

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
Pyraflufen-Ethyl

Chemical Code # 5865, Document Processing Number # 52951

20 November 2003
Revised: 21 June 2004

I. DATA GAP STATUS

Combined, rat	No data gap, possible adverse effect:
Chronic toxicity, dog:	No data gap, no adverse effects
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, no adverse effects
Teratology, rat:	No data gap, no adverse effects
Teratology, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, possible adverse effect
Chromosome effects:	No data gap, no adverse effects
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 211809 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

indicates a study in review.

File name: T040621

Prepared by H. Green, 12/12/03

Revised by T. Moore, 6/21/04

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

****52951-0062, 063 205935, 205936**, "ET-751: Combined Oncogenicity and Toxicity Study by Dietary Administration to CD Rats for 104 Weeks", (S.Patel, Huntingdon Life Sciences Ltd., Eye, Suffolk, England, Report number 96/NHH060/0448, 24 December 1996). 70 CD rats per sex per group received ET-751 Technical (97.6% pyraflufen-ethyl) in the diet at 0 (basal diet), 80, 400, 2000, and 10000 ppm for 104 weeks. 20 rats per sex per group were necropsied after 52 weeks of treatment for evaluation of chronic toxicity. Average dose levels were 3.4, 17.2, 86.7, and 468.1 mg/kg/day for males and 4.4, 21.8, 111.5, and 578.5 mg/kg/day for females at 80, 400, 2000, and 10000 ppm respectively. At 10000 ppm, perigenital staining was increased for females from week 22. Bodyweights were reduced 14% to 17% in males compared to controls during treatment. Anemia (spherocytosis and reduced packed cell volumes, hemoglobin concentration, mean cell volume, and mean cell hemoglobin) was noted for males throughout treatment. High urine volumes and low specific gravities were also noted for both sexes. Possible adverse effect: treatment related renal changes included papillary necrosis/sloughing (both sexes); acute papillitis (males); and papillary transitional cell hyperplasia and dilatation/hyperplasia of collecting ducts (females). In liver, electron-lucent vacuoles within the mitochondria from both centriacinar and peri-acinar hepatocytes of both sexes were seen at 52 weeks. Electron-lucent vacuoles from the centriacinar hepatocytes were present in all animals at 104 weeks. These liver changes were also seen at 2000 ppm. Chronic NOEL = 2000 ppm (anemia, bodyweight, perigenital staining). No neoplastic changes. Acceptable. (Green and Gee, 10/23/03)

CHRONIC TOXICITY, DOG

****52951-0059 205931**, "ET-751: Toxicity Study By Oral (Capsule) Administration To Beagle Dogs For 52 Weeks", (A. Broadmeadow, Huntingdon Life Sciences Ltd., Eye, Suffolk, England, Report number 96/NHH083/0883, 19 December 1996). 4 Beagle dogs per sex per group received ET-751 Technical (97.3% pyraflufen-ethyl) orally in gelatin capsules at 0 (empty capsule), 40, 200, and 1000 mg/kg/day for 52 weeks (7 days per week). No treatment related effects were indicated for clinical signs, food and water consumption, ophthalmology, blood, bone marrow, urine, organ weight, macroscopic and microscopic pathology. All dogs survived to study termination. Chronic NOEL = 1000 mg/kg/day. No adverse effects. Acceptable. (Green and Gee, 10/15/03).

ONCOGENICITY, MOUSE

****52951-0061 205934**, "ET-751: 78-Week Oral Oncogenicity Study in Mice", (Maki Kuwahara, The Institute of Environmental Toxicology, Suzuki-cho, Kodaira-shi, Tokyo, Japan, Study number IET 93-0100, 4 December 1996). 60 ICR (Crj:CD-1) mice per sex per group received ET-751 Technical (97.6% pyraflufen-ethyl) in the diet at 0 (basal diet), 200, 1000, and 5000 ppm for 78 weeks. 10 per sex per group were necropsied after 13 weeks. Average intake of ET-751 Technical was 20.99, 109.7, and 546.8 mg/kg/day for males and 19.58, 98.3, and 523.7 mg/kg/day for females at 200, 1000, and 5000 ppm respectively. At 1000 ppm, increases in liver masses were noted at terminal necropsy. Statistically significant increases in centrilobular hepatocellular swelling and hepatocellular vacuolation were recorded in males at 13 weeks. At terminal microscopy, in liver, significant increases were noted for foci of cellular alterations (acidophilic and clear cell foci) and brown pigment deposition of Kupffer cells in males; and for centrilobular hepatocellular swelling and single cell necrosis in females.

At 5000 ppm, anemia (decreased hematocrit, hemoglobin, and erythrocyte count in males and increased platelets in both sexes) was apparent after 13 weeks of treatment. Relative liver

weights were increased for males and females at the interim and terminal necropsies. Significant increases in pale color and accentuated lobular pattern in liver were noted for males and females at the 13 week interim sacrifice. An increased incidence of spots and masses in liver of both sexes was identified at terminal necropsy. Coarse liver surface was also increased in males at terminal sacrifice. Increases in non-neoplastic lesions were generally confined to liver: Increased Centrilobular hepatocellular swelling, hepatocellular vacuolation, increased brown pigment deposition of Kupffer cells, and micro-granuloma were noted at the interim and terminal sacrifice. Possible adverse effect: hepatocellular adenomas were significantly increased for males at 1000 and 5000 ppm and in females at 5000 ppm at terminal sacrifice. No treatment related changes were reported at 200 ppm. Chronic NOEL = 200 ppm (liver changes). No treatment related increases in malignant neoplasms. Acceptable. (Green and Gee, 10/10/03).

REPRODUCTION, RAT

**52951-0074 205950, "ET-751: Two-Generation Reproduction Study in Rats" (Sakiko Fujii, The Institute of Environmental Toxicology, Tokyo, Japan, Study Number IET 93-0102, 19 March 1996). 24 Crj:CD (SD) rats per sex per group received ET-751 in the diet at 0 (basal diet), 100, 1000, and 10000 ppm through two generations (1 litter per generation). Treatment began 10 weeks prior to mating in each generation. Average mg/kg/day for F0 animals were 6.84-7.78, 70.8-80.1, and 721.0-813.0 mg/kg/day; values for F1 parents were 8.10-9.06, 82.3-91.2, 844.0-901.0 mg/kg/day at 100, 1000, and 10000 ppm respectively. Bodyweights were reduced for F0 and F1 males (statistically significant) and F1 females at 10000 ppm. Significant increases in relative liver weights for F0 and F1 females and relative kidney weights for F0 and F1 males and for F1 females were noted at the high dose level. Necropsy revealed liver and kidneys dark in color in F0 and F1 males and females at 10000 ppm. Single cell necrosis, inflammatory cell infiltration, and increased brown pigment deposition in Kupffer cells were noted (statistically significant) in liver of F0 males and F1 males and females at microscopy. In kidney, increased brown pigment deposition in proximal tubular cells (statistically significant) was recorded in F0 and F1 males and females. Decreased bodyweight gains were noted for F1 and F2 offspring. Parental NOEL = 1000 ppm (bodyweight reduction, histological changes in liver and kidney). There were no treatment related effects on F0 and F1 parental reproduction performance at any dose level. Reproductive NOEL = 10000 ppm. Acceptable. (Green and Gee, 10/24/03).

52951-0075 205951, "Two-Generation Reproduction Study in Rats with ET-751, Preliminary Study", (Sakiko Fujii, The Institute of Environmental Toxicology, Tokyo, Japan, Study # ET 93-0101, 18 November 1994). 8 Crj:CD (SD) rats per sex per group received ET-751 Technical (97.6% pyraflufen-ethyl) in the diet at 0 (basal diet), 30, 300, 3000, and 10000 ppm from 3 weeks prior to mating, through breeding, until weaning of the F1 pups (3 weeks of age). Bodyweight (statistically significant) and food consumption were reduced for males at 10000 ppm throughout the treatment period. Necropsy revealed dark liver and kidneys in both sexes at 10000 ppm. Relative kidney weights were increased at the high dose level in both sexes and relative liver weights were increased in females. Pups weights at the high dose level were decreased (not statistically significant) during lactation. No treatment induced effects were noted at the other treatment levels. (Green and Gee, 10/24/03). No worksheet.

TERATOLOGY, RAT

**52951-0072 205945, "ET-751: Teratology Study Following Oral (Gavage) Administration in the Rat", (L.M. Burns, Huntingdon Life Sciences Ltd., Eye, Suffolk, England, Report 95/NHH082/0849, 11 December 1995). 22 mated female CD rats per group received ET-751 Technical (97% pyraflufen-ethyl) by oral gavage at 0 (0.5% (w/v) methylcellulose), 100, 300, and 1000 mg/kg/day on gestation days 6 through 15. No treatment related maternal effects. No treatment effects on implantation count and fetal growth and development. No adverse effects.

No teratogenicity. Maternal and Developmental NOEL = 1000 mg/kg/day. Acceptable. (Green and Gee, 10/15/03).

52951-0072 205946, "ET-751: Preliminary Teratology Study Following Oral (Gavage) Administration in the Rat", (L.M. Burns, Pharmaco LSR, Toxicology Services Worldwide, Eye, Suffolk, England, Report # 95/NHH079/0443, 18 October 1995). 6 mated female CD rats per group received ET-751 Technical (97% pyraflufen-ethyl) by oral gavage at 0 (0.5% w/v methylcellulose), 100, 300, and 1000 mg/kg/day on gestation days 6 through 15. No treatment-related effects on maternal mortality, clinical signs, bodyweight, food and water consumption, and macroscopic pathology. No teratogenicity, no treatment effect on implantation count, *in utero* growth or development. (Green and Gee, 10/15/03) (No worksheet).

TERATOLOGY, RABBIT

**52951-0073 205947, "ET-751: Teratology Study in the Rabbit", (L. M. Burns, Huntingdon Life Sciences Ltd., Eye, Suffolk, England, Report 95/NHH085/1329, 24 April 1996). 15 mated female New Zealand White rabbits per group received ET-751 Technical (97.0% pyraflufen-ethyl) by oral gavage at 0 (0.5% (w/v) aqueous methylcellulose), 20, 60, and 150 mg/kg/day on gestation days 6 through 19. At 150 mg/kg, two animals were sacrificed *in extremis* and 3 for humane reasons between gestation days 16 and 24. Reduced food intake and fecal output preceded the sacrifices. Necropsy revealed varying degrees of treatment-related disturbance in the gastrointestinal tract. All 5 were pregnant, but only 3 had viable fetuses. Post-implantation loss was greater than in the study controls and was above the upper limit of the historical control data range, but not statistically significant (27.1% versus 8.8% in controls). Mean live litter size was 5.8 at 150 mg/kg/day versus 8.9 in controls. One female at 60 mg/kg/day was found dead, two were sacrificed. Reduced food intake and fecal output preceded the deaths and, at necropsy, the gastro-intestinal disturbance was similar to that seen at the high dose. All 3 were pregnant, but only two had viable litters. Fetal and placental weights were unaffected by treatment. Maternal NOEL = 20 mg/kg/day (mortality). Developmental NOEL 150 mg/kg/day. No teratogenicity. Acceptable. (Green and Gee, 10/24/03).

GENE MUTATION

**52951-0076 205952, "ET-751: Assessment of Mutagenic Potential in Amino-Acid Auxotrophs of *Salmonella typhimurium* and *Escherichia coli* (the Ames Test)", (K. May, Pharmaco LSR Ltd., Eye, Suffolk, England, Report # 94/NHH072/0379, 17 August 1994). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* strain WP2 *uvrA* were exposed to ET-751 Technical (97.6% pyraflufen-ethyl), in the presence and absence of S9 rat liver fraction, at 0 (DMSO), 156.3, 312.5, 625, 1250, 2500, and 5000 $\mu\text{g}/\text{plate}$ for 48 hours in two separate assays. No increase in the reversion rate. Positive controls were functional. Acceptable. (Green and Gee, 10/31/03).

**52951-0080 205956, "ET-751: L5178Y TK +/- Mouse Lymphoma Mutation Assay Using the Methodology Recommended by the O.E.C.D. (1984)", (J. M. Lloyd, Pharmaco LSR Ltd., Eye, Suffolk, England, Report No. 94/NHH074/0600, 15 September 1994). Duplicate cultures of L5178Y TK +/- mouse lymphoma cells were exposed for 4 hours to ET-751 Technical (97.6% pyraflufen-ethyl), in the presence and absence of rat liver S9 (induced with Aroclor 1254), in two assays. In the first assay, concentrations were 0 (DMSO), 10, 20, 40, 60, and 80 $\mu\text{g}/\text{ml}$ in the absence of S9 and 0, 20, 40, 80, 120, and 160 $\mu\text{g}/\text{ml}$ with S9 mix present. Assay two used concentrations of 0, 20, 40, 60, 80, and 100 $\mu\text{g}/\text{ml}$ without S9, and 0, 40, 80, 120, 160, and 200 $\mu\text{g}/\text{ml}$ with S9 mix. After treatment, cells were resuspended in non-selective medium. Cell concentration of each culture was counted daily and maintained at 2×10^5 cells per ml. After 3 days, cells were plated in selective or non-selective medium triplicate plates for each per initial culture. Survival was estimated from non-selective plates and mutants were scored in selective plates containing trifluorothymidine (TFT). After 11-12 days of incubation at 37°C in 5% CO₂

atmosphere, the resultant colonies (diameter > 0.05 mm) were counted. No increase in mutagenic activity was recorded in the absence of S9 mix. Forward gene mutations were increased in the presence of S9 activation at weakly cytotoxic concentration levels with a control value of 13.3 (per 10⁵ survivors) versus 30.4 at 200 µg/ml and 22.8 at 160 µg/ml in the second assay. No similar increase was seen in the first assay + S9. Acceptable. (Green and Gee, 12/3/03).

**52951-0081 205957, "Report on Mutation Assay of ET-751 Using L5178Y Mouse Lymphoma Cells", (Noriho Tanaka, Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan, HRI Report No. 10-426, 14 July 1998). Duplicate cultures (50 ml tubes) of L5178Y mouse lymphoma cells (4 x 10⁶ cells) were exposed to ET-751 Technical (97.0% pyraflufen-ethyl) for 4 hours at 0 (DMSO), 10, 20, 30, 40, and 50 µg/ml without rat liver S9 (induced with phenobarbital and 5,6-benzoflavone) and at 0, 150, 200, 250, 300, and 350 µg/ml with S9 in two assays. Mutation frequency was determined using the multiwell technique and colonies were sized into large (> 1/4 diameter of well) and small (<1/4) No increase in forward gene mutation was indicated. Positive controls were functional. Acceptable. (Green and Gee, 12/3/03).

CHROMOSOME EFFECTS

**52951-0079 205955, "ET-751: An *In Vitro* Test for Induction of Chromosome Damage: Cytogenetic Study in Cultured Human Peripheral Lymphocytes", (Carol A. Dance, Pharmaco LSR Ltd., Eye, Suffolk, England, Report No. 94/NHH073/0786, 10 October 1994). Duplicate cultures of human (male) whole blood lymphocytes (stimulated for 48 hours with PHA) were exposed to ET-751 Technical (97.6% pyraflufen-ethyl), in the presence and absence of rat liver S9, at 0 (DMSO), 650, 1300, and 2600 µg/ml in two assays. Non-activated cultures were exposed for 19 or 43 hours; cultures with activation for 3 hours and harvested 16 or 40 hours later. One thousand lymphocytes per culture were scored for metaphases (mitotic index) and one hundred metaphases were examined for aberrations. Test article precipitation was reported in all cultures treated at 2600 µg/ml. No increase in aberrant metaphases. Positive controls were functional. Acceptable. (Green and Gee, 10/31/03).

**52951-0084 205960, "ET-751: Mouse Micronucleus Test", (C. N. Edwards, Pharmaco LSR Ltd., Eye, Suffolk, England, Report No. 94/NHH075/0658, 24 August 1994). Five CD-1 mice per sex per group received a single dose of ET-751 Technical (97.6% pyraflufen-ethyl) by oral gavage at 0 (corn oil), 1250, 2500, and 5000 mg/kg followed by bone marrow sampling 24, 48, and 72 hours later. Additional groups were dosed twice (24 hour interval) at 0, 2500, and 5000 mg/kg with sampling 24 hours later. One female at 2500 mg/kg was found dead 19 hours after the second dose. One male at 5000 mg/kg showed piloerection and hunched posture from shortly after the second dose until scheduled sacrifice. Three smears of bone marrow were prepared per animal. At least one was scored for micronuclei in polychromatic erythrocytes and in mature erythrocytes. At least 2000 cells were scored per animal. The ratio of PCE:NCE was calculated. No increase in micronucleated polychromatic erythrocytes. Positive control (chlorambucil) was functional. Acceptable. (Green and Gee, 12/5/03).

DNA DAMAGE

52951-0082 205958, "ET-751: DNA Repair Test (Rec-Assay) with *Bacillus subtilis*", (Koichi Miyahama, Nihon Nohyaku Co., Ltd., Research Center, Osaka, Japan, Study # GA-08,94-0093, 10 November 1994). Triplicate cultures of *Bacillus subtilis* strains M45 (rec⁻) and H17 (rec⁺) were exposed to ET-751 Technical for 24 hours, in the presence and absence of S9, at 0 (DMSO), 343.75, 687.5, 1375, 2750, and 5500 µg/disk in a disk diffusion assay. No indication of growth inhibition (DNA damage/repair) in either strain. Negative (kanamycin) and positive controls (mitomycin C and 2-aminoanthracene) were functional. Unacceptable but possibly upgradeable (dosing level rationale, activation source, rationale for using a diffusion assay with a compound

with low water solubility). (Green and Gee, 12/4/03).

**52951-0083 205959, "Rat Liver DNA Repair (UDS) Test", (Ricarda A. Grant and Raymond Proudlock, Huntingdon Life Sciences Ltd., Cambridgeshire, England, Report no. NHH099/982777, 30 April 1998). Five Hsd/Ola Sprague-Dawley male rats per group received a single dose of ET-751 Technical by oral gavage at 0 (1% aqueous methylcellulose), 600, or 2000 mg/kg. Hepatocytes from 4 animals per group were isolated 2 and 14 hours post-dosing. Viability was determined by trypan blue dye exclusion and ranged from 91% to 100%. After attachment, cells were exposed to (methyl-³H) thymidine for 4 hours followed by 24 hours with unlabelled thymidine. Twelve replicate cultures were initiated per animal and six autoradiographs were prepared. Three slides per animal were scored, 50 cells per slide. No increase in the gross or net nuclear grain counts at any dose level or sampling time. Positive controls were functional. Dimethylnitrosamine was used for the 2 hour expression time and 2-acetylaminofluorene for the 14 hour expression time. Acceptable. (Green and Gee, 12/4/03).

METABOLISM

**52951-0085 205961, "Absorption, Distribution, Metabolism and Excretion of [Pyrazole-5-¹⁴C] ET-751 Following a Single Oral Administration to Male and Female Rats", (Kazuhiko Motoba, Nihon Nohyaku Co., Ltd., Research Center, Osaka, Japan, Report no. LSRC-M96-002A(translated), Study no. GB-01, 95-0044, 25 September 1996). 5 Sprague-Dawley rats per sex per group received a single dose of [Pyrazole-5-¹⁴C] ET-751 at 5 or 500 mg/kg followed by sacrifice 3, 6, 9, 24, 96, or 168 hours later. Absorption, tissue distribution, and excretion were evaluated. The majority (66% to 69% at 5 mg/kg and 88% to 90% at 500 mg/kg in males and females respectively) of the administered radioactivity was excreted in feces during 24 hours post-dosing. Excretion in urine accounted for 28% and 32% at 5 mg/kg and 3% and 6% at 500 mg/kg of dosed radioactivity in males and females respectively during 24 hours. Blood and plasma radioactivity concentrations reached maximum levels for males 3 hours (5 mg/kg) and 9 hours (500 mg/kg) post-dosing and for females 3 hours (both dose levels) post-dosing and fell below the detection limit at 48 to 72 hours post-dosing. Highest concentrations of radiolabel were found in plasma (2 times those in whole blood). Elimination from blood was more rapid in females.

6 hours after treatment with 5 mg/kg, peak concentrations of dosed radioactivity (%) were observed in large intestine (3%, males and 0.5% females), small intestine (0.8%, males and 5%, females), liver (4% to 5%), kidney (0.17%, males and 1.4% females), and gastrointestinal contents (44%, males and 37%, females). At 500 mg/kg, peak concentrations were also seen after 6 hours where all tissues contained less than 1% of administered radioactivity except for gastrointestinal contents (31%, males and 35% females).

Metabolites were identified in urine, plasma, and feces (see review or study). Proposed metabolic pathways were ester hydrolysis and N-demethylation on the pyrazole ring 1 position and hydrolysis of the ether bond on the 5 position of the phenyl ring to phenol and methylation to the methoxy moiety. Acceptable. (Green and Gee, 12/2/03).

**52951-0086 205962, "Metabolism and Excretion of [Phenyl-5-¹⁴C] ET-751 Following a Single Oral Administration to Male and Female Rats", (Kazuhiko Motoba, Nihon Nohyaku Co., Ltd., Research Center, Osaka, Japan, Report no. LSRC-M96-056A, Study no. GB-01, 96-0109, 2 December 1996). 5 Sprague-Dawley rats per sex received a single oral gavage dose of [Phenyl-¹⁴C] ET-751 at 5 mg/kg. Immediately after dosing, each animal was transferred to a glass metabolism cage. Urine and feces were collected at 24 hour intervals through 96 hours. Expired air was collected during the first 24 hour interval post-dosing. All animals were sacrificed 96 hours after treatment. Blood, plasma, aorta, eyeballs, brain, salivary gland, thyroid, thymus, heart, lung, liver, kidneys, adrenal gland, spleen, pancreas, fat, muscle, urinary bladder, stomach, small

intestine, large intestine, testes, prostate, ovaries, uterus, bone, bone marrow and gastrointestinal contents samples were quantified for radioactivity by liquid scintillation counting. Metabolites in urine and feces were evaluated by high performance liquid chromatography (HPLC) and 2-D thin layer chromatography. Radioactivity in expired air was below the limits of detection. Group average concentrations of radioactivity in tissues and organs (including gastrointestinal contents) at 96 hours were at or below the minimum detection level. The majority of dosed radioactivity was excreted into urine and feces during 24 hours post-dosing. 16.09% (males) and 20.67% (females) was excreted into urine and 78.86% (males) and 78.88% (females) was found in feces during the time period. Metabolites were identified in urine and feces (see review below or refer to study for specifics). As with [Pyrazole-5-¹⁴C] ET-751, the proposed metabolic pathways were ester hydrolysis and N-demethylation on the pyrazole ring 1 position and hydrolysis of the ether bond on 5 position of the phenyl ring to phenol and methylation to the methoxy moiety. Acceptable. (Green and Gee, 11/18/03).

**52951-0087 205963, "Absorption, Distribution, Metabolism, and Excretion of a Single Oral Dosing of [Pyrazole-5-¹⁴C] ET-751 Following Repetitive Oral Dosing of Non-Radiolabeled Test Substance to Rats", (Kazuhiko Motoba, Nihon Nohyaku Co., Ltd., Research Center, Osaka, Japan, Report No. LSRC-M96-033A (Translated), Study No. GB-01, 96-0008, 10 October 1996). 20 male Sprague-Dawley rats received non-labelled ET-751 Technical by oral gavage at 5 mg/kg/day for 14 days. On the 15th day, they received a single oral gavage dose of [Pyrazole-5-¹⁴C] ET-751 at 5 mg/kg. For the absorption phase, blood samples were obtained from 5 males 1, 3, 6, 9, 12, and at 24 hour intervals thereafter, through 168 hours after the radiolabel dose. In the excretion study, 5 males were transferred to glass metabolism cages after the radiolabel dose. Urine and feces were collected at 24 hour intervals through 96 hours. Then the animals were sacrificed for organ/tissue sampling as part of the distribution study. For the distribution study, 5 animals per group were sacrificed 6 and 24 hours following the radiolabel dose. Blood, aorta, eyeballs, brain, salivary gland, thyroid, thymus, heart, lung, liver, kidneys, adrenal gland, spleen, pancreas, fat, muscle, urinary bladder, stomach, small intestine, large intestine, testes, prostate, bone, bone marrow, and gastrointestinal contents were sampled. Liquid scintillation counting was used to quantify radioactivity in samples. Metabolites in urine, feces, plasma, and gastrointestinal contents were identified using high performance liquid chromatography (HPLC).

Radioactivity in blood and plasma reached peak concentrations 3 hours after dosing and declined to the detection limit at 96 hours. Radioactivity concentrations in plasma were higher (>two times) than those in whole blood.

25.83% (urine) and 61.64% (feces) of dosed radioactivity was excreted during 24 hours post dosing. At 96 hours, the total values were 26.66% and 64.44% for urine and feces respectively.

The highest concentrations of radioactivity at 6 hours were in gastrointestinal contents (70.23%), liver (4.535%), large intestine (1.997%), small intestine (0.640%), and kidney (0.203%). At 24 hours, gastrointestinal contents (1.260%) and liver (0.235%) showed the only notable concentrations. At 96 hours, concentrations were at or below the limits of detection in all samples.

Metabolites in urine, feces, and plasma were identified (see study or review for details). The proposed metabolic pathways were ester hydrolysis and N-demethylation on the pyrazole ring 1 position and hydrolysis of the ether bond on 5 position of the phenyl ring to phenol and methylation to the methoxy moiety. Acceptable. (Green and Gee, 12/3/03).

**52951-0088 205964, "Metabolism and Excretion of [Pyrazole-5-¹⁴C] ET-751 into Bile Following a Single Oral Administration to Rats", (Kazuhiko Motoba, Nihon Nohyaku Co., Ltd., Research Center, Osaka, Japan, Report No. LSRC-M96-017A, Study No. GB-01, 96-0019, 22 October 1996). 6 male Sprague-Dawley rats with cannulated bile ducts received a single oral gavage dose of [Pyrazole-5-¹⁴C] ET-751 at 5 mg/kg. They were then placed under restraint in ballman

cages to collect bile, urine, and feces. Bile was collected 0-6, 6-12, 12-24, 24-36, and 36-48 hours post-dosing. Urine and feces were collected every 24 hours. Animals were sacrificed at 48 hours to measure residual radioactivity in the gastrointestinal tract and gastrointestinal contents. Liquid scintillation was used to measure radioactivity in samples. High performance liquid chromatography was used to identify metabolites. 17.90% (feces), 19.66% (urine) and 36.09% (bile) of dosed radioactivity were excreted during 48 hours post-dosing. 13.86% was found in the gastrointestinal contents and 2.86% in the gastrointestinal tract at 48 hours. Absorption from oral administration was estimated as 55.75% (urine and bile content combined). Metabolites in urine, feces, gastrointestinal tract, and gastrointestinal contents were identified (see study or review for details). Proposed metabolic pathways were ester hydrolysis and N-demethylation on the 1 position of the pyrazole ring and hydrolysis of the ether bond on the 5 position of the phenyl ring to the phenolic hydroxy moiety. Acceptable. (Green and Gee, 12/3/03).

SUBCHRONIC STUDIES

4-Week Rat Feeding Study

0053; 205925; "ET-751: Preliminary Toxicity Study by Dietary Administration to CD Rats for Four Weeks" (Broadmeadow, A., Pharmaco LSR Ltd, Eye, Suffolk, England, Pharmaco LSR Report No. 93/NHH057/0824, 04/06/94). ET-751 (Lot No. 3AM0011N, purity = 96.8%) was admixed to the diet and fed to 10 CD rats per sex per dose at dose levels of 0 (untreated diet), 20, 200, 2000, or 20000 ppm (0, 2.4, 23.2, 230.4, 2619.0 mg/kg/day, respectively for males and 0, 2.3, 22.2, 223.2, 2296.4 mg/kg/day, respectively for females) for 4 weeks. 2 males and 2 females at 20000 ppm were sacrificed *in extremis* or found dead between days 12 and 15. Clinical signs exhibited by the mortalities included thin build, underactive behavior, pallor, partially closed eyelids, hunched posture, and respiratory distress. No treatment-related clinical signs were observed at dose levels less than 20000 ppm. A treatment-related decrease mean body weight gain was observed in both sexes at 20000 ppm. Treatment-related decreases in mean hemoglobin and hematocrit levels and increases in the mean white blood cell level and reticulocytes were observed in both sexes at 20000 ppm. Treatment-related increases in mean urea, total protein, and alkaline phosphatase levels in males at 20000 ppm and treatment-related increases in mean total bilirubin, total cholesterol, alanine aminotransferase, and aspartate aminotransferase levels in both sexes at 20000 ppm were observed. Treatment-related increases in mean relative liver (males at 2000 and 20000 ppm, females at 20000 ppm), spleen (both sexes at 20000 ppm), and kidney (males at 20000 ppm) weights were observed. Macroscopic examination revealed treatment-related enlarged and/or swollen spleens in surviving males at 20000 ppm and examination of bone marrow smears revealed a reduction in the myeloid to erythroid ratio in males at 20000 ppm. Microscopic examination revealed treatment-related extramedullary hemopoiesis in the spleen of surviving males at 20000 ppm. **No adverse effects.** NOEL (M) = 230.4 mg/kg/day (2000 ppm) and NOEL (F) = 223.2 mg/kg/day (2000 ppm) (based on an increase in mean relative spleen weight in both sexes and histological changes in the spleen and bone marrow in males). **Supplemental** because 1) no ophthalmological examinations were performed and 2) the animals were treated for only 4 weeks. (Corlett, 01/02/04)

90-Day Rat Feeding Study

0055, 0056; 205927, 205928; "ET-751: Toxicity Study by Dietary Administration to CD Rats for 13 Weeks Followed by an 8 Week Reversibility Period" (Broadmeadow, A., Huntingdon Life Sciences Ltd, Eye, Suffolk, England, Pharmaco LSR Report No. 93/NHH058/1093, 12/11/96). 821. ET-751 (Lot No. 3AM0011N, purity = 96.8%) was admixed to the diet and fed to 10 CD rats per sex per dose at dose levels of 0 (untreated diet), 200, 1000, 5000, or 15000 ppm (0, 17.8, 85.6, 455.5, and 1489.4 mg/kg/day, respectively for males and 0, 19.4, 95.4, 499.0, and 1502.9 mg/kg/day, respectively for females) [with 5 additional rats per sex per dose level at the 0, 5000, and 15000 ppm dose levels to test reversibility (8-week reversibility period used)] for 13 weeks. 1 male at 15000 ppm was sacrificed *in extremis* on Day 9 and 2 males at 15000 ppm died on Day 12. Treatment-related clinical signs exhibited by some animals at 15000 ppm included thin build,

underactive behavior, pallor, partially closed eyelids, hunched posture, irregular respiration, fast respiration, abdominal distension, and piloerection with all signs clearing in all surviving animals after Week 7. No treatment-related clinical signs were observed at dose levels less than 15000 ppm. A treatment-related decrease mean body weight gain was observed in males at 15000 ppm during Weeks 0-13; no treatment-related effect was observed during Weeks 13-21. Treatment-related decreases in mean hemoglobin and hematocrit levels (reversibility demonstrated in both after 7 weeks) and an increase in the mean white blood cell level (reversibility demonstrated after 5 weeks) were observed in both sexes at 15000 ppm. Treatment-related increases in mean alkaline phosphatase, alanine aminotransferase (reversibility demonstrated after 3 weeks), aspartate aminotransferase (reversibility demonstrated after 3 weeks), and cholesterol levels were observed in males at 15000 ppm. Urinalysis revealed a treatment-related increase in mean urine volume and a treatment-related decrease in mean specific gravity in both sexes at 15000 ppm (reversibility demonstrated in both after 3 weeks). Treatment-related increases in mean relative spleen (in males and females (not statistically significant) at 15000 ppm), and kidney (in males at 15000 ppm) weights were observed with reversibility demonstrated in the spleen but not the kidneys. Macroscopic examination revealed no treatment-related abnormalities. Microscopic examination revealed treatment-related periportal hepatocytic hypertrophy in the main group surviving males at 15000 ppm but not in the reversibility group animals. **No adverse effects.** NOEL (M) = 455.5 mg/kg/day (5000 ppm) based on increased liver enzyme levels and periportal hepatocytic hypertrophy; NOEL (F) = 499.0 mg/kg/day (5000 ppm) based on decreased hemoglobin and hematocrit levels and increased mean relative spleen weight (not statistically significant). **Acceptable.** (Corlett, 01/15/04)

14-Day Rat Repeated-Dosing Dermal Toxicity Study

0089; 205965; "14-Day Dermal Range-Finding Study with ET-751 2.5% EC (N) in Rats" (Biegel, L.B., Covance Laboratories, Inc., Madison, WI, Project Identification: Covance 6283-109 T-5112, 09/08/00). ET-751 (Lot No. 9AM8302F, 2.6% a.i.) was applied (neat except where noted) to the clipped dorsal area of the trunk of 3 CrI:CD®(SD)IGS BR rats per sex per dose at dose levels of 0 (reverse osmosis water), 50 (neat Days 1 and 2, in reverse osmosis water Days 3 through 8), 100 (neat Days 1 through 8, in reverse osmosis water Days 9 through 14), 250, 500, or 1000 mg/kg/day for 6 to 7 hours per day, 7 days per week, for 15 days (0, 100, and 250 mg/kg/day dose levels), for 5 days (500 and 1000 mg/kg/day dose levels), or for 8 days (50 mg/kg/day dose level). No animals died during the study. No treatment-related clinical signs were observed. Treatment-related erythema and edema were observed at all dose levels during the treatment period. No treatment-related effects on body weight and food consumption could be determined from the data presented. **No adverse effects.** NOEL (M/F, skin and systemic) not determined. **Supplemental** because 1) no ophthalmological examinations were performed, 2) the animals were treated for only 5 to 15 days, 3) only 3 animals per sex per dose level were used, 4) hematology and serum chemistry were not conducted, 5) no macroscopic or microscopic examinations were conducted on the test animals, and 6) the test article was a formulated product and not the technical grade active ingredient. (Corlett, 01/30/04)

28-Day Rat Repeated-Dosing Dermal Toxicity Study

52951-0090; 205966, 211809; "28-Day Dermal Toxicity Study with Pyraflufen-ethyl Technical in Rats" (Biegel, L.B., Covance Laboratories Inc., Madison, WI, Laboratory Study Identification Covance 6283-107, 9/21/00). 822. Pyraflufen-ethyl technical (Lot No. 6AM0038I, purity = 98.67%) was applied (neat) to the clipped dorsal area of the trunk of 10 CrI:CD®(SD)IGS BR rats per sex per dose at dose levels of 0 (reverse osmosis water), 100, 300, or 1000 mg/kg/day for 6 to 7 hours per day, 7 days per week, for 29 days. No animals died during the treatment period. No treatment-related skin effects at the test site or treatment-related systemic clinical signs were observed. Body weight and organ weight determinations along with hematology and serum chemistry revealed no treatment-related effects. Macroscopic and microscopic examinations on the animals revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F, systemic and skin) = 1000 mg/kg/day based on no effects at the highest dose tested. Study

previously unacceptable but possibly upgradable with documentation that moistening the test site on the test animal with reverse osmosis water, and then applying the test article, a powder, to this test site and covering with porous gauze dressing sufficiently moistened the test article to ensure good contact with the skin; requested documentation submitted under record no. 211809; stated that the test material, a powder, had adhered to the skin of the animals as a consequence of moistening. **Study acceptable.** (Corlett, 01/28/04, upgraded, Moore, 6/21/04)

0091; 205967; "28-Day Dermal Toxicity Study with ET-751 2.5% EC (N) in Rats" (Biegel, L.B., Covance Laboratories Inc., Madison, WI, Laboratory Study Identification: Covance 6283-108, 9/21/00). ET-751 2.5% EC (N) (Lot No. 9AM8302F, 2.6% a.i.) was applied (neat except where noted) to the clipped dorsal area of the trunk of 10 CrI:CD®(SD)IGS BR rats per sex per dose at dose levels of 0 (reverse osmosis water), 25 (in reverse osmosis water), 100 (in reverse osmosis water), or 250 mg/kg/day for 6 to 7 hours per day, 7 days per week, for 30 days. 1 female at 100 mg/kg/day died on Day 28. No treatment-related systemic clinical signs were observed. Treatment-related dermal irritation including erythema, edema, atonia, desquamation, and fissuring were observed at 100 and 250 mg/kg/day. Body weight and organ weight determinations along with hematology and serum chemistry revealed no treatment-related effects. Microscopic examination revealed treatment-related acanthosis of the treated skin in both sexes at 25, 100, and 250 mg/kg/day, hyperkeratosis of the treated skin in males at 100 and 250 mg/kg/day and in females at 25, 100, and 250 mg/kg/day, and ulceration and acute inflammation of the treated skin in males at 250 mg/kg/day and in females at 100 and 250 mg/kg/day. **No adverse effects.** NOEL (M/F, skin and systemic) not determined. **Supplemental** because the test article was a formulated product and not the technical grade active ingredient. (Corlett, 02/05/04)

Supplemental Dog Oral Toxicity Study

0057; 205929; "ET-751: Preliminary Toxicity Study by Oral (Capsule) Administration to Beagle Dogs" (Broadmeadow, A., Pharmaco LSR Ltd, Eye, Suffolk, England, Pharmaco LSR Report No. 94/NHH070/0668, 11/04/94). ET-751 (Lot No. 4AM00210, no information on the purity of the material provided) was administered by gelatin capsule(s) once each day to 1 beagle dog per sex at a dose level of 20 mg/kg on Days 1 to 3, 40 mg/kg on Days 4 to 7, 80 mg/kg on Days 8 to 10, 160 mg/kg on Days 11 to 14, 320 mg/kg on Days 22 to 24; a second group of dogs consisting of 1 male and 1 female received 1000 mg/kg for 14 days. No animals died during the study. No treatment-related clinical signs were observed. No treatment-related effects on body weight, hematology, serum chemistry, urinalysis, and organ weight could be determined from the data presented. Macroscopic examination revealed no treatment-related abnormalities. **No adverse effects.** NOEL not determined. **Supplemental** because 1) no ophthalmological examinations were performed, 2) the animals were treated for only 14 to 24 days, 3) only 1 animal per sex per dose level was used, and 4) no negative control group was used. (Corlett, 01/20/04)

90-Day Dog Oral Toxicity Study

0058; 205930; "ET-751: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 13 Weeks" (Broadmeadow, A., Huntingdon Life Sciences Ltd, Eye, Suffolk, England, Report No. 95/NHH071/0275, 9/19/96). 821. ET-751 (Lot No. 4AM0024D, purity = 97.0%) was administered orally in gelatin capsule(s) once each day 7 days per week to 4 beagle dogs per sex per dose at dose levels of 0 (empty capsules), 40, 200, or 1000 mg/kg/day for 13 weeks. No animals died during the treatment period. No treatment-related clinical signs were observed. No treatment-related effects on body weight and food consumption were observed. No treatment-related hematological changes were observed. No treatment-related changes in serum chemistry were observed. Urinalysis revealed no treatment-related changes. Organ weight determinations revealed no treatment-related effects. Macroscopic and microscopic examination of organs and tissues revealed no treatment-related abnormalities. Electron microscopy conducted on samples of liver tissue revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F) = 1000 mg/kg/day based on no effects at the highest dose tested. **Acceptable.** (Corlett, 01/23/04)