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

SUMMARY OF TOXICOLOGY DATA

Amicarbazone

Chemical Code # 6104, **Document Processing Number (DPN)** # 53197
SB 950 # NA
2/4/14, revised: 8/5/2014, 9/29/14

DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
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:	No data gap, possible adverse effect

Toxicology one-liners are attached.

All record numbers for the above study types through 277985 (Document No. 53197-0116) were examined. This includes all relevant studies indexed by DPR as of 9/29/14

In the 1-liners below:
indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.

File name: T140929
Revised by T. Moore, 2/4/14, by Pan R. and Leung, P., 8/5/2014, T. Moore, 9/29/14

NOTE: The following symbols may be used in the Table of Contents which follows:
* = data adequately address FIFRA requirement
† = study(ies) flagged as “possible adverse effect”
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

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METABOLISM AND PHARMACOKINETICS

0092; 0116; 269128, 277985; "Weight of evidence analysis for the absorption of amicarbazone in the rat metabolism study submitted in support of amicarbazone registration". Heilman, J. 05/22/2014, E^{x}ponent®, Inc., Washington, DC 20036. Project ID: Amicarbazone 14-01, 1401542.000-4238. The author submitted supplemental information on metabolism study of Amicarbazone submitted by Bayer Corporation: "The Metabolism of [Triazolinone-3-^{14}C] MKH 3586 in Rats" (Krolski, M.E. and Bosnak, L.L., Bayer Corporation, Agriculture Division, Research and Development Department, Stilwell, KS, Bayer Study No. M6041801, Bayer Report No. 107540, 05/27/1997.) In the study, radiolabelled [Triazolinone-3-^{14}C] MKH 3586 was administered as a single oral dose by gavage to 4 male Fischer rats at a dose rate of 5 mg/kg. No mortalities occurred. 64% of the radioactive residue was excreted in the urine and 27% was excreted in the feces. No significant part of radioactive residue was found in the tissues, residual carcass, or respired gases. 17 metabolites of the test article were detected in the excreted urine and feces. The main pathways for degradation and excretion of the test article were direct conjugation with glucuronic acid to form MKH 3586 GA, which was excreted mainly in the feces and deamination to form DA MHK 3586 with subsequent oxidation to form a variety of hydroxylated metabolites that were excreted in the urine. The study was previously deemed **Supplemental** due to absorption of the test material was not adequately characterized. In light of the additional information submitted by the author, the 10% of glucuronidated conjugates of MKH 3586 in feces indicates the 10% of the administered dose was absorbed and excreted as glucuronidated Amicarbazone in the feces. The remaining 17% of the administered dose was excreted directly into the feces. Therefore the study is upgraded to **Acceptable**. (Corlett and Moore, 11/07/2013, upgraded, Pan &Leung, 8/5/2014)

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat

0059, 269086; "Acute Oral Toxicity Study with Technical Grade MKH 3586 in Rats" (Zorbas, M.A., Miles Inc., Agriculture Division, Toxicology, Stilwell, KS, Study Number 94-012-ZH, 12/12/1994). 870.11. Technical Grade MKH 3586 (Batch number 17004/93, purity = 98.2%), suspended in 0.5% (w/v) methylcellulose and 0.4% (v/v) Tween 80 in deionized water, was administered in a single dose by gavage to 5 Sprague-Dawley rats per sex per dose at dose levels of 0 (vehicle only), 98.4, 252, 816, 978 (males only), 1218 (females only), 1334 (males

only), 1710, 3388, and 5210 mg/kg. Mortalities occurred as follows- males: 0/5, 0/5, 0/5, 0/5, 1/5, 2/5, 5/5, 5/5, 4/5, respectively; females: 0/5, 0/5, 0/5, 2/5, 3/5, 5/5, 5/5, 5/5, respectively. Treatment-related clinical signs were observed in all dose groups in both sexes except at 98.4 mg/kg. These signs included decreased activity, oral staining, salivation, red or clear nasal staining, convulsions, urine staining, and jumping when touched. Necropsy on the animals found dead revealed signs of salivation and lacrimation, urine staining, reddened lungs, and black zones on the glandular mucosa of the stomach; necropsy on the survivors revealed no abnormalities except for serosal masses on the uterus of one female at 816 mg/kg. LD_{50} (M) = 1300 (1085-1639) mg/kg, LD_{50} (F) = 1015 (653-1395) mg/kg. NOEL (M/F) = 98.4 mg/kg (based on absence of clinical signs). Toxicity Category III. **Acceptable**. (Corlett, 02/26/2013)

Acute dermal toxicity

0061, 269088; "Acute Dermal Toxicity Study with Technical Grade MKH 3586 in Rats" (Zorbas, M.A., Miles Inc., Agriculture Division, Toxicology, Stilwell, KS, Study Number 94-022-AP, 12/09/1994). 870.1200. Technical Grade MKH 3586 (Batch number 17004/93, purity = 98.2%), moistened with deionized water, was applied to the clipped back of 6 Sprague-Dawley rats per sex per dose at dose levels of 0 and 2000 mg/kg for 24 hours using an occlusive dressing. No mortalities occurred. No treatment-related clinical signs were observed. Necropsy revealed no gross lesions. LD_{50} (M/F) > 2000 mg/kg. Toxicity Category III. **Acceptable**. (Corlett, 02/27/2013)

Acute inhalation toxicity, rat

0063, 269090; "Acute Four-Hour Inhalation Toxicity Study with MKH 3586 in Rats" (Warren, D.L. and Berry, L.A., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Study Number 94-042-BT, 04/27/1995). 870.1300. Technical Grade MKH 3586 (Batch number 17004/93, 98.2% a.i.) was aerosolized and administered in a nose-only manner under dynamic conditions to 6 Sprague-Dawley rats per sex per dose at dose levels of 0 (air only) and 2.24 mg/l (mean gravimetric concentration with mean MMAD (GSD) of 3.72 um (2.03)) for 4 hours. No mortalities occurred. Treatment-related oral and nasal staining (in both sexes) and lacrimation (in males only) were observed; complete recovery was observed after 1 day in all animals. Necropsy revealed no gross lesions. LC_{50} (M/F) > 2.24 mg/l. Toxicity Category IV. **Acceptable**. (Corlett, 03/04/2013)

Primary eye irritation, rabbit

0065, 269092; "Primary Eye Irritation Study with Technical Grade MKH 3586 in Rabbits" (Zorbas, M.A., Miles Inc., Agriculture Division, Toxicology, Stilwell, KS, Study Number 94-335-YG, 10/18/1994). 870.2400. 52 mg (0.1 ml weight equivalent) of Technical Grade MKH 3586 (Batch number 17004/93, purity = 97.8%) was placed into the conjunctival sac of one eye of each of 6 New Zealand White rabbits. No corneal opacity or iritis was observed. Grade 1 conjunctival irritation was observed in all 6 treated eyes 24 hours after treatment with all signs of conjunctival irritation clearing in all treated eyes 72 hours after treatment. Toxicity Category IV. **Acceptable**. (Corlett, 03/06/2013)

Primary dermal irritation

0070, 269097; "Primary Dermal Irritation Study with Technical Grade MKH 3586 in Rabbits" (Zorbas, M.A., Miles Inc., Agriculture Division, Toxicology, Stilwell, KS, Study Number 94-325-YH, 10/18/1994). 870.2500. 0.5 g of Technical Grade MKH 3586 (Batch number 17004/93, purity = 97.8%), moistened with tap water, was applied to the clipped skin of the back of each of 6 New Zealand White rabbits for 4 hours using an occlusive dressing. No erythema or edema was observed at any test site in any animal at any time during a 72-hour observation period following test material removal. Toxicity Category IV. **Acceptable**. (Corlett, 03/07/2013)

Dermal sensitization

0078, 269105; "Dermal Sensitization Study with Technical Grade MKH 3586 in Guinea Pigs" (Zorbas, M.A., Bayer Corporation. Agriculture Division, Toxicology, Stilwell, KS, Study Number 94-324-BO, 05/16/1995). 870.2600. The Buehler method was used to assess the skin sensitization potential of Technical Grade MKH 3586 (Batch number 17004/93, purity = 98.2%). 20 Hartley albino guinea pigs (10 males and 10 females) were treated with the test material. For induction, 0.4 g of the test article, moistened with deionized water, was applied to the clipped dorsal area adjacent to the left shoulder of each animal for 6 hours, once per week for 3 weeks (days 0, 7, and 14) using an occlusive Webril patch. On day 28, a challenge dose of 0.4 g of the moistened test article was applied to the clipped dorsal area adjacent to the left hip of each animal for 6 hours in the same manner as during the induction phase. A concurrent challenge control group consisting of 5 males and 5 females was also included in the study. The positive control was functional. Observations after the removal of the challenge phase patches revealed no erythema in the induced animals. **The results of the study indicate that the test material is not a sensitizer when using this modified version of the method of Buehler. Acceptable**. (Corlett, 07/03/2013)

SUBCHRONIC STUDIES (units of mg/kg/day unless specified)

Oral toxicity, rat:

0106; 270326; "Technical Grade MHK 3586: A Subchronic Toxicity Testing Study in the Rat" (Wahle, B.S. and Christenson, W.R., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Laboratory Project Study ID 94-172-BP, Report No. 107753, 11/11/1997). 870.3100. Technical Grade MKH 3586 (test batch No. 17004/93, purity = 98.2%) was admixed to the diet and fed to 15 CDF[F-344]/BR rats per sex per dose at dose levels of 0 (diet only), 100, 250, 500, 1000, 2500, or 5000 ppm (0, 6.9, 17.8, 32.9, 67.2, 181.8, and 353.8 mg/kg/day, respectively for males and 0, 7.9, 20.5, 38.0, 77.7, 200.6, and 396.8 mg/kg/day, respectively for females) for approximately 13 weeks with 15 additional rats per sex used at the 0 and 5000 ppm dose levels to assess recovery over a subsequent ~4-week treatment-free period. 5 animals/sex/dose group in the main study were euthanized after 5 weeks of treatment and necropsied. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. A treatment-related decrease in mean body weight was observed in females at 1000 ppm and in both sexes at 2500 and 5000 ppm; this observed effect persisted in both male and female recovery group animals. A treatment-related decrease in mean food consumption was observed in both sexes at 2500 and 5000 ppm; this observed effect persisted in both male and female recovery group animals. Hematological investigations revealed a treatment-related increase in the mean platelet count in males at dose levels of 1000 ppm and above (persisting in the recovery group animals), and a treatment-related increase in reticulocyte count in males at dose levels of 1000 ppm and above and in females at 5000 ppm (persisting in both recovery group sexes), treatment-related decreases in the mean red blood cell count (at 1000 ppm and above in males and at 5000 ppm in females), and treatment-related decreases in mean hemoglobin, hematocrit, mean cell volume, and mean cell hemoglobin in both sexes at 1000 ppm and above (most effects persisting in recovery group males and some persisting in recovery group females). Treatment-related increases in the mean cholesterol level in both sexes at 1000 ppm and above (persisting in both recovery group sexes) and the mean total protein level in both sexes at 5000 ppm (reversibility exhibited in both recovery group sexes) were observed; a treatment-related increase was observed in the mean triiodothyronine level in males at 1000 ppm and above and in females at 2500 ppm and above (recovery exhibited by the females but not the males). Determinations of mean liver enzyme activities at study termination revealed a treatment-related decrease in mean N-demethylase activity in males at

1000 ppm and above and a treatment-related increase in O-demethylase activity in males at 5000 ppm and in females at 1000 ppm and above. Urinalysis revealed a treatment-related increase in leukocytes in both sexes at 2500 and 5000 ppm (recovery observed in females but not males). Treatment-related increases in mean relative liver weight (at 1000 ppm and above in both sexes), brain weight (at 2500 ppm and above in males and at 1000 ppm and above in females), and testes weight (at 2500 and 5000 ppm) were observed with all of the weight increases persisting in the recovery group animals. Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination revealed treatment-related hepatocytomegaly in males at 1000 ppm and above and in females at 2500 ppm and above, cytoplasmic vacuolization in the pancreas in both sexes at 5000 ppm, increased incidence of follicular cell hyperplasia in the thyroid in both sexes at 2500 ppm and above, an increased incidence of pigmentation in the spleen in males at 1000 ppm and in both sexes at 2500 and 5000 ppm, atrophy of bone marrow cells in females at 2500 ppm and above, and atrophy in the femur in females at 2500 ppm and above; microscopic examination of the recovery group animals revealed no treatment-related abnormalities. **Possible adverse effect indicated: Atrophy of bone and bone marrow.** NOEL (M) = 32.9 mg/kg/day (500 ppm) and NOEL (F) = 38.0 mg/kg/day (500 ppm) based on increased relative liver weight and microscopic findings in the liver. **Acceptable.** (Corlett and Moore, 05/01/2013)

Oral toxicity, mouse

0086 (part 7); 269122; "Technical Grade MKH 3586: A 6-Week Range-Finding Study in the Mouse" (Wahle, B.S., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Laboratory Project Study ID 94-971-BU, Agricultural Division Report Number 109261, 10/01/1999). The data in review were presented in Document No. 53197-0086 on pages 2270-2392 and consisted of a summary of a study and tables; this study was conducted to determine the dose levels to be used in a 18-month oncogenicity study. Technical Grade MKH 3586 (no lot number and no information on test article purity were provided) was administered continuously in the feed to 10 CD-1 mice per sex per dose at dose levels of 0 (feed only), 25, 50, 100, 250, 500, 1000, 2500, 5000, and 7000 ppm for 6 weeks. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. The mean body weights of both sexes in the 7000 ppm group were less than those of the control group. Hematological investigations revealed dose-related decreases in mean red blood cell count, hemoglobin, and hematocrit levels in males at 2500, 5000, and 7000 ppm and a dose-related increase in mean reticulocyte count in both sexes at 2500, 5000, and 7000 ppm. Clinical chemistry revealed a dose-related increase in the mean cholesterol level in both sexes at 2500, 5000, and 7000 ppm and a statistically significant increase in the mean alanine aminotransferase level in females at 7000 ppm. A treatment-related increase in mean relative liver weight was observed in both sexes at doses of 500 ppm and above. Compound-related hepatocytomegaly (500-7000 ppm), thyroid follicular cell hypertrophy (5000-7000 ppm), and splenic pigmentation (5000-7000 ppm) were reported. **No adverse effects. NOEL (M/F)** = 250 ppm based on liver histopathology and increased mean relative liver weight. **Supplemental study** (Animals were dosed for only 6 weeks, only 10 animals per sex per dose were used, and individual histopathology data were not presented in the study report). (Corlett and Leung, 10/02/2013)

Oral toxicity, non-rodent:

Study not submitted.

Dermal toxicity, 21/28-day or 90-day:

0105; 270325; "Repeated-Dose 21-Day Dermal Toxicity Study with Technical Grade MKH 3586 in Rats" (Sheets, L.P. and Gastner, M.E., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Study Number 97-122-MH, Agriculture Division Report No. 108043, 07/27/1998). 870.3200. Technical Grade MKH 3586 (Batch No. 05362/0005, purity = 98.2-98.5%) was moistened with deionized water and applied to the clipped skin of the back of 10 Sprague-Dawley rats per sex per dose at dose levels of 0 (vehicle only), 200, 500, or 1000 mg/kg/day for 6 hours per day, 17 dose applications for females and 18 dose applications for males over a period of 21 and 22 days, respectively. The test sites were covered with a gauze with plastic backing secured with a bandage that was secured with tape. No treatment-related mortalities occurred. No treated-related clinical signs or dermal effects at the test sites were observed. No effects on body weights or on food consumption were observed. Hematology and serum chemistry investigations revealed no treatment-related effects. No treatment-related effects on liver tissue enzyme activities were observed. No treatment-related effects on organ weights were observed. Macroscopic and microscopic examination of the organs of the test animals revealed no treatment-related abnormalities. **No adverse effects indicated.** NOEL (M/F, systemic and skin) = 1000 mg/kg/day (based on no effects at the highest dose tested). **Acceptable.** (Corlett and Leung, 05/16/2013)

CHRONIC STUDIES

Chronic, rat

0085; 269121;** "Technical Grade MHK 3586: A Combined Chronic Toxicity/Oncogenicity Testing Study in the Rat" (Wahle, B.S., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Agriculture Division Report Number 109260, Toxicology Study ID Number 95-272-DK, 10/08/1999). 870.4300. Technical Grade MKH 3586 (Batch No. 05362/0005, purity = 98.1-98.6%) was dissolved in ethanol and admixed to the diet (to which corn oil had been added) and fed continuously to 50 Fischer 344 rats per sex per dose at dose levels of 0 (diet, corn oil, and ethanol only), 50, 500, or 1250 (males)/1000 (females) ppm (0, 2.3, 25, and 67 mg/kg/day, respectively for males and 0, 2.7, 30, and 65 mg/kg/day, respectively for females) for approximately 104 weeks. Also, the test article was administered continuously to an additional 10 to 20 rats per sex per dose at dose levels of 0 (20/sex), 50 (10/sex), 500 (10/sex), or 1250 (males)/1000 (females) (20/sex) ppm for 1 year (1-year sacrifice group). No mortalities related to treatment occurred. No treatment-related clinical signs were observed. The mean body weight of the 1250 ppm males and the 500 ppm and 1000 ppm females was less than that of the control group animals over the course of the study. Hematology revealed no treatment-related effects in the 2-year group. A treatment-related increase in the mean cholesterol level in both sexes in the 2-year group at 500 and 1000/1250 ppm was observed. Urinalysis revealed no treatment-related effects in the 2-year group. A treatment-related increase in mean relative liver weight was observed in both sexes in the 1-year group at 500 and 1000/1250 ppm; no treatment-related effects on organ weights were observed in the 2-year group. Macroscopic examination revealed no treatment-related gross lesions in the 1-year and 2-year groups. Microscopic examination revealed a statistically significant decrease in vacuolization of the cytoplasm in the liver of males at 1250 ppm in the 1-year group. Neuropathological examination of 1-year group control and high-dose animals revealed no treatment-related abnormalities. No treatment-related tumors were observed**. No adverse effects. NOEL (M)** = 2.3 mg/kg/day (50 ppm) and **NOEL (F)** = 2.7 mg/kg/day (50 ppm) based on lower mean body weight, increased mean relative liver weight, and increased mean serum cholesterol**. Acceptable.** (Corlett and Moore, 09/19/2013)

Chronic, dog

**** 0084; 269111**; "Technical Grade MHK 3586: A Chronic Toxicity Feeding Study in the Beagle Dog" (Jones, R.D., Bayer Corporation (formerly Miles Inc.), Agriculture Division, Toxicology, Stilwell, KS, Study Number 97-276-MU, 08/05/1999). 870.4100. Technical Grade MKH 3586 (Batch No. 05362/0005, purity = 98.2-98.6%) was admixed to the diet (to which 1% corn oil had been added) and fed to 4 Beagle dogs per sex per dose at dose levels of 0 (diet and corn oil only), 75, 100, 300, or 1200 ppm (0, 1.6, 2.5, 8.9, and 31.5 mg/kg/day, respectively for males and 0, 1.8, 2.3, 8.7, and 34.6 mg/kg/day, respectively for females) for 1 year. No mortalities occurred. No treated-related clinical signs were observed. No treatment-related effects on mean body weight or on mean food consumption were observed. Quantitative electroencephalography indicated no treatment-related effects. Electrocardiography and blood pressure data revealed no abnormalities. Neurological examinations revealed a treatment-related barely detectable to mild deficient in backward stepping-foot placement/proprioceptive foot positioning in one or both rear legs in 3 females at 1200 ppm after 6 months of treatment; this sign was observed in 1 female at 1200 ppm after 12 months of treatment. Treatment-related decreases in mean hemoglobin and mean hematocrit were observed in males at 100, 300, and 1200 ppm, a treatment-related increase in mean platelet count was observed in both sexes at 1200 ppm, and a treatment-related decrease in mean % of eosinophils was observed in females at 1200 ppm. Treatment-related increases in mean cholesterol (in males at 300 and 1200 ppm and in females at 100, 300, and 1200 ppm), in mean triglycerides (in males at 100, 300, and 1200 ppm and in females at 300 and 1200 ppm), in mean gamma-glutamyl transpeptidase (in females at 1200 ppm), in mean lactate dehydrogenase (in males at 1200 ppm), in mean bile acids (in males at 1200 ppm), and mean globulin (in females at 300 and 1200 ppm) levels, and a treatment-related decrease in mean albumin (in males at 1200 ppm and in females at 100, 300, and 1200 ppm) were observed. No treatment-related effects on cortisol levels were observed. Urinalysis revealed no treatment-related effects. Cell phenotyping data revealed no dose-related effects. Immunoglobulin analyses revealed no treatment-related effects. Determinations of mean liver enzyme activities at study termination revealed treatment-related increases in O-demethylase activity in males at 100, 300 and 1200 ppm and in females at 1200 ppm and in cytochrome P-450 activity in males at 1200 ppm. A treatment-related increase in mean relative liver weight was observed in males at 300 and 1200 ppm and in females at 1200 ppm (not statistically significant). Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination revealed treatment-related diffuse thymic atrophy in all four males and one female at 1200 ppm. **Possible adverse effect:** thymic atrophy. **NOEL (M)** = 1.6 mg/kg/day (75 ppm) and **NOEL (F)** = 1.8 mg/kg/day (75 ppm) based on clinical pathology changes**. Acceptable.** (Corlett and Leung, 05/28/2013)

Oncogenicity, rat

See Chronic Toxicity, rat above.

Oncogenicity, mouse

** 0086; 269122; "Technical Grade MHK 3586: An Oncogenicity Testing Study in the Mouse" (Wahle, B.S., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Agriculture Division Report Number 109261, Toxicology Study ID Number 95-271-EU, 10/08/1999). 870.4200. Technical Grade MKH 3586 (test batch No. 05362/0005, purity = 98.1-98.6%) was dissolved in ethanol and admixed to the diet (to which corn oil had been added) and fed continuously to 50 CD-1[ICR]/BR mice per sex per dose at dose levels of 0 (diet, corn oil, and ethanol only), 100, 1500, or 4000 ppm (0, 15.7, 245, and 709 mg/kg/day, respectively for males and 0, 17.9, 275, and 806 mg/kg/day, respectively for females) for approximately 18 months. No mortalities related to treatment occurred. No treatment-related clinical signs were observed.

A treatment-related decrease in mean body weight was observed in males at 4000 ppm. Hematology revealed a treatment-related increase in mean hemoglobin distribution width in both sexes at 4000 ppm and an increase in mean reticulocyte count in males at 4000 ppm. A treatment-related increase in mean relative liver weight was observed in both sexes at 1500 and 4000 ppm. Macroscopic examination revealed no treatment-related gross lesions. Microscopic examination revealed a treatment-related increase in hepatocellular hypertrophy in both sexes at all dose levels and a treatment-related increase in hemoglobin-derived splenic pigment in males at 1500 and 4000 ppm and in females at 4000 ppm. **No adverse effects**. **Mouse Chronic Dietary NOEL (M)** < 15.7 mg/kg/day (100 ppm) and **NOEL (F)** < 17.9 mg/kg/day (100 ppm) based on dose-related hepatocellular hypertrophy. No treatment-related neoplastic changes were observed. **Acceptable.** (Corlett and Moore, 10/17/2013)

GENOTOXICITY

Gene mutation

** 0089, 269125; "MKH 3586: Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HPRT Assay *In Vitro*" (Brendler-Schwaab, S. Department of Cancerogenicity and Genotoxicity, Toxicology, Bayer AG, Wuppertal, Germany, Study No. T 9053849, Report No. 26582, 08/29/1997). 870.5300. Cultures of the Chinese hamster lung (V79) cell line (HPRT locus) were treated with MKH 3586 (Batch number 05362/0005 (mixed batch), purity = 98.1%), in the presence and absence of rat liver S-9 activation system, 2 trials for each, for 5 hours at concentrations of 0 (media only), 0 (media and not more than 1% DMSO), 250, 500, 1000, 2000, and 4000 ug/ml. Positive controls of ethyl methanesulphonate (EMS) (non-activated) and benzo(a)pyrene (B(a)P) (activated) were used. No evidence of mutagenic effects was observed in this mammalian forward mutation system**. No adverse effects indicated. Acceptable**. (Corlett and Leung, 08/16/2013)

** 53187-0114; 276070; "MKH 3586: Salmonella/Microsome Test, Plate Incorporation and Preincubation Method"; (B. Herbold; Bayer AG, Fachbereich Toxicology, D-42096 Wuppertal, F.R.G.; Study No. T 3054103; 9/13/95); *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were incubated with MKH 3586 technical (batch no. 17004/93; purity: 98.4%) at concentrations ranging from 16 to 5000 µg/plate under conditions of (-/+) activation by means of the plate incorporation method (1st trial) or preincubation with the test material for 20 minutes prior to incorporation into the agar (2nd trial) and incubated for 48 hours at 37° C. Each treatment was cultured in quadruplicate. An Aroclor 1254-induced rat liver S9 fraction was used to metabolize the test material. There was no apparent treatment-related increase in the incidence of reverse mutation. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 9/26/14)

Chromosome damage

Note: the Mouse Micronucleus Study fulfilled the data requirement for an *in vivo* mammalian cytogenetic study.

DNA damage or miscellaneous effects

** 53197-0113; 276069; "MKH 3586: Micronucleus Test on the Mouse"; (B. Herbold; Bayer AG, Fachbereich Toxicology, D-42096 Wuppertal, F.R.G.; Study No. T 8061542; 6/5/97); Fifteen Hsd/Win mice/sex were dosed by intraperitoneal (ip) injection with 100 mg/kg of MKH 3586 technical (batch nos. 05362/0005; purity: 98.1%). Five control animals/sex were dosed ip with the vehicle, aqueous 0.5% Cremophor. Five treated animals/sex/time point were euthanized at 16, 24 and 48 hours post-dose. The control animals were euthanized at 24 hours post-dose. A

positive control group of five animals/sex 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. No deaths resulted from the treatment. Treatment-related signs included apathy, spasms, twitching, rolling over, hind limbs spread apart, and difficulty breathing. There was no treatment-related increase in the number of micronuclei per 1000 polychromatic erythrocytes. The positive control was functional. **No adverse effect indicated. Study acceptable.** (Moore, 9/29/14)

REPRODUCTIVE TOXICITY, RAT

** 0083, 269110; "A Two Generation Reproductive Toxicity Study with MKH 3586 Technical in the Sprague-Dawley Rat" (Astroff, A.B. and Freshwater, K.J., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Study Number 95-672-FY, Agriculture Division Report Number 108188, 09/17/1998). 870.3800. MKH 3586 Technical (Batch No. 05362/0005, purity = 98.2%) was administered in the diet to 30 Sprague-Dawley rats (P generation) per sex per dose for a 10-week pre-mating phase and a mating phase, and then to the mated females through gestation (approximately 20 days) and lactation (approximately 21 days) at dose levels of 0 (diet only), 100, 500, or 1000 ppm ((M) 0, 5.0-8.7, 26.6-45.0, and 57.3-88.6 mg/kg/day, (F), premating: 0, 6.2-8.2, 32.4-40.2, and 70.7-87.1 mg/kg/day, gestation: 0, 6.5-6.7, 34.5-35.8, and 70.2-79.2 mg/kg/day, lactation: 0, 9.8-18.1, 52.6-106.5, and 87.0-245.6 mg/kg/day). Weanlings from the F_1 litters (30 per sex per dose level, usually one male and one female per litter) were selected randomly to become parents of the next generation. These animals were treated from weaning for a 10-week premating period, a mating phase and then to the mated females through gestation and lactation at dose levels of 0 (diet only), 100, 500, or 1000 ppm ((M) 0, 5.4-10.6, 28.9-54.7, and 64.0-120.2 mg/kg/day, (F) premating 0, 6.8-10.2, 36.0-53.4, and 77.6-115.1 mg/kg/day, gestation: 0, 6.6-7.2, 38.3-40.6, and 78.5-85.6 mg/kg/day, lactation: 0, 10.9-17.9, 57.0-99.6, and 134.7-230.2 mg/kg/day). The mean body weight of the P generation 1000 ppm females was less than that of the control group throughout their treatment period. The mean body weights of both sexes in the 500 and 1000 ppm groups of the F1 generation were less than that of the control group throughout their treatment period as well. Except for a lower mean food consumption at the initiation of the P generation premating treatment for both sexes in the 500 and 1000 ppm treatment groups, no effect was apparent. No effects on the mating, fertility and gestation indices were observed in the P and F_1 generations. A treatment-related decrease in mean F_1 and F_2 pup weight gain was observed at 500 and 1000 ppm. A treatment-related increase in mean relative liver weight was observed in P generation adult males and females at 1000 ppm, and in F_1 generation adult males at 1000 ppm and F_1 generation adult females at 500 and 1000 ppm. Microscopic examination of the liver of F_1 generation adult animals revealed treatment-related hepatocytomegaly at 1000 ppm in both sexes; no treatment-related microscopic findings were observed in the P generation adult animals. **No adverse effect indicated. Parental NOEL (M/F)** 100 ppm ((M) 5.4-10.6 mg/kg/day, (F) 6.8 to 10.2 mg/kg/day) (based on a lower mean body weight during F_1 pre-mating interval) **Reproductive NOEL:** 1000 ppm (70.7 to 115.1 mg/kg/day), (based on no treatment-related effects on the 1000 ppm treatment groups of both generations) **Developmental NOEL**:100 ppm (9.8 to 18.1 mg/kg/day) (based on lower mean body weights for pups in the 500 and 1000 ppm groups of both generations); **Study acceptable**. (Corlett and Moore, 1/15/14)

0082, 269109; "A Pilot Reproductive Toxicity Study with MKH 3586 Technical in the Sprague-Dawley Rat" (Astroff, A.B., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Study Number 95-972-DL, 04/22/1996). MKH 3586 Technical (Batch No. 17004/93, purity =

98.2%) was administered in the diet to 10 Sprague-Dawley rats per sex per dose for a 4-week pre-mating phase and a mating phase, and then to the mated females through gestation and lactation at dose levels of 0, 100, 500, 1000, or 5000 ppm ((M) 0, 6.2-9.5, 32.5-47.7, 66.1-93.7, and 351.0-468.2 mg/kg/day, (F) premating: 0, 7.5-9.2, 37.4-44.5, 72.6-110.8, and 158.6-487.5 mg/kg/day, gestation: 0, 6.9-8.2, 36.2-41.7, 80.8-86.9, and 500.2-707.1 mg/kg/day, lactation: 0, 11.0-20.0, 53.5-104.5, 116.5-241.4, and 576.1-1526.6 mg/kg/day). With the exception of one male at 5000 ppm that was sacrificed in moribund condition during the pre-mating phase of the study, no mortalities occurred in the parental animals. The mean body weights of both sexes in the 5000 ppm group and the females in the 1000 ppm group were less than those of the control group throughout the treatment period. The mean food consumption at the initiation of the study was reduced for both sexes in the 5000 ppm group and for the females in the 1000 ppm group. Thereafter, no effect was apparent. A treatment-related increase in the mean estrous cycle length was observed at 5000 ppm. No effects on the mating, fertility and gestation indices were observed. The mean body weights of the pups in the 500, 1000 and 5000 ppm groups were less than those of the control pups ($p<0.05$ or 0.01). The pups in the 5000 ppm group demonstrated a reduced viability. A treatment-related increase in mean relative liver weight was observed for both sexes in the 500, 1000, and 5000 ppm. **No adverse effects. Parental NOEL** (M/F) 100 ppm ((M) 6.2-9.5 mg/kg/day, (F) 6.9 to 20.0 mg/kg/day) (based on increased mean relative liver weight), **Reproductive NOEL**: 1000 ppm (72.6 to 110.8 mg/kg/day) (based on an increase in the mean estrous cycle length for the dams in the 5000 ppm group); **Offspring NOEL:** 100 ppm (11.0 to 20.0 mg/kg/day) (based on a lower mean body weight for the pups in the 500 ppm group); **Supplemental study** (only 10 parental animals per sex per dose level were used, the animals were dosed for only 4 weeks prior to mating, no histopathology was performed, and the F1 pups were sacrificed immediately after weaning). (Corlett and Moore, 1/14/14)

DEVELOPMENTAL TOXICITY

Rat

** 0077, 269104; “A Developmental Toxicity Study with MKH 3586 Technical in the Sprague-Dawley Rat” (Astroff, A.B. and Young, A.D., Bayer Corporation, Agricultural Division, Toxicology, Stilwell, KS, Study Number 96-612-KJ, Agriculture Division Report Number 108653, 07/08/1999). 870.3700. MKH 3586 Technical (Batch No. 05362/0005, purity = 98.2%), suspended in an aqueous 0.5% carboxymethylcellulose and 0.4% Tween 80 solution, was administered by gavage to 30 mated female Sprague-Dawley rats per dose at dose levels 0 (vehicle only), 15, 100, or 300 mg/kg on days 6 through 19 of gestation. One dam at 300 mg/kg was found dead. Treatment-related hard stool at 100 and 300 mg/kg/day and clonic convulsions at 300 mg/kg/day were observed. A treatment-related decrease in maternal mean body weight gain was observed at 15, 100, and 300 mg/kg/day during the gestation day 0 to gestation 20 interval. A treatment-related increase in mean relative maternal liver weight was observed at 15, 100, and 300 mg/kg/day. No treatment-related effects on fetus viability and posimplantation loss were observed. A treatment-related decrease in mean fetal weight was observed at 300 mg/kg/day; no effect on mean placental weight was observed. Examination of the fetal skeletal structures revealed treatment-related incidences in litters of incompletely ossified squamosal bones, zygoma, and ribs at 100 and 300 mg/kg/day, enlarged sagittal suture, enlarged posterior font, enlarged squamosal suture, and with wavy/curved ribs at 100 and 300 mg/kg/day, and incompletely ossified nasal bones, parietal bones, frontal bones, lumbar arches, metatarsals, and sternebrae (segments 2 and 3) at 300 mg/kg/day. **No adverse effects. Maternal NOEL** < 15 mg/kg/day (based on decreased mean body weight gain and

increased mean relative liver weights), **Developmental NOEL** = 15 mg/kg/day (based on incompletely ossified skeletal structures). **Acceptable.** (Corlett and Leung, 07/19/2013)

0079, 269106; "A Supplemental Developmental Toxicity Study with MKH 3586 Technical in the Sprague-Dawley Rat" (Astroff, A.B. and Young, A.D., Bayer Corporation, Agricultural Division, Toxicology, Stilwell, KS, Study Number 99-T12-EH (supplemental submission to Bayer Report 108653, Study #96-612-KJ), Agriculture Division Report Number 108653-1, 02/25/2000). MKH 3586 Technical (Batch No. 05362/0005, purity = 97.8%), suspended in an aqueous 0.5% carboxymethylcellulose and 0.4% Tween 80 solution (w/v), was administered by gavage to 30 mated female Sprague-Dawley rats per dose at dose levels 0 (vehicle only), 5, and 15 mg/kg on days 6 through 19 of gestation. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. No treatment-related effects on maternal mean body weight gain and maternal mean food consumption were observed. No treatment-related effect on mean relative liver weight was observed. Macroscopic examination of the dams revealed no treatment-related changes. No treatment-related effects on fetus viability, posimplantation loss, placental weights and fetal body weights were observed. No treatment-related fetal external malformations, visceral malformations, or skeletal malformations and variations were observed. **No adverse effects**. **Maternal NOEL =** 15 mg/kg/day (based on no treatment-related effects on the dams at the highest dose tested)**, Developmental NOEL =** 15 mg/kg/day (based on no treatment-related effects on the litters at the highest dose tested). **Supplemental study** (only two treatment levels were used). (Corlett and Moore, 07/19/2013)

Rabbit

0081, 269108; "MKH 3586 Developmental Toxicity Study in Rabbits After Oral Administration" (Holzum, B., Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH-28605, Bayer AG Study No. T4061728, 03/25/1999). 870.3700. MKH 3586 (Mixed Batch No. 05362/0005, purity = 98.2%), prepared in a suspension of 0.5% carboxymethylcellulose in demineralized water, was administered by gavage to 22 (23 at 5 mg/kg) mated female Himalayan CHBB:HM rabbits per dose at dose levels of 0 (vehicle only), 5, 20, or 70 mg/kg on days 6 through 28 *post coitum*. No spontaneous deaths occurred. Two females at 70 mg/kg exhibited treatment-related clinical signs including cold to the touch ears, discolored urine (light yellow), decreased urination, and reduced feces; one of these animals aborted on day 21 *post coitum* and was sacrificed. A treatment-related decrease in maternal mean body weight gain at 20 and 70 mg/kg/day during the gestation day 6 to gestation day 29 interval was observed. No treatment-related effects on fetus viability and posimplantation loss were observed. A treatment-related decrease in mean fetal weight (not statistically significant) and an increased incidence of coarse-grained placentas were observed at 70 mg/kg/day. Examination of the fetal skeletal structures revealed a possible treatment-related incidence of incompletely ossified medial phalanges of 5th digits at 70 mg/kg/day. No adverse effects. **Maternal NOEL** = 5 mg/kg/day (based on decreased mean body weight gain), **Developmental NOEL** = 20 mg/kg/day (based on incompletely ossified medial phalanges of 5th digits). **Acceptable.** (Corlett and Moore, 07/30/2013)

0080, 269107; "MKH 3586: Additional Developmental Toxicity Study in Rabbits After Oral Administration" (Holzum, B., Department of Toxicology Pharma of the Institute of Toxicology, Wuppertal, Bayer AG, Germany, Report No. PH-28606, Study Number T6067139 (supplemental submission to Bayer AG Study No. T4061728), 03/25/1999). MKH 3586 (Mixed Batch No. 05362/0005, purity = 98.2%), prepared in a suspension of 0.5% carboxymethylcellulose in demineralized water, was administered by gavage to 22 (at 0 mg/kg) or 23 (at 70 mg/kg) mated female Himalayan CHBB:HM rabbits per dose at dose levels of 0 (vehicle only) or 70 mg/kg on days 6 through 28 *post coitum*. No spontaneous deaths

occurred. Treatment-related decreased urination, decreased water consumption, reduced feces, light yellow urine, and ears cold to touch were observed. Treatment-related decreased maternal food consumption and maternal body weight loss (days 6 to 12) were observed. No treatment-related effects on fetus viability and posimplantation loss were observed. A treatment-related decrease in mean fetal weight and a treatment-related increased incidence of coarse-grained placentas were observed. Examination of the fetal skeletal structures revealed treatment-related incidences of incompletely ossified medial phalanges of 5th digits and 5th toes, incompletely ossified 1st cervical vertebral bodies, and incompletely ossified 13th caudal vertebral bodies. **No adverse effects. Maternal NOEL** not determined (decreased body weight gain and reduced food consumption at the only dose (70 mg/mg) tested), **Developmental NOEL** not determined (incompletely ossified skeletal structures at the only dose (70 mg/kg) tested). **Supplemental study** (only one treatment level was used). (Corlett and Moore, 08/02/2013)

NEUROTOXICITY

Acute neurotoxicity, rat

0103, 270322; "An Acute Oral Neurotoxicity Screening Study with Technical Grade MKH 3586 in Fischer 344 Rats" (Sheets, L.P. and Hamilton, B.F., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Study Number 94-412-BF, 05/21/1996). 870.62. Technical Grade MKH 3586 (Batch No. 17004/93, purity = 98.2%), prepared in 0.5% methylcellulose-0.4% Tween 80 in deionized water, was administered by gavage in a single dose at concentrations of 0 (vehicle), 20, 100 (females only), 150 (males only), 400 (females only), and 600 (males only) mg/kg to 12 Fischer 344 CDF(F-344)/BR rats per sex per dose level. 5 males at 600 mg/kg and 1 female at 400 mg/kg died; these were the only mortalities that occurred prior to study termination. Treatment-related clinical signs observed included salivation and clear nasal discharge in the high-dose males and females, red nasal staining and oral staining at the mid- and high-dose levels of both sexes, urine staining in females at the mid- and high-dose levels, and ptosis in both sexes at the high-dose level. FOB assessments conducted on Day 0, 3 hours after treatment, revealed treatment-related ptosis at all dose levels in both sexes and tremors in males at the high-dose during home cage observations; treatment-related salivation in both sexes at the high-dose level, red nasal staining in both sexes at the mid- and high-dose levels, and palpebral closure in males at the high-dose level during the handling observations; treatment-related tremors in males at the high-dose level and ptosis in males at all dose levels and in females at the mid- and high-dose levels during the open field observations, treatment-related no reaction during approach response reflex testing in both sexes at the high-dose level; a treatment-related decrease in body temperature in both sexes at the mid- and high-dose levels. FOB assessments conducted on days 7 and 14 revealed no treatment-related effects. Treatment-related decreases in both motor activity and locomotor activity during the first 10-minute interval of testing was observed in both sexes at the mid- and high-dose levels during day 0 testing; motor activity and locomotor activity assessments conducted on days 7 and 14 revealed no treatment-related effects. No treatment-related gross lesions were observed in animals that survived to the study termination. Micropathological examination revealed slight neuronal necrosis in the midline of the thalamus in the region of the reuniens thalamic nucleus in one high-dose male and in one high-dose female. **Possible adverse effect:** Slight neuronal necrosis in the midline of the thalamus in the region of the reuniens thalamic nucleus**. NOEL (M/F)**< 20 mg/kg (based on FOB results)**. Acceptable**. (Corlett and Leung, 04/05/2013)

0104; 270323; "An Acute Oral Neurotoxicity Screening Study with Technical Grade MKH 3586 in Fischer 344 Rats" (Sheets, L.P., Bayer Corporation, Agriculture Division, Toxicology,

Stilwell, KS, Study Number 96-912-GY, Agricultural Division Report Number 107431-1, 05/09/1997). This study was conducted to supplement the data in a previously reviewed acute neurotoxicity study (Study Number 94-412-BF) located in Document No. 53197-0103 (Record No. 270322) where a NOEL could not be determined since clinical signs were observed at the lowest dose level tested (20 ppm) during the cageside observations and during the FOB assessments at day 0 (3 hours after treatment). Technical Grade MKH 3586 (Batch No. 17004/93, purity = 98.3%), suspended in 0.5% methylcellulose-0.4% Tween 80 in deionized water, was administered in a single dose by gavage to 10 Fischer 344 CDF(F-344)/BR rats per sex per dose at dose levels of 0 (vehicle only), 2, 5, and 10 mg/kg. No mortalities occurred. No treatment-related clinical signs were observed. FOB assessments 3 hours after treatment revealed no treatment-related effects. **NOEL (M/F)** = 10 mg/kg (based on no observed signs of toxicity at the highest dose tested). **Supplemental study** (no motor activity tests were conducted and no neuropathological examinations were conducted). (Corlett and Leung, 04/09/2013)

0104; 270324; "Original: Acute Oral Neurotoxicity Screening Study with Technical Grade MKH 3586 in Fischer 344 Rats, Supplemental: A Supplemental Acute Oral Neurotoxicity Study to Investigate Neuropathology and Possible qEEG Changes with Technical Grade MKH 3586 in Fischer 344 Rats " (Sheets, L.P. and Hamilton, B.F., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Study Number 95-912-GH, Agricultural Division Report Number 107431-2, 07/14/1997). This study was conducted to supplement the data in a previously reviewed acute neurotoxicity study (Study Number 94-412-BF) located in Document No. 53197-0103 (Record No. 270322). Technical Grade MKH 3586 (Batch No. 17004/93, purity = 98.3%), suspended in 0.5% methylcellulose-0.4% Tween 80 in deionized water, was administered in a single dose by gavage to 7 sets of 6 control and 8 treated female Fischer 344 CDF(F-344)/BR rats (0 (vehicle only) and 400 mg/kg, respectively). All rats were evaluated by detailed physical examinations for clinical signs 1, 2, 4, and 8 hours after treatment and once daily thereafter until terminal sacrifice. For neuropathology, 6 sets of control and treated animals (6 rats per dose level per time point) were sacrificed at 8 hours or 1, 3, 7, 15, or 28 days following treatment. The 7th set of control and treated animals was used for quantitative electroencephalography (qEEG) investigations at the time of peak neurobehavioral effect (3 to 4 hours after treatment) and again approximately 8 hours, 1, 3, 7, 15, and 28 days after treatment. 3 of the treated animals died within 8 hours of treatment. Treatment-related clinical signs including partially closed eyes, decreased activity, salivation, repetitive pawing in the rearing position and/or repetitive jaw chewing, clear lacrimation, and red or clear nasal discharge were observed with all of these signs clearing in the surviving animals after the 8-hour observation point; treatment-related red nasal staining, red staining of the fore-paws, oral staining, and urine staining were observed up to 9 days after treatment. Treatment-related qEEG effects (primarily an increase in power of delta, theta, and alpha waves under normotonic, auditory, visual, and somatosensory stimulus conditions) generally resolved 3 days after treatment. Necropsy revealed no gross observations. Micropathological examination of brain tissue revealed slight neuronal necrosis in the midline of the thalamus in the region of the reuniens thalamic nucleus in one treated animal sacrificed one day after treatment. **Possible adverse effect:** Slight neuronal necrosis in the midline of the thalamus in the region of the reuniens thalamic nucleus. **NOEL (M/F)** cannot be determined from the data presented. **Supplemental study** (Only one dose level was used, only female animals were used, and no motor activity tests were conducted). (Corlett and Leung, 04/16/2013)

90-day neurotoxicity, rat

** 53197-0107, -0115; 270328, 276557, 276558; "A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade MKH 3586 in Fischer 344 Rats" (Sheets, L.P. and Cain, D.M., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Laboratory Project Study ID 94-472-ZQ, Report No. 108655, 08/05/1999). 870.6200. Technical Grade MKH 3586 (test batch No. 17004/93, purity = 98.5%) was admixed to the diet and fed to 12 Fischer 344 CDF(F-344)/BR rats per sex per dose at dose levels of 0 (diet only), 100, 500, or 1000 ppm (0, 6.7, 33.4, and 66.5 mg/kg/day, respectively, for males and 0, 7.8, 38.2, and 75.8 mg/kg/day, respectively, for females) for 13 weeks. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. Treatment-related decreases in mean body weight and mean food consumption were observed in females at 500 and 1000 ppm. FOB and motor activity assessments revealed no treatment-related effects. No treatment-related effect on brain weight was observed. Macroscopic examination revealed no treatment-related gross lesions. Micropathological examination revealed no treatment-related abnormalities. **No adverse effects indicated. Rat Subchronic Neurotoxicity NOEL:** (M/F) 1000 ppm ((M) 66.5 mg/kg/day, (F) 75.8 mg/kg/day (based on no neurotoxic effects in either sex of the 1000 ppm treatment group; Study previously unacceptable but possibly upgradable with submission of the analysis of the dosing material. The requested information was submitted under record nos. 276557, 276558; **Study acceptable** (Corlett and Leung, 05/10/2013, upgraded, Moore, 9/26/14)

Developmental neurotoxicity, rat

** 53197-0090, -0115, 269126, 276559; "A Developmental Neurotoxicity Screening Study with Technical Grade MKH 3586 in Wistar Rats" (Sheets, L.P. and Lake, S.G., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Study No. 98-472-VL, Agricultural Division Report No. 109811, 03/01/2001). 870.6300. Technical Grade MKH 3586 (Mixed Batch Number 05362/0005, purity = 97.8%) was admixed to the diet and fed to 30 female presumed-pregnant Wistar Crl:W(HAN)BR rats per dose at dietary levels of 0 (diet only), 100, 500, or 1000 ppm (0, 7.4 to 7.8, 37.4 to 41.7, and 84.7 to 102.6 mg/kg/day, respectively, during gestation and 0, 14.4 to 19.0, 64.4 to 96.5, and 140.1 to 212.0 mg/kg/day, respectively, during lactation) from gestation day (GD) 0 through lactation day (LD) 21. No effects on the mating, fertility, and gestation indices were observed. No treatment-related clinical signs were observed in the P females during gestation and lactation. Treatment-related decreases in mean body weight gain during gestation and in mean body weight during lactation were observed in P females at 1000 ppm. No effects on the live birth, viability, and lactation indices were observed. A treatment-related decrease in mean body weight was observed in pups at 1000 ppm through post-natal day 21 and F1 generation males and females at 500 and 1000 ppm until termination. Pup developmental landmark observations revealed no treatment-related effects. Modified Functional Observational Battery (FOB) assessments conducted on the dams on gestations days 6, 13, and 20 and on lactation days 4, 11, and 21 and on the F1 offspring on post-natal days 4, 11, 21, 35, 45, and 60 revealed no treatment-related effects. Motor activity and locomotor activity assessments on the F1 generation male and female animals conducted on post-natal days 13, 17, 21, and 60 revealed no treatment-related effects. Acoustic startle response habituation assessments on F1 generation male and female animals conducted on post-natal days 22, 38, and 60 revealed no treatment-related effects. Passive avoidance (learning and retention phases, conducted on post-natal days 24 and 31, respectively) assessments, conducted on F1 generation male and female animals revealed no treatment-related effects. Assessments of learning and memory using a water maze apparatus on post-natal day 60 and 7 days later on F1 generation male and female animals revealed no treatment-related effects. No treatment-related effects on serum T3 and T4 levels of pups sacrificed on post-natal day 11 and on serum T3 and T4 levels of F1 generation animals sacrificed at the

study termination were observed. No treatment-related effects on brain, thyroid, and liver weights of the non-perfused F1 generation animals sacrificed at the study termination were observed. Measurements of the brains of pups sacrificed on post-natal day 11 and the brains of F1 generation animals sacrificed at the study termination revealed no treatment-related effects. No treatment-related necropsy findings were found in the pups sacrificed on post-natal day 11 or in the F1 generation animals sacrificed at study termination. Microscopic examination of brain and thyroid tissues of pups sacrificed on post-natal day 11 revealed no treatment-related abnormalities. Microscopic examination of the brain, other neural tissues, the thyroid, and muscle tissue of F1 generation male and female animals sacrificed on post-natal day 70 revealed no treatment-related abnormalities. **No adverse neurotoxic effects. Maternal NOEL** = 37.4 to 41.7 mg/kg/day (during gestation), 64.4 to 96.5 mg/kg/day (during lactation) (500 ppm) (based on a decrease in maternal body weight), **Developmental NOEL** = 100 ppm (based on a decrease in mean pup/F1 animal body weights). Study previously unacceptable but possibly upgradable with submission of the analysis of the dosing material; the information under record no. 276559 was sufficient to document the concentration of the test material in the dosing preparations. **Study acceptable** (Corlett and Leung, 06/05/2013, upgraded, Moore, 9/29/14)

Delayed neurotoxicity, hen

Not required.

IMMUNOTOXICITY

**** 0094, 269130**; "Amicarbazone- A 28-Day Dietary Immunotoxicity Study in Female Fischer 344 Rats" (Hurley, J.M., WIL Research Laboratories, LLC, Ashland, OH and ImmunoTox, Inc., Richmond, VA, Study No. WIL-476011, 02/01/2011). 870.7800. Amicarbazone (Lot no. 0709AMZ120-1, purity = 99.3%) was admixed to the diet and fed continuously to 10 F344/DuCRL (CDF) female rats per dose at dose levels of 0 (diet/corn oil/ethanol only), 250, 1000, or 2500 ppm (0, 20, 81, and 195 mg/kg/day, respectively) for 28 consecutive days. No mortalities occurred. No treatment-related clinical signs were observed. No treatment-related effect on body weight was observed. A treatment-related decrease in mean absolute spleen weight was observed at 2500 ppm; no treatment-related effects on the mean relative spleen and mean relative thymus weights were observed. Macroscopic examination revealed no treatment-related abnormalities. A treatment-related decrease in mean spleen cell numbers ($p \leq 0.05$), a decrease in mean antibody-forming (AFC)/10^6 spleen cells (specific activity) (not statistically significant), and a decrease in mean AFC/spleen (total spleen activity) (not statistically significant) were observed. The positive control (cyclophosamide monohydrate) was functional. NOEL (F) = 81 mg/kg/day (1000 ppm) (based on a decrease in spleen cell numbers, a decrease in AFC/10^6 spleen cells (specific activity), and a decrease in AFC/spleen (total spleen activity). **Possible adverse effect:** Significant reduction in IgM AFC in the spleen. **Acceptable**. (Corlett and Moore, 10/30/2013)

ENDOCRINE DISRUPTOR STUDIES

Not required at this time.

SUPPLEMENTAL STUDIES

STUDIES ON METABOLITES

MKH 3586 Oxadiazolinon

Primary Eye Irritation

0067, 269094; "Acute Eye Irritation Study of MKH 3586 Oxadiazolinon by Instillation into the Conjunctival Sac of Rabbits" (Leuschner, J., LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany, LPT Report No. 9301/344/95, Bayer Study No. T8067195, 04/26/1999). 870.2400. 0.1 ml of MKH 3586 Oxadiazolinon (an intermediate for MKH 3586, Batch no. LVK 2302/3/1, purity = 99.8%) was instilled into the conjunctival sac of one eye of each of 3 Himalayan rabbits. No conjunctival irritation was observed in the treated eyes. Grade 3 cornea opacity and grade 2 iritis were observed in all 3 treated eyes 24 hours after treatment. Grade 1 corneal opacity and grade 1-2 iritis were observed in all 3 treated eyes 7 days after treatment. Grade 1 corneal opacity and grade 2 iritis were observed in 2 of 3 treated eyes on day 16 through day 21. Toxicity Category I. **Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett, 08/22/2013)

Primary Dermal Irritation

0072, 269099; "Acute Skin Irritation Test (Patch Test) of MKH 3586 Oxadiazolinon in Rabbits" (Leuschner, J., LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany, LPT Report No. 9300/344/95, Bayer Study No. T8067195, 04/26/1999). 870.2500. 0.5 ml of MKH 3586 Oxadiazolinon (an intermediate for MKH 3586, Batch no. LVK 2302/3/1, purity = 99.8%) was applied to the shaved dorsal skin of each of 3 Himalayan rabbits for 4 hours covered with a gauze patch and held in place using a semi-occlusive dressing. No edema was observed in any test animal. Grade 1 erythema was observed in one animal 24 hours after patch removal with all signs of irritation clearing in all animals 48 hours after patch removal. Toxicity Category IV. **Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett, 08/26/2013)

Dermal Sensitization

0076, 269103; "MKH 3586 Oxadiazolinon, Study for the Skin Sensitization Effect in Guinea Pigs (Guinea Pig Maximization Test According to Magnusson and Kligman)" (Stropp, G., Bayer AG, Department of Industrial Toxicology, Wuppertal, Germany, Study number T 9067484, Report No. PH-28870, 06/16/1999). 870.26. The Magnusson and Kligman maximization assay was used to assess the potential of MKH 3586 Oxadiazolinon (an intermediate for MKH 3586, Batch no. LVK 2302/3/1, purity = 99.8%) to elicit delayed contact hypersensitivity in the guinea pig. 20 Hsd:Poc:DH guinea pigs were treated with the test material at induction (intradermal injections followed by a topical application 1 week later) and at challenge (a topical application 2 weeks after the topical induction). A concurrent negative control group consisting of a group of 10 animals was also included in the study. The control animals were treated identically to the test animals, except that during induction, the test material was replaced with the vehicle (polyethylene glycol 400). The positive control was functional. The challenge sites were examined for erythema 48 and 72 after the start of application; no skin effects were observed in the treatment group or in the control group. The results of the study indicate that the test material is not a sensitizer when using the Magnusson and Kligman maximization assay. **Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett, 08/30/2013)

Mutagenicity

0087, 269123; "MKH 3586 Oxadiazolinon: Salmonella/Microsome Test Plate Incorporation and Preincubation Method" (Herblod, B., Bayer AG, PH-PD Toxicology, Carcinogenicity and Genotoxicity, Wuppertal, Germany, Study No. T 2059963, Report No. 28997, 07/20/1999). 870.5100. Triplicate cultures of *S. typhimurium* strains TA1535, TA100, TA1537, TA98, and TA102 were exposed, first by the plate incorporation method then by pre-incubation method, to MKH 3586 Oxadiazolinon (an intermediate of MKH 3586, Batch number LVK 2302/3/1, purity = 99.8%), in the presence and absence of S9 rat liver fraction, at 0 (ethyl glycol dimethylether), 16, 50, 158, 500, 1581, and 5000 ug/plate and incubated for 48 hours at 37°C. Positive controls were functional. There was no treatment-related increase in mutation frequency. Negative results indicate that under these test conditions the test article is not mutagenic in these tested strains. **No adverse effects indicated. Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett and Moore, 08/21/2013)

MKH 3586-Triazolinon

Primary Eye Irritation

0068, 269095; "Acute Eye Irritation Study of MKH 3586-Triazolinon by Instillation into the Conjunctival Sac of Rabbits" (Leuschner, J., LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany, LPT Report No. 9301/348/95, Bayer Study No. T2067199, 04/27/1999). 870.2400. 100 mg of MKH 3586-Triazolinon (an intermediate for MKH 3586, Batch no. LVK 2304/11, purity = 99.9%) was instilled into the conjunctival sac of one eye of each of 3 Himalayan rabbits. No iritis or conjunctival irritation was observed in any of the treated eyes. Grade 1 cornea opacity was observed in all 3 treated eyes 24 hours after treatment, clearing in 2 of the treated eyes 48 hours after treatment and clearing in all treated eyes 5 days after treatment. Toxicity Category III. **Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett, 08/26/2013)

Primary Dermal Irritation

0073, 269100; "Acute Skin Irritation Test (Patch Test) of MKH 3586-Triazolinon in Rabbits" (Leuschner, J., LPT Laboratory of Pharmacology and Toxicology, Hamburg, Germany, LPT Report No. 9300/348/95, Bayer Study No. T2067199, 04/27/1999). 870.2500. 500 mg of MKH 3586-Triazolinon (an intermediate for MKH 3586, Batch no. LVK 2304/11, purity = 99.9%), moistened with sufficient water to ensure good contact with the skin, was applied to the shaved dorsal skin of each of 3 Himalayan rabbits for 4 hours covered with a gauze patch and held in place using a semi-occlusive dressing. No erythema or edema was observed at any test site in any animal at any time during a 72-hour observation period following test material removal. Toxicity Category IV. **Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett, 08/27/2013)

Dermal Sensitization

0075, 269102; "MKH 3586-Triazolinon, Study for the Skin Sensitization Effect in Guinea Pigs (Guinea Pig Maximization Test According to Magnusson and Kligman)" (Klimetzek, V., Bayer AG, Institute of Toxicology, Wuppertal, Germany, Study number T 8068266, Report No. PH-29139, 09/09/1999). 870.26. The Magnusson and Kligman maximization assay was used to assess the potential of MKH 3586-Triazolinon (an intermediate for MKH 3586, Batch no. LVK 2304/11, purity = 99.9%) to elicit delayed contact hypersensitivity in the guinea pig. 10 Hsd:Poc:DH guinea pigs were treated with the test material at induction (intradermal injections followed by a topical application 1 week later) and at challenge (a topical application 2 weeks after the topical induction). A concurrent negative control group consisting of a group of 5

animals was also included in the study. The control animals were treated identically to the test animals, except that during induction, the test material was replaced with the vehicle (polyethylene glycol 400). The positive control was functional. The challenge sites were examined for erythema 48 and 72 after the start of application; no skin effects were observed in the treatment group or in the control group. **The results of the study indicate that the test material is not a sensitizer when using the Magnusson and Kligman maximization assay. Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett, 09/03/2013)

Mutagenicity

0088, 269124; "MKH 3586-Triazolinon: Salmonella/Microsome Test Plate Incorporation and Preincubation Method" (Herblod, B., Bayer AG, PH-PD Toxicology, Carcinogenicity and Genotoxicity, Wuppertal, Germany, Study No. T 4059965, Report No. 29069, 08/06/1999). 870.5100. Triplicate cultures of *S. typhimurium* strains TA1535, TA100, TA1537, TA98, and TA102 were exposed, first by the plate incorporation method then by pre-incubation method, to MKH 3586-Triazolinon (an intermediate of MKH 3586, Batch number LVK 2304/11, purity = 99.9%), in the presence and absence of S9 rat liver fraction, at 0 (DMSO), 16, 50, 158, 500, 1581, and 5000 ug/plate and incubated for 48 hours at 37°C. Positive controls were functional. There was no treatment-related increase in mutation frequency. Negative results indicate that under these test conditions the test article is not mutagenic in these tested strains. **No adverse effects indicated. Supplemental study** (the test article is an intermediate of MKH 3586). (Corlett and Leung, 08/21/2013)

4-Methyl DA MKH 3586

Rat Metabolism

0091; 269127; "The Metabolism of [Triazolinone-3-^{14}C] 4-Methyl DA MKH 3586 in Rats" (Krolski, M.E. and Bosnak, L.L., Bayer Corporation, Agriculture Division, Research and Development Department, Stilwell, KS, Bayer Study No. M6041802, Bayer Report No. 108807, 02/23/1999). 870.7485. [Triazolinone-3-^{14}C] 4-Methyl DA MKH 3586 (an aerobic soil degradate of MKH 3586) (Vial No. C-803) (no lot number presented), purity > 99%, specific activity of 22.7 mCi/mmol (209700 dpm/ug), dissolved in tap water, was administered as a single oral dose by gavage to 4 male Fischer rats at a dose rate of 5 mg/kg. No mortalities occurred. Approximately 79% of the administered dose was excreted in the urine (52% in the first 6 hours) and 8% was excreted in the feces (virtually all in the first 24 hours). No significant part of radioactive residue was found in the tissues, residual carcass, or respired gases. 17 metabolites of the test article were detected in the excreted urine and 9 in the excreted feces. Based on the metabolites identified, the exclusive route of detoxification for the test article was through oxidative processes and demethylation. **Supplemental study** (the test article is an aerobic soil degradate of the active ingredient MKH 3586). (Corlett and Leung, 11/12/2013)