CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Fluensulfone

Chemical Code #6091, **Tolerance** # 53183
SB 950 # NA

2/28/13

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect indicated
Oncogenicity, rat:	No data gap, no adverse effect indicated
Oncogenicity, mouse:	No data gap, non-neoplastic adverse effect
Reproduction, rat:	No data gap, non-reproductive adverse effect
Teratology, rat:	No data gap, no adverse effect indicated
Teratology, rabbit:	No data gap, no adverse effect indicated
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effect indicated
DNA damage:	No data gap, no adverse effect indicated
Neurotoxicity:	Data gap, no adverse effect indicated

Toxicology one-liners are attached.

All record numbers through #266323 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T022813
Revised by T. Moore, 2/28/13

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

**** 0054, 266294**; "104-Weeks Combined Chronic Toxicity and Oncogenicity (Feeding) Study in the Rat" (St. Kaiser, Harlan Laboratories Ltd., Itingen, Switzerland, Harlan Laboratories Study B80188, 07/19/2011). 870.4300. MCW-2 TECH (Batch Number 36372130-291-PF1, purity = 96.7%) was admixed to the diet and fed to 50 HanRcc:WIST(SPF) rats per sex per dose at dose levels of 0 (untreated diet), 30, 200, or 1200 ppm (0, 1.6, 11.0, and 66.3 mg/kg/day, respectively, after 1 year and 0, 1.4, 9.6, and 57.7 mg/kg/day, respectively, after 2 years for males and 0, 1.9, 13.1, and 75.2 mg/kg/day, respectively, after 1 year, and 0, 1.7, 11.6, and 69.3 mg/kg/day, respectively, after 2 years for females) continuously for at least 104 weeks and to 20 additional animals per sex per dose (satellite group animals) for at least 52 weeks. Mortality totals (main group animals) were as follows- males: 11 (3 spontaneous, 8 sacrificed *in extremis*)/50, 8 (4 spontaneous, 4 sacrificed *in extremis*)/50, 15 (8 spontaneous, 7 sacrificed *in extremis*)/50, 12 (4 spontaneous, 8 sacrificed *in extremis*)/50, respectively; females: 14 (2 spontaneous, 12 sacrificed *in extremis*)/50, 14 (2 spontaneous, 12 sacrificed *in extremis*)/50, 17 (10 spontaneous, 7 sacrificed *in extremis*)/50, 12 (4 spontaneous, 8 sacrificed *in extremis*)/50, respectively. Mortality totals (satellite group animals) were as follows- males: no mortalities; females: 2/20 (spontaneous), 0/20, 0/20, 0/20, respectively. No treatment-related clinical signs were observed. No treatment-related increase in incidence of palpable masses occurred. Functional Observational Battery (FOB), conducted on each satellite group animal at week 52 of treatment, revealed no treatment-related effects. A treatment-related decrease in mean body weight was observed in males at 200 and 1200 ppm; no effect on body weight was observed in females. A treatment-related decrease in mean food consumption in males at 1200 ppm was observed; no effect on food consumption was observed in females. Hematology investigations of the satellite group animals revealed a treatment-related increase in the mean red blood cell count in males (at weeks 26 and 52) at 1200 ppm, treatment-related decreases in mean corpuscular hemoglobin in males (at weeks 13, 26, and 52) at 1200 ppm, in mean hemoglobin in females (at weeks 13, 26, and 52) at 1200 ppm, and in mean corpuscular hemoglobin concentration in males (at weeks 13, 26, and 52) and in females (at weeks 13 and 26) at 1200 ppm, a treatment-related increase in mean reticulocyte count in males (at week 26) at 1200 ppm, and a treatment-related decrease in mean white cell count in females (at week 52) at 1200 ppm; investigations of the main group animals revealed treatment-related increases in the mean red blood cell count (at week 104) and in mean reticulocyte count (at week 78) in males at 1200 ppm, a treatment-related decrease in mean hemoglobin in females (at weeks 78 and 104) at 1200 ppm, and a treatment-related decrease in the mean corpuscular hemoglobin concentration (at weeks 78 and 104) in both sexes at 1200 ppm. Clinical biochemistry investigations in the males revealed treatment-related increases in mean cholesterol, triglycerides, and phospholipids (at weeks 13, 26, and 52) at 1200 ppm, in mean urea (at week 26) at 200 and 1200 ppm, in mean total bilirubin (at week 52) at 1200 ppm, in mean protein and albumin (at weeks 26 and 52) at 1200 ppm, in mean aspartate aminotransferase, creatine kinase, and alkaline phosphatase (at week 52) at 1200 ppm, and in lactate dehydrogenase (at weeks 26 and 52) at 1200 ppm; investigations in the females revealed treatment-related increases in mean urea and triglycerides (at week 52) and in mean phospholipids (at weeks 26 and 52) at 1200 ppm. Urinalysis revealed a treatment-related increase in mean ketones level (at weeks 13 and 26) in males at 1200 ppm. Treatment-related increases in mean relative liver, kidney, and adrenal weights were observed in satellite group males at 1200 ppm and a treatment-related increase in mean relative kidney weight was observed in satellite group females at 1200 ppm; in the main group, treatment-related increases in mean relative liver and kidney weights in both sexes at 1200 ppm were observed. Determinations of mean liver enzyme activities at week 52 (satellite group animals) revealed treatment-related increases in mean uridine diphospho-glucuronosyl transferase (in both sexes at 1200 ppm), in mean cytosolic glutathione-S-transferase (in males at 1200 ppm and in females

at 200 and 1200 ppm), and in mean microsomal epoxide hydrolase (in males at 1200 ppm and in females at 200 and 1200 ppm). A treatment-related increase in the concentration of fluoride in bone of the main group animals in both sexes at all dose levels was observed; a treatment-related increase in the concentration of fluoride in bone of satellite group animals in both sexes at 200 and 1200 ppm was observed. A treatment-related increase in the concentration of fluoride in teeth of both the main and satellite group animals in both sexes at 200 and 1200 ppm was observed. Macroscopic examination revealed a treatment-related increase in the number of foci in the lungs in both sexes at 1200 ppm. In the main group animals, microscopic examination revealed a treatment-related increased incidence of non-neoplastic hyperkeratosis in the esophagus of both sexes at 200 and 1200 ppm and a treatment-related increased incidence of non-neoplastic chronic interstitial inflammation in the lungs associated with increased severity of intra-alveolar macrophages in females at 200 ppm and in both sexes at 1200 ppm. No treatment-related tumors were observed. **Possible adverse effect:** fluorosis of the bones. NOEL (M) < 1.4 mg/kg/day (30 ppm) and NOEL (F) < 1.7 mg/kg/day (30 ppm) based on a treatment-related increase in the concentration of fluoride in the bones of the main group animals. **Acceptable.** (Corlett and Leung, 02/20/2013)

CHRONIC TOXICITY, RAT

See Combined, Rat above.

CHRONIC TOXICITY, DOG

** 0046; 266286; “MCW-2 TECH : 52-Week Oral (Feeding) Toxicity Study in the Beagle Dog with an 8-week Recovery Period” (Braun, L., Harlan Laboratories Ltd., Itingen, Switzerland, Harlan Laboratories Study C57031, 07/14/2011). 870.4100. MCW-2 TECH (Batch No. 36372130-291-PF1, purity = 96.5%) was admixed to the diet and fed to 4 beagle dogs per sex per dose at dose levels of 0 (diet only), 5, 50, 100, or 500 ppm (0, 0.1, 1.5, 3.1, and 16.0 mg/kg/day, respectively for males and 0, 0.1, 1.5, 3.3, and 16.2 mg/kg/day, respectively for females) for 52 weeks with 4 additional dogs per sex used at 0 and 500 ppm to assess recovery over a subsequent 8-week treatment-free period. No treatment-related mortalities occurred. A treatment-related decrease in mean body weight was observed in males at 500 ppm; no treatment-related effect on mean body weight in females was observed. No treatment-related effect on mean food consumption in either sex was observed. Treatment-related decreases in mean hemoglobin (in males only), mean corpuscular hemoglobin (in males only), and mean corpuscular hemoglobin concentration (in both sexes) and treatment-related increases in mean reticulocytes (in both sexes), white blood cell count (in females only), platelet count (in females only), red cell volume distribution width (in both sexes), and hemoglobin concentration distribution width (in both sexes) were observed at 500 ppm with the recovery group animals exhibiting reversibility of these effects. Treatment-related decreases in mean protein and albumin levels were observed in both sexes at 500 ppm with the recovery group animals exhibiting reversibility of these effects. Analyses of total triiodothyronine (T3), total thyroxine (T4), and thyroid-stimulating hormone levels in the serum of the test animals revealed no treatment-related effects. Analysis of liver tissue revealed an increase in mean microsomal epoxide hydrolase activity in both sexes at 500 ppm. Urinalysis revealed no treatment-related effects. A treatment-related increase in mean relative liver weight was observed in females at 100 ppm with recovery group animals exhibiting reversibility of this effect. Analytical determinations of fluorine in the teeth of the test animals revealed a treatment-related increase in fluorine in both sexes at 500 ppm with recovery group animals exhibiting reversibility of this effect; analytical determinations of fluorine in the bone of the test animals also revealed a treatment-related increase in fluorine in both sexes at 500 ppm with this effect persisting in the recovery group animals. Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination revealed treatment-related hepatocellular hypertrophy and brown pigment in the sinusoidal cells of the liver in both sexes at 500 ppm with reversibility of the hepatocellular hypertrophy effect observed (except in one female) in the recovery group animals but with the brown pigment in the sinusoidal cells of the liver effect persisting in the recovery group animals. **No adverse effects.** NOEL (M) = 3.1

mg/kg/day (100 ppm) and NOEL (F) = 3.3 mg/kg/day (100 ppm) based on hepatocellular hypertrophy and brown pigment in the sinusoidal cells of the liver. **Acceptable**. (Corlett and Leung, 12/07/2012)

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

**** 0055, 266295**; "MCW-2 TECH: 78-Weeks Oncogenicity (Feeding) Study in CD-1 Mice" (St. Kaiser, Harlan Laboratories Ltd., Itingen, Switzerland, Harlan Laboratories Study B80190, 08/31/2011). 870.4200. MCW-2 TECH (Batch Number 36372130-291-PF1, purity = 96.67%) was admixed to the diet and fed to 50 CD-1 mice per sex per dose at dose levels of 0 (untreated diet), 30, 200, or 1200 ppm (0, 4.2, 27.6, and 152.3 mg/kg/day, respectively for males and 0, 6.4, 39.0, and 188.4 mg/kg/day, respectively for females) for 78 weeks with 6 additional animals per sex per dose that were treated for 13 weeks and then sacrificed for liver enzyme determination. Mortality totals (main group animals) were as follows- males: 14 (10 spontaneous, 4 sacrificed *in extremis*)/50, 8 (6 spontaneous, 2 sacrificed *in extremis*)/50, 4 (3 spontaneous, 1 sacrificed *in extremis*)/50, 9 (8 spontaneous, 1 sacrificed *in extremis*)/50, respectively; females: 7 (6 spontaneous, 1 sacrificed *in extremis*)/50, 13 (9 spontaneous, 4 sacrificed *in extremis*)/50, 14 (12 spontaneous, 2 sacrificed *in extremis*)/50, 14 (8 spontaneous, 6 sacrificed *in extremis*)/50, respectively. No treatment-related clinical signs were observed. No treatment-related increase in incidence of palpable masses occurred. A treatment-related decrease in mean body weight was observed in males at 200 and 1200 ppm and females at 1200 ppm. A treatment-related decrease in mean food consumption in both sexes at 1200 ppm was observed. Hematology investigations revealed a treatment-related decrease in the mean red blood cell count in both sexes at week 52 at 1200 ppm and a treatment-related decrease in the mean white blood cell count in females at week 52 at 1200 ppm; no treatment-related hematological effects were observed at week 78. Clinical biochemistry investigations at week 78 revealed no treatment-related effects. Determinations of mean liver enzyme activities at week 13 revealed treatment-related increases in several enzymes including microsomal 7-ethoxyresorufin O-dealkylase, microsomal lauric acid 12-hydroxylase, and microsomal uridine diphospho-glucuronosyl transferase in females at 1200 ppm, and in cytosolic glutathione-S-transferase and microsomal epoxide hydrolase in both sexes at 1200 ppm. Macroscopic examination revealed treatment-related nodules in the lungs in females at 200 and 1200 ppm. Microscopic examination revealed treatment-related alveolar/bronchiolar tumors in the lungs (benign and/or malignant) in females at 200 and 1200 ppm. Microscopic examination of the lungs revealed treatment-related non-neoplastic bronchiolization in both sexes at 200 and 1200 ppm. The lung was identified as the target organ. **Possible adverse effect:** treatment-related bronchiolization in the lungs at the mid- and high-doses in both sexes. NOEL (M) = 4.2 mg/kg/day (30 ppm) and NOEL (F) = 6.4 mg/kg/day (30 ppm) based on bronchiolization in the lungs. **Acceptable.** (Corlett and Leung, 01/31/2013)

REPRODUCTION, RAT

**** 53183-0059; 266299**; "MCW-2 Tech: Two-Generation Reproduction Toxicity Study"; (R. Ceccatelli; Harlan Laboratories Ltd, 4414 Fullinsdorf, Switzerland; Study No. B92654; 5/20/11); Twenty four HanRcc:WIST rats/sex/group were treated in the diet with 0, 30, 250, or 1800 ppm of MCW-2 Technical (batch no. 36372130-291-PF1; purity: >96.0%) for two generations. The treatment included 10 weeks prior to mating, mating, 3 weeks of gestation and 3 weeks of lactation for the P generation. At that time, 24 F1 animals/sex/group were selected as parents and treated for 13 weeks prior to mating, followed by mating and 3 weeks each of gestation and lactation of the F2 generation. Both sexes in the 1800 ppm group of both P and F1 generations had lower mean body weights than those of the controls at the end of the premating period ($p<0.01$) or during the gestation and/or lactation periods (females, $p<0.05$ or 0.01). The mean

food consumption of these animals was lower than that of the control animals as well The mean absolute and/or relative liver and kidneys weights of both sexes in the 1800 ppm group for both generations were greater than those of the control group (p<0.01). The relative testes weights of the 1800 ppm group in both generations were greater than the control values (p<0.05 or 0.01). The mean absolute and relative adrenal weights of the 1800 ppm P females and the mean absolute adrenal weights of the F1 1800 ppm females were less than the control values (p<0.05 or 0.01). In the histopathological evaluation, centrilobular hypertrophy was noted in the livers of the 1800 ppm males in both generations. In the kidneys of these animals, hyaline droplet formation, tubular basophilia and tubular castes were evident. Follicular cell hypertrophy was also noted in the thyroids of these males. Centrilobular hypertrophy in the liver was the only treatment-related lesion which was noted for the F1 1800 ppm females (0: 0/24 vs. 1800: 3/24). The estrous cycling of the 1800 ppm females was more irregular than that of the control, especially for the F1 generation. The mean time to confirmed mating was delayed by approximately a day for the 1800 ppm group when compared to that of the control group. The mean time to sexual maturation was likewise delayed by approximately a day for the 1800 ppm F1 generation. There was no treatment-related effect on sperm motility, morphology or sperm head count for the P or F1 generation adult males. Evaluation of ovarian staging in the control and 1800 ppm females in the P and F1 generations did not reveal any apparent treatment-related effect. There were no treatment-related effects upon the fertility or gestation indices. The mean litter sizes were not affected by the treatment. There was no treatment-related effect upon pup viability. Mean pup weights for the 1800 ppm group of both generations were lower than those of the control group on days 14 and 21 of the lactation period (p<0.05 or 0.01). The fluoride content of the bones and teeth of the adult animals in both sexes of the F1 30 ppm treatment group and above was elevated above that of the control group (p<0.05). The fluoride content in the bones and teeth of the F2 weanling pups was elevated for the males in the 1800 ppm group and for the females in the 250 and 1800 ppm groups. **Possible adverse effect:** fluorosis in the bones (non-reproductive). **No adverse reproductive effect indicated. Parental NOEL:** (M/F) <30 ppm (M: <2.1 to 2.3 mg/kg/day, F: <2.1 to 4.8 mg/kg/day) (based upon increased fluoride levels in the bones of the adults animals of the 30 ppm group), **Reproduction NOEL:** 1800 ppm (M:125.1 to 148.6 mg/kg/day, F: 137.7 to 293.2 mg/kg/day) (based upon the lack of a treatment-related effect at the highest treatment level), **Developmental NOEL:** 250 ppm (F: 37.9 to 40.8 mg/kg/day) (based upon lower mean pup weights during lactation and increased levels of fluoride in the bones of the 1800 ppm group of both generations); **Study acceptable.** (Moore, 12/20/12)

TERATOLOGY, RAT

** 53183-0060; 266300; “Technical Grade BYI01921: A Prenatal Developmental Toxicity Study in the Wistar Rat”; (A.D. Young; Bayer CropScience LP Toxicology, Stilwell, KS; Study ID No. 02-T12-LD; 9/4/03); Thirty mated female Wistar rats/group were dosed orally by gavage with 0 (vehicle: aqueous 2% Cremophor, 0.5% carboxymethyl cellulose), 8, 50, or 300 mg/kg/day of BYI 01921 technical; batch no. NLL6692-75; purity: 97.0% (5/6/02), 96.1% (8/14/02)) from day 6 through day 19 of gestation. Three dams in the 8 mg/kg group and four dams in the 300 mg/kg group died during the study. The deaths were deemed to be incidental and were due to technical errors. The mean body weights of the 300 mg/kg dams were lower than that of the control animals over the course of the treatment period (p<0.01). The mean food consumption of the 300 mg/kg dams was less than that of the control group from gestation day 6 through 12 (p<0.01). The mean relative liver and kidney weights of the 300 mg/kg dams were greater than the control values (p<0.01). The mean weight of the 300 mg/kg female fetuses was less than that of the control group (p<0.05). There were no apparent treatment-related abnormalities in fetal development. **No adverse effect indicated. Maternal NOEL:** 50 mg/kg/day (based upon lower body weight gain and food consumption and greater mean relative liver and kidneys weights for the 300 mg/kg dams); **Developmental NOEL:** 50 mg/kg/day (based upon the lower mean weight

of the 300 mg/kg female fetuses); **Study acceptable.** (Moore, 12/12/12)

TERATOLOGY, RABBIT

** 53183-0061; 266301; "MCW-2 Tech: Prenatal Developmental Toxicity Study in the Himalayan Rabbit"; (R. Ceccatelli; Harlan Laboratories Ltd, 4414 Fullinsdorf, Switzerland; Study No. B92687; 11/10/09); Twenty-mated female Himalayan rabbits/group were dosed orally by gavage with 0, 2.5, 10 or 40 mg/kg/day of MCW-2 Technical; batch no. 36372130-291-PF1; purity: 96.67%) from gestation day 6 through gestation day 27. No unscheduled deaths occurred during the study. There was a slight mean body weight loss for the does in the 40 mg/kg group between day 6 and day 8. The mean food consumption of the study animals was not apparently affected by the treatment. The mean body weight of the fetuses in the 40 mg/kg group was less than that of control group ($p<0.01$). There were no other treatment-related effects upon the fetuses. **No adverse effect evident. Maternal NOEL:** 10 mg/kg/day (based upon mean body weight loss on gestation days 7 and 8 for the does in the 40 mg/kg treatment group); **Developmental NOEL:** 10 mg/kg/day (based upon lower mean body weights for the fetuses in the 40 mg/kg treatment group); **Study acceptable.** (Moore, 12/14/12)

GENE MUTATION

** 53183-0048; 266288; "Reverse Mutation Assay using Bacteria (*Salmonella typhmurium* and *Escherichia coli*)"; (C. Donath; BSL Bioservice, Scientific Laboratories GmbH, 82152 Planegg, Germany; Study No. 110567; 4/5/11); *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 uvrA were exposed for 48 hours at 37° C to MCW-2 Tech (batch no. 411-33-1; purity: 96.8%) at concentrations ranging from 10.0 to 5000 ug/plate with and w/o activation in the first trial, using plate incorporation as the exposure procedure. In the second trial, cells were exposed to concentrations of the test material ranging from 3.16 to 5000 ug/plate, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material. There was no increase in reverse mutation in any of the strains tested under conditions of either non-activation and activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 10/31/12)

** 53183-0049; 266289; "*Salmonella typhmurium* and *Escherichia coli* Reverse Mutation Assay with McW-2 Tech"; (A. Sokolowski; RCC Cytotest Cell Research GmbH (RCC-CCR), D-64380 Rossdorf, Germany; Study No. 1176200; 8/1/08); *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 uvrA were exposed for 48 hours at 37° C to MCW-2 Tech (batch no. 36372130-291-PF1; purity: 96.2%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first trial, using plate incorporation as the exposure procedure. In the second trial, cells were exposed to concentrations of the test material ranging from 33 to 5000 ug/plate, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material. There was no increase in reverse mutation in any of the strains tested under conditions of either non-activation and activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 11/1/12)

**** 53183-0050; 266290**; "BYI 01921*Salmonella/*Microsome Test, Plate Incorporation Method"; (B. Herbold; Bayer AG, PH-PD Toxicology, Rodents and Genotoxicity, D42096 Wuppertal, Germany; Study No. T 7070901; 5/27/02); *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 strains were incubated with BYI 01921 technical (trials 1 and 2: batch no. NLL6692-5, purity: 98.9%; trial 3: batch no. NLL6692-13.1; purity: 98.4%) at levels ranging from 16 to 5000 μg/plate

in the 1st trial and 1000 to 6000 ug/plate in the 2nd trial under conditions of (-/+) activation and incubated for 48 hours at 37° C. In the 3rd trial, the bacterial strains were exposed to 375 to 5200 ug/plate of the test material under the same exposure conditions w/o activation. In the 1st two trials, each treatment was incubated in triplicate. A single incubation was performed for each treatment level in the 3rd trial. An Aroclor 1254-induced rat liver S9 fraction was used to metabolize the test material. There was an apparent treatment-related increase in the incidence of reverse mutation with and w/o activation for the TA100 strain. The positive controls were functional. **Possible adverse effect indicated. Study acceptable.** (Moore, 11/1/12)

CHROMOSOME EFFECTS

** 53183-0051; 266291; "*In Vitro* Assessment of the Clastrogenic Activity of MCW-2 Technical in Cultured Human Peripheral Lymphocytes"; (J. Leuscher; Laboratory of Pharmacology and Toxicology (LPT) GmbH & Co. KG, 21147 Hamburg, Germany; Report No. 24556; 6/7/10); Primary human lymphocyte cultures in whole blood, procured from healthy volunteers, were treated with MCW-2 Technical (batch no. 36372130-291-PF1; purity: 96.5%). In both trials, the cells were exposed to concentrations of the test material ranging from 62.5 to 1000 ug/ml with and w/o activation. For the non-activated assay, the cell were exposed for 4 hours and incubated for an additional 20 hours in the 1st trial. In the 2nd trial, the cells were exposed for 24 hours. In the activation assay, the cells were exposed for 4 hours and incubated for an additional 20 hours in both trials. An Aroclor 1254-induced rat liver S9 fraction was used to metabolize the test material. Two hundred metaphases/treatment level were examined for structural abnormalities. No treatment-related increase in chromosomal aberrations was evident under conditions of nonactivation or activation unless excessive cytotoxicity was evident. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/2/12)

DNA DAMAGE

** 53183-0053; 266293; "BYI 01921 Micronucleus-Test on the Male Mouse"; (B. Herbold; Bayer Healthcare, PH-PD P Health Care Toxicology, Molecular and Genetic Toxicology, D-42096 Wuppertal, Germany; Report No. AT00208; 1/15/03); Five male Hsd/Win mice/group were dosed twice by intraperitoneal (ip) injection with 0, 75, 150 or 300 mg/kg of BYI 01921 technical (batch no. NLL 6692-13.1; purity: 98.4%) with a 24-hour interval between injections. The animals were euthanized 24 hours after the second injection. The vehicle was 0.5% aqueous Cremophor. A positive control group of five males also received a single ip injection with 20 mg/kg of cyclophosphamide and was euthanized at 24 hours post-dose. The femoral bone marrow was harvested and evaluated for the presence of micronuclei in both polychromatic and normochromatic erythrocytes. One thousand polychromatic erythrocytes were evaluated per animal. Treatment-related signs included apathy, roughened fur, spasms, slitted eyes and difficulty breathing. There was no treatment-related increase in the number of micronuclei per 2000 polychromatic erythrocytes. The positive control was functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/6/12)

NEUROTOXICITY

Acute Neurotoxicity

0062; 266302; "Acute Oral Toxicity Peak-Effect Study in Rats" (Sommer, E.W., Harlan Laboratories Ltd., Itingen, Switzerland, Harlan Laboratories Study C57413, 10/30/2009). MCW-2 TECH (Batch Number 36372130-291-PFI, purity = 98.65%), prepared in polyethylene glycol (PEG-) 300, was administered in a single dose by gavage to 3 RccHan:WIST (SPF) rats per sex per dose at dose levels of 0 (vehicle only), 100, 500, 1000, and 2000 mg/kg. All animals dosed with 2000 mg/kg died within 24 hours of dose administration; all of the other animals survived until the scheduled termination (8 days after dose administration). No treatment-related clinical signs were observed in animals dose with 100, 500, and 1000 mg/kg. Prior to death, somnolent

behavior, decreased activity, salivation, absence of push-off reflex (males only), hunched posture, prostration, and ruffled fur were observed in animals dosed with 2000 mg/kg with the peak effect observed at 5 hours. No treatment-related effects on body weight and food consumption were observed at 100, 500, and 1000 mg/kg. Macroscopic examination revealed no abnormalities. NOEL (M/F) = 1000 mg/kg (based on clinical observations). **Supplemental study** (1. only 3 animals per sex per dose group were used, 2. no motor activity tests were conducted, and 3. no neuropathological examinations were conducted). (Corlett, 12/18/2012)

0063, 266303; "Acute Oral Neurotoxicity (Gavage) Study in Rats" (Sommer, E.W., Harlan Laboratories Ltd., Itingen, Switzerland, Harlan Laboratories Study C56895, 04/08/2010). 870.62. MCW-2 TECH (Batch Number 36372130-291-PF1, purity = 98.65%), prepared in polyethylene glycol (PEG-) 300, was administered in a single dose by gavage to 10 RccHan:WIST (SPF) rats per sex per dose at dose levels of 0 (vehicle only), 100, 400, and 1200 mg/kg. After dosing, one female at 1200 mg/kg died within 24 hours; no other mortalities occurred. General cageside observations revealed decreased activity in one female at 400 mg/kg and in one male and in one female at 1200 mg/kg; detailed clinical observations revealed no treatment-related clinical signs. No effects on body weight were observed. FOB assessments conducted on day 1 (approximately 1 to 4 hours after dosing) revealed treatment-related signs including decreased activity, decreased rearing, and reduced righting reflex at 400 and 1200 mg/kg in females and at all dose levels in males; FOB assessments revealed no treatment-related effects on days 7 and 14. Locomotor activity assessments revealed treatment-related decreases in mean total distance and mean total rearing in males at 400 and 1200 mg/kg and in females at all dose levels; locomotor activity assessments revealed no treatment-related effects on days 7 and 14. Macroscopic examination revealed no treatment-related abnormalities. Microscopic examination of nervous system tissues revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F) < 100 mg/kg (based on decreased activity, decreased rearing, and reduced righting reflex during FOB (males) and decreased locomotor activity (females)). **Unacceptable but possibly upgradable** with the submission of current positive control data that provides evidence of the ability of the observational methods used to detect major neurotoxic endpoints including limb weakness or paralysis, tremor, and autonomic signs, that demonstrates the sensitivity and reliability of the activity-measuring device and testing procedures, and the submission of positive control groups exhibiting central nervous system and peripheral nervous system pathology; also required is a submission of a clear, detailed explanation of the scoring system used in the general cageside observations and in the detailed clinical observations and an explanation of the data presented in the Functional Observational Battery summary tables (Corlett and Leung, 01/02/2013)

Subchronic Neurotoxicity

0064; 266304; "MCW-2 TECH: 13-Week Neurotoxicity (Feeding) Study in Rats" (Sommer, E.W., Harlan Laboratories Ltd., Itingen, Switzerland, Harlan Laboratories Study C95347, 06/15/2011). 870.6200. MCW-2 TECH (Batch Number 36372130-291-PF1, purity = 95.7%) was admixed to the diet and fed to 12 RccHan:WIST(SPF) rats per sex per dose at dose levels of 0 (diet only), 100, 500, or 2500 ppm (0, 6, 31, and 153 mg/kg/day, respectively for males, and 0, 7, 34, and 162 mg/kg/day, respectively for females) daily for 92 days. No treatment-related mortalities occurred. General cageside observations and detailed clinical observations revealed no treatment-related clinical signs. A treatment-related decrease in mean body weight was observed in males at 2500 ppm. A treatment-related decrease in mean food consumption was observed in both sexes at 2500 ppm. FOB and motor activity assessments revealed no treatment-related effects. No treatment-related effect on mean relative brain weight was observed. Macroscopic examination revealed no abnormalities. Neuropathological examination of the central and peripheral nervous system tissues revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M) = 31 mg/kg/day (500 ppm) and (F) = 34 mg/kg/day (500 ppm) (based on decreases in mean body weight and mean food consumption in males and a decrease in mean food consumption in females). **Unacceptable but possibly upgradable** with the

submission of current positive control data that provides evidence of the ability of the observational methods used to detect major neurotoxic endpoints including limb weakness or paralysis, tremor, and autonomic signs, that demonstrates the sensitivity and reliability of the activity-measuring device and testing procedures, and the submission of positive control groups exhibiting central nervous system and peripheral nervous system pathology. (Corlett and Leung, 01/11/2013)

RAT METABOLISM

53183-0034; 266258; "The Metabolism and Excretion of [^{14}C] MCW-2 in the Rat upon Administration of Single Oral High and Low Doses"; (G.B. Quistad, A. Kovatchev, J. La Mar; Pacific BioLabs, Hercules, CA and Analytical Phase, PTRL West, Inc., Hercules, CA; PBL Study No. 10B0104G-X01G; 2/2/11); Four Wistar rats/sex/group were dosed orally by gavage with 5 or 500 mg/kg of [Thiazole-4-^{14}C]MCW-2 (lot no. TJBIOS-NB21-33, specific activity: 56.7 mCi/mmol, radiochemical purity: 98.7-99.3%) or [Trifluorobutene-1,2-^{14}C]MCW-2 (lot no. TJBIOS-NB18-44, specific activity: 57.7 mCi/mmol; radiochemical purity: 96.8-98.8%). Unlabeled MCW-2 (lot no. 411-12, purity: 99.9%) was used to adjust the specific activity of the dosing preparations. Urine and feces were collected for up to 120 hours post-dose. Exhaled carbon dioxide samples were collected for up to 72 hours post-dose. Radiolabeled metabolites were isolated and identified from the urine, cage wash and feces. Only the urinary and limited cage wash data were presented for evaluation in the summary. Of the administered dose, 84 to 88% (includes cage wash as well as urine) of thiazole- labeled and 75 to 83% of the butene-labeled test material was recovered in the urine by 120 hours post-dose. Approximately 4% of the administered dose was recovered as radiolabeled carbon dioxide when the butene-labeled test material was administered. Increased dosage did not affect the excretion profile. Hydrolysis of the sulfur-carbon bond and substitution of the cysteinyl sulfur of glutathione on the thiazole ring in the conjugation reaction were surmised to be the initial steps in the metabolic process. Further metabolic steps included the hydrolysis of the sulfur bond and removal of the glutathione moiety from the thiazole ring. Glucuronide conjugates were also identified which bound to the thiazole thiol group. Butene sulfinic and sulfonic acids were the most prominent metabolites resulting from the butene-labeled test material. **Study supplemental.** (Moore, 10/19/12)

53183-0036; 262261; "The Pharmacokinetics of [^{14}C] MCW-2 in the Rat upon Administration of Single Oral High and Low Doses"; (G.B. Quistad, A. Kovatchev, J. La Mar; Pacific BioLabs, Hercules, CA and Analytical Phase, PTRL West, Inc., Hercules, CA; PBL Study No. 10174G-X01G; 7/26/11); Eight Wistar rats/sex/group were dosed orally by gavage with either 5 or 500 mg/kg of [Thiazole-4-^{14}C]MCW-2 (lot no. TJBIOS-NB21-33, specific activity: 56.7 mCi/mmol, radiochemical purity: 100%) or [Trifluorobutene-1,2-^{14}C]MCW-2 ((low) lot no. TJBIOS-NB18-44, specific activity: 57.7 mCi/mmol, (high) lot no. TJBIOS-NB21-52, specific activity: 58.7 mCi/mmol; radiochemical purity: 96.5-97%). Unlabeled MCW-2 (lot no. 411-12, purity: 99.9%) was used to adjust the specific activity of the dosing preparation. Blood was drawn using a surgically implanted jugular cannula up to 12 times over a 2-week period. The concentration of the radiolabel was determined in the whole blood and plasma. The site of radiolabeling directly influenced the pharmacokinetic profile of the test material. Dosing with the thiazole-labeled moiety resulted in the prolonged retention of compound in the blood as compared to the plasma. The T1/2 values for the blood ranged from 146 to 208 hours for the low dose and 134 to 162 hours for the high dose group. These values contrasted with the plasma values of 14.2 and 15 hours for the low dose group and the 58 and 67 hours for the high dose group. The Cmax and Area Under the Curve values were likewise increased for the animals in the high dose group based on this increased retention. The k elimination rate constant for the blood reflected a prolonged retention time as well. The pharmacokinetic profile of the butene-radiolabel moiety did not demonstrate any difference between the blood and plasma. For the thiazole-label, the Tmax values varied from 2 to 8 hours post-dose for the low dose and from 1 to 48 hours for the high

dose. For the butene-label, the Tmax values were 4 hours for the low dose and 24 hours for the high dose. The increased retention time of the thiazole-label was attributed to the formation of a covalent linkage of the thiazole moiety to hemoglobin and displacement of the butene sulfinic acid moiety. **Study supplemental.** (Moore, 10/23/12)

53183-0037; 266262; "The Tissue Distribution of [^{14}C] MCW-2 in the Rat upon Administration of Single Oral High and Low Doses"; (G.B. Quistad, A. Kovatchev; Pacific BioLabs, Hercules, CA and Analytical Phase, PTRL West, Inc., Hercules, CA; PTRL West Study No. 1791W; 9/23/11); Nine Wistar rats/sex/group were dosed orally by gavage with 5 or 500 mg/kg of [Thiazole-4-^{14}C]MCW-2 (lot no. TJBIOS-NB21-33, specific activity: 56.7 mCi/mmol, radiochemical purity: 99.2%, chemical purity: 99.1%) or [Trifluorobutene-1,2-^{14}C]MCW-2 (lot no. TJBIOS-NB18-44, specific activity: 57.7 mCi/mmol; radiochemical purity: 99.0%, chemical purity: 98.5%). Unlabeled MCW-2 (lot no. 411-12, purity: 99.9%) was used to adjust the specific activity of the dosing preparations. The recovery of radiolabel over the course of the sampling period primarily demonstrated the movement of the radiolabel down the gastrointestinal tract. For the most part, there was no specific tissue/organ which had a high concentration of the radiolabel over the course of the assay period. The position of the radiolabeling did not distinctively alter the distribution profile. There was no difference in the distribution in regard to sex or dose level. Only in the whole blood and red blood cells was the radiolabel retained over the course of the sampling period. This was particularly true for the thiazole-labeled test material. The increased retention time of the thiazole-label had been attributed to the formation of a covalent linkage of the thiazole moiety to hemoglobin and displacement of the butene sulfinic acid moiety. When the tissue content of the radiolabel was determined based on the percentage of administered dose, the stomach, small intestine and liver demonstrated the highest percentages regardless of the site of radiolabeling. By the time the one tenth of the Cmax level had been achieved in the blood, all sites assayed had at most only a fraction of a percentage of the administered dose. **Study supplemental.** (Moore, 10/30/12)

53183-0038; 266263; "The Metabolism, Excretion, and Tissue Distribution of [Thiazole-^{14}C] MCW-2 in the Rat upon Administration of Repeated Oral Doses (5 mg/kg)"; (G. Quistad, A. Kovatchev, J. LaMar; Pacific BioLabs, Hercules, CA and Analytical Phase, PTRL West, Inc., Hercules, CA; PBL study no. 10L0405G-X01G; 9/15/11); Two cohorts of 4 Wistar rats/sex were dosed orally by gavage with 5 mg/kg/day of unlabeled MCW-2 (lot no. 411-12, purity: 99.9%) for 14 days. On the fifteenth day, each animal was dosed with 5 mg/kg of [Thiazole-4-^{14}C]MCW-2 (lot no. TJBIOS-NB21-33, specific activity: 56.7 mCi/mmol, radiochemical purity: >95.0%). The first cohort was euthanized at 24 hours post-final dose and the distribution of radioactivity in select tissues/organs was determined. Urine, feces and cage wash samples were collected from the 4 animals/sex in the cohort up to 7 days post-final dose. A tissue distribution assessment was also performed on these animals at 7 days post-final dose. Eighty five to 87% of the administered dose was recovered in the urine (urine and cage wash). Eighty eight to 89% of the radiolabel was recovered within the first 24 hours post-final dose. In the tissue distribution assessment, 24 hours post-final dose, the test material was predominantly located within the gastrointestinal tract with absorption via the hepatic portal system elevating the levels in the liver. By 7 days post-final dose, these levels had greatly diminished. Radiolabel was retained the most in the whole blood. The increased retention time of the radiolabel in the blood has been attributed to the formation of a covalent linkage of the thiazole moiety to hemoglobin. Other tissues which were well perfused also demonstrated a higher relative retention of the radiolabel (i.e. spleen and lungs). Conjugation of the thiazole-moiety with glutathione resulted in the formation of thiazole mercapturate and ultimately thiazole sulfonic acid and thiazole glucuronides. **Study supplemental.** (Moore, 10/31/12)

IMMUNOTOXICITY

Mouse 4-Week Immunotoxicity

** 53183-0068; 266318; " Immunotoxicity Evaluation of MCW-2 in a 28-Day Dietary Study in CD-1 Female Mice: Evaluation of Anti-Sheep Red Blood Cell (SRBC) Response"; (P.S. Struve; Burleson Research Technologies, Inc., (BRT), Morrisville, NC; Study No. BRT 20100420; 2/22/11, revised, 4/11/11); Ten female CD1 mice/group received 0, 100, 500, or 2500/1500 ppm of MCW-2 Technical (lot no. 36372130-291-PF1; purity: 96.70%) in the diet for 32 days. After one week of treatment during which the animals in the high dose group fed very little and lost weight, the treatment for this group was discontinued from study day 8 to 11 and then resumed at 1500 ppm from study day 11 through 32. On study day 27, the study animals received an iv injection of 0.2 ml of $1x10^8$ sheep red blood cells (SRBC)/ml. Primary humoral function was evaluated by analyzing for the SRBC-specific IgM levels in the serum by means of an enzyme-linked immunosorbent assay (ELISA). One death in the 2500 ppm treatment group on day 8 resulted from the treatment. The mean body weights of the 500 and 2500/1500 ppm groups were lower than those of the control group at various time points during the study ($p<0.05$, 0.0001). The mean food consumption of the 2500/1500 ppm group was lower than that of the control group throughout the treatment period (NS, $p<0.01$, 0.001). There was no apparent treatment-related effect upon the mean absolute or relative spleen or thymus weights. The treatment did not suppress the primary humoral response to SRBC in a treatment-related manner. **No adverse effect indicated. Mouse 28-Day Immunotoxicity NOEL:** (F) 1500 ppm (based upon the lack of treatment-related effects at the highest treatment level); **Study acceptable.** (Moore, 11/8/12)

MECHANISTIC STUDIES

53183-0056; 266296; "MCW-2 TECH: 3- and 7-Day Oral (Feeding) Mechanistic Lung Toxicity Study in Mice"; (St. Kaiser; Harlan Laboratories Ltd., 4452 Itingen, Switzerland; Study No. D26983; 11/8/11); Ten female CD-1 mice/group received 0 or 1200 ppm of MCW-2 Technical (batch no. 36372130-291-PFI, purity: 96.17%) in the diet for up to 7 days. A positive control group of 10 female mice received 1305 ppm of isoniazid (batch no. 060M0090, purity: $\geq$ 99%) for up to 7 days as well. Five animals/group/time point were euthanized on study days 3 and 7. At approximately 14 and 2 hours prior to sacrifice, each animal received an ip injection of 0.1 ml of a bromodeoxy-uridine (BrdU) solution (10 mg/ml) for an assessment of cellular proliferation. In the necropsy, the lungs and jejunum were dissected, processed, embedded, and cut into sections. Immuno-chemical techniques were used to identify BrdU positive cells in the tissues. The jejunum samples were used to confirm that the injection of BrdU was properly administered. An increased cell proliferation in the lungs was noted for both the MCW-2 and isoniazid-treated mice after 3 days of treatment. This proliferative response was no longer evident after 7 days of treatment for either test material. **No adverse effect apparent. Study supplemental.** (Moore, 11/6/12)

53183-0057; 266297; "Comparative Biotransformation of MCW-2 in Human and Mice Lung Microsomes"; (M. Jager; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1388500; 12/15/11); Microsomes were isolated from human and mouse lung tissue and used to assess the metabolism of MCW-2 Technical (batch no. 326-115-02; purity: 98.6%). The standard substrate, chlorzoxazone, was used to assess the relative enzyme activity of the lung microsomes derived from humans (pooled samples from non-smoking individuals) and from male and female CD-1 mice. The activities of the microsomes from male and female mice were 315 and 400 times, respectively, that of the human microsomes. Specific inhibitors of

cytochrome enzymes CYP 2E1 and Cyp 2f2 (a mouse-specific isoform), 4-methyl pyrazole and 5-phenyl-1-pentyne, respectively, were used to differentiate which enzymes contributed to the metabolism of the MCW-2. When the test material at a concentratioon of 2 uM was incubated for 120 minutes at 37° C, no metabolism was apparent in the human lung microsomal preparation. With the mouse lung microsomes, over 95% of the parent material was metabolized. Addition of 4-methyl pyrazole to incubation preparation still resulted in 91 to 94% of the test material being metabolized. When 5-phenyl-1-pentyne was added, 73 to 77% of the test material was metabolized. Based on these results, MCW-2 is not readily metabolized by human lung microsomes. Cyp 2f2 appears to be a greater factor than CYP 2E1 in the metabolism of MCW-2 in the mouse lung microsomes. **No adverse effect indicated. Study supplemental.** (Moore, 11/7/12)

53183-0071; 266321; "MCW-2 Tech: 2-Week Oral (Feeding) Mode of Action (Effects on Alanine Amino Transferase – ALAT) Study in the Beagle Dog*";* (L. Braun; Harlan Laboratories Ltd., 4452 Itingen, Switzerland; Study No. C59044; 7/14/11); Four male beagle dogs/group received 0 or 500 ppm of MCW-2 TECH (batch no. 36372130-291-PF1; purity: 96.70%) in the diet for 2 weeks (0, 21.1 mg/kg/day). There was no treatment-related effect upon food consumption or body weight gain. There were no treatment-related clinical signs. The necropsy examination or histopathological evaluation did not reveal any treatment-related lesions. Over the course of the treatment period, plasma alanine aminotransferase (ALAT) activity declined to 26% of the pretest control value in the 500 ppm treatment group. The hepatic ALAT activity was 14% of the concurrent control at the conclusion of the treatment period for the 500 ppm group. In an effort to elucidate the cause of this decline, pyridoxal 5'-phosphate (a cofactor for ALAT) was measured in both the plasma and liver. ALAT1 isozyme mRNA expression was measured in the liver by polymerase chain reaction. ALAT1 protein expression in the liver was determined by Western Blot techniques. The pyridoxal 5'-phosphate level was less in the plasma of the 500 ppm group animals but greater in their liver in comparison to the control values. This results indicated that treatment did not specifically affect pyridoxal 5'-phosphate. The ALAT1 isozyme mRNA expression was reported to be 3.36 and 3.07 times greater in the liver of the 500 ppm group for the two experiments. The ALAT1 protein expression was not affected in the liver in the liver of the treated group. Although ALAT activity in the plasma and liver of the treated animals was reduced, the concentration of the pyridoxal 5'-phosphate cofactor in the plasma or liver and the expression of the ALAT1 mRNA or ALAT1 protein expression in the liver were not affected by the treatment. **Supplemental Study.** (Moore, 12/4/12)

53183-0072; 266322; "MCW-2 Tech: Mode of Action on Alanine Amino Transferase (ALAT) *In* Vitro in Dog Liver Homogenate"; (P. Sagelsdorff; Harlan Laboratories Ltd., 4414 Fullinsdorf, Switzerland; Study No. C58796; 3/30/10); The metabolism of MCW-2 technical (batch no. 36372130-291-PF1, purity: 96.7%) and the inhibition of alanine aminotransferase (ALAT) activity were evaluated in dog liver homogenates. ALAT activity and the concentration of the test material in the medium were measured periodically during the one-hour incubation period. The concentration of MCW-2 declined over the course of the incubation. However, no inhibition of ALAT was evident. **Study supplemental.** (Moore, 12/6/12)

53183-0073; 266323; "MCW-2 Tech: Mode of Action on Inhibition of Alanine-Aminotransferase"; (P. Sagelsdorff; Harlan Laboratories Ltd., 4414 Fullinsdorf, Switzerland; Study No. C15686; 11/11/09); Serum and liver tissue recovered from dogs fed for 4 weeks with 0 or 900 ppm of MCW-2 Technical in the diet (Study No. B89087; vol. 53183-0042, rec. no. 266282) were assayed for alanine aminotransferase (ALAT) activity. In an *in vitro* assay, aliquots of serum and liver cytosol from both control and treated animals were mixed in varying ratios and analyzed for ALAT activity. Immunoblotting techniques were employed to quantify ALAT-specific protein

content in the serum and liver cytosol. There was no apparent effect on the ALAT activity in the various ratios of mixtures of serum or liver cytosol derived from control and treated animals. The treatment did not affect the ALAT protein content in the serum or liver cytosol. **Study supplemental.** (Moore, 12/7/12)

STUDIES ON METABOLITES

Metabolite #3625

Mutagenicity

** 53183-0065; 266306; "*Salmonella typhmurium* and *Escherichia coli* Reverse Mutation Assay with MCW-2 Metabolite #3625"; (A. Sokolowski; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1317202; 7/5/10); *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 uvrA were exposed for 48 hours at 37° C to MCW-2 Metabolite #3625 (batch no. 231PAL071; purity: 93.6%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first trial, using plate incorporation as the exposure procedure. In the second trial, cells were exposed to concentrations of the test material ranging from 33 to 5000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material. There was no increase in reverse mutation in any of the strains tested under conditions of either non-activation and activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 11/9/12)

Chromosomal Aberration

** 53183-0065; 266307; "*In Vitro* Chromosome Aberration Test in Chinese Hamster V79 Cells with MCW-2 Metabolite #3625"; (C. Hall; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1345801; 12/22/10); V79 Chinese hamster cells were exposed to concentrations of MCW-2 Metabolite #3625 (batch no. 231PAL071; purity: 93.6%) ranging from 9.3 to 2370 ug/ml with and w/o activation for 4 hours, followed by an additional 14 hours of incubation in the first trial. In the 2nd trial, the cells were exposed to concentrations ranging from 148.1 to 2370 ug/ml under conditions of activation for 4 hours and incubated for an additional 14 hours. Under conditions of non-activation, the cells were exposed for 18 hours to concentrations ranging from 9.3 to 2370 ug/ml. A phenobarbital/beta-naphthoflavone-induced rat liver S9 fraction was used to metabolize the test material. Duplicate cultures were performed at each treatment level. One hundred metaphases per culture were evaluated (200 metaphases per treatment level). There was no treatment-related increase in chromosomal cell aberrations under conditions of either activation or non-activation. Positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/13/12)

** 53183-0065; 266308; "Micronucleus Assay in Bone Marrow Cells of the Rat with MCW-2 Metabolite #3625"; (M. Merker; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1345802; 7/6/11); Seven male Wistar rats/group were dosed orally by gavage with 0, 500, 1000 or 2000 mg/kg of MCW-2 Metabolite #3625 (batch no. 231PAL080; purity: 93.6%). These animals were euthanized 24 hours post-dose. An additional group of 7 males were dosed in the same manner with 2000 mg/kg and euthanized at 48 hours post-dose. The vehicle was sterile water. A positive control group of seven males were dosed orally with 20 mg/kg of cyclophosphamide and was euthanized at 24 hours post-dose. The femoral bone marrow was harvested and evaluated for the presence of micronuclei in polychromatic erythrocytes (PCE). Two thousand polychromatic erythrocytes were evaluated per animal. The number of PCE per 2000 erythrocytes was also determined. Treatment-related signs included apathy and ruffled fur. There was no treatment-related increase in the number of

micronuclei per 2000 polychromatic erythrocytes. The positive control was functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/14/12)

DNA Damage

** 53183-0065; 266308; "Micronucleus Assay in Bone Marrow Cells of the Rat with MCW-2 Metabolite #3625"; (M. Merker; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1345802; 7/6/11); Seven male Wistar rats/group were dosed orally by gavage with 0, 500, 1000 or 2000 mg/kg of MCW-2 Metabolite #3625 (batch no. 231PAL080; purity: 93.6%). These animals were euthanized 24 hours post-dose. An additional group of 7 males were dosed in the same manner with 2000 mg/kg and euthanized at 48 hours post-dose. The vehicle was sterile water. A positive control group of seven males were dosed orally with 20 mg/kg of cyclophosphamide and was euthanized at 24 hours post-dose. The femoral bone marrow was harvested and evaluated for the presence of micronuclei in polychromatic erythrocytes (PCE). Two thousand polychromatic erythrocytes were evaluated per animal. The number of PCE per 2000 erythrocytes was also determined. Treatment-related signs included apathy and ruffled fur. There was no treatment-related increase in the number of micronuclei per 2000 polychromatic erythrocytes. The positive control was functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/14/12)

Metabolite #3626

Mutagenicity

**** 53183-0066; 266310**; "*Salmonella typhmurium* and *Escherichia coli* Reverse Mutation Assay with MCW-2 Metabolite #3626"; (A. Sokolowski; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1317203; 8/23/10); *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 uvrA were exposed for 48 hours at 37° C to MCW-2 Metabolite #3626 (batch no. 231PAL021; purity: 98.1%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first experiment, using plate incorporation as the exposure procedure. In the second experiment, cells were exposed to concentrations of the test material ranging from 33 to 5000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. In Experiment 2A, strains TA100 and WP2 uvrA were exposed to concentrations of the test material ranging from 100 to 5000 ug/ml w/o activation. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material. There was an increase in reverse mutation in the TA100 strain under conditions of non-activation. **Possible adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 11/16/12)

** 53183-0066; 266311; "Gene Mutation Assay in Chinese Hamster V79 Cells *In Vitro* with MCW-2 Metabolite #3626"; (C. Hall; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1388400; 6/14/11); Chinese hamster V79 cells were exposed for 4 hours at 37° C to MCW-2 Metabolite #3626 (batch no. 231PAL052; purity: 98.7%) at concentrations ranging from 3.8 to 60 μg/ml (non-activation) and 37.5 to 1000 ug/ml (activation) in the first trial. In the second trial, the cells were exposed for 24 hours under conditions of non-activation at concentrations ranging from 5.5 to 175 ug/ml and for 4 hours under conditions of activation at concentrations ranging from 87.5 to 1000 ug/ml. A phenobarbital and beta-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the mutation frequency in either of the trials. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 11/16/12)

Chromosomal Aberration

** 53183-0066; 266312; "Micronucleus Assay in Bone Marrow Cells of the Rat with MCW-2 Metabolite #3626"; (M. Merker; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1345902; 7/6/11); Seven male Wistar rats/group were dosed orally by gavage with 0, 125, 250 or 500 mg/kg of MCW-2 Metabolite #3626 (batch no. 231PAL052; purity: 98.8%). These animals were euthanized 24 hours post-dose. An additional group of 7 males were dosed in the same manner with 500 mg/kg and euthanized at 48 hours post-dose. The vehicle was corn oil. A positive control group of seven males were dosed orally with 20 mg/kg of cyclophosphamide and was euthanized at 24 hours post-dose. The femoral bone marrow was harvested and evaluated for the presence of micronuclei in polychromatic erythrocytes (PCE). Two thousand polychromatic erythrocytes were evaluated per animal. The number of PCE per 2000 erythrocytes was also determined. Treatment-related signs included apathy and ruffled fur. There was no treatment-related increase in the number of micronuclei per 2000 polychromatic erythrocytes. The positive control was functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/20/12)

DNA Damage

** 53183-0066; 266313; "*In Vivo* Unscheduled DNA Synthesis in Rat Hepatocytes with MCW-2 Metabolite #3626"; (M. Merker; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1345901; 7/6/11); Eight Sprague-Dawley male rats/group received a single oral dose of 0 (corn oil), 250 or 500 mg/kg of MCW-2 Metabolite #3626 (batch no. 231PAL052; purity: 98.8%) by gavage. Hepatocytes from 4 animals/group/time point were isolated at 4 and 16 hours post-dosing. Viability was determined by trypan blue dye exclusion and ranged from 70% to 90%. After attachment, cells were exposed to (methyl- 3 H) thymidine for 4 hours followed by an overnight incubation with unlabelled thymidine. Two slides per animal were scored, 50 cells per slide. No increase in the net nuclear grain counts was evident at any dose level or sampling time. **No adverse effect indicated** . Positive controls were functional. **Study Acceptable** . (Moore, 11/20/12)

53183-0067; 266314; "MCW-2 Metabolite #3627: Acute Oral Toxicity Study in Rats"; (G. Arcelin; Harlan Laboratories Ltd, 4414 Fullinsdorf, Switzerland; Study No. C81230; 6/28/10); Six female RccHan: WIST rats were dosed orally by gavage with 2000 mg/kg of MCW-2 Metabolite #3627 (batch no. 215PAL44; purity: 99.5%) in the Up and Down Procedure. No deaths resulted from the treatment. There were no apparent clinical signs. No treatment-related lesions were evident in the necropsy examination. LD50 (F) > 2000 mg/kg; Toxicity Category III; **Study acceptable.** (Moore, 11/20/12

Metabolite #3627

Mutagenicity

** 53183-0067; 266315; "*Salmonella typhmurium* and *Escherichia coli* Reverse Mutation Assay with MCW-2 Metabolite #3627"; (A. Sokolowski; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1317201; 6/25/110); *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2 uvrA were exposed for 48 hours at 37° C to MCW-2 Metabolite #3627 (batch no. 215PAL44; purity: 99.5%) at concentrations ranging from 3 to 5000 ug/plate with and w/o activation in the first experiment, using plate incorporation as the exposure procedure. In the second experiment, cells were exposed to concentrations of the test material ranging from 33 to 5000 ug/plate with and w/o activation, using the pre-incubation procedure in which cells were exposed to the test material for 60 minutes prior to plating and incubated for another 48 hours. Each treatment level was plated in triplicate. A phenobarbital and beta-naphthoflavone- induced rat liver S9 fraction was used to metabolize the test material.

There was no increase in reverse mutations for any of the strains under conditions of activation or non-activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 11/21/12)

Chromosomal Aberration

** 53183-0067; 266316; "*In Vitro* Chromosome Aberration Test in Chinese Hamster V79 Cells with MCW-2 Metabolite #3627"; (C. Hall; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1328701; 7/12/10); V79 Chinese hamster cells were exposed to concentrations of MCW-2 Metabolite #3627 (batch no. 215PAL44; purity: 99.5%) ranging from 8.3 to 2131 ug/ml with and w/o activation for 4 hours, followed by an additional 14 hours of incubation in the first trial. In the 2nd trial, the cells were exposed to concentrations ranging from 133.2 to 2131 ug/ml under conditions of activation for 4 hours and incubated for an additional 14 hours. Under conditions of non-activation, the cells were exposed for 18 hours to concentrations ranging from 8.3 to 2131 ug/ml. A phenobarbital/beta-naphthoflavone-induced rat liver S9 fraction was used to metabolize the test material. Duplicate cultures were performed at each treatment level. One hundred metaphases per culture were evaluated (200 metaphases per treatment level). There was no treatment-related increase in chromosomal cell aberrations under conditions of either activation or non-activation. Positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/26/12)

DNA Damage

** 53183-0067; 266317; "**:** Micronucleus Assay in Bone Marrow Cells of the Rat with MCW-2 Metabolite #3627"; (M. Merker; Harlan Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1328702; 10/21/10); Seven male Wistar rats/group were dosed orally by gavage with 0, 500, 1000 or 2000 mg/kg of MCW-2 Metabolite #3627 (batch no. 215PAL44; purity: 99.5%). These animals were euthanized 24 hours post-dose. An additional group of 7 males were dosed in the same manner with 2000 mg/kg and euthanized at 48 hours post-dose. The vehicle was sterile water. A positive control group of seven males were dosed orally with 20 mg/kg of cyclophosphamide and was euthanized at 24 hours post-dose. The femoral bone marrow was harvested and evaluated for the presence of micronuclei in polychromatic erythrocytes (PCE). Two thousand polychromatic erythrocytes were evaluated per animal. The number of PCE per 2000 erythrocytes was also determined. Treatment-related signs included apathy and ruffled fur. There was no treatment-related increase in the number of micronuclei per 2000 polychromatic erythrocytes. The positive control was functional. **No adverse effect indicated. Study acceptable.** (Moore, 11/14/12)