hydrochloride Technical (80.6%) in the diet at 0 (basal diet), 200, 1000, and 6000 ppm through 2 generations with 1 litter in the first generation and 2 litters in the second generation. Group mean test article intake during the 10-week premating interval was 13.56, 69.99, and 421.80 mg/kg/day for F0 males; 16.31, 84.86, and 505.17 mg/kg/day for F0 females; 13.87, 70.68, and 428.74 mg/kg/day for F1 males; and 16.08, 80.92, and 501.71 mg/kg/day for F1 females at 200, 1000, and 6000 ppm, respectively. Treatment-related clinical observations were limited to red and swollen skin around the anal opening in 7/25 F0 males, 25/25 F0...
females, 21/25 F1 males, and all F1 females at 6000 ppm. Significant reductions in F0 group mean bodyweight were noted for males at 1000 ppm for weeks 4 through 9 and 11 and, at 6000 ppm, for weeks 6 through 9 compared to controls. Group mean bodyweight for F0 males was slightly lower than controls (ns) through the remainder of the F0 generation. Group mean bodyweight for F0 females was comparable to controls during the premating period. Group mean bodyweight for F1 males and females was comparable to controls during the F2a and F2b pre- and postmating periods, including the gestation and lactation periods for F1 females. No treatment-related effects on food consumption were noted for F0 and F1 males and females during the study. There were no treatment-related effects on F0 male or female reproductive performance or fertility at any dose level. A treatment-related decrease in F1 male fertility was noted at 6000 ppm. Significantly fewer pregnant females resulted at both the F2a and F2b matings at the high dose compared to controls. None of the 8 F1 males at 6000 ppm that failed to sire a litter in the F2a mating sired a litter in the F2b mating. Additionally, the 16 F1 males that successfully sired litters in the F2a mating, sired only 9 litters in the F2b mating at the high dose. Furthermore, the mean cohabitation days for the F2b mating were significantly longer (5.73 days) vs the controls (2.23 days) indicating a possible libido effect. At necropsy, treatment related changes were noted in the rectum of F0 and F1 males and females at 6000 ppm. At terminal necropsy, areas of red foci in the rectum were noted for 20/25 F0 and F1 males and 25/25 F0 and F1 females at 6000 ppm (the incidence for respective controls was 0). Also, increased incidence of small testes (9/24) and testes containing fluid (16/24) was noted for F1 males at 6000 ppm vs controls (0/25). Histology for F0 and F1 adults at 6000 ppm included increased incidence of ulceration of the mucosa of the rectum at the rectal-anal junction in 25/25 F0 males and 22/25 F0 females and 24/25 F1 males and 23/25 F1 females along with chronic-active inflammation of the submucosa of the rectum in 25/25 F0 males and 24/25 F0 females and 24/24 F1 males and 25/25 F1 females vs controls (0 incidence for both sexes). Additionally, bilateral atrophy/degeneration of the testes characterized by complete loss of germinal epithelium, atrophy of seminiferous tubules, and persistence of sertoli cells (variable amounts of intertubular edema was also frequently present) was noted in 15/24 F1 males at 6000 ppm vs controls (0/24). No treatment-related effects were noted for clinical signs, gestation, viability, weaning indices, number of pups per litter, sex ratios, necropsy or histology findings for the F1, F2a, and F2b pups/litters at any treatment level. Parental NOEL for males = 200 ppm (13.7 mg/kg/day) based on reduced bodyweight and bodyweight gain. Parental NOEL for females = 1000 ppm (82.9 mg/kg/day) based on clinical observations (red and swollen skin around the anal opening). Pup NOEL (M/F) = 6000 ppm (425.3 mg/kg/day for males and 503.4 mg/kg/day for females). Reproductive NOEL (M/F) = 1000 ppm (70.3 mg/kg/day for males and 82.9 mg/kg/day for females) based on reduced fertility and fecundity in F1 parents for both litters and increased cohabitation interval for the F2b litter. No adverse reproductive effect. Acceptable. (Green and Leung, 11/25/14).

**DEVELOPMENTAL TOXICITY**

**Rat**

**53293-0028 280638, “Teratogenicity study in rats with Kasugamycin”; 833; rats; The Institute of Environmental Toxicocology, Tokyo, Japan. Study No.: TMN-0136, IET 89-0085, 4/1991; Fujii, S.; Kasugamycin, Lot No. KP-834, a fine brown powder, 64.5% pure Kasugamycin**
hydrochloride. Groups of 24 pregnant SD rats were treated with the test substance at daily oral gavage doses of 0, 40, 200 or 1000 mg/kg/day from day 6 to 15 of gestation. No mortality. Loose stool was observed in 7 females in 1000 mg/kg/day group during the dosing period. Reduced food consumption in 200 and 1000 mg/kg/day group rats, reduced body weight gain in 1000 mg/kg/day group was observed in the treated maternal rats. Distention in cecum with stool in one female of 200 mg/kg/day group, and 5 females of 1000 mg/kg/day group, subcutaneous mass in one 200 mg/kg/day group female, and emaciation, distention with gas in small intestine in one female of 1000 mg/kg/day group was observed at maternal rats necropsy. No treatment related effects were observed in fetuses of all dosing groups. No observed adverse effect was observed in fetuses at 1000 mg/kg/day. NOEL: Maternal: 40 mg/kg/day due to food consumption reduction in 400 mg/kg/day group rats; Developmental: 1000 mg/kg/day due to no treatment related effects at HDT. Study Acceptable. (Pan& Leung, 1/22/2015).

Rat Developmental Dose-Range-Finding Study

53293-0027 280637, “Teratogenicity study in rats with Kasugamycin”; supp; rats; Eurofins/Product Safety Laboratories, Dayton, NJ. Lab Project ID: TMN-0135, IET 89-0084 6/1990; Fujii, S.; Kasumin 2L, Lot No.7290, a dark blue-green liquid, 2.60% w/v Kasugamycin hydrochloride. Groups of 7 pregnant SD rats were treated with the test substance at daily oral gavage doses of 0, 10, 100, 300 or 1000 mg/kg/day from day 6 to 15 of gestation. No mortality. Loose stool was observed in 4 females in 1000 mg/kg/day group during dosing period. Reduced food consumption in 300 and 1000 mg/kg/day group rats, reduced body weight gain in 1000 mg/kg/day group was observed in the treated maternal rats. No treatment related effects were observed in fetuses of all dosing groups. The recommended dose levels for main teratogenicity study in rats are selected to be 300 and 1000 mg/kg/day to produce signs of toxicity. No observed adverse effect was observed in fetuses at 1000 mg/kg/day. Study Supplemental. (Pan& Leung, 11/24/2014).

Rabbit

**53293-0031 280641, “Kasugamycin: Teratogenicity study in the rabbit”; 833; rabbits; Life Science Research, Suffolk, IP23 7PX, England. Lab Project ID: TMN-0134, 86/HKC004/114; Ross, F. W.; Kasugamycin, Lot No. KP-834, a fine brown powder, 64.5% pure Kasugamycin hydrochloride. Groups of 15 inseminated New Zealand White female rabbits were treated with the test substance at daily oral gavage doses of 0, 1, 3 or 10 mg/kg/day from day 6 to 19 of gestation. Animals were euthanized on day 29 of gestation and examined for uterine content. One female in group 4 (10 mg/kg/day) died on day 7. Reduced food intake and fecal output was observed of it before death. One female in group 3 (3 mg/kg/day) was killed in extremis on day 19 due to suspected tracheal intubation. Necropsy findings in these rabbits showed respiratory tract disorder. Two females in groups 2 (1 mg/kg/day) aborted on gestation days 23, and two females in groups 4 (10 mg/kg/day) aborted on gestation days 21 and 21. Necropsy findings in these rabbits were consistent with reduced food consumption (group 2) and respiratory and gastro-intestinal tracts disorder (group 4). One female in group 4 had a total loss of litter. No treatment related observations in maternal survival and pregnancy status, as well as offspring data. NOEL (No Observed Effect Level): Maternal NOEL=1 mg/kg/day (mortalities and total
litter loss); Developmental NOEL = 10 mg/kg/day (no effect at HDT). Study acceptable. (Pan& Leung, 1/13/2015)

**Rabbit Preliminary Teratology Study**

53293-0032 280642, “Kasugamycin: Preliminary teratology study in the rabbits”; supp; rabbit; Life Science Research, Eye, Suffolk, IP23 7PX. Lab Project ID: 85/HK003/783, 2/20/1986; Tesh, J.M., Ross, F.W., and Wright, P.C.; Kasugamycin, Lot No. KP-570, 67.1% pure, a light brown powder. Groups (1-4) of 4 New Zealand White rabbits were treated with the test substance at daily oral gavage doses of 0, 250, 500, or 1000 mg/kg/day from gestation days 6 to 19, Group 1 received distilled water only. Reduction of food consumption, bodyweight and body weight gain, clinical signs were observed in all treated animals, before they were killed in extremis on gestation days of 14, 15 or 16. The study restarted with dosages of 0, 10, 30 and 100 mg/kg/day for groups 5, 6, 7 and 8, respectively, for the same duration of treatment. One female in 30 mg/kg/day group aborted on day 22 after insemination. Three females in the 100 mg/kg/day group aborted on days 18, 19 or 27 after insemination. One female from 10 mg/kg/day group was killed in extremis on day 27 after insemination. Reduction of food consumption, bodyweight loss, and stained or matted fur on part of the body surfaces were observed before their deaths. No abnormalities were noted in necropsy except for pale kidney, and/or liver, distended gastro-intestinal tract. Females receiving 10 and 30 mg/kg/day exhibited weight loss during early stage of dosing period followed by some degree of recovery through the remaining treatment period. Lower mean fetal weight was observed in group 7 offspring. For the main teratology study in rabbit, 10 mg/kg/day was selected as the high dose based on mortality, body weight, food consumption, and fetal data. Study supplemental. (Pan& Leung, 1/9/2015).

**GENOTOXICITY**

**Gene Mutation**

**53293-0040 280650, “Evaluation of Kasugamycin (Lot No. KP-570) in the V79/HGPRT Forward Mutation Assay,” (R.R. Young, Litton Bionetics, Inc., Kensington, MD., LBI Project No. 22207, August 1985). Single cultures of 4 x 10^6 V79 cells were treated (in 250 ml flasks) with Kasugamycin technical (67.1%) for 4 hours at 37 ± 2°C, in the presence and absence of rat liver S9 mix, at 0 (Eagle’s Minimal Essential Medium (EMEM)), 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/ml. Two independent non-activated and 3 independent activated trials were performed using single cultures in each trial. Cytotoxicity was increased in activated cultures at 10 mg/ml (relative survival was 50% of negative control values). Relative survival in non-activated cultures was comparable to negative control values. An increase in forward gene mutations was not indicated with or without rat liver S9 mix. Positive controls were functional. No adverse effect. Acceptable (Green and Leung, 12/17/14).

53293-0042 280652, “Mutagenicity Testing on Kasugamycin-HCL in Microbial Systems,” (Y. Shirasu et al., The Institute of Environmental Toxicology, Tokyo, Japan, Laboratory Project ID. IET-11-15-1976, November 15, 1976). This submission contains summary results of mutagenicity testing with Kasugamycin hydrochloride (80%) in (1) a rec-assay using the repair proficient (H17) and deficient (M45) strains of B. subtilis in a disk diffusion assay performed in
duplicate with overnight exposure to Kasugamycin hydrochloride at 0 (distilled water), 100, 200, 1000, and 2000 µg/disk in the absence of activation. The inhibition zone for the repair deficient strain (M45) was up to twice as large as that for the wild type strain (M17) at 1000 and 2000 µg/dish and up to 3 times the vehicle control value. Dosing level rationale was not provided and metabolic activation was not included. (2) Summary results were also provided for a bacterial reverse mutation assay. Duplicate cultures of Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 and Escherichia coli strain WP2 hcr were exposed to Kasugamycin hydrochloride, in the presence and absence of rat liver S9 mix, at 0 (distilled water), 5, 10, 50, 100, and 200 µg/plate. Additionally, single cultures of S. typhimurium strain G46 were exposed to Kasugamycin hydrochloride without activation at 0, 10, 50, 100, and 500 µg/plate. There was no increase in the number of revertants per plate under the test conditions. Dosing level rationale and treatment times were not provided. (3) In a host mediated assay, 6 male ICR mice per group received 2 equal doses of Kasugamycin hydrochloride 24 hours apart at 0 (distilled water), 500, and 2000 mg/kg by oral gavage. Immediately after the second dose, 2 ml (5.8 x 10^8 cell/ml) of S. typhimurium G46 (his-) in logarithmic growth phase was inoculated intraperitoneally. Animals in each group were sacrificed 3 hours later. Triplicate cultures of the S. typhimurium G46 containing peritoneal fluid were plated and incubated at 37°C for 2 days, after which the survivors and revertants were counted. There was no increase in the number of revertants per plate or in cell toxicity at any treatment level compared to vehicle controls. These summaries are supplemental data. (Green and Leung, 12/23/14).

**53293-0044 280654, “Bacterial Reverse Mutation Assay,” (V.O. Wagner and M.R. VanDyke, BioReliance Rockville, MD., BioReliance Study No. AC29CA.503.BTL, November 4, 2009). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvrA* were exposed by direct plate incorporation to Kasugamycin technical (71.5%), in the presence and absence of rat liver S9 mix, at 0 (sterile distilled water), 1.5, 5.0, 15, 50, 150, 500, 1500, or 5000 µg/plate for 48 to 72 hours. Two trials were performed. Toxicity (reduced number of revertants per plate) was noted beginning at 150 µg/plate (strains TA100 and WP2 *uvrA* (-S9)), 500 µg/plate (strains TA98, TA1535, and WP2 *uvrA* (+S9)), or 1500 µg/plate (strain TA1537) in the initial toxicity-mutation assay and at 500 and 1500 µg/plate (strains TA98, TA100, TA1535, and WP2 *uvrA*) and at 1500 and 5000 µg/plate in strain TA1537 in the confirmatory assay with and without rat liver S9 mix. There was no increase in the number of revertants per plate in the initial toxicity-mutation assay or the confirmatory assay, with or without activation, compared to solvent controls. Positive controls were functional. No adverse effect. Acceptable. (Green and Leung, 12/15/14).

**Chromosome Effects**

53293-0039 280649, “Mutagenicity Evaluation of Kasugamycin Technical (Purity 67.1%, Lot No. KP-570) in an In Vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells,” (J.L. Ivett, Litton Bionetics, Inc., Kensington, MD., LBI project No. 20990, May 1985). Duplicate cultures of Chinese hamster ovary (CHO-WBL) cells were exposed to Kasugamycin technical (67.1%), in the presence and absence of rat liver S9 mix, at 0 (McCoy’s 5a medium, negative control), 0 (McCoy’s 5a medium, solvent control), 2.0, 3.0, 4.0, and 5.0 mg/ml for 2 hours with activation and for 7.25 hours without. Cultures were treated with colcemid (1.0 µg/ml) 2.5 hours before harvest (10 hours after the start of treatment). There was no increase in metaphases with aberrations after the 2 hour treatment with rat liver S9
mix or after the 7.25 hour treatment without activation vs solvent controls. Positive controls were functional. The time from the start of treatment to cell harvest was inadequate for aberration expression. Data are supplemental. (Green and Leung, 12/16/14).

**53293-0043 280653, “Kasugamycin: Assessment of Clastogenic Action on Bone Marrow Erythrocytes in the Micronucleus Test,” (G. Hodson-Walker, Life Science Research, Eye, Suffolk, England, LSR Report No. 85/HKC001/207, March 30, 1985). Five CD-1 mice per sex per group received a single oral gavage dose of Kasugamycin technical (67.1%) at 0 (distilled water), 200, 1000, and 5000 mg/kg followed by bone marrow sampling 24, 48, and 72 hours later. Bone marrow from 5 mice per sex per group was sampled at 24 hours after a single oral dose of 200 and 1000 mg/kg. Bone marrow from five control and 5000 mg/kg mice per sex was sampled at 24, 48, and 72 hours after treatment. Two thousand polychromatic erythrocytes (PCEs) per animal were evaluated for the presence of micronuclei. All animals survived to scheduled bone marrow sampling times. At 5000 mg/kg, six males and 1 female had brown anal staining and evidence of diarrhea on the day of dosing. No treatment-related increases in micronucleated polychromatic erythrocytes (MN-PCEs) were noted at any treatment level or sampling time compared to vehicle controls, and polychromatic to normochromatic erythrocyte ratios (PCE:NCE) were comparable to vehicle controls. Positive controls were functional. No adverse effect. Acceptable. (Green and Leung, 12/22/14).

**53293-0045 280655, “In Vitro Mammalian Chromosome Aberration Test,” (R. Gudi and M. Jois, BioReliance, Rockville, MD., BioReliance Study No. AC29CA.331.BTL, November 9, 2009). Duplicate cultures of Chinese hamster ovary cells (CHO-K1) were exposed to Kasugamycin technical (71.5%) at 0 (water), 542.5, 1085, 2170, 4340 ug/ml for 4 hours with and without rat liver S9 mix and for 20 hours without activation. Cells were harvested 20 hours after treatment initiation. Colcemid® at 0.1 ug/ml was added to each culture 2 hours prior to harvest to arrest dividing cells in metaphase. At the high dose level, cell growth was reduced 12% and 20% after the 4 hour and 20 hour treatment periods without activation, respectively, relative to the solvent controls. There was no toxicity (cell growth inhibition) in cultures treated for 4 hours with rat liver S9 mix at any treated level compared to solvent controls. No reduction in the mitotic index was noted at any treated level after the 4 hour treatment period with and without activation compared to solvent controls. The mitotic index was reduced 14% at 4340 ug/ml after the 20 non-activated treatment compared to the solvent control. There was no increase in structural or numerical chromosome aberrations after the 4 hour treatment period with and without rat liver S9 activation and after the 20 hour non-activated treatment period at any treated level compared to solvent controls. Positive controls were functional. No adverse effect. Acceptable. (Green and Leung, 12/18/14).

**53293-0041 280651, “Kasugamycin: Unscheduled DNA Synthesis in Human Cells, Cell line: Hela S3,” (A. H. Seeberg, Life Science Research, Roma Toxicology Center, Roma, Italy, LSRC-RTC Report No. 161001-M-01885, April 29, 1985). Cultured human cells (Hela 3) were treated in triplicate with Kasugamycin technical (67.1%) in the presence of 3H-thymidine, with and without rat liver S9 mix, at 0 (Eagle’s Minimal Essential Medium (EMEM)), 0.156, 0.313, 0.625, 1.25, 2.50, 5.00, or 10.0 mg/ml for 3 hours at 37°C. There was no treatment-related increase in the incorporation of 3H-thymidine compared to vehicle controls. An increase in the
induction of unscheduled DNA synthesis (UDS) was not indicated. Positive controls were functional. Acceptable. (Green and Leung, 12/19/14).

NEUROTOXICITY

Acute neurotoxicity, rat

**53293-0046, 0047 280656, 280657, “An oral (Gavage) Acute Neurotoxicity Study of Kasugamycin Technical in Rats,” (M. J. Beck, WIL Research Laboratories, LLC., Ashland, OH., Study No. WIL-476009, December 28, 2009). Twelve Crl:CD(SD) rats per sex per group received a single oral gavage dose of Kasugamycin technical (71.5%) at 0 (deionized water), 500, 1000, and 2000 mg/kg followed by a 2-week observation period. All animals survived to the scheduled necropsy. There were no treatment-related effects on clinical observation findings, bodyweights, bodyweight gains, functional observational battery evaluation results, locomotor activity measures, brain weights and measurements, macroscopic pathology results, or neurohistopathology findings at any treatment level. Record 280656 is a rat dose range-finding acute neurotoxicity study. Systemic NOEL (M & F) = 2000 mg/kg. No adverse effect. Acceptable. (Green, 12/8/14).

Acute Dose Range-Finding Neurotoxicity Study

53293-0046 280656, “An Oral (Gavage) Dose Range-Finding Acute Neurotoxicity Study of Kasugamycin Technical  in Rats,” (C.S. Roegge, WIL Research Laboratories, LLC., Ashland, OH., Study No. WIL-476007, August 6, 2009). Five Crl:CD(SD) rats per sex per group received a single oral gavage dose of kasugamycin technical (71.5%) at 0 (deionized water), 500, 1000, and 2000 mg/kg. Detailed clinical observations of each animal were performed at 1, 2, 4, and 8 hours post-treatment. All animals were sacrificed and discarded without macroscopic evaluation after completion of the 8 hour post treatment observations. All animals survived to scheduled sacrifice with no treatment-related clinical findings. Based on the maximum plasma concentration in male and female rats at 1 hour following a single dose (Tmax) (Cheng, 1998, record 280660), one hour post-dosing was selected as the time to peak effect for study day 0 functional observational battery (FOB) and locomotor activity evaluations in the main study. Supplemental data. (Green, 12/8/14). No worksheet.

90-day dietary neurotoxicity, rat

**53293-0048 280658, “A 90-Day Dietary Neurotoxicity Study of Kasugamycin Technical in Rats,” (M. J. Beck, WIL Research Laboratories, LLC., Ashland, OH., Study No. WIL-476008, December 21, 2009). Twelve Crl:CD(SD) rats per sex per group received Kasugamycin technical (71.5%) in the diet at 0 (basal diet), 300, 3000, and 6000 ppm for 90 consecutive days. Group mean daily intake of kasugamycin technical during the study was 21, 210, and 439 mg/kg/day for males and 23, 238, and 486 mg/kg/day for females at 300, 3000, and 6000 ppm, respectively. All animals survived to scheduled necropsy. No treatment-related effects were noted for clinical observations or for detailed physical examinations. Group mean bodyweight gains were significantly reduced for males (during weeks 1 to 2, 3 to 4, 4 to 5, and 0 to 13) and for females (during weeks 3 to 4) at 6000 ppm vs controls. Additionally, at 6000 ppm, group
mean bodyweight was reduced for males (up to 14.1% during study weeks 2 to 13) and for females (up to 10.3% during study weeks 4 to 13) vs controls (values were statistically different for males during weeks 2 to 13 and for females at week 4). Treatment-related changes in bodyweight were not indicated for males and females at 300 and 3000 ppm. No treatment-related changes were noted for group mean food consumption, functional observational battery (FOB) and locomotor activity results, macroscopic pathology findings (including brain weights and measurements), and neurohistopathology results at 300, 3000, and 6000 ppm. Systemic NOEL = 3000 ppm (210 mg/kg/day for males and 238 mg/kg/day for females) based on reduced bodyweight and bodyweight gain. Neurotoxicity NOEL = 6000 ppm (439 mg/kg/day for males and 486 mg/kg/day for females). No adverse effect. Acceptable. (Green, 12/5/14).

**IMMUNOTOXICITY**

**53293-0051  280661, “A 28-Day Dietary Immunotoxicity Study of Kasugamycin Technical in Female CD-1® Mice,” (S.D. Seidel, WIL Research Laboratories, LLC., Ashland, OH., Study No. WIL-476010, May 24, 2010). Two assays were performed, an antibody forming cell (AFC) assay and a natural killer (NK) cell function assay. In each assay, ten female Crl:CD-1 mice per group received Kasugamycin technical (71.5%) in the diet at 0 (basal diet), 300, 3000, and 10,000/7000 ppm for 28 days. The dietary concentration at 10,000 ppm was reduced to 7000 ppm from day 15 (NK assay) or 16 (AFC assay) to the end of the treatment period due to excessive bodyweight loss. Ten positive control females in each assay received basal diet throughout the study. The ten positive control females in the AFC assay were administered an intraperitoneal injection of cyclophosphamide at 50 mg/kg/day once daily for 4 consecutive days (study days 24 to 27). In the NK cell function assay, the ten positive control females received a single intravenous injection of anti-asialo GM1 twenty-four hours prior to scheduled necropsy. All AFC assay animals were immunized with sheep red blood cells (sRBC) (1 x 10^8 sRBC) via a tail vein injection 96 hours prior to scheduled necropsy. Group mean test article consumption was 70, 755, and 2468 mg/kg/day for AFC assay group animals and 68, 691, and 2531 mg/kg/day for NK assay group animals at 300, 3000, and 10,000/7000 ppm, respectively. Three 7000 ppm AFC assay group animals were euthanized in extremis on study days 21, 23, and 25 due to treatment-related bodyweight losses. At 10,000/7000 ppm, Group mean bodyweight was significantly reduced for NK cell assay group animals for study weeks 1 to 4 and for AFC assay group animals for study weeks 2 to 4 compared to vehicle controls. Additionally, at the high dose level, group mean cumulative bodyweight change was significantly reduced for NK assay group animals for study weeks 0 to 1, 0 to 2, 0 to 3, and 0 to 4 and for AFC assay group animals for study weeks 0 to 2, 0 to 3, and 0 to 4 compared to vehicle controls. At 3000 ppm, group mean bodyweight for NK cell assay animals was significantly reduced for study weeks 1 and 2 compared to vehicle controls. In the AFC assay, group mean spleen weights (absolute) and total spleen activity (AFC/spleen) for the 10,000/7000 ppm animals were significantly lower than the vehicle controls. However, when evaluated as AFC specific activity (AFC/10^6 spleen cells), the mean response for the high dose animals was lower than the vehicle controls but with no dose response and no statistical difference. In the NK cell assay, there was no effect on natural killer cell activity at any dose level compared to vehicle controls. There were no treatment-related immunological effects at 300 and 3000 ppm. Positive controls were functional. Immunotoxicity NOEL (F) = 3000 ppm (691 mg/kg/day (NK assay) and 755 mg/kg/day (AFC assay)) based on lower spleen weights and total spleen activity (AFC/spleen) in the AFC assay. Systemic NOEL
(F) = 300 ppm (68 mg/kg/day (NK assay) and 70 mg/kg/day (AFC assay)) based on reduced bodyweight. No adverse effect. Acceptable. (Green, 12/23/14).