

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
Cyflufenamid

Chemical Code # 6003, Tolerance # 53086

31 August 2010

I. DATA GAP STATUS

Combined toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no 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, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 245133 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T100831 prepared by H. Green.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

****53086-0027, 0040 245013, 245026**, "NF-149 Combined Carcinogenicity and Toxicity Study by Dietary Administration to CD Rats for 104 Weeks", (S. Cooper, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. N0D010/002653, 5 January 2001). 60 CD rats per sex per group received NF-149 in the diet at 0 (basal diet), 100, 500, 2000 (females only), and 5000 ppm (males only) for 104 weeks. Additional groups of 20 animals per sex per group (toxicity phase) were necropsied after 52 weeks of treatment. Group mean achieved dosages during treatment (52 weeks) for toxicity phase animals were 5.2, 27, and 277 mg/kg/day for males at 100, 500, and 5000 ppm and 6.7, 34, and 133 mg/kg/day for females at 100, 500, and 2000 ppm, respectively. Group mean values for carcinogenicity phase animals were 4.4, 22, and 229 mg/kg/day for males and 5.5, 28, and 115 mg/kg/day for females during the 104 week treatment period, respectively.

Treatment-related clinical signs recorded for carcinogenicity phase animals included: increased incidence of thin build in both sexes at the high dose levels; reduced incidence of hairloss on the ventral body surface in mid and high dose females; increased incidence of hunched posture in high dose males; lower incidences of general pallor and pale eyes in mid and high dose females; increased incidences of brown staining of the ventral body surface and the perigenital area in high dose males; and increased incidence of yellow staining of the perigenital area in high dose males and females vs controls. Additionally, the number of carcinogenicity phase animals with palpable swellings and the number of palpable swellings per animal were increased for high dose males and females vs controls. Group mean onset times in treated groups were comparable to control values.

Group mean bodyweight gain was significantly reduced for toxicity phase males at 5000 ppm for weeks 0-1, 7-8, 8-9, and 14-18 relative to controls (overall bodyweight gain for the entire period (weeks 0-50) was 92% (ns) of the control value). Toxicity phase female bodyweight gain values at treated levels were comparable to controls. For carcinogenicity phase animals, group mean bodyweight gain was significantly reduced for mid and high dose males during week 0-1 and for high dose females for weeks 0-1, 1-2, and 2-3. For weeks 0-104, at the high dose, overall group mean bodyweight gain was 91% (males, ns) and 85% (females, ns) of control values. Group mean food consumption was reduced (ns) for high dose males during most weeks of the toxicity and carcinogenicity phases of the study (the overall reduction was 5% for each phase of the study (weeks 1 to 50 and 1 to 104, respectively) vs controls). Food consumption for high dose females was slightly reduced in toxicity phase animals through week 10 and in the carcinogenicity phase through week 18, while overall food consumption for both phases showed no treatment effect.

Treatment-related group mean hematology changes included: reduced cell hemoglobin for high dose females; slightly increased platelet counts in high dose females; reduced mean cell volumes in both sexes at the high dose; and slightly increased erythrocyte counts in high dose males vs controls. Treatment-related group mean serum chemistry changes included: reduced alanine aminotransferase activity for mid and high dose males and females; reduced aspartate aminotransferase activity in both sexes at the high dose; increased gamma glutamyl transpeptidase activity in both sexes at the high dose level; reduced total bilirubin concentrations in both sexes at the high dose level; increased cholesterol in high dose females; increased total protein concentrations for mid and high dose males and high dose females; increased albumin concentrations in high dose males; and reduced albumin/globulin ratios in mid and high dose

females and high dose males compared to controls (changes were recorded at one of more sample times and attained statistical significance occasionally). Significant increases in group mean urine volume and reductions in specific gravity (at weeks 12, 77, and 103) along with increases in urinary pH (at weeks 25, 51, and 103) were noted for high dose males vs controls. Marginal increases in group mean urine volume and pH were recorded for high dose females at week 103 compared to controls.

At necropsy of the toxicity phase animals after 52 weeks of treatment, significant increases in group mean relative values for liver and kidney weights were noted in both sexes at the high dose level and for thyroid/ parathyroid weights in high dose males compared to controls. At necropsy of carcinogenicity phase animals (week 104), group mean relative liver and thyroid/parathyroid weights were significantly increased for both sexes at the high dose level vs controls (group mean relative epididymides weights were also significantly increased for high dose males). Treatment-related macroscopy in toxicity phase animals sacrificed after 52 weeks included areas of change (pale and/or dark areas) in the liver of females (the incidence was 0/20, 2/20 (10%), 1/19 (5%), and 3/18 (17%) at 0, 100, 500, and 2000 ppm, respectively, (ns)). Treatment-related macroscopy results for carcinogenicity phase animals that were found dead or sacrificed prior to terminal sacrifice included: increased incidence of swollen livers in both sexes at the high dose level and in mid dose females; increased incidence of areas of change (pale and/or dark areas) in livers and dark lachrymal glands in high dose females; increased incidence of areas of change (pale or dark areas) on lungs of high dose males; reduced incidence of foot abrasions in high dose males; increased incidence of swollen tails in high dose females; and reduced obesity in mid and high dose males compared to controls. Treatment-related necropsy results for carcinogenicity phase animals sacrificed at 104 weeks included: increased incidence of hydronephrosis of the kidneys in high dose females; increased incidence of swollen livers in high dose males; increased incidence of skin/subcutis masses in high dose males; and reduced incidence of areas of change in the lachrymal glands of high dose males compared to controls.

Treatment-related non-neoplastic histopathology for toxicity phase animals included: increased chronic myocarditis of the heart ventricle in high dose females; increased cortical tubular casts in kidneys of high dose females; increased cortical tubular hyaline droplets and pigmented cortical tubules in kidneys of mid and high dose males; increased periacinar hepatocytic hypertrophy in liver of high dose males and females and increased centriacinar/panacinar hypertrophy and panacinar hepatocytic vacuolation in high dose males; increased follicular epithelial hypertrophy in thyroid glands of high dose males; and increased hyperplasia of the parathyroids (ns) for low, mid, and high dose males compared to controls. Treatment-related neoplastic histology was not indicated for toxicity phase animals. Treatment-related non-neoplastic findings for carcinogenicity phase animals were noted in the heart ventricle, adrenal glands, kidneys, liver, thyroids, pancreas, lungs, and lachrymal glands relative to controls. In the heart ventricle, an increased incidence of moderate chronic myocarditis was noted for high dose females (ns). In adrenals, cortical hemorrhagic degeneration was significantly increased for high dose males. The incidence of pigmented cortical tubules in kidney was increased for high dose males ($p < 0.001$) and females (ns). Centrilobular hepatocytic hypertrophy in liver was increased for high dose males ($p < 0.001$) and females (ns). Follicular cell cystic hyperplasia in the thyroid gland was increased for high dose males (ns). Significant increases in acinar atrophy with chronic inflammation of the pancreas were noted for high dose females. Increased incidence of accumulation of alveolar macrophages in lungs of high dose males was noted (ns). Inflammatory cell infiltrate in lachrymal glands of high dose males was increased (ns). Treatment-related neoplastic findings for carcinogenicity phase animals were noted in thyroid and pancreas. In males, the test for trend including all dose groups was statistically significant for benign thyroid follicular cell adenoma ($p = 0.034$) and for benign follicular cell adenoma and malignant follicular carcinoma combined ($p = 0.004$). (the trend test was not significant when the high dose group was excluded ($p > 0.08$ in both cases)). Pairwise comparison of the combined incidence for the high dose group with that for the study control was also statistically significant ($p = 0.028$) (the

incidence was also above the historical control incidence range). In pancreas, the test for trend including all treatment groups was statistically significant for benign islet cell adenoma and malignant islet cell carcinoma combined in females ($p = 0.013$) (excluding the high dose females, the trend test was not significant ($p > 0.3$), and comparisons of each treatment group with the control were also not significant). Compared to historical control data provided for pancreatic tumors, the incidence of adenomas was within the background incidence, while the incidence of carcinomas was slightly above the range.

Volume 53086-0027, record 245013 contains the 4 week preliminary dietary toxicity study in rats.

Chronic NOEL = 100 ppm (4.4 to 5.2 mg/kg/day for males and 5.5 to 6.7 mg/kg/day for females) based on liver, kidney, and thyroid histology. **Possible adverse effect:** increased follicular cell adenomas and follicular cell carcinomas in the thyroid (males) and increased islet cell adenomas and islet cell carcinomas in the pancreas (females). Acceptable. (Green, 1/28/10).

CHRONIC TOXICITY, DOG

**53086-0034 245020, "NF-149 Toxicity Study by Dietary Administration to Beagle Dogs for 52 Weeks", (M. Bellringer, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD 066/002463, 20 December 2000). 4 Beagle dogs per sex per group received NF-149 (92.5%) in the diet at 0 (basal diet), 30, 120, and 480 ppm for 52 weeks. All animals survived to termination. Group mean daily achieved dosages of NF-149 were 1.04, 4.14, and 17.29 mg/kg/day for males and 1.08, 4.41, and 17.32 mg/kg/day for females at 30, 120, and 480 ppm, respectively. No treatment-related changes in clinical signs, bodyweight, food consumption, ophthalmology, neurological evaluation results, hematology, urinalysis, organ weights, or macroscopic pathology were recorded. Significant increases in group mean alkaline phosphatase activity were noted for both sexes at the high dose level at treatment weeks 13, 26, and 52 vs controls (and for mid dose males at week 26). Histopathology revealed treatment-related effects in the adrenal glands of females. Slight diffuse cortical hypertrophy was increased in adrenal glands of mid and high dose females vs controls (the incidence was 0/4, 0/4, 1/4, and 2/4 at 0, 30, 120, and 480 ppm, respectively) and focal vacuolation in the *zona fasciculata* was increased for high dose females (the incidence was 2/4, 3/4, 2/4, and 4/4, respectively). No adverse effect. NOEL = 30 ppm (1.04 mg/kg/day (males) and 1.08 mg/kg/day (females)) based on serum chemistry and adrenal histology. Acceptable. (Green, 3/11/10).

53086-0060 245046, "NF-149 (Cyflufenamid) Re-evaluation of the No Observed adverse Effect Level (NOAEL) in the Dog Chronic Toxicity Study", (H. Takaori, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan, Report No. RD-1102239, 20 August 2002). This discussion is a re-evaluation of the NOAEL (based on liver toxicity) cited in the chronic dog study based on results from a mechanistic alkaline phosphatase (ALP) isoenzyme investigation report (record 245050). In this study, increased ALP activity resulting from dietary treatment of male Beagle dogs with NF-149 at 4000 ppm (112.3 mg/kg/day) for 14 days was determined to have resulted from liver derived isoenzyme fraction and was, therefore, considered liver toxicity. Consequently, the author cites the original findings in the chronic dog study (52 week dietary treatment, with significantly increased ALP activity at the high dose level (480 ppm (17.29 mg/kg/day for males and 17.32 mg/kg/day for females)) where the NOAEL was 480 ppm (in the absence of liver weight or histologic change) and suggests the NOAEL should be 120 ppm (4.14 mg/kg/day (males) and 4.41 mg/kg/day (females)) based on the significantly increased ALP activity (representing liver toxicity) at the high dose level in both sexes in view of the mechanistic study findings. The NOAEL for the subchronic dietary toxicity study in dogs (record 245019) remained unchanged at 150 ppm (6.5 mg/kg/day (males) and 7.5 mg/kg/day (females)) based on liver changes (hepatic fat deposition plus increased ALP activity). Supplemental data. (Green, 4/8/10).

ONCOGENICITY, MOUSE

****53086-0025, 0039 245011, 245025**, "NF-149 Carcinogenicity Study by Dietary Administration to CD-1 Mice for 78 Weeks", (S. Cooper, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD022/002230, 20 December 2000). 50 CD-1 mice per sex per group received NF-149 (95.1%) in the diet at 0 (basal diet), 60, 500, and 4000 ppm (reduced to 2000 ppm from week 20) for 78 weeks. Group mean achieved dosages were 7, 63, and 325 mg/kg/day for males and 9, 76, and 404 mg/kg/day for females at 60, 500, and 4000/2000 ppm, respectively during the 78 week treatment period. The number of animals found dead, sacrificed *in extremis*, and/or sacrificed for humane reasons during the 78 week treatment period was 19, 15, 19, and 10 males and 17, 13, 12, and 14 females at 0, 60, 500, and 4000/2000 ppm, respectively (0, 0, 2, and 2 males and 0, 1, 0, and 7 females at 0, 60, 500, and 4000 ppm, respectively were sacrificed/died during weeks 1 to 19). Also, during weeks 1 to 19, thin build was recorded in 0, 0, 1, and 1 males and 1, 4, 1, and 11 females at 0, 60, 500, and 4000 ppm, respectively. Group mean bodyweight gain for treatment weeks 0 through 78 was significantly reduced for both sexes at the high dose level compared to controls (values were 75% (males) and 77% (females) of control values). Group mean food consumption at the high dose level was generally comparable to controls for both sexes during treatment weeks 1 through 19 (96% (both sexes) of control values) and 1 through 78 (98% (males) and 99% (females) of control values). No treatment-related hematology results. Group mean relative liver weights were significantly increased for high dose males and females and for 500 ppm females vs controls. Macroscopy of all animals (including unscheduled deaths) revealed significant increases in liver masses (9/50, 10/50, 15/50, and 23/50 at the control, low, mid, and high dose level) and areas of change (6/50, 3/50, 5/50, and 17/50, respectively) in high dose males vs controls. Additionally, slightly increased incidences (ns) of lung masses (6/50, 8/50, 7/50, and 12/50 at the control, low, mid, and high dose levels) and mottled spleens (4/50, 4/50, 6/50, and 10/50, respectively) were noted for high dose females vs controls. Histopathology results (all animals) for neoplastic changes indicated significant increases in hepatocellular adenomas in livers of high dose males compared to controls. The test for trend, including all groups (6/50, 8/50, 12/50, and 17/50 at 0, 60, 500, and 4000/2000 ppm, respectively), was significant ($p = 0.016$) and, it was also significant excluding the high dose group ($p = 0.043$). Also, the combined incidence of benign hepatocellular adenoma and malignant hepatocellular carcinoma was significant for trend ($p = 0.038$) in males (the incidence of hepatocellular carcinoma was 1/50 for each of the male groups (including controls)). The historical control incidence range was 4 to 16 for hepatocellular adenoma and 1 to 5 for hepatocellular carcinoma (11 studies, with 50 to 56 livers examined per study). No hepatocellular tumors were reported in female mice. In lungs, the incidence of bronchiolar-alveolar adenoma in females was statistically increased by trend analysis ($p = 0.039$) (a pairwise comparison with the control was not significant) (the incidence was 13/50, 9/50, 10/50, and 12/50 for males and 4/50, 3/50, 5/50, and 8/50 for females at 0, 60, 500, and 4000/2000 ppm, respectively). Additionally, the combined incidence of benign bronchiolar-alveolar adenoma and malignant bronchiolar-alveolar carcinoma (incidence was 13/50, 12/50, 13/50, and 16/50 for males and 5/50, 6/50, 6/50, and 11/50 for females, respectively) in females was significant for trend ($p = 0.014$) (malignant bronchiolar-alveolar carcinoma incidence was 0/50, 3/50, 3/50, and 4/50 for males and 1/50, 3/50, 1/50, and 3/50 for females, respectively). Exclusion of the high dose group using the trend test was non-significant in both cases (with the adenomas alone ($p > 0.3$) and with the adenomas and carcinomas combined ($p > 0.5$)). Treatment-related non-neoplastic histopathology included: significant increases in fat deposition in various zones of the liver (Oil red 'O' stained (ORO)) in both sexes at 4000/2000 ppm; significant increases in loss of hepatocytes and pooling of blood in livers of high dose males; significant increases in fine vacuolation of myocytes and in fine fat deposition in ORO stained sections of heart in high dose females (primarily present in unscheduled deaths during the first 19 weeks of treatment prior to reduction from 4000 ppm to 2000 ppm); and significant increases in fine vacuolation of cortical tubular epithelium and fat deposition in cortical tubular epithelium of ORO stained sections of kidney in high dose females (primarily in unscheduled deaths during the first 19 weeks of treatment - the lesions were not present in mice sacrificed after 78 weeks of treatment) (the incidence of fine vacuolation was 1/19, 0/15, 1/21, and 3/11 for males and 0/17, 0/14, 0/12, and 8/15 for females and, ORO fat deposition incidence

was 6/19, 3/15, 2/21, and 1/11 for males and 2/17, 2/14, 3/12, and 10/15 for females at 0, 60, 500, and 4000/2000 ppm, respectively). Chronic NOEL for males = 500 ppm (63 mg/kg/day) based on reduced bodyweight gain and for females = 60 ppm (9 mg/kg/day) based on liver weight. Oncogenicity NOEL = 500 ppm based on increased hepatocellular adenomas in liver of males. **Possible adverse effect:** increased hepatocellular adenomas in the liver and increased pulmonary neoplasms in the lungs. Acceptable. (Green, 2/22/10).

REPRODUCTION, RAT

**53086-0041, 0042, 0043, 0062 245027, 245028, 245029, 245048 "NF-149 Study of Effects on Reproductive Performance in CD Rats Treated Continuously Through Two Successive Generations by Dietary Administration", (R. Patten, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD067/002313, 18 December 2000). 32 F0 and 28 F1 Sprague-Dawley CD rats per sex per group received NF-149 (95.1%) in the diet at 0 (basal diet), 80, 250, and 800 ppm through 2 generations with 1 litter per generation. Group mean achieved dosages during the pre-mating period were 5.8, 18.0, and 57.4 mg/kg/day for F0 males; 6.5, 19.9, and 66.2 mg/kg/day for F0 females; 7.4, 23.0, and 75.2 mg/kg/day for F1 males and 7.8, 24.1, and 78.2 mg/kg/day for F1 females at 80, 250, and 800 ppm, respectively. Three F0 females were sacrificed *in extremis* (1 on gestation day 9, and 2 on *post-partum* day 23) and 1 was sacrificed for humane reasons on gestation day 15 at 800 ppm and one 250 ppm female was sacrificed on gestation day 8 with trauma to the muzzle (no treatment-related pathology was indicated for any of the animals).

For F0 animals, no treatment-related effects were indicated for clinical signs, bodyweight changes, food consumption, mating performance and fertility, gestation length, estrous cycles, or sperm parameters.

One F0 female at 80 ppm, 4 at 250 ppm, and 2 at 800 ppm experienced total litter death between lactation days 2 and 4. At 800 ppm, significant increases in group mean F0 relative thyroid weights for males and females and in relative liver weight for females were recorded compared to controls. Additionally, a significantly decreased incidence of increased glycogen was noted in liver of males at 800 ppm vs controls. No treatment-related histopathology.

No treatment-related effects were noted for F1a litter size, pup sex ratios, or pup sexual maturation. At 800 ppm, significantly increased group mean relative F1a liver weights (both sexes) were noted for unselected pups vs controls. Group mean relative F1a thyroid weights were also increased for unselected female pups (ns) at 800 ppm.

At 800 ppm, two F1 females were sacrificed *in extremis* (one on lactation day 1, the other on day 2 (treatment-related pathology was not indicated) and 1 male was sacrificed for humane reasons during week 12 with trauma to a hindlimb. Additionally, one F1 control male was found dead during week 10 (macroscopy was unremarkable).

No treatment-related effects were indicated for F1 clinical signs, bodyweight changes, food consumption, mating performance and fertility, gestation length, estrous cycles, or sperm parameters.

One F1 control female, two at 80 ppm, 4 at 250 ppm, and three at 800 ppm experienced total litter death between lactation days 2 and 4.

F1 parental macroscopy results for treated groups were generally unremarkable, except group mean relative thyroid weights for F1 females at 800 ppm were marginally increased vs controls (109% of the control value, ns). No treatment-related histology was indicated for F1 parents.

No treatment-related effects were noted for F2a litter size, pup sex ratios, or pup sexual

maturation. Group mean F2a pups weights were reduced at 800 ppm vs controls on lactation day 21 (with statistical significance for males). Group mean F2a relative liver weights for female pups at 800 ppm were significantly increased vs controls. F2a macroscopy and histopathology results were unremarkable. Parental NOEL = 250 ppm (18.0 to 23.0 mg/kg/day for males and 19.9 to 41.2 mg/kg/day for females) (increased thyroid weights). Pup NOEL = 250 ppm (reduced pup weight). Reproductive NOEL = No effect at the HDT. No adverse reproductive effect. Acceptable. (Green, 1/7/10).

53086-0041 245027, "NF-149 Preliminary Study of Effects on Reproductive Performance in CD Rats by Dietary Administration", (R. Patten, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD 018/980131, 8 December 2000). 6 Sprague-Dawley CD rats per sex per group received diets containing NF-149 (95.2%) at 0 (basal diet), 250, 1000, and 4000 ppm for 4 weeks before mating of F0 animals. F1a pups selected as F1 parents received treated diets from weaning. F1 animals at 1000 and 4000 ppm were terminated at 6 weeks of age. F1 control and 250 ppm parents continued on treatment (at sponsor's request), were mated at 10 to 11 weeks of age, and their F2a offspring were terminated at weaning (21 days of age). F0 group mean achieved dosages during the pre-mating period were 22.6, 88.1, and 357.1 mg/kg/day for males and 23.2, 87.9, and 357.7 mg/kg/day for females at 250, 1000, and 4000 ppm, respectively. F0 group mean achieved dosages for females during gestation were 22.1, 85.3, and 359.1 mg/kg/day and during lactation were 45.1, 150.1, and 810.3 mg/kg/day, respectively at 250, 1000, and 4000 mg/kg/day. One F0 female at 4000 ppm was sacrificed *in extremis* on gestation day 23 with dystocia/prolonged parturition, pallor, and hunched posture (delivered offspring and retained fetuses were dead). Group mean F0 bodyweight gains were slightly reduced (ns) for males at 1000 and 4000 ppm during the treatment period and for females at 4000 ppm during the pre-mating period compared to controls. Group mean F0 food consumption for both sexes during the pre-mating period was comparable to controls. F0 females at 4000 ppm had marginally longer gestation lengths compared to controls (22.5 to 23.5 days vs 22 to 23 days). F0 necropsy results included an increase in females with dark livers at 1000 ppm (2/4) and 4000 ppm (2/4) and increased incidence of swollen livers and accentuated liver lobular pattern in 2/4 females at 4000 ppm compared to controls (one high dose female was noted with a perforated jejunum adhering to the liver). Group mean relative liver weights for both sexes at 4000 ppm were increased relative to controls. In high dose males, significant decreases were noted in liver for cytochrome P450 concentrations and increases in p-nitrophenol UDP-glucuronyltransferase activity and cytosolic hydroxysteroid sulfotransferase activity compared to controls. F0 male sperm parameters and hormone analyses (testosterone, luteinizing hormone and follicle stimulating hormone) in treated groups were generally comparable to control values. One F0 female at 1000 ppm and 2 at 4000 ppm had total litter death between lactation days 2 and 4 (poor maternal care (unfed pups) and cannibalized pups were indicated). F1a litter size was reduced at 4000 ppm on day 1 and on day 21 at 1000 and 4000 ppm relative to controls. Group mean pup weights were reduced for F1a males and females at 4000 ppm and for males at 1000 ppm on lactation days 1 and 21 compared to controls. The number of unselected F1a pups observed as missing (cannibalized) or dead or sacrificed prior to termination was increased at 1000 ppm and 4000 ppm relative to controls (macroscopy showed an increase in the number of pups without milk in the stomach at the time of death with a treatment-related increase in the number of litters effected).

F1 group mean achieved dosages for weeks 1-2 were 43.7, 184.8, and 796.8 mg/kg/day for males and 42.1, 173.1, and 719.9 mg/kg/day for females at 250, 1000, and 4000 ppm, respectively. F1 group mean achieved dosages during the pre-mating period at 250 ppm were 31.9 mg/kg/day for males and 32.9 mg/kg/day for females. During gestation and lactation, group mean achieved dosages for F1 females at 250 ppm were 21.8 mg/kg/day and 31.6 mg/kg/day, respectively. F1 mating performance, fertility, gestation length, parturition, and macroscopy were unaffected by treatment at 250 ppm. F2a litter size at 250 ppm was reduced at weaning compared to controls. NOEL not determined. Supplemental data. (Green, 12/16/09). No worksheet.

53086-0042 245028, "NF-149 Preliminary Study of Effects on Reproductive Performance in CD Rats by Dietary Administration", (R. Patten, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD 040/980208, 8 December 2000). 12 Sprague-Dawley CD rats per sex per group received diets containing NF-149 (95.2%) at 0 (basal diet), 400, 600, and 800 ppm for 4 weeks before mating of F0 animals. 16 F1 animals per sex per group received the same dietary levels as F0 animals from weaning through the production of 1 litter (F1 parents were mated at 10 to 11 weeks of age). F2a offspring were terminated at weaning (21 days of age). F0 group mean achieved dosages during the pre-mating period were 36.2, 54.3, 71.6 mg/kg/day for males and 39.0, 57.7, and 78.3 mg/kg/day for females at 400, 600, and 800 ppm, respectively. During gestation and lactation, F0 group mean achieved dosages for females were 36.3, 54.0, and 71.4 mg/kg/day and 64.5, 97.3, and 144.0 mg/kg/day at 400, 600, and 800 mg/kg/day, respectively. Two F0 control females were sacrificed for humane reasons (1 on gestation day 22 (preceding signs included dystocia, hunched posture, cyanosis) and the other on lactation day 2 (preceded by thin build, hunched posture, piloerection, dull eyes)) and one 400 ppm female was sacrificed *in extremis* prior to lactation day 1 with dystocia, hunched posture, and cyanosis. F0 group mean bodyweight gain was comparable to controls for both sexes during the pre-mating period and for females during gestation (during lactation, it was increased at 600 and 800 ppm (statistically significant) compared to controls). F0 group mean food consumption for treated groups was comparable to controls. Total litter death occurred for 2 F0 females each at 600 ppm (1 female with total litter death occurring during lactation days 1 to 4, the litter from the second dam was cannibalized prior to lactation day 1) and 800 ppm (one female with total litter death during lactation days 1 to 4, the other with dead or missing pups during lactation days 2 to 6). F0 group mean relative liver weight was significantly increased at 800 ppm compared to controls. F1a litter size and pup survival were not effected by treatment with NF-149. F1a group mean lactation day 1 and day 21 pup weights were reduced at 600 and 800 ppm compared to controls (statistically significant on day 1 for both sexes at 800 ppm and for males at 600 ppm).

F1 group mean achieved dosages for the pre-mating period were 41.7, 65.4, and 86.1 mg/kg/day for males and 43.7, 67.7, and 88.6 mg/kg/day for females at 400, 600, and 800 ppm, respectively. F1 females had group mean achieved dosages of 33.6, 50.9, and 68.7 mg/kg/day during gestation and 62.1, 95.0, and 121.3 mg/kg/day during lactation and 400, 600, and 800 ppm, respectively. F1 bodyweight and food consumption in treated groups were comparable to controls. One, 2, 2, and 2 F1 females at 0, 400, 600, and 800 ppm, respectively, had total litter deaths that occurred between lactation days 1 to 4. One F2a litter at 600 ppm was sacrificed for humane reasons and 1 litter at 800 ppm died prior to lactation day 1 (pups were characterized as cold, unfed, and/or underactive). No treatment-related effects on F2a litter size and survival. Group mean F2a pup weights in treated groups were comparable to controls on lactation day 1 and slightly lower (ns) than controls on day 21. F1 group mean relative liver weight was increased for 800 ppm males relative to controls. F1 and F2a offspring necropsy results were unremarkable. NOEL not determined. Supplemental data. (Green, 12/21/09). No worksheet.

TERATOLOGY, RAT

**53086-0035 245021, "NF-149 Study of Effects on Embryo-Fetal Toxicity in CD Rats by Oral Gavage Administration", (R. Patten, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD023/983327, 1 December 2000). 22 mated female Sprague-Dawley CD rats per group received NF-149 (95.2%) by oral gavage at 0 (5% gum arabic plus 0.01% Tween 80), 100, 300, and 1000 mg/kg/day on gestation days 6 to 19. No maternal deaths. Increased post-dosing salivation was noted at 300 (3 females) and 1000 mg/kg/day (all females). At 1000 mg/kg/day, the incidence of brown staining (particularly on the head), piloerection, and ungroomed coat was increased compared to controls. At necropsy, an increased incidence of brown staining on the heads, limbs, and body surface of high dose females was also noted. Significant increases in absolute and relative liver weights were noted at 300 and 1000 mg/kg/day (relative liver weights were marginally but statistically increased at

100 mg/kg/day) compared to controls. No treatment-related effects on maternal bodyweight, food consumption, reproductive outcomes, implantation/fetal/litter parameters, and fetal alterations. Maternal NOEL = 100 mg/kg/day (liver effects, salivation). Developmental NOEL = 1000 mg/kg/day. No teratogenicity. Acceptable. (Green, 11/16/09).

TERATOLOGY, RABBIT

**53086-0036, 0037, 0061 245022, 245023, 245047, "NF-149 Embryo-Fetal Toxicity Study in the Rabbit by Oral Gavage Administration", (R. Patten, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD069/993528, 1 December 2000). 26 mated female New Zealand white rabbits (HsdIf NZW) per group received NF-149 (95.1%) by oral gavage at 0 (5% (w/v) gum arabic and 0.01% Tween 80 (w/v) in water), 10, 60, and 300 mg/kg/day on gestation days 6 to 28. No maternal deaths. At 300 mg/kg/day, 7 females aborted (1 female aborted on gestation day 18, two on day 23, one on day 26, and three on day 28). One 60 mg/kg/day female had total litter resorption. One, 1, 2, and 3 females were not pregnant at 0, 10, 60, and 300 mg/kg/day, respectively. Significant reductions in group mean bodyweight gain were noted for females at 10, 60, and 300 mg/kg/day during the treatment period compared to controls (group mean maternal bodyweights were comparable to controls during the entire gestation period). A dose-related decrease in group mean maternal food consumption was noted at 10, 60, and 300 mg/kg/day compared to controls (statistical significance was noted for days 13-16). Values for total food consumed during the gestation period were 90%, 87%, and 73% of the control values during the period at 10, 60, and 300 mg/kg/day, respectively. Mean fetal weights were significantly reduced at 300 mg/kg/day compared to controls and marginally lower at 60 mg/kg/day. Fetal skeletal evaluation revealed an increased incidence of enlarged anterior fontanelle, incompletely ossified cervical vertebrae, and unossified epiphyses and metacarpals/phalanges at 300 mg/kg/day relative to controls. A slightly increased incidence of incompletely ossified epiphyses and metacarpals/phalanges was also observed at 60 mg/kg/day compared to controls. Maternal NOEL < 10 mg/kg/day (reduced food consumption and bodyweight gain). Developmental NOEL = 10 mg/kg/day (delayed development). No teratogenicity. Acceptable. (Green, 12/1/09).

53086-0037, 0038 245023, 245024, "NF-149 Study of Effects on Embryo-Fetal Toxicity in the Rabbit by Oral Gavage Administration", (R. Patten, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD172/004737, 5 April 2001). 24 mated female New Zealand white rabbits (HsdIf NZW) per group received NF-149 (95.2%) by oral gavage at 0 (5% (w/v) gum arabic and 0.01% Tween 80 (w/v) in water), 5, and 10 mg/kg/day on gestation days 6 to 28. Two control females (one on gestation day 8 with ulceration in cervical region and the other on day 24 with pale pinnae, slow respiration, and rales) and one 10 mg/kg/day female (on day 9 with red staining in cervical region) were sacrificed *in extremis*. One 5 mg/kg/day female (day 29) and one 10 mg/kg/day (day 27) aborted. No treatment-related effects were indicated for mortality, maternal bodyweight/bodyweight gain, maternal food consumption, maternal necropsy results, or litter responses (*corpora lutea*, implantations, number of live offspring, resorptions, pre and post implantation loss). Fetuses were not evaluated. Maternal NOEL = 10 mg/kg/day. Supplemental data. (Green, 12/7/09).

53086-0036 245022, "NF-149 Preliminary Embryo-Foetal Toxicity Study in the Rabbit by Oral Gavage Administration", (R.Patten, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD020/980186, 6 December 2000). 4 mated female New Zealand white rabbits per group received NF-149 (95.2%) by oral gavage at 0 (5% (w/v) gum arabic and 0.01% Tween 80 (w/v) in water), 100, 300, 700, and 1000 mg/kg/day on gestation days 6 to 28. 3 high dose females were sacrificed in extremis. Clinical signs prior to sacrifice included bodyweight loss, pale and/or loose feces, reduced feces, and reduced food consumption (2 females). One 700 mg/kg/day female was found dead on gestation day 11 (loose feces (for 2 days) and bodyweight loss (from the start of treatment) were noted prior to

death) (the animal was pregnant). Another 700 mg/kg/day female aborted on gestation day 29 (pale or loose feces (from the start of treatment), reduced food intake (days 24-28), and bodyweight loss (days 26-28) were noted for this female). At 300 mg/kg/day, one female had pale/loose feces during the entire treatment period, another had loose feces for 3 days, and a third female had pale feces on gestation days 28-29. Group mean maternal bodyweights and bodyweight gains were reduced during the treatment period at 100, 300, and 700 mg/kg/day compared to controls. Group mean maternal food consumption was reduced during the treatment period at 300 and 700 mg/kg/day compared to controls and at 100 mg/kg/day through gestation day 23. Maternal necropsies were unremarkable. Implantation/fetal/litter parameters at 100, 300, and 700 mg/kg/day were generally comparable to controls (except pre-implantation loss at 300 and 700 mg/kg/day was higher). Teratogenicity was not indicated. Supplemental data. (Green, 11/17/09). (No worksheet).

53086-0061 245047, "Study of Tolerance in the Rabbit by Oral Gavage Administration", (R.J. Patten, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD019/980120, 6 December 2000). Two unmated female New Zealand White rabbits (staircase phase animals) received NF-149 (95.2%) by oral gavage at 50 mg/kg on days 1 and 2, 100 mg/kg on days 3 and 4, 200 mg/kg on days 5 and 6, 400 mg/kg on days 7 and 8, 800 mg/kg on days 9 and 10, and 1000 mg/kg on days 11 and 12. Two additional females were mated and received NF-149 by oral gavage at 1000 mg/kg for seven consecutive days beginning on post-mating day 6. All animals were necropsied following their respective treatment schedules.

Both staircase phase females showed reduced food intake and fecal output from treatment day 5 until necropsy. Both females showed a large overnight loss in bodyweight before dosing on day 5 (i.e., after receiving the second dose of 100 mg/kg) and a subsequent cumulative loss through the end of treatment. Necropsy results were unremarkable.

Both mated females showed a large overnight bodyweight loss after receiving their second dose at 1000 mg/kg/day and fluctuations in bodyweight continued through the remainder of the treatment period. Necropsy results were unremarkable. Supplemental data (Green, 4/7/10). (No worksheet).

GENE MUTATION

**53086-0044 245030, "NF-149 Bacterial Mutation Assay", (J. Kitching, Huntington [sic] Life Sciences, Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD 096/993078, 14 July 2000). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain CM891 WP2 *trp uvrA* were exposed (preincubation for 30 minutes) to NF-149 (92.5%), in the presence and absence of S9 mix, at 0 (DMSO), 5, 15, 50, 150, 500, 1500, or 5000 µg/plate for 72 hours (at 37°C) after plate incorporation. There were no increases in the number of revertants per plate. Cytotoxicity (incomplete background lawn) was noted in both trials at 5000 µg/plate (in the presence and absence of S9 mix). Precipitation was noted at 1500 and 5000 µg/plate in the first trial and at 5000 µg/plate in the second. Positive controls were functional. Acceptable. (Green, 3/22/10).

**53086-0045 245031, "NF-149: Mutation at the Thymidine Kinase (tk)[®] Locus of Mouse Lymphoma L5178Y Cells (MLA) Using the Microtitre[®] Fluctuation Technique", (M. Fellows, Covance Laboratories, Ltd., North Yorkshire, England, Study No. 1537/1, 21 August 1997). Duplicate cultures of L5178Y TK^{+/-} mouse lymphoma cells were exposed for 3 hours to NF-149 (96.4%), in the presence and absence of rat liver S9 mix, at 0 (DMSO), 12.5, 25, 50, 100, and 200 µg/ml. No treatment-related increase in the mutant frequency was indicated. Positive controls were functional. Acceptable. (Green, 10/29/09).

CHROMOSOME EFFECTS

**53086-0046 245032, "NF-149 In Vitro Mammalian Chromosome Aberration Test in Human Lymphocytes", (L. Akhurst, Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, Report No. NOD 114/992861, 31 July 2000). Duplicate cultures of male whole human blood were exposed, in the presence and absence of rat liver S9 mix, to NF-149 (95.2%) concentrations of 0 (DMSO), 0 (untreated), 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/ml for 3 hours in experiment 1. In experiment 2, duplicate cultures were exposed, in the absence of S9, at 0 (DMSO), 0 (untreated), 31.3, 62.5, 125, 250, 375, 500, 750, and 1000 µg/ml for 21 hours, and, at 0 (DMSO), 0 (untreated), 250, 500, and 1000 µg/ml, for 3 hours in the presence of S9 mix. Cells were harvested 21 hours after the start of treatment (colcemid was added 2 hours prior to harvest). One hundred metaphase cells were examined from each culture. In experiment 1, precipitation was present at 62.5, 125, 250, 500, and 1000 µg/ml both with and without S9 mix. Precipitation was present in cultures in experiment 2 at 62.5, 125, 250, 375, 500, 750, and 1000 µg/ml in the absence of S9 mix and at 500 and 1000 µg/ml with activation. In experiment 1, the mitotic index relative to the solvent control was 73%, 46%, and 41% in the absence of S9 mix and 64%, 80%, and 82% with S9 mix at 250, 500, and 1000 µg/ml (the treatment levels chosen for metaphase analysis), respectively. Relative mitotic index values for experiment 2 at metaphase evaluation levels were 99%, 77%, and 47% (at 31.3, 62.5, and 125 µg/ml, respectively) without S9 mix and 65%, 47%, and 44% (at 250, 500, and 1000 µg/ml, respectively) with S9 mix. 100 metaphases per duplicate culture were scored. No increases in polyploidy or structural chromosome aberrations were indicated. Positive controls were functional. Acceptable. (Green, 11/4/09).

**53086-0047 245033, "NF-149 Mouse Micronucleus Test", ©. Mason, Huntingdon Life Sciences, Ltd., Eye, Suffolk, England, Report No. NOD 128/993514, 14 July 2000). 10 CD-1 outbred albino mice per sex per group received a single oral gavage dose of NF-149 (95.2%) at 0 (1% methylcellulose), 500, 1000, and 2000 mg/kg with bone marrow sampling (5 per sex) 24 and 48 hours later. 2000 polychromatic erythrocytes per animal were evaluated and scored by light microscopy. There was no increase in micronucleated polychromatic erythrocytes. Positive controls were functional. Acceptable. (Green, 11/5/09).

DNA DAMAGE

**53086-0048 245034, "NF-149 Rat Liver DNA Repair (UDS) Test", ®. Proudlock, Huntingdon Life Sciences, Ltd., Cambridgeshire, England, Report No. NOD 003/970962, 30 June 1997). 5 male outbred albino Hsd/Ola Sprague-Dawley rats per group received a single oral gavage dose of NF-149 (96.4%) at 0 (5% Gum Arabic), 600, and 2000 mg/kg. Hepatocytes were sampled 2 and 14 hours after treatment. Viability of the hepatocytes was determined using trypan blue and ranged from 90% to 99%. After attachment, cells were exposed to ³H thymidine for 4 hours followed by 24 hours of incubation with unlabelled thymidine. Six cultures were initiated per animal (4 animals per group were evaluated) and 3 autoradiographs were prepared. 150 cells per treated animal were analyzed. There was no increase in net nuclear grain counts. Positive controls were functional. Acceptable. (Green, 11/9/09).

MUTAGENICITY STUDIES WITH METABOLITES OF CYFLUFENAMID

GENE MUTATION

**53086-0073 245059, "149-F Bacterial Mutation Assay", (J. Kitching, Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, Report No. NOD 087/992978, 6 November 2000). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain CM891 WP2 *trp uvrA* were exposed (preincubation for 30 minutes) to 149-F (99.8%) (*N*-cyclopropylmethoxy-2,3-difluoro-6-trifluoromethylbenzamidine), in

the presence and absence of S9 mix, at 0 (DMSO), 5, 15, 50, 150, 500, 1500, or 5000 µg/plate for 72 hours (at 37°C) after plate incorporation. There were no increases in the number of revertants per plate. Cytotoxicity (no background lawn, incomplete background lawn) was observed in both trials at 1500 and 5000 µg/plate. Positive controls were functional. Acceptable. (Green, 10/22/09).

**53086-0074 245060, "149-F1 Bacterial Mutation Assay", (J. Kitching, Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, Report No. NOD 117/993246, 6 November 2000). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain CM891 WP2 *trp uvrA* were exposed (preincubation for 30 minutes) to 149-F1 (99.1%) (2,3-difluoro-6-trifluoromethylbenzamidine), in the presence and absence of S9 mix, at 0 (DMSO), 5, 15, 50, 150, 500, 1500, or 5000 µg/plate for 72 hours (at 37°C) after plate incorporation. There were no increases in the number of revertants per plate. Cytotoxicity (incomplete background lawn) was noted in both trials at 5000 µg/plate. Positive controls were functional. Acceptable. (Green, 10/22/09).

**53086-0075 245061, "149-F6 Bacterial Mutation Assay", (J. Kitching, Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, Report No. NOD 131/993335, 6 November 2000). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain CM891 WP2 *trp uvrA* were exposed (preincubation for 30 minutes) to 149-F6 (99.9%) (2,3-difluoro-6-trifluoromethylbenzamide), in the presence and absence of S9 mix, at 0 (DMSO), 5, 15, 50, 150, 500, 1500, or 5000 µg/plate for 72 hours (at 37°C) after plate incorporation. There were no increases in the number of revertants per plate. Cytotoxicity (reduced number of revertants in the *E. coli* strain) was noted in the second trial at 5000 µg/plate (+/- S9 mix). Positive controls were functional. Acceptable. (10/26/09).

**53086-0076 245062, "149-F11 Bacterial Mutation Assay", (J. Kitching, Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, Report No. NOD 132/993430, 6 November 2000). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain CM891 WP2 *trp uvrA* were exposed (preincubation for 30 minutes) to 149-F11 (98.2%) ((Z)-N-(α-cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)cabamoylacetic acid), in the presence and absence of S9 mix, at 0 (DMSO), 5, 15, 50, 150, 500, 1500, or 5000 µg/plate for 72 hours (at 37°C) after plate incorporation. There were no increases in the number of revertants per plate. Marginal cytotoxicity (slightly reduced revertant count compared to the solvent control and below the low end of the reversion range in the historical control data) was indicated for *S. typhimurium* strain TA100 (with S9 mix) at the high dose level in the second trial. Positive controls were functional. Acceptable. (Green, 10/27/09).

**53086-0077 245063, "149-(E)-FB Bacterial Mutation Assay", (J. Kitching, Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, Report No. NOD 088/992981, 6 November 2000). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain CM891 WP2 *trp uvrA* were exposed (preincubation for 30 minutes) to 149-(E)-FB (99.5%) ((E)-N-(α-cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-phenylacetamide), in the presence and absence of S9 mix, at 0 (DMSO), 5, 15, 50, 150, 500, 1500, or 5000 µg/plate for 72 hours (at 37°C) after plate incorporation. There were no increases in the number of revertants per plate. Precipitate was noted in 5000 µg/plate cultures in both trials. Cytotoxicity was not indicated. Positive controls were functional. Acceptable. (Green, 10/27/09).

SUBCHRONIC STUDIES

Dog 4 Week Dietary Toxicity Study

53086-0032 245018, "NF-149 Toxicity Study by Dietary administration to Beagle Dogs for 4 Weeks", (M. Bellringer, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD 025/983491, 22 December 1999). 3 Beagle dogs per sex per group received NF-149 (95.2%) in the diet at 0 (basal diet), 1000, 2000, and 4000 ppm for 4 weeks. Group mean daily achieved NF-149 intake values were 45, 97, and 152 mg/kg/day for males and 48, 93, and 142 mg/kg/day for females at 1000, 2000, and 4000 ppm, respectively. All animals survived to terminal necropsy. Thin appearance was noted for 2 males and 1 female at 4000 ppm from treatment week 3 and for 1 mid dose female during week 3. During treatment weeks 0 through 4, group mean bodyweight gains for high dose males and females were significantly reduced compared to controls (mid dose values were also reduced (ns) for both sexes vs controls). Group mean food consumption was significantly reduced for high dose females during the 4 week treatment period (and (ns) for high dose males) vs controls. Significant increases were noted in group mean alkaline phosphatase activities (both sexes at the mid and high dose levels); urea nitrogen concentrations (both sexes at the high dose level and mid dose males); and cholesterol concentrations (low, mid, and high dose males) vs controls. Group mean glucose concentrations were significantly reduced for both sexes at the high dose level vs controls. Group mean urine volume was significantly reduced for both sexes at 4000 ppm (and (ns) for mid dose females) and urine pH was significantly increased for both sexes at the mid and high dose levels vs controls. Significant increases in group mean relative organ weights were noted for liver (both sexes at the mid and high dose levels) and brain (high dose males) vs controls. Group mean relative weights were significantly reduced for thymus in mid and high dose males and for uterus in high dose females vs controls. Treatment-related necropsy results included: reduced thymus size in all males and 2/3 females at 4000 ppm (and in 1/3 mid dose females); small prostate in all high dose males and 2/3 mid dose males; thin uterus and small cervix in all high dose females and 1/3 mid dose females; and hindlimb muscle atrophy in 1 high dose male vs controls. Treatment-related histology included: areas of diffuse vacuolation in the neuropil (in the region of the thalamus (cerebrum)) in all high dose males; involution/atrophy of the thymus in all males and females at 4000 ppm and in 1 mid dose female; retarded acinar development and the absence of colloid in the prostate of all high dose males and in 2 mid dose males; arrested follicular development in ovaries of all high dose females and in 1 mid dose female; reduced numbers of endometrial glands and reduced width of the endometrium and myometrium in uterus of all high dose females and 1 mid dose female; and decreased cervical diameter due to a reduction in stroma in all high dose females and in 1 mid dose female vs control (control incidence for all of these findings was 0). Supplemental data. (Green, 3/2/10). No worksheet.

Dog 13 Week Subchronic Dietary Toxicity Studies

**53086-0032, 0033 245018, 245019, "NF-149 Toxicity Study by Dietary Administration to Beagle Dogs for 13 Weeks", (M. Bellringer, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD 039/983796, 23 December 1999). 4 Beagle dogs per sex per group received NF-149 (95.2%) in the diet at 0 (basal diet), 150, 500, and 1500 for 13 weeks. Group mean daily achieved dosages of NF-149 were 6.5, 23.2, and 76.2 mg/kg/day for males and 7.5, 24.4, and 70.5 mg/kg/day for females at 150, 500, and 1500 ppm, respectively. All animals survived to termination. No treatment-related clinical signs were indicated. During treatment weeks 0 through 13, group mean bodyweight gain was significantly reduced for both sexes at the high dose level vs controls. Group mean food consumption was significantly reduced for high dose females during the treatment period (weeks 1 through 13) vs controls. Treatment-related group mean serum chemistry changes that attained statistical significance vs controls included: increased alkaline phosphatase activity for high dose males and females (weeks 6 and 13) and for mid dose females (week 13); increased urea nitrogen

concentrations for mid dose males (week 13) and high dose males (week 6 and 13) and females (week 13); increased cholesterol concentrations for mid dose males (week 13) and high dose males (week 13) and females (week 6 and 13); increased potassium levels for mid dose females (week 6) and for both sexes (week 6 and 13) at the high level; and reduced calcium levels for mid dose females (week 6) and high dose males (week 13) and females (week 6). Significant group mean relative organ weight changes compared to controls that were recorded at necropsy include: increased liver weights for low, mid, and high dose females and for mid and high dose males; increased brain weights for high dose males; increased thyroid weights in high dose males; increased heart weights for high dose females; and increased kidney weights for low, mid, and high dose females. Macroscopy was unremarkable. Treatment-related histopathology included: significantly increased vacuolation in the brain, involving areas of grey matter and areas of white matter (mainly in cerebrum and thalamus) of females (4/4) and males (ns, 3/4) at 1500 ppm; increases in enlarged, finely vacuolated hepatocytes in various zonal areas of the liver (sections stained with Oil red O (ORO) revealed the presence of fat) in both sexes at the mid and high dose level; increased (ns) involution/atrophy in the thymus of high dose males and females; increases in the incidence and degree of abnormal spermatogenic cells within ducts ($p \leq 0.05$) and of reduced numbers of spermatozoa (ns) in epididymides in high dose males; slightly retarded acinar development in prostate of low and high dose males; arrested follicular development in ovaries of one 1500 ppm female; and reduced width of myometrium and endometrium in the uterus and decreased cervical diameter in 2 high dose females compared to controls. NOEL < 150 ppm (6.5 mg/kg/day (males) and 7.5 mg/kg/day (females)) based on liver weight in females). No adverse effect. Acceptable. (Green, 3/8/10).

53086-0057 245043, "NF-149 Reversibility Study by Dietary Administration to Female Beagle Dogs for 13 Weeks Followed by a 13 Week Recovery Period", (M. Bellringer, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD 124/993983, 17 November 2000). This study was performed to evaluate the reversibility of the brain lesions noted at the high dose level in the subchronic dietary toxicity 13 week dog study (treatment levels were 0, 150, 500, and 1500 ppm, volume 53086-0033, record 245019). Two (control) or 3 or 5 female Beagle dogs per group received NF-149 (95.2%) in the diet at 0 (basal diet), 150, and 1500 ppm for 13 weeks. Subsequently, 2 control and 3 high dose females received untreated diet during a 13 week recovery period. There were no unscheduled deaths during the treatment or recovery periods. The overall group mean NF-149 intakes were 6.33 and 65.06 mg/kg/day for females at 150 and 1500 ppm, respectively, during the 13 week treatment period. No treatment-related changes were noted for clinical signs, bodyweight, food consumption, neurological examination results, electrocardiography results, or macroscopic pathology. Microscopic pathology revealed vacuolation in the cerebrum and thalamus regions of the brain in 2/3 females at 1500 ppm sacrificed after the 13 week treatment period, and in all high dose females sacrificed after the 13 week recovery period (severity was slightly decreased for recovery animals). No treatment-related effects were noted for control animals. Electron microscopic evaluations were performed on brain samples from animals which showed a treatment-related effect by light microscopy. Electron microscopy results included; one of the 2 high dose females (sacrificed at the end of the treatment period) with brain lesions showed numerous large vacuoles, many with a thin myelin membrane, and the other female showed numerous small areas of myelin edema on many sheaths; 1/3 high dose females sacrificed at the end of the recovery period had occasional small vacuoles with a thin myelin wall and several areas of possible myelin edema and another high dose recovery female had numerous small areas of myelin edema on many myelin sheaths. Supplemental data. (Green, 3/17/10).

53086-0058 245044, "NF-149 Reversibility Study by Dietary Administration to Female Dogs for 13 Weeks Followed by a 26 Week Recovery Period", (M. Bellringer, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD 125/993984, 4 December 2000). This study was performed to evaluate the reversibility of the brain lesions noted at the high dose level in the subchronic dietary toxicity 13 week dog study (treatment levels were 0, 150, 500, and 1500 ppm, volume 53086-0033, record 245019). 2 (control) or 3 female

Beagle dogs per group received NF-149 (95.2%) in the diet at 0 (basal diet) and 1500 ppm for 13 weeks. Subsequently, all animals received untreated diet during a 26-week recovery period. The overall group mean NF-149 intake was 64.48 mg/kg/day for females at 1500 ppm during the 13 week treatment period. There were no unscheduled deaths. No treatment-related changes were noted for clinical signs, bodyweight, food consumption, neurological examination results, electrocardiography results, macroscopic pathology, or histology. Supplemental data. (Green, 4/6/10).

53086-0059 245045, "NF-149 Brain Vacuolation in Dogs", (C. Gopinath, Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. RD-1102311, 16 August 2002). This position paper characterizes the vacuolation of the white and/or grey matter of the brain reported in: the 4 week dietary study in Beagle dogs (record 245018) at the high dose level (4000 ppm, 152 mg/kg/day (males) and 142 mg/kg/day (females)); the 13 week dietary study in Beagle dogs (record 245019) at the high dose level (1500 ppm, 76.2 mg/kg/day (males) and 70.5 mg/kg/day (females)); and the 13 week dietary/13 week reversibility study (record 245043) in female Beagle dogs at the high dose level (1500 ppm, 65.06 mg/kg/day). Brain vacuolation did not result in the 13 week dietary/26 week reversibility study (record 245044) in female Beagle dogs with a treatment level of 1500 ppm (64.48 mg/kg/day) or the 52 week chronic dietary study in Beagle dogs (record 245020) where the high dose level was 480 ppm (17.29 mg/kg/day (males) and 17.32 mg/kg/day (females)). The brain vacuolation was identified by electron microscopy as myelin edema affecting white matter of the cerebrum and thalamus. There were no associated glial or neuronal changes and no evidence of any demyelination or axonal changes (the peripheral nervous system was unaffected). Additionally, the brain lesions appear to be species specific since the effect was not seen in mice and rats treated with NF-149. The myelin edema reported in the 4 and 13 week dog studies with NF-149 was similar to that reported (in the literature) for compounds such as vigabatrin (an anti-convulsant agent). Other compounds mentioned, known to induce myelin edema (although more severe than that seen with NF-149 and vigabatrin), include bromethalin (used in rodenticides), CDTD (2'-chloro-2,4-dinitro-5',6-di(trifluoromethyl)-diphenylamine, an acaricide), isoniazid (used for treatment of tuberculosis), hexachlorophene (an anti-microbial agent), and triethyltin (TET) (used as an industrial chemical and biocide). Additionally, *in vitro* assays for effects of NF-149 on uncoupling activity (gamma-aminobutyric acid (GABA)-transaminase or monoamine oxidase (MOA)) in dog's brain (performed by the sponsor-summaries were provided) reported negative results. Supplemental information. (Green, 4/12/10).

Rat 3 Month Subchronic Oral Toxicity Study

**53086-0028 245014, "Subchronic Oral Toxicity Study of 26-4966* in Rats (*Code No. NF-149)", (K. Goto, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan, Report No. H080, 28 August 1997). 10 Crj:CD (SD) rats per sex per group received 26-4966 (NF-149) (96.4%) in the diet at 0 (basal diet), 50, 300, 1800, and 10800 ppm for 3 months. Group mean achieved dosages during the treatment period were 3.3, 20.1, 117.4, and 673.3 mg/kg/day for males and 4.1, 24.7, 143.9, and 782.6 mg/kg/day for females at 50, 300, 1800, and 10800 ppm, respectively. All animals survived to termination. Significant increases in slight abdominal distension (days 16 to 56) and periurethral urinary staining (days 70 to 87) were noted for high dose females compared to controls. No treatment-related clinical signs were noted for males at any treatment level. Group mean bodyweight and bodyweight gain were significantly reduced for both sexes at 10800 ppm vs controls during the treatment period (days 1 to 91) and for females at 1800 ppm for days 56 to 84. Group mean food consumption (g/kg/day) was significantly reduced at the beginning of treatment at 1800 ppm (day 1) and at 10800 ppm (days 1 and 7) for both sexes vs controls (values were comparable to controls during the remainder of the treatment period). Hematology results at 10800 ppm included significant increases in platelet counts for both sexes, and significant decreases in hematocrit, hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin for females vs controls. Serum chemistry results at 10800 ppm included: significant increases in urea nitrogen, total cholesterol,

total protein, potassium, inorganic phosphorus, and gamma glutamyltransferase activity in both sexes (significant decreases in glucose were also noted); significant increases in albumin, calcium, and glutamic oxaloacetic transaminase activity in males; and significant decreases in total bilirubin, albumin/globulin ratio, chloride, and cholinesterase activity in females vs controls. Urea nitrogen and glutamic oxaloacetic transaminase activity were significantly increased for females at 1800 ppm. Group mean relative liver, kidney, testes, thyroid, and cecum weights were significantly increased at 10800 ppm for both sexes vs controls. At 1800 ppm, significant increases in relative weights were noted for kidneys and thyroid glands in both sexes, and for liver in females vs controls. Treatment-related histopathology results included significant increases in myocarditis (males) and myocardial vacuolation (females) of the heart, bile duct hypertrophy and centrilobular hypertrophy in the liver (both sexes), hyaline droplets in tubular epithelium (males) and tubular vacuolization (females) in kidney, follicular hypertrophy of the thyroid gland (both sexes), and Leydig cell hyperplasia in the testes (males) at 10800 ppm vs controls. A significant increase in centrilobular hypertrophy in liver was also noted for females at 1800 ppm. NOEL = 300 ppm (20.1 mg/kg/day for males and 24.7 mg/kg/day for females) (bodyweight reduction and histological changes). No adverse effect. Acceptable. (Green, 1/13/10).

Rat 4 Week Subchronic Dietary Toxicity Study

53086-0027 245013, "NF-149 Preliminary Toxicity Study by Dietary Administration to CD Rats for 4 Weeks", (S. Cooper, Huntingdon Life Sciences, Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD009/972496). 5 CD rats per sex per group received NF-149 (94.7%) in the diet at 0 (basal diet), 300, 1800, 6000 (males only), 3000 (females only), and 10800 ppm for 4 weeks. All animals survived to termination. Group mean achieved dosages were 34, 199, 698, and 1193 mg/kg/day for males and 35, 211, 375, and 1212 mg/kg/day for females at 300, 1800, 6000 (males)/3000 (females), and 10800 ppm, respectively, during the treatment period. Treatment-related clinical signs included increases in hairloss on the ventral body surface (for males at 6000 and 10800 ppm), fast respiration (after overnight fasting prior to termination for males and females at 10800 ppm), and piloerection, underactivity, and hunched posture for females at 10800 ppm vs controls. Bodyweight gain was significantly reduced at 10800 ppm for both sexes and for females at 3000 ppm for days 0 through 28 (reduced bodyweight gain was pronounced during the first week of treatment, thereafter bodyweight gain was generally comparable to controls). Food consumption was reduced (ns) through treatment day 3 for both sexes at 1800 ppm and higher and was generally comparable to controls thereafter. Treatment-related serum chemistry included: slightly reduced alanine aminotransferase activity for males at 1800 ppm and above; significantly increased alkaline phosphatase activity for females at 10800 ppm; significantly increased gamma glutamyl transferase activity in both sexes at 6000/3000 and 10800 ppm; significantly increased cholesterol, triglycerides, and total protein concentrations for both sexes at 10800 ppm and for males at 6000 ppm; and significantly reduced plasma sodium and chloride concentrations for both sexes at 10800 ppm compared to controls. Group mean relative organ weights were significantly increased: for liver in both sexes at 6000 (males)/3000 (females) and 10800 ppm and for 1800 ppm females; and for kidneys in both sexes at 6000 (males)/3000 (females) and 10800 ppm vs controls. Group mean relative weights for seminal vesicles were significantly reduced in males at the high dose level. Necropsy results were unremarkable. Treatment-related histopathology was noted in the liver, heart, kidneys, and prostate gland. In liver, significant increases in the incidence of panacinar hepatocytic vacuolation was noted for 6000 and 10800 ppm males (Oil Red 'O' staining identified this at the high dose as the accumulation of fine fat within the hepatocytes) and of centriacinar/panacinar hypertrophy in 6000 and 10800 ppm males and high dose females compared to controls. A significant increase of myocarditis was noted in heart ventricles of 6000 and 10800 ppm males and 3000 ppm females and of myocardial vacuolation in the hearts of high dose females compared to controls. In kidney, cortical tubular hyaline droplets were increased (ns) at 1800 ppm and higher for males and cortical tubular vacuolation was significantly increased for high dose females vs controls.

Epithelial atrophy in the prostate was increased (ns) for high dose males compared to controls. Supplemental data. (Green, 1/14/10) (no worksheet).

Rat 4 Week Subchronic Dermal Toxicity Study

53086-0029 245015, "NF-149 Toxicity Study by Dermal Administration to CD Rats for 4 Weeks", (S. Cooper, Huntington [sic] Life Sciences Ltd., Alconbury, Huntington [sic], Cambridgeshire, England, Report No. NOD119/993979, 21 December 2000). 5 CD rats per sex per group were dermally treated (clipped, unabraded, occluded) with NF-149 (95.1%) at 0 (5% Gum arabic/0.01% Tween 80 in water), 100, 300, and 1000 mg/kg/day for 4 weeks (6 hours per day, 7 days per week). All animals survived to termination. No treatment-related changes were recorded for clinical signs, food consumption, ophthalmology, hematology, organ weights, macroscopy, or histopathology. No treatment-related changes were reported for skin treatment sites. Bodyweight gain was slightly reduced (ns) in mid and high dose males for weeks 0 through 4 vs controls. Group mean creatine phosphokinase activity was slightly increased (ns) for high dose males vs controls. Urinalysis indicated slightly increased pH (ns) and slightly reduced specific gravity (ns) in urine from high dose males compared to controls. Unacceptable, not upgradeable (incomplete observations, necropsy, and histology). (Green, 2/26/10).

90-Day Dermal Toxicity Study

Not submitted.

Mouse 3 Month Subchronic Oral Toxicity Study

**53086-0026 245012, "Subchronic Oral Toxicity Study of 26-4966* in Mice (*Code No. NF-149)", (K. Goto, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan, Report No. H081, 31 March 1998). 10 Crj: CD-1 (ICR) mice per sex per group received 24-4966 (NF-149) (96.4%) in the diet at 0 (basal diet), 100, 400, 1600, and 7000 ppm for 3 months. Group mean achieved dosages during the treatment period were 14.0, 50.7, 218.1, and 808.0 mg/kg/day for males and 17.6, 70.8, 295.1, and 940.2 mg/kg/day for females at 100, 400, 1600, and 7000 ppm, respectively. All animals survived to scheduled termination. The incidence of abdominal distention was significantly increased for high dose females on days 21 to 32 compared to controls (5/10 vs 0/10). Group mean bodyweight gains were significantly reduced for both sexes at the high dose level during treatment week 1 compared to controls (bodyweight gains for all treated groups were generally comparable to control values for days 0 to 91). Group mean food consumption (g/kg/day) was slightly reduced (ns) during the entire treatment period at the high dose level for both sexes (significant reductions were noted on days 1, 7, and 14 for both sexes, and for females on days 63 and 70) vs controls. Group mean hematocrit values were significantly decreased for high dose males relative to controls. The following significant changes in group mean serum chemistry values were noted at the high dose level: increased total cholesterol (both sexes); increased glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities (males); decreased albumin/globulin ratios (A/G) in males (also in 1600 ppm females); and increased urea nitrogen (BUN) and total protein (females) compared to controls. Group mean relative liver weights were significantly increased for both sexes at 1600 and 7000 ppm vs controls (group mean relative spleen weights were also significantly increases for high dose males). The incidence of dark brown liver was significantly increased for high dose males (5/10) vs controls (0/10) and (ns) for 1600 ppm males (3/10). Statistically significant treatment-related histopathology included: increased centrilobular hepatocellular hypertrophy in liver of both sexes at 1600 (4/10 each sex) and 7000 ppm (10/10 each sex); increases in focal necrosis of hepatocytes and yellow pigment in Kupffer cells in liver of males (10/10) and females (10/10) at 7000 ppm; increased nucleolar hypertrophy in liver of high dose males (10/10); myocardial vacuolation (slight) of the heart was noted in high dose males (8/10) and females (2/10, ns); increased testicular Leydig cell hyperplasia (slight) in high dose males (10/10); and decreased secretory granules in the submaxillary glands of high dose

males (7/10) compared to controls. NOEL = 400 ppm (50.7 mg/kg/day (males) and 70.8 mg/kg/day (females) based on liver histology. No adverse effect. Acceptable. (Green, 2/11/10).

NEUROTOXICITY STUDIES

**53086-0031, 0145, 0146 245017, 253819, 253820, 253821, "NF-149 Neurotoxicity Study by Dietary Administration to CD Rats for 13 Weeks", (S. Cooper, Huntingdon [sic] Life Sciences Ltd., Alconbury, Huntingdon [sic], Cambridgeshire, England, Report No. NOD174/012398, 8 June 2001). 10 CD rats per sex per group received NF-149 (95.4%) in the diet at 0 (basal diet), 200, 1000, and 5000 ppm for 13 weeks. The overall achieved dosages during the 13 week treatment period were 17.5, 88.0, and 453.1 mg/kg/day for males and 21.3, 98.0, and 571.8 mg/kg/day for females at 200, 1000, and 5000 ppm, respectively. One high dose male was sacrificed for humane reasons during treatment week 13 - not treatment related (signs observed prior to death included piloerection, thin build, decreased fecal pellets, and a swollen area on the left ventral abdomen; reduced food consumption and bodyweight were noted in the week before death; histology revealed a malignant lymphoma). No treatment-related clinical signs were recorded. Bodyweight gain was significantly reduced at the high dose for both sexes during the first week of treatment vs controls. Bodyweight gains for both sexes at the high dose during the rest of the treatment period were variable with overall gains for weeks 0 through 13 at 91% (males, ns) and 82% (females, $p < 0.05$) of control values for the period. Food consumption was generally comparable to controls except for slight reductions for mid and high dose females at weeks 1 and 2 vs controls (ns). No treatment-related effects were noted for function observational battery results, motor activity results, brain weights, anatomical measurements, necropsy findings, or histology. Systemic NOEL = 1000 ppm (88.0 mg/kg/day for males and 98.0 mg/kg/day for females, respectively) based on reduced bodyweight gain. Neurotoxicity NOEL = 5000 ppm (453.1 mg/kg/day (males) and 571.8 mg/kg/day (females)). No adverse effect. Acceptable. (Green, 2/3/10).

53086-0030 245016, "Cyflufenamid: Acute Neurotoxicity Study Waiver Request", (H. Takaori and Y. Fujii, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan, Report No. RD-01465, 5 November 2008). This position paper proposes a waiver for acute neurotoxicity testing of cyflufenamid (NF-149) based on results of existing studies. Results from an acute oral toxicity study (5000 mg/kg) (record 245005) and a 90-day neurotoxicity study (500 mg/kg/day) (record 245017) in rats indicate neurotoxic signs limited to waddling/unsteady gait and piloerection at 5000 and 3200 mg/kg in the acute study and no clinical signs of neurotoxicity or neuropathological lesions in the 90-day study. The only finding in a developmental toxicity study in rats (record 245021) indicating an effect on the nervous system was piloerection. Clinical and pathological effects on the developing nervous system were not noted in the developmental toxicity study (record 245021) nor in the reproduction study (record 245029) (offspring brain histology was included in the reproduction study). (Green 5/12/10).

IMMUNOTOXICITY STUDIES

**53086-0052 245038, "NF-149: 4 Week Dietary Immunotoxicity Study in the Mouse", (A. Bottomley, Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England, Report No. NOD 0307, 31 October 2008). 10 Crl:CD1(ICR) mice per sex per group received NF-149 (95.6%) in the diet at 0 (basal diet), 400, 1600, and 7000 ppm for 4 weeks. A splenic plaque forming cell (PFC) assay was used to determine the immune system response. All treated animals received a single dose of Sheep Red Blood Cells (SRBC) at 2×10^9 cells per ml at a dose volume of 0.2 ml/animal by intravenous injection on treatment day 25. Positive control animals received cyclophosphamide (in 0.9% saline) by oral gavage at 20 mg/kg/day for 5 consecutive days (starting 7 days prior to termination). Group mean achieved dose during the 4 week treatment period was 50, 298, and 1916 mg/kg/day for males and 63, 388, and 2542 mg/kg/day for females at 400, 1600, and 7000 ppm, respectively. One high dose female was

sacrificed in poor condition on treatment day 23 (underactivity, reduced body temperature, pallor of the whole body, and fast breathing were reported prior to sacrifice and necropsy revealed a pale, enlarged liver). Significantly reduced group mean bodyweight gains were noted for both sexes at 7000 ppm for treatment days 1 through 4 compared to negative controls (bodyweight gains for days 1 through 29 were generally comparable to controls). Group mean food consumption was increased for high dose males ($p < 0.05$) and females (ns) during the treatment period. Necropsy revealed enlarged liver in both sexes (1/10 and 10/10 males and 1/10 and 7/10 females at the mid and high dose, respectively), pale areas in the liver in both sexes (1/10 and 3/10 males at the mid and high dose, respectively, and 1/10, 1/10, and 3/10 females at the low, mid, and high dose, respectively), pale livers in one high dose male and female, an accentuated liver lobular pattern in 2/10 high dose females, and pallor of the spleen in 2/10 high dose males vs controls. There were no significant changes in the numbers of PFCs/ 10^6 cells or PFCs/spleen at 400, 1600, and 7000 ppm vs the negative control. The positive control was functional (a significant reduction in PFCs/ 10^6 cells and PFCs/spleen was noted vs the negative control). No immunotoxicity, no adverse effect. Immunotoxicity NOEL = 7000 ppm (1916 mg/kg/day for males and 2542 mg/kg/day for females). Acceptable. (Green, 3/24/10).

**53086-0053 245039, "NF-149: 4 Week Dietary Immunotoxicity Study in the Rat", (C. Brennan, Huntingdon Life Sciences, Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD 0306, 29 October 2008). 10 Crl:CD(SD) rats per sex per group received NF-149 (95.6%) in the diet at 0 (basal diet), 300, 1800, and 10800 ppm for 4 weeks. A splenic plaque forming cell (PFC) assay was used to determine the immune system response. All treated animals received a single antigenic challenge with 2×10^8 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 dose during the 4 week treatment period was 24, 150, and 942 mg/kg/day for males and 25, 150, and 927 mg/kg/day for females at 300, 1800, and 10800 ppm, respectively. All animals survived to termination. No treatment-related clinical signs or organ weight changes were reported. Group mean bodyweight gain was significantly reduced for both sexes at the high dose level for days 1 through 29. Food consumption for treated groups was generally comparable to controls during the treatment period. Macropathology revealed an increased incidence of enlarged liver (5/10) and stomach corpus mucosa depressions (2/10) in high dose females compared to negative controls. A significant decrease in the group mean number of PFCs/spleen and cells/spleen for high dose females compared to the negative controls was indicated (the group mean value for PFCs/ 10^6 splenocytes was also reduced (without statistical significance)). The decreases for treated females indicated a weak response compared to the magnitude of the positive control results. Values for high dose females were 36% (PFCs/spleen), 66% (cells/spleen), and 67% (PFCs/ 10^6 splenocytes) of negative control values, while group mean values for the positive control females were 0.1% (PFCs/spleen), 37% (cells/spleen), and 0.3% (PFCs/ 10^6 splenocytes) of negative control values. Additionally, decreases in the group mean values for high dose females were attributed to 3 animals (individual results showed only 3 animals with values below the lowest negative control value for both PFCs/spleen and cells/spleen). In view of these comparisons, the significance of the changes indicated for PFCs/spleen, cells/spleen, and PFCs/ 10^6 splenocytes in high dose females remains inconclusive. There were no treatment-related changes in PFCs/spleen, cells/spleen, or PFCs/ 10^6 splenocytes for males compared to negative controls. Immunotoxicity NOEL = 10800 ppm (942 mg/kg/day for males and 927 mg/kg/day for females). Acceptable. (Green, 3/30/10).

METABOLISM STUDIES

**53086-0049 245035, " ^{14}C -NF-149 Metabolism in Rats", (A. Langford-Pollard, Huntingdon Life Sciences, Ltd., Alconbury, Huntingdon, Cambridgeshire, England, Report No. NOD 021/994500, 19 December 2000). 2, 3, 4, 15, or 16 Sprague-Dawley (Crl:CD[®]BR) rats received a single oral gavage dose of [Fluorinated phenyl- ^{14}C]NF-149 at 10 or 200 mg/kg. Additional groups of 4 and

8 rats per sex per group received 14 consecutive daily oral gavage doses at 10 mg/kg/day. Also, 4 bile duct cannulated rats per sex per group received a single dose by stomach cannula at 10 and 200 mg/kg.

Non-Radiolabeled NF-149 Pre-Test Observation (Group 1a)

2 Sprague-Dawley (CrI:CD[®]BR) rats per sex received a single oral gavage dose of non-radiolabeled NF-149 at 200 mg/kg followed by a 48-hour observation period. No clinical signs were indicated during 48 hours post-dosing.

Preliminary Excretion Balance (Group 1b)

3 male rats received a single oral gavage dose of ¹⁴C-NF-149 at 10 mg/kg. 27.4% and 74.4% of dosed radioactivity was excreted in urine and feces (mean values for 3 animals), respectively, during 72 hours after dosing. Most excretion occurred during the 0 to 24 hour interval (24.46% in urine and 65.17% in feces). At sacrifice (72 hours after dosing), ~2% of the radioactive dose remained in the carcass. The proportion of dose excreted as volatiles during 72 hours post-dosing was below the limit of detection.

Excretion Balance/Tissue Distribution (Groups 1c and 1d)

Group 1c

Group mean urinary excretion values (% of dose) during 168 hours after a single oral dose of ¹⁴C-NF-149 at 10 mg/kg (4 rats per sex per group) were 31.1% (males) and 17.9% (females). Most radioactivity was excreted during 0 to 12 hours post-dosing (20.1% (males) and 10.4% (females)). Group mean excretion in feces was 66.3% (males) and 80.8% (females) during 0 to 168 hours post-dosing with most excretion occurring during 0 to 24 hours post-dosing (54.7% (males) and 57.2% (females)). At sacrifice (168 hours post-dosing), 0.5% (males) and 0.4% (females) of dose remained in the carcass, 0.4% (males) and 0.2% (females) in muscle, and 0.3% (both sexes) remained in liver. Concentrations of radioactivity (μg equivalents of ¹⁴C-NF-149 per g of fresh tissue (μg equiv/g)) were highest in liver (0.481 μg equiv/g (males) and 0.664 μg equiv/g (females)) and kidneys (0.261 μg equiv/g (males) and 0.218 μg equiv/g (females)). 0.08 to 0.12 μg equiv/g were detected in adrenal glands, bone marrow, heart, pituitary, and red blood cells. <0.07 μg equiv/g were found in all other tissues.

Group 1d

23.6% (males) and 10.5% (females) of dose (group mean values) were excreted in urine during 168 hours after a single oral dose of ¹⁴C-NF-149 at 200 mg/kg (4 rats per sex per group) (most was excreted during 0 to 12 hours post-dosing (12.2% (males) and 3.7% (females))). Group mean fecal excretion was 76.9% (males) and 88.4% (females) during 0 to 168 hours post-dosing with most excretion occurring during 0 to 24 hours post-dosing (68.2% (males) and 70.0% (females))). At sacrifice (168 hours post-dosing), 0.2% of dose remained in the carcass (both sexes), 0.1% (males) and 0.2% (females) in liver, and 0.1% (both sexes) in muscle. Concentrations of radioactivity were highest in liver (2.87 μg equiv/g (males) and 5.92 μg equiv/g (females)), kidneys (1.46 μg equiv/g (males) and 1.30 μg equiv/g (females)), fat (1.57 μg equiv/g (females) and 0.32 μg equiv/g (males)), and red blood cells (1.03 μg equiv/g (both sexes))). All other tissues showed concentrations either < 0.07 μg equiv/g or below the limit of detection.

Plasma and Red Blood Cell Radioactivity Kinetics (Groups 2a and 2b)

Group mean peak plasma radioactivity concentrations (Plasma C_{max}) of 1.35 μg equiv/ml (males) and 0.804 μg equiv/ml (females) were reached at (T_{max}) 4 hours (males) and 1 hour (females) after a single oral gavage dose of ¹⁴C-NF-149 at 10 mg/kg (Group 2a, 15 rats per sex). After a

single oral gavage dose of ^{14}C -NF-149 at 200 mg/kg (Group 2b, 15 rats per sex), group mean plasma C_{max} levels of 17.7 μg equiv/ml (males) and 6.24 μg equiv/ml (females) were reached at (T_{max}) 12 hours (males) and 6 hours (females). Group mean peak red blood cell radioactivity concentrations (RBC C_{max}) of 0.656 μg equiv/ml (males) and 0.400 μg equiv/ml (females) were reached at (T_{max}) 4 hours (males) and 1 hour (females) after 10 mg/kg. After a 200 mg/kg dose, RBC C_{max} values of 9.49 μg equiv/ml (males) and 3.64 μg equiv/ml (females) were reached at (T_{max}) 12 hours (males) and 24 hours (females) post-dosing.

The extent of systemic exposure of rats to radioactivity, characterized by plasma radioactivity concentration-time curve through 120 hours post-dose (AUC_{120}), increased proportionately with increasing dose (10 and 200 mg/kg). Plasma AUC_{120} values were 28.7 μg equiv.h/ml for males and 15.2 μg equiv.h/ml for females at 10 mg/kg and 574 μg equiv.h/ml for males and 285 μg equiv.h/ml for females at 200 mg/kg. The Red Blood Cell AUC_{120} also appeared to increase proportionately with increasing dose from 10 to 200 mg/kg, however statistically significant non-proportionality was indicated ($p < 0.001$). RBC AUC_{120} values were 25.1 μg equiv.h/ml for males and 21.2 μg equiv.h/ml for females at 10 mg/kg and 530 μg equiv.h/ml for males and 331 μg equiv.h/ml for females at 200 mg/kg. The RBC AUC_{120} values in males were ~ 1.1 fold higher, and those in females were $\sim 22\%$ lower, than predicted from a linear relationship. C_{max} and AUC_{120} values for females were $\sim 50\%$ lower for plasma and $\sim 40\%$ lower for red blood cells than corresponding values in males and the differences were statistically significant ($p < 0.001$). The plasma elimination half-lives (plasma $t_{1/2}$) were calculated: 15.5 hours for males and 14.2 hours for females at 10 mg/kg and 19.4 hours for males and 34.1 hours for females at 200 mg/kg. Calculated red blood cell elimination half-lives (RBC $t_{1/2}$) were 478 hours for males and 70.8 hours for females at 10 mg/kg and 276 hours for males and 144 hours for females at 200 mg/kg (however, the standard error associated with both male values and the high dose female value was large).

Quantitative Tissue Distribution (Groups 3a and 3b)

16 rats per sex per group received a single oral gavage dose of ^{14}C -NF-149 at 10 mg/kg (Group 3a) or 200 mg/kg (Group 3b). 4 rats per sex were sacrificed at T_{max} (4 hours for Group 3a animals and 12 (males) and 6 hours (females) for Group 3b animals), 24, 48, and 72 hours post-dosing.

Group 3a

Group means of 92.5% (males) and 86.0% (females) for dosed radioactivity were found in the gastrointestinal tract (plus contents) at the 4 hour sacrifice and 0.6% (males) and 0.4% (females) of dose were detected at the 72 hour sacrifice. At 72 hours, the largest proportion of dose was in liver (0.6% of dose for both sexes), muscle (0.4% (males) and 0.2% (females)), fat (0.2% (males) and 0.5% (females)), and skin (0.2% of dose for both sexes)).

At the 4-hour sacrifice (T_{max}), group mean concentrations of radioactivity were highest in the gastrointestinal tract plus contents (95.1 μg equiv/g (males) and 99.6 μg equiv/g (females)) and liver (7.91 μg equiv/g (males) and 5.52 μg equiv/g (females)). Moderate concentrations of radioactivity (0.686 μg equiv/g to 3.00 μg equiv/g) were detected in adrenal glands, fat, kidneys, lungs, ovaries, pancreas, and skin. Other tissues with concentrations of radioactivity higher than plasma levels (0.799 μg equiv/g (males) and 291 μg equiv/g (females)) included heart, muscle, spleen, thyroid, and uterus.

At the 24-hour sacrifice, group mean radioactivity concentrations had declined in all tissues except fat (2.56 μg equiv/g) and bone marrow (0.409 μg equiv/g) in females. Tissue concentrations were highest in the gastrointestinal tract plus contents (5.95 μg equiv/g (males) and 12.5 μg equiv/g (females)) and liver (2.93 μg equiv/g (males) and 2.64 μg equiv/g (females)). Other tissues with radioactivity concentrations higher than plasma (0.248 μg equiv/g (males) and

0.103 µg equiv/g (females)) included adrenal glands, bone marrow, epididymis, kidneys, lungs, ovaries, pancreas, prostate, skin, whole blood, uterus, and red blood cells.

At the 48-hour sacrifice, group mean radioactivity concentrations were decreased in all tissues compared to the 24-hour sacrifice values. Concentrations remained highest in the gastrointestinal tract (1.19 µg equiv/g (males) and 2.14 µg equiv/g (females)), fat (0.332 µg equiv/g (males) and 1.51 µg equiv/g (females)), and liver (1.57 µg equiv/g (males) and 1.49 µg equiv/g (females)). Tissues with radioactivity concentrations equal or higher than plasma levels (0.107 µg equiv/g (males) and 0.047 µg equiv/g (females)) included adrenal glands, bone marrow, heart, kidneys, lungs, ovaries, muscle, pancreas, skin, uterus, whole blood, and red blood cells.

At the 72-hour sacrifice, group mean concentrations of radioactivity had declined in all tissues except thyroid compared to the 48-hour sacrifice time. Concentrations remained highest in liver (1.02 µg equiv/g (males) and 1.13 µg equiv/g (females)), fat (0.192 µg equiv/g (males) and 0.542 µg equiv/g (females)), and gastrointestinal tract (0.429 µg equiv/g (males) and 0.405 µg equiv/g (females)). Radioactivity concentrations greater than plasma values (0.068 µg equiv/g (males) and 0.032 µg equiv/g (females)) were detected in adrenal glands, bone marrow, epididymis, heart, kidneys, lungs, muscle, ovaries, pancreas, skin, thyroid, uterus, whole blood, and red blood cells.

Group mean elimination half-life ($t_{1/2}$) values for the decline of radioactivity in tissues after a single oral gavage dose of ^{14}C -NF-149 at 10 mg/kg ranged from 7.0 to 30.4 hours for males and 8.0 to 56.8 hours for females (values were longest for red blood cells in males (30.4 hours) and fat in females (56.8 hours)). Group mean plasma $t_{1/2}$ values were 15.3 hours (males) and 16.9 hours (females).

Group 3b

At the T_{max} sacrifice times (12 hours for males and 6 hours for females after a single oral gavage dose of ^{14}C -NF-149 at 200 mg/kg), group mean values for the proportion of dosed radioactivity in the gastrointestinal tract plus contents were 33.1% of dose for males and 77.5% of dose for females, at the 72-hour sacrifice, group mean values were 0.2% (males) and 0.4% (females) of dose. The highest group mean values for retained radioactivity in tissues at 72-hours were in fat (0.2% (males) and 0.9% (females)), liver (0.2% for both sexes), and skin (0.14% (males) and 0.15% (females)).

At the T_{max} sacrifice times (12-hours for males and 6-hours for females), group mean concentrations of radioactivity were highest in the gastrointestinal tract plus contents (722 µg equiv/g (males) and 2350 µg equiv/g (females)), fat (89.7 µg equiv/g (males) and 98.7 µg equiv/g (females)), liver (85.0 µg equiv/g (males) and 69.5 µg equiv/g (females)), and adrenal glands (12.4 µg equiv/g (males) and 53.5 µg equiv/g (females)). Tissues with group mean concentrations of radioactivity greater than plasma values (11.2 µg equiv/g (males) and 5.52 µg equiv/g (females)) included brain, epididymis, heart, kidneys, lungs, muscle, ovaries, pancreas, prostate, seminal vesicles, skin, spleen, thyroid, and uterus.

At the 24-hour sacrifice, group mean concentrations of radioactivity had declined in all tissues except bone marrow (5.08 µg equiv/g (males) and 3.26 µg equiv/g (females)) and fat (42.3 µg equiv/g (males) and 115.0 µg equiv/g (females)). Group mean tissue concentrations of radioactivity were highest in gastrointestinal tract plus contents (129 µg equiv/g (males) and 212 µg equiv/g (females)) and liver (28.3 µg equiv/g (males) and 21.8 µg equiv/g (females)). Tissues with group mean radioactivity concentrations greater than plasma values (5.27 µg equiv/g (males) and 1.37 µg equiv/g (females)) included adrenal glands, epididymis, heart, kidneys, lungs, ovaries, muscle, pancreas, prostate, skin, spleen, uterus, and red blood cells.

At the 48-hour sacrifice, group mean concentrations of radioactivity had declined in all tissues compared to the 24-hour sacrifice except in ovary (12.4 µg equiv/g), uterus (4.36 µg equiv/g), whole-blood (2.24 µg equiv/g (males) and 1.51 µg equiv/g (females)), and red blood cells (2.78 µg equiv/g (males) and 1.99 µg equiv/g (females)). The highest group mean concentrations were found in fat (15.5 µg equiv/g (males) and 90.1 µg equiv/g (females)), gastrointestinal tract plus contents (10.7 µg equiv/g (males) and 40.5 µg equiv/g (females)), liver (12.7 µg equiv/g (males) and 20.6 µg equiv/g (females)), and ovaries (12.4 µg equiv/g). Group mean tissue concentrations that remained greater than plasma values (1.97 µg equiv/g (males) and 1.19 µg equiv/g (females)) were detected in adrenal glands, bone marrow, epididymis, heart, kidneys, lungs, muscle, pancreas, prostate, and skin.

At the 72-hour sacrifice, group mean concentrations of radioactivity had declined in all tissues except pituitary (females) compared to the 48-hour sacrifice time. Group mean tissue concentrations were highest in fat (4.49 µg equiv/g (males) and 24.7 µg equiv/g (females)), gastrointestinal tract plus contents (3.97 µg equiv/g (males) and 10.1 µg equiv/g (females)), and liver (7.76 µg equiv/g (males) and 9.30 µg equiv/g (females)). Tissues with group mean radioactivity concentrations greater than plasma values (0.92 µg equiv/g (males) and 0.34 µg equiv/g (females)) included adrenal glands, epididymis, heart, kidneys, lungs, muscle, ovaries, pancreas, pituitary, prostate, skin, spleen, uterus, whole-blood, and red blood cells.

Group mean elimination half-life ($t_{1/2}$) values for tissues after a single oral gavage dose of ^{14}C -NF-149 at 200 mg/kg ranged from 6.0 to 29.6 hours for males and 7.3 to 51.7 hours for females (values were longest for red blood cells in both sexes (males (29.6 hours) and females (51.7 hours) (uterus values also matched the RBC values in females)) . Group mean plasma $t_{1/2}$ values were 14.7 hours (males) and 19.9 hours (females).

Quantitative Tissue Distribution (Group 4a) and Excretion Balance/Tissue Distribution (Group 4b) After 14 Consecutive Daily Doses

Group 4a

4 rats per sex were sacrificed at 4 hours (plasma T_{max}) after the last of 14 consecutive daily oral gavage doses of ^{14}C -NF-149 at 10 mg/kg/day. Group mean concentrations of radioactivity were highest in the gastrointestinal tract plus contents (142 µg equiv/g (males) and 129 µg equiv/g (females)), liver (16.2 µg equiv/g (males) and 12.9 µg equiv/g (females)), and fat (3.28 µg equiv/g (males) and 9.98 µg equiv/g (females)). Tissues with group mean concentrations of radioactivity greater than plasma values (1.59 µg equiv/g (males) and 0.684 µg equiv/g (females)) included adrenal glands, bone marrow, heart, kidneys, lungs, ovaries, pancreas, pituitary, prostate, skin, spleen, thyroid, uterus, red blood cells, and whole-blood.

Following the sacrifice at 24 hours after the final dose, group mean concentrations of radioactivity had declined in all tissues except pituitary (females). Group mean concentrations remained highest in the gastrointestinal tract plus contents (15.2 µg equiv/g (males) and 18.4 µg equiv/g (females)), liver (7.60 µg equiv/g (males) and 6.99 µg equiv/g (females)), and fat (2.00 µg equiv/g (males) and 7.65 µg equiv/g (females)). Other tissues with group mean concentrations of radioactivity greater than plasma values (0.749 µg equiv/g (males) and 0.286 µg equiv/g (females)) included adrenal glands, heart, kidneys, lungs, muscle, ovaries, pancreas, pituitary, skin, spleen, thyroid, uterus, red blood cells, and whole-blood.

At 168 hours after the final dose, group mean concentrations of radioactivity had declined in all tissues compared to the 24 hour sacrifice. Tissue concentrations of radioactivity were highest in liver (1.34 µg equiv/g (males) and 2.07 µg equiv/g (females)). Tissue concentrations of radioactivity that were higher than plasma concentrations (0.069 µg equiv/g (males) and 0.044 µg equiv/g (females)) were detected in adrenal glands, bone marrow, brain, epididymis, fat, gastrointestinal tract plus contents, heart, kidneys, lungs, muscle, pancreas, pituitary, skin,

spleen, thyroid, uterus, red blood cells, and whole-blood.

Group mean elimination half-life ($t_{1/2}$) values for the decline of radioactivity in tissues after 14 consecutive oral gavage doses of ^{14}C -NF-149 at 10 mg/kg/day ranged from 18.0 to 133.3 hours for males and 19.4 to 231.0 hours for females (values were longest for red blood cells in males (133.3 hours) and females (231.0 hours). Group mean plasma $t_{1/2}$ values were 38.1 hours (males) and 45.3 hours (females).

Group 4b

Animals received 14 consecutive daily oral gavage doses of ^{14}C -NF-149 at 10 mg/kg/day. Excreta were collected for 24 hour periods after the first, fifth, and tenth dose and for the 0 to 168 hour period after the 14th dose. Urinary excretion during these sampling periods accounted for 16.34% to 35.15% (males) and 10.05% to 17.54% (females) of daily dose. Elimination in feces accounted for 72.79% to 93.37% (males) and 68.02% to 111.14% (females) of daily dose during the sampling periods. The highest percentages of dose for both urine and feces in each sex represented the 0 to 168 hour periods.

Biliary Excretion (Groups 5a and 5b)

Group 5a

4 bile duct and stomach cannulated rats per sex received single doses of ^{14}C -NF-149 *via* stomach cannula at 10 mg/kg. During 48 hours post-dosing, 60.6% (males) and 77.4% (females) of dose were excreted in bile; 8.3% (males) and 5.4% (females) of dose in urine; and 24.2% (males) and 15.6% (females) of dose in feces. Radioactivity remaining in the carcass at 48 hours post-dosing accounted for 0.9% (males) and 1.9% (females) of dose.

Group 5b

During 48 hours after single doses of ^{14}C -NF-149 *via* stomach cannula at 200 mg/kg (4 rats per sex), 33.5% (males) and 43.2% (females) of dose were excreted in bile; 61.5% (males) and 54.3% (females) in feces; and 5.8% (males) and 3.4% (females) in urine. Radioactivity remaining in the carcass at 48 hours accounted for 0.8% (males) and 3.8% (females) of dose.

Metabolites/Components in Pooled Urine Samples

Following single oral gavage doses of ^{14}C -NF-149 at 10 mg/kg and 200 mg/kg, metabolite/component profiles of treated (β -glucuronidase/sulphatase) and untreated urine were qualitatively similar at both doses. 18 metabolites/components were found and 2 were identified (reverse phase HPLC and normal phase TLC). The 2 identified metabolites were the major components of the pooled urine samples: U3 (149-F1) (2,3-difluoro-6-trifluoromethylbenzamidine) accounted for 13.6% (10 mg/kg) and 10.7% (200 mg/kg) of dose in males and 8.3% (10 mg/kg) and 5.1% (200 mg/kg) of dose in females and U7 (149-F6) (2,3-difluoro-6-trifluoromethylbenzamide) at 0.9% (10 mg/kg) and 1.0% (200 mg/kg) of dose in males and 0.3% (10 mg/kg) and 0.1% (200 mg/kg) in females. After consecutive daily oral doses of ^{14}C -NF-149 at 10 mg/kg for up to 14 days, metabolite profiles in urine were qualitatively similar to those following a single oral dose. Metabolite U3 (149-F1) accounted for 7.2% (males) and 4.5% (females) at day 1; 9.5% (males) and 4.1% (females) at day five; 9.8% (males) and 7.5% (females) at day 10; and 14.4% (males) and 6.3% (females) during 0-96 hours after the last dose. The other identified metabolite, U7 (149-F6) accounted for 0.9% (males) and 0.2% (females) at day one; 1.1% (males) and 0.2% (females) at day five; 1.7% (males) and 0.4% (females) at day 10; and 3.0% (males) and 0.6% (females) during 0-96 hours after the last dose.

Metabolites/Components in Pooled Fecal Extracts

18 metabolites/components were found in pooled fecal extracts following single oral gavage doses of ^{14}C -NF-149 at 10 mg/kg and 200 mg/kg. Similar metabolite profiles were obtained for males and females. After a 10 mg/kg dose, the major identified metabolite FE11 (149-F-3-OH-B) (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(3-hydroxyphenyl)acetamide) accounted for 10.9% (males) and 27.3% (females) of dose. Other identified metabolites/components included FE16 (the parent compound, NF-149) at 3.7% (males) and 4.6% (females); FE12 (149-F-4-OH-B) (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(4-hydroxyphenyl)acetamide) at 1.9% (males) and 6.2% (females); FE8 (149-F) (N-cyclopropylmethoxy-2,3-difluoro-6-trifluoromethylbenzamidine) at 4.4% (males) and 2.8% (females); and FE1/2 (149-F1) (2,3-difluoro-6-trifluoromethylbenzamidine) at 1.0% (males) and 1.1% (females). After a 200 mg/kg dose, FE16 (unchanged NF-149) was the major component in fecal extracts at 42.0% (males) and 50.5% (females) of dose. FE11 accounted for 5.2% (males) and 7.9% (females) of dose and FE12 for 3.4% to 5.3% (males) and 8.9% to 18.5% (females).

After consecutive daily oral doses of ^{14}C -NF-149 at 10 mg/kg, the major component in fecal extracts was FE16 (unchanged NF-149) at all sampling times (values ranged from 27.3% to 39.1% (males) and 19.6% to 35.8% (females)). Metabolite FE11 accounted for 6.7% to 11.2% (males) and 13.7% to 16.9% (females) across the sampling times.

Metabolites/Components in Pooled Bile Samples

Following single doses of ^{14}C -NF-149 at 10 mg/kg and 200 mg/kg, metabolite profiles of untreated (no enzymatic treatment) bile were qualitatively similar. 2 of the 18 metabolites/components found in pooled samples were identified. B15 (149-F-4-OH-B) (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(4-hydroxyphenyl)acetamide) accounted for 0.4% (males) and 0.5% (females) of dose after 10 mg/kg and 0.2% (both sexes) after 200 mg/kg. B16 (149-F- α -OH-B) (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-hydroxy-2-phenylacetamide) accounted for 0.2% of dose (both sexes) after 10 mg/kg and 0.1% (males) and <0.1% (females) after 200 mg/kg. The major fraction, B12 (not structurally identified), accounted for 31.0% (males) and 51.3% (females) of dose after 10 mg/kg and 16.6% (males) and 30.1% (females) after 200 mg/kg. Following incubation with β -glucuronidase/sulphatase, metabolite B15 increased to 14.9% (males) and 36.9% (females) of dose after 10 mg/kg and 8.6% (males) and 19.8% (females) after 200 mg/kg and B16 accounted for 3.5% (males) and 3.4% (females) of dose after 10 mg/kg and 3.1% (males) and 3.6% (females) after 200 mg/kg. B12 accounted for 6.3% (males) and 5.7% of dose (females) after 10 mg/kg and 2.9% (males) and 4.2% (females) after 200 mg/kg following enzyme treatment.

Metabolites/Components in Pooled Plasma Samples

Pooled plasma samples obtained from rats sacrificed 4 hours (T_{max}) after single oral gavage doses of ^{14}C -NF-149 at 10 mg/kg contained 12 metabolites/components. 3 components were identified: P1 (149-F1) (2,3-difluoro-6-trifluoromethylbenzamidine) at 2.8% (males) and 1.2% (females) of dose; P7 (149-F) (N-cyclopropylmethoxy-2,3-difluoro-6-trifluoromethylbenzamidine) at 5.4% (males) and 6.2% (females); and P11 (unchanged NF-149) at 7.1% (males) and 2.3% (females). Two major metabolites/components remained unidentified (one accounted for 23.2% (males) and 23.0% (females) of dose and other for 22.7% (males) and 26.8% (females) of dose). Following single oral gavage doses at 200 mg/kg and sacrifice at T_{max} (12 hours (males) and 6 hours (females)), P1 accounted for 19.7% (males) and 9.6% (females) of dose; P7 for 7.2% (males) and 6.9% (females) of dose; and P11 for 2.4% (males) and 39.3% (females) of dose. Following 14 consecutive daily oral doses of ^{14}C -NF-149 at 10 mg/kg, plasma samples were obtained from rats sacrificed at 4 hours after the last dose. The major identified metabolites in pooled plasma samples included P1 (149-F) at 31.0% (males) and 8.5% (females) of dose and P11 (unchanged NF-149) at 0.7% (males) and 13.4% (females) of dose.

Metabolites/Components in Pooled Liver Samples

Following single oral gavage doses of ^{14}C -NF-149 at 10 mg/kg and 200 mg/kg, the liver metabolic/component profiles were qualitatively similar at T_{max} (4-hours for both sexes at 10 mg/kg and 12 hours (males) and 6 hours (females) at 200 mg/kg). After 10 mg/kg, L2 (149-F1) accounted for 28.1% (males) and 22.6% (females) of dose; L12 (149-F- α -OH-B) for 2.5% (males) and 4.4% (females) of dose; and L13 (unchanged NF-149) for 1.7% (males) and 6.9% (females). After 200 mg/kg, L2 accounted for 34.4% (males) and 21.1% (females) of dose; L12 for <0.7% (males) and <1.3% (females) of dose; and L13 for 1.7% (males) and 27.8% (females). Following 14 consecutive daily oral gavage doses of ^{14}C -NF-149 at 10 mg/kg/day, liver samples were obtained from rats sacrificed 4 hours after the last dose. Major metabolites/components in pooled liver extracts included L2 at 17.8% (males) and 8.8% (females) and L13 at 2.1% (males) and 13.1% (females) of dose.

Metabolites/Components in Pooled Kidney Samples

After single oral gavage doses of ^{14}C -NF-149 at 10 mg/kg and 200 mg/kg, kidney metabolite/component profiles were qualitatively similar at T_{max} (4-hours for both sexes at 10 mg/kg and 12 hours (males) and 6 hours (females) at 200 mg/kg). After 10 mg/kg, metabolite K2 (149-F1) accounted for 35.6% (males) and 47.2% (females) of dose and K11 (unchanged NF-149) accounted for 32.9% (males) and 18.7% (females). After 200 mg/kg, metabolite K2 was 35.5% (males) and 15.4% (females) of dose and K11 accounted for 1.5% (males) and 27.4% (females). Radioactive metabolites/components in kidney samples obtained 4 hours after the last of 14 consecutive daily oral gavage doses of ^{14}C -NF-149 at 10 mg/kg included K2 (149-F1) at 23.1% (males) and 22.4% (females) of dose; K4 (149-F6) (2,3-difluoro-6-trifluoromethylbenzamide) at 15.3% (males) and 14.0% (females); and K11 (unchanged NF-149) at 3.9% (males) and 13.4% (females).

Metabolites/Components in Pooled Fat Samples

Following single oral gavage doses of ^{14}C -NF-149 at 10 mg/kg and 200 mg/kg and sacrifices at T_{max} (4 hours for both sexes at 10 mg/kg and 12 hours (males) and 6 hours (females) at 200 mg/kg), fat metabolite/component profiles were similar. 3 metabolites/components were found. After 10 mg/kg, F1 (149-F- α -OH-B) (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-hydroxy-2-phenylacetamide) was 9.8% (males) and 3.4% (females) of dose; F2 (unchanged NF-149) was 53.4% (males) and 49.7% (females) of dose; and F3 (unidentified) accounted for 2.0% (males) and 1.0% (females) of dose. After 200 mg/kg, F1 was 7.6% (males) and 1.2% (females) of dose; F2 was 67.2% (males) and 65.1% (females); and F3 accounted for <0.1 (males) and 2.8 (females) of dose. Following 14 consecutive daily oral gavage doses of ^{14}C -NF-149 at 10 mg/kg/day with sacrifice 4 hours after the final dose, profiles for the 3 metabolites/components in fat were F1 at 3.4% (males) and 2.8% (females) of dose; F2 at 49.1% (males) and 30.6% (females); and F3 at <0.1 (males) and 5.9% (females).

The proposed metabolic pathway for NF-149 in the rat is by two main pathways. In one pathway, NF-149 is hydrolyzed to 149-F (N-cyclopropylmethoxy-2,3-difluoro-6-trifluoromethylbenzamide) and then reduced to the amidine 149-F1 (2,3-difluoro-6-trifluoromethylbenzamide). 149-F1 is then deaminated to form 149-F6 (2,3-difluoro-6-trifluoromethylbenzamide). In the other main pathway, 149-F-3-OH-4-OH-B (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(3,4-dihydroxyphenyl)acetamide) is formed by di-hydroxylation of the non-fluorinated phenyl ring and is subsequently converted to the methoxy derivative. The intermediate mono-hydroxylated metabolites 149-F-3-OH-B (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(3-hydroxyphenyl)acetamide) and 149-F-4-OH-B (N-(α -cyclopropylmethoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(4-hydroxyphenyl)acetamide) are also present. Hydroxylation of the α position occurs as a minor pathway. The minor pathway

proceeds *via* cleavage of the cyclopropylmethoxy moiety to form 149-F4B (N-phenylacetyl-2,3-difluoro-6-trifluoromethylbenzamidoxime) which is then converted to a glucuronide conjugate or cyclized to the oxadiazole 149-F12 (3-(2,3-difluoro-6-trifluoromethylphenyl)-5-benzyl-1,2,4-oxadiazole. Acceptable. (Green, 5/5/10).

**53086-0050 245036, “[Cyclopropyl-2,3-¹⁴C]NF-149 - Metabolism Study in Rat. Balance Study of Rat at Low Dose Level”, (H. Mori, Nippon Soda Co., Ltd., Odawara Research Center, Metabolism & Chemistry Laboratory (NSM), Odawara, Kanagawa, Japan, Report No. NSM01-021, 8 February 2002). 4 Sprague-Dawley strain (SD) rats per sex received a single oral gavage dose of [Cyclopropyl-2,3-¹⁴C]NF-149 at 10 mg/kg. Urine samples were collected at 12 and 24 hours post-dosing and at 24 hour intervals through 168 hours thereafter. Feces were collected at 24 hours post-dosing and at 24 hour intervals through 168 hours thereafter. Whole blood was collected at sacrifice (168 hours post-dosing) and a portion separated into plasma and erythrocytes by centrifugation (15 minutes at 3000 rpms). During 168 hours post-dosing, average values of 31.7% (males) and 19.7% (females) of dose were excreted in urine (most was excreted during 0 to 12 hours post-dosing - 22.4% for males and 10.2% for females). 5.2% (males) and 4.7% (females) of dose was excreted in urine during 12 to 24 hours post-dosing and 2.5% (males) and 2.8% (females) during 24 to 48 hours. Fecal excretion (average values) accounted for 56.9% (males) and 69.5% (females) during 0 to 168 hours post-dosing (42.7% (males) and 47.5% (females) of dose was excreted during 0 to 24 hours and 11.9% (males) and 17.1% (females) during 24 to 48 hours post-dosing). 4.2% (males) and 3.1% (females) of dose remained in the carcass and fat contained 1.5% (males) and 1.0% (females) of dose at sacrifice.

Group average concentrations of radioactivity in tissues (µg equivalents of ¹⁴C-NF-149 per g of fresh tissue (µg equiv/g) at sacrifice were highest in fat (2.30 µg equiv/g (males) and 1.82 µg equiv/g (females)); thyroid (0.97 µg equiv/g (males) and 0.92 µg equiv/g (females)); and epididymis (0.86 µg equiv/g). Intermediate concentrations of radioactivity were detected in adrenal gland (0.473 µg equiv/g (males) and 0.541 µg equiv/g (females)); liver (0.341 µg equiv/g (males) and 0.593 µg equiv/g (females)); ovary (0.570 µg equiv/g); pancreas (0.642 µg equiv/g (males) and 0.414 µg equiv/g (females)); prostate (0.537 µg equiv/g); and skin with hair (0.302 µg equiv/g (males) and 0.148 µg equiv/g (females)). Average concentrations of radioactivity in other tissues were <0.3 µg equiv/g.

The major metabolite in urine collected during 0 to 72 hours post-dosing was CPCA-Gly (2-(cyclopropylcarbonylamino)acetic acid) at average values of 30.0% (males) and 18.0% (females). The major metabolite in feces was 149-F-3-OH-4-OH-B (N-(α-cyclopropyl-methoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(3,4-dihydroxyphenyl)acetamide) at average values of 33.4% (males) and 41.4% (females) of dose. Other fecal metabolites included 149-F-3-OH-B (N-(α-cyclopropyl-methoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(3-hydroxyphenyl)acetamide) at 3.2% (males) and 1.6% (females) and 149-F-4-OH-B (N-(α-cyclopropyl-methoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-(4-hydroxyphenyl)acetamide) at 4.5% (males) and 9.8% (females). Unchanged NF-149 was found at trace levels (males) and 3.24% (females) of dose and metabolite 149-F-α-OH-B (N-(α-cyclopropyl-methoxyimino-2,3-difluoro-6-trifluoromethylbenzyl)-2-hydroxy-2-phenylacetamide) at trace levels (both sexes).

A diagram of the proposed metabolic pathway of [cyclopropyl-2,3-¹⁴C]NF-149 in rats was included. Acceptable. (Green, 5/6/10).

**53086-0051 245037, “[Cyclopropyl-2,3-¹⁴C] NF-149 - Metabolism Study in Rat. Blood Concentration of Rat at Low Dose Level”, (H. Mori, Metabolism & Chemistry Laboratory (NSM) Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan, Report No. NSM01-004, 29 June 2001). 4 Sprague-Dawley strain (SD) rats per sex received a single oral gavage dose of [Cyclopropyl-2,3-¹⁴C]NF-149 at 10 mg/kg. A sample of blood (~30 mg) was collected from each animal at 0.25, 0.5, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, and 120 hours after

dosing. Animals were sacrificed after the last sampling. Whole blood was centrifuged to separate plasma and erythrocytes. In plasma, average C_{max} values (μg equivalents of [Cyclopropyl-2,3- ^{14}C]NF-149 per g (μg equiv/g)) of 1.30 μg equiv/g for males and 0.91 μg equiv/g for females were reached at 2 hours (T_{max}) after dosing for both sexes. Average elimination half-lives ($t_{1/2}$) were 6.48 hours (males) and 7.92 hours (females). Average plasma values for the area under the radioactivity concentration time curve (AUC_{120}) were 12.34 μg equiv.h/g for males and 10.00 μg equiv.h/g for females. In erythrocytes, average C_{max} values of 0.31 μg equiv/g (males) and 0.35 μg equiv/g (females) were determined at 2 hours post-dosing (T_{max}) for both sexes. Average values for $t_{1/2}$ were 25.29 hours for males and 9.03 hours for females and average AUC_{120} values were 9.88 μg equiv.h/g (males) and 4.64 μg equiv.h/g (females). Acceptable. (Green, 5/11/10).

53086-0064 245050, "Mechanistic Investigation of the Increment of Serum ALP Activity in Dogs Treated with NF-149", (M. Oh-hira, Nippon Soda Co., Ltd., Odawara Research Center, Toxicologist Laboratory, Odawara, Kanagawa, Japan, Report No. P588, 5 July 2002). The study was performed to determine the origin of the increased serum alkaline phosphatase (ALP) activity resulting from dietary intake of NF-149. Three male Beagle dogs per group received NF-149 (95.9%) in the diet at 0 (basal diet) and 4000 ppm for 14 days (400 g of diet were offered to each animal from 11 am to 4 pm daily). Blood samples were collected (cephalic vein) and serum total ALP activity was measured (Hitachi 7070-type automatic analyzer) on days 0 (just before feeding), 7, and 14 (the net change of activity for days 0 - 7 and 0 - 14 were also calculated). For analysis of ALP isoenzymes, serum samples collected pretreatment (day 0) and on treatment day 14 were subjected to polyacrylamide gel electrophoresis (constant current at 3mA per sample for 110 minutes). Group mean achieved dietary intake of NF-149 at 4000 ppm was 112.3 mg/kg/day. No treatment-related changes were noted for clinical signs, bodyweights, and food consumption. Group mean total serum ALP activity calculated for days 0 - 7 and 0 - 14 were slightly decreased for the control animals and significantly increased for 4000 ppm animals (the increased activity was determined to be due to the liver derived isoenzyme fraction). Supplemental data. (Green, 4/7/10).

53086-0065 245051, "Effect of NF-149 on the Peroxidase Activity", (T. Sasaki, Odawara Research Center, Nippon Soda Co., Ltd., Odawara, Kanagawa, Japan, Study No. H209, 18 January 2001). The study was performed to investigate the inhibitory effect of NF-149 on porcine thyroid peroxidase activity. Porcine thyroid glands (purchased from a slaughter house) were homogenized and centrifuged to obtain the microsomal fraction (6.6 mg protein/ml). The pellet was resuspended in 0.2M phosphate buffer (pH 7.4) and stored for 64 days at -80°C . Subsequently, the microsomal fraction was assayed (in triplicate) for oxidation of guaiacol after treatment with NF-149 (in 90% DMSO solution) at $1 \times 10^{-5}\text{M}$, $1 \times 10^{-4}\text{M}$, $5 \times 10^{-4}\text{M}$, and $1 \times 10^{-3}\text{M}$ (concentrations ranged from approximately 4.1 to 412 ppm) by measuring (with a spectrophotometer at 470 nm) the suppression of absorbance (Δ Abs/min) compared to the vehicle control. Average values of triplicate assays were used to calculate 50% inhibition concentrations (IC_{50}) by the Probit method. 6-propyl-2-thiouracil (PTU) in 0.1N NaOH solution was used as the positive control. No inhibitory effect on peroxidase activity after treatment with NF-149 was noted (i.e., no suppression of absorbance was noted). The positive control was functional. Supplemental data. (Green, 4/12/10).

53086-0066 245052, "Effect of NF-149 on Hepatic Drug Metabolizing Enzymes in Male Mice", (Y. Kanaguchi, Odawara Research Center, Odawara, Kanagawa, Japan, Report No. H202, 30 November 2000). 5 Crj: CD-1 (ICR) SPF male mice per group received NF-149 (95.9%) in the diet at 0 (basal diet), 60, and 2000 ppm for 14 days (positive control animals received phenobarbital at 500 ppm). All animals survived the treatment period. No treatment-related changes were noted for clinical signs, bodyweight changes, or food consumption. At sacrifice after 14 days of treatment, no treatment-related liver weight change or hepatic lesions were noted. Significant increases in cytochrome P-450 activity were noted at 2000 ppm vs negative controls (UDP-glucosyltransferase activity and sulfotransferase activity were comparable to

negative controls). At 60 ppm, liver enzyme activities were all comparable to negative control values. In the positive control group (phenobarbital and 500 ppm), significant increases were noted for cytochrome P-450 activity and sulfotransferase activity (UDP-gluconyltransferase activity was not affected by treatment) vs the negative controls. Supplemental data. (Green, 2/8/10).

53086-0054 245040, "Consideration of the Metabolism and E-Fate of the Phenylacetic Acid Moiety of NF-149", (T. Kawai, Nippon Soda Co., Ltd., Odawara Research Center, Metabolism & Chemistry Laboratory (NSM), Odawara, Kanagawa, Japan, Report No. RD-II 02241, 22 August 2002). This document includes brief results summaries of 3 studies with NF-149: a rat metabolism study (record 245035), an aerobic soil metabolism study, and a wheat metabolism study relative to the environmental fate of the phenylacetic acid moiety (PAA) of NF-149 after treatment. PAA was indicated as a naturally occurring compound since (1) it was found at the in rat urine from animals treated with NF-149 and from untreated control animals; (2) it was found in higher concentrations in untreated soil than in NF-149 treated soil; and (3) it was present in grain in very low amounts in wheat treated with NF-149 (and the cleavage of the amide group in the parent molecule was not indicated as a major metabolic pathway). Supplemental information. (Green, 5/11/10).

53086-0055 245041, "NF-149 (Cyflufenamid), [Cyclopropyl-¹⁴C] NF-149 Metabolism and E-Fate Studies", (T. Kawai, Nippon Soda Co., Ltd., Odawara Research Center, Metabolism & Chemistry Laboratory (NSM), Odawara, Kanagawa, Japan, Report No. RD-II 02241, 22 August 2002). This document cites the results of an aerobic soil metabolism study, a wheat metabolism study, and a rat metabolism study in arguing that further studies with [Cyclopropyl-¹⁴C] NF-149 would not contribute more information to the risk profile. In the aerobic soil metabolism study, the cyclopropyl group decomposed rapidly to CO₂ and no metabolites derived from the cyclopropyl moiety could be detected, therefore, cyclopropanecarboxylic acid and its related compounds would not be present in significant amounts in the environment. There were no significant differences in residue profiles and residue levels between wheat metabolism studies conducted with [fluorinated phenyl-¹⁴C] NF-149 and those with [cyclopropyl-¹⁴C] NF-149 (residue levels in grain were very low and the main component in other matrices was parent compound only). The rat metabolism study with [cyclopropyl-¹⁴C] NF-149 was also cited (see record 245036). Supplemental information. (Green, 5/12/10).

53086-0063, 0067 245049, 245053, "Effect of NF-149 on the Carnitine Palmitoyltransferase" and NF-149 (Cyflufenamid) Heart Lesions in Rat and Mouse Toxicity Studies", (H. Takaori, Odawara Research Center, Odawara, Kanagawa, Japan, Report No. P590 and RDII02240, 5 July 2002 and 20 August 2002). The effect of NF-149 on heart carnitine palmitoyltransferase (CPT) from rat and mouse was investigated *in vitro*. Heart mitochondrial fraction from 3 IGS male rats (13 weeks of age), 2 IGS male rats (8 weeks of age), and 20 Crj:CD-1 (ICR) male mice (6 weeks of age) was collected. Positive controls included amiodarone, oxfenicine, and adriamycin and inhibited CPT activity *in vitro* at similar concentrations. NF-149 (95.9%) inhibited both rat (52% inhibition) and mouse (51% inhibition) CPT activity *in vitro* at 1 mM compared to the vehicle control (DMSO). In the discussion, the author proposes that fatty acids are the major oxidation fuel for the heart (while glucose and lactate provide the remaining need) and since CPTs are a transporter of long chain fatty acids into the mitochondrial matrix, inhibition of CPT may result in depression of myocardial metabolism and contribute to heart lesions (chronic myocarditis (rats), myocardial vacuolation (rats and mice), and fat deposition (mice)) seen at the highest dose in long term studies with NF-149. Supplemental data. (Green, 2/4/10).