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

SUMMARY OF TOXICOLOGY DATA

Penthiopyrad

Chemical Code # 6020 **Tolerance** # 53104

13 December 2011, Revised 8/25/15

I. 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, possible adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, possible adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	No data gap, no adverse effect

Toxicology one-liners are attached.

All record numbers through 278777 (vol. no. 53104-0527) were examined. This includes all relevant studies indexed by DPR as of 8/25/15.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T150824
Revised by T. Moore, 8/25/15

Toxicology one-liners are attached.

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.

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

**53104-0238 251121, "Metabolism of [^{14}C]MTF-753 in Rats", (K. S. Gupta, *et al*., Ricerca Biosciences, LLC., Concord, OH., Ricerca LLC Document No. 015188-1-2, 1 November 2005, amended 1 September 2009). 3 or 4 Wistar Hanover GALAS™ rats per sex per group received a single oral gavage dose of [Pyrazolyl-5-^{14}C]MTF-753 or [Thienyl-4-^{14}C]MTF-753 at 10 and 100 mg/kg.

Pharmacokinetic Experiment

Jugular cannulae were surgically implanted into the rats to allow for collection of blood samples at 0.25, 0.5, 1, 2, 6, 12, 24, 48, and 72 hours after a single oral dose at 10 and 100 mg/kg. Subsequently, the time to maximum plasma radiolabel concentration (Tmax), the maximum radiolabel concentration (Cmax), the area under the curve (radiolabel concentration-time curve) (AUC), and the elimination half-life ($t_{1/2}$) were determined. At both dose levels and in both sexes, the elimination of both the pyrazole (P) and thienyl (T) labels in plasma was rapid. T1/2 was 13.6 to 16.8 hours (P label) and 14.1 to 21.4 hours (T label). Plasma concentrations of both species reached their maximum levels (Tmax) at 0.4 to 0.5 hours post-dosing at 10 mg/kg (both sexes) and at 1.0 to 1.3 hours at 100 ppm post-dosing (both sexes). Cmax values were 1.5 to 1.6 ug equiv/g for males and 3.3 to 3.4 ug equiv/g for females at 10 mg/kg (both species) and 14.3 to 15.2 ug equiv/g for males and 28.4 to 31.9 ug equiv/g for females at 100 mg/kg (both species). The area under the curve values ($AUC_{o\text{-}inf}$), as with Cmax values, were proportional to dose level. The $AUC_{0\text{-}inf}$ was 21.9 ug equiv/g.h (males) and 27.8 ug equiv/g.h (females) after 10 mg/kg and 228.6 ug equiv/g.h (males) and 322.2 ug equiv/g.h (females) after 100 mg/kg of P label and 21.4 ug equiv/g.h (males) and 27.4 ug equiv/g.h (females) at 10 mg/kg and 224.8 ug equiv/g.h (males) and 324.4 ug equiv/g.h (females) at 100 mg/kg of T label).

Excretion Experiment

After a single oral dose at 10 and 100 mg/kg, urine and cage wash samples were collected 6, 12, and 24 hours post-dosing and at 24 hour intervals, thereafter, through the end of the 96 hour exposure period . Feces were collected at 24 hour intervals through the end of the exposure period. The gastrointestinal tract (GI) and contents and the carcass were sampled at termination. The percentage recovery of administered radiolabel was determined and metabolites were identified. The majority of dosed radiolabel (both species) was excreted in feces over the 96 hour post-dosing period (77.06% (males) and 69.61% (females) at 10 mg/kg

and 81.99% (males) and 73.69% (females) at 100 mg/kg with the P label and 78.97% (males) and 71.97% (females) at 10 mg/kg and 84.31% (males) and 72.25% (females) at 100 mg/kg with the T label). 10.91% (100 mg/kg) to 13.24% (10 mg/kg) for males and 18.18% (100 mg/kg) to 21.79% (10 mg/kg) of administered dose was excreted in urine with the P label and 7.34% (100 mg/kg) to 11.80% (10 mg/kg) for males and 15.87% (10 mg/kg) to 16.33% (100 mg/kg) for females was excreted with the T label during 96 hours. Cage wash contained 1.24% to 2.73% of administered P label and 1.50% to 3.72% of T label. The majority of administered radiolabel (both species) was excreted 24 hours post dosing. Gastrointestinal (GI) tract and contents contained 0.05% to 0.06% of administered P label and 0.06% to 0.10% of T label at the end of the exposure period. Carcass contained 0.24% to 0.29% of P label and 0.32% to 0.41% of T label after the exposure period. The major ^{14}C component (both species) in male and female feces was unchanged MTF-753 (3.11% to 8.06% of administered dose (AD) at 10 mg/kg and 12.26% to 30.38% at 100 mg/kg. Major metabolites present at 5% or more of AD in feces (at both dose levels and in both sexes) included DM-A-COOH (2-methyl-4-[3-[(3-trifluoromethyl-1*H*-pyrazole-4-carbonyl)amino]thiophen-2-yl)pentanoic acid), PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide), 753-A-OH (N-[2-(3-hydroxy-1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide), and 753-A-COOH (2-methyl-4-[3-[(1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carbonyl)amino]thiophen-2-yl]pentanoic acid). Major metabolites in urine following P label dosing included PCA (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid), PAM, 753-A-COOH (two isomers), DM-A-COOH (two isomers) and DM-PAM (3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) at 0.21% to 2.14% for males and 0.34% to 3.07% for females at 10 mg/kg and at 0.33% to 1.47% for males and 0.37% to 3.19% for females at 100 mg/kg (MTF-753 was detected at low levels (0.01% of AD) for low dose males only). After a single T labeled dose, major urinary metabolites included DM-A-COOH (two isomers) and 753-A-COOH (two isomers) at 0.44% to 2.33% for males and 0.29% to 3.51% for females at 10 mg/kg and at 0.41% to 1.68% for males and 0.62% to 3.99% for females at 100 mg/kg (MTF-753 was not detected).

<u>Biliary Excretion Experiment</u>

Biliary and duodenal cannulae were surgically implanted into the rats to allow for continuous collection of bile and infusion of a replacement bile salt solution. After a single oral dose at 10 and 100 mg/kg, urine, cage wash, and bile were collected 6, 12, and 24 hours after dosing and at 24 hour intervals, thereafter, through the end of the 72 hour exposure period. Feces were collected at 24 hour intervals through 72 hours. GI tract and contents and carcass were sampled at termination. Percentage recovery of administered dose was determined for each and metabolites were characterized. The excretion of radioactivity in bile, urine, and feces was dose dependent. At both dose levels, the majority of dose was excreted in bile (65.69% to 74.62% of AD after the P labeled dose and 62.8% to 81.07% after the T labeled dose). Urine contained 13.84% to 18.27% of AD (P label) and 5.02% to 16.75% (T label) and feces had 9.69% to 13.25% of AD (P label) and 7.97% to 11.23% (T label) after 72 hours. GI tract and contents contained 0.03% to 0.09% of AD (P label) and 0.04% to 0.17% (T label) and residual carcass had 0.22% to 1.20% (P label) and 0.52% to 0.82% (T label). Major metabolites in pooled bile samples during 24 hours post treatment with the P and T radiolabel at 10 and 100 mg/kg (present at 0.98% to 7.4% of AD of both species) included metabolites 42.45 min. and 44.63 min., 753-A-OH, 753-A-COOH (two isomers), and DM-A-COOH (two isomers). Major urinary metabolites (accounting for $\geq$ 1% of AD) for both sexes during 24 hours post-dosing with the P radiolabel at 10 mg/kg included DM-A-COOH (2 isomers), 753-A-COOH (2 isomers), PAM, and 753-A-OH (> 1% in females only). At 100 mg/kg (P label), PCA, 753-A-COOH (1 isomer), 753-A-OH (males only), and DM-A-COOH (1 isomer) (females only) were present at $\geq$ 1% of AD. After treatment with the T radiolabel, major urinary metabolites (> 1% of AD) included DM-A-COOH (1 isomer) (both sexes) and 753-A-OH (females only) at 10 mg/kg and

DM-A-COOH (2 isomers) and 753-A-OH in females only at 100 mg/kg. Major fecal metabolites during 24 hours post treatment with P radiolabel included MTF-753 (0.8% to 1.18% (males) and 0.9% to 1.06% (females) of AD), DM-A-COOH (1 isomer) (0.88% to 1.22% (females) and 0.58% to 1.14% (males) of AD), and 753-A-COOH (1 isomer) + DM-A-OH (N-[2-(3-hydroxy-1,3-dimethylbutyl)thiophen-3-yl]-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (0.41% to 0.51% (males) and 0.61% to1.32% (females) of AD) at 10 and 100 mg/kg. After a single dose with T radiolabel, the major components in feces included MTF-753 (0.40% to 0.64% (males) and 0.53% to 0.71% (females) of AD) DM-A-COOH (1 isomer) (0.50% to 0.78% (males) and 1% to 1.19% (females) of AD) at 10 and 100 mg/kg.

Tissue Distribution Experiment

After a single oral dose at 10 and 100 mg/kg, 3 per sex per group were sacrificed at 1, 24, 48, and 72 hours post-dosing (sacrifice times were based on pharmacokinetic data (Tmax, Cmax, and $t_{1/2}$)). The percentage of residual administered radiolabel was determined for adrenals, blood, bone, bone marrow, brain, eye, fat, heart, stomach, stomach contents, intestine, intestinal contents, kidney, liver, lung, lymph nodes (mesenteric), muscle, ovaries, packed cells, pancreas, pituitary, plasma, prostate, skin, spleen, testes, thymus, thyroid, urinary bladder, uterus, and carcass at each time point. Radioactivity was widely distributed in all tissues collected. Tissue radioactivity concentrations (ppm MTF-753 equivalents) peaked at 1 hour after dosing with concentrations in adrenals, fat, kidney, liver, lymph nodes, pancreas, and urinary bladder higher than those in plasma (liver had the highest ratio to plasma concentration at 4.535 to 12.225). Radiolabel half-lives in tissues decreased rapidly (except in packed cells and blood) with digressive values ranging from 3.2 to 24.7 hours (packed cell and blood half-lives were 33.5 to 390.1 hours and 21.8 to 46.4 hours, respectively).

A proposed metabolic pathway schematic for MTF-753 (both the pyrazole and the thienyl labels) was included (page 39). The main metabolic reactions proposed include N-dealkylation, side-chain hydroxylation, side-chain hydroxylation followed by dehydration, oxidation of the methyl group to the carboxylic group, glucuronidation, thienyl ring cleavage and ring opening, and hydrolysis of the amide group. Acceptable. (Green, 3/10/11).

**53104-0240 251123, "Multiple Dose Excretion and Tissue Distribution Study of [^{14}C]MTF-753 in Rats", (J.F. Shah and R.H. McClanahan, Environmental Sciences Department, Ricerca Biosciences, LLC., Concord, OH., Ricerca Biosciences LLC Document No. 020780-1, 24 February 2009). 3 Wistar Han GALAS^{TM} rats per sex per group received [Pyrazolyl-5-^{14}C]MTF-753 by oral gavage at 10 mg/kg/day for 4 or 7 consecutive days.

Group 1, Excretion, Mass Balance

Following each dose, urine, feces, and cage wash samples from each animal were collected and weighed during 24-hour intervals, and the percentage of administered dose/radiolabel (AD) was determined by liquid scintillation counting (LSC). At termination, radiolabel in whole blood from individual animals was measured (LSC). Carcass and gastrointestinal (GI) tract contents from each animal were weighed, homogenized, and the radiolabel content measured (LSC). Additionally, the metabolite profile was determined (high performance liquid chromatography) (HPLC) in pooled urine and feces samples collected on study days 2, 5, 8, and 10. The majority of administered dose (AD) of radiolabel was excreted in feces (group means of 71.84% (males) and 64.98% (females) of AD) through study day 11. Group means of 17.15% (males) and 22.77% (females) of AD were excreted in urine during the same time period. Cagewash contained group means of 2.29% (males) and 3.12% (females) of AD. Group mean radioactive residue levels in the GI tract and contents were 0.3% of AD for both sexes and carcass averaged 0.27% (males) and 0.22% (females). The main ^{14}C component in feces was

unchanged MTF-753 (at 2.23%, 9.12%, and 5.00% of AD for males and at 0.85%, 4.76%, and 1.54% of AD for females at days 2, 5, and 8, respectively). Major metabolites in feces included DM-A-COOH (2-methyl-4-{3-[(3-trifluoromethyl-1*H*-pyrazole-4-carbonyl)amino]thiopen-2-yl}pentanoic acid) (isomer a) at 4.8% to 7.50% of AD in males and 4.54% to 12.58% of AD in females and (isomer b) at 4.5% to 6.06% of AD in males and 3.71% to 7.28% of AD in females; PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxyamide) at 6.23% to 7.13% of AD in males and 0.99% to 4.40% of AD in females; 753-A-COOH (2-methyl-4-{3-[(1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carbonyl)amino]thiopen-2-yl}pentanoic acid) (isomer a) at 2.05% to 2.38% of AD in males and 4.71% to 7.04% of AD in females; and 753-A-OH (N-[2-(3-hydroxy-1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) at 3.01% to 4.38% of AD in males and 1.23% to 2.65% of AD in females. Main urinary metabolites included PCA (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) at 0.48% to 3.07% of AD in males and 0.64% to 1.55% of AD in females; 753-A-COOH (isomer b) at 1.20% to 1.63% of AD in males and 1.78% to 2.39% of AD in females; DM-PAM (3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) at 1.23% to 2.58% of AD in males and 2.19% to 2.75% of AD in females; PAM at 2.82% to 8.51% of AD in males and 2.93% to 4.07% of AD in females; DM-A-COOH (isomer a) at 0.24% to 0.37% of AD in males and 1.94% to 2.13% of AD in females; DM-A-COOH (isomer b) at 0.57% to 0.87% of AD in males and 2.63% to 2.90% of AD in females; and DM-PCA (3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) at 0% to 1.59% of AD in males and 1.04% to 1.71% of AD in females.

<u>Groups 2 through 4, Tissue Distribution, Time Course</u>

Group 2, 3, and 4 animals were sacrificed on study days 5, 8, and 10, after 4, 7, and 7 consecutive doses, respectively. The % of AD was measured (LSC) for pooled plasma, red blood cells, adrenals, fat, kidneys, liver, lung, lymph nodes (mesenteric), ovaries, pancreas, and thyroid samples. Additionally, the metabolite profile for plasma (pooled samples) was determined (HPLC and LSC) for groups 2 and 3 at study days 5 and 8, respectively. Results were compared to results of the single dose rat metabolism study (record 251121). Group mean whole blood residues (% of AD) were 0.12% to 0.07% for males and 0.11% to 0.06% for females for study days 5, 8, and 10. Other tissues contained no more than 0.32% of AD (female liver contained the highest % of AD on study day 5 (0.31% of AD)). Concentrations of radioactivity in tissues generally increased from study day 5 to 8 and then decreased from day 8 to 10 (except for liver and kidney which had maximum levels on day 5). Tissues with tissue:plasma concentration ratios greater than 1 (a possible indication of accumulation potential) included blood, kidney, liver, and red blood cells (highest ratios were in liver, 5.2:1 for males on day 8 and 5.6:1 for females on day 5). The main ^{14}C components in pooled plasma samples on study days 5 and 8 included PAM, DM-PAM, 753-A-OH, 753-A-COOH (b isomer) + DM-A-OH (N-[2-(3-hydroxy-1,3-dimethylbutyl)thiophen-3-yl]-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide), unchanged MTF-753, 753-T-DO (N-[5-hydroxy-5-(1,3-dimethylbutyl)-2-oxo-2,5-dihydrothiophen-4-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide), PTU (N-[2-(1,3-dimethyl-1-butenyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) + DM-753 (N-[2-(1,3-dimethylbutyl)thiophen-3-yl]-1*H*-pyrazole-4-carboxamide), DM-A-COOH (isomers a and b), 753-A-COOH (isomer a), and DM-PCA. The proposed metabolic pathway schematic for MTF-753 provided in record 251121 was also included with this study. Acceptable. (Green, 3/17/11).

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat

**53104-0241 251124, "MTF-753: Acute Oral Toxicity Study in Rats", (G. Arcelin, RCC Ltd., Toxicology Division, Füllinsdorf, Switzerland, RCC Study No. 783900, 31 October 2000). 3 male and 3 female HanIbm: WIST (SPF) rats received a single oral gavage dose of MTF-753 (penthiopyrad technical) (99.8%) at 2000 mg/kg followed by a 14-day observation period. All animals survived the 14 day post-dosing observation period. No treatment-related clinical signs were noted during the study period. One female had a 4% bodyweight loss during days 8 to 15. One male showed a 20.4% bodyweight loss during days 1 to 8 and recovered by day 15. Necropsy results were unremarkable. LD 50 > 2000 mg/kg. Toxicity category III. Acceptable. (Green, 10/12/10).

Acute dermal toxicity

**53104-242 251125, "MTF-753: Acute Dermal Toxicity Study in Rats", (G. Arcelin, RCC Ltd., Toxicology Division, Füllinsdorf, Switzerland, RCC Study No. 783900, 23 January 2001). 5 HanIbm: WIST (SPF) rats per sex received a single 24 hour dermal exposure (clipped, unabraded, semi-occluded skin) to MTF-753 (penthiopyrad technical) (99.8%) at 2000 mg/kg followed by a 14-day observation period. No deaths occurred and no treatment-related clinical signs were recorded. At the treatment site, no erythema, edema, or other dermal effects were noted at the end of the 24-hour exposure period and during the 14 day post-treatment observation period. Necropsy results were unremarkable. LD 50 > 2000 mg/kg. Toxicity category III. Acceptable. (Green, 10/12/10).

Acute inhalation toxicity, rat

**53104-0243 251126, “MTF-753: 4-Hour Acute Inhalation Toxicity Study in Rats”, (U. Decker, *et al*., RCC Ltd., Toxicology Division and Environmental Chemistry & Pharmanalytics Division, Füllinsdorf, Switzerland, RCC Study No. 783292, 20 December 2001). 5 HanIbm: WIST (SPF) rats per sex were exposed to MTF-753 (penthiopyrad technical) (99.8%) by nose-only inhalation at 5.669 mg/l analytical (with a mass median aerodynamic diameter (MMAD) range of from 2.60 µm with a geometric standard deviation (GSD) of 2.91 to 2.81 µm (MMAD) and 3.01 (GSD)) for 4 hours followed by a 14-day observation period. All animals survived to termination. No clinical symptoms were noted during exposure. One hour after the end of the exposure period, a moderate decrease in spontaneous activity and hunched posture were seen in all animals (additionally, one male had moderate alopecia, all other animals had ruffled fur). All animals recovered and were free from clinical signs from 2 days after exposure (test day 3) through necropsy (test day 15). At necropsy, all animals showed bodyweight gains. Necropsy results were unremarkable. LC_{50} > 5.669 mg/l. Toxicity category IV. Acceptable. (Green, 10/14/10).

Primary eye irritation, rabbit

**53104-0245 251128, “MTF-753: Eye Irritation Study in Rabbits”, (H. Ueda, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 00-0114, 2 April 2001). The left eyes of 3 female New Zealand White rabbits per group were treated once with MTF-753 (penthiopyrad technical) (99.8%) at 0.1 g per eye followed by a 72 hour observation period (treated eyes of Group A females remained unrinsed through the 72-hour observation period, while those of Group B animals were irrigated with deionized water for 30 to 60 seconds after treatment). No irritation of the cornea or iris was indicated at any time point. One hour after treatment, treated eyes were noted with conjunctival redness (score of 1) (all Group A and B animals), chemosis (score of 2 for all Group A animals and 2 Group B animals and score of 1 for the remaining Group B animal), and discharge with moistening of the lids (all Group A animals and 1 Group B animal). All treated eyes in Groups A and B had scores of 0 at 48 hours after treatment. Toxicity category III. Acceptable. (Green, 10/18/10).

Primary dermal irritation

**53104-0244 251127, "MTF-753: Dermal Irritation Study in Rabbits", (H. Ueda, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 00-0115, 15 March 2001). 3 female New Zealand White rabbits (Kbl:NZW) received a single 4-hour dermal exposure (clipped, unabraded, occluded skin (applied under a 2.54 cm^2 gauze pad) to MTF-753 (penthiopyrad technical) (99.8%) at 0.5 g moistened with deionized water and followed by a 72-hour observation period. No dermal response was noted (all dermal irritation scores were 0.0 at all time points). All animals gained bodyweight during the study. Toxicity category IV. Acceptable. (Green, 10/18/10).

Dermal sensitization

**53104-0246, 0477 251129, 256689, "MTF-753: Skin Sensitization Study in Guinea Pigs, Maximization Test", (H. Ueda, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 00-0116, 2 April 2001). 20 female Hartley guinea pigs (Group A) received six intradermal injections (0.1 ml each, as 3 pairs of injections, 1 on each side of the midline to clipped, intact skin of the shoulder region (2 x 4 cm area)) on the first day of induction. The three pairs of injections included: (a) 50% v/v Freund's Complete Adjuvant (FCA) in physiological salt solution (SPS); (b) 5% w/w MTF-753 (penthiopyrad technical) (99.8%) in paraffin oil; and (c) 5% w/w MTF-753 in 50% FCA in SPS (10 positive control females (Group C) received the same injections with 2,4-dinitrochlorobenzene (DNCB) in place of MTF-753). Additionally, on the first day of induction, 10 MTF-753 negative control animals (Group B) and 5 DNCB negative control animals (Group D) received 3 pairs of injections as above without MTF-753 or DNCB, *ie*: (a) 50% v/v FCA in SPS; (b) 100% paraffin oil; and (c) 50% FCA in SPS. Seven days after the intradermal injections, the topical induction phase was conducted. 0.4 g of MTF-753 mixed with white petrolatum (50% w/w mixture) was applied to the intradermal injection area of Group A animals using a 2 cm x 4 cm filter paper patch (covered with plastic wrap and secured in place with adhesive tape) (Group C animals were treated with DNCB in the same manner and Group B and D animals were treated with white petrolatum alone) (sites on group A and B animals were pretreated with 10% sodium laurel sulfate in white petrolatum). The patches were removed after a 48 hour exposure period. On test day 21, the right and left flank of each animal were clipped and shaved and a challenge filter paper patch (2 cm^2) treated with 0.2 g of MTF-753 mixed with white petrolatum (50% w/w) was applied (occluded) to the left flanks of each animal in Groups A and B (Groups C and D animals were treated with 0.2 g of DNCB mixed with white petrolatum). The right flanks of each animal in Groups A, B, C, and D were treated with white petrolatum alone. Patches were removed after the 24-hour exposure period. Scoring of the sites for a sensitization response was performed 24 and 48 hours after patch removal. All test article treated animals (Group A), test article negative control animals (Group B), and negative control animals for DNCB (Group D) had skin reaction scores of 0 (no reaction) at 24 and 48 hours after the challenge patch removal. Positive control (DNCB) treated animals (Group C) all had scores of 3 (intense redness and swelling) at 24 and 48 hours after patch removal. All animals in all groups gained bodyweight during the study. Record 256689 contains a discussion of the rationale for the dosing levels used for topical induction and challenge. The test article was not a dermal sensitizer using the Guinea pig maximization test. Acceptable. (Green, 2/28/11).

SUBCHRONIC STUDIES

Rat 4-Week Dermal Toxicity Study

**53104-0253 251136, "MTF-753: 4-Week Dermal Toxicity Study in the Rat", (C.J. Groom, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: LDA0079/073706, 15 September 2008). 10 Crl:CD® (SD) rats per sex per group were dermally

treated (clipped, unabraded, occluded) with MTF-753 (penthiopyrad technical) (98.6%) at 0 (untreated), 40, 200, and 1000 mg/kg/day for 4 weeks (6 hours per day, 7 days per week). There were no unscheduled deaths and no treatment-related clinical signs at any treatment level. There were no treatment-related dermal reactions at the application sites of any animals. No treatment-related effects were noted for bodyweight, bodyweight gain, food consumption, ophthalmology, hematology, urinalysis, necropsy results, organ weights, or histopathology. A statistically significant increase in total serum protein was recorded for high dose males (values were within the historical control range). Dermal NOEL (m/f) and Systemic NOEL (m/f) = 1,000 mg/kg/day. No adverse effect. Acceptable. (Green, 11/4/10).

Rat 90-Day Oral (Feeding) Toxicity Study

**53104-0257, 0258, 0261, 0262 251140, 251141, 251144, 251145, "MTF-753: Subchronic 90-Day Toxicity Study (Feeding) in the Rat", (E. Rosner, *et al*., RCC Ltd., Toxicology, Itingen and Füllinsdorf, Switzerland, RCC Study No. 781503, 17 January 2005). 10 Hanlbm:WIST (SPF) rats per sex per group received MTF-753 (penthiopyrad technical) (99.8%) in the diet at 0 (basal diet), 40, 100, 250, and 625 mg/kg/day for 90 days. Additionally, 10 per sex per group (recovery groups) received MTF-753 at 0 (basal diet) and 625 mg/kg/day for 90 days followed by a 29-day recovery period receiving basal diet. One high dose male was found dead on test day 91 (uncoordinated movements, labored respiration, ruffled fur, and poor condition were reported prior to death) and another was accidently killed during blood sampling (all other animals survived to scheduled necropsy). Treatment-related cageside observations were limited to increased incidences of soft feces in all high dose males and females between treatment weeks 4 and 7compared to controls. Treatment-related decreases in locomotor activity during 60 minutes were noted for high dose males and females compared to controls (decreases were also noted for males treated at 100 and/or 250 mg/kg/day after 45 and/or 60 minutes). Group mean bodyweight and bodyweight gain were significantly reduced for high dose males from treatment week 2 through the end of the treatment period compared to controls. Group mean hemoglobin, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (MCHC) were significantly reduced and activated partial thromboplastin time (APTT) significantly increased for both sexes at the high dose level at the end of the 13-week treatment period. Decreased MCHC and increased APTT were also noted at 100 and 250 mg/kg/day in males and at 250 mg/kg/day in females after 13 weeks. Significant increases in group mean total cholesterol, phospholipid values, gamma-glutamyl transferase activity, alkaline phosphatase activity, and triglycerides (females only) were noted for both sexes at the high dose level at the end of the 13-week treatment period compared to controls. Significant increases in total cholesterol in females at 250 mg/kg/day and in phospholipids at 100 mg/kg/day (males) and at 250 mg/kg/day (both sexes) were also recorded vs controls. After 13-weeks of treatment, group mean relative liver weights were significantly increased at 100, 250, and 625 mg/kg/day compared to controls. At the end of the recovery period, group mean relative liver, testes, and epididymides weights were significantly increased for high dose males vs controls. Treatment-related necropsy findings following the 13-week treatment period included an accentuated lobular liver pattern in 3/10 males at 250 mg/kg/day and in 5/10 males at the high dose level compared to controls (0/10). At the end of the recovery period, 1 high dose male was noted with an enlarged liver and another with a thickened liver. Treatment-related statistically significant histopathology findings included: increased minimal to moderate hepatocellular hypertrophy of the liver at 40 mg/kg/day (1 male and 3 females), 100 mg/kg/day (6 males and 5 females), 250 mg/kg/day (10 males and 8 females), and 625 mg/kg/day (9 males and 10 females). At the end of the recovery period, hepatocellular hypertrophy was still present in 10 high dose males and 6 females. Additionally, macrovesicular fatty change (1 female at 100 mg/kg/day, 4 females at 250 mg/kg/day, and 10 per sex at 625 mg/kg/day), hepatocellular degeneration (1 male at 100 mg/kg/day, 2 males at 250 mg/kg/day, and 10 males

and 7 females at 625 mg/kg/day), and Kupffer cell proliferation (3 males at 100 mg/kg/day, 2 males at 250 mg/kg/day, and 10 males and 6 females at 625 mg/kg/day) were noted in liver after 13 weeks. Macrovesicular fatty change (1 per sex), hepatocellular degeneration (1 male), and Kupffer cell proliferation (1 male) were noted for high dose animals at the end of the recovery period. No treatment-related effects were noted for food consumption, functional observational battery results, grip strength, ophthalmology, or urinalysis. NOEL < 40 mg/kg/day (liver weights, liver histology). No adverse effect. Record 251140 contains independent (AICOS Technologies AG) statistical analyses of results data for bodyweights, organ weights, hematology, serum chemistry, and urinalysis. Records 251144, 251145 contain a 28-day dose-range finding feeding study in rats used to set treatment levels for this study. Acceptable. (Green, 11/19/10).

Dog 28-Day Dietary Toxicity Study

53104-0259 251142, "MTF-753: 28-Day Dose Range-Finding Study in Dogs", (N. Nakashima, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 00-0050, 5 April 2001). One Beagle dog per sex per group received MTF-753 (penthiopyrad technical) (99.8%) in the diet at 0 (basal diet), 1000, 3000, 10000, and 32000 ppm for 4 weeks. Group average weekly MTF-753 intakes were 27.1, 79.6, 269, and 920 mg/kg/day for males and 29.1, 94.1, 316, and 982 mg/kg/day for females at 1000, 3000, 10000, and 32000 ppm, respectively. All animals survived to the end of the treatment period. At 32000 ppm, bodyweight gain was decreased in both sexes (0.4 kg) vs controls (0.8 kg (male) and 0.7 kg (female)) and increased alkaline phosphatase activity and total cholesterol were noted for both sexes at treatment week 2 and 4. Additionally, in the high dose male, slight reductions in hematocrit, hemoglobin concentration, and erythrocyte count at treatment week 2 and increases in glutamic oxaloacetic transaminase activity and glutamic pyruvic transaminase activity at week 4 were noted. Relative liver weight was increased for both sexes at 32000 ppm (relative thyroid weight was also increased for the female), for the female at 10000 ppm, and for the 3000 ppm male vs the controls. Diffuse hepatocellular hypertrophy was noted in the liver of the high dose male and female, the 10000 ppm female, and the 3000 ppm male compared to the controls. Mucosal edema of the lamina propria of the gallbladder was observed in both sexes at 3000 ppm and higher compared to controls (ordinary histology of the gallbladder was precluded for the high dose male because of hypoplastic change (atrophy)). Additionally, an increase in the severity of vacuolation of the tubular cells in the pars recta of the kidney was noted for the high dose male and female vs the control animals. No treatment-related changes were noted for clinical observations, food consumption, ophthalmology, urinalysis, or necropsy results. Supplemental data. (Green, 1/25/11). No worksheet. Based on these results, a dose level of 30,000 ppm was selected as the highest dose level for the 90-day oral toxicity study.

Dog 90-Day Oral (Dietary) Toxicity Study

**53104-0256, 0259 251139, 251142, "MTF-753: 90-Day Oral (Dietary) Toxicity Study in Dogs", (N. Nakashima, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 00-0119, 1 May 2001). 4 Beagle dogs per sex per group received MTF-753 (penthiopyrad technical) (99.8%) in the diet at 0 (basal diet), 300, 3000, and 30000 ppm for 91 days. Group average weekly MTF-753 intakes were 8.01, 76.7, and 811 mg/kg/day for males and 8.18, 80.9, and 864 mg/kg/day for females at 300, 3000, and 30000 ppm, respectively. Group mean bodyweights were reduced (-3.4% to -9.3%) during the treatment period for both sexes at the high dose level (statistical significance was reached only for males at week 12) vs controls. Group mean food consumption was slightly reduced (ns) at 30000 ppm for males during weeks 1 through 3, 5, and 7 and for females during weeks 3 and 4 vs controls. Group mean activated partial thromboplastin time was significantly decreased in both sexes at the high dose level and in mid dose females at week 7. Significant increases in group mean total

bilirubin and alkaline phosphatase activity were noted for both sexes at 30000 ppm at weeks 7 and 13 (not significant in females at week 13) vs controls. At the high dose level, group mean relative liver weights in both sexes and relative thyroid weights in females were significantly increased. Histopathology revealed a significantly increased incidence of diffuse hepatocellular hypertrophy in the liver of high dose males (4/4 vs 0/4) and females (4/4 vs 0/4) compared to controls. The incidence of cholecystitis (characterized by infiltration of foamy macrophages into the lamina propria mucosae and loss of long mucosal folds) was increased in high dose males (3/4 vs 0/4 (ns)) and females (4/4 vs 0/4 ($p \leq 0.05$)) vs controls. The incidence of cortical cell hypertrophy in the adrenal was significantly increased for high dose males (4/4 vs 0/4) relative to controls. Record 251142 is a 28-day dietary dose range-finding study in dogs used to set treatment levels. NOAEL = 3000 ppm (76.7 mg/kg/day (males) and 80.9 mg/kg/day (females)) based on reduced bodyweight, increased liver weight, and liver histology). No adverse effect. Acceptable. (Green, 1/26/11).

Mouse 4-Week Dietary Toxicity Study

53104-0260 251143, “MTF-753: 28-Day Dose Range-Finding Study in Mice”, (N. Nakashima, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 00-0020, 15 June 2001). Six (SPF) ICR (Crj:CD-1) mice per sex per group received MTF-753 (penthiopyrad technical) (99.8%) in the diet at 0 (basal diet), 30, 100, 300, and 1000 mg/kg/day for 29 days (males)/30 days (females). All animals survived to scheduled necropsy. Significant decreases in hemoglobin concentration and erythrocyte count were noted for high dose females vs controls. Group mean albumin levels were decreased in males and females at 1000 mg/kg/day (significant in males) and in males at 300 mg/kg/day (ns) vs controls. Group mean globulin values were increased at the high dose level in males and females (significant in females) relative to controls. The resulting albumin/globulin ratios were significantly decreased for high dose males and females and for 300 mg/kg/day males. Additionally, group mean triglyceride values were significantly increased in males at 100, 300, and 1000 mg/kg/day vs controls. Significantly increased group mean relative liver weights were noted for both sexes at 300 and 1000 mg/kg/day vs controls. Diffuse hepatocellular hypertrophy was noted in the livers of all high dose males (6/6) and females (6/6). No treatment-related effects were noted for clinical observations, bodyweights, food consumption, or gross pathology. No adverse effect was indicated. NOEL = 30 mg/kg/day for males (serum chemistry) and 100 mg/kg/day for females (serum chemistry, liver weights). Unacceptable, possibly upgradeable with complete histopathology. (Green, 2/3/11).

Mouse 90-Day Dietary Toxicity Study

**53104-0255, 0260 251138, 251143, “MTF-753: 90-Day Oral (Dietary) Toxicity Study in Mice for Dose Range Finding”, (N. Nakashima, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 00-0101, 21 October 2002). 10 ICR mice (Crj:CD-1) per sex per group received MTF-753 (penthiopyrad technical) (99.8%) in the diet at 0 (basal diet), 30, 100, 300, and 1000 mg/kg/day for 90 days. Significant reductions in group mean erythrocyte counts in both sexes and in hemoglobin concentrations in females were noted at the high dose level compared to controls. Significant increases in group mean blood urea nitrogen levels were noted for males at 300 and 1000 mg/kg/day (the increase was greater at 300 mg/kg/day) vs controls. Additionally, the group mean albumin/globulin ratio was significantly reduced for high dose males vs controls (albumin was decreased and globulin increased (ns) vs controls). Group mean relative liver weights were significantly increased for both sexes at 300 and 1000 mg/kg/day vs controls. Additionally, significant increases were noted for group mean relative thyroid weights in high dose males vs controls. Gross pathology was unremarkable. At 1000 mg/kg/day, significant increases in the incidence of diffuse hepatocellular hypertrophy were noted in males (5/10) and females (5/10) vs controls (0/10 both sexes) and of hypertrophy of the

follicular epithelial cells in the thyroid of males (8/10) and females (6/10) vs controls (0/10 both sexes). NOEL = 100 mg/kg/day (based on serum chemistry and liver weights). No adverse effect. Record 251143 is a 28-day dose range-finding study in mice. Acceptable. (Green, 2/3/11).

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

**53104-0253 251136, "MTF-753: 4-Week Dermal Toxicity Study in the Rat", (C.J. Groom, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: LDA0079/073706, 15 September 2008). 10 Crl:CD ® (SD) rats per sex per group were dermally treated (clipped, unabraded, occluded) with MTF-753 (penthiopyrad technical) (98.6%) at 0 (untreated), 40, 200, and 1000 mg/kg/day for 4 weeks (6 hours per day, 7 days per week). There were no unscheduled deaths and no treatment-related clinical signs at any treatment level. There were no treatment-related dermal reactions at the application sites of any animals. No treatment-related effects were noted for bodyweight, bodyweight gain, food consumption, ophthalmology, hematology, urinalysis, necropsy results, organ weights, or histopathology. A statistically significant increase in total serum protein was recorded for high dose males (values were within the historical control range). Dermal NOEL (m/f) and Systemic NOEL (m/f) = 1,000 mg/kg/day. No adverse effect. Acceptable. (Green, 11/4/10).

CHRONIC STUDIES

Chronic, rat

**53104-0263 251146, “MTF-753: 52-Week Oral Toxicity (Feeding) Study in the Rat”, (E. Rosner and K. Blumbach, RCC Ltd., Itingen, Switzerland, RCC Study No. 851873, 27 April 2006). 20 HanBrl:WIST (SPF) rats per sex per group received MTF-753 (penthiopyrad technical) (98.8%) in the diet at 0 (basal diet), 6.25, 25, 100, and 400 mg/kg/day for 52 weeks (an additional 10 interim sacrifice animals per sex per group were treated at the same levels for 26 weeks followed by necropsy). At 25 mg/kg/day, 1 interim group male died following blood sampling on the day of necropsy (26 weeks) and 1 main group male was sacrificed with paresis during week 46 (histology revealed vertebral osteosarcoma). Significant reductions in main group high dose male bodyweight gain during the first 15 weeks of treatment were noted compared to controls. Treatment-related group mean hematology after 26 weeks of treatment included: significantly increased partial thromboplastin time (PPT) and relative prothrombin time (PT) in both sexes at 400 mg/kg/day and significantly increased hemoglobin distribution width and methemoglobin concentration in 100 and 400 mg/kg/day females vs controls. Treatment-related group mean hematology findings after 52 weeks of treatment included: significantly reduced hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration, and higher hemoglobin distribution width; significantly reduced absolute and relative reticulocyte counts; significantly increased PTT; and significantly decreased PT in high dose males vs controls (high dose females showed significantly reduced MCV and MCH and significantly increased PT compared to controls). Treatment-related significant group mean serum chemistry effects after 26 weeks of treatment included: reduced bilirubin concentration in both sexes at 100 and 400 mg/kg/day; increased cholesterol and phospholipid concentration in both sexes at 400 mg/kg/day; reduced glutamate dehydrogenase activity (GLDH) in both sexes at 400 mg/kg/day; significant gamma-glutamyl transferase (GGT) activity in both sexes at 400 mg/kg/day; and increased alkaline phosphatase activity in 400 mg/kg/day males. Significant group mean serum chemistry changes after 52 weeks included: reduced bilirubin concentration in both sexes at 100 and 400 mg/kg/day; increased cholesterol and phospholipid concentration in both sexes at 400 mg/kg/day (and in females at 100 mg/kg/day); reduced GLDH activity in both sexes at 400 mg/kg/day (statistically significant in males only); significant GGT activity in both sexes at 400

mg/kg/day; increased alkaline phosphatase activity in males at 400 mg/kg/day; and reduced glucose levels in both sexes at the high dose compared to controls. Group mean relative liver weights were significantly increased for both sexes at 100 and 400 mg/kg/day after 26 weeks of treatment vs controls (relative kidney and adrenal gland weights were also significantly increased for males at 100 and 400 mg/kg/day). After 52 weeks of treatment, group mean relative liver weight was significantly increased for both sexes at 100 and 400 mg/kg/day and group mean relative kidney weight was increased for high dose males and relative adrenal gland weight significantly increased for high dose females relative to controls. After 52 weeks of treatment, significant histopathology changes vs controls included: increased incidence and/or severity of adrenal cortical lipid vacuolation in both sexes at 100 and 400 mg/kg/day; increased incidence and severity of diffuse hypertrophy of the adrenal zona glomerulosa in males at 100 mg/kg/day (12/20 vs 4/20) and in both sexes at 400 mg/kg/day (17/20 vs 4/20 for males and 16/20 vs 1/20 for females); increased incidence and severity of foci of basophilic (regenerating) tubules in kidneys of high dose males (14/20 vs 8/20); increased incidence and severity of hypertrophy of ovarian interstitial cells in high dose females (17/20 vs 5/20); and increased incidence and severity of diffuse hypertrophy of the thyroid follicular epithelium in both sexes at the high dose level (all animals of both sexes vs 9/20 control males and 1/20 females). There were no treatment-related clinical signs, masses or nodules, food consumption changes, ophthalmology, urinalysis, or necropsy results. NOEL = 25 mg/kg/day (based on relative liver weights and liver and adrenal gland histology). No neoplastic changes were indicated. Acceptable. (Green and Leung, 12/7/10)

Chronic, dog

**53104-0254 251137, “MTF-753: Chronic Oral (Dietary) Toxicity Study in Dogs”, (N. Nakashima, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 02-0105, 8 March 2006). 4 Beagle dogs per sex per group received MTF-753 (penthiopyrad technical) (98.8%), in the diet at 0 (basal diet), 310, 2150, and 15000 ppm for 52 weeks. Group average weekly MTF-753 intakes were 7.91, 54.4, and 461 mg/kg/day for males and 8.10, 56.6, and 445 mg/kg/day for females at 310, 2150, and 15000 ppm, respectively. All animals survived to scheduled termination. At 15000 ppm, just before feeding on treatment day 2, two males and one female showed clonic convulsions with salivation. Subsequently, one of the 2 males showed clonic convulsions with salivation (lasting 1 to 3 minutes) once per week during treatment weeks 37, 39-41, 43, 47-49, and 51. Group mean bodyweights were reduced for high dose males and females during treatment weeks 1 through 52 (reductions were statistically significant for males during weeks 7 through 28) compared to controls. At week 52, group mean bodyweights were 21.7% (males) and 16.7% (females) lower than controls. Group mean food consumption was slightly reduced for high dose males at treatment weeks 1 through 3 and for high dose females at weeks 1 through 13 and 40 (statistically different from control at week 1) compared to controls. At 15000 ppm, significant reductions in erythrocyte counts (at treatment weeks 13, 26, and 52), hemoglobin concentration (week 52), mean corpuscular hemoglobin concentration (weeks 13 and 52), and activated partial thromboplastin time (weeks 13, 26, and 52) were noted for males (activated partial thromboplastin time was also significantly decreased for females). Platelet counts were significantly increased for high dose males at weeks 13, 26, and 52 (basophil counts were also increased at week 13). At the high dose level, significant increases in group mean alkaline phosphatase activity (weeks 13, 26, and 52 for both sexes) and gamma-glutamyl transpeptidase activity (weeks 13, 26, and 52 for males and weeks 13 and 52 for females) were noted compared to controls. Group mean total cholesterol and triglycerides were significantly increased for high dose males and females at weeks 13, 26, and 52 vs controls. Group mean globulin was significantly increased for high dose males and females and for mid dose females at week 13 compared to controls. At necropsy, an increased incidence of ascites (ns) was noted for high dose males vs controls (2/4

vs 0/4). At 15000 ppm, significant increases in group mean relative liver weights (males) and adrenal gland weights (both sexes) were noted compared to controls. Group mean relative liver weights were also increased for high dose females (ns) vs controls. Histology revealed a significantly increased incidence of diffuse hepatocellular hypertrophy in the liver and cortical cell hypertrophy in the adrenal gland for both sexes at 15000 ppm vs controls (4/4 vs 0/4 in both organs and both sexes). In gallbladder, at the high dose level, mucosal epithelial hyperplasia was increased for males (3/4 (ns)) and females (4/4 ($p \leq 0.05$)) vs controls (0/4 both sexes) (cholecystitis was also increased in gallbladder (1/4 in each sex (ns)) at the high dose vs controls (0/4 in each sex). NOEL males = 2150 ppm (54.4 mg/kg/day) (reduced bodyweight, hematology, serum chemistry, liver and adrenal histology). NOEL females = 310 ppm (8.10 mg/kg/day) (blood chemistry). Adverse effects were not indicated. Acceptable. (Green and Leung, 2/2/11).

Oncogenicity, rat

****53104-0265, 0410 251148**, **254577**, "MTF-753: 104-Week Oncogenicity (Feeding) Study in the Rat", (E.Rosner and K. Blumbach, RCC Ltd., Itingen, Switzerland, RCC Study No. 847154, 7 June 2006). Fifty HanBrl:WIST rats per sex per group received MTF-753 (penthiopyrad technical) (98.8%) in the diet at 0 (basal diet), 9, 27, 83, and 250 mg/kg/day for 104 weeks. Group mean female bodyweights were significantly reduced at 83 mg/kg/day from treatment week 26 and at the high dose level for weeks 88 to 94 compared to controls (group mean bodyweight gain was also statistically reduced for females at 83 mg/kg/day for weeks 82 to 92 and at the high dose level for weeks 90 and 92 vs controls). Group mean relative liver weight was significantly increased for both sexes at 250 mg/kg/day vs controls. Treatment-related macroscopic findings were noted in the liver and thyroid of males and in the lung and adrenal glands of females. At 250 mg/kg/day, the incidence of an accentuated lobular pattern in the liver of males was significantly increased compared to controls (8/50 vs 1/50). Additionally, in high dose males, the incidence of thyroid gland nodules was increased vs controls (5/50 vs 2/50) (the difference was not statistically different, but there was a histology correlated change (follicular neoplasia)). In females, the incidence of foci in the lungs was significantly increased at 83 mg/kg/day (13/50) and 250 mg/kg/day (13/50) vs controls (5/50). Also, in high dose females, adrenal discoloration was significantly increased compared to controls (5/50 vs 0/50). Non-neoplastic histology included: significantly increased hepatocellular hypertrophy (mainly centrilobular) in liver for both sexes at 250 mg/kg/day (11/50 vs 1/50 for males and 7/50 vs 0/50 for females) and for females at 83 mg/kg/day (5/50) and 27 mg/kg/day (5/49) vs controls and significant increases in periportal fatty degeneration in the liver of males at 83 mg/kg/day (7/50) and 250 mg/kg/day (33/50) vs controls (0/50). Treatment-related neoplastic changes included a significantly increased incidence of follicular adenomas in the thyroid gland of high dose males sacrificed at scheduled termination vs controls (9/49 (18.4%) vs 3/50 (6%)) (the incidence of follicular carcinoma was also increased (ns) (3/49 (6.1%) vs 2/50 (4%))). RCC Ltd. historical control data incidences in males were 0% to 14.29% for follicular adenomas and 0% to 6% for carcinomas. Record 254577 contains additional historical control data for thyroid follicular adenomas from Harlan Laboratories, Ltd. Chronic NOEL = 27 mg/kg/day (reduced female bodyweight, periportal fatty degeneration in male liver). Carcinogenicity NOEL = 83 mg/kg/day (thyroid adenomas in males). Possible adverse effect: increased thyroid follicular adenomas in males. Acceptable. (Green and Leung, 2/15/11).

Oncogenicity, mouse

****53104-0266, 449 251168, 254578,** "MTF-753: Carcinogenicity Study in Mice", (A. Enomoto, The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID: IET 02-0007, 14 June 2006). Fifty-two ICR (Crj:CD-1) mice per sex per group received MTF-753 (penthiopyrad technical) (98.8%) in the diet at 0 (basal diet), 20, 60, 200, and 600 mg/kg/day for

78 weeks. Group mean bodyweights were significantly reduced for high dose males (from treatment week 16) and females (from week 13) vs controls. Food consumption across treated groups was generally comparable to controls. Significant increases were noted for group mean relative thyroid weights in both sexes at 600 mg/kg/day vs controls and for group mean relative liver weights in males at 200 and 600 mg/kg/day (group mean relative liver weights were slightly increased (ns) for high dose females vs controls). Necropsy results included significantly increased incidences of dark colored liver (5/34 vs 0/36) and spots in the liver (6/34 vs 0/36) for high dose males at terminal sacrifice (dark colored liver was also significantly increased when scheduled and unscheduled deaths were combined (6/52 vs 0/52)) vs controls. Additionally, at 200 mg/kg/day, a significantly increased incidence of masses in the liver was seen in terminal sacrifice males. Treatment-related neoplastic changes were noted in the liver. Significant increases in the incidence of hepatocellular adenomas were recorded for 200 mg/kg/day (11/31) and 600 mg/kg/day males (12/34) at terminal sacrifice vs controls (5/36) (combining the incidence for scheduled and unscheduled deaths, statistical significance was noted at 600 mg/kg/day only (15/52 vs 7/52)). The combined incidence of hepatocellular adenoma and carcinoma was statistically increased at 200 mg/kg/day (13/31) and 600 mg/kg/day (13/34) for terminal sacrifice males vs controls (6/36) and, after adding scheduled and unscheduled male deaths together, at 600 mg/kg/day (19/52 vs 9/52). Non-neoplastic changes were seen in the thyroid. The incidence of hypertrophy of follicular cells in the thyroid was significantly increased at 200 mg/kg/day (14/31 vs 1/36 for males and 11/40 vs 0/42 for females) and 600 mg/kg/day (25/34 for males and 32/42 for females) at terminal sacrifice and after combining scheduled and unscheduled deaths (14/52 vs 1/52 for males and 12/52 vs 0/52 for females at 200 mg/kg/day and 28/52 for males and 34/52 for females at 600 mg/kg/day). Additionally, a significantly increased incidence of altered colloid in the thyroid was seen in both sexes at 600 mg/kg/day and in 200 mg/kg/day males at terminal sacrifice (31/34 vs 3/36 for males and 38/42 vs 2/42 for females at 600 mg/kg/day and 11/31 for 200 mg/kg/day males) and for scheduled and unscheduled deaths combined (41/52 vs 3/52 for males and 44/52 vs 2/52 for females at the high dose level and 11/52 for 200 mg/kg/day males). Furthermore, brown pigment deposition in thyroid follicular cells was significantly increased at 600 mg/kg/day for both sexes (at scheduled sacrifice (19/34 vs 0/36 for males and 27/42 vs 0/42 for females) and after combining scheduled and unscheduled deaths (19/52 vs 0/52 for males and 28/52 vs 0/52 for females) vs controls. Record 254578 contains historical control data for hepatocellular adenoma and carcinoma incidence in ICR (Crj:CD-1) mice (both sexes). Chronic NOEL = 60 mg/kg/day (increased liver weights in males, thyroid follicular cell hypertrophy in both sexes). Carcinogenicity NOEL = 60 mg/kg/day (neoplastic liver changes in males). **Possible adverse effect:** increased incidence of hepatocellular adenomas and carcinomas in males. Acceptable. (Green and Leung, 2/8/11).

GENOTOXICITY

Gene mutation

**53104-0251 251134, “Bacterial Reversion Assay with MTF-753", (M. Nakajima, Biosafety Research Center, Foods, Drugs, and Pesticides (An-Pyo Center), Shizuoka, Japan; Experiment No. 4847 (077-014), 1 August 2000). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed (cultures were preincubated for 20 minutes in test tubes prior to plating) to MTF-753 (Penthiopyrad Technical) (99.8%), in the presence and absence of rat liver S9 mix, at 0 (DMSO), 2.34, 4.69, 9.38, 18.8, 37.5, 75.0, 150, 300, 600, or 1200 *u*g/plate for 48 hours (at 37° C). There was no increase in the number of revertants per plate compared to the solvent controls. Growth inhibition of the background lawn was noted for *S. typhimurium* strains TA100 (-S9) and TA1535 (-S9) at 37.5 *u*g/plate; for strains TA100 (+/- S9), TA1535 (-S9), and TA1537

(+/- S9) at 75.0 ug/plate and higher; for TA98 (-S9) at 300 ug/plate and higher; and for TA98 (+S9) and *E. Coli* strain WP2 *uvr*A (+/- S9) at 600 ug/plate and higher. Test article precipitation was noted at 600 ug/plate and higher. No adverse effect. Positive controls were functional. Acceptable. (Green and Leung, 7/13/10).

**53104-0248 251131, "Gene Mutation Assay with MTF-753 in Mouse Lymphoma Cells (MLA)", (S. Masumori, Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shizuoka, Japan, Experiment No. 4849 (077-016), 1 August 2000). Duplicate cultures of L5178Y $TK^{+/-}$ mouse lymphoma cells were exposed to MTF-753 (penthiopyrad technical) (99.8%), in the presence and absence of rat liver S9 mix for 3 hours, at 0 (DMSO), 4.32, 6.18, 8.82, 12.6, 18.0, 25.7, 36.8, 52.5, or 75.0 *u*g/ml. Substantial cytotoxicity (less than 50% relative survival vs solvent controls) was demonstrated at 36.8 *u*g/ml and higher with and without activation. No treatment-related increase in the mutant frequency was indicated. No adverse effect. Positive controls were functional. Acceptable. (Green and Leung, 8/23/10)

Chromosome damage

**53104-0249 251132, "Micronucleus Study with MTF-753 in Mice", (M. Nakajima, Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo-Center), Shizuoka, Japan, Experiment No. 4850 (077-017), 1 August 2000). 6 BDF_1 (C57BL/6 x DBA/2) [SPF] male mice per group received MTF-753 (penthiopyrad technical) (99.8%) by oral gavage, once daily on 2 consecutive days with a 24-hour interval, at 0 (0.5% carboxymethylcellulose), 500, 1000, and 2000 mg/kg/day with bone marrow sampling 24 hours after the last dose. 2000 polychromatic erythrocytes (PCEs) per animal were analyzed for 5 animals per group. All males survived to sacrifice. The mean percentage of PCEs to normochromatic erythrocytes was significantly decreased at 2000 mg/kg/day. There was no increase in micronucleated polychromatic (immature) erythrocytes. Positive controls were functional. Acceptable. (Green and Leung, 9/15/10).

****53104-0247 251130**, "Cytogenetic Assay with MTF-753 in Chinese Hamster Lung (CHL/IU) Cells", (M. Nakajima, Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shizuoka, Japan, Experiment No. 4848 (077-015), 1 August 2000). Duplicate cultures of Chinese hamster lung (CHL) cells were exposed to MTF-753 (penthiopyrad technical) (99.8%), in the presence and absence of rat liver S9 mix for 6 hours, and, in the absence of activation for 24 hours, at 0 (DMSO), 52.4, 65.5, 81.9, 102, 128, 160, 200, or 250 *u*g/ml. Cells were harvested 24 hours after the start of treatment. 2 hours prior to harvest, colcemid solution (0.2 *u*g/ml) was added to each culture to arrest dividing cells in metaphase. 100 metaphases per plate were evaluated microscopically. The cell survival rate relative to the solvent control was below 50% at 102 *u*g/ml (5.8% and 7.5%) (-S9 mix) and at 160 *u*g/ml (41.0% and 39.3%) (+S9 mix) after the 6-hour treatment period, and at 52.4 *u*g/ml (49.3% and 49.8%) (-S9 mix) after the 24 hour treatment. Structural chromosome aberrations were not increased after treatment for 6 and 24 hours without activation. After the 6-hour treatment period with S9 mix at 160 *u*g/ml (the highest evaluated dose due to toxicity at the 2 higher concentrations (200 and 250 *u*g/ml)), an increase in structural chromosome aberrations was noted. **Possible adverse effect:** increased incidence of chromosomal aberrations in the presence of activation. Positive controls were functional. Acceptable. (Green and Leung, 9/28/10).

DNA damage or miscellaneous effects

**53104-0250 251133, "*In Vivo/In Vitro* Unscheduled DNA Synthesis (UDS) Assay with MTF-753 in Rat Hepatocytes", (S. Masumori, Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo-Center), Shizuoka, Japan, Experiment No. 4851 (077-018), 1 August 2000). 4 Crj:CD(SD)IGS [SPF] male rats per group received a single oral gavage dose of MTF-753

(penthiopyrad technical) (99.8%) at 0 (0.5% carboxymethylcellulose), 1000, and 2000 mg/kg. Hepatocytes were sampled 2 and 16 hours after treatment. No mortality/toxicity was recorded. Viability of the hepatocytes was determined using trypan blue (the mean ranged from 67% to 80%). After attachment, cells were exposed to ^{3}H thymidine for 4 hours followed by 18 hours of incubation with unlabelled thymidine. 3 animals per group were evaluated (3 autoradiographs per animal were prepared with 50 cells/autoradiograph and 150 cells per treated animal analyzed). There was no increase in net nuclear grain counts and mean cell viability was comparable to the vehicle control group. Positive controls were functional. Acceptable. (Green and Leung, 9/22/10).

**53104-0252 251135, "DNA Repair Assay (Rec-Assay) with MTF-753 in *Bacillus Subtilis* Spores", (M. Nakajima, Biosafety Research Center, Foods, Drugs, and Pesticides (An-Pyo Center), Shizuoka, Japan, Experiment No. 4846 (077-013), 1 August 2000). *Bacillus subtilis* strains H 17 Rec+ and M 45 Rec- were exposed to MTF-753 (penthiopyrad technical) (99.8%) in triplicate, in the absence of rat liver S9 mix, at 0 (DMSO), 177, 354, 708, 1416, 2831, 5663, 11325, and 22650 *u*g/disk and, in the presence of activation at 0 (DMSO), 88.5, 177, 354, 708, 1416, 2831, 5663, and 11325 *u*g/disk for 24 hours. Transparent or white crystalline precipitation was noted on the disks at 177 *u*g/disk and higher (+/- S9 Mix) at the end of the exposure period. No increase in DNA damage was indicated (growth inhibition zones were comparable in both the repair deficient (M45) and repair proficient strain (H17) with and without activation). Negative and positive controls were functional. Acceptable. (Green and Leung, 10/11/10).

REPRODUCTIVE TOXICITY, RAT

**53104-0270, 0271 251172, 251173, "A Reproduction Toxicity Study in Rats with MTF-753", (Shoji Teramoto, The Institute of Environmental Toxicology, Mitsukaido-shi, Ibaraki, Japan, Laboratory Project ID: IET 03-0085, 14 October 2005). 24 F0 and F1 Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats per sex per group received MTF-753 (penthiopyrad technical) (98.8%) in the diet at 0 (basal diet), 200, 1000, and 5000 ppm through 2 generations with 1 litter per generation. Group mean MTF-753 intakes for parental animals over the entire study period were, for F0 animals: 11.0 (males) and 18.1 mg/kg/day (females) at 200 ppm; 54.0 (males) and 90.5 mg/kg/day (females) at 1000 ppm; and 278 (males) and 439 mg/kg/day (females) at 5000 ppm. Values for F1 animals were: 12.8 (males) and 19.0 mg/kg/day (females) at 200 ppm; 64.2 (males) and 95.6 mg/kg/day (females) at 1000 ppm; and 340 (males) and 480 mg/kg/day (females) at 5000 ppm. Two high dose F0 females were found dead (one at treatment week 2 (necropsy revealed calculus in the urinary bladder and nephritis and cystitis), the other on lactation day 11 (histology revealed massive necrosis of the hepatocytes)).

Group mean bodyweight gain at the high dose level was significantly reduced for F0 males at treatment weeks 0 to 2 and for F0 females at weeks 0 to 6 vs controls. Group mean bodyweight gain was significantly increased for F0 females at 5000 ppm during lactation days 0 to 21.

At F0 necropsy, the incidence of dark-colored liver in high dose females was significantly increased vs control (15/24 vs 0/24). Additionally, a significantly increased incidence of thyroid enlargement was noted for high dose F0 males vs controls (8/24 vs 2/24) (the incidence for F0 females was also increased (ns) (6/24 vs 2/24)). Group mean relative liver, adrenal gland, and thyroid gland weights were significantly increased for F0 males and females at 5000 ppm vs controls (relative kidney weights were also significantly increased for males). Additionally, group mean relative liver weights were significantly increased for F0 females at 1000 ppm compared to controls.

F0 histopathology revealed significantly increased incidences of hypertrophy of the centrilobular hepatocytes in liver of males (9/24 vs 0/24) and females (11/24 vs 0/24) at 5000 ppm, hypertrophy of adrenal cortical cells in high dose females (20/24 vs 2/24) , and thyroid follicular cell hypertrophy in both sexes (11/24 vs 0/24) at the high dose level vs controls.

21, 24, 21, and 22 live F1a litters were produced with lactation day 0 group mean live litter sizes of 10.8, 11.2, 11.6, and 11.8 at 0, 200, 1000, and 5000 ppm, respectively. Group mean F1a pup weights were significantly reduced at the high dose level for males at lactation days 4 and 7 and for both sexes at days 14 and 21 vs controls. Group mean relative F1a spleen weights were significantly reduced for both sexes at 5000 ppm compared to controls. Histology results for thymus and spleen of high dose F1a pups (both sexes) were unremarkable.

All F1 animals survived to termination. Group mean bodyweight gain for F1 males was significantly decreased at 1000 ppm for treatment weeks 0 to 10 and at 5000 ppm for weeks 0 to 18 compared to controls.

At necropsy of F1 animals, an increased incidence of thyroid enlargement (ns) was noted for high dose males (7/24 vs 3/24) and females (5/24 vs 1/24) vs controls. Also, at 5000 ppm, the incidence of dark-colored liver was significantly increased for F1 females vs controls (16/24 vs 0/24). F1 group mean relative brain, pituitary gland, testes, epididymides, liver, and adrenal gland weights were significantly increased for males at 5000 ppm vs controls (relative brain and pituitary weights were also significantly increased in males at 1000 ppm). F1 females had significant increases in relative liver, adrenal gland, and thyroid gland weights at the high dose level and in liver and adrenals at 1000 ppm vs controls.

F1 histology results indicated significant increases in the incidence of hypertrophy of the centrilobular hepatocytes in liver of males (7/24 vs 0/24) and females (14/24 vs 0/24) at 5000 ppm, hypertrophy of adrenal cortical cells in mid dose females (10/24 vs 3/24) and high dose females (23/24 vs 3/24) , and thyroid follicular cell hypertrophy in males (8/24 vs 0/24) and females (11/24 vs 0/24) at the high dose level vs controls.

23, 23, 22, and 23 live F2a litters were produced with lactation day 0 group mean live litter sizes of 11.6, 11.1, 11.3, and 12.1 at 0, 200, 1000, and 5000 ppm, respectively. Group mean F2a pup weights were significantly reduced at the high dose level for both sexes at lactation days 14 and 21 vs controls. Group mean relative F2a thymus (males and females) and spleen weights (males) were significantly reduced at the high dose level vs controls. Group mean F2a relative brain weights were significantly increased for high dose males and females and for mid dose females vs controls. Thymus and spleen histology results for F2a pups (both sexes) were unremarkable.

Parental NOEL = 200 ppm (11.0 to 12.8 mg/kg/day (males) and 18.1 to 19.0 mg/kg/day (females)) (reduced bodyweight gain, increased relative liver and adrenal weights). Pup NOEL = 1000 ppm (54.0 to 64.2 mg/kg/day (males) and 90.5 to 95.6 mg/kg/day (females)) (reduced pup weight gain). Reproductive NOEL = 5000 ppm (278 to 340 mg/kg/day (males) and 439 to 480 mg/kg/day). No adverse reproductive effects. Acceptable. (Green and Leung, 1/24/11).

53104-0270 251172, “A Reproduction Toxicity Study in Rats with MTF-753, Preliminary study”, (Shoji Teramoto, The Institute of Environmental Toxicology, Mitsukaido-shi, Ibaraki, Japan, Laboratory Project ID: IET 03-0085, 24 December 2004). 8 Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats per sex per group received MTF-753 (penthiopyrad technical)

(98.8%) in the diet at 0 (basal diet), 680, 1360, 3400, and 8500 ppm from 3 weeks prior to mating through weaning of the F1a offspring. F0 animals (both sexes) and F1a pups were necropsied after weaning of the F1a offspring. Group mean F0 achieved dosages during the treatment period were 37.9, 73.2, 187, and 477 mg/kg/day for males and 75.0, 139.6, 358, and 829 mg/kg/day for females at 680, 1360, 3400, and 8500 ppm, respectively. All F0 animals survived to scheduled termination with no treatment-related clinical signs. Group mean F0 male bodyweight gain at the high dose level and group mean F0 female food consumption at 3400 and 8500 ppm were significantly reduced during treatment week 1 (premating) compared to controls (F0 female bodyweight gain was also reduced at the high dose level (ns)). At F0 necropsy, group mean relative liver weights were significantly increased for both sexes at 8500 ppm and for males at 3400 ppm vs controls. Additionally, at the high dose level, significant increases were noted for F0 group mean relative thyroid weights in males and decreases for group mean relative spleen weights in females. No signs of reproductive toxicity were indicated for F0 animals at any treatment level. Group mean F1a pup bodyweight gain was significantly decreased for both sexes during lactation days 4 through 21. At the high dose level, significant increases in F1a group mean relative brain weights were noted in both sexes and group mean relative spleen weights were significantly reduced in F1a males vs controls. F0 Parental NOEL = 1360 ppm (73.2 mg/kg/day for males and 139.6 mg/kg/day for females) (reduced food consumption, increased liver weights). Pup NOEL = (187 mg/kg/day for males and 358 mg/kg/day for females) (reduced bodyweight gain). Reproductive NOEL = No effect at the HDT. Supplemental data. (Green, 1/10/11). No worksheet.

DEVELOPMENTAL TOXICITY

Rat

**53104-0272, 0273, 0274 251174, 251175, 251176, “MTF-753: Embryo-Fetal Toxicity Study by Gavage Administration to Han Wistar Rats”, (R. Patten, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: MTU353/053059, 1 March 2006). 22 mated female HsdBrl Han:Wist (Han Wistar) rats per group received MTF-753 (penthiopyrad technical) (98.6%) by oral gavage at 0 (aqueous carboxymethylcellulose, 0.5% w/v/Tween 80, 0.1% w/v), 62.5, 250, and 1000 mg/kg/day on gestation days 6 through 19. All females survived to scheduled necropsy and no treatment-related clinical signs were noted. At 1000 mg/kg/day, significant reductions were noted for group mean maternal bodyweight gain (through gestation day 9) and group mean maternal food consumption (for gestation days 6 to 9) compared to controls (values were comparable to controls, thereafter). Maternal necropsy results were unremarkable. One high dose female was not pregnant. Group mean live litter size was slightly smaller than controls at the high dose level (ns) (litter sizes were 11.6, 11.5, 10.8, and 10.2 at 0, 62.5, 250, and 1000 mg/kg/day, respectively). . Early resorptions and post-implantation loss were significantly increased at the high dose level compared to controls (2 females with 3 resorptions, 1 dam with 4 resorptions (3 early and 1 late), and a third female with 8 resorptions at 1000 mg/kg/day vs 1 control litter with 3 resorptions). No treatment-related fetal skeletal or visceral changes were indicated. Record 251174 is a preliminary embryo-fetal toxicity study used to set dosing levels. Record 251176 is a validation of the analytical method used to verify homogeneity and stability of dosing solutions. Maternal NOEL = 250 mg/kg/day (reduced bodyweight gain and food consumption). Developmental NOEL = 1000 mg/kg/day. Teratogenicity was not indicated. Acceptable. (Green and Leung, 1/6/2011).

53104-0272 251174, MTF-753: Preliminary Embryo-Fetal Toxicity Study by Gavage (Once Daily) Administration to Han Wistar Rats”, (R. Patten, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: MTU354/050107, 15 November 2005). Six mated female HsdBrl Han:Wist (Han Wistar) rats per group received MTF-753

(penthiopyrad technical) (98.6%) by oral gavage at 0 (aqueous carboxymethylcellulose, 0.5% w/v/Tween 80, 0.1% w/v), 250, 500, and 1000 mg/kg/day on gestation days 6 through 19. All dams survived to scheduled necropsy. No treatment-related effects were noted for maternal clinical signs, bodyweight and bodyweight gain, or food consumption during the treatment period. Necropsy results were unremarkable. Litter size, numbers of *corpora lutea*, implantations, numbers of resorptions and live young, sex ratios, and pre and post-implantation loss in treated groups were all comparable to control results. Teratogenicity was not indicated. Supplemental data. (Green, 1/5/2011). (No worksheet).

Rabbit

**53104-0274, 0275, 0276 251176, 251177, 251178, "MTF-753: Embryo-Fetal Toxicity Study in the Rabbit by Gavage Administration", (R. Patten, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: MTU351/053490, 1 March 2006). 24 mated female New Zealand White rabbits per group received MTF-753 (penthiopyrad technical) (98.6%) by oral gavage at 0 (aqueous carboxymethylcellulose, 0.5% w/v/Tween 80, 0.1% w/v), 25, 75, and 225 mg/kg/day on gestation days 6 through 28. One high dose female aborted on gestation day 26 and was sacrificed (a marked reduction in food consumption was noted for this animal from day 14). One, 2 and two females from the control, 75, and 225 mg/kg/day groups, respectively, were not pregnant on gestation day 29. No treatment-related effects were noted at any dosing level for maternal clinical signs, bodyweight gain, or food consumption, during the treatment period. Maternal necropsy results were unremarkable. At 225 mg/kg/day, group mean fetal weight was reduced 7.8% vs controls (mainly as a result of significantly reduced group mean female fetal weights (12.1% lower than controls; $p<0.05$) (group mean male fetal weights were 3.6% lower than controls (ns)). Although the group mean combined (males and females) fetal weight was not statistically significant, both the group mean combined value and the group mean female value were outside the historical control range. Record 251176 is a validation of the analytical method used to verify homogeneity and stability of dosing solutions. Record 251177 is a preliminary embryo-fetal toxicity study in the rabbit used to establish dosing levels. Maternal NOEL = 75 mg/kg/day (abortion). Fetal NOEL = 75 mg/kg/day (reduced fetal weight). Developmental NOEL = 225 mg/kg/day. No teratogenicity. Acceptable. (Green and Leung, 1/4/2011).

53104-0275 251177, "MTF-753: Preliminary Embryo-Fetal Toxicity Study in the Rabbit by Gavage Administration", (R. Patten, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: MTU352/050139, 1 March 2006). 6 mated female New Zealand White rabbits per group received MTF-753 (penthiopyrad technical) (98.6%) by oral gavage at 0 (aqueous carboxymethylcellulose, 0.5% w/v/Tween 80, 0.1% w/v), 250, 500, and 1000 mg/kg/day on gestation days 6 through 28. At 1000 mg/kg/day, 2 females were sacrificed (one on gestation day 21, the other on day 27) with evidence of abortion. At 500 mg/kg/day, 1 female was sacrificed for humane reasons on gestation day 21 (terminal signs included bodyweight loss, inappetance, low food and water consumption, abnormal feces, and abnormal gait), another female was found dead on day 27 with evidence of abortion, and a third female was sacrificed on day 28 with evidence of abortion. Treatment-related group mean increases in low water consumption, low food consumption, and abnormal feces were noted at 250 mg/kg/day and higher vs controls, and 3/6 females at 500 and 1000 mg/kg/day were observed with a thin build. Group mean bodyweight gain was reduced at 250 (-66%), 500 (-56%), and 1000 mg/kg/day (-98%) for gestation days 6 through 29 compared to controls. Group mean food consumption was reduced at 250 (-19%), 500 (-21%), and 1000 mg/kg/day (-28%) vs controls for gestation days 6 through 28. At scheduled termination, group mean pre-implantation loss increased at 250 (13.8%), 500 (29.3%), and 1000 mg/kg/day (33.0%) vs controls (1.8%) (there was no effect on post-implantation loss). Resultant total live litter sizes

were 11.5, 9.3, 7.3, and 7.0 at 0, 250, 500, and 1000 mg/kg/day, respectively. Group mean fetal weights were reduced at 1000 mg/kg/day (-15%) vs controls. Supplemental data. (Green, 12/29/10). (No worksheet).

NEUROTOXICITY

Acute neurotoxicity, rat

**53104-0277, 0278 251179, 251180, "MTF-753, Acute Neurotoxicity Study by a Single Oral Administration to the Rat", (M.J. Chapman, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, HLS Project ID LDA/0077/072863, 18 December 2008). Ten Crl:CD® (SD)IGS BR rats per sex per group received a single oral gavage dose of MTF-753 (penthiopyrad technical) (98.6%) at 0 (aqueous 0.5% w/v carboxymethylcellulose and 0.1% w/v Tween 80), 125, 500, and 2000 mg/kg followed by a 14 day observation period. All animals survived to terminal sacrifice. Group mean bodyweight gain between study days 1 and 8 was slightly reduced (ns) at 2000 mg/kg (-9% for males and -10% for females) vs controls. No treatment-related changes were noted for clinical signs (routine observations) or food consumption. Functional observation battery (FOB) results were as follows (all effects were noted at the time of peak effect after dosing (4-hours post-dosing) on study day 1). No treatment-related findings were recorded for home cage observations at any dose level. In-the-hand observations noted included: piloerection in 6 males and 5 females at 2000 mg/kg vs controls; reduced reactivity to handling (severity grade) in 500 and 2000 mg/kg females, and, to a lesser extent, in high dose males compared to controls and to pre-treatment values; reduced body tone in 7 males and 6 females at 2000 mg/kg and in one 500 mg/kg female vs controls. Arena observations included: an increased incidence of abnormal gait in high dose males and females and in mid-dose females; hunched posture in 5 per sex at 2000 mg/kg and in 2 males and 1 female at 500 mg/kg; 7 high dose females had slight, whole body tremors; 5 high dose females showed chewing mouth movements; and 3 high dose males had slow breathing. Manipulation observations included: significant reductions in group mean body temperature in both sexes at 500 and 2000 mg/kg and in 125 mg/kg females (the effect was most marked in females); and increases in group mean landing foot splay values in both sexes at 500 and 2000 mg/kg (statistically significant in males only). Motor activity observations included: group mean high beam scores (rearing activity) and, to a lesser extent, low beam scores (cage floor activity) that were significantly reduced at 500, and 2000 mg/kg (the total score and some six minute interval scores (particularly in the first half of the 1-hour test period) were significantly reduced at 500 and 2000 mg/kg and at 125 mg/kg for females (values for low dose females, although statistically significant, were generally within the historical control range)). All day 8 and 15 FOB results were comparable to controls. No treatment-related changes were noted for brain weights, brain measurements, gross pathology results, or histopathology results at any dose level. Neurotoxicity was not indicated. Record 251179 in an acute dose ranging/time to peak effect study in rats used to set dosing levels/observation times. Systemic NOEL = 125 mg/kg (transient FOB alterations). Neurotoxicity NOEL = 2000 mg/kg. Acceptable. (Green, 3/24/11).

53104-0277 251179, MTF-753: Dose Range and Time to Peak Effect in Rats by Acute Oral Administration, (M.J. Chapman, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, HLS Project ID LDA/0076/070069, 22 December 2008). Three Crl:CD (SD)IGS BR rats per sex per group received a single oral gavage dose of MTF-753 (penthiopyrad technical) (98.6%) at 0 (aqueous 0.5% w/v carboxymethylcellulose and 0.1% w/v Tween 80), 25, 125, 500, and 2000 mg/kg followed by an 8 day observation period. Functional observational battery (FOB) evaluations were performed 1, 2, 4, and 6/8 hours post-dosing. Treatment-related FOB results included hunched posture, abnormal gait, reduced arousal, extended bouts of grooming, and chewing mouth movements at 2000 mg/kg. Also at 500 and

2000 mg/kg, body temperature was reduced at all observation times (the greatest reduction occurred at 4 hours with values in 2 per sex at 500 mg/kg and all values at 2000 mg/kg < 37o C). At 125 mg/kg, body temperature was minimally reduced for all males at 4 hours post-dosing and 1 female had a temperature of < 37o C. No treatment related changes were recorded for bodyweight gains and food consumption at any dose level and no treatment related FOB results were recorded at 25 mg/kg. NOEL = 25 mg/kg (body temperature reduction). Time to peak-effect after a single dose is considered to be 4 hours. Supplemental information. (Green, 3/23/11). No worksheet.

90-day neurotoxicity, rat

**53104-0258, 0279 251141, 251181, "MTF-753, 13-Week Dietary Neurotoxicity Study in Rats", (C.J. Groom, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: LDA0078/073100, 2 October 2008). 10 Crl:CD(SD) rats per sex per group received MTF-753 (penthiopyrad technical) (98.6%) in the diet at 0 (basal diet), 10, 40, 160, and 640 mg/kg/day for 13 weeks. Overall group mean achieved doses were 11.0, 43.8, 177.0, and 711.8 mg/kg/day for males and 10.7, 42.5, 170.4, and 686.2 mg/kg/day for females at 10, 40, 160, and 640 mg/kg/day, respectively. Group mean bodyweight gains were decreased for females at 160 mg/kg/day (13%) and for both sexes at the high dose level (17% for males and 11% for females) compared to controls (statistically significant for high dose males only).
No treatment-related effects were noted for mortality, clinical signs, food consumption, neurobehavioral observations (FOB and motor activity), brain weights and brain measurements, necropsy results, or histology results. Systemic NOAEL = 40 mg/kg/day for females and 160 mg/kg/day for males (based on reduced bodyweight gain). Neurotoxicity NOEL = 640 mg/kg/day. No adverse effect. Acceptable. (Green, 12/14/10).

Developmental neurotoxicity, rat

**53104-0280, 0281, 0452, 0453, 0454, 0455, 0456, 0457 251182, 251183, 255006, 255007, 255008, 255033, 255034, 255035, "MTF-753: Developmental Neurotoxicity Study in the CD Rat by Oral (Gavage) Administration", (D.R. Stannard, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Project ID: MTU0419, 14 July 2009). 22 mated female Crl:CD® (SD) IGS BR rats per group received MTF-753 (penthiopyrad technical) (98.6%) by oral gavage at 0 (aqueous carboxymethylcellulose 0.5% w/v/Tween 80 0.1% w/v), 100, 250, and 500 mg/kg/day from gestation day 6 through lactation day 6, inclusive. F1a pups were dosed at the same levels from lactation days 7 through 21. One F0 control female and 2 F0 females at 100 mg/kg/day were sacrificed during parturition due to dystocia. No treatment-related effects were noted for F0 female clinical signs, bodyweight and bodyweight gain, food consumption, functional observational battery results, gestation length, litter size, macropathology, or brain weights.

Functional observational battery testing (FOB) was performed on F1a pups on post partum days 4, 11, 21, 35, 45, and 60. F1a motor activity was measured (using low and high beam infra-red light automated monitoring over 10 successive 6-minute intervals) on post partum days 13, 17, 22, and 59. F1a pre-pulse inhibition of startle response was tested (automated) on days 23/24 and 61/62 of age. Pup learning and memory were both assessed on days 23/24 and 61/62 of age using the Morris swimming maze (a different set of animals was evaluated at each time point). Sacrifice and brain examinations (measurements/morphometry) were performed on post partum days 21 and 66. F1a day 1 pup weights were decreased at 250 and 500 mg/kg/day for both sexes vs controls (with statistical significance for females). Additionally, group mean F1a bodyweight gain for male and female pups at 500 mg/kg/day and for males at 250 mg/kg/day was significantly lower than controls during lactation days 1 through 4. During the F1a treatment period (lactation days 7 through 21), yellow/brown/red staining of the perianal region

(with a low incidence of associated skin reddening/encrustations) was noted in a majority of male and female pups at 500 mg/kg/day compared to controls. Additionally, group mean pup bodyweight gain was significantly reduced for lactation days 7 through 21 for both sexes at 250 and 500 mg/kg/day vs controls. Seven males and 3 females at the high dose level had yellow and/or brown perianal staining on day 21 compared to controls during the FOB in the hand observations (results were comparable to controls at the subsequent observation timepoints). Treatment-related FOB arena observations included increased whole body tremors on day 21 of age for 5/22 pups per sex at the high dose level vs controls (males: 0/21, females: 1/21). Motor activity high and low beam scores (rearing and cage floor activity, respectively) were increased for both sexes at 500 mg/kg/day (with increased frequency of statistically significant low beam scores) and for mid-dose males on lactation day 17 during the latter part of the 1 hour session vs controls (both high and low beam scores were generally above the historical control ranges as well). No treatment-related effects on F1a pup sexual maturation, auditory startle response, learning and memory, brain weights and morphometry, or macropathology and histopathology at all sample times (perfused and non-perfused). Record 251182 is a preliminary developmental neurotoxicity study in rats used to establish dosing levels. Records 255006, 255007, 255008, 255033, 255034, and 255035 contain positive control validation studies for neurotoxicity testing procedures. Maternal NOEL = 500 mg/kg/day (no effect at the HDT). Systemic offspring NOEL = 100 mg/kg/day (perianal staining, reduced bodyweight gain). Offspring Functional Neurotoxicity NOEL = 100 mg/kg/day (increased motor activity, whole body tremors at day 21 of age). Offspring Neurohistomorpholigical NOEL = 500 mg/kg/day (no effect at the HDT). No adverse effect. Acceptable. (Green, 12/28/10).

Delayed neurotoxicity, hen

Study not submitted nor required.

IMMUNOTOXICITY

**53104-0308 251210, “MTF-753: 4-Week Dietary Immunotoxicity Study in the Rat”, (H.C. Kilpatrick, Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, England, Study No. LDA0080/073719, 3 February 2009). 10 male Crl:CD (SD) rats per group received MTF-753 (penthiopyrad technical) (98.6%) in the diet at 0 (basal diet), 45, 175, and 700 mg/kg/day for 4 weeks. A splenic plaque forming cell (PFC) assay was used to determine the immune system response. All treated and negative control animals received a single antigenic challenge with sheep red blood cells (SRBC) in 0.9% saline, administered by intravenous injection 4 days prior to termination. Positive control animals received a single intraperitoneal injection of cyclophosphamide at 50 mg/kg in 0.9% saline, 2 days prior to termination. Group mean achieved doses during the 4 week treatment period were 46, 178, and 710 mg/kg/day at 45, 175, and 700 mg/kg/day, respectively. All animals survived to the end of the study and no treatment -related clinical signs were noted at any level (including positive control animals). Group mean bodyweight gain was significantly reduced for high dose animals for study days 1 through 29 compared to controls (bodyweight loss was noted during the first 4 days of treatment and reduced bodyweight gain was recorded between days 4 and 8, and bodyweight gain was slightly lower than controls during the remainder of treatment). Slightly reduced group mean bodyweight gain was also recorded through day 8 at 45 and 175 mg/kg/day vs controls (ns), but overall (days 1 through 29) bodyweight gain was comparable to controls. Group mean food consumption was generally comparable to controls during the treatment period (except, an 11.9% reduction was noted during the first week at 700 mg/kg/day vs controls). Group mean water consumption values at the high dose level were consistently higher than controls (17.6%) throughout the treatment period. No treatment-related changes were noted for total and differential leukocytes and bone marrow composition. At 700 mg/kg/day, statistically significant increases in group mean relative liver weights and decreases in group mean relative spleen

weights were recorded compared to controls. Histology revealed a dose-related increase in cortical tingible body macrophages in the thymus at 175 and 700 mg/kg/day. The immunotoxicology assay indicated a decrease in the total number of spleen cells at 700 mg/kg/day vs controls, which correlated with the reduction in spleen weight in these animals. There was no treatment-related decrease in the number of antibody secreting cells in the Plaque-Forming Cell (PFC) assay measured as total PFCs/spleen and PFCs/10^6 cells (values for PFCs/spleen were decreased 20% vs controls while PFCs/10^6 were increased 10% vs controls ($p>0.05$ in both cases)) (positive control values were decreased 99.5% (PFCs/spleen) and 97.5% (PFCs/10^6) compared to controls). Subacute NOEL = 175 mg/kg/day (178 mg/kg/day) based on reduced bodyweight gain, increased water consumption, and increased liver weight. Immunotoxicity NOEL = 700 mg/kg/day. Acceptable. (Green, 11/1/10).

****53104-0309 251211**, "MTF-753: 4-Week Dietary Immunotoxicity Study in the Mouse", (L.J. Webley, Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, UK., Project ID: MTU0418, 26 March 2009). 10 male Crl:CD-1™ (ICR)BR mice per group received MTF-753 (penthiopyrad technical) (98.6%) in the diet at 0 (basal diet), 62.5, 250, and 1000 mg/kg/day for 4 weeks. A splenic plaque forming cell (PFC) assay was used to determine the immune system response. All vehicle control and MTF-753 treated animals received a sensitizing intravenous dose (by bolus injection) of sheep red blood cells (SRBC) in 0.9% saline 4-days prior to termination. Positive control animals were treated orally with cyclophosphamide at 20 mg/kg/day for 5 consecutive days. The SRBC intravenous dose was given after the fourth cyclophosphamide dose (animals were sacrificed 3 days after the last cyclophosphamide dose). Group mean achieved doses during the 4 week treatment period were 74.9, 301, and 1136 mg/kg/day at 62.5, 250, and 1000 mg/kg/day, respectively. All animals survived to the end the study and no treatment -related clinical signs were noted at any level. All animals gained bodyweight during the study. Group mean food consumption for treated groups was comparable to control values. Group mean water consumption was increased at 250 mg/kg/day (21%) and 1000 mg/kg/day (25%) compared to controls. No treatment-related changes were noted for total and differential leukocytes and bone marrow composition. Group mean relative liver weights were increased at 250 and 1000 mg/kg/day vs controls (values were 5.747% and 6.296% vs 5.153 %, respectively). Necropsy results were unremarkable. Treatment-related histology results of the spleen were noted at 1000 mg/kg/day and included: increased incidence of red pulp extramedullary hemopoiesis (with no increase in severity) compared to controls (10/10 vs 5/10). Also, a higher incidence of increased cellularity/size of the periarteriolar lymphocyte sheath (PALS) (minimal severity grade) was noted at 1000 mg/kg/day compared to controls (6/10 vs 2/10). A statistically significant decrease in both the number of plaque-forming cells expressed as PFCs/10^6 cells and PFCs/spleen was noted at 1000 mg/kg/day (values were decreased 55.2% and 59.4%, respectively compared to controls) (positive control values were decreased 98% and 98.9%, respectively compared to treatment controls). Immunotoxicity NOEL = 250 mg/kg/day (301 mg/kg/day) (decreased PFCs/10^6 cells and PFCs/spleen). Acceptable. (Green, 10/27/10).

ENDOCRINE DISRUPTOR STUDIES

Study not submitted nor required at this time.

SUPPLEMENTAL STUDIES

MUTAGENICITY STUDIES WITH METABOLITES OF PENTHIOPYRAD

GENE MUTATION

**53104-0285 251187, "A Reverse Mutation Assay of DM-PCA in Bacteria", (M. Sarada, Laboratory, BML, Inc., Saitama, Japan, Project ID. 10613, 13 December 2005). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to DM-PCA (3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) (99.71%), in the presence and absence of rat liver S9 mix, at 0 (DMSO), 313, 625, 1250, 2500, and 5000 *u*g/plate for 48 hours (at 37° C) after plate incorporation (cultures were preincubated in test tubes for 20 minutes with test solutions prior to plating). There was no increase in the number of revertants per plate compared to solvent controls. Cytotoxicity/growth inhibition was not indicated. No adverse effect. Positive controls were functional. DM-PCA is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 7/15/10).

**53104-0287 251189, "Mouse Lymphoma TK Assay (MLA) of DM-PCA", (S. Masumori, Biosafety Research Center, Foods, Drugs and Pesticides, Shizuoka, Japan, Experiment No. A453 (077-064), 26 December 2007). Single cultures of L5178Y TK$^{+/-}$ mouse lymphoma cells were exposed to DM-PCA (3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) (99.66%), in the presence and absence of rat liver S9 mix for 3 hours and in the absence of activation for 24-hours, at 0 (DMSO), 1.76, 3.52, 7.03, 14.1, 28.1, 56.3, 113, 225, 450, 900, and 1800 *u*g/ml. Cytotoxicity was not indicated. The color of the medium at the start of treatment was changed to orange or yellow at 450 *u*g/ml and higher after the 3-hour treatment (+/- S9 mix) and at 900 *u*g/ml and higher in the 24-hour treatment cultures (-S9 mix). At the end of the treatment period, the color of the medium was changed to orange or yellow at 900 *u*g/ml and higher in the 3-hour treatment cultures (+/- S9 mix). The pH values of the medium at 1800 *u*g/ml were as much as 0.9 units lower than solvent controls (6.5 vs 7.4) at the start of treatment and up to 0.8 units lower (7.8 vs 7.0) at the end of treatment. No treatment-related increase in the mutant frequency was indicated. No adverse effect. Positive controls were functional. DM-PCA is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 8/25/10).

**53104-0290 251192, "A Reverse Mutation Assay of PCA in Bacteria" , (H. Mochizuki, Bozo Research Center Inc., Tokyo, Japan, Project ID. M-1220, 10 November 2005). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to PCA (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) (99.92%), in the presence and absence of S9 mix, at 0 (DMSO), 156, 313, 625, 1250, 2500, and 5000 *u*g/plate for 48 hours (at 37° C) after plate incorporation (cultures were preincubated in test tubes for 20 minutes with test solutions prior to plating). There was no increase in the number of revertants per plate compared to the solvent controls. Cell growth inhibition and test article precipitation were not indicated. At 5000 ug/plate, the number of revertants per plate across the strains were generally at or slightly lower than the bottom of the range for solvent historical control values with and without activation. Positive controls were functional. PCA is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 9/22/10).

****53104-0292 251194**, "Mouse Lymphoma TK Assay (MLA) of PCA", (J. Tanaka, Biosafety Research Center, Foods, Drugs and Pesticides, Shizuoka, Japan, Experiment No. B068 (077-071), 23 March 2009). Single cultures (duplicate cultures for vehicle controls) of L5178Y TK$^{+/-}$ mouse lymphoma cells were exposed to PCA (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) (99.92%), in the presence and absence of rat liver S9 mix for 3 hours, and, in the absence of activation for 24 hours, at 0 (DMSO), 3.79, 7.58, 15.2, 30.3, 60.7, 121, 243, 485,

971, and 1941 *u*g/ml. The color of the medium was changed to orange or yellow in the 3 hour treatment cultures (+/- S9 mix) and in the 24 hour cultures (-S9 mix) at 243 *u*g/ml or higher at the start of the treatment period and in the 3 hour treatment cultures at 971 *u*g/ml or higher at the end of the treatment period (pH values were determined and found to be reduced up to 1.2 at 1941 *u*g/ml compared to the solvent controls (7.7 vs 6.5)). Cytotoxicity (reduced relative suspension growth) was noted at 1941 *u*g/ml with activation (3-hour treatment) and at 971 and 1941 *u*g/ml after the 24-hour treatment. An equivocal increase in the mutant frequency (2.25 x the solvent control value) was noted at 485 *u*g/ml after the 24-hour treatment period (the positive control value was 6.5 x the solvent control value). No increase in the mutant frequency was noted in cultures treated for 3 hours (-/+ S9 mix). Positive controls were functional. PCA is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 8/18/10).

**53104-0296 251198, "A Reverse Mutation Assay of PAM in Bacteria", (M. Sarada, Laboratory, BML, Inc., Saitama, Japan, Project ID. 10614, 27 December 2005). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (99.97%), in the presence and absence of rat liver S9 mix, at 0 (DMSO), 313, 625, 1250, 2500, or 5000 *u*g/plate for 48 hours (at 37° C) after plate incorporation (cultures were preincubated in test tubes for 20 minutes with test solutions prior to plating). There was no increase in revertants per plate compared to vehicle controls. Growth inhibition of test strains and test article precipitation were not indicated. No adverse effect. Positive controls were functional. PAM is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 7/16/10).

****53104-0298 251200,** "Mouse Lymphoma TK Assay (MLA) of PAM", (S. Masumori, Biosafety Research Center, Foods, Drugs, and Pesticides, Shizuoka, Japan, Experiment No. B067 (077-070), 24 March 2009). Single cultures (duplicate cultures were used for vehicle controls) of L5178Y TK$^{+/-}$ mouse lymphoma cells were exposed to PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (99.97%), in the presence and absence of rat liver S9 mix for 3 hours and in the absence of activation for 24 hours, at 0 (DMSO), 3.77, 7.54, 15.1, 30.2, 60.3, 121, 241, 483, 966, and 1931 *u*g/ml. A weak, but statistically significant, dose dependent increase in the mutation frequency was indicated after the 24-hour treatment (-S9 mix) compared to the solvent controls (values were also above the historical control range for solvent controls). Relative total growth values were severely reduced at 966 and 1931 *u*g/ml compared to solvent controls (5.1% and 0.3% of solvent controls, respectively) and the % small colonies was increased at 1931 *µ*g/ml. Since the relative total growth was less than 1% at 1931 *µ*g/ml, the increase in % of small colonies was considered a result of test article cytotoxicity and was probably not due to chromosomal aberration. Increases in mutation frequency results for all 3-hour treatment groups, with and without activation, were within the historical solvent control range, less than twice the concurrent solvent control value, and not statistically significant. Positive controls were functional. PAM is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 10/5/10).

**53104-0301 251203, "A Reverse Mutation Assay of 753-A-OH in Bacteria", (M. Sarada, Laboratory, BML, Inc., Saitama, Japan, Report No. 10615, 5 January 2006). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to 753-A-OH (98.91%), in the presence and absence of S9 mix, at 0 (DMSO), 39, 78, 156, 313, 625, 1250, 2500, or 5000 *u*g/plate for 48 hours (at 37° C) after plate incorporation (cultures were preincubated in test tubes for 20 minutes with test solutions prior to plating). There were no increases in the number of revertants per plate compared to solvent controls. In both trials, in the presence and absence of S9 mix, cell growth inhibition was noted for all *Salmonella typhimurium* strains at 1250 *u*g/plate, and precipitation of

the test article was noted at 1250 *u*g/plate and above. Positive controls were functional. 753-A-OH is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 7/1/10).

**53104-0303 251205, "Gene Mutation Test with 753-A-OH Using Mouse Lymphoma L5178Y Cells (MLA)", (V. Stone, Covance Laboratories Ltd., Harrogate, North Yorkshire, England, Covance Study No. 8200859, 7 July 2009). Duplicate cultures of L5178Y $TK^{+/-}$ mouse lymphoma cells were exposed to 753-A-OH (N-[2-(3-hydroxy-1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (98.91%) for 3 hours, in the presence and absence of rat liver S9 mix, and for 24 hours without activation, at concentrations ranging from 10 to 500 *u*g/ml. No treatment-related increase in the mutant frequency was indicated. No adverse effect. Positive controls were functional. 753-A-OH is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 7/20/10).

**53104-0304 251206, "Reverse Mutation Test of 753-T-DO in Bacteria", (L. Williams, Covance Laboratories Ltd., Harrogate, North Yorkshire, England, Covance Study No. 8200851, 26 June 2009). Triplicate cultures (solvent controls were tested in quintuplicate) of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to 753-T-DO (99.84%), in the presence and absence of rat liver S9 mix, at 0 (DMSO), 0.32, 1.6, 8.0, 31.25, 40.0, 62.5, 125.0, 156.25, 200.0, 250.0, 312.5, 500.0, 625.0, 1000, 2500, or 5000 *u*g/plate for 72 hours (at 37° C). Direct plate incorporation was used in the first trial. In the second trial, cultures treated in the presence of activation were preincubated for 1 hour prior to plating. There were no increases in the number of revertants per plate compared to solvent controls in trials 1 and 2. In trial 1, reduced background lawn was noted for *S. typhimurium* strains TA98 (+/- S9 mix), TA 100 (+/- S9 mix), TA1535 (-S9 mix), and TA1537 (+S9 mix) at 1000 ug/plate. Also, in trial 1, additional testing of *E. Coli* strain WP2 *uvr*A resulted in reduced revertant numbers in the absence of S9 mix at 1000 ug/plate and toxicity (no revertants) at 5000 ug/plate in the presence and absence of activation. In trial 2, reduced background lawn was noted for *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 in the absence and presence of activation at 250, 500, and 1000 ug/plate and complete toxicity (no revertant colonies) was noted for *E. Coli* strain WP2 *uvr*A at 2500 ug/plate and higher with and without activation. Precipitation was noted at 1000 ug/plate and above in both trials. Positive controls were functional. 753-T-DO is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 7/9/10).

**53104-0306 251208, "Gene Mutation Test with 753-T-DO Using Mouse Lymphoma L5178Y Cells (MLA)", (M. Lloyd, Covance Laboratories Ltd., Harrogate, North Yorkshire, England, Covance Study No. 8200898, 8 July 2009). Duplicate cultures of L5178Y $TK^{+/-}$ mouse lymphoma cells were exposed to 753-T-DO *(N*-[5-(1,3-dimethylbutyl)-5-hydroxy-2-oxo-2,5-dihydrothiophen-4-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (99.84%), in the presence and absence of rat liver S9 mix for 3 hours, and, in the absence of activation for 24 hours, at concentrations ranging from 5 *u*g/ml to 120 *u*g/ml. No treatment-related increase in the mutant frequency was indicated. No adverse effect. Cytotoxicity was demonstrated and positive controls were functional. 753-T-DO is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 8/11/10).

CHROMOSOME EFFECTS

**53104-0286 251188, "Chromosome Aberration Test with DM-PCA in Mammalian Cultured Cells", (M. Nakajima, Biosafety Research Center, Foods, Drugs and Pesticides, Shizuoka, Japan, Experiment No. A452 (077-063), 26 December 2007). Duplicate cultures of Chinese hamster lung (CHL) cells were exposed to DM-PCA (3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) (99.71%), in the presence and absence of rat liver S9 mix for 6 hours, and, in the absence

of activation for 24 hours, at 0 (DMSO), 450, 900, and 1800 *u*g/ml. Cells were harvested 24 hours after the start of treatment. 2 hours prior to harvest, colcemid solution (0.2 *u*g/ml, final concentration) was added to each culture to arrest dividing cells in metaphase. At the start of treatment, the pH of the culture medium was reduced (0.3 to 1.1 units) at 900 and 1800 *u*g/ml compared to the solvent controls with and/or without activation for the 6 and 24 hour treatment assays. No increase in structural chromosome aberrations was indicated. No adverse effect. Positive controls were functional. DM-PCA is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 8/4/10).

****53104-0291 251193**, "*In Vitro* Chromosome Aberration Test in Chinese Hamster V79 Cells with PCA", (H. Hoffmann, RCC Ltd., Cytotest Cell Research GmbH (RCC-CCR), Rossdorf, Germany, Study No. 1128202, 14 May 2008). Duplicate cultures of Chinese hamster lung fibroblast V79 cells were exposed to PCA (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) (99.1 $\pm$ 0.2%), in the presence and absence of rat liver S9 mix for 4 hours, and, in the absence of activation for 18 and 28 hours, at 0 (DMSO), 62.5, 125, 250, 500, 1000, and/or 2000 *u*g/ml. Cells were harvested 18 hours (4 and 18 hour exposure periods) and 28 hours after the start of treatment. Colcemid (0.2 *u*g/ml) was added to each culture 2.5 hours prior to harvest. 100 metaphases per culture were evaluated. A weak increase in structural chromosome aberrations was noted in the initial trial (at the low (500 *u*g/ml) and mid dose (1000 *u*g/ml*)*) and was reproduced in a repeat trial (at the mid dose) with an 18-hour treatment and harvest interval in the absence of rat liver S9 mix (high dose results were comparable to solvent control values). The increase was slightly above the historical solvent control range and lower than the positive control response. There was no cytotoxicity and no increase in the rate of polyploid metaphases in any of the assays. Positive controls were functional. PCA is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 10/1/10).

**53104-0293 251195, "Micronucleus Test of PCA in Mice", (J. Tanaka, Biosafety Research Center, Foods, Drugs and Pesticides, Shizuoka, Japan, Experiment No. B342 (077-083), 19 May 2009). 6 B6D2F_1 (BDF_1) [SPF] male mice per group received PCA (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxylic acid) (99.86%) administered by oral gavage, once daily on 2 consecutive days with a 24-hour interval, at 0 (0.5% methylcellulose), 500, 1000, and 2000 mg/kg/day with bone marrow sampling 24 hours after the last dose. 2000 polychromatic erythrocytes per animal (from 5 animals per group) were evaluated. No mortalities. There was no increase in micronucleated polychromatic (immature) erythrocytes. Positive controls were functional. PCA is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 9/2/10).

****53104-0297 251199**, "Chromosome Aberration Test with PAM in Mammalian Cultured Cells", (M. Nakajima, Biosafety Research Center, Foods, Drugs and Pesticides, Shizuoka, Japan, Experiment No. B066 (077-069), 26 March 2009). Duplicate cultures of Chinese hamster lung (CHL) cells were exposed to PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (99.97%), in the presence and absence of rat liver S9 mix for 6 hours, and, in the absence of activation for 24 hours, at 0 (DMSO), 483, 966, 989, 1236, 1545, and/or 1931 *u*g/ml. Cells were harvested 24 hours after the start of treatment. 2 hours prior to harvest, colcemid solution (0.2 *u*g/ml final concentration) was added to each culture to arrest dividing cells in metaphase. 100 metaphases per plate were evaluated microscopically. Structural chromosome aberrations were not increased after treatment for 6 hours with and without activation at 0 (DMSO), 483, 966, and 1931 *u*g/ml. In the confirmatory assay, **structural chromosome aberrations were increased** at 1931 *u*g/ml after treatment for 24 hours in the absence of S9 mix. In the re-confirmatory assay (24 hour treatment without activation), a dose dependent increase in cells with structural chromosome aberrations was noted at 989, 1236, 1545, and 1931 *u*g/ml. **Possible adverse effect:** increased incidence of chromosomal aberrations in the

absence of activation. Positive controls were functional. PAM is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 8/6/10).

**53104-0299 251201, "Micronucleus Test of PAM in Mice", (S. Masumori, Biosafety Research Center, Foods, Drugs and Pesticides, Shizuoka, Japan, Experiment No. B341 (077-082), 27 May 2009). Six B6D2F$_1$ (BDF$_1$) [SPF] mice per sex group received PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (100.00%) administered by oral gavage, once daily on 2 consecutive days with a 24-hour interval, at 0 (0.5% methylcellulose), 125 (males only), 250 (males only), 500, 1000 (females only), or 2000 mg/kg/day (females only) with bone marrow sampling 24 hours after the last dose. Subsequently, an additional study (using the same protocol as described above) was performed dosing only females (6 or 7 per group) at 0, 250, 500, and 1000 mg/kg/day (the additional study was performed because of female mortality at 2000 mg/kg/day in the first micronucleus test). 2000 polychromatic erythrocytes per animal (from 5 animals per group) were evaluated. 4/6 female mice died at 2000 mg/kg/day in the first micronucleus test (all male mice treated up to the high dose (500 mg/kg/day) survived). No treatment-related clinical signs were noted for males in the first micronucleus test (1 of the 2 surviving high dose females had tremors and piloerection). All females treated up to the high dose (1000 mg/kg/day) in the second micronucleus test survived (piloerection was noted in 1/7 females treated at 1000 mg/kg/day). There were no increases in micronucleated polychromatic (immature) erythrocytes. Positive controls were functional. PAM is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 9/14/10).

**53104-0302 251204, "Chromosome Aberration Test with 753-A-OH in Cultured Chinese Hamster Lung (CHL) Cells", (M. Lloyd, Covance Laboratories Ltd., Harrogate, North Yorkshire, England, Covance Study No. 8200858, 11 June 2009). Duplicate cultures (1.081 x 10^6 to 1.604 x 10^6 average cell count per culture) of Chinese hamster lung (CHL) cells were exposed to 753-A-OH (N-[2-(3-hydroxy-1,3-dimethylbutyl)thiophen-3-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (98.91%), in the presence and absence of rat liver S9 mix for 3 hours, and, in the absence of activation for 20 hours, at concentrations ranging from 6.25 to 1000 *u*g/ml. Cells were harvested 20 hours after the start of treatment. 1.5 hours prior to harvest, colchicine (1 *u*g/ml) was added to each culture to arrest dividing cells in metaphase. Precipitate was present at 200 *u*g/ml and higher in the presence and absence of S9 mix in both trials after treatment. In trial 1, cytotoxicity (52% to 100%) was noted at 600 *u*g/ml and higher (except 800 *u*g/ml (0%)) in the presence of S9 mix. In trial 2, marked cytotoxicity (49% to 74%) was noted at 200 *u*g/ml and higher after the 20-hour treatment without activation. In both trials, cultures at 50, 100, and 200 *u*g/ml were analyzed for chromosomal aberrations. No increase in structural chromosome aberrations was indicated. No adverse effect. Positive controls were functional. 753-A-OH is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 7/27/10).

**53104-0305 251207, "Chromosome Aberration Test with 753-T-DO in Cultured Chinese Hamster Lung (CHL) Cells", (M. Lloyd, Covance Laboratories Ltd., Harrogate, North Yorkshire, England, Covance Study No. 8200860, 17 June 2009). Duplicate cultures (1.081 x 10^6 to 1.604 x 10^6 average cell count per culture) of Chinese hamster lung (CHL) cells were exposed to 753-T-DO (*N*-[5-(1,3-dimethylbutyl)-5-hydroxy-2-oxo-2,5-dihydrothiophen-4-yl]-1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxamide) (99.84%), in the presence and absence of rat liver S9 mix for 3 hours, and, in the absence of activation for 20 hours, at 0 (DMSO), 2.5, 5.0, 10.0, 12.50, 15, 17.50, 20.0, 22.50, 25, 30, 35, 40, 45, 50, 60, 70, 75, 80, 85, 90, 95, 100, 105, 110, or 120 *u*g/ml. Cells were harvested 20 hours after the start of treatment. 1.5 hours prior to harvest, colchicine (1 *u*g/ml) was added to each culture to arrest dividing cells in metaphase. In trial 1, cytotoxicity (59% to 100% relative to vehicle controls) was noted at 45 *u*g/ml through 80 *u*g/ml (highest concentration tested), in the absence of activation, and, at 90 *u*g/ml through 120

*u*g/ml (73% to 100%) with S9 mix (precipitation was noted at 105 through 120 *u*g/ml). In trial 2, cytotoxicity greater than 50% was noted at 10 *u*g/ml (55%) (values at 12.5 and 15.0 *u*g/ml were 28% and 40%, respectively) and at 17.5 *u*g/ml through 40 *u*g/ml (highest concentration tested) (59% to 100%) in the absence of S9 mix. In trial 2 with activation, cytotoxicity was noted at 20 *u*g/ml (60%) and at 75 *u*g/ml through 120 *u*g/ml (55% to 100%). No increase in structural chromosome aberrations was indicated. Positive controls were functional. 753-T-DO is a metabolite of penthiopyrad. Acceptable. (Green and Leung, 8/2/10).

SUBCHRONIC STUDIES WITH METABOLITES OF PENTHIOPYRAD

Rat 13-Week Dietary Toxicity Study with DM-PCA

**53104-0283, 0284 251185, 251186, "DM-PCA: 13 Weeks Dietary Toxicity Study in the Rat", (E.L. Moore, Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Alconbury, Huntingdon, Cambridgeshire, UK., HLS study No. MTU0421, 20 May 2009). 10 HsdRccHan™:WIST (Han Wistar) rats per sex per group received DM-PCA (3-trifluoromethyl-1H-pyrazole-4-carboxylic acid) (98.1%) in the diet at 0 (basal diet), 1000, 4000, and 16000 ppm for 13 weeks. The overall mean achieved dosages were 66, 258, and 1038 mg/kg/day for males and 77, 306, and 1200 mg/kg/day for females at 1000, 4000, and 16000 ppm, respectively. All animals survived to scheduled termination. No treatment-related effects on clinical signs; grip strength, sensory reactivity, motor activity; ophthalmology; organ weights; necropsy results; or histology. Group mean bodyweight gain was significantly reduced (24%) for high dose males during the 13-week treatment period compared to controls. Group mean food consumption for high dose males was slightly decreased (7%) compared to controls (ns) during the 13-week treatment period. Bodyweight gain and food consumption for all other treatment groups including high dose females was comparable to controls. A minimal but statistically significant reduction in group mean hematocrit (≤ 4.0%) was noted in high dose males and females at treatment week 13 compared to controls. In males, a concomitant marginally lower group mean erythrocyte count (2.5% lower, ns) and significantly decreased mean number of reticulocytes was also noted compared to controls. Additionally, a slight reduction, compared to controls, in lymphocyte, monocyte, and large unstained cell counts resulted in a significant reduction in group mean total leukocyte counts for high dose males (high dose females were not affected). Statistically significant group mean serum chemistry changes in treatment week 13 included: slightly increased alkaline phosphatase and aspartate amino-transferase activities in high dose males, reduced calcium concentration in high dose males, and reduced phosphorus concentrations in mid and high dose males compared to controls. Group mean urine evaluation results in treatment week 13 indicated statistically significant reductions in pH for mid dose females and high dose males and females and slight increases in protein for high dose males. NOAEL for males = 4000 ppm (258 mg/kg/day) (reduced bodyweight gain). NOAEL for females = 16000 ppm (1200 mg/kg/day). No adverse effect. Record 251185 is the 2-week preliminary dietary study used to set treatment levels for the 13-week study. Acceptable. (Green, 11/10/10).

Rat 2-Week Dietary Toxicity Study with DM-PCA

53104-0283 251185, "DM-PCA: Preliminary Study by Dietary Administration to Han Wistar Rats for 2 Weeks", (E. Moore, Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Alconbury, Huntingdon, Cambridgeshire, UK., Study No. MTU0422, 19 December 2008). 5 HsdRccHan™:WIST (Han Wistar) rats per sex per group received DM-PCA (98.1%) in the diet at 0 (basal diet), 3200, 8000, 20000 ppm for 2 weeks. Overall mean achieved dosages were 288, 718, and 1731 mg/kg/day for males and 310, 742, and 1785 mg/kg/day for females at 3200, 8000, and 20000 ppm, respectively. There were no unscheduled deaths or treatment-related

clinical signs at any dose level. Overall group mean bodyweight gain for females was reduced at 8000 ppm (22%) and 20000 ppm (25%) compared to controls. Additionally, group mean food consumption was reduced for 11% vs control for high dose females during treatment week 1. No treatment-related organ weight changes or necropsy results were recorded. NOAEL = 20000 ppm (1731 mg/kg/day) for males and 3200 ppm (310 mg/kg/day) for females. Supplemental information. (Green, 11/8/10). (No worksheet).

Rat 28-Day Oral (Gavage) Toxicity Study with PCA

**53104-0289 251191, "PCA: 28-Day Oral Toxicity (Gavage) Study in the Wistar Rat", (W.H. Braun, RCC Ltd., Itingen/Switzerland, RCC Study No. B57352, 29 May 2008). 5 HanRcc:WIST(SPF) rats per sex per group received PCA (1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxylic acid) (99.1%) by oral gavage at 0 (0.5% aqueous carboxymethylcellulose and 0.1% Tween 80), 100, 300, and 1000 mg/kg/day for 28 days. No treatment-related effects were recorded for mortality, clinical signs, functional observational battery results, grip strength, locomotor activity, food consumption, bodyweights, hematology, serum chemistry, urinalysis, organ weights, necropsy results, or histology. PCA is a metabolite of penthiopyrad. No adverse effect. NOEL and NOAEL = 1000 mg/kg/day. Acceptable. (Green, 11/29/10).

Rat 28-Day Dietary Toxicity Study with PAM

0527; 278777; "PAM: Toxicity Study by Dietary Administration to Han Wistar Rats for 4 Weeks" (Cocker, C.Y., Huntingdon Life Sciences, Eye Research Centre, Eye, Suffolk, UK, Huntingdon Life Sciences Project Identity: MCW0055, 02/07/2014). 870.3050. PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxyamide, a metabolite of the active ingredient penthiopyrad, Lot No. 018-080227-1, purity = 99.97%) was admixed to the diet and fed to 10 Han Wistar rats per sex per dose at overall achieved doses of 0 (untreated diet), 7.3, 21.0, 64.4, and 137.5 mg/kg/day (males) and 0, 7.4, 22.2, 86.8, and 203.9 mg/kg/day (females) continuously for 4 weeks. No mortalities occurred. Brown staining of the head (in 3/10 males and in 3/10 females) and impaired locomotion (in 2/10 males and in 1/10 females) were observed at the high-dose level (males: 156 mg/kg/day, females: 270 mg/kg/day). No treatment-related sensory reactivity and no grip strength assessment effects were observed. Motor activity assessments revealed no treatment-related effects. Treatment-related decreases in mean terminal body weight and food consumption were observed at the high-dose (males: 156 mg/kg/day, females: 270 mg/kg/day). Hematological investigations revealed an increase in the mean white blood cell level in males at 64.4 and 137.5 mg/kg/day and in females at 86.8 and 203.9 mg/kg/day, and a decrease in the mean platelet level in both sexes at the high-dose. Blood chemistry investigations revealed increases in mean creatine phosphokinase, cholesterol, and phospholipids levels in males at 21.0, 64.4, and 137.5 mg/kg/day, an increase in the mean alkaline phosphatase level in both sexes at the high-dose, and increases urea, aspartate aminotransferase, and creatine phosphokinase in females at 203.9 mg/kg/day. Macroscopic examination revealed treatment-related femoro-tibial joint enlargement in both sexes at the high-dose and marked testes enlargement in males at the high-dose. Microscopic examination revealed treatment-related inflammation of the femoro-tibial joint in males at 21.0, 64.4, and 137.5 mg/kg/day and in females at 86.8 and 203.9 mg/kg/day, hyperostosis of the femur including joint in males at 64.4, and 137.5 mg/kg/day and in females at 209.3 mg/kg/day, increased granulopoiesis of bone in the sternum in both sexes at the high-dose, hyperplasia of the bile ducts in males at 64.4 and 137.5 mg/kg/day and in females at 22.2, 86.8 and 203.9 mg/kg/day, lymphoid cell infiltration in the liver (portal) in females at the high-dose, edema/inflammatory cell infiltration in the submucosa of the nonglandular region of the stomach in females at the high-dose, inflammation/degeneration/necrosis of Brunner's glands in the duodenum in females at the high-dose, tubular vacuolation, dilation, and degeneration/atrophy in the testes at the high-dose. **Possible adverse effect: inflammation and hyperostosis of**

the femoro-tibial joint resulting in impaired locomotion. NOEL (M) = 7.3 mg/kg/day and NOEL (F) = 7.4 mg/kg/day based on femoro-tibial joint inflammation (males) and bile duct hyperplasia (females). **Supplemental study** (the test article, PAM (1-methyl-3-trifluoromethyl-1*H*-pyrazole-4-carboxyamide), is a metabolite of the active ingredient penthiopyrad). (Corlett and Leung, 07/30/2015)