CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
ACTIVE INGREDIENT FLUPYRADIFURONE

Chemical Code # 6098, Document Processing Number (DPN) # 53190
Original date: May 7, 2013
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DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
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 270394 (Document No. 53190-0066) were examined. This includes all relevant studies indexed by DPR as of 5/2/2013.

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: t20130507
Original by C. Aldous and T. Moore

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

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METABOLISM AND PHARMACOKINETICS (Accepted test series)

53190-0059 267989 Klempner, A., "[Pyridinylmethyl-^{14}C] BYI 02960 - absorption, distribution, excretion, and metabolism in the rat," Bayer CropScience AG, Monheim am Rhein, Germany, Jan. 12, 2012. Laboratory Study # M1824559-4, Bayer Report MEF-11-747. Groups of 4 Wistar Hsd/Cpf: WU rats/sex were dosed with flupyradifurone at 2 and 200 mg/kg (single dose), with label placed on the methylene carbon between the pyridinyl group and the tertiary amino nitrogen. Chemical and radiochemical purity were each > 99%. An additional 4 males were dosed with 2 mg/kg of this test article iv. Rats were monitored for excretion patterns for 72 hours prior to sacrifice, with evaluation of tissues for radiolabel. Major metabolites were analyzed. Urinary label comprised 75% to 90% of administered dose for either sex and both routes. Females had slightly higher urinary percentages than males. Feces comprised nearly all of the balance (23-26% in males, and 7-10% in females in gavage studies, and 15% in the males dosed by iv). Nearly all of label was collected within the first 24 hours by urinary or fecal route regardless of sex or route of exposure. Peak plasma radioactivity was at or about 1 hr for low dose oral or iv administration, and at 2-4 hrs following high dose oral treatment. T½ for absorption was calculated to be 0.13 to 0.21 hrs in male and female gavage groups, with no obvious difference between dose levels. Elimination t½ was 3-4 hrs for all groups except for high dose females, which had an 8.1-hr elimination t½. About 0.1 to 0.3% of administered dose was found in tissues at 72 hours. Concentration of label in tissues at 72-hr sacrifice tended to be highest in blood cells and gastro-intestinal tract (both sexes) and eyes (females), intermediate in most commonly sampled organs, with brain being typically the lowest concentration. Parent flupyradifurone was the dominant metabolite in urine, regardless of sex, dose, or route. In gavage-treated males, 36-38% of administered dose was excreted as parent in urine, compared to 61-73% of administered dose in females. In all cases, the second most common residue was BYI 02960-OH, the hydroxyl residing on the furan. BYI 02960-OH in urine comprised 9-18% of administered dose in all groups. The only other abundant metabolite was BYI 02960-hippuric acid, derived from an initial cleavage to produce a carboxylic acid residue of the pyridinyl group (designated BYI 02960-CNA), most of which was conjugated to BYI 02960-hippuric acid. In gavage-treated males, this urinary metabolite comprised 7-10% of administered dose, compared to only 1-2% of administered dose in females. Small amounts of glucuronides and sulfate conjugates of BYI 02960-OH, plus one additional glucuronide and a desisofluoroethyl product were also quantified in urine. In feces, males excreted primarily BYI 02960-OH (7-11% of administered dose), plus lesser amounts of parent flupyradifurone. Females, which as noted above excreted most of label in the urine, had parent flupyradifurone as the largest residue (about 4%), and 2-3% of administered dose as BYI 02960-OH. This was a major study in the series of reports on metabolism and pharmacokinetics, which collectively address such data requirements. Aldous, 4/30/2013.

53190-0059 267990 Koester, J., "Quantitative whole body autoradiography of [pyridinylmethyl-^{14}C] BYI 02960 in male and female rats: distribution of total radioactivity and elimination from blood, organs and tissues after single oral administration including determination of radioactivity in the excreta and exhaled $^{14}CO_2$," Bayer CropScience AG, Monheim am Rhein, Germany, 5/30/2011. Study # M1821837-0, Bayer Report # MEF-11/276. All treatments were by gavage at 5 mg/kg. Investigators conducted serial sacrifices of one rat/sex at 1, 4, 8, 24, 48, 72, 120, and 168 hours. Tissues were assessed by whole-body autoradioluminography on sagittal sections. The last 4 rats/sex were used for ethanolamine/EtOH scrubbing for CO_2 and other volatiles. Peak tissue contents were observed at 1 hr in all sampled tissues except for nasal mucosa in both sexes and for peri-renal fat at 4 hours in females (these peaked at 4 hours at comparatively low concentrations). Concentrations in all tissues were declining sharply by 24 hours, and had approached or reached limit of quantification by 48 hours. Liver, renal medulla, and adrenals had about 2x higher concentrations than blood at t_{max} (at 1 hour). Most tissues had concentrations similar to blood. Brain, spinal cord, and peri-renal fat had exceptionally low concentrations. Rapid excretion through urine and to a lesser extent through feces was consistent with more detailed examinations in Record No. 267989. Excretion of volatiles, including CO_2, accounted for no more than 0.09% and 0.03% of administered dose in males and females, respectively. Useful supplementary data. Aldous, 1/23/13.

53190-0059 267991 Klempner, A., "[Furanone-4-^{14}C] BYI 02960 - absorption, distribution, excretion, and metabolism in the rat," Bayer CropScience AG, Monheim am Rhein, Germany, 12/22/2011 Laboratory Study # M1824560-6. Bayer Report MEF-11-556. Four Wistar rats/sex were dosed once by gavage with 2 mg/kg [furanone-4-^{14}C] BYI 02960. Periodic sampling of urine, feces, and plasma preceded organ and tissue sampling at 168-hour termination. As was the case with pyridinylmethyl-^{14}C label in Record No. 267989, radioactivity was recovered primarily in urine (79% of administered dose in males, and 91% of administered dose in females), and feces (17% of administered dose in males, and 10% of administered dose in females). Tissues, including g.i. tract, comprised only 0.49% of administered dose in males, and 0.18% in females. Absorption was efficient and clearance was rapid. Modeled kinetic values for male and female parameters were 1.60 and 1.34 hours for t_{max}, 0.232 and 0.166 hours for $t_{1/2\ abs}$, 3.07 and 2.88 hours for $t_{1/2\ elim\ 1}$, and 53 and 54 hours for $t_{1/2\ elim\ 2}$. After 7 days, no organ or tissue contained remarkable amounts of radiolabel. Highest concentrations in males were thyroid gland > harderian gland > adrenal gland. Highest concentrations in females were thyroid gland > adrenal gland > harderian gland. Thyroid gland concentrations were about 4x above plasma and the majority of other tissues. Metabolite profiles for males and females compared favorably to analogous profiles of low dose males and females with the pyridinylmethyl-^{14}C label reported in Record No. 267989. Most major metabolites do not break the parent molecule between the two label sites (at the C-N bond). When such cleavage occurred, the pyridinylmethyl-^{14}C label yielded a carboxylic acid residue of the pyridinyl group (designated BYI 02960-CNA), which was primarily conjugated to BYI 02960-hippuric acid, whereas the complementary cleavage product observed after furanone-4-^{14}C dosing was BYI 02960 - difluoroethylamino-furanone (the only identified product of C-N bond cleavage). The small yield (3.4%) of BYI 02960 - difluoroethylamino-furanone in the present study was appreciably less than that of the complementary cleavage products of the C-N bond breakage noted in Record No. 267989 (over 9%). The study investigator judged that CO_2 and biomolecules presumably derived from BYI 02960 - difluoroethylamino-furanone was represented in part by the unidentified highly polar metabolite(s) of peak No. 1 (5.2% of administered dose). No such

polar peak was found after treatment with the pyridinylmethyl-^{14}C label in Record No. 267989. This is a valid component of the metabolism and pharmacokinetics series. Aldous, 4/30/13.

53190-0059 267992 Koester, J., "Quantitative whole body autoradiography of [furanone-4-^{14}C] BYI 02960 in male and female rats: distribution of total radioactivity and elimination from blood, organs and tissues after single oral administration including determination of radioactivity in the excreta and exhaled $^{14}CO_2$," Bayer CropScience AG, Monheim am Rhein, Germany, 5/30/2011. Laboratory Study # M1821760-5, Bayer Report # MEF-11/275. Eight Wistar Hsd/Cpf: WU rats/sex were dosed once by gavage with [Furanone-4-^{14}C] BYI 02960, of chemical and radiochemical purity> 99%, at about 5 mg/kg. There were serial sacrifices of one rat/sex at 1, 4, 8, 24, 48, 72, 120, and 168 hours. The last 4 rats/sex were used for ethanolamine/EtOH scrubbing for CO_2 and other volatiles. With initial sacrifices at 1, 4, 8, and 24 hours, peak tissue contents were observed at 1 hr in all sampled tissues except for the vitreous body (peak at 4 hours in males only, at a comparatively low concentration). Concentrations in all tissues were declining rapidly by 24 hours. In contrast to the parallel study with [pyridinylmethyl-^{14}C] BYI 02960 treatment, there were still small but measurable levels of radioactivity in all assessed organs and tissues in treated males after 7 days. Females likewise had measurable residues in most assessed organs and tissues at 7 days, although the quantities were much smaller than in males. Liver, renal medulla, and adrenals had about 2x higher concentrations than blood at t_{max} (generally at 1 hour). Most tissues had concentrations on the same order as blood. Brain, spinal cord, and especially peri-renal fat had much lower concentrations than blood. Rapid excretion through urine and to a lesser extent through feces was consistent with other studies on flupyradifurone. Excretion of volatiles, including CO_2, accounted for about 3% and 0.75% of administered dose in males and females, respectively. This is markedly higher than was observed in the [pyridinylmethyl-^{14}C] BYI 02960 study (Record No. 267990). This was justifiably considered by the investigator as evidence for extensive degradation of the furanone ring after cleavage of the parent molecule at C-N bond. This is a valid component of the metabolism and pharmacokinetics series. Aldous, 1/24/13.

53190-0060 267993 Koester, J., "[Furanone-4-^{14}C] BYI 02960 - Metabolism in organs and tissues of male and female rats," Bayer CropScience AG, Monheim am Rhein, Germany, 2/2/12 (amended). Bayer Report No. MEF-11/271. This focused study involved four Wistar rats/sex, dosed with 3 mg/kg [pyridinylmethyl-^{14}C] BYI 02960 six hours before sacrifice, with assessment of total label and metabolite characterization in urine, plasma, and selected tissues. Balance at 6 hrs in males and females, respectively, was: 37% and 43% in urine, 24% and 27% in carcass, 24% and 13% in g.i. tract plus feces, 9% and 9% in skin, and lesser amounts in assessed tissues. Concentrations in liver and kidneys were about twice those of plasma. Urinary residues were mainly parent (22.1% and 37.6% of administered dose in males and females, respectively), with much lesser respective amounts as BYI 02960-OH (6.9% and 3.3%), and cleavage product BYI 02960 - difluoroethylamino-furanone (1.5% and 0.2%), these being the only identified metabolites comprising over 1% of administered dose in either sex. Parent flupyradifurone constituted 83% and 96% of total recovered residues (TRR) in urine of males and females, with BYI 02960-OH comprising 5.2% and 1.9% in M and F, and BYI 02960-difluoroethylamino-furanone comprising 7.7% and 0.9% of TRR, respectively. Liver and kidney followed similar patterns (parent as dominant residue, particularly in females), with the above metabolites plus 1-3% glucuronides and about 1% des-difluoroethyl product (as in other studies, metabolites being most abundant in males). This is a valid component of the metabolism and pharmacokinetics series. Aldous, 2/28/13.

53190-0060 267994 Koester, J., "[Ethyl-1-^{14}C] BYI 02960 - Metabolism in organs and tissues of male and female rats," Bayer CropScience AG, Monheim am Rhein, Germany, 10/10/2011. Bayer Report No. MEF-11/555. This focused study involved four male Wistar rats, dosed with 2 mg/kg [Ethyl-1-^{14}C] BYI 02960 72 hours before sacrifice, with assessment of total label in urine, feces, expired air, plasma, and selected tissues; and metabolite characterization in urine and feces. Balance (% dose administered) was 82% in urine, 14% in feces, 0.2% in expired air, and 4% in body plus g.i. tract. Most (74%) of urinary label was recovered during the first 24 hrs. No tissues exceeded plasma label concentrations at sacrifice. A unique contribution of ethyl label in this study was the ability to measure urinary difluoroacetic acid (DFA), which constituted 5.28% of administered label. Urinary excretion of DFA was nearly constant over the 3 days, compared to rapidly declining activity of parent and of other metabolites over time. This is a valid component of the metabolism and pharmacokinetics series. Aldous, 4/29/2013.

53190-0060 267995 Koester, J., "[Ethyl-1-^{14}C] BYI 02960 - Metabolism in organs and tissues of male and female rats (3 time-points)," Bayer CropScience AG, Monheim am Rhein, Germany, 2/2/12 (amended). Bayer Report No. MEF-11/270. This focused study involved four Wistar rats/sex/sacrifice time at a fixed dosed of 3 mg/kg [Ethyl-1-^{14}C] BYI 02960. Sacrifice times were 1, 6, and 24 hrs. Assessment included total label in urine, feces, plasma, and selected tissues over time, and metabolite characterization in urine and feces, and tissues (liver, kidneys, muscle, and fat). Balance study results mirrored guideline pyridinylmethyl- and furanone-label studies, which showed rapid clearance, largely as parent flupyradifurone and a few familiar metabolites, with clearance most rapid in females, which also produced fewer metabolites than males. Urinary difluoroacetic acid (DFA) constituted about 2% of administered label in both sexes. Urinary DFA was not quantifiable at 1 hr, but was evident at 0.1-0.2% of administered dose in 0-6-hr urine collection, and 1.91-1.70% of administered dose in the 0-24-hr collection. This suggests that initial disposition of flupyradifurone involves mostly clearance of parent and of small amounts of metabolites which keep the main structure of the compound intact. After distribution to various body compartments, however, residues which survive first-pass excretion undergo appreciable cleavage of the ethyl group to form DFA. In plasma, DFA constituted only 2-3% of plasma total recovered residues (TRR) in the 1-hr sacrifice group (at which time over 90% of TRR in either sex was parent). By 24 hrs, 91% and 82% of TRR in plasma in males and females was DFA, with most of the balance being parent. Also, by 24 hrs, DFA constituted over 50% of TRR in both sexes in all assessed organs/tissues. Parent flupyradifurone was the second most abundant labeled constituent at 24 hrs in each tissue. This is a valid component of the metabolism and pharmacokinetics series. Aldous, 2/28/13.

Conclusions from metabolism/pharmacokinetics studies: Combined reports adequately assess the disposition of flupyradifurone in the rat. Absorption is rapid. Elimination is efficient. Both sexes excrete predominantly in the urine. The most abundant urinary component is parent flupyradifurone, particularly so in females, with BYI 02960-OH [the hydroxyl residing on the furan] being the second most abundant labeled urinary component. About 9% of administered dose undergoes cleavage between the two ring components, yielding predominantly BYI 02960-hippuric acid from the pyridinyl portion. The furanone portion is either excreted as BYI 02960-difluoroethylamino-furanone or as one or more very polar residues, with up to 3% of administered dose recovered as CO_2 and perhaps other volatiles in males. The small amounts of flupyradifurone which are not excreted in the initial few hours after dosing are subject to cleavage of the N-difluoroethyl bond, manifest as difluoroacetic acid (DFA) in studies using label on the ethyl group. Collectively these studies fill the data requirements for disposition of flupyradifurone in the rat. Aldous, 1/24/13.

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat (Acceptable)

53190-0043; 267934; "Acute Toxicity in the Rat after Oral Administration"; (U. Gillissen; Bayer Schering Pharma AG, GDD-GED General Toxicology, 42096 Wuppertal, Germany; Study No. T 4080150; 6/8/09); Six female Wistar rats/group were dosed orally by gavage with 300 or 2000 mg/kg of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%). The vehicle was aqueous 2% Cremophor EL. Four of the 6 animals in the 2000 mg/kg group died within 3 hours of dosing. Clinical signs included tremors, decreased motility, piloerection, labored breathing and clonic cramps. In the necropsy examination of the animal which died, the liver was black or black spotted and the lungs were hemorrhagic. Otherwise, no treatment-related lesions were evident in the other animals; 300 mg/kg <LD50 (F) < 2000 mg/kg; Toxicity Category II; **Study acceptable.** (Moore, 2/28/13)

Acute dermal toxicity (Unacceptable, possibly upgradeable)

53190-0043; 267939; "Acute Toxicity in the Rat after Dermal Application"; (U. Gillissen; Bayer Schering Pharma AG, GDD-GED General Toxicology, 42096 Wuppertal, Germany; Study No. T 5080151; 6/8/09); The skin of five Wistar rats/sex was exposed to 2000 mg/kg of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) for 24 hours under a semi-occlusive wrap. The test material was placed on top of a wet gauze layer prior to placement on the skin. No deaths resulted from the treatment. No treatment-related clinical signs were evident. In the necropsy examination, no treatment-related lesions were noted. Reported LD50 (M/F) > 2000 mg/kg; Toxicity Category not assigned; **Study unacceptable**, possibly upgradeable to acceptable with the assurance from the laboratory staff that the test material was adequately moistened to permit good contact between the material and the skin. (Moore, 3/4/13)

Acute inhalation toxicity, rat (Acceptable)

53190-0043; 267940; " Acute Inhalation Toxicity in Rats"; (A. Folkerts; Bayer Schering Pharma AG, BSP-GDD-GED-GT-Inhalation, 42096 Wuppertal, Germany; Study No. T4080033; 1/7/10); Five rats/sex/group were exposed nose-only to 0 (PEG 400 alone) or 4671 mg/m^3 (analytical) of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) for 4 hours. The test material was prepared as a 50% solution (w/w) in PEG 400. The mean MMAD (GSD) was 2.04 (1.86) um. No deaths resulted from the exposure. Clinical signs among the animals exposed to the test material included increased and irregular breathing patterns, piloerection, reduced motility, increased motility, anxiety, tremor, high-legged gait, exophthalmia, stridor, and abdominal position with uncoordinated movements.. The body temperature of the exposed animals was lower when measured at the end of the exposure ((M) 0: 37.9 vs. 4671: 33.6^o C, (F) 0: 38.2 vs. 33.4^o C). No treatment-related lesions were noted in the necropsy examination. LC50 (M/F) > 4671 mg/m^3; Toxicity Category IV; **Study acceptable.** (Moore, 3/5/13)

Primary eye irritation, rabbit (Acceptable)

53190-0043; 267941; "Acute Eye Irritation on Rabbits, 1st Amendment to Report AT05341"; (C. Gmelin; Bayer Schering Pharma AG, GDD-GED General Toxicology, 42096 Wuppertal, Germany; Study No. T 0079824; 7/8/09, amended, 10/29/09); The eyes of 3 New Zealand White rabbits were treated by ocular instillation with 0.1 g/eye of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%). Neither corneal opacity nor iritis were noted during the 72-hour observation period. Conjunctival redness, grades 2 (1/3) and 1 (2/3), was

evident at 24 hours post-dose, clearing by 48 hours. Chemosis, grade 1 (1/3), was noted at 24 hours, clearing by 48 hours. Toxicity Category III; **Study acceptable.** (Moore, 3/5/13)

Primary dermal irritation (Acceptable)

53190-0043; 267942; "Acute Skin Irritation/Corrosion on Rabbits"; (C. Gmelin; Bayer Schering Pharma AG, GDD-GED General Toxicology, 42096 Wuppertal, Germany; Study No. T 9079823; 7/8/09); The skin of 3 New Zealand White rabbits were exposed to 0.5 g/site, one site/animal, of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) for 4 hours under a semi-occlusive wrap. The test material was moistened with water. No erythema or edema was evident throughout the 72-hour observation period. Toxicity Category IV; **Study acceptable.** (Moore, 3/6/13)

Dermal sensitization (Unacceptable)

53190-0043; 267943; "Local Lymph Node Assay in Mice"; (H.-W. Vohr; Bayer Schering Pharma AG, BSP-GDD-GED-GT-Special Toxicology, 42096 Wuppertal, Germany; Study No. T 4079413; 6/29/09); The dorsal skin on the ears of 6 female NMRI mice/group was treated by topical application with 25 µl/ear/day of 0 (vehicle: dimethylformamide), 2, 10, or 50% of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) for 3 days. On day 4, each animal was euthanized. The draining auricular lymph nodes were removed. The nodes were weighed and then crushed through a sieve and the number of cells/ml was determined. An 8 mm diameter section of ear was punched out and weighed. A stimulus index (SI) was determined by dividing the mean node and ear weights, change in ear thickness and nodal cell counts of the treated groups by the mean values of the vehicle control. There was no treatment-related increase in any of the parameters which were evaluated. No positive control study data were included in the study. **Study unacceptable**, not upgradeable (the LLNA protocol (OPPTS 870.2600) specifies that either tritiated thymidine or ^{125} I-uracil and fluorodeoxyuridine should be used to characterize cellular proliferation). (Moore, 3/6/13)

SUBCHRONIC STUDIES

Oral toxicity, rat: (Acceptable)

**53190-0045 267950 Odin-Feurtet, M., "BYI 02960: Study type: 90-day oral toxicity - rat," Bayer Cropscience, Sophia Antipolis, France, 3/21/12. Bayer Study SA 07294. Ten Wistar Rj:WI(IOPS HAN) rats/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 99.5%, Lot No. NLL 7780-44-6, for at least 95 days at 0, 100, 500, or 2500 ppm in a subchronic study. An additional 10/sex/group at 0 or 2500 ppm comprised a one-month recovery study segment. Overall compound consumption was 6, 30, and 156 mg/kg/day in treated males, and 7.6, 38, and 186 mg/kg/day in females. NOEL = 100 ppm in males, based on increased relative thyroid weights. NOEL = 500 ppm in females, due primarily to markedly decreased body weight associated with decreased food consumption, increased relative liver weights, and diffuse liver centrilobular hypertrophy. All of the latter findings were also observed in high dose males. At the high dose, both sexes had significantly reduced clinical chemistry values (total bilirubin and glucose), and females had significantly elevated cholesterol. Study is acceptable, and supports dose regimen for the chronic/oncogenicity study which followed. Aldous, 11/28/12.

53190-0062 267998 Blanck, M., "BYI 02960: Exploratory 28-day toxicity study in the rat by dietary administration," Bayer Cropscience, Sophia Antipolis, France, Feb. 1, 2008. Bayer

Study SA 07047. Groups of 5 male Wistar rats/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 99.7%, Batch No. 7780-27-1, in a 28-day pilot short subchronic study. Achieved dose levels were 34 and 385 mg/kg/day in treated groups. There was no NOEL in this study, as "prominent lobulation" of the liver was dose-related at 500 to 5000 ppm. There was a 45 g body weight decrement at 5000 ppm during the first week, and of 72 grams at day 29. Food consumption at 5000 ppm was significantly less than controls ($p < 0.01$) at all study weeks. Clinical chemistry seemed to reflect mainly altered liver function and poor nutritional state, with marked reductions in total bilirubin and glucose at 5000 ppm, and increased cholesterol at that dose level. Urea was increased significantly at 5000 ppm, with no alteration of creatinine. Thyroxin was non-significantly reduced and TSH non-significantly elevated at sacrifice. It is not possible to rule out an alteration in such enzymes with small sample sizes and a single sampling point. There were significant increases in relative liver, kidney, and thyroid weights at 5000 ppm. Centrilobular hepatocellular hypertrophy and thyroid follicular cell hypertrophy were observed in all high dose rats, and in no other rats. Liver microsomal enzymes showed some induction, particularly of BROD activity. UDPGT induction was comparable to major inducers such as phenobarbital and beta-naphthoflavone. Useful supplementary data. Aldous, 4/18/13.

53190-0062 267997 Capt, A., "BYI 02960: Exploratory 28-day toxicity study in the rat by gavage," Bayer Cropscience, Sophia Antipolis, France, 2/24/2009 (amended). Bayer Study SA 06075. Groups of 5 Wistar rats/sex/group were dosed by gavage with 0, 75, 200, or 350 mg/kg/day BYI 02960 (Flupyradifurone), purity 98.3%, Batch No. NLL 7780-16-5 in a 28-day pilot short subchronic study. There were no absolute NOEL's. Clinical signs of "increased salivation" and clinical chemistry change of reduced bilirubin, neither of which was necessarily "adverse," were observed in both sexes at all dose levels. Findings affecting both of the highest two dose levels in one or both sexes included premature deaths (F), increased alanine aminotransferase (F), markedly decreased plasma glucose (M), slightly increased creatinine (F), increased triglycerides (F), increased liver weights (both sexes), diffuse liver centrilobular hypertrophy (both sexes), diffuse thyroid follicular cell hypertrophy in (M), and erosion and/or necrosis of the glandular stomach (M). Liver microsomal fractions were evaluated for effects on metabolic enzymatic activities. Total P-450 specific content was not greatly altered by treatment. Activities toward particular substrates (notably BROD, EROD, and PROD) were generally elevated by an order of magnitude, with the dose-response seeming to plateau at 200 mg/kg/day in several instances. Useful supplementary data. Several of the responses at 200-350 mg/kg/day would be unsustainable in a long-term study, hence might be characterized as "adverse." Aldous, 4/18/13.

Oral toxicity, mouse: (Supplementary data)

53190-0046 267951 Odin-Feurtet, M., "BYI 02960: 90-day toxicity study in the mouse by dietary administration," Bayer Cropscience, Sophia Antipolis, France, 3/22/12 (amended). Bayer Study SA 07295. Ten C57BL/6J mice/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 99.5%, at 0, 100, 500, or 2500 ppm for 93-95 days. Mean achieved dose levels were 16, 81, and 407 mg/kg/day for males, and 19, 98, and 473 mg/kg/day for females. This was a range-finding study for longer term studies, and included most parameters of a standard subchronic study. Clinical signs were not remarkable. NOEL = 500 ppm. Body weight decrements were evident at 2500 ppm in both sexes at 1 week. At termination, high dose males weighed 3 g below controls, and high dose females trailed by 1 gram. Food consumption was commonly slightly reduced at 2500 ppm in both sexes, especially early in the study.

Additional high dose changes included clinical chemistry changes (mainly decreased cholesterol and increased urea), increased liver weight, pale liver at necropsy (mainly females), and increased degree of liver vacuolation in both sexes. Plasma samples from 5/sex/group were collected at 90 days for flupyradifurone, yielding 1.94, 10.8, and 42.8 mg/L for treated males, and 1.23, 8.72, and 34.0 mg/L for females. Useful supplementary data, supportive of the dose range used in the oncogenicity study which followed. Aldous, 1/29/13.

53190-0062 267999 Blanck, M., "BYI 02960: Preliminary 28-day toxicity study in the mouse by dietary administration," Bayer Cropscience, Sophia Antipolis, France, 11/23/07. Bayer Study SA 07013. Groups of 5 C57BL/6J mice/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 99.7%, Batch No. 7780-27-1, in a 28-day pilot short subchronic study. Achieved dose levels were 50, 98, and 207 mg/kg/day for treated males, and 59, 122, and 240 mg/kg/day for treated females. Other than a slight body weight gain decrement during the first week in high dose males, there were no definitive treatment effects. Useful supplementary data. Aldous, 2/14/13 (no DPR worksheet for this elective study).

Oral toxicity, non-rodent: (Acceptable)

**53190-0048 267961 Eigenberg, D. A., "A 90-day toxicity feeding study in the beagle dog with Technical Grade BYI 02960," Xenometrics LLC, Stilwell, KS, 4/22/10. Laboratory Study #: 09-S76-QQ. Groups of 4 beagle dogs were dosed in diet with BYI 02960 [Flupyradifurone, purity 96.2%, Lot No. 2009-000239] for 90 days in a standard subchronic study. Original dose levels were 0, 400, 1200, and 3600 ppm. Highest dose was reduced from 3600 ppm to 2400 ppm beginning study week 9 due to excessive toxicity ("unsteady and stiff back legs and lower back" in one high dose dog/sex). Estimated achieved dose levels were 12, 33, and 102/85 mg/kg/day for treated males (highest dose level achieved reflects first the initial 8 study weeks, followed by the last 5 weeks). Treated females received 12, 41, and 107/78 mg/kg/day, respectively. NOEL = 400 ppm. Focal myofiber atrophy/degeneration was the most sensitive response, affecting at least 2/group of both sexes at 1200 ppm and above (usually of "minimal" grade). Creatine phosphokinase was elevated at day 56 in mid- to high-dose males and females, possibly related to muscle pathology. Liver appears to have been affected at these dose levels, since ALT and AST were elevated at day 56 at mid- to high-dose levels in both sexes, although microscopic change at termination was limited to brown pigment accumulation in Kupffer cells in two high dose females. Liver weights were elevated in high dose males and females. Body weights were significantly reduced in both sexes at highest dose, and marginally reduced in mid-dose males. Hemoglobin, hematocrit, and RBC counts were reduced at high dose in both sexes on most assessments. Acceptable. Aldous, 4/29/13.

53190-0062 268000 Odin-Feurtet, M., "BYI 02960: Preliminary 28-day toxicity study in the dog by dietary administration," Bayer Cropscience, Sophia Antipolis, France, 12/9/2008. Bayer Study SA 07290. Two beagles/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 99.5%, Batch No. 7780-44-6, at 0, 500, 2000, or 4000 ppm for 28 days. Achieved dose levels were 16, 62, and 118 mg/kg/day in treated males, and 18, 77, and 131 mg/kg/day in females. Slight body weight decrement was reported at 4000 ppm in association with slight food consumption reduction, and slightly increased platelet counts were indicated at 4000 ppm. Small decreases in glycogen storage in liver were observed in 2000 ppm males, and in both sexes at 4000 ppm. No findings were "adverse," nor were subchronic NOEL's affected by this elective supplementary study, hence no DPR worksheet was created. Aldous, 2/15/13.

Dermal toxicity, 21/28-day or 90-day: (Acceptable)

**53190-0049 267963 Cada, A., "A subacute dermal toxicity study in rats with Technical Grade BYI 02960," Xenometrics LLC, Stilwell, KS, June 5, 2012. Study No. 11-S22-US. Ten Wistar CRL: WI (Han) rats/sex/group were dosed (by dermal application to shaved skin under 3 x 3-in gauze pads wetted with water) for 6 hrs/day on 28 consecutive days with BYI 02960 (Flupyradifurone), purity 96.2% at 0, 50, 150, or 500 mg/kg/day. Investigator noted that "The high-dose of 500 mg/kg body weight was selected after consideration of the amount of test material that can be reliably applied to the skin." NOEL = 500 mg/kg/day (highest dose tested). Acceptable. Aldous, 1/30/13.

CHRONIC STUDIES

Combined chronic and oncogenicity, rat (Acceptable)

**53190-0054 267969 Garcin, J. C., "BYI 02960: Study type: Combined chronic / carcinogenicity feeding - rat (835)," Bayer Cropscience, Sophia Antipolis, France, March 5, 2012. Bayer Study SA 08337. Sixty Wistar Rj:WI(IOPS HAN) rats/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 96.2%, Lot No. 2009-000239, for at least 2 years at 0, 80, 400, or 2000 ppm in an oncogenicity study. An additional 10/sex/group comprised a one-year chronic study segment. Overall compound consumption was 3.2, 16, and 81 mg/kg/day in treated males, and 4.5, 22, and 120 mg/kg/day in females. NOEL = 80 ppm (M) and 400 ppm (F). Survival was generally high, and notably higher in 2000 ppm females than in other female groups during the last six months of the study. Body weights were significantly decreased in 2000 ppm males (up to 7%) and markedly decreased in 2000 ppm females (up to 17%), the latter associated with slight food consumption decrements. Males had slightly reduced bilirubin, and females had consistently reduced bilirubin levels and elevated cholesterol. Relative liver weights were elevated at 2000 ppm in both sexes. In liver, centrilobular hypertrophy and centrilobular vacuolation were sharply elevated in high dose males, to a lesser extent in high dose females and in mid-dose males at chronic study sacrifice. The same groups were affected among oncogenicity study rats. Additional histopathology changes were observed in oncogenicity study rats. Local hepatocellular brown pigment was markedly elevated in high dose females only. High dose females showed an accumulation of brown pigment in Kupffer cells in liver. Eosinophilic foci of hepatocellular alteration were elevated in high dose males. Lung responses were limited to high dose females, which showed focal alveolar foamy macrophages, focal chronic interstitial inflammation, and focal perivascular inflammation. Colloid alteration in thyroids of mid- to high dose males was clearly treatment-related, with no dose-response in that range, and no corresponding change in females. There were no observed neoplasia effects. Study is acceptable, with no adverse effects. Aldous, 4/11/13.

Chronic, dog (Acceptable)

**53190-0052 267967 Cada, A., "A chronic toxicity feeding study in the beagle dog with Technical Grade BYI 02960," Xenometrics LLC, Stilwell, KS, 2/17/12. Xenometrics Study No. 09-C76-RZ. Groups of four dogs/sex/group were dosed in diet with BYI 02960

(Flupyradifurone), purity 96.2%, Lot No. 2009-000239 for one year in a guideline chronic study. Achieved doses in treated males were 4.6, 7.8, and 28.1 mg/kg/day, and in females: 4.1, 7.8, and 28.2 mg/kg/day. NOEL = 300 ppm, based on muscle degeneration in both sexes, observed in 2 dogs/sex in gastrocnemius and in 3 dogs/sex in biceps femoris. Mean grade was 1.0 to 1.5. There were no other chronic effects. Study is acceptable, with no adverse effects indicated. Aldous, 12/12/12.

Oncogenicity, rat (See Combined chronic and oncogenicity, rat, above)

(See Combined chronic and oncogenicity, rat: above)

Oncogenicity, mouse (Acceptable)

53190-0053 267968 Kennel, P., "BYI 02960: Study type: carcinogenicity feeding - mouse," Bayer Cropscience, Sophia Antipolis, France, 2/24/12. Bayer Study No. SA 08338. Fifty C57BL/6J mice/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 96.2%, for at least 552 days at 0, 70, 300, or 1500 ppm in an oncogenicity study. An additional 10/sex/group comprised a one-year chronic study segment. Overall compound consumption was 10.0, 43, and 224 mg/kg/day in treated males, and 12.2, 53, and 263 mg/kg/day in females. Hematology was performed at weeks 53-54, and at weeks 79-80. All mice were necropsied, however only oncogenicity segment mice were examined microscopically. Survival was uniformly high. There were no treatment-related clinical signs. NOEL = 300 ppm: 43 mg/kg/day in males and 53 mg/kg/day in females. High dose findings were modestly reduced body weights (both sexes), reduced kidney weights and modestly increased numbers of atrophic or small kidneys (males), marked **decreases in normal age-related kidney pathology in males (basophilic tubules, cortical mineralization, cortico-epithelial vacuolation), and an increase in the extent of hepatocellular vacuolation in males. High dose females had a decided **decrease** in a common liver finding (hepatocellular macrovacuolation, mainly periportal, diffuse). There was no treatment-related neoplasia. Acceptable, with no adverse effects. Aldous, 4/12/13.

GENOTOXICITY

Gene mutation (Acceptable)

** 53190-0055; 267970; "Salmonella/Microsome Test, Plate Incorporation and Preincubation Method"; (B. Herbold; Bayer Schering Pharma AG, GDD-GED-GTOX Genetic Toxicology, 42096 Wuppertal, Germany; Study No. T 8080064; 6/24/09); *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 strains were incubated with BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) at levels ranging from 16 to 5000 µg/plate (both trials) under conditions of (-/+) activation and incubated for 48 hours at 37^{o} C by means of the plate incorporation method. In the 2nd trial, the bacterial strains were preincubated with the test material for 20 minutes prior to incorporation into the agar. Each treatment was incubated in triplicate. An Aroclor 1254-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the incidence of reverse mutation. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 3/7/13)

** 53190-0055; 267971; "Salmonella Typhimurium Reverse Mutation Assay, 1st Amendment to Report"; (A. Sokolowski; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1425802; 8/23/11, amended, 10/17/11); *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed for 48 hours at 37° C to BYI 02960 (Flupyradifurone technical) (batch no. PFV107N005; purity: 97.2%) at concentrations ranging from 3 to 5000 µg/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 µg/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 treatment-related increase in reverse mutations with or w/o activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/7/13)

** 53190-0055; 267976; "V79/HPRT-Test *In Vitro* for the Detection of Induced Forward Mutations"; (G. Entian; Bayer Schering Pharma AG, GDD-GED-GTOX Genetic Toxicology, 42096 Wuppertal, Germany; Study No. T 1080067; 10/29/09); Chinese hamster V79 cells were exposed to BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) for 5 hours at 37° C at concentrations ranging from 46 to 2944 µg/ml with and w/o activation. Two trials were performed with duplicate cultures for each treatment level. An Aroclor 1254-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the mutation frequency in either of the trials. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/11/13)

Chromosome damage (Acceptable)

** 53190-0055; 267979; "*In Vitro* Chromosome Aberration Test with Chinese Hamster V79 Cells"; (M. Thum; Bayer Schering Pharma AG, GDD-GED-GTOX Genetic Toxicology, 42096 Wuppertal, Germany; Study No. T9080065; 11/11/09); Chinese hamster V79 were exposed to concentrations of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) ranging from 500 to 3000 µg/ml with and w/o activation for 4 hours at 37° C. Cells were harvested at 18 or 30 hours after the beginning of the treatment. In addition, cells were exposed to the test material for 18 hours at concentrations ranging from 200 to 800 µg/ml w/o activation. One trial was performed. Duplicate cultures were incubated for each treatment level. An Aroclor 1254-induced rat liver S9 fraction was used for activation. There was no treatment-related increase in the percentage of aberrations with or w/o activation. **No adverse effect indicated**. The positive controls were functional. **Study acceptable**. (Moore, 3/12/13)

DNA damage or miscellaneous effects (Acceptable)

** 53190-0055; 267982; "Micronucleus Test on the Male Mouse"; (B. Herbold; Bayer Schering Pharma AG, GDD-GED-GTOX Genetic Toxicology, 42096 Wuppertal, Germany; Study No. T 0080066; 7/9/09); Five male Hsd/Win mice/group were dosed twice by intraperitoneal (ip) injection with 0, 10, 20 or 40 mg/kg of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) with a 24-hour interval between injections. The animals were euthanized 24 hours after the second injection. The vehicle was corn oil. 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. Two

thousand polychromatic erythrocytes were evaluated per animal. Treatment-related signs included apathy, spasm, sternal recumbency, and breathing difficulties. 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, 3/13/13)

** 53190-0055; 267983; "Micronucleus Assay in Bone Marrow Cells of the Mouse with BYI 02960-a.i."; (J. Wieland; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1425801; 11/10/11). Five female Hsd/Win mice/group were dosed twice by intraperitoneal (ip) injection with 0, 12.5, 25 or 50 mg/kg of BYI 02960 (Flupyradifurone technical) (batch no. 2009-000239; purity: 96.2%) with a 24-hour interval between injections. The animals were euthanized 24 hours after the second injection. The vehicle was DMSO/corn oil (1/9). A positive control group of five males also received a single ip injection with 40 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. Two thousand polychromatic erythrocytes were evaluated per animal. Clinical signs were occurred in a dose-related manner and included reduced spontaneous activity, ruffled fur and abdominal positioning. 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, 3/13/13)

REPRODUCTIVE TOXICITY, RAT (Acceptable)

**53190-0051 267966 Milius, A. D., "Technical Grade BYI 02960: A two-generation reproductive toxicity study in the Wistar rat," Xenometrics LLC, Stilwell, KS, 10/17/11. Study No. 09-R72-SA. Groups of 30 Wistar rats/sex/group were dosed with 0, 100, 500, or 1800 ppm flupyradifurone for 2 generations in a standard reproduction study. Achieved dose levels during F0 pre-mating were 6.6, 32, and 117 mg/kg/day for males, and 7.7, 39, and 137 mg/kg/day for F0 females. F1 achieved levels were similar for F1 pre-mating rats. Achieved levels during gestation were similar to respective pre-mating levels, except that F1 gestational a.i. intake was elevated about 20-30% above F0 intake, associated with delayed body weight attainment in F1 dams. Investigators reduced a.i. concentrations to 50% of normal during lactation to maintain near constant compound intake. Parental systemic toxicity NOEL = 100 ppm, based on body weight decrements in the F1 pre-mating period (statistically significant in females, a continuation of delayed growth during lactation and early post-weaning period of F1 pups). Parental reproductive effects NOEL = 500 ppm, based on equivocal effects: reduction in mean number of estrous cycles in the last 3 weeks of F1 pre-mating period, and non-significant reduction in mean live litter size for F1 parental generation, associated with lower implantation counts. Offspring viability and growth NOEL = 100 ppm, based on diminished F2 pup body weights, statistically significant during lactation from PND 7 onward, and continuing into the pre-mating growth period. Acceptable, with no adverse effects indicated. Aldous, 4/12/2013.

DEVELOPMENTAL TOXICITY

Rat (Acceptable)

**53190-0050 267964 Langrand-Lerche, C., "BYI 02960: Developmental toxicity study in the rat by gavage," Bayer Cropscience, Sophia Antipolis, France, 2/22/10. Bayer Study SA 08347. Twenty-three pregnant Crl:CD (SD) rats/sex/group were dosed by gavage with BYI 02960 (Flupyradifurone), purity 96.2%, Lot No. 2009-000239, on gestation days 6-20 in a guideline study at 15, 50, or 150 mg/kg/day. Maternal NOEL = 15 mg/kg/day, based on very slight body weight decrements in the first few days of treatment. Developmental NOEL = 50 mg/kg/day, based on slight fetal body weight decrements, and on slight ossification delays. Clinical signs finding of "increased salivation" was noted in 20/23 at the highest dose level, compared to none in any other groups. There was a decrement of body weights of high dose dams of about 10 g from gestation day 8 and throughout the dosing period. Liver weights were significantly elevated in high dose dams. Study is acceptable, with no adverse effects indicated. Aldous, 4/17/2013.

Rabbit (Acceptable)

**53190-0050 267965 Kennel, P., "BYI 02960: Study type: prenatal developmental toxicity study - rabbit," Bayer Cropscience, Sophia Antipolis, France, 1/26/12. Bayer Study SA 10314. Twenty three mated NZW does/group were dosed by gavage with BYI 02960 (Flupyradifurone), purity 96.2%, Lot No. 2009-000239, on gestation days 6-28 in a guideline study at 7.5, 15, or 40 mg/kg/day. Maternal toxicity NOEL = 15 mg/kg/day, based on modest food consumption and body weight gain decrements. Developmental toxicity NOEL = 40 mg/kg/day (no developmental toxicity at the highest dose level). Study is acceptable, with no adverse effects indicated. Aldous, 4/17/13.

NEUROTOXICITY

Acute, rat [Acceptable. Possible adverse effect (transient clinical signs in acute phase)]

****53190-0056 267986** Garcin, J. C., "BYI 02960: An acute neurotoxicity study in the rat by oral administration," Bayer Cropscience, Sophia Antipolis, France, 9/30/2011. Bayer Study SA 10096. NOEL = 35 mg/kg. Twelve Wistar rats/sex were dosed once with BYI 02960 (Flupyradifurone), purity 96.2%, Lot No. 2009-000239, at 0, 50, 200, or 800 mg/kg in the primary study. A follow-up study was conducted in 12 females/group at 0, 20, or 35 mg/kg to provide a NOEL for peak (day 1) effects. Both sexes showed increased piloerection at 50 mg/kg on day 1, and 50 mg/kg females on day 1 had increased incidence of dilated pupils. One 800 mg/kg female died on day 1 after showing characteristic clinical symptoms. Numerous day 1 (2 hrs post-dose) FOB findings demonstrated a marked effect at 800 mg/kg and commonly a dose-response at 200-800 mg/kg, including decreased rearing behavior (only females), flaccid muscle tone, rats "cold to touch" (and decreased rectal temperatures of 1.5 to 2.5 °C), piloerection (a basis of NOEL), rapid respiration, low arousal state, incoordination, tremors and other involuntary movements including myoclonic jerks and chewing (plus convulsions in one high

dose female), gait abnormality observations of "no movements," "slightly flattened posture" or "hunched posture" at 800 mg/kg only, dilated pupils (including 50 mg/kg in females), exaggerated flexion of paws, surface righting reflex "uncoordinated or slow," and weak tail-pinch response in high dose females. None of these findings were elicited in the low dose follow-up study. Day 1 exploratory motor activity was significantly reduced at 200-800 mg/kg in both sexes during the first 10 minutes in the apparatus (significant, $p < 0.01$). Overall (1-hr) activities were significantly reduced in 800 mg/kg males and females. Accommodation patterns were normal in both sexes. Subsequent assessment days were uneventful. Possible adverse effects: relatively low NOEL for transient effects such as dilated pupils, slowed righting reflex, incoordination, and tremors. There were no effects on brain weights, gross pathology, or neurohistopathology. Acceptable, C. Aldous, Jan. 4, 2013.

Subchronic, rat (Acceptable)

**53190-0057 267987 "BYI 02960: A 90-day neurotoxicity study in the rat by oral administration," Bayer Cropscience, Sophia Antipolis, France, 6/28/2011. Bayer Study SA 09283. Groups of 12 Wistar Rj:WI (IOPS HAN) rats/sex/group were dosed in diet with BYI 02960 (Flupyradifurone), purity 96.2%, Lot No. 2009-000239 at 0, 100, 500, or 2500 ppm in a standard subchronic neurotoxicity study design, with all rats subjected to periodic FOB and motor activity assessments, and 6/group of controls and high dose groups perfusion-fixed and evaluated for neurohistopathology at termination. Mean achieved dose levels were 5.7, 29, and 143 mg/kg/day in treated males and 6.9, 35, and 173 mg/kg/day in females. NOEL = 500 ppm for both sexes. Body weight and food consumption decrements were observed in both sexes, most markedly at week 1. Modest but occasionally statistically significant food consumption decrements were observed occasionally at later intervals, and body weights of both sexes were still reduced at termination of study. There were no neurotoxicity responses at any dose level. Study is acceptable, with no adverse effects indicated. Aldous, 4/12/13.

Neurotoxicity Validation Studies, rat (Positive control studies)

53190-0066 270393 Garcin, J. C., "Trimethyltin: an acute neurotoxicity study after a single i.p. administration in rats," Bayer CropScience, Sophia Antipolis, France, 6/20/2011. Laboratory Study **#:** SA 09296. This study was submitted in support of flupyradifurone, but may be used to support other studies provided by the test facility. Pathologist: L. Elies. Twelve male Wistar rats per group were dosed once ip with 0, 6, or 10 mg/kg trimethyltin (97%). Rats were observed for at least 9-10 days prior to FOB and motor activity assessment, and were sacrificed at day 13-15 with perfusion prior to examination of central and peripheral nervous system. Key clinical signs, limited almost entirely to 10 mg/kg, included piloerection, hyper-reactivity to external stimuli, and resistance to handling; each in nearly all high dose rats. Less common high dose findings were abnormal skin color, reduced motor activity, aggression, uncoordinated movements, tremors, "wasted" appearance, general pallor, and clonic convulsions. The only treatment effect at 6 mg/kg was piloerection (3/12 rats). Body weights were markedly reduced at 10 mg/kg only. Common FOB findings upon handling were "squirming, twisting or attempting to bite, with or without vocalizations." "Rigid" muscle tone was evident in 3/6 high dose rats, and "flaccid" tone in 1/6 high dose rats. Arousal level of "somewhat high" was observed in 3/6 high dose males. Tremors were seen in 1/6 high dose rats in the open field. Incoordination, "slipping hindlimb," and "slightly flattened body" were each observed in at least 2/6 high dose

rats, and uncommonly at 6 mg/kg. Sensory reactivity tests found constricted pupils in 5/6 high dose rats, and in 1/6 of the 6 mg/kg rats. Paw reflex: flexion was exaggerated at 10 mg/kg. Tail pinch response was exaggerated at 10 mg/kg. Motor activity at 10 mg/kg was initially much elevated above other groups, and accommodation was limited. Main observations at termination were "thin" or "emaciated" in 5/11 surviving 10 mg/kg rats, compared to no such observations in other groups. Brain neurohistopathology found neuronal necrosis at the olfactory bulb, piriform cortex, hippocampus, amygdaloid nucleus, entorhinal cortex, and the granular layer of the dentate gyrus. Focal gliosis was commonly observed at brain levels 2-6. Several nuclei in levels 6 to 8 showed chromatolysis (red nucleus, reticular nucleus, spinal trigeminal nucleus). Ventral horns of the spinal cord at all three levels likewise showed chromatolysis. Focal myelin degeneration was observed in the reticular formation and all evaluated levels of the spinal cord. Myelin degeneration was observed commonly at 10 mg/kg in all assessed peripheral nerves, and rarely at 6 mg/kg. This is a useful validation study, showing coherent changes in behavior and in neurohistopathology. Aldous, April 10, 2013.

53190-0066 270394 Garcin, J. C., "Carbaryl: an experimental functional observation battery validation study in rats," Bayer CropScience, Sophia Antipolis, France, 6/20/2011. Laboratory Study #: SA 09246. This study was submitted in support of flupyradifurone, but may be used to support other studies provided by the test facility. Some measurements (mostly quantitative) were assessed in rats prior to treatment to evaluate inter-observer variability. These results indicated that all observers gave similar values, except for one observer whose readings were slightly outside the norm for the other three, and who was determined to benefit from targeted additional training. In the main validation study, groups of 10 male Wistar rats were dosed once ip with carbaryl at 0, 15, or 30 mg/kg. Rats were then observed in an FOB within 1 hr of dosing to test overall ability to assess expected changes, and to assess inter-observer variability (4 observers). The lead observer normally handled the rats, hence had the closest opportunity to see subtle treatment effects. Common dose-related findings were piloerection, involuntary motor movements such as chewing, muscle fasciculation, and tremors, and postural change (several rats lying with flattened body position), low muscle tone, increased lacrimation, and increased salivation, convulsions (one rat), reduced arousal, uncoordinated gait, walking on tiptoes, and body or limb(s) dragging when rats sought to move. Respiration was often rapid and occasionally labored. Sensory response assessments indicated as occasional non-responsiveness of auditory response or corneal reflex, reduced or lacking flexion of hind-paws, absent pupil reflex, pinpoint pupils, delayed righting reflex, and absent tail response. Quantitative measures slightly suggested decreased grip strength and increased foot splay in high dose rats. Results show the facility's ability to detect major behavioral changes, and indicate adequate inter-technician abilities to identify key findings. Useful validation study. Aldous, 4/11/13.

Developmental neurotoxicity, rat (Acceptable)

**53190-0058 267988 Gilmore, R., "A developmental neurotoxicity study with Technical Grade BYI 02960 in Wistar rats," Xenometrics LLC, Stilwell, KS, July 9, 2012. Study No. 11-D72-UW. Thirty Wistar Han females/group were administered BYI 02960 (Flupyradifurone), purity 96.2%, in the diet from gestation day 6 through lactation day 21. Dietary levels of 0, 120, 500, and 1200 ppm during gestation were reduced to 0, 60, 250, and 600 ppm during lactation in an effort to normalize achieved maternal exposure through the treatment period. Estimated achieved doses during gestation days 13-20 for treated dams were 9.4, 40, and 93 mg/kg/day. Exposures were similar at other segments of the treatment period. Investigators retained 23

usable litters per group for standard developmental neurotoxicity tests. Maternal NOEL = 500/250 ppm (gestation/lactation), based on body weight decrements: up to 7% in late gestation. Pup developmental neurotoxicity NOEL = 500/250 ppm, based on non-significant increase in motor activity and locomotor activity in high dose males at PND 13 only; and on a statistically significant increase in auditory startle reflex peak amplitude in high dose PND 60 females. Acceptable, with no adverse effects (apparent developmental findings each limited to one sex and one time period at a high dose level). Aldous, 4/29/2013.

Delayed neurotoxicity, hen (Not required at this time)

This study type is not required at this time.

IMMUNOTOXICITY (Acceptable)

**53190-0061 267996 Repetto, M., "BYI 02960: 28-day immunotoxicity study in the female Wistar rat by dietary administration," Bayer Cropscience, Sophia Antipolis, France, 9/22/11. Bayer Study SA 10353. Ten female Wistar rats/group were dosed in diet with flupyradifurone (96.2%) at 0, 125, 600, or 3000 ppm for 28 days. Estimated achieved dose levels in treated rats were 10, 50, and 230 mg/kg/day in treated rats. A positive control group received cyclophosphamide (3.5 mg/kg/day) daily by gavage for 28 days. Each rat received 2.5 x 10^8 sheep red blood cells (SRBC's) by tail-vein injection 4 days before necropsy. Rats were bled on study day 30 (just before sacrifice) by retro-orbital sinus. Sera were evaluated for SRBC-specific IgM by ELISA. No significant difference was noted in anti-SRBC IgM up to 3000 ppm in comparison to concurrent controls. The highest dose elicited body weight gain decrements during the first week, with transient food consumption decrements, but no notable clinical signs. There were no changes in T-cell-dependent antibodies due to flupyradifurone, whereas cyclophosphamide rats had significantly ($p < 0.01$) reduced counts. Flupyradifurone rats showed no changes in spleen or organ weights, whereas cyclophosphamide rats had diminished weights of both organs ($p < 0.01$). There were no systematic gross changes in flupyradifurone groups, whereas thymus and spleen were "atrophic/small" in 5/10 cyclophosphamide rats, and in 6/10 cyclophosphamide rats, respectively. Study is acceptable, and negative for immunotoxicity. Aldous, 4/17/13.

ENDOCRINE DISRUPTOR STUDIES (Not required at this time)

This test series is not required at this time.

STUDIES ON METABOLITES (Supplemental data)

BYI 02960-difluoroethyl amino-furanone

Rat Acute Oral Toxicity Study

53190-0043; 267936; "Acute Oral Toxicity in Rats "Acute Toxic Class Method"; (N. Rokh; CIT, BP 563, 27005 Evreux, France; Study No. 37503; TAR; 5/19/11); Three female Sprague-Dawley rats were dosed orally by gavage with 300 mg/kg and 6 females were dosed orally with 2000 mg/kg of BYI-02960-difluoroethyl-amino-furanone (Flupyradifurone metabolite) (batch no. NLL 8671-61; purity: 98.5%) (vehicle: purified water). No deaths resulted from the treatment. Hypoactivity and piloerection were noted for as clinical signs for some of the animals. No treatment-related lesions were evident in the necropsy examination. LD50 (F) > 2000 mg/kg; Toxicity Category III; **Study acceptable.** (Moore, 3/4/13)

Rat 28-Day Dietary Toxicity Study

53190-0044 267949 Kubaszky, R., "BYI 02960-difluoroethyl aminofuranone: A 28-day dietary toxicity study in Wistar rats," CiToxLAB Hungary Ltd., Szabadságpuszta, Hungary, 2/29/12. Bayer Study 11/116-100P. Groups of 20 Wistar rats/sex/group were dosed in diet with BYI 02960-difluoroethyl aminofuranone (a metabolite of flupyradifurone) for 28 days in a supplementary feeding study at 200, 800, or 3000 ppm. Achieved dose of test article was 17, 67, and 244 mg/kg/day for treated males, and 19, 76, and 273 mg/kg/day for females. Body weight data were erratic, with the most likely treatment effect being a slight decrement in body weight gain in males during the first week, followed by normal gains thereafter. There were no coherent findings in clinical signs or in a single abbreviated FOB assessed once near termination. There were no remarkable or statistically significant hematology, clinical chemistry, or pathology changes. This supplementary study indicates that this metabolite does not cause adverse effects at about twice the molar dose that is employed in subchronic and chronic studies with flupyradifurone. Aldous, 11/29/12.

Mutagenicity Study

** 53190-0055; 267973; "Salmonella Typhimurium Reverse Mutation Assay with BYI-02960-Difluoroethyl- Amino-Furanone (Metabolite of BYI 02960)"; (A. Sokolowski; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1399701; 5/24/11); *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed for 48 hours at 37^{o} C to BYI-02960-difluoroethyl-amino-furanone (Flupyradifurone metabolite) (batch no. NLL 8671-6-1; purity: 98.5%) at concentrations ranging from 3 to 5000 µg/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 µg/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 treatment-related increase in reverse mutations with or w/o activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/7/13)

** 53190-0055; 267978; "Harlan, Cytotest Cell Research GmbH (H. Wollny; Harlan CCR), 64380 Rossdorf, Germany; Study No. 1399702; 12/20/11); In the first trial, Chinese hamster V79 cells were exposed for 4 hours at 37^{o} C to BYI-02960-difluoroethyl-amino-furanone

(Flupyradifurone metabolite) (batch no. NLL 8671-6-1; purity: 98.5%) at concentrations ranging from 51.3 to 1640 µg/ml (+/-) activation). In the second trial, the cells were exposed for 24 hours under conditions of non-activation and for 4 hours under conditions of activation to concentrations ranging from 51.3 to 1640 µg/ml. A phenobarbital and beta-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the mutation frequency in either of the trials. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/11/13)

Chromosomal Aberration Study

**** 53190-0055; 267981**; "*In* Vitro Chromosome Aberration Test in Chinese Hamster V79 Cells"; (C. Hall; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1399703; 10/7/11); V79 Chinese hamster cells were exposed to concentrations of BYI-02960-difluoroethyl-amino-furanone (Flupyradifurone metabolite) (batch no. NLL 8671-6-1; purity: 98.5%) ranging from 6.4 to 1636 µg/ml with and w/o activation for 4 hours, followed by an additional 14 hours of incubation. 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 an increase in the incidence of chromosomal aberrations at all treatment levels in the non-activated assay, albeit not in a treatment-related manner. Positive controls were functional. **Possible adverse effect indicated. Study acceptable.** (Moore, 3/13/13)

DNA Damage Study

** 53190-0055; 267984; "Micronucleus Assay in Bone Marrow Cells of the Mouse with BYI-02960-difluoroethyl-amino-furanone (Metabolite of BYI-02960)" (C. Hall; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1421401; 11/28/11); Five male Hsd/Win mice/group were dosed twice by intraperitoneal (ip) injection with 0, 125, 250 or 500 mg/kg of BYI-02960-difluoroethyl-amino-furanone (Flupyradifurone metabolite) (batch no. NLL 8671-6-1; purity: 98.5%) with a 24-hour interval between injections. The animals were euthanized 24 hours after the second injection. The vehicle was sterile water. A positive control group of five males also received a single ip injection with 40 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. Two thousand polychromatic erythrocytes were evaluated per animal. Clinical signs were occurred in a dose-related manner and included reduced spontaneous activity, ruffled fur and abdominal positioning. 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, 3/13/13)

** 53190-0055; 267985; "*In* Vivo Unscheduled DNA Synthesis in Rat Hepatocytes with BYI-02960-difluoroethyl-amino-furanone (Metabolite of BYI-02960)" (C. Hall; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1421402; 10/26/11); Four male Wistar rats/group/time point were dosed with 0 (sterile water), 1000 or 2000 mg/kg of BYI-02960-difluoroethyl-amino-furanone (Flupyradifurone metabolite) (batch no. NLL 8671-6-1; purity: 98.5%) and euthanized at 4 or 16 hours after dosing. For positive controls, 4 males/group were treated with 80 mg/kg of N,N'-dimethylhydrazine and euthanized 4 hours post-dose or 100 mg/kg of 2-acetylaminofluorene and euthanized at 16 hours after dosing. Upon recovery of the hepatocytes, a primary culture was established and the cells were exposed to ^{3}H-thymidine (10 µCi/ml) for 4 hours, followed by further incubation overnight with

unlabeled thymidine. Two cultures/animal in each trial, 50 cells/culture, were evaluated for the number of net grains/nucleus. There was no treatment-related increase in unscheduled DNA synthesis. The positive controls were functional. **No adverse effect indicated. Study acceptable.** (Moore, 3/14/13)

6-Chloro-3-pyridyl) methanol

Rat Acute Oral Toxicity Study

53190-0043; 267937; "IM-0: Acute Oral Toxicity Study in Rats"; (N. Mochizuki, K. Goto; Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, 250-02, Japan; Project No. G-0887; 4/28/93; amended, 9/30/97); Five Crj:CD rats/sex/group were dosed orally by gavage with 1000, 1500, 2000, or 3000 mg/kg of **:** IM-0 ((6-chloro-3-pyridyl)- methanol) (Flupyradifurone metabolite) (lot no. NK-2327'-6, purity: 98.65%) (vehicle: ion exchange water). An additional group of 5 females was dosed with 1300 mg/kg of the test material. The following mortality resulted from the treatment; (1000 (M/F): 0/.5, 1300 (F): 0/5, 1500 (M): 1/5, (F): 3/5, 2000 (M): 3/5, (F): 5/5, 3000 (M/F): 5/5). Clinical signs included loss of righting reflex and motor activity, hypotonia, and ataxia. In the necropsy examination, hemorrhage in the stomach was noted for some of the animals which died during the study. LD50 (95% confidence intervals): (M) 1842 mg/kg (1389 to 2622 mg/kg), (F) 1483 mg/kg (not reported); Toxicity Category III; **Study acceptable.** (Moore, 3/4/13)

Rat Subchronic Dietary Toxicity Study

53190-0049 267962 Nukui, T. and S. Ikeyama, "IM-0 - Thirteen-week dietary subchronic toxicity study in rats," Odawara Research Center, Kanagawa, Japan, 11/28/97. Project No. G-0889. Groups of ten Crj:CD (SD) rats were dosed in diet with (6-chloro-3-pyridyl) methanol for 13 weeks in a subchronic study at 0, 160, 800, 4000, or 20000 ppm. Estimated corresponding dose levels of test article were 10, 49, 250, and 1247 mg/kg/day in treated males, and 11, 56, 276, and 1174 mg/kg/day in females. This study may be relevant because the product of oxidation of its methanol moiety to the carboxylic acid is an observed metabolite of flupyradifurone (designated BYI 02960-CNA). Body weights of high dose males and females at termination were 78% and 77% of controls, respectively. Food consumption was markedly reduced at several intervals in these rats. Alkaline phosphatase was significantly elevated in 20000 ppm females. Eosinophilic intra-nuclear inclusions in the proximal tubular epithelium were observed in 4000 to 20000 ppm males (with strong dose-response in degree of effect), and in 20000 ppm females. Apparent NOEL's for males and females of this test article were 800 ppm (males) and 4000 ppm (females). Useful supplementary data, indicating that this product has only minor toxicity. Aldous, 4/29/13.

Mutagenicity Study

** 53190-0055; 267974; "IM-0 – Reverse Mutation Study on Bacteria"; (N. Mochizuki, Y. Kanaguchi; Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan 100; Project No. G-949; 6/6/94, amended, 9/30/97); *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA were exposed to IM-0 ((6-chloro-3-pyridyl)methanol) (Flupyradifurone metabolite) (lot no. NK-3120; purity: 99.14%) at concentrations ranging from 313 to 5000 µg/plate. The cultures were preincubated for 20 minutes before plating under conditions of (+/-) activation. The S9 fraction was derived from the liver of male Sprague-Dawley rats treated with phenobarbital/5,6 benzoflavone.. There were 2 trials with 3 plates per treatment level. There were no treatment-related increases in reversion

frequencies with or w/o activation. Positive controls were functional. **Study acceptable.** (Moore, 3/8/13)

Difluoroacetic Acid

Rat Acute Oral Toxicity Study

53190-0043; 267935; Acute Oral Toxicity in Rats "Acute Toxic Class Method"; (C. Gerbeix; CIT, BP 563, 27005 Evreux, France; Study No. 37066 TAR; 10/22/10); Six female Sprague-Dawley rats were dosed orally by gavage with 300 mg/kg and 3 females were dosed orally with 2000 mg/kg of BCS-AA56716 (Flupyradifurone metabolite, difluoroacetic acid) (batch no. BCOO 5984-4-11, purity: 99.6%). Two of the 3 animals in the 2000 mg/kg treatment group died within 1 hour of dosing. The third animal survived the 14-day observation period. All of the animals in the 300 mg/kg group survived. Clinical signs included hypoactivity, dyspnea and lateral recumbency. No lesions were evident for the surviving animals in the necropsy examination. 300 mg/kg <LD50 (F) < 2000 mg/kg; Toxicity Category II; **Study acceptable.** (Moore, 3/4/13)

Rat 14-Day Dietary Toxicity Study

53190-0063 268001 Kennel, P., "BCS-AA56716 (Difluororacetic acid): preliminary 14-day toxicity study in the rat by dietary administration," Bayer Cropscience, Sophia Antipolis, France, 9/19/11. Bayer Study SA 10323. Groups of 5 Wistar rats/sex/group were dosed in diet with this metabolite of flupyradifurone], 96.7% purity, in a 14-day pilot study at 0, 500, 2000, or 8000 ppm. Achieved dose levels of difluororacetic acid were 48, 187, and 745 mg/kg/day in treated males, and 51, 201, and 800 mg/kg/day in females. As in the subsequent 90-day study, all dose levels elicited reduced serum glucose and increased serum urea. There were neither deaths nor clinical signs at any dose level. Useful supplementary data, with no impact on subchronic NOEL's. Aldous, 2/15/13 (no DPR worksheet for this elective pilot study).

Rat Subchronic Dietary Toxicity Study

53190-0047 267960 Kennel, P., "B.C.S.-AA56716 (Difluororacetic acid): study type: 90-day oral toxicity - rat," Bayer Cropscience, Sophia Antipolis, France, 2/2/2012. Bayer Study SA 10324. Groups of 10 Wistar rats/sex/group were dosed in diet with B.C.S.-AA56716 [Difluororacetic acid, a metabolite of flupyradifurone], 97.1% purity, in a standard subchronic study. Treated rats received 200, 1000, or 6000 ppm of the test article, with achieved dose levels of 12.7, 66, and 380 mg/kg/day in respective males, and 15.6, 79, and 472 mg/kg/day in females. No NOEL was established for this compound. There was a clear dose-response for greatly decreased serum glucose in both sexes over the whole range tested. Although less marked, there was also increased urea in sera of all groups of both sexes, with flat dose-response. Total bilirubin was decreased in all groups of males, and at 1000 ppm and above in females. Despite generally high urinary volume and low urinary refractive index at 1000 ppm and above in both sexes, urine of males in this dose range had conspicuously elevated ketones, and females has lesser but clearly treatment-related increases in ketones. Occasional erosion or necrosis of the glandular stomach was observed in both sexes at 1000 ppm and above. Body weights were reduced in 1000-6000 ppm males and in 6000 ppm females. Hemoglobin and HCT reductions in 1000-6000 ppm females were statistically significant, yet lacked dose-response in that range. Study is relevant because about 2% of orally administered flupyradifurone is metabolized to produce this product, according to rat metabolism studies (see Summary of Toxicological Data).

Note that an analog, fluoroacetic acid, is a potent metabolic poison. Useful supplementary data. Aldous, 4/29/2013.

Mutagenicity Study

53190-0055; 267972; "Salmonella Typhimurium Reverse Mutation Assay with BCS-AA56716 (Metabolite of BYI 02960)"; (A. Sokolowski; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1351101; 9/30/10); *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed for 48 hours at 37^{o} C to BCS-AA56716 (Flupyradifurone metabolite); Batch No. BCOO 5984-4-11; purity: 99.6%) at concentrations ranging from 3 to 5000 µg/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 µg/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 treatment-related increase in reverse mutations with or w/o activation. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/7/13)

** 53190-0055; 267977; "Gene Mutation Assay in Chinese Hamster V79 Cells *In Vitro* (V79/HPRT)"; (C. Hall; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1351102; 12/20/10); In the first trial, Chinese hamster V79 cells were exposed for 4 hours at 37^{o} C to BCS-AA56716 (Flupyradifurone metabolite) (Batch No. BCOO 5984-4-11; purity: 99.6%) at concentrations ranging from 30 to 960 µg/ml (+/-) activation). In the second trial, the cells were exposed for 24 hours under conditions of non-activation and for 4 hours under conditions of activation to concentrations ranging from 30 to 960 µg/ml. A phenobarbital and beta-benzoflavone-induced rat liver S9 fraction was used to metabolize the test material. There was no treatment-related increase in the mutation frequency in either of the trials. **No adverse effect indicated.** The positive controls were functional. **Study acceptable.** (Moore, 3/11/13)

Chromosomal Aberration Study

** 53190-0055; 267980; "*In Vitro* Chromosome Aberration Test in Chinese Hamster V79 Cells"; (C. Hall; Harlan, Cytotest Cell Research GmbH (Harlan CCR), 64380 Rossdorf, Germany; Study No. 1351103; 12/15/10); V79 Chinese hamster cells were exposed to concentrations of BCS-AA56716 (Flupyradifurone metabolite) (Batch No. BCOO 5984-4-11; purity: 99.6%) ranging from 3.8 to 960 µg/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 60 to 960 µg/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 60 to 960 µg/ml as well. A phenobarbital/beta-naphthoflavone-induced rat liver S9 fraction was used to metabolize the test material. Duplicate cultures were performed at each treatment level. Generally, 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, 3/12/13)

6-Chloronicotinic Acid

Rat Acute Oral Toxicity Study

53190-0043; 267938; "IC-0: Acute Oral Toxicity Study in Rats"; (N. Mochizuki, K. Goto; Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, 250-02, Japan; Project ID. G-0941; 11/30/93, amended, 9/30/97); Five animals/sex/group were dosed orally by gavage with 2000 or 5000 mg/kg of IM-0 ((6-chloronicotinic acid) (Flupyradifurone metabolite) (lot no. Batch Nr.5, purity: 99.4%) (vehicle: Tween 80 in ion exchange water). No deaths resulted from the treatment. No treatment-related clinical signs were evident. No treatment-related lesions were noted in the necropsy. LD50 (M/F) > 5000 mg/kg; Toxicity Category IV; **Study acceptable.** (Moore, 3/4/13)

Mutagenicity Study

** 53190-0055; 267975; "IC-0 – Reverse Mutation Study on Bacteria"; (N. Mochizuki, Y. Kanaguchi; Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan 100; Project No. G-942; 6/6/94, amended, 9/30/97); *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA were exposed to IC-0 ((6-chloronicotinic acid) (Flupyradifurone metabolite) (lot no. Batch Nr.5; purity: 99.4%) at concentrations ranging from 313 to 5000 µg/plate. The cultures were preincubated for 20 minutes before plating under conditions of (+/-) activation. The S9 fraction was derived from the liver of male Sprague-Dawley rats treated with phenobarbital/5,6-benzoflavone. There were 2 trials with 3 plates per treatment level. There were no treatment-related increases in reversion frequencies with or w/o activation. Positive controls were functional. **Study acceptable.** (Moore, 3/8/13)