CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Ethaboxam

Chemical Code #6105, **Tolerance #** 53198
SB 950 # NA

7/9/13

I. DATA GAP STATUS

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

Toxicology one-liners are attached.

All record numbers through #268962 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T130709
Revised by T.Moore, 7/9/13

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

**** 53198-0031; 268915**; ”LGC-30473: Combined Carcinogenicity and Toxicity Study by Dietary Administration to CD Rats for 104 Weeks”; (R.A. Paffett; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LKF 002/984932; 8/28/02); Sixty Crl:CD BR rats/sex/group received 0, 100, 300 or 650 ppm of LGC-30473 (ethaboxam technical) (lot no. P980622; purity: 99.0%;) in the diet for 2 years (carcinogeneity phase) ((M) 0, 5.5, 16.4, 35.8 mg/kg/day, (F) 0, 7.0, 21.0, 45.5 mg/kg/day). An additional 20 animals/sex/group received the treatment for 52 weeks (toxicity cohort). The survival of the study animals was not affected by the treatment. The mean body weight gain and food consumption of both sexes in the 650 ppm group were less than the control values over the course of the study. Although some of the parameters evaluated in the hematology demonstrated statistical significance when the results of the treated groups were compared to that of the control group, there was no consistent pattern of effect that appeared to be treatment-related. For the clinical chemistry, only the serum cholesterol level of the females in the 650 ppm group were consistently greater than that of the control group over the course of the study (NS, $p<0.01$). Other parameters were statistically different between the treated and control animals. However no consistent treatment-related effect was discernible. The urinalysis did not reveal any apparent treatment-related effects. No treatment-related effects were noted in the functional observational battery or motor activity assessments. The ophthalmological examination did not reveal any apparent treatment-related effects on the eyes. In the necropsy examination, the mean weights of the epididymides and seminal vesicles of the 650 ppm males were less than those of the control group after 104 weeks of treatment (note: no effect was evident after 52 weeks of treatment) ($p<0.01$ or 0.05). The mean kidney and uterine weights of the 650 ppm females were greater than those of the controls after 52 weeks of treatment (note: no effect was evident after 104 weeks of treatment) ($p<0.05$ or 0.01). In the histopathology examination, there was an increased incidence of no spermatozoa or abnormal spermatogenic cells in the ducts of the epididymides of the 650 ppm males throughout the study. The incidence of leydig cell adenoma in the testes was noted for all of the treatment group males in the carcinogenicity phase (0: 1/60, 100: 4/60, 300: 6/60, 650: 7/60). There was also an increased incidence of bilateral atrophy of the seminiferous tubules in the testes of the 650 males over the course of the study. Interestingly, there was a significant decrease in the incidence of unilateral atrophy of the seminiferous tubules in the testes of the 650 ppm males in comparison with the control group. The incidence of hyperplasia in the pars distalis of the pituitary of both sexes in the 650 ppm group was increased after either 52 or 104 weeks of treatment ($p<0.05$). However, no consistent treatment-related effect was evident. Clear acinar cell atrophy was noted in the pancreas of the 650 ppm females after 52 weeks of treatment ($p<0.01$). The effect was not evident after 104 weeks of treatment. An increase in the incidence of no corpora lutea in the ovaries was noted for all of the treatment groups over the course of the study (0: 19/80, 100: 26/55, 300: 28/53, 650: 41/80). However, the incidence for the 100 and 300 ppm females was within the range of the historical control. **Possible adverse effect indicated:** leydig cell adenomas in the testes, abnormal spermatogenic cells in the epididymides; **Rat Chronic Toxicity NOEL: (M)** 100 ppm (5.5 mg/kg/day) (based upon the incidence of abnormal spermatogenic cells in the ducts of the epididymides of the males in the 300 ppm group); **(F)** 300 ppm (21.0 mg/kg/day) (based upon the increased incidence of no corpora lutea in the ovaries of the females in the 650 ppm group); **Oncogenicity:** incidence of leydig cell adenomas. **Study acceptable.** (Moore, 4/2/13)

CHRONIC TOXICITY, RAT

See Combined, Rat above.

CHRONIC TOXICITY, DOG

** 53198-0030; 268914; "LGC-30473: Toxicity Study by Oral Capsule Administration to Beagle Dogs for 52 Weeks"; (M.H. Barker; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LKF 015/002317; 12/14/01); Four beagle dogs/sex/group received 0, 5, 10 or 30 mg/kg/day of LGC-30473 (ethaboxam technical) (lot no. P980622; purity: 99.0%) orally in gelatin capsules for 12 months. One male in the 10 mg/kg group was euthanized for humane reasons during week 41 due to debilitating hernias. The mean body weight gain of the 30 mg/kg females was less than that of the control females over the course of the study ($p<0.05$). There was no apparent treatment-related effect on the food consumption. There was no treatment-related effect on the motility, viability or count of the ejaculated sperm from the male dogs over the course of the study. Examination of individual sperm revealed a high incidence of abnormalities even in the control group. The hematological evaluation did not reveal any treatment-related effects on any of the parameters which were evaluated. No treatment- related effects were evident in the urinalysis, ophthalmological examination or bone myelogram. In the clinical chemistry evaluation, the serum alkaline phosphatase activity was increased for both sexes in the 30 mg/kg group over the course of the study (NS, $p<0.05$ or 0.01). The serum glucose and cholesterol levels were elevated for both sexes in the 30 mg/kg group in comparison to the control group (NS, $p<0.05$ or 0.01). The mean liver weights of both sexes in the 30 mg/kg were greater than those values for the controls ($p<0.05$). In the histopathology examination, there was a dose-related increase in the severity of hepatocytic hypertrophy in the livers of both sexes in the 10 and 30 mg/kg groups, advancing from a centrilobular to a generalized positioning within the liver. Likewise, cytoplasmic pigmentation of hepatocytes was increased in the livers of all of the treated groups in a dose-related manner. This response was indicatve of bilirubin pigment accumulation. **No adverse effect indicated**. **Dog Chronic Oral Toxicity NOEL:** (M/F) < 5 mg/kg/day (based upon the increased level of pigmentation in the liver hepatocytes of both sexes in the 5 mg/kg/day group); **Study acceptable.** (Moore, 3/22/13)

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

** 53198-0033; 268918; "LGC-30473: Carcinogenicity Study by Dietary Administration to CD-1 Mice for 78 Weeks"; (P.R. Chambers; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LKF 012/012194; 7/10/02); Fifty CD-1 mice/sex/group received 0, 100, 300 or 900 ppm of LGC-30473 (ethaboxam technical) (lot no. P980622; purity: 99.0%) for 78 weeks ((M) 0, 11.8, 35.0, 117.4 mg/kg/day, (F) 0, 13.8, 43.8, 135.4 mg/kg/day). There were no treatment-related effects upon the animals' survival. The mean body weight gain of both sexes in the 900 ppm group was less than that of the control group over the course of the study (NS, $p<0.01$). The mean food consumption was not affected by the treatment. The differential distribution of the white blood cell population was not affected by the treatment. The mean liver weights of both sexes were greater than those of the control group (NS, $p<0.01$). The histopathological examination did not reveal any treatment-related non-neoplastic or neoplastic lesions. **Oncogenicity was not evident**. **No adverse effect noted. Mouse Chronic Dietary Toxicity NOEL:** (M/F) 300 ppm ((M) 35 mg/kg/day, (F) 43.8 mg/kg/day) (based upon the lower mean body weight gain and increased liver weights for both sexes in the 900 ppm group. **Study acceptable.** (Moore, 3/25/13)

REPRODUCTION, RAT

** 53198-0029 268908, “LGC-30473: Study of reproductive performance in CD rats treated continuously through two successive generations by dietary administration”, CD rats, Willoughby, C., Huntingdon Life Sciences Ltd., Cambridgeshire, PE28 4HS, England, 2/25/02. Project ID: LKF 009/012244. LGC-30473, Batch No. P980622, a white powder was stored in 4°C in the dark. Groups of 32/sex rats received test substance at 0, 65, 200 or 650 ppm in diet for 10 weeks before pairing and throughout mating, gestation, littering and lactation. F1 generation comprised of 28/sex/group was treated with test diets for 10 weeks before pairing and throughout mating, gestation, littering and lactation. Mean test substance intake in 65, 200 and 650 ppm groups: for F0 generation males before pairing: 5.2, 16.2 and 52.6 mg/kg/day, respectively; for F1 generation males before pairing: 5.8, 17.7 and 60.4 mg/kg/day, respectively; for F0 generation females before pairing: 5.7, 17.6 and 56.1 mg/kg/day, respectively; for F0 generation females during gestation: 5.1, 15.5, and 51.2 mg/kg/day, respectively; for F0 generation females during lactation: 11.7, 35.1 and 112.8 mg/kg/day, respectively; for F1 generation females before pairing: 6.2, 18.5 and 60.7 mg/kg/day, respectively; for F1 generation females during gestation: 5.0, 15.6 and 50.8 mg/kg/day, respectively; for F1 generation females during lactation: 9.7, 27.2 and 76.7 mg/kg/day, respectively. Offsprings of F0 and F1 generations were evaluated for survival, growth and development until weaning. The following effects were seen in Group 4 animals over 2 generations: decreased number of F1 males mated and numbers of induced pregnancies in female partners, decreased F1 females pregnant with live litter, and number of young reared to weaning; decreased F1 offspring viability and bodyweight, bodyweight gain and food consumption; decreased implantation sites and live litter size, viability, bodyweight and bodyweight gain in F2 offspring; decreased percentages of motile and progressively motile sperm, weight of testis and cauda epididymis, and number of sperm in cauda epididymis, decreased percentage of normal sperm, increased percentage of decapitate and abnormal sperm in F1 generation males; decreased percentages of motile and progressively motile sperm, weight of cauda epididymis, decreased percentage of normal sperm, increased percentage of decapitate and abnormal sperm in F0 generation males. Histopathological examinations revealed increased incidences in Group 4 F1 generation males: reduced number of spermatozoa and abnormal spermatogenic cells in ducts of right epididymis, tubules with depletion of all germ cells and abnormal spermatids in occasional tubules in the right testis. **Parental/Reproductive NOEL (No Observed Effect Level):** 200 ppm (16.2 mg/kg/day for F0 males, 17.7 mg/kg/day for F1 generation males), 650 ppm (56.1 mg/kg/day) for F0 generation females, 200 ppm (18.5 mg/kg/day) for F1 generation females, due to reduced bodyweight before pairing and reduced mating performance in F1 males, reduced sperm count and motility in F0 and F1 males, abnormal histopathological findings of F1 right epididymis and testes. **Offspring NOEL**: 200 ppm (F1 and F2 generations), 15.5 to 35.1 mg/kg/day due to reduced pup survival and weight. **Acceptable.** (Pan & Leung, 3/7/2013)

TERATOLOGY, RAT

** 53198-0027 268901, “LGC-30473: Repeat study for effects on embryofoetal development in the rat by gavage administration”, rat, Gardner, T., Huntingdon Life Sciences Ltd., Cambridgeshire, PE18 6ES, England, 8/5/97. Project ID: LKY 58. LGC-30473, Batch No. 4-1, a light brown powder, 97.5% pure was stored in 4°C in the dark. Groups of 25 pregnant rats received test substance at 0, 10, 30, 100, or 300 mg/kg in 1% methylcellulose once daily from day 6 to day 19 post coitum inclusive. The rats were observed for clinical signs, body weight, food and water consumption during the treatment period. On day 20 post coitum, all rats were killed by CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine: the number of corpora lutea, number and distribution of live young and embryofetal deaths, fetal weight and gross abnormalities. No maternal mortality. A transient reduction of weight gain was noted in 300

mg/kg females during day 8 and 12. Transient food consumption reduction in the high dose group, followed with incidences of increased food consumption in all treated groups was observed. Increased number of litters with pre-implantation loss in 100 and 300 mg/kg females was observed. Increased incidences and durations of yellow staining on undercage tray paper, fur loss and skin alopecia was observed in 100 and 300 mg/kg/day groups. **Maternal and developmental NOEL (No Observed Effect Level):** 30 mg/kg/day due to clinical and autopsy finding [alopecia] and bodyweight change. **Acceptable.** (Pan & Leung, 2/28/2013)

** 53198-0028 268902, "LGC-30473: Study for effects on embryofoetal development in the rat by gavage administration", rat, Gardner, T., Huntingdon Life Sciences Ltd., Cambridgeshire, PE18 6ES, England, 8/5/97. Project ID: LKY 36. LGC-30473, batch no. 4-1, a light brown powder, 97.5% pure was stored in 4°C in the dark. Groups of 25 pregnant rats received test substance at 0, 100, 300 or 1000 mg/kg in 1% methylcellulose once daily from day 6 to day 19 post coitum inclusive. The rats were observed for clinical signs, body weight, food and water consumption during the treatment period. On day 20 post coitum, all rats were killed by CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine: the number of corpora lutea, number and distribution of live young and embryofetal deaths, fetal weight and gross abnormalities. No maternal mortality. Reduced weight gain and food consumption was noted in 1000 mg/kg females. Statistically significantly decreased mean gravid uterine and litter weight in 300 and 1000 mg/kg/day groups, reduced fetal weights in all treated groups were observed. Increased percentage of fetuses per litter with malformations, visceral and skeletal [irregular and incomplete ossification] anomalies in 1000 mg/kg/day group, increased percentage of fetuses per litter with visceral anomalies [thin diaphragm with protrusion of liver, abnormal lobation of liver] in 100 and 300 mg/kg/day groups were observed. **Maternal NOEL** = 300 mg/kg/day due to reduced body weight gain and food consumption. **Developmental NOEL**: <100 mg/kg/day due to reduced pup weight. **ACCEPTABLE.** (Pan & Leung, 2/26/2013)**

53198-0027 268900, "LGC-30473: A dose range finding study in the pregnant rat by gavage administration", rat, Gardner, T., Huntingdon Life Sciences Ltd., Cambridgeshire, PE18 6ES, England, 11/5/96. Project ID: LKY 35. LGC-30473, a light brown powder, 90.4% pure was stored in 4°C in the dark. Groups of 10 pregnant rats received test substance at 0, 100, 300 or 1000 mg/kg in 1% methylcellulose once daily from day 6 to day 19 post coitum inclusive. The rats were observed for clinical signs, body weight, food and water consumption during the treatment period. On day 20 post coitum, all rats were killed by CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine: the number of corpora lutea, number and distribution of live young and embryofetal deaths, fetal weight and gross abnormalities. No maternal mortality. A transient weight loss and reduced food consumption was noted in 1000 mg/kg females during the first week. Reduced number of corpora lutea, implantation, live young and litter weights were observed in the offsprings of the 300 mg/kg females. The data for the control and high dose females were comparable with respect to pre-treatment corpora lutea and implantation counts. Decreased mean litter and fetal weights were observed in the high dose group offspring compared with the control group offspring. The low dose at 100 mg/kg produced no test treatment related adverse effects. The same dosage sequence was selected for the main teratology study. **Supplemental.** (Pan & Leung, 2/19/2013)

TERATOLOGY, RABBIT

** 53198-0028 268905, "LGC-30473: Study for effects on embryofoetal development in the New Zealand White Rabbit by gavage administration", New Zealand White Rabbit, Gardner, T., Huntingdon Life Sciences Ltd., Cambridgeshire, PE18 6ES, England, 4/10/97. Project ID: LKY 37.

LGC-30473, Batch No. 4-1, a light brown powder, 97.5% pure was stored in 4°C in the dark. Groups of 20 pregnant rabbits received test substance at 0, 25, 75 or 125 mg/kg in 1% methylcellulose once daily from day 6 to day 28 post coitum inclusive. The rabbits were observed for clinical signs, body weight, food and water consumption during the treatment period. On day 29 post coitum, all rabbits were killed by Pentobarbitone sodium injection, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine: the number of corpora lutea, number and distribution of live young and embryofetal deaths, fetal weight and gross abnormalities. There were 5 deaths during the study, 2 in 25 mg/kg/day group, 3 in 125 mg/kg/day group. One female rabbit in 25 mg/kg/day group was killed on day 27 of pregnancy due to unsteadiness and lethargy following early delivery of 2 dead fetuses on day 26. Another female in this group was killed following dosing error on day 23. Two females in the 125 mg/kg/day group were killed on day 15 and 16 due to inappetence and thin appearance. The two deaths were considered treatment related. Another female in this group was killed following dosing error on day 6. Orange colored urine was observed in all treated groups. Transiently reduced weight gains in the 75 and 125 mg/kg/day groups at days 8 and 10 were observed followed with comparable weight gain the rest of the study period. Reduced food consumption was noted among the dams with live young in the 75 and 125 mg/kg/day groups. **Maternal NOEL (No Observed Effect Level):** 25 mg/kg/day due to mortality, bodyweight gain and food consumption reductions in the 75 and 125 mg/kg/day group. **Developmental NOEL:** 125 mg/kg/day due to lack of effect on the reproductive and fetal development data. **Acceptable.** (Pan & Leung, 3/1/2013)

53198-0028 268904, “LGC-30473: A dose range finding study of effects in the pregnant New Zealand White Rabbit by gavage administration”, rabbit, Gardner, T., Huntingdon Life Sciences Ltd., Cambridgeshire, PE18 6ES, England, 11/5/96. Project ID: LKY 38. LGC-30473, batch no. 2-3, a light brown powder, 90.4% pure was stored in 4°C in the dark. Groups of 6 pregnant rabbits received test substance at 0, 75, 150, or 300 mg/kg in 1% methylcellulose once daily from day 6 to day 28 post coitum inclusive. The rabbits were observed for clinical signs, body weight, food and water consumption during the treatment period. On day 29 post coitum, all rabbits were killed by Pentobarbitone sodium injection, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine: the number of corpora lutea, number and distribution of live young and embryofetal deaths, fetal weight and gross abnormalities. One rabbit in the high dose group was sacrificed due to poor physical condition, another 2 rabbits from this group were withdrawn from treatment due to persistent inappetence. A control group animal was sacrificed due to dosing accident. Orange colored urine was observed in all treated groups. One high dose group rabbits showed signs of abortion before being taken off the treatment. Weight loss occurred in 300 mg/kg/day groups until stabilizing at day 12, significant initial weight loss in 150 mg/kg/day group until partial recovery from day 8 to 28. Transient weight loss in 75 mg/kg/day group was followed with comparable or superior gains compared with the control group. Food consumption in high dose and 150 mg/kg/day groups was suppressed consistently during the study period, that of the 75 mg/kg/day was lower than the control group initially followed with comparable or higher values later in the study. Decreased numbers of corpora lutea, and implantation, as well as decreased litter weights in all treated groups, decreased fetal weights in high dose group were observed. Due to these findings, dosage of 0, 25, 75 and 125 mg/kg/day was selected for the main study. **Supplemental.** (Pan & Leung, 2/28/2013)

GENE MUTATION

** 53198-0034**,** 268921 “LGC-30473: Mammalian cell mutation assay”, 842, mammalian cell mutation assay. Clare, M., Huntingdon Life Sciences Ltd., Cambridgeshire, PE28 4HS, England. 12/21/01. Laboratory Project LKF/038. LGC-30473, white powder, Lot no. P980622, 99.0% pure,

stored at 4°C in the dark. Test substance up to 300 ug/ml in DMSO was tested in for potential mutagenicity for 3 and 24 hours with or without metabolic activation of rat liver S9-mix. Positive control materials induced expected responses with or without metabolic activation. Test substance showed negative responses over the dose range tested in all test conditions with or without metabolic activation. The test substance is not mutagenic. No adverse effects. **Acceptable**. (Pan & Leung, 3/18/13)

** 53198-0034**,** 268920 "LGC-30473: Bacteria mutation test", 842, Bacteria reverse mutation assay. May, K., Huntingdon Life Sciences Ltd., Cambridgeshire, PE28 4HS, England. 8/23/01. Laboratory Project LKF/037. LGC-30473, white powder, Lot no. P980622, 99.0% pure, stored at 4°C in the dark. Test substance up to 5000 ug/plate in DMSO was tested in *Salmonella Typhimurium* TA 1535, TA1537, TA100 and TA98; *Escherichia Coli* WP_2uvrA for potential mutagenicity with or without metabolic activation of rat liver S9-mix. Precipitation of test material was seen in all strains at 5000 ug/plate. Strain specific positive control materials induced expected responses in both assays with or without metabolic activation. Test substance showed negative responses over the dose range tested in all tester strains, with or without metabolic activation. The test substance is not mutagenic. No adverse effects. **Acceptable**. (Pan & Leung, 3/13/13)

CHROMOSOME EFFECTS

** 53198-0034**,** 268923 "LGC-30473: In Vitro Mammalian Chromosome aberration test in Human Lymphocytes, Amended Report", 843, Chromosomal aberration study. Allais, L., Huntingdon Life Sciences, Cambridgeshire, PE28 4HS, England. 10/11/2002. Laboratory Project LKF 039/013151. Cultured human lymphocytes were exposed to LGC-30473 (white powder, Lot no. P980622, 99.0% pure, stored at 4°C in the dark), *in vitro* for 3 or 19 hours in the absence or presence of rat liver S-9 mix. Mitotic index and chromosome aberrations were analysed for all or selected dose levels. Positive control materials induced large and statistically significant increases of chromosome aberration under all conditions. The test substance did cause significant increase in aberrant cells compared with solvent control in 250 ug/plate without S9 and in 125 and 250 ug/plate with S9 in the first test, as well as in 100 ug/plate for 19 hours without S9 in second test, all of which were at or exceeded the 99% upper confidence limit from the historical control data. These statistically significant increases in numbers of aberrant cells were without dose response relationship, and higher relative mitotic indices were observed in these dose levels. Therefore the test results are equivocal in determining if the test material is mutagenic in inducing chromosome aberration in vitro. No adverse effects. **Study acceptable** (Pan &Leung, 3/21/13).

**** 53198-0036, 268931** "Ethaboxam: Induction of micronuclei in the cultured human peripheral blood lymphocytes", 842, In vitro micronucleus test. Whitwell, J., Covance Laboratories, Harrogate, North Yorkshire HG3 1 PY England. 10/6/08. Laboratory Project 1405/51. ETHABOXAM, white powder, batch no. P980622, purity: 102.5%, was stored at 4°C in the dark. The test article in DMSO, at concentrations up to 35 ug/ml, was administered to cultured human peripheral blood lymphocytes, at 24 (Experiment 1) or 48 hours (Experiment 2) after PHA (Phytohaemagglutinin, mitogen) treatment, in the absence of metabolic activation, for 20 hours followed with a recovery time of 28 hours with addition of cytochalasin B. The cells were examined for micronucleated binucleated cells (MNBN) as well as micronucleated mononucleate cells (MNMON). 4-nitroquinoline 1 –oxide (NQO) and vinblastine (VIN) were used as clastogenic and aneugenic positive control chemicals respectively. In Experiment 2, statistically significant increase of MNBN in 10, 12.5, 15, 17.5 and 27.5 ug/ml groups, statistically significant increase of MNMON in 9, 10, 12.5, 15, 17.5 and 27.5 ug/ml groups were observed. In Experiment 1, statistically significant increase of MNBN in 35 ug/ml group, statistically significant increase of

MNMON in 15, 25 and 35 ug/ml groups were observed. Mechanistic analysis with FISH staining using probes for centromeres indicated the micronuclei formation due to Ethaboxam treatment was via an aneugenic (whole chromosome loss) mechanism. Non Disjunction analysis using chromosome specific DNA probes estimate of NOEL to be approximately 6-7 ug/ml. **Study acceptable** (Pan, 4/11/13).

**** 53198-0036, 268935** "LGC-30473: In vitro micronucleus test in cultured human lymphocytes, Final report", 842, In vitro micronucleus test. Hynes, G., Huntingdon Life Sciences, Cambridgeshire, PE28 4HS, England. 6/29/06. Laboratory Project VFX 0049/062770. LGC-30473, white powder, batch no. P980622, purity: 99.7%, was stored at 4°C in the dark. The test article in DMSO was administered to cultured human peripheral blood lymphocytes, at 24 or 48 hours after PHA (Phytohaemagglutinin, mitogen) treatment, for 3 (in the presence of metabolic activation) or 20 hours (in the absence of metabolic activation) followed with a recovery time of 28 hours with addition of cytochalasin B. The cells were examined for micronucleated binucleated cells (MNBN) as well as micronucleated mononucleate cells (MNMON). Mitomycin C, colchicine and Cyclophosphamide were used as positive control chemicals in the absence or presence of metabolic activation, respectively. Test substance was tested for its possible cytotoxicity by measuring Proliferative Index of treated cultures comparing those of the control cultures. Toxicity test was repeated due to insufficient scorable dose levels or insufficient toxicity from the first test. Statistically significantly increase of mononucleate cells in 10, 15 and 20 ug/ml group without S9 at 24 hour after PHA treatment, statistically significantly increase of binucleate cells in 10 and 20 ug/ml group without S9 at 24 hour after PHA treatment, statistically significantly increase of mononucleate cells in 75 ug/ml group with S9 at 24 hour after PHA treatment was observed. Mechanistic analysis with FISH staining using probes for centromeres indicated the micronuclei formation due to LGC-30473 treatment was via an aneugenic (whole chromosome loss) mechanism. **Study acceptable** (Pan, 4/12/13).

** 53198-0036**,** 268936 "LGC-30473: In vitro micronucleus test in cultured human lymphocytes", 842, In vitro micronucleus test. May, K., Huntingdon Life Sciences, Cambridgeshire, PE28 4HS, England. 4/3/09. Laboratory Project VFX 0055/073082. LGC-30473, white powder, batch no. P980622, purity: 102.5%, was stored at 4°C in the dark. The test article in DMSO, at concentrations of 0.5, 1, 2, 3,4, 5, 6, 7, 8, 9, and 10 ug/ml (24 h PHA) or 0.5, 1, 2, 3, and 4 ug/ml (48 h PHA) was administered to cultured human peripheral blood lymphocytes, at 24 or 48 hours after PHA (Phytohaemagglutinin, mitogen) treatment, for 20 hours (in the absence of metabolic activation) followed with a recovery time of 28 hours with addition of cytochalasin B at 6 ug/ml. The incidence of micronucleated binucleated cells (MNBN) as well as micronucleated mononucleate cells (MNMON) and polynucleate cells per 500 cells was assessed per culture. Mitomycin C and colchicine were used as positive control chemicals in the absence of metabolic activation. They induced statistically significant increases in the number of mononucleate (Colchicine) and binucleate cells (Mitomycin C) containing micronuclei. Statistically significantly ($p<0.01$) increase of mononucleate cells containing micronucleus at 5, 6, 7, 8, 9 and 10 ug/ml and binucleate cells at 7, 8, 9 and 10 ug/ml without S9 at 24 hour after PHA treatment, statistically significantly ($p<0.01$) increase of mononucleate cells and binucleate cells containing micronucleus in 4 ug/ml group without S9 at 48 hour after PHA treatment was observed. Mechanistic analysis with FISH staining using probes for centromeres indicated the micronuclei formation due to LGC-30473 treatment was via an aneugenic (whole chromosome loss or gain), not non-disjunction, mechanism. **Study acceptable** (Pan, 4/12/13).

53198-0040; 268951; "LGC-30473: Spermatogonial Chromosome Aberration Test"; (J. Kwon; Korea Institute of Technology, Yuseong-gu, Daejeon 305-600; Korea; Project ID. No. N02041; 2/27/03); Five male ICR mice/group were dosed orally by gavage with 0 (aqueous 0.5% methyl cellulose), 125, 250, 500 or 1000 mg/kg/day of LCG-30473 technical (ethaboxam technical); lot

no. 02-011, purity: 98.2%) for 5 days. A positive control group of 5 male mice received a single ip dose of 120 mg/kg of cyclophosphamide in saline. At 24 hours post-final dose, the animals were euthanized and spermatogonia were recovered from the tubules of the testes. Each animal had previously received an ip injection of colchicine, 10 mg/kg, at 5 hours prior to sacrifice. One hundred metaphases from each animal were evaluated for the presence of aberrations. There was no treatment-related increase in the number of aberrant metaphases. **No adverse effect indicated.** The positive control was functional. **Study unacceptable**, possibly upgradeable with the submission of an appropriate rationale for the multiple dosing regimen which was employed in this study. (Moore, 4/11/13)

DNA DAMAGE

** 53198-0035**,** 268926 “LGC-30473: Rat micronucleus test”, 842, mammalian erythrocyte micronucleus test. Mehmood, Z., Huntingdon Life Sciences Ltd., Cambridgeshire, PE28 4HS, England. 11/15/01. Laboratory Project LKF/046. LGC-30473, 99.0% pure, solid white powder, batch no. P980622, was stored at 4°C in the dark. The test article in 0.5% methyl cellulose, at 0, 500, 1000 or 2000 mg/kg was given by a single oral gavage. Preliminary toxicity test was conducted on 2 rats/sex/group at 500, 1000 or 2000 mg/kg. Fast respiration, flat posture, abnormal gait, prominent eyes were observed in 2000 mg/kg LGC-30473 treated male and female rats after dosing. Micronucleus test was done on 14, 7, 7, 14 and 5 male rats for vehicle control, 500, 1000, 2000 mg/kg test substance and positive control group (cyclophosphamide, 20 mg/kg), respectively. The animals were sampled 24 hours (7, 7, 7, 6 and 5 for vehicle control, 500, 1000, 2000 mg/kg test substance and positive control group (cyclophosphamide, 20 mg/kg), respectively) or 48 hours (7 in vehicle control and 7 in high dose group) after the administration. Bone marrows were evaluated for percentage of polychromatic erythrocytes (PCE) and micronucleated PCE at 24 and 48 hours. Fast respiration, flat posture, piloerection, underactive, nervous behavior, abnormal gait, prominent eyes were observed in LGC-30473 treated male rats after dosing. In addition, hunched posture, partially closed eyelids and coat and tail staining were observed in 2000 mg/kg group rats. One high dose group animal was found dead 21 hours after dosing. Weight loss was observed in treated animals at 24 and 48 hours. One high dose group animal was found dead 21 hours after dosing. Statistically significant reduction of percentage of premature erythrocytes and increased micronucleated premature erythrocytes were observed in the positive control group rats. No changes in proportion of immature erythrocytes as well as micronucleated premature erythrocytes were observed in test substance treated groups at either sampling time. No adverse effects. **Study acceptable** (Pan, 4/4/13).

**** 53198-0035, 268925** “Ethaboxam: Induction of micronuclei in the bone marrow of treated mice and subsequent FISH staining”, 842, mammalian erythrocyte micronucleus test. Whitwell, J., Covance Laboratories, Harrogate, North Yorkshire HG3 1 PY England. 3/13/09. Laboratory Project 1405/52. ETHABOXAM, white powder, Lot no. P980622, was stored at 4°C in the dark. The test article in 1% methyl cellulose and 0.1% Tween 80, at 0, 150, 200, 250 or 300 mg/kg/day was given to three mice/group, as two administrations of intraperitoneal injection 24 hours apart. The animals were sampled 24 or 48 hours after the final administration. Bone marrows were evaluated for percentage of polychromatic erythrocytes (PCE) and micronucleated PCE at 24 and 48 hours. FISH (Fluorescence In Situ Hybridization) with Star*FISH mouse pan-centromeric DNA probe-FITC labeled was used to stain bone marrow smear slides to detect the presence of the centromeres. Flow cytometry was used to examine peripheral blood micronucleus. Satellite group animals were dosed as per the main group and sacrificed at selected timepoints for bioanalysis study. Blood and bone marrows from satellite animals were used to analyze toxicokinetics of Ethaboxam. Erythropoietin (EPO) was analysed from blood samples of satellite animals. Reduced percentage of PCE in test substance treated groups indicated slight bone marrow toxicity. Possible adverse effect: The 300 mg/kg/day group at 24 hours showed

increased micronucleus percentage. Mechanistic FISH analysis showed the increased C+ MN PCE (centromere positive) in the 300 mg/kg/day group slides. This indicates that the micronuclei induced were via whole chromosome loss or aneugenic mechanism. Concentration of test substance in bone marrow and plasma was determined in satellite group animals. The high concentration of erythropoietin in 300 mg/kg/day group at 24 hours coincides with high MN PCE in this group at this time point. The test substance induced micronuclei in the polychromatic erythrocytes of the bone marrow 24 hours after a two day dosing at 300 mg/kg/day. **Study acceptable** (Pan, 3/29/13)

** 53198-0036**,** 268929 "LGC-30473 Mouse micronucleus test", 842, mammalian erythrocyte micronucleus test. Hynes, G., Huntingdon Life Sciences Ltd., Cambridgeshire, PE28 4HS, England. 4/23/09. Laboratory Project VFX/0054/073067. LGC-30473, white powder, 102.5% pure, Lot no. P980622, was stored at 4°C in the dark. The test article in 1% methyl cellulose and 0.1% Tween 80, at 400 or 300 mg/kg/day was given to three male mice/group, as two administrations of intraperitoneal injection 24 hours apart in the Preliminary toxicity test. The animals were examined for clinical signs or mortalities for 48 hours after the final administration. In the Micronucleus test, seven, 7, 7, 31 (including 24 for Toxicokinetics study, Satellite group) and 5 males were administered to vehicle control, 50, 150, 300 mg/kg/day test substance and 12 mg/kg/day mitomycin C, respectively, as two administrations of intraperitoneal injection 24 hours apart. Bone marrow smears were evaluated for percentage of polychromatic erythrocytes (PCE) in 1000 erythrocytes and micronucleated PCE in 2000 polychromatic erythrocytes per animal at 24 and 48 hours. Satellite group animals were sacrificed at selected timepoints for bioanalysis study. Blood and tissue samples from satellite animals were used to analyze toxicokinetics of LGC-30473. No clinical signs were observed in the vehicle and positive control group animals. Clinical signs including underactive behavior, piloerection, flattened posture, hunched posture, irregular respiration, partially closed eyelids and ungroomed coat in the 50 and 150 mg/kg/day groups. In addition, unsteady gait in 150 mg/kg/day group, writhing, prostrate posture, slow respiration, cyanosis, and thin build were observed in 300 mg/kg/day group animals. Three animals were found dead prior to scheduled termination, one animal was moribund and was killed in extremis prior to scheduled termination. One spare animal was dosed to replace one of the animals found dead in the 24 hour group. As a result, 6 animals at 24 hour, 5 animals at 48 hour sampling time were available for bone marrow micronucleus analysis. Reduced bodyweight was observed in 150 and 300 mg/kg/day group mice at 24 and 48 hours after dosing. Concentration of test substance in tissues and plasma was determined in satellite group animals. Reduced percentage of PCE in test substance treated groups indicated slight bone marrow toxicity. The test substance induced statistically significantly increased micronuclei in the polychromatic erythrocytes of the bone marrow 24 and 48 hours after a two day dosing at 300 mg/kg/day. The incidences of micronucleated PCE in the 300 mg/kg/day test substance treated group were within historical vehicle control values from March 2005 to March 2007. Therefore the test results are equivocal in determining if the test material is mutagenic in inducing micronucleus in premature erythrocytes in mice. **Study acceptable** (Pan, 4/9/13).

NEUROTOXICITY

Rat Acute Neurotoxicity

** 53198-0038; 268945; "Ethaboxam: Neurotoxicity Study by a Single Oral Administration to Sprague-Dawley Rats followed by a 14-Day Observation Period"; (G. Goddard; Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, and Eye Research Centre, Eye, Suffolk, IP23, 7PX, England; Project ID No. VRY0023; 8/19/11); Ten Crl:CD (SD) rats/sex/group were dosed orally by gavage with 0 (vehicle: aqueous 1% (w/v) methylcellulose with 0.1% Tween 80), 300, 1000 or 2000 mg/kg of Ethaboxam technical (batch

no. AS2293b; purity: 98.9%). Functional Observational battery and motor activity evaluations were performed prior to dosing and at 6 hours post-dose (time to peak effect) and on days 8 and 15. Five animals/sex/dose were perfused whole body and pertinent nervous tissues were examined histologically. No deaths resulted from the treatment. The mean body weight gains of both sexes in the 1000 and 2000 mg/kg groups were lower than those of the control group between day 1 and day 8 (NS, $p<0.05$ or 0.01). There was some apparent treatment-related effect on the activity of the animals at 6 hours post-dose. The 1000 and 2000 mg/kg males demonstrated increased activity and rearing in the arena observation and but decreased motor activity. The females in the 1000 and 2000 mg/kg groups did not display a statistically significant reduction in the two arena parameters, but demonstrated a reduced level of motor activity ($p<0.05$). No parameters in other domains were apparently affected. These results could not be attributed to a particular neurotoxic etiology. There were no treatment-related lesions noted in the histological evaluation of the nervous tissue. **No adverse effect indicated. Rat Acute Oral Neurotoxicity NOEL:** (M/F) > 2000 mg/kg; **Study acceptable** (Moore, 4/4/13)

53198-0038; 268944; "Ethaboxam: Dose Range and Time to Peak Effect Study in Male Sprague-Dawley Rats by Acute Oral Administration"; (G. Goddard; Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, and Eye Research Centre, Eye, Suffolk, IP23, 7PX, England; Project ID. No. VRY0017; 8/19/11); Three Crl:CD (SD) rats/sex/group were dosed orally by gavage with 300, 1000 or 2000 mg/kg of Ethaboxam Technical (batch no. AS2293b; purity: 98.9%). The vehicle was aqueous 0.1% Tween 80 in 1% methylcellulose. Animals were examined for signs of toxicity on the day of dosing in order to establish the time to peak effect. Body weights were recorded on the day of dosing and on days 4 and 7. A functional observational battery (FOB) was performed prior to the initiation of the study and at 1, 2, 4 and 6 hours post-dose. No animals died as a consequence of the treatment. In the FOB, several animals demonstrated a reduced level of arousal at up to 6 hours post-dose. One of these animals also exhibited slight whole body tremor at 4 hours post-dose. No treatment-related lesions were evident in the necropsy examination at 7 days post-dose. **Study supplemental.** (Moore, 4/2/13)

Rat Subchronic Neurotoxicity

** 53198-0037; 268937; "A 90-Day Oral Dietary Neurotoxicity Study of Ethaboxam in Rats"; (M. Beck; WIL Research Laboratories, Ashland, OH; Study No. WIL-69002; 8/11/09); Twelve Crl:CD (SD) rats/sex/group received 0, 250, 600 or 1500 ppm of Ethaboxam technical (lot no. P 980622; purity: 99.6%) in the diet for 13 weeks ((M) 0, 18, 43, 106 mg/kg/day, (F) 0, 21, 50, 122 mg/kg/day). No deaths resulted from the treatment. The mean body weights of the 1500 ppm males were less than those of the control group over the course of the study ($p<0.05$). Likewise the mean food consumption of these animals was less than that of the control group ($p<0.05$, 0.01). There were no treatment-related effects noted in the various FOB assessments over the course of the study. The motor activity measurements did not reveal any treatment-related effects. No ocular lesions were noted in the ophthalmological examination. No treatment-related lesions were evident in the microscopic examination of the pertinent nervous tissue. **No adverse effect was evident. Rat Subchronic Neurotoxic NOEL:** (M/F) 1500 ppm ((M) 106 mg/kg/day, (F) 122 mg/kg/day) (based upon the lack of neurotoxic effects at the 1500 ppm treatment level); **Study acceptable.** (Moore, 4/9/13)

IMMUNOTOXICITY

** 53198-0040; 268947; "Ethaboxam: Preliminary 4-Week Dietary Immunotoxicity Study in the Male Sprague-Dawley Rat"; (E. Moore; Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS England; Project ID No. VRY0020; 8/15/11); Eight male Crl:CD (SD) rats/group received 0, 500, 1000 or 2000 ppm of Ethaboxam technical (batch no. AS 2293b; purity: 98.9%) in the diet for 4 weeks (0, 40, 75, 155 mg/kg/day). Another 8 males were dosed by

intraperitioneal injection with 50 mg/kg of cyclophosphamide in 0.9% saline on day 27 as the positive control group. On day 25, five days before necropsy on day 29, each animal received an iv injection of $2x10^8$ sheep red blood cells (SRBC). SRBC-specific IgM plaques were determined for each animal by incubating a spleen cell suspension preparation with guinea pig complement and SRBC. No deaths occurred during the treatment period. The mean body weight gains of the 1000 and 2000 ppm animals were less than that of the control group over the course of the study (p<0.05, 0.01). The food consumption of these two groups was also less than that of the control group (p<0.01). There were no treatment-related lesions noted in the necropsy examination. The mean relative liver weights for the 1000 and 2000 ppm groups were greater than that of the control group (p<0.01). There was no treatment-related effect evident in the plaque-forming cell assay. **No adverse effect indicated.** The positive control was functional. **Study acceptable.** (Moore, 4/10/13)

** 53198-0040; 268948; "Ethaboxam: 4-Week Dietary Immunotoxicity Study in the Male Sprague-Dawley Rat"; (E. Moore; Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS England; Project ID No. VRY0027; 8/12/11);); Ten male Crl:CD (SD) rats/group received 0, 250, 650 or 1500 ppm of Ethaboxam technical (batch no. AS 2293b; purity: 98.9%) in the diet for 4 weeks (0, 21, 52, 121 mg/kg/day). Another 8 males were dosed by intraperitioneal injection with 50 mg/kg of cyclophosphamide in 0.9% saline on day 27 as the positive control group. On day 25, five days before necropsy on day 29, each animal received an iv injection of $2x10^8$ sheep red blood cells (SRBC). SRBC-specific IgM plaques were determined for each animal by incubating a spleen cell suspension preparation with guinea pig complement and SRBC. No deaths occurred during the treatment period. The mean body weight gains of the 650 and 1500 ppm animals were less than that of the control group over the course of the study (p<0.05, 0.01). The food consumption of these two groups was also less than that of the control group (p<0.01). There were no treatment-related lesions noted in the necropsy examination. There was no treatment-related effect evident in the plaque-forming cell assay. **No adverse effect indicated.** The positive control was functional. **Study acceptable.** (Moore, 4/10/13)

METABOLISM STUDIES

Metabolism, Rat

** 53198-0039**,** 268941 "LGC-30473: Metabolism in rats", 851, Metabolism study. Langford-Pollard, A. D., Huntingdon Life Sciences, Cambridgeshire, PE28 4HS, England. 6/30/03. Laboratory Project LKF/019. LGC-30473, white powder, batch number P980622, 98.3% pure, stored at approximately 4°C in the dark. [^{14}C-thiazole] radiolabel: Lot number: SK-09-LG (Groups 1, 3, 5, 9, 11, 3.09MBq/mg) and SK-03-HR (Groups 7, 8, 13 and 14, 3.236 MBq/mg), radiochemical purity: ≥ 95%, stored at <-15°C in the dark. [^{14}C-thiophene] radiolabel: lot number SK-08-LG, specific activity 3.218 MBq/mg, radiochemical purity: ≥ 95%, stored at <-15°C in the dark. A series experiments were done to define the metabolism of LGC-30473 in rats. Majority of the experiments was conducted with both [^{14}C-thiazole] and [^{14}C-thiophene] radiolabeled LGS-30473, at low (10 mg/kg) and high doses (150 mg/kg), bile cannulation experiment (low and high doses) and repeat dosing experiments (low dose only) were done with [^{14}C-thiazole] radiolabeled LGS-30473 only. Both male and female rats were dosed with oral gavage or stomach cannula. Total recovery of radioactivity in urine, cagewash, expired air, feces and carcass were close to 100% at 120 hours after single dosing for both radiolabels in both low and high concentrations and in both sexes. The percentage of total dose dropped in urine (~30% to ~15%), increased in feces (~70% to 80-90%) in high dose treated groups, compared with low dose groups, regardless of radiolabel type. The total retention of radioactivity in carcass at 120 hours was less than 1% in all treated group rats. At 120 hours, the tissues with the highest radioactivity were thyroid and liver following single doses of [^{14}C-thiazole] radiolabeled

LGS-30473, liver, blood cells and kidney following single doses of [^{14}C-thiophene] radiolabeled LGS-30473. Bile duct cannulation was performed to examine the biliary excretion of radiolabel during the first 48 hours following the low and high doses of [^{14}C-thiazole] radiolabeled LGS-30473. The average total biliary excretion for male rats was 45.00% in the low dose group, 25.86% in the high dose group, for female rats was about 35-37% in the low and high dose groups. The total absorption in the low dose treated groups (71-73%) was higher than that for the high dose treated groups (48.48% for males and 61.14% for females). The low dose [^{14}C-thiazole] radiolabeled LGS-30473 treated groups reached Tmax faster (1 and 2 hours) than the high dose groups (3 - 4 and 6 hours) in plasma and blood cells. The Cmax in plasma and blood cells for the rats treated with high dose were about 6 times of that of the low dose. The AUC_{120} in plasma and blood cells for the rats treated with high dose were about 10-11 times of that of the low dose. The low dose [^{14}C-thiophene] radiolabeled LGS-30473 treated groups reached Tmax faster (2 and 1 hours) than the high dose groups (4 hours) in plasma and blood cells. The Cmax in plasma and blood cells for the rats treated with high dose were about 8-11 times of that of the low dose. The AUC_{120} in plasma and blood cells for the rats treated with high dose were about 9-11 times of that of the low dose. Repetitive dosing of low dose [^{14}C-thiazole] radiolabeled LGS-30473 had a similar pattern of excretion as the single oral doses, urinary excretion was much lower than fecal excretion, with no substantial sex differences. The tissues with the highest concentration of radioactivities were thyroid, liver, blood cells, kidney and whole blood. The major radioactive components in urine, fecal extracts, bile and liver extracts were quantified using reverse phase HPLC. The following metabolites were identified: LGC-32794 (TzB22), LGC-32800 (U17), LGC-32801 (U13), LGC-32802 (FE17) and LGC-32803 (FE15). The proposed biotransformation pathway for LGC-30473 in rats showed the test substance underwent N-deethylation followed by oxidation of the thiazole sulfur to LGC-32800. It also underwent enolisation, the enol form was hydrolysed to LGC-32801, or conjugated with sulfate to LGC-32802, or underwent hydroxylation/sulfate conjugation to LGC-32803. **Study acceptable** (Pan &Leung, 4/26/13).

SUBCHRONIC STUDIES

Rat 4-Week Dietary Toxicity Study

53198-0024; 268894; "LGC-35523: Toxicity Study by Dietary Administration to CD Rats for 4 Weeks"; (D. Colman; Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID. No. LKF 122/033024; 8/14/03); Ten Sprague-Dawley rats/sex/group received 0, 650, 2000, or 13000 ppm of LGC-35523 (ethaboxam metabolite) (lot no. P030325, purity: 92.6%) for 4 weeks ((M) 0, 56.5, 170.9, 1104.1 mg/kg/day, (F) 0, 56.5, 170.7, or 1155.8 mg/kg/day). No deaths occurred during the treatment period. The mean body weight gain of the males in the 13000 ppm group was less than that of the control group over the course of the treatment ($p<0.05$). Food consumption of the males in the 13000 ppm group was less than that of the control during the 1st week. Thereafter their food consumption was comparable.. There were no treatment-related effects upon the FOB parameters. Although the motor activity measurements for the males in the treated groups were greater than those of the control group, this apparent effect did not demonstrate a treatment-related response and the females did not demonstrate a comparable effect. There were no apparent treatment-related effects upon the hematology or urinalysis parameters. In the clinical chemistry evaluation, the mean serum cholesterol value for the 13000 ppm females was greater than the control group value ($p<0.01$). The mean serum albumin levels of both sexes in the 13000 ppm group were greater than the control group values ($p<0.05$). There was no treatment-related effect noted in the necropsy examination or on the mean absolute or relative organ weights. No histopathological examination was performed. **No adverse effect indicated. Rat 4-Week Dietary NOEL:** (M/F) 2000 ppm ((M) 170.9 mg/kg/day, (F) 170.7 mg/kg/day) (based upon treatment-related effects on clinical chemistry parameters and/or body weight gain for both sexes in the 13000 ppm group) **Study supplemental.** (Moore, 2/26/13)

Rat Subchronic Dietary Toxicity Study

**** 53198-0024; 268895**; "LGC-30473: Toxicity to Rats by Dietary Administration for 13 Weeks, Amended Report"; (G.T. Higgs; Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LKY 26/963670; 7/30/03); Ten Crl:CD rats/sex/group received 0, 200, 650 or 2000 ppm of LGC-30473 (ethaboxam technical) (batch no. 6-1; purity: 99.2%) in the diet for 13 weeks ((M) 0, 163., 49.7, 154 mg/kg/day, (F) 17.9, 58.0, 164 mg/kg/day). No deaths resulted from the treatment. The mean body weight gain and food consumption of both sexes in the 2000 ppm group and the males in the 650 ppm group were less than the control values (NS, p<0.01 or 0.05). There was no treatment-related effect upon the hematology parameters, ophthalmology or urinalysis parameters. The serum levels of one or the other of the electrolytes, potassium, calcium and phosphorus, were reduced for the both sexes in the 2000 ppm groups and the males in the 650 ppm group (p<0.01 or 0.05). The serum cholesterol levels of both sexes in the 2000 ppm group were elevated above those of the control group (p<0.01). The mean relative liver weights of both sexes in the 2000 ppm group and the females in the 650 ppm group were greater than the control values (p<0.01 or 0.05). The mean relative testes weight of the males in the 2000 ppm group was less than that of the control (p<0.01). The mean absolute epididymides weights of the males in the 650 and 2000 ppm groups were less than that of the control group (p<0.01). The mean absolute prostate weight of the males in the 2000 ppm group was less than that of the control group (p<0.05). Histopathological evaluation of the epididymides revealed the absence of spermatozoa at 2000 ppm or the presence of abnormal spermatogenic cells in the ducts at 650 ppm. The testes of the 2000 ppm group were severely atrophied and abnormal spermatids were noted in the testicular tubules of the 650 ppm group. Very slight hypertrophy of the hepatocytes in the livers of both sexes in the 2000 ppm group was noted as well. **Possible adverse effect:** abnormal spermatogenesis; **Rat Subchronic Dietary NOEL:** (M/F) 200 ppm ((M) 16.3 mg/kg/day, (F) 17.9 mg/kg/day) (based on lower mean body weight gain and abnormal spermatogenesis in the males and increased mean liver weight of the females in the 650 ppm treatment group); **Study acceptable**. (Moore, 3/15/13)

Rat 28-Day Repeated Dosing Dermal Toxicity Study

53198-0027; 268898; "LGC-30473: Twenty-Eight Day Dermal Toxicity Study in the Rat, Amended Report"; (P. Higgs; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LFK 34/963103; 7/30/03); The skin of 5 CD rats/sex/group was treated with 0, 100, 300 or 1000 mg/kg/day of LGC-30473 (ethaboxam technical) (batch no. 4-1; purity: 97.5%) for 6 hours/day for 28 days. The test material was suspended in 1.0% (w/v) aqueous methylcellulose. No mortality resulted from the treatment. The mean body weight gain of the males in the 1000 mg/kg group over the course of the study was less than that of the control group (p<0.05). There was no apparent treatment-related effect on the food consumption. There were no treatment-related effects on either hematological or clinical chemical parameters. The mean organ weights were not affected by the treatment. There were no treatment-related lesions in the histological examinations related to systemic exposure. There was an increased incidence of epidermal hyperplasia, hyperkeratosis, ulcers and dermal inflammation of the skin for the males in the 300 and 1000 mg/kg groups. **No adverse effect indicated. Reported Rat 28-Day Repeated Dosing Dermal NOEL: (Systemic) (M)** 300 mg/kg/day (based upon the reduced body weight gain of the males in the 1000 mg/kg group) **(F)** 1000 mg/kg/day (based upon the lack of a treatment-related effect in the 1000 mg/kg group); **(Dermal) (M)** 100 mg/kg/day (based upon the increased incidence of dermal lesions for the males In the 300 mg/kg group) **(F)** 1000 mg/kg/day (based upon the lack of treatment-related effects in the highest dose treated). **Study unacceptable**, upgradeable (ophthalmology was not performed**).** (Moore, 3/20/13)

Dog Subchronic Oral Toxicity Study

** **53198-0026; 268897**; "LGC-30473: Toxicity Study by Oral Capsule Administration to Beagle Dogs for 13 Weeks"; (T. Gardner; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LKF 014/993350; 5/3/02); Four beagle dogs/sex/group received 0, 15, 40 or 100 mg/kg/day of LGC-30473 (ethaboxam technical) (batch no. P980622; purity: 99.0%) orally in capsules for 13 weeks. One male and one female in the 100 m/kg group and one female in the 40 mg/kg group were euthanized *in extremis*. These deaths occurred during weeks 2, 7 and 10; respectively. The male was suffering from meningitis and the two females demonstrated signs of severe anemia. Histopathological evaluation revealed changes in the spleen and bone marrow. The mean body weight gains of the animals of both sexes in all of the treatment groups were reduced in a dose-related manner in comparison with the control group values (NS). The mean food consumption of both sexes in the 100 mg/kg treatment group was less than that of the control group over the course of the study ($p<0.01$). In the hematology evaluation, the mean red blood cell counts, hematocrit percentage and hemoglobin concentrations of both sexes of the 100 mg/kg group were less than the control values during the treatment period (NS, $p<0.05$, or 0.01). In the clinical chemistry evaluation, the serum glucose levels were increased for the males in the males in the 40 and 100 mg/kg groups ($p<0.05$ or 0.01). The serum cholesterol levels were increased for both sexes in 100 mg/kg group (NS, $p<0.05$). In the urinalysis, the mean urine volumes for both sexes In the 100 mg/kg group were less than those of the control group at both of the time points assayed (NS, $P<0.05$). The mean absolute and relative liver weights of all of the treatment groups were increased in a dose-related manner in relation to the control group values. The mean absolute and relative thymus weights of both sexes in the 100 mg/kg group were less than the control group values (NS). Hepatocytic hypertrophy was noted in the livers of the males in the 15, 40 and 100 mg/kg and in the females of the 40 and 100 mg/kg treatment groups. The lesion was noted in the centrilobular portion of the liver, advancing to a more generalized positioning as the dose increased. Atrophy and involution of the thymus was noted for 1 of 3 males and for 2 of 3 females in the 100 mg/kg group. **Possible adverse effect:** anemia; **Dog Subchronic Toxicity NOEL:** (M/F) < 15 mg/kg/day (based upon treatment-related effects on the livers of both sexes in the 15 mg/kg treatment group); **Study acceptable.** (Moore, 3/19/13)

Mouse Subchronic Dietary Toxicity Study

53198-0025; 268896; "LGC-30473: Preliminary Toxicity Study by Dietary Administration to CD-1 Mice for 13 Weeks"; (P.R. Chambers; Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LKF 011/993345; 8/15/02); Ten Crl:CD-1 mice/sex/group received 0, 200, 450, 1000, or 2500 ppm of LGC-30473 (ethaboxam technical) (batch no. P980622; purity: 99.0%) in the diet for 13 weeks ((M) 0, 33, 74, 163, 405 mg/kg/day, (F) 41, 93, 195 or 483 mg/kg/day). One male in the 2500 ppm group died during week 8. The mean body weight gains of the males in the 1000 and 2500 ppm groups were less than that of the control group over the course of the study ($p<0.05$). The mean food consumption of the females in the 2500 ppm group was less than the control group value over the course of the study ($p<0.05$). The mean relative liver weights of both sexes in the 1000 and 2500 ppm group and the males in the 450 ppm group were greater than the control group values ($p<0.05$ or 0.01). Centrilobular hepatocytic hypertrophy was noted in the livers of both sexes of the 1000 and 2500 ppm groups and the males in the 450 ppm group. **No adverse effect indicated. Mouse Subchronic Dietary NOEL:** (M) 200 ppm (33 mg/kg/day) (based upon the incidence of hypertrophy in the livers of the males in the 450 ppm group), (F) 450 ppm group (93 mg/kg/day) (based upon the incidence of hypertrophy in the livers of the females in the 1000 ppm group); **Study supplemental** (the clinical assessments were not performed and only the liver and testes were examined in the histopathology). (Moore, 3/18/13)

MECHANISTIC

53198-0030 268913, “LGC-30473: Investigation of reproductive hormone levels and genital tract pathology following dietary administration to male rats”, rat, Webley, L.J., Huntingdon Life Sciences Ltd., Cambridgeshire, PE28 4HS, England, 10/29/2002. Project ID: LKF/066. LGC-30473, a light brown powder, 90.4% pure was stored in 4°C in the dark. Groups of 10 male rats received test substance at 0, 650 or 2000 ppm in diet for 13 weeks [mean test substance uptake: 0, 34.8 and 114.3 mg/kg/day for 0, 650 and 2000 ppm groups, respectively]. The rats were observed for clinical signs, body weight, and food consumption during the treatment period. Blood samples for analysis of luteinizing hormone, testosterone and follicle stimulating hormone were obtained before treatment commenced and on days 7, 14, 28 and 91 of treatment. On completion of the treatment period, all rats were killed and necropsied, reproductive tissues were weighed and processed for histopathological examination. Yellow staining of cage paper in the treated groups, reduced bodyweight and food consumption in the treated groups, statistically significantly reduced body weight gain in the 2000 ppm group throughout the study was observed. Blood chemistry evaluation showed statistically significantly decreased level of blood testosterone at days 7, 14 and 28, statistically significantly increased levels of luteinizing hormone and follicle stimulating hormone at day 91 in 2000 ppm group. Statistically significantly reduced absolute and relative weights of epididymides and testes in 2000 ppm group, statistically significantly reduced absolute weights of epididymides in 650 ppm group and prostate gland in 2000 ppm group was observed. Final necropsy discovered small epididymides, small and flaccid testes in the high dose group animals. Histopathology examinations revealed increased inflammation, cellular debris in ducts and reduced numbers of spermatozoa in epididymides, increased bilateral germ cell depletion/degeneration and increased bilateral interstitial cell hyperplasia in testes of high dose rats. **Supplemental.** (Pan & Leung, 3/11/2013)

53198-0041; 268959; “Effect of Ethaboxam on Testosterone Production”; (K. Mikata; Sumitomo Chemical Company, Konohana-ku, Osaka, Japan; Project ID No. X0209; 11/15/10); Human adrenocortical NCI-H295R cells were exposed to concentrations of ethaboxam technical (lot no. NS-07G13A, purity: 99.9%) ranging from 100 pM to 100 uM for 24 hours. Temperature of the incubation was not reported. The data sheet for the cell line recommended 37°C. The concentration of testosterone in the culture medium was measured at the conclusion of the incubation period, using an enzyme immunoassay procedure. There was no treatment-related effect on the testosterone concentration in the medium. **Study supplemental.** (Moore, 4/11/13)

53198-0041; 268962; “Evaluation of Effects of Ethaboxam on Human Estrogen Receptor and Human Androgen Receptor using *In Vitro* Reporter Gene Assays”; (K. Saito; Sumitomo Chemical Company, Konohana-ku, Osaka, Japan; Project ID No. RGA-116; 11/25/10); Two Hela cell lines were modified to express either the human estrogenic receptor (a(hERa)) or human androgenic receptor (hAR). A plasmid which incorporated the luciferase-bound reporter gene was introduced into both of the cell lines. The agonist, 17b-estradiol (E2) and antagonist, 4-hydroxytamoxifen (HTM) demonstrated appropriate responses in the transcription activation assay for the a(hERa) receptor. Ethaboxam did not demonstrate any interaction with the receptor up to a concentration of 1 uM (a control study had demonstrated diminished luciferase activity at a concentration of 10 uM). Likewise, in the transcription activation assay with hAR, the agonist, dihydroxytestosterone (DHT) and the antagonist, hydroxyfultamide (HFL) demonstrated the appropriate responses. However, ethaboxam did not interact with the receptor up to a concentration of 1 uM. **Study supplemental.** (Moore, 4/12/13)

STUDIES ON METABOLITES

Rat Acute Oral Toxicity Study

** 53198-0023; 268887; "LGC-35523: Acute Oral Toxicity to the Rat (Acute Toxic Class Method); (P. Chandler; Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. LKF 125/033177/AC; 8/1/03); Six female CD rats were dosed orally by gavage with 5000 mg/kg of LGC-35523 (lot no. P030325, purity: 92.6%) (vehicle: aqueous 1% methycellulose). No deaths resulted from the treatment. Clinical signs included increased urination and discolored urine. No treatment-related lesions were noted in the necropsy examination. LD50 (F > 5000 mg/kg; Toxicity Category IV; **Study acceptable.** (Moore, 2/21/13)

Rat 4-Week Dietary Toxicity Study

53198-0024; 268894; "LGC-35523: Toxicity Study by Dietary Administration to CD Rats for 4 Weeks"; (D. Colman; Huntingdon Life Sciences, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID. No. LKF 122/033024; 8/14/03); Ten Sprague-Dawley rats/sex/group received 0, 650, 2000, or 13000 ppm of LGC-35523 (ethaboxam metabolite) (lot no. P030325, purity: 92.6%) for 4 weeks ((M) 0, 56.5, 170.9, 1104.1 mg/kg/day, (F) 0, 56.5, 170.7, or 1155.8 mg/kg/day). No deaths occurred during the treatment period. The mean body weight gain of the males in the 13000 ppm group was less than that of the control group over the course of the treatment ($p<0.05$). Food consumption of the males in the 13000 ppm group was less than that of the control during the 1st week. Thereafter their food consumption was comparable.. There were no treatment-related effects upon the FOB parameters. Although the motor activity measurements for the males in the treated groups were greater than those of the control group, this apparent effect did not demonstrate a treatment-related response and the females did not demonstrate a comparable effect. There were no apparent treatment-related effects upon the hematology or urinalysis parameters. In the clinical chemistry evaluation, the mean serum cholesterol value for the 13000 ppm females was greater than the control group value ($p<0.01$). The mean serum albumin levels of both sexes in the 13000 ppm group were greater than the control group values ($p<0.05$). There was no treatment-related effect noted in the necropsy examination or on the mean absolute or relative organ weights. No histopathological examination was performed. **No adverse effect indicated. Rat 4-Week Dietary NOEL:** (M/F) 2000 ppm ((M) 170.9 mg/kg/day, (F) 170.7 mg/kg/day) (based upon treatment-related effects on clinical chemistry parameters and/or body weight gain for both sexes in the 13000 ppm group) **Study supplemental.** (Moore, 2/26/13)

Mutagenicity

** 53198-0034**,** 268919 "LGC-35523: Bacteria reverse mutation test", 842, Bacteria reverse mutation assay. May, K., Huntingdon Life Sciences Ltd., Cambridgeshire, PE28 4HS, England. 7/23/03. Laboratory Project LKF 124/033098. LGC-35523, brownish powder, Batch PO30325, 92.6% pure, stored at 4°C in the dark. Test substance up to 5000 ug/plate in DMSO was tested in *Salmonella Typhimurium* TA 1535, TA1537, TA100 and TA98; *Escherichia Coli* WP_2uvrA for potential mutagenicity with or without metabolic activation of rat liver S9-mix. Strain specific positive control materials induced expected responses in both assays with or without metabolic activation. Test substance showed negative responses over the dose range tested in all tester strains, with or without metabolic activation. The test substance is not mutagenic. No adverse effects. **Acceptable.** (Pan & Leung, 3/13/13)

Chromosomal Aberration

** 53198-0034**,** 268924 "LGC-35523: In Vitro Mammalian Chromosome aberration test in Human Lymphocytes", 843, Chromosomal aberration study. Pritchard, L., Huntingdon Life Sciences,

Cambridgeshire, PE28 4HS, England. 04/04/2003. Laboratory Project LKF 123/033246. Cultured human lymphocytes were exposed to LGC-35523 (brownish powder, Lot no. P030325, 92.6% pure, stored at 4°C in the dark), *in vitro* for 3 or 20 hours in the absence or presence of rat liver S-9 mix. Mitotic index and chromosome aberrations were analyzed for all or selected dose levels. Positive control materials induced large and statistically significant increases of chromosome aberration under all conditions. The test substance did not cause significant increase in aberrant cells compared with solvent control. The test material is not mutagenic in inducing chromosome aberration in vitro. No adverse effects. **Study acceptable** (Pan &Leung, 3/22/13).