CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION

MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

PYRIDALYL (previously designated as S-1812)

Chemical Code # 5991, **Document Processing Number (DPN)** # 53074
SB 950 # (Not applicable)
Original date: 7/28/09

I. DATA GAP STATUS

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

Toxicology one-liners are attached.

All record numbers for the above study types through 242050 (Document No. 19) were examined. This includes all relevant studies indexed by DPR as of 7/15/09.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: t20090728.wpd
Revised by: (This is the initial summary by Aldous, 7/28/09)

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

****53074-0014 242022** Enomoto, A., "S-1812: Combined chronic toxicity and carcinogenicity study in rats," The Institute of Environmental Toxicology, Ibaraki, Japan, 3/22/02. IET # 99-0011. Groups of 50 rats/sex/group were dosed in diet with pyridalyl [Lot PS-98041G, 93.7% purity] for 104 weeks in the lifetime study, and 20/sex/group in the interim sacrifice chronic group. Dose levels were 0, 30, 100, 500, and 1000 ppm. Corresponding achieved dose levels in the lifetime study were 1.0, 3.4, 17, and 34 mg/kg/day for treated males, and 1.2, 4.1, 21, and 43 mg/kg/day for treated females. NOEL = 100 ppm (temporary food consumption decrements and sustained body weight in both sexes). Body weights of lifetime study 500 and 1000 ppm males at week 76 were 7% and 6% below controls, respectively: corresponding groups of females had body weight decrements of 8% and 12%. High dose rats typically had increased motor activity compared to controls at week 49 (the only assay time: statistically significant in females). Uncommon tumor types, rhabdomyosarcoma and oligodendroglioma (the latter all cerebral), were non-significantly elevated in 1000 males (lesions were each observed in two high dose males, and not in any other males). One high dose female also had an oligodendroglioma. Published background incidences of these tumors are on the order of 0.1% to 0.2%, and this reviewer considers incidences of 2/50 to indicate "possible adverse effects." Brown pigment in the spleen (evidently hemosiderin) was elevated in both sexes at 1000 ppm. Study is acceptable. Aldous, 5/19/09.

CHRONIC TOXICITY, RAT

See COMBINED, RAT, above.

CHRONIC TOXICITY, DOG

**53074-0012 242020 Uchida, H., "12-Month oral toxicity study with S-1812 in dogs," Panapharm Laboratories Co., Ltd, Kumamoto, Japan, 7/13/02. Laboratory Project ID: 29917. Groups of four beagles/sex/group were dosed by gelatin capsule with Pyridalyl (S-1812), 93.7% purity, for 1 year in a standard chronic study. Dose levels were 0, 1.5, 5, 20, and 80 mg/kg/day. NOEL = 20 mg/kg/day, based on modest increases in alkaline phosphatase in 80 mg/kg/day males, and on modest increases in liver weights in 80 mg/kg/day females. Acceptable, with no adverse effects. Aldous, 5/26/09.

ONCOGENICITY, RAT

See COMBINED, RAT, above.

ONCOGENICITY, MOUSE

****53074-0013 242021** Enomoto, A., "S-1812: Carcinogenicity study in mice," The Institute of Environmental Toxicology, Ibaraki, Japan, 3/29/02, Laboratory Project No. IET 99-0012. Groups of 52 SPF ICR (Crj:CD-1) mice/sex/group were dosed in diet with pyridalyl [Lot PS-98041G, 93.7% purity] for 78 weeks in the lifetime study. An additional 12/sex were assigned to 52-week interim sacrifice groups. Dose levels were 0, 15, 50, 1000, and 2500 ppm. Corresponding achieved dose levels in the lifetime study were 1.6, 5.0, 103, and 267 mg/kg/day for treated males, and 1.5, 4.8, 99, and 264 mg/kg/day for treated females. NOEL = 50 ppm (dose-related body weight decrements at 1000 to 2500 ppm in both sexes). Findings at 2500 ppm included slightly diminished food consumption (both sexes) and slightly increased relative liver weights (both sexes: statistically significant in satellite study females only). Thyroid follicular cell adenomas were observed in one male and in two females at 2500 ppm, but not in controls. Background incidence in females is low enough that this reviewer considers this possibly treatment-related, and a "possible adverse effect." Acceptable, C. Aldous, 4/15/09.

REPRODUCTION, RAT

**53074-0012 242018 and 242019 Shimizu, N., "A two-generation reproduction study in rats with S-1812: Amended report," The Institute of Environmental Toxicology, Ibaraki, Japan, 4/15/02, IET Study No. 99-0077. Groups of 24 SPF Sprague-Dawley (Crj:CD (SD)) rats were dosed in diet with Pyridalyl (S-1812), Lot PS-98041G, 93.7% purity. Dosing was continuous for 10 weeks pre-mating, mating (up to 2 weeks), gestation, and lactation for F0 and F1 generations. In addition, selected F2 females were maintained on treatment for 5 weeks after weaning for a follow-up assessment of age at vaginal opening. Parental systemic toxicity NOEL = 40 ppm for males and 200 ppm for females, based primarily on pre-mating body weight decrements. A more precise NOEL for male body weight decrements may be derived from the combined rat study (Record No. 242022), where there were no body weight effects were observed at 100 ppm. Parental reproductive effects NOEL = 1000 ppm (no effects). Offspring viability and growth NOEL = 40 ppm (modest pup body weight decrements during lactation). Thymic weights were somewhat lower in 200 to 1000 ppm weanlings (PND 26) than in controls (primarily in males). There was no associated histopathology (examined in ten F2 males each at 0 and 1000 ppm). Study is acceptable, with no adverse effects. Aldous, 5/26/09.

53074-0012 242019 is the brief histopathology report for thymus in weanlings (considered as part of the above record). No additional DPR worksheet. Aldous, 4/21/09.

53074-0012 242017 is the range-finding study for study 53074-0012 242018, above. Eight rats/sex/group were dosed in diet with pyridalyl at 0, 100, 500, or 1000 ppm. Based on body weight decrements, some changes in organ weights, and hepatocyte single cell necrosis; investigators selected the dosing regimen for the primary study. Useful supplementary data. No DPR worksheet. Aldous, 4/21/09.

TERATOLOGY, RAT

**53074-0011 242016 Shimizu, N. "Teratogenicity study in rats with S-1812," The Institute of Environmental Toxicology, Ibaraki, Japan, Dec. 5, 2001. Laboratory Study # IET 00-0095. Groups of 24 Crj:CD (SD) rats were dosed daily by gavage (in 1 ml/kg corn oil vehicle) on gestation days 6-19 in a standard developmental toxicity study. Dose levels were 0, 10, 50, and 250 mg/kg/day. Maternal toxicity NOEL = 10 mg/kg/day, based on very small reduction of body weight gain indicated at gestation day 15-18 at 50 mg/kg/day. No developmental toxicity was evident (developmental NOEL = 250 mg/kg/day, highest dose tested). Study is acceptable, with no adverse effects. Aldous, 7/22/09.

53074-0011 242015 Pilot study to 53074-0011 242016 above. Dose levels were 0, 50, 100, 200, and 300 mg/kg/day, (N = 7). Treatment at the highest dose level of 300 mg/kg/day led to statistically significant food consumption decrements between gestation days 6 and 12. One female at that dose had ventricular dilatation and associated enlarged cerebrum. That female had one fetus with anal atresia and rudimentary tail (pp. 76, 91). It is not clear whether this single gross finding and associated malformations were treatment-related. The highest dose in the definitive study (250 mg/kg/day) was justifiable, based on this pilot study. Aldous, supplementary data. No DPR worksheet. June 1, 2009.

TERATOLOGY, RABBIT

**53074-0011 242014 Hojo, H., "Teratogenicity study in rabbits with S-1812," The Institute of Environmental Toxicology, Ibaraki, Japan, 12/27/01. Laboratory Study # IET 00-0097. Groups of 25 mated SPF Japanese White Rabbits (Kbl:JW) (30 does in highest dose group) were dosed by gavage (0.5ml/kg corn oil vehicle) from PND days 6-27 with pyridalyl [93.7% purity] in a standard developmental toxicity study (necropsy PND 28). Maternal toxicity NOEL = 100 mg/kg/day (three abortions, one premature delivery, and one death of a pregnant female, all at 150 mg/kg/day). Maternal body weight gains from about PND 12 onward were reduced at 150 mg/kg/day, with associated decrement in food consumption. Developmental toxicity NOEL =

100 mg/kg/day, based on body weight decrements in fetuses (statistically significant in female fetuses). Acceptable, with no adverse effects. Aldous, 7/22/09. See below for pilot study: (Record No. 242013).

53074-0011 242013 Pilot study for 53074-0011 242014, above. Doses up to 150 mg/kg/day pyridalyl were administered to groups of 6 pregnant rabbits. The highest dose was associated with maternal decrement in body weight gain. There was one 150 mg/kg/day doe which aborted, and one at 100 mg/kg/day (the next lower dose) had a premature delivery. Investigators justifiably determined that 150 mg/kg/day would be a suitable highest dose for the primary study. No DPR worksheet for this pilot study. Aldous, 5/28/09.

GENE MUTATION

**53074-0015 242027 Kitamoto, S., "Reverse mutation test of S-1812 in bacterial systems," Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, 8/27/01 (as amended). Laboratory Study # 3376. *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537; and *E. coli* strain WP2*uvrA* were employed in a standard reverse mutation test [pre-incubation] with Pyridalyl [S-1812], Lot No. PS-98041G, 93.7% purity. Dose concentrations were: non-activated at 0, 9.77, 19.5, 39.1, 78.1, 156, and 313 µg/plate (precipitate noted at 156+ µg/plate), and activated at 0, 39.1, 78.1, 156, 313, 625, and 1250 µg/plate (precipitate noted at 1250+ µg/plate). Activated systems used commercial S-9 mix from SD rats exposed to phenobarbital (4 consecutive days), and 5,6-benzoflavone (once, 2 days before sacrifice). Positive controls were all functional, and within laboratory historical control range. Pyridalyl did not cause cytotoxicity at concentrations tested. S-1812 did not show any dose-dependent increases in numbers of revertant colonies in the presence or absence of S-9. Acceptable, with no adverse effects. Aldous, 7/28/09. (Note: Record No. 242026 is an earlier version of this record).

**53074-0015 242028 Cifone, M. A., "CHO HGPRT forward mutation assay with S-1812 with a confirmatory assay and duplicate cultures," Covance Laboratories Inc., Vienna, VA, 3/15/00. Laboratory Study # 6311-215. Cell line CHO-K_1-BH_4 was used to assess forward mutation to $HGPRT^-$ phenotype, hence selection for ability to survive in media containing 6-thioguanine. Pyridalyl [S-1812], Lot No. PS-98041G, 93.7% purity, was tested in two independent trials, each with and without S-9. Study used a 4-hr exposure, a 7-day expression period with subcultures to maintain exponential growth, and 7-10 days in selection medium with 6-thioguanine. Test article concentrations were 0, 9.4, 18.8, 37.5, 75, 150, and 300 µg/ml without S-9 (with precipitation at 300 µg/ml): and with S-9 at 0, 2, 4, 5, 6, 7, and 8 µg/ml (trial 1), or 0, 2, 4, 5, 6, 7, 8 and 10 µg/ml (trial 2) (with excessive cytotoxicity in the range of 8 to 10 µg/ml, and marginal but variable alterations in survival and/or population growth emerging at 5-6 µg/ml in the first trial, and at about 7 µg/ml in the second trial). Positive controls, BrdU (without S-9), and methylcholanthrene (with S-9), gave robust responses. Investigators considered tests with and without S-9 to be negative. Tests without S-9 were clearly negative. Trial 1 with S-9 had four of twelve sub-sets with statistically significant increases in mutant frequencies compared to concurrent controls, whereas Trial 2 with S-9 was negative at all pyridalyl concentrations. The overall results with S-9 should be considered negative, considering that (1) the minimum laboratory threshold for a positive response was not even approached at any time, (2) the statistically significant pyridalyl mutant frequencies in the first trial were comparable to mutant frequencies in negative controls of the second trial, and to Covance pooled negative and vehicle controls for studies with S-9 (3) there was no dose-response in intermediate concentrations in Trial 1. Acceptable, with no adverse effects. Aldous, 7/28/09.

53074-0015 242029 Yamada, T., "L5178Y/$TK^{+/-}$ Mouse lymphoma mutagenesis assay of S-1812," Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, 5/15/02. Laboratory Study # M0991. L5178Y/$TK^{+/-}$ cells were evaluated for potential mutation to allow survival in presence of trifluorothymidine after 4 hrs exposure to Pyridalyl (S-1812),

98.6% purity. There were two trials with S-9 and two without S-9. Non-activated exposures were 3.13, 6.25, 12.5, 25, 50, and 100 µg/ml in first trial, then 3.13, 6.25, 12.5, 25, 37.5, and 50 µg/ml in second trial. Activated exposures were 2.5, 5, 7.5, and 10 µg/ml in first trial, then 2.5, 5, 7.5, 10, and 12.5 µg/ml in second trial. None of the treatments achieved criteria for a positive test. This study was not conducted according to an authorized protocol, and that the study was not inspected by a QA unit. Raw data were determined by the QA to be consistent with the report. Study is considered supplementary useful data. Aldous, 6/26/09.

CHROMOSOME EFFECTS

****53074-0015 242034** Odawara, K., "In vitro chromosomal aberration test on S-1812 in Chinese hamster lung cells (CHL/IU)," Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, 1/24/00. Laboratory Study # 3426. Cells were exposed without S-9 for 6, 24, or 48 hrs up to precipitation levels, consistently with negative results. Cells with S-9 were treated with 0, 15, 20, or 25 µg/ml pyridalyl (93.7% purity) in two trials. Each pyridalyl treatment with S-9 gave a modest increase in cells with chromosomal aberrations compared to concurrent controls. Treatment with S-9 should be considered mildly positive, in agreement with investigators' determination. Acceptable, with "possible adverse effect." Aldous, 7/28/09.

**53074-0015 242039 Odawara, K., "Micronucleus test on S-1812 in CD-1 mice," Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, Oct. 1, 1999. Laboratory Study # 3421. Groups of 5 male mice were dosed once with Pyridalyl [S-1812], 93.7% purity, by corn oil gavage 24 hrs before sacrifice at 0, 500, 1000, or 2000 mg/kg. Bone marrow cells were collected at termination from femurs, and 2000 PCE's per mouse were examined for micronuclei. Cyclophosphamide was an effective positive control (60 mg/kg, treatment 24-hr before sacrifice). Additional groups of 5 male mice were treated 48 hrs before sacrifice with 0 or 2000 mg/kg pyridalyl. All pyridalyl groups were clearly negative. Acceptable study, with no adverse effects. Aldous, 6/30/09.

DNA DAMAGE

**53074-0015 242038 Cifone, M. A., "*In vivo/In vitro* unscheduled DNA synthesis in rat primary hepatocyte cultures at two timepoints with S-1812 technical," Covance Laboratories Inc., Vienna, VA, March 10, 2000. Laboratory Study # 6311-214. Sprague-Dawley (Crl:CD (SD) IGS BR) rats were treated *in vivo* with Pyridalyl [S-1812], Lot No. PS-98041G, 93.7% purity in a single gavage dose of 0, 500, 1000, or 2000 mg/kg. Primary liver cell cultures were prepared from tissues collected of rats killed after 2-4 hours, or alternatively after 15-16 hours from the time of dosing. Investigators exposed cells to ^{3}H-TdR for 4 hours for labeling, then evaluated them for UDS. Dimethylnitrosamine (DMN) was a potent primary control. Results for the shorter exposure time were negative. The 15-16 hour treatment found "% cells with $\geq$ 5 mean net nuclear grain counts" to exceed the pre-determined criteria for a positive response at 1000 mg/kg. Investigators justifiably considered this study to be negative, since only the one intermediate group met the criterion for a positive response. Aldous, 7/28/09.

NEUROTOXICITY

Not required at this time.

METABOLISM

53074-0015 242040 Johnson, T. L., "The pharmacokinetics of [^{14}C]S-1812 (two radiolabels) in the rat upon administration of single oral high dose and low doses," PTRL East, Inc., Richmond, KY, Feb. 6, 2002. Laboratory Study # 1469. Groups of five Sprague-Dawley (Crl:CDBR) rats/sex/group were dosed with pyridalyl, labeled at either the phenyl or propenyl substituent. Test articles were typically about 99% purity, and were dosed by gavage in corn oil (5 ml/kg) at radioactive doses of about 25 µCi/rat. Each of three groups/sex/test article were bled 5 times, providing an overall profile over 15 sampling periods: 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72,

96, and 120 hrs. There were no apparent sex effects on results. As is often the case, high doses were slower to achieve T_{max} than lower doses, and elimination $T_{½}$ tended to be larger with high doses. Propenyl label was associated with slightly higher C_{max} and slightly delayed T_{max} compared to phenyl label. Further, propenyl label had a substantially elevated elimination phase half-life ($T_{½}$) than phenyl label (18 hrs for low dose phenyl label, compared to 52-60 hours for low dose propenyl label). Results of this study, which only involved pharmacokinetics in blood (including fractional analysis of RBC's and plasma), were used to set sampling times for metabolism, disposition, and tissue distribution studies in this series. Acceptable component of the metabolism study series. Aldous, June 3, 2009.

53074-0016 242041 Kimmel, E. C., T. L. Johnson, and A. Bautista, "Tissue distribution of [^{14}C]S-1812 (two radiolabels) in the rat upon administration of single oral high dose and low doses," PTRL West, Inc., Hercules, CA and PTRL East, Inc., Richmond, KY, July 5, 2002 (amended). Laboratory Study # 986W/1471E. Groups of 3 Sprague-Dawley (Crl:CDBR) rats/sex/group were dosed with pyridalyl of high purity, to which labeled pyridalyl (either phenyl- or propenyl-labeled) pyridalyl of at least 97% purity was added to achieve 5 or 500 mg/kg single gavage doses at 50 µCi/rat. The study evaluated radioactivity in many tissues sampled at 4 times (based on results of Record No. 242040): ½ C_{max} (absorption), C_{max}, ½ C_{max} (elimination), and 1/10 C_{max} (elimination)**.** These sampling times were 4, 8, 24, and 48 hrs for 5 mg/kg phenyl label, 6, 8, 24, and 48 hrs for 500 mg/kg phenyl label, 4, 12, 48, and 120 hrs for 5 mg/kg propenyl label, and 6, 12, 72, and 120 hrs for 500 mg/kg propenyl label. Five tissues were evaluated for extractable residues: liver, kidney, lung, blood, and fat. Major metabolites were evaluated in these tissues. Whole blood label diminished an order of magnitude as expected, from C_{max} to 1/10 C_{max} (elimination) in the case of low dose males and females administered phenyl label, and in low dose males administered the propenyl label. In other cases, it appeared that the estimate of 1/10 C_{max} (elimination) was premature. Label persisted longest in fat (regardless of gender, dose, or label position): often showing a higher concentration at estimated 1/10 C_{max} (elimination) than at C_{max}. In general, adrenal radiolabel was higher than in tissues other than fat during the elimination phase. Some other tissues, such as thyroids, pancreas, and ovaries, often had radiolabel content higher than most other tissues during elimination phase, but differences were typically small and variable. Of the five tissues evaluated for extractable residues, fat was unique in that label was consistently highly extractable, and composed nearly all of parent pyridalyl, regardless of gender, dose, or label placement. Initially [½ C_{max} (absorption)], over 91% of label in each of the 5 assessed tissues was extractable following phenyl-label treatment. Extractability for low-dose phenyl groups at 1/10 C_{max} (elimination) for tissues other than fat ranged from 69% upward without obvious differences between tissues. Percent extractability for high-dose phenyl groups at 1/10 C_{max} (elimination) was generally over 90%. Percent extractability of propenyl label at ½ C_{max} (absorption) ranged from 74% to 95%, but dropped for all tissues over time, so that extractability at 1/10 C_{max} (elimination) was 37% to 53% for liver and kidney, 53-67% for lung, but very low for blood (21-22% extractable for low dose propenyl groups, and a constant 0% for high dose propenyl groups). Parent pyridalyl was the major component of residues in liver, kidney, lung, blood, and fat during the early absorption process of phenyl-labeled test article. The percentage of pyridalyl label during elimination varied greatly. Liver retained only small amounts of pyridalyl at 1/10 C_{max} (less than 10% of radiolabel), whereas fat still contained 95-100% of radiolabel as pyridalyl at that time. Parent pyridalyl was typically the dominant component of lung and kidney through 1/10 C_{max}. The major metabolite in liver by 1/10 C_{max} was the product of degradation of the dichloropropenyl group to yield the corresponding phenoxyacetic acid (designated S-1812-PhCH_2COOH). This same acid was typically a major component of blood, particularly in the elimination phase. Blood also carried large and variable amounts of uncharacterized polar components. One more characterized metabolite was relatively important in phenyl labeling experiments: S-1812-DP (loss of the dichloropropenyl group to yield a phenol). S-1812-DP occasionally constituted over 20% of extracted radiolabel, and had no strong proclivity for any one tissue. A comparatively minor characterized metabolite

was HPHM [loss of the trifluoropyridine group, with the balance of parent unchanged]. HPHM rarely exceeded a few percent of extractable radiolabel, and often was below detection limits. Label placement in the propenyl substituent led to increased percent of recovered label in the highly polar fraction. This is consistent with finding in Record 242049 that over 10% of a propenyl-label administration was released as $^{14}C\text{-}CO_2$, suggesting that propenyl label was incorporated into the carbon pool. Acceptable component of the metabolism study series. Aldous, 7/16/09.

53074-0018 242049 Ruzo, L., K. Aldcroft, A. Bautista, T. Johnson, and N. Gibson, "The disposition and metabolism of [^{14}C]S-1812 (three radiolabels) in the rat upon administration of single oral high and low doses," PTRL West, Inc., Hercules, CA, 4/16/02 (amended). Laboratory Study # 807W/1221E. Groups of 4 Sprague-Dawley (Crl:CDBR) rats/sex were dosed once by gavage in corn oil vehicle. There were low-dose and high dose groups at 5 and 500 mg/kg/day, respectively, with ^{14}C label placement in phenyl and propenyl groups, and 5 mg/kg/day only for label placement in the pyridyl group. Investigators evaluated excreta for metabolites, and quantified expired CO_2 over time. Tissues were examined for radioactivity after 168 hrs. About 90% of recovered radioactivity following phenyl- or pyridyl-labeled pyridalyl was found in feces, with about 2% in urine, and the balance of recovered radioactivity was found in tissues (about 1%), and carcass (up to 4%). There was no remarkable dose effect. No CO_2 was obtained from phenyl- or pyridyl-labeled material. In contrast, propenyl-label led to 11-12% in expired air as CO_2, in addition to 55-59% of label in feces, 10-18% in urine, 2% in tissues, and 4-8% in carcass. Analyses of excreted products did not indicate sex differences in disposition, however the extent of metabolism of pyridalyl was typically reduced at the higher dose levels. Low dose exposure to either phenyl or pyridyl label led to 31-39% of administered label in parent pyridalyl, 42-51% of label as S-1812-DP (loss of the dichloropropenyl group to yield a phenol), about 2% of label as S-1812-Py-OH (hydroxy substituent added to pyridyl ring), and up to 4-7% of administered dose as HPHM (loss of the pyridyl ring: relevant in the case of low dose phenyl-label). High dose exposure to phenyl label led to about 50% of administered label as parent pyridalyl and only 25-29% of label as S-1812-DP. Cleavage of the pyridyl ring after pyridyl-label administration yielded 2-hydroxy-5-trifluoromethylpyridine (HTFP) or conjugation products of 3-hydroxy-5-trifluoromethylpyridone (HPDO): each of the latter comprising about 1% of administered dose. Urine following administration of propenyl label consisted of many polar products, none of which was abundant. The majority of excreted radioactivity was obtained within the first 48 hrs after dosing, regardless of label position. Acceptable component of the metabolism study series. Aldous, 7/20/09.

53074-0017 242042 Ruzo, L., A. Bautista, and T. Johnson, "The biliary excretion of [^{14}C]S-1812 in the rat," PTRL West, Inc., Hercules, CA and PTRL East, Inc., Richmond, KY, 1/14/02. Laboratory Study # 985W/1470E. Sixteen non-fasted Sprague-Dawley (Crl:CD BR) rats (7 M and 9 F), provided with biliary cannulae, were dosed once at 5 mg/kg with pyridalyl (99.7% purity) containing sufficient phenyl-[^{14}C]S-1812 to provide about 44-49 µCi/rat. Of these, 4 per sex were usable after removal of rats with insufficient urine or bile collection, or poor radiolabel accountability. Feces, urine, cage-wash, and bile were collected over 48 hrs for assessment of radioactivity and determination of major metabolites. Kidney, liver, and g.i. contents were similarly assessed at 48-hr termination. Distribution as % administered dose for male and female rats, respectively were feces (76% and 55%), urine (0.8% and 0.5%), cage wash (0.9% and 1.3%), bile (8.2% and 7.9%), g.i. tract contents (3.2% and 17.5%), liver (0.3% and 0.5%), kidney (0.0% and 0.0%), and carcass (5.2% and 10.5%). Rats suitable for evaluation lost an average of 42 g (M) and about 32 g (F) during the evaluation period. The principal component of bile was the glucuronide of S-1812-DP (loss of the dichloropropenyl group to yield a phenol). In males, there were also small quantities of unconjugated S-1812-DP plus uncharacterized metabolites (each less than 1% of administered dose). Females yielded only the glucuronide. The main liver metabolite in both sexes was S-1812-$PhCH_2COOH$, consistent with Record No. 242041. Investigators considered the "absorption rate" of pyridalyl to be 65-66%, based among

other things on the presumption that any pyridalyl which was metabolized had been absorbed. Acceptable component of the metabolism study series. Aldous, June 23, 2009.

Summary of metabolism data: Pyridalyl is moderately well-absorbed. Studies with propenyl [^{14}C]-label indicated at least 30% absorption, based on findings of radiolabel in urine, expired air, and tissues. Since parent pyridalyl was not detected in urine or bile, pyridalyl in feces may be considered not to have been absorbed. Thus the observation that about 35% of radiolabel was excreted as parent pyridalyl after phenyl-[^{14}C] or pyridyl-[^{14}C] administration suggests that about 65% is the upper limit of plausible absorption. The predominant point of metabolic attack is the dichloropropenyl group, as shown by the appreciable amount of radiolabel (11-12%) in expired air as CO_2, and as polar metabolites (10-18%) in urine after propenyl [^{14}C]-label administration. Similarly, the predominance of S-1812-DP (loss of the dichloropropenyl group to yield a phenol) in feces following phenyl-[^{14}C] or pyridyl-[^{14}C] labeling attests to the propenyl substituent being the most vulnerable part of the molecule. Secondarily, changes to the pyridyl component through hydrolytic cleavage or hydroxylation of the intact pyridyl ring typically account for a few percent of administered dose. The lipophilic parent compound is capable of appreciable accumulation, primarily unchanged, in body fats. In other tissues, parent molecule is metabolized and excreted, with a median elimination α phase $T_{½}$ of 2 days. Aldous, 6/23/09.

53074-0019 242050 Matsunaga, H., “Metabolism of [phenyl-^{14}C]S-1812 in rats (14-day repeated oral administration),” Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, 3/28/01. Laboratory Study # 3515. Groups of 3 Crj:CD (SD) rats/sex per sacrifice interval were dosed daily by gavage with [phenyl-^{14}C]S-1812 at 5 mg/kg/day for up to 14 days, with some groups maintained beyond the treatment sequence for evaluation of clearance. Sacrifices were held on days 1, 6, 10, 14, 17, 20, and 27 after the first dose [i.e. first dose = Day 0]. ^{14}C-tissue distribution was evaluated in all rats. Metabolite analysis in tissues was performed on the day 14 sacrifice group (limited to blood, peri-renal fat, kidneys, liver, and lungs). Urine and feces were collected over intervals from the group sacrificed on day 27, and major metabolites in these excreta were identified. There were no obvious sex differences in any observations. By the end of day 27, 92-94% of administered label had been collected in feces, and 2-4% in urine. Rate of fecal excretion fell to about 10% of steady state levels by the third day after treatment cessation, by which time urinary excretion was insignificant. Tissue levels tended to accumulate for several days during treatment, usually appearing to have achieved a steady state by about day 10. Accumulation was highest in body fat, as had been observed in single-dose studies (see Record No. 242041). In general, tissues which had relatively high levels in single-dose studies likewise accumulated more than most other tissues in this multiple-dose study. Notable concentrations at day 14 in units of µg a.i. equivalents/g tissue were (males/females) brown fat (57.50/33.70), peri-renal fat (43.11/32.36), liver (5.60/3.74), adrenals (1.55/2.00), and thyroids (3.76/1.83): other internal organs/tissues had lower concentrations. Post-treatment tissue elimination $T_{½}$ was presented as α and β phases where biphasic reductions were indicated. The median α phase $T_{½}$ was 2 days, and the β phase tended to be several days. Liver was comparatively efficient in clearing label after cessation of dosing. Fecal metabolite analysis revealed the major species (in comparable amounts) to be parent pyridalyl and S-1812-DP (loss of the dichloropropenyl group to yield a phenol). Much less abundant in feces were other metabolites previously characterized in single-dose studies: S-1812-PhCH_2COOH (product of degradation of the dichloropropenyl group to yield the corresponding phenoxyacetic acid), HPHM (loss of the trifluoropyridine group, with the balance of parent unchanged), and slightly lesser amounts of pyridalyl which had been hydroxylated on the pyridine ring. The total of the non-characterized fecal metabolites and unextractable residues each constituted about one-fifth of the amount of radiolabel of parent pyridalyl or S-1812-DP. Urinary metabolites were mainly uncharacterized polar products. Comparative tissue metabolite abundances after 14 days of dosing were comparable to single-dose results of Record No. 242041 (total radioactivity of fat >>liver>kidneys and lungs>>blood).

Parent pyridalyl was the main component of fat, and was present in appreciable amounts in other tissues. S-1812-$PhCH_2COOH$ was the primary characterized constituent in liver, kidney, and blood. Study is an acceptable component of the metabolism study series. Aldous, June 19, 2009.

NEUROTOXICITY

Not required at this time.

SUBCHRONIC AND SUBACUTE STUDIES

Rat 2-Week Dietary Toxicity Study

53074-0009; 242002; "Two-Week Oral Toxicity Study of S-1812 in Rats"; (T. Kunimatsu; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Kasugade-naka, Konohana-ku, Osaka, Japan; Project ID. S0397; 4/4/02); Six male Crj:CD (SD) rats/group received 0 or 7000 ppm of S-1812 Technical (lot no. TH95030701, purity: 95.1%) in the diet for 2 weeks (0, 603 mg/kg/day). No deaths resulted from the treatment. The mean body weight of the treated animals was less than that of the control group throughout the treatment period (p<0.01 or 0.05). The food consumption the 7000 ppm animals was significantly less than that of the control group (p<0.01). The mean red blood cell count, hemoglobin concentration and hematocrit of the 7000 ppm animals were elevated in comparison to the control animals (p<0.01). The reticulocyte count was lower than that of the control group (p<0.01). The prothrombin and activated partial thromboplastin times were prolonged for the 7000 ppm animals (p<0.01). In the clinical chemistry evaluation, the total serum cholesterol, triglycerides, phospholipids and blood urea nitrogen were elevated for the 7000 ppm animals (p<0.01or 0.05). Alanine aminotransferase activity in the serum of the 7000 ppm animals was increased (p<0.01). The inorganic phosphate level was greater than the control value (p<0.01). The serum potassium level was reduced for these animals in comparison to the control group (p<0.05). The mean relative liver, testes and adrenal weights were greater for the 7000 ppm animals than the control group (p<0.01 or 0.05). The livers of the 7000 ppm animals were dark in appearance. In the histopathological examination of the lungs of the 7000 ppm group, edema was noted for three of the animals. Medial arterial thickening was noted for 3 of these animals. Foamy, eosinophilic cells were noted in the alveoli of all of the animals. **Possible adverse effect:** medial arterial thickening in the lungs; **Study supplemental.** (Moore, 4/21/09)

Rat 4-Week Dietary Toxicity Study

53074-0009; 242009; "Four-Week Oral Toxicity Study of S-1812 in Rats"; (T. Kunimatsu; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Kasugade-naka, Konohana-ku, Osaka, Japan; Project ID. S0418; 4/4/02); Six Crj:CD(SD) rats/sex/group received 0, 70, 200, 700 or 2000 ppm of S-1812 Technical (lot no. NSA-950525, purity: 98.7%) in the diet for 4 weeks (reported a.i. uptake: (M) 0, 7.05, 19.5, 64.6, 182 mg/kg/day, (F) 0, 7.21, 19.6, 66.4, 188 mg/kg/day). An additional group of 6 rats/sex/group received 0 or 2000 ppm of the test material in the diet for 2 weeks. No deaths resulted from the treatment. The mean body weights of the males in the 2000 ppm group were less than those of the control animals (p<0.01 or 0.05). The mean food consumption of the 700 and 2000 ppm males was less than the control values during the 1st 2 weeks of the study (p<0.01). This effect persisted to the termination of the study for the 2000 ppm animals. No treatment-related effects were apparent in the urinalysis or hematology. In the clinical chemistry evaluation, total cholesterol and phospholipid levels were elevated for both sexes in the 2000 ppm groups after two weeks of treatment (p<0.01 or 0.05). After 4-weeks of treatment, the total cholesterol level of the 700 and 2000 ppm males was statistically elevated above that of the control group (p<0.01or 0.05). The relative mean liver weights for both sexes in the 700 and 2000 ppm groups and the 200 ppm females were greater than the control group after 4 weeks of treatment (p<0.01 or 0.05). The relative mean lung weights of both sexes in the 2000 ppm group were greater than those of the control group (p<0.01 or 0.05). The mean absolute and relative ovarian weights of the 2000 ppm females were greater than the control values (p<0.01). In the histopathology examination,

degeneration and/or vacuolation of the adrenal cortex was noted in the 2000 ppm males after 2 weeks of treatment and in the 2000 ppm females after both 2 and 4 weeks of treatment. In the ovaries of the 2000 ppm females, degeneration and/or vacuolation of the interstitial cells was noted after both 2 and 4 weeks of treatment. Persistent corpora luteum were also observed in the group at both time points. In the tissue samples examined by electron microscopy, slightly increased fatty vacuolation in the zona reticularis of the adrenal glands from one male and two females of the 2000 ppm group was observed after 2 weeks of treatment. After 4 weeks of treatment, vacuolation was also noted in the zona reticularis of one male and two females of the 2000 ppm group. Slight swelling of the mitochondria in the zone reticularis was evident for these two females as well. **Possible adverse effect:** degeneration of adrenal cortex and interstitial cells in the ovaries; **Rat Four-Week Dietary NOEL:** (M/F) 200 ppm ((M) 19.5 mg/kg/day, (F) 19.6 mg/kg/day) (based upon the lower food consumption noted for both sexes in the 700 ppm group); **Study supplemental**. (Moore, 4/23/09)

Rat Subchronic Dietary Toxicity Studies

**** 53074; 241996**; "S-1812: 90-Day Subchronic Oral Toxicity in Rats"; (A. Enomoto; The Institute of Environmental Toxicology, Mitsukaido-shi, Ibaraki 303-0043, Japan; Project ID. IET 98-0075; 9/29/99); Ten Sprague-Dawley rats/sex/group received 0, 100, 1000 or 2000 ppm of S-1812 (lot no. PS-98041G; purity: 93.7%) in the diet for 13 weeks ((M) 0, 5.56, 56.0, 111.3 mg/kg/day, (F) 0, 6.45, 64.0, 128.6 mg/kg/day). One female in the 2000 ppm group died during the 9th week of treatment. The mean body weights of both sexes in the 1000 and 2000 ppm groups were less than the control group throughout the study ($p<0.01$, 0.05, NS). The mean food consumption was also less for these two groups throughout the study ($p<0.01$, 0.05, NS). No treatment-related effects were noted in the ophthalmology, urinalysis or hematology. In the clinical chemistry evaluation, the mean serum cholesterol levels for both sexes in the 2000 ppm group were greater than the control values ($p<0.01$). In the necropsy, the mean relative liver weights of both sexes in the 2000 ppm group and the females in the 1000 ppm group were greater than those of the control group ($p<0.01$). In the liver, centrilobular hypertrophy of the hepatocytes was noted for both sexes in the 2000 ppm group ((M) 0: 0/10 vs. 2000: 8/10, (F) 0: 0/10 vs. 2000: 5/10). An increase the severity of single cell necrosis was noted in the livers of the 1000 and 2000 ppm females (single cell necrosis, grade 1: 0: 4/10 vs. 1000: 5/10, 2000: 5,/10, grade 2: 0: 0/10 vs. 1000: 2/10, 2000: 4/10). The tenth female in the 2000 ppm group demonstrated centrilobular hepatic necrosis. In the ovaries, vacuolation of the interstitial gland cells in the 1000 and 2000 pppm groups was also identified (0: 0/10 vs. 1000: 2/10, 2000: 8/10). **Possible adverse effect:** hepatic necrosis. **Rat Subchronic dietary NOEL:** (M/F) 100 ppm ((M) 5.56 mg/kg/day, (F) 6.45 mg/kg/day) (based upon the lower mean body weights for both sexes in the 1000 ppm group and the treatment-related effects on the livers of the 1000 ppm females); **Study acceptable.** (Moore, 4/20/09)

**53074-0009 241998 Kimura, J., "Three-month oral toxicity study of S-1812 in rats," Environmental Health Science Laboratory, Osaka, Japan, 9/30/97. Laboratory Study # S0450. Groups of ten Crj:CD (SD) rats/sex/group were dosed in diet with Pyridalyl (S-1812), Lot No. KOBE5951006, purity 98.4% for 13 weeks in a subchronic study at 0, 70, 700, 2000, and 3500 ppm. Estimated achieved dose levels in treated groups were 4.7, 47, 133, and 233 mg/kg/day in increasing male groups, and 5.4, 56, 153, and 256 mg/kg/day in increasing female groups. NOEL = 70 ppm, based mainly on liver responses such as single-cell hepatocellular necrosis and associated mononuclear cell infiltration (statistically significant in females only); "dark" livers (males); elevated albumin/globulin ratio, cholesterol, and phospholipids (all in males); and elevated γ-glutamyl transpeptidase (females). Typically above signs seen in only one sex at 700 ppm were observed in both sexes at 2000 and 3500 ppm. Body weights and food consumption were reduced, dose-related, at 2000 and 3500 ppm. Vacuolation of ovarian interstitial cells was increased in 2000 and 3500 ppm females. Vacuolation of the zona reticularis of the adrenals was elevated in 2000-3500 ppm females and in 3500 ppm males. Vacuolation of the zona fasciculata was elevated in 3500 ppm females. Grossly "pale" adrenals

were observed in 2000 and 3500 ppm females. Foamy or eosinophilic cells were observed in lung alveoli of both sexes at 3500 ppm. A few control and 3500 ppm tissues were prepared for EM evaluation: these examinations showed proliferation of the SER in all high dose rats examined, and confirmed adrenal cortical vacuolation changes noted above (but only in 1 of the 4 high-dose rats examined). Six rats/sex, of controls and high dose groups only, were evaluated for serum hormones: this satellite analysis found 3500 ppm males significantly lower than controls in testosterone, and estradiol in 3500 ppm females was significantly lower than respective controls. The latter was likely related to chance variation in estrous cycle stage at sampling in normally-cycling rats. Acceptable, with no adverse effects. Aldous, 7/16/09.

Rat 2-Week Repeated Dosing Dermal Toxicity Study

53074-0009; 242001; "7-Day Repeated Dose Dermal Toxicity Study of S1812 in Rats"; (H. Ogata; Safety Assessment Laboratory, Panapharm Laboratories Co., Ltd., Uto-shi, Kumamoto 869-0425, Japan; Project ID. 20046; 3/28/01); The skin of five Crj:CD (SD) rats/sex was exposed to 0, 100, 300 or 1000 mg/kg/day of S-1812 Technical (lot no. PS-98041G; purity: 93.7%), 6 hours/day, for 7 days. No apparent treatment-related effects were noted in the hematology or clinical chemistry evaluations. No treatment-related lesions were noted in the necropsy examination. **No adverse effect indicated.** 1000 mg/kg/day selected for high dose in guideline 28-day repeated dosing dermal toxicity study. **Study supplemental.** (Moore, 4/20/09)

Rat 4-Week Repeated Dosing Dermal Toxicity Study

** 53074-0009; 242000; "28-Day Repeated Dose Dermal Toxicity Study of S1812 in Rats"; (H. Ogata; Safety Assessment Laboratory, Panapharm Laboratories Co., Ltd., Uto-shi, Kumamoto 869-0425, Japan; Project ID. 20047; 4/3/02); The skin of 10 Sprague-Dawley rats/sex/group was exposed to 0, 30, 100 or 1000 mg/kg/day of S-1812 Technical (lot no. PS-98041G; purity: 93.7%), 6 hours/day, for 28 days. No deaths resulted from the treatment. No clinical signs were noted. The mean body weights and food consumption were not affected in a dose-related manner. No treatment-related effects were noted in the ophthalmology, urinalysis, hematology, urinalysis, necropsy or histopathology. **No adverse effect indicated. Rat 28-Day Repeated Dosing Dermal Toxicity NOEL:** (M/F) 1000 mg/kg/day (based upon the lack of treatment-related effects in the 1000 mg/kg group); **Study acceptable.** (Moore, 4/20/09)

Rat 28-Day Inhalation Toxicity Study

53074-0009; 242011; "S-1812 - A 28-Day Inhalation Toxicity Study, A Nebulized Aerosol Formulation in the Albino Rat"; (W. Lee; CTBR Bio-Research Inc. (CTBR), Senneville, Quebec H9X 3R3, Canada; Project ID. 78255; 9/29/03); Ten Sprague-Dawley rats/sex/group were exposed nose-only to 0, 0.023, 0.157, or 0.931 mg/l (gravimetric) of S-1812 technical (PS-98041G; purity: 93.70%) for 6 hours/day, 5 days/week for 4 weeks (the nominal target concentrations were 0.025, 0.16 and 1.0 mg/l). The mean MMAD (GSD) values were 2.8 (1.7), 2.8 (1.8), and 2.8 (1.8) um, respectively. One female in the intermediate exposure group was found dead on day 22. The mean body weights of both sexes in the intermediate and high exposure groups were less than the control values during the study period ($p<0.01$ or 0.05). The mean food consumption of these study animals was less than that of the control group at various times during the study. No treatment-related effects were noted in the ophthalmology or hematology evaluations. In the clinical chemistry, the mean sodium and chloride levels in the serum of both sexes in the intermediate and high exposure groups were greater than the control values ($p<0.001$, 0.01 or 0.05). The mean serum globulin of both sexes in the high exposure group were less than the control values ($p<0.001$ or 0.05). The mean serum inorganic phosphate levels of both sexes in the high exposure group were greater than those of the control group ($p<0.01$ or 0.05). The mean cholesterol and albumin levels of the males in the high exposure group were greater than the control values ($p<0.001$ or 0.05). The mean relative liver and lung weights of both sexes in the high exposure group were greater than those of the control group ($p<0.001$). The mean relative testis weight of the high exposure males was

greater than the control value (p<0.001). The mean relative thymus weights of both sexes in the high exposure group and the mean absolute thymus weight of the high exposure group females were less than the control values (p<0.01 or 0.05). The mean relative adrenal weight of the high exposure females was greater than that of the control (p<0.05). In the histopathological examination, a higher incidence of macrophage accumulation was noted in the lungs of the high exposure group males (0: 1/10 vs. 0.931: 8/10). Atrophy/necrosis was observed in the lymphoid tissue of the thymus of the high exposure group males (0: 0/10 vs. 0.931: 4/10). **Possible adverse effect:** necrosis in the lymphoid tissue of the thymus. **Rat 28-Day Inhalation Exposure NOEL:** (M/F) 0.023 mg/l (based upon treatment-related effects on clinical chemistry parameters of both sexes in the 0.157 mg/l exposure group); **Study supplemental.** (Moore, 4/27/09)

Mouse 2-Week Dietary Toxicity Study

53074-0009; 242010; "Two-Week Oral Toxicity Study of S-1812 in Mice"; (K. Bando; Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Kasugade-naka, Konohana-ku, Osaka, Japan; Project ID. S023; 10/1/97); Six Crj:CD-1 mice/sex/group received 0, 700, 2000 or 7000 ppm of S-1812 technical (lot no. KOBE951221; purity: 93.3%) in the diet for 2 weeks (reported a.i. uptake, (M) 0, 116, 332, 1140 mg/kg/day, (F) 0, 121, 356, 1240 mg/kg/day). No deaths occurred during the study. The mean body weights of both sexes in the 7000 ppm group were less than those of the control group (p<0.01 or NS). The food consumption of these animals was less than that of the control group. No treatment-related effects were apparent in the hematology. In the clinical chemistry, the total cholesterol and phospholipids in the serum of both sexes in the 7000 ppm group and of the males in the 2000 ppm group were increased in comparison to the control group values (p<0.01). The serum alkaline phosphatase and leucine aminopeptidase activities was slightly elevated for both sexes in the 7000 ppm group (p<0.01). Although sodium, calcium and/or chloride levels were greater in the serum of some of the treated animals, there was no consistent effect in both sexes. Serum cholinesterase activity was increased for the males in the 7000 ppm group. The blood urea nitrogen was elevated for the females in the 7000 ppm group (p<0.05). In the necropsy, the mean absolute liver weights of the 7000 ppm males and the mean relative liver weights of both sexes in the 7000 ppm group and the males in the 2000 ppm group were greater than the control values (p<0.01). In the histopathological examination, vacuolation was noted in the zona reticularis/fasciculata of the adrenal gland of the 7000 ppm males (0: 0/6 vs. 7000: 5/6). In the liver of the 7000 ppm males, slight and mild centrilobular hypertrophy (0: 0/6 vs. 7000: 4/6) and slight and mild centrilobular vacuolation (0: 0/6 vs. 7000: 6/6) were observed. No apparent treatment-related lesions were noted for the females. **No adverse effect apparent. Mouse 2-Week Dietary NOEL: (M) 700 ppm (**116 mg/kg/day) (based upon increased total cholesterol and phospholipids in the serum and increased relative liver weight of the 2000 ppm males), **(F) 2000 ppm** (356 mg/kg/day) (based upon increased total cholesterol and phospholipids in the serum and increased relative liver weight of the 7000 ppm females); **Study supplemental.** (Moore, 4/24/09)

Mouse Subchronic Dietary Toxicity Study

**53074-0009 241997 Enomoto, A., "S-1812: 90–day subchronic oral toxicity study in mice," The Institute of Environmental Toxicology, Ibaraki, Japan, Oct. 4, 1999. Laboratory Study # IET 98-0076. Groups of 12 ICR (Crj:CD-1) mice/sex/group were dosed in diet with Pyridalyl [Lot PS-98041G, 93.7% purity] for 13 weeks in a subchronic study at 0, 70, 700, 3500, or 7000 ppm. Mean estimated achieved dose levels were 8.2, 82, 378, and 721 mg/kg/day in treated males, and 9.5, 87, 415, and 879 mg/kg/day in females. NOEL for males = 70 ppm, based on small increases in plasma cholesterol. NOEL for females = 700 ppm, based on decreased body weight and food consumption; slightly decreased hematocrit; elevated plasma cholesterol and creatinine; increased relative liver weight; and ovarian atrophy. Findings at the LOEL for females were also found at 3500-7000 ppm in males except for serum creatinine and ovarian pathology. Other key findings at 3500-7000 ppm in males included centrilobular hypertrophy

(incidences in males of 0, 0, 0, 9, and 12 in control through high dose), associated gross finding of accentuated lobular pattern in liver, and significant increases in alkaline phosphatase. Study is acceptable, with no adverse effects. Aldous, 7/16/09.

Dog Subchronic Oral Toxicity Study

**53074-0009 241999 Suetake, K., "90-Day oral toxicity study with S-1812 in dogs," Panapharm Laboratories Co., Ltd., Kumamoto, Japan, 1/18/00. Laboratory Study # 29814. Groups of 4 beagle dogs/sex/group were dosed by capsule with Pyridalyl (S-1812), 93.7% purity, in a subchronic study. Dose levels were 0, 10, 100, and 300 mg/kg/day. Initially the highest dose was 1000 mg/kg/day, but due to deaths of one male and one female by day 3, the highest dose was reduced to 300 mg/kg/day (on day 8 for females, and on day 15 for males), and the two decedents were replaced. NOEL = 10 mg/kg/day (females), and 100 mg/kg/day (males). Findings in 100 mg/kg/day females included vacuolation of the zona fasciculata of the adrenal cortex in 3/4 females (this finding was observed in all 300 mg/kg/day survivors). Lower frequency observations, considered treatment-related findings in 100 and 300 mg/kg/day females, included liver mid-zonal hepatocellular vacuolation, and mid-zonal hepatocellular eosinophilic inclusions; thickening of arterial and arteriolar walls in the lungs; and brown pigmentation in the proximal tubules of the kidneys. Above lung findings were also observed in one high dose male. There was a slight (non-significant) decrement in body weight gain in both sexes at 300 mg/kg/day. Tachypnea and/or wheezing commonly occurred in females, being observed in 3 of the 4 females at 1000 mg/kg/day, also in two of these females after dose reduction to 300 mg/kg/day, and in one 100 mg/kg/day female on days 10-11 only. One high dose male (apparently during the 1000 mg/kg/day treatment phase) also had tachypnea and wheezing of 2 days' duration. Study is acceptable, with no adverse effects. Aldous, 5/27/09.

53074-0011 242012 Dohi, T., "Stability test of S-1812 in capsule," Sumika Chemical Analysis Services, Ltd., Osaka, Japan, 1/25/99. Page 12 shows that storage in capsules for up to 14 days has no perceptible effect on stability. Supplementary data (relates to dog capsule administration studies). No DPR worksheet. Aldous, 5/27/09.

53074-0017 242045 Kunimatsu, T., "Four-week hormone study of S-1812 in rats (non-GLP)," Sumitomo Chemical Co., Ltd., Osaka, Japan, Sept. 6, 2002. Laboratory Study # S0998. Groups of 8 males or16 females per sex per group were dosed in diet with pyridalyl, 93.7% purity, for 4 weeks in a supplementary study to evaluate several possible hormonal effects (suggested by reproduction study findings and other studies). Dose levels were 0, 100, 500, 1000, and 2000 ppm. Special features of this study included analyses of hormones (testosterone and corticosterone in males; estradiol, progesterone, and corticosterone in females); organs weights of liver, kidneys, and adrenals in both sexes, plus dorso-lateral prostate, ventral prostate, testes, epididymides, and seminal vesicles in males, or uterus and ovaries in females; and histopathology of ovaries. Usually N = 8 for any particular measure. Body weights were reduced significantly in males at ≥ 500 ppm and in females at ≥ 1000 ppm, with decrements of food consumption at those dose levels. Hormones were examined at termination only, and were sufficiently variable that only major differences could have been perceived in this study: all hormone values were negative. Supplementary data of limited usefulness. Aldous, 7/13/09.

53074-0017 242043 This is the analysis report for concentrations of pyridalyl in Record No. 242045 above. No DPR review is needed. Aldous, 7/13/09.

In vitro MECHANISTIC STUDIES

53074-0017 242044 Nagahori, H., "Effects of S-1812 on sex steroid biosynthesis in rats," Sumitomo Chemical Co., Ltd., Osaka, Japan, Sept. 5, 2002. Laboratory Study # X0091. Primary Leydig cell or ovary cell culture media were exposed to Pyridalyl to evaluate effects on production of hormones or activities of enzymes related to steroid hormone biosynthesis.

Analyses by RIA of Leydig cell cultures for three hormones (17α-OH-progesterone, androstenedione, and testosterone) at pyridalyl levels of 0, 1, 3, 10, and 30 µM found significant increases in androstenedione at 10 and 30 µM (increases over control levels of 22% and 64%, respectively). A time-course assay found androstenedione concentrations in the Leydig cell cultures to be consistently about twice control levels after exposure to 10 µM pyridalyl from 5 to 48 hrs. Concentrations of hormones 17α-OH-progesterone and testosterone were unchanged in Leydig cell culture media. Activity of the enzyme 17β-hydroxysteroid dehydrogenase, which forms testosterone from androstenedione, was slightly (but statistically significantly) reduced at 3 and 30 µM in Leydig cell cultures, but 10 µM levels were unaffected. This is weak evidence of a relevant inhibition of this enzyme. Assessment of primary ovarian cell cultures to the same pyridalyl concentrations yielded no changes in levels of progesterone, 17α-OH-progesterone, androstenedione, or estradiol. Also, activities of enzymes 17β-hydroxysteroid dehydrogenase and aromatase were unaffected in ovarian cell cultures. Investigators concluded that a slight inhibition of 17β-hydroxysteroid dehydrogenase in males was the cause of a slight loading of androstenedione at 10 to 30 µM. Useful non-guideline supplementary study. Aldous, 7/14/09.

53074-0017 242045 Kunimatsu, T., “Four-week hormone study of S-1812 in rats (non-GLP),” Sumitomo Chemical Co., Ltd., Osaka, Japan, Sept. 6, 2002. Laboratory Study # S0998. Groups of 8 males or16 females per sex per group were dosed in diet with pyridalyl, 93.7% purity, for 4 weeks in a supplementary study to evaluate several possible hormonal effects (suggested by reproduction study findings and other studies). Dose levels were 0, 100, 500, 1000, and 2000 ppm. Special features of this study included analyses of hormones (testosterone and corticosterone in males; estradiol, progesterone, and corticosterone in females); organs weights of liver, kidneys, and adrenals in both sexes, plus dorso-lateral prostate, ventral prostate, testes, epididymides, and seminal vesicles in males, or uterus and ovaries in females; and histopathology of ovaries. Usually N = 8 for any particular measure. Body weights were reduced significantly in males at ≥ 500 ppm and in females at ≥ 1000 ppm, with decrements of food consumption at those dose levels. Hormones were examined at termination only, and were sufficiently variable that only major differences could have been perceived in this study: all hormone values were negative. Supplementary data of limited usefulness. Aldous, 7/13/09.

53074-0017 242043 This is the analysis report for concentrations of pyridalyl in Record No. 242045 above. No DPR review is needed. Aldous, 7/13/09.

ACUTE STUDIES ON METABOLITES OR IMPURITIES OF PYRIDALYL

53074-0017 242047 Kunimatsu, T., “Acute oral toxicity study of S-1812 impurity (propargyl ether) in rats (non-GLP),” April 10, 2002. Presumably the same unspecified propargyl ether impurity tested in mice above was evaluated in this rat acute oral toxicity test. Groups of 5/sex Crj:CD(SD) rats were dosed once with this propargyl ether (Lot No. Y-42-98, 99.4% purity) at 2000 mg/kg (aq. methylcellulose vehicle, 10 ml/kg). Rats were observed frequently shortly after dosing, then daily for 14 days. Rats were necropsied at termination. There were no deaths, no treatment-related clinical signs, and no treatment-related gross findings. Potentially useful supplementary data, although the particular ether was not specified. No DPR worksheet. Aldous, 7/15/09.

GENOTOXICITY TESTS ON METABOLITES OR IMPURITIES OF PYRIDALYL

53074-0015 242023 Ota, M., “Reverse mutation test of HPDO in bacterial systems,” Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, Feb. 5, 2002. Lab Project ID M2777. This minor metabolite of pyridalyl was tested in a bacterial reverse mutation test using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537;

and *E. coli* strain WP2*uvrA* (pre-incubation, with and without S-9). Test article gave a definitive dose-related increase in the number of revertant colonies with WP2*uvrA* over the range of 156-2500 µg/plate with and without S-9. TA1535 also increased mutation frequencies 3- to 4-fold over controls in absence of S-9 in the range of 156-625 µg/plate, above which the compound was cytotoxic. Useful supplementary data, suggesting a "possible adverse effect" of a minor metabolite. Aldous, no DPR worksheet, 7/28/09.

53074-0015 242024 Ota, M., "Reverse mutation test of HTFP in bacterial systems," Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, Feb. 5, 2002. Lab Project ID M2508. This minor metabolite of pyridalyl (see Ruzo *et al.* in DPR Document No. 53074-0018, Record No. 242049) was tested in a bacterial reverse mutation test using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537; and *E. coli* strain WP2*uvrA* (pre-incubation, with and without S-9). HTFB was not cytotoxic and did not precipitate at concentrations up to the limit test of 5000 µg/plate. There were clear, dose-related increases in revertants with WP2*uvrA* at 1250 µg/plate with and without S-9: furthermore, 625 to 1250 µg/plate were associated with approximately 2-fold increase in revertants (without dose-response in this range). TA 1535 showed a dose-related increase in revertants at 2500 and 5000 µg/plate, with and without S-9. The other strains were negative. Useful supplementary data, suggesting a "possible adverse effect" of a minor metabolite. Aldous, no DPR worksheet, July 6, 2009.

53074-0015 242025 Kitamoto, S. "Reverse mutation test of dehydrochlorinated derivative of S-1812 in bacterial systems," Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka, Japan, March 11, 2002. Lab Project ID M3158. Test article was 2,6-dichloro-4-(3-chloroprop-2-ynyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether, 99.4% purity, Lot No. Y-4298. Material was tested in a bacterial reverse mutation test using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537; and *E. coli* strain WP2*uvrA* (pre-incubation, with and without S-9). There was a range-finding assay, followed by a single trial of the main assay. Test article was not cytotoxic, but precipitated at concentrations of 500 µg/plate and above without S-9 and at or above 1500 µg/plate with S-9. Author considered that TA 1535 showed a dose-related increase in revertants in presence of S-9, based on subtle increases in revertants in both the range-finding trial and in the primary assay. Actual revertant numbers for the primary assay at 0, 156, 313, 625, 1250, 2500, and 5000 µg/plate were 7, 15, 12, 16, 14, 7, and 8, respectively. No other strains indicated test criteria for a positive test. Historical control range for TA 1535 was 8.4 ± 3.1, which appears to indicate a mild treatment response in this study. Useful supplementary data, suggesting a "possible adverse effect" of a minor metabolite. Aldous, no DPR worksheet, July 28, 2009.

53074-0015 242030 Sasaki, K. "*In vitro* gene mutation test of HPDO in V79 Chinese hamster cells," Hatano Research Center, Kanagawa, Japan, March 14, 2002. Lab Project ID G-01-055. This minor metabolite of pyridalyl was tested at 0.11 to 1.8 mg/ml with and without S-9 to detect mutation to 6-thioguanine resistance. There were two independent trials. The highest dose level in the first trial with S-9 obtained a mutation frequency 2.1 x higher than the concurrent control (also out of the historical negative control range out of 14 studies). All other values were uneventful. There was no indication of a response in the second trial. This study was appropriately considered negative by investigators. Aldous, no DPR worksheet, July 9, 2009.

53074-0015 242032 Sasaki, K. "*In vitro* gene mutation test of HTFP in V79 Chinese hamster cells," Hatano Research Center, Kanagawa, Japan, March 14, 2002. Lab Project ID G-01-053. This minor metabolite of pyridalyl was tested at 0.10 to 1.6 mg/ml with and without S-9 to detect mutation to 6-thioguanine resistance. There were two independent trials. In the first trial without S-9, mutation frequencies at 0.4 and 1.6 mg/ml were elevated over concurrent controls by 3.8 and 7.5-fold, respectively. The latter values were within range of historical negative controls. In the second trial without S-9, all HTFP values were below concurrent control. There

was no consistent change in the trials with S-9. This study was appropriately considered negative by investigators. Aldous, no DPR worksheet, July 9, 2009.

53074-0015 242033 Kitamoto, S. "*In vitro* gene mutation test of S-1812 impurity (propargyl ether) in V79 Chinese hamster cells," Hatano Research Center, Kanagawa, Japan, 4/26/02. Lab Project ID G-01-093. This unspecified propargyl ether contaminant in pyridalyl was tested at 0.01 to 0.1 mg/ml as in Record No. 242032, above. The highest dose level was highly cytotoxic with S-9, but had no evident toxicity without S-9. There was no increase in mutation frequency with or without S-9 in the single trial undertaken. Aldous, no DPR worksheet, July 9, 2009.

53074-0015 242035 Yamakage, K., "Chromosomal aberration test of HPDO in cultured Chinese hamster lung cells," Hatano Research Center, Kanagawa, Japan, 3/14/02. This minor metabolite of pyridalyl was tested with and without S-9 in two trials. Exposure durations were 6 hrs and 24 hrs, respectively, without S-9; and 6 hrs in both trials with S-9. There were minor increases in cells with chromosomal aberrations with S-9 after 6 hrs treatment, but without dose-response. In the 24-hr exposure groups without S-9, there was a dose-related increase in aberrations (statistically significant 0.23 and 0.45 mg/ml: the highest concentrations which could be assessed due to cytotoxicity at higher levels). Assays with S-9 were not remarkable. Chromosomal aberrations observed without S-9 indicate a "possible adverse effect" on this metabolite. Aldous, no DPR worksheet, July 9, 2009.

53074-0015 242036 Yamakage, K., "Chromosomal aberration test of HTFP in cultured Chinese hamster lung cells," Hatano Research Center, Kanagawa, Japan, 3/14/02. This minor metabolite of pyridalyl was tested with and without S-9 in two trials. Exposure durations were 6 hrs and 24 hrs, respectively, without S-9; and 6 hrs in both trials with S-9. Concentrations were 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml in all cases. There were no treatment effects on chromosomal aberrations in any trials. Numbers of polyploid cells were significantly increased in the 6-hr study with S-9 at 1.6 mg/ml, and there were non-significant increases in polyploid cells at that dose level in the 6-hr trial without S-9 and in the second 6-hr trial with S-9. Investigators considered this to be a negative study, however this DPR reviewer considers the general increase in polyploid cells to be a plausible treatment effect. Thus this is considered to represent a "possible adverse effect" for this metabolite. Aldous, no DPR worksheet, July 9, 2009.

53074-0015 242037 Matsumoto, K., "HPDO: Micronucleus test in mice," The Institute of Environmental Toxicology, Ibaraki, Japan, Oct. 7, 2002. This was a negative micronucleus test with this minor metabolite of pyridalyl. Aldous, no DPR worksheet, July 9, 2009.

53074-0017 242046 Mizuhashi, F., "Micronucleus test of S-1812 impurity (propargyl ether) in mice," Biosafety Research Center, Shizuoka, Japan, 4/30/02. An unspecified propargyl ether impurity was tested in the mouse micronucleus assay at 0 (corn oil), 500, 1000, or 2000 mg/kg (6 male Crj: Cd-1 (ICR) [SPF] mice/group), with a 24-hr pre-treatment time. Additional groups of 6 males received 2 mg/kg MMC (positive control) for 24 hrs, or 0 or 2000 mg/kg propargyl ether with a 48-hr pre-treatment time. Toxicity was not limiting at any dose, based on absence of clinical signs or body weight effects. Five mice/group were assessed for the micronucleus assay. All propargyl ether tests were negative, compared with a clearly functional positive control. There was no change in ratio of PCE's to total erythrocytes. Potentially useful supplementary data, although the particular ether was not specified. No DPR worksheet. Aldous, 7/14/09.

53074-0017 242048 Nakajima, M., "*In vivo/in vitro* unscheduled DNA synthesis (UDS) assay with HTFP in rat hepatocytes," Biosafety Research Center, Shizuoka, Japan, Sept. 1, 2005. Lab project ID 8781 (028-049). HTFP (minor metabolite of pyridalyl), 97% purity, was

administered to male Sprague-Dawley (Crl:CD (SD) IGS BR) rats at 0, 1000, or 2000 mg/kg (0.5 % methylcellulose suspension, 10 ml/kg b.w.) by gavage. Groups of 4 were dosed either 2 hrs or 16 hrs before sacrifice. Positive controls were 5 mg/kg DMN (2-hr pre-treatment) or 100 mg/kg 2-AAF (16-hr pre-treatment). Primary liver cell cultures were prepared at respective termination times. Investigators exposed cells to ^{3}H-TdR for 4 hours for labeling, then evaluated them (blind to treatment) for UDS. A preliminary toxicity study in both sexes found that both sexes could tolerate at least 2000 mg/kg, hence males only were utilized in the UDS study. All pyridalyl groups were negative for UDS in the primary study. Positive controls were functional. Useful supplementary data on a minor metabolite. No DPR worksheet. Aldous, 7/15/09.