CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
ORTHOSULFAMURON (designated as IR5878 in laboratory reports)

Chemical Code # 5904, Document Processing Number (DPN) # 52976
SB 950 # N/A
February 5, 2007
Revised date (N/A)

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect (oncogenicity)
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, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers for the above study types through 224244 (Document No. 52976-0108) were examined. This includes all relevant studies indexed by DPR as of January 11, 2007.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: t20070205.wpd
Revised by Aldous, 2/5/07.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

****52976-0031 224151** Webley, L., "IR5878: Combined carcinogenicity and toxicity study by dietary administration to HAN Wistar rats for 104 weeks," Huntingdon Life Sciences Ltd., 7/20/04. Laboratory Study #: AGR 131/033063. Two batches of orthosulfamuron (purity 98.6 to 98.8%) were used, with diets adjusted weekly to provide constant mg/kg/day dosages of 0, 1, 5, 500, and 1000 mg/kg/day. Fifty Han Wistar rats/sex/group were assigned to a 2-yr oncogenicity study. Also at those dose levels, an additional twenty rats/sex/group were assigned to a 1-yr chronic toxicity study. Mean achieved dosages were within ± 5% of target. NOEL = 5 mg/kg/day. Body weights were reduced in both sexes at 500 to 1000 mg/kg/day during the second year of the oncogenicity study. Liver was a primary target, with statistically significant relative liver weight increases in both sexes at 500 and 1000 mg/kg/day at 1-yr toxicity study termination, and in males at termination of the oncogenicity study. Hepatocytic centrilobular vacuolation and hypertrophy were highly significantly elevated in toxicity study males at these dose levels, as was vacuolation in oncogenicity study males. Altered liver function may account for elevated plasma protein levels, and secondarily for elevated circulating calcium, each at 1000 mg/kg/day. Kidneys showed treatment effects in the oncogenicity study only, with significantly elevated degree and/or incidence of hyperplasia and mineralization of the pelvic/papillary epithelium in females, and a small (non-significant but plausibly treatment-related) increase in chronic progressive nephropathy in 500 and 1000 mg/kg/day males. Chronic progressive nephropathy incidence was highly significantly elevated in 1000 mg/kg/day females among oncogenicity study rats. Females at 500 and 1000 mg/kg/day displayed "exfoliation of the tail" during the latter half of the toxicity study. Both sexes at those dose levels had perigenital yellow staining beginning in the same time period. Modest, dose-related depression of RBC parameters (decreased RBC counts, Hb, and HCT, with some compensatory increase in platelet counts) was observed at 500 and 1000 mg/kg/day in both sexes. Statistically significant elevations in degree of hemosiderosis in spleen were noted in 500 and 1000 mg/kg/day females, likely related to associated mild anemia. Possibly associated with altered liver function, thyroid cystic follicular cell hyperplasia was significantly elevated in 1000 mg/kg/day males, and follicular cell hypertrophy was non-significantly elevated in 500 and 1000 mg/kg/day males. Thyroid follicular cell adenoma incidences in males were 1, 2, 1, 7*, and 10** among oncogenicity study rats. Study is **acceptable**. Thyroid responses, including adenomas, should be considered as "**possible adverse effects**," although enhanced liver metabolism of thyroid hormones is often the basis of such tumors. At least one mechanistic study has been submitted to address liver metabolic influence on thyroid hormones and thus on the thyroid gland: see Document No. 52976-0045 of the present submission. Aldous, 1/23/07.

52976-0045 224166 Taylor, L. M., [Supplemental to 52976-0031 224151, combined rat study]. "IR5878: Investigation of the potential effects on thyroid function in male rats using the perchlorate discharge and thyroxine clearance tests," Huntingdon Life Sciences Ltd., 11/24/04. Laboratory Study #: IGA 037/043176. Young male Han Wistar rats were used throughout in this

study. Rats were dosed in diet with orthosulfamuron at 0, 5, or 1000 mg/kg/day for 90 days. Positive controls received either phenobarbital (75 mg/kg/day, for 14 days prior to sacrifice), or propylthiouracil (200 mg/kg/day, for 16 days prior to sacrifice). Generally N = 6. The low dose of 5 mg/kg/day orthosulfamuron had no effects in these studies. Hormone assays: Plasma was evaluated for untreated and orthosulfamuron groups on days 30 and 90 for reverse-T3, T3, T4, and TSH. The only plausible treatment effect for these hormones was a slight but statistically significant increase in TSH on day 90 only at 1000 mg/kg/day orthosulfamuron, with a larger TSH increase in the phenobarbital positive controls. Thyroxine pharmacokinetics were evaluated after iv dosing with ^{125}I-thyroxine, followed by whole blood radioactivity monitoring. High dose orthosulfamuron blood label was virtually identical to controls for the first 6 hr, then subsequently appeared to clear the label slightly more quickly than untreated controls. This appears to this reviewer to be too small of a difference to attribute to treatment. Rats were administered or propylthiouracil (200 mg/kg/day, for 16 days prior to sacrifice) to block formation of thyroid hormones. Six hours before sacrifice, all rats were dosed ip with ^{125}I-iodide. Half of the rats at each dose were administered perchlorate (a competitive inhibitor for iodine transport), and the rest received the same volume of saline. Rats were killed 2.5 minutes after the latter injections, with rapid removal of thyroids for analysis of radioactivity by gamma counting. Some definitive findings were (1) thyroid glands in the propylthiouracil group weighed about 3 times those of other groups, (2) the propylthiouracil group had about 25% or less of the thyroid-to-blood ratio of label in the saline subgroup compared to other groups, and (3) administration of perchlorate to the propylthiouracil group reduced the thyroid/blood ratio to less than 25% of the saline sub-group. Clearly propylthiouracil greatly inhibited thyroid accumulation of iodine. Since most of the label found in the thyroids of propylthiouracil rats was easily displaced by a competitive substrate, iodine was presumably not incorporated into organic molecules in propylthiouracil rats. This propylthiouracil/perchlorate study indicates that orthosulfamuron does not block the incorporation of iodine into organic molecules in the thyroid. Most other measures compared effects of orthosulfamuron to the potent hepatocyte inducer, phenobarbital. Liver weights were significantly enlarged (9% for orthosulfamuron, 18% with phenobarbital). Thyroid plus parathyroid weights were elevated with phenobarbital only (14%). Centrilobular hepatocyte hypertrophy was elevated in 4/6 orthosulfamuron rats (all “minimal” degree), and in 6/6 phenobarbital rats (all “slight” degree). Hepatocyte microsomal protein and microsomal cytochrome P450 levels increased (phenobarbital > orthosulfamuron). There was a profound increase in hepatic microsomal 7-pentoxyresorufin O-depentylase activity (31-fold for orthosulfamuron, and 136-fold for phenobarbital), and an increase in hepatic microsomal UDP-glucuronosyltransferase [66% for orthosulfamuron (significant), 19% for phenobarbital (not significant)]. Evidence of the above induction supports, although does not prove, the thesis that orthosulfamuron elicits thyroid tumors by greatly increasing liver metabolism thyroid hormones, leading to over-stimulation of the thyroid. Useful supplementary data. Aldous, 2/2/07.

CHRONIC TOXICITY, RAT

(see “Combined, Rat” above)

CHRONIC TOXICITY, DOG

**52976-0105 224241 Whittaker, R., "IR5878: Toxicity study by oral capsule administration to beagle dogs for 52 weeks," Huntingdon Life Sciences Ltd., Jan. 12, 2004. Laboratory Study #: IGA 022/033476. Four beagle dogs/sex/group were dosed daily by oral capsule with orthosulfamuron, Batch G009/02, purity 98.56% for 1 year in a chronic study at 0, 75, 300, and 1000 mg/kg/day. One high dose female was placed off treatment after declining health (especially inappetence and pale feces) at week 32, then sacrificed moribund at week 40. Clinical chemistry of this female included great increases in alkaline phosphatase and alanine aminotransferase at week 32, signaling major liver injury at that time (attributable to treatment). NOEL = 75 mg/kg/day (dose-related incidence and degree of hepatocellular hypertrophy, associated at 300 mg/kg/day with about 30% increase in relative liver weight; prominent pigmented Kupffer cells, and sharp, dose-related increases in alkaline phosphatase activity). Decreased albumin levels in both sexes was also consistent with altered liver function, but at 300 mg/kg/day this was limited to 1 of 3 sampling intervals (week 26). Common signs at high dose levels included modest body weight decrements, slightly decreased food consumption (females only), modest decrease in RBC parameters (RBC count, Hb, HCT) and associated increase in platelet counts, decreased creatinine (males), increased relative kidney and adrenal weights, decreased prostate weight, slight increase in cortical vacuolation in kidneys (males), and gall bladder epithelial hyperplasia (in 2/4 males). Study is acceptable, with no adverse effects. Aldous, 2/2/07.

ONCOGENICITY, RAT

See Combined, Rat, above.

ONCOGENICITY, MOUSE

**52976-0030 224150 Webley, L., "IR5878: carcinogenicity study by dietary administration to CD-1 mice for 78 weeks," Huntingdon Life Sciences Ltd., 12/22/03. Laboratory Study # AGR 130/032070. Fifty Crl: CD-1® (ICR) BR mice/sex/group were dosed in diet with Orthosulfamuron (IR5878), purity 98%, Batch No. FCF/T/172-00 for 78 weeks in a standard oncogenicity study. Dietary concentrations were adjusted weekly to achieve dose levels of 0, 100, 500, and 1000 mg/kg/day. NOEL for males = 100 mg/kg/day (strong dose-response for centrilobular hepatocyte hypertrophy and vacuolation at 500 and 1000 mg/kg/day). NOEL for females = 1000 mg/kg/day (no apparent treatment effects). Liver weights were slightly but statistically significantly elevated in 1000 mg/kg/day males at termination. Study is acceptable, with no adverse effects. Aldous, 1/23/07.

REPRODUCTION, RAT

**52976-0010 211741 Marburger, A., "IR5878: Two-generation reproduction toxicity study in the rat," RCC Ltd., Itingen and Füllinsdorf facilities, Switzerland, 10/17/03. RCC Study No. 841291. Groups of 24 pairs of HanBrl: WIST rats/generation were dosed with orthosulfamuron (designated IR5878 technical, approximately 98.5% purity) in a standard 2 generation study (1

mating period per generation) at 0, 350, 1400, or 5600 ppm (treatments reduced to 225, 900, and 3600 ppm during lactation), achieving pre-mating treated group exposures of 22, 89, and 354 mg/kg/day in F0 males, 26, 102, and 409 mg/kg/day in F0 females, 24, 97, and 388 mg/kg/day in F1 males, and 28, 111, and 445 mg/kg/day in F1 females. Parental systemic toxicity NOEL = 1400 ppm (elevated absolute and relative liver weights in F0 males, associated with hepatocellular hypertrophy in the majority of F0 males, and a lesser but statistically significant increase in hepatocellular hypertrophy in F1 males; elevated incidence and/or degree of tubular mineralization and urothelial hyperplasia in kidneys of F0 and F1 females). Parental reproductive effects NOEL = 5600 ppm (no effects at the HDT). Offspring viability and growth NOEL = 1400 ppm: marked reduction in motor activity in F1 5600 ppm males at 58 to 72 days of age, followed by normal habituation. There were no associated changes in FOB nor in clinical signs over the course of the study. Acceptable, with no adverse effects. Aldous, 10/7/04.

52976-0009 211739, RCC Study No. 841176, Marburger, A., C. Knuppe, and D. Nehrbass, "Preliminary reproduction toxicity study in the rat," RCC Ltd., Itingen and Füllinsdorf facilities, Switzerland, 7/15/02. Basic dietary dose levels of orthosulfamuron (designated IR5878 technical, 98.44% purity) were 0, 1400, 7000, and 14000 ppm, administered to 5 HanBrl: WIST (SPF quality) rats/sex/group for a 3-week pre-mating period. For males, these dose levels continued until sacrifice at the end of the weaning period. For females, these dose levels were continued through gestation. Treatment levels for females were reduced to 0, 900, 4500, and 9000 ppm during lactation. F1 pups were maintained for one week after PND 21 weaning at those reduced dietary concentrations. At termination, F0 rats were necropsied and subjected to histopathology, whereas pups received only gross necropsy. Parental systemic toxicity NOEL = 1400 ppm (non-significant increase in incidence/degree of kidney tubular mineralization in females, increased incidence/degree of hepatocellular hypertrophy in males and females, decreased glycogen storage in males). There was a slight decrease in food consumption during the first week of the study in both sexes. There was a statistical increase in incidence/degree of hepatocellular fatty change in 14000 ppm males. The primary study identified similar liver and kidney responses as treatment effects. Aldous, 10/7/04 (no independent worksheet for pilot study: essential histopathology data from pilot study is incorporated into worksheet of primary study).

TERATOLOGY, RAT

**52976-0008 211738 Comotto, L., "IR5878: teratogenesis study in rats by oral route," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, 1/19/01. Laboratory Study #: R06790. Twenty-five mated CRL:CD® (SD) BR rats/group were dosed with orthosulfamuron (purity 98.54%) by gavage in 10 ml/kg of 0.5% aq. methylcellulose during gestation days 6-15 in a standard developmental toxicity study at 100, 300, or 1000 mg/kg/day. Maternal NOEL = 300 mg/kg/day (minor body weight gain decrement during gestation days 6-8, associated with minor decrement in food consumption, at 1000 mg/kg/day). Developmental toxicity NOEL = 1000 mg/kg/day (HDT: no treatment effects). Acceptable, with no adverse effects. Aldous, Nov. 4, 2004.

52976-0008 211737 Comotto, L., "IR5878: preliminary teratogenesis study in rats by oral route," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, 8/29/00. Laboratory Study #: R06780. There were 8 assigned dams/dose at 0, 250, 500, and 1000 mg/kg/day. Seven pregnant dams were present in each group. Aside from a modest (not statistically significant) body weight gain decrement at 1000 mg/kg/day (p. 38 of pilot study), there was no evident maternal toxicity in that study, and no effects on gestational growth or viability (pp. 44-49). These fetuses were only examined externally in accordance with the protocol, and no external malformations nor anomalies were observed. Useful supplementary data. No DPR worksheet. Aldous, 9/10/04.

TERATOLOGY, RABBIT

**52976-0007 211733 Comotto, L., "IR5878: teratogenesis study in New Zealand White rabbits by oral route," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, Aug. 9, 2001. Laboratory Study #: R06810. Twenty mated New Zealand White does per group were dosed by gavage in 4 ml/kg of 0.5% aq. methylcellulose with Orthosulfamuron (IR5878), purity 98.01% during gestation days 6-18 in a standard developmental toxicity study. Dose levels were 0, 25, 75, or 250 mg/kg/day. Maternal NOEL = 75 mg/kg/day (modest body weight decrement at 250 mg/kg/day). Developmental NOEL = 75 mg/kg/day (increase in bilateral punctate ribs at 250 mg/kg/day). There was no remarkable trend otherwise related to 13th ribs. No other soft tissue or skeletal findings were noteworthy. No adverse effects in the primary study (see also summary of pilot study, below). Aldous, 9/17/04.

52976-0007 211732 Comotto, L., "IR5878: preliminary teratogenesis study in rabbits by oral route," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, 10/19/00. Laboratory Study #: R06800. There were 8 mated does/dose at 0, 250, 500, and 1000 mg/kg/day. Seven does were pregnant in each group. Five 1000 mg/kg/day does died prematurely, as did 2 of the eight 500 mg/kg/day does. No fetuses survived to termination at 1000 mg/kg/day, the majority due to early resorptions. Body weight gains were significantly reduced at 1000 mg/kg/day during PND's 10-14. Body weight gains were also depressed significantly during the treatment period (measured days 6-19) in 250 and 500 mg/kg/day does (pages 43-44). Food consumption appeared to be reduced meaningfully among surviving does of all treated does having litters at termination, particularly after about PND 14, with some possibly treatment-related rebound after PND 24 (pp. 57-58). There was a significant increase in early resorptions among does surviving to term at 500 mg/kg/day (p. 61). The majority of decedents at 500 and 1000 mg/kg/day had histopathology in the stomach mucosa, such as hyperplasia, hypertrophy, edema, and/or excessive mucus production. Such findings were considered to reveal intrinsic irritation of test article (pp. 30 and 78). Fetuses received only external observation. The only clear malformation noted was a single fetus at 500 mg/kg/day with cleft palate and probable acrania (pp. 29 and 31). Probable acrania was observed in one other litter at that dose level. Fetal findings are "possible adverse effects," developmental toxicity being limited to dose levels producing frank toxicity in does. Results support the dose selection range for the primary developmental toxicity study. Useful supplementary data. No DPR worksheet. Aldous, 9/17/04.

52976-0022 224142 Winkler, V., [a supplemental evaluation to the primary rabbit developmental toxicity study (52976-0007 211733)]. This report notes that the original study author considered the maternal NOEL for that study to be less than 25 mg/kg/day, based on maternal body weight decrements. This supplementary by Winkler, however, considers that the body weight data do not show a meaningful dose-response, and that the maternal NOEL should be 75 mg/kg/day. The original DPR review also placed the maternal NOEL at 75 mg/kg/day, in agreement with Winkler. There is thus no need for a change in the disposition of the study at DPR. Aldous, 12/11/06 (no worksheet).

GENE MUTATION

**52976-0011 211746 Fassio, F., "Study on the ability of the test article IR5878 to induce gene mutations in strains of *Salmonella typhimurium* and *Escherichia coli*," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, 9/14/99. Study # 990501. Orthosulfamuron (IR5878), purity 93.72%, was tested with *Salmonella typhimurium* strains TA98, TA 100, TA1535, and TA1537, and *E. coli* strain WP2 uvrA$^-$ in standard reverse mutation studies, with and without S-9. *E. coli* tolerated up to 5000 µg/plate without cytotoxicity, and was negative in each of the 2 independent assays undertaken, with and without S-9, using triplicate samples at each dose level (about 3-fold concentration steps). In contrast, all *Salmonella typhimurium* strains were limited by extreme cytotoxicity, such that in most cases, statistically significant reductions in revertants were observed at 5 µg/plate (with or without S-9), and higher exposures had few or no surviving colonies. Two additional trials were conducted with all *Salmonella typhimurium* strains in the range of 0.05 to 5 µg/plate. The latter *Salmonella* trials were likewise negative for mutagenicity. This study is acceptable (no adverse effects), since all strains were exposed to the limit test (*E. coli*) or to the limits of cytotoxicity (*Salmonella*), however the mutagenicity challenge in the latter case was minor. Aldous, 11/3/04.

**52976-0011 211754 Golzio, L., "Study of the capacity of the test article IR5878 to induce gene mutation in mouse lymphoma L5178Y cell line," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, 8/30/00. Laboratory Study #: R06400. Mouse lymphoma L5178Y cells were evaluated for mutations affecting the thymidine kinase locus, based on acquired 5-trifluorothymidine resistance. Based on preliminary cytotoxicity experiments, concentrations of 30, 100, 300, or 1000 µg/ml orthosulfamuron (IR5878), purity 98.54%, were used in the two mutagenicity trials (two cultures per concentration), with or without S-9. Exposure periods were 3 hr, followed by 3 days in suspension under favorable growing conditions for expression. Cell densities were then adjusted to 1 x 10^4 cells/ml, treated with 5-trifluorothymidine (3 µg/ml), and pipetted to microtiter plates for at least one week for colony development. There were no treatment-related increases in mutations in treated groups, whereas positive controls were functional. Study is acceptable, with no adverse effects. Aldous, 11/3/04.

(Gene Mutation Studies with Metabolites or Contaminants of Orthosulfamuron): *Salmonella*

52976-0041 224162 Krüger, I., "Reverse mutation assay using bacteria (*Salmonella typhimurium*) with IR8181," BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany, 4/28/04. Study No. 040382. IR8181 (O-desmethyl metabolite of orthosulfamuron) was tested with 5 strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537, and TA 102) by plate incorporation and by pre-incubation, each with 3 reps per dose level with and without S9, at 0, 32, 100, 316, 1000, 2500, and 5000 µg/plate. There were functional positive controls in all cases. There were no effects on mutation frequencies. Remarkable cytotoxicity was confined to TA 102 at 5000 µg/plate with S9, with slight indication of toxicity without S9 at that dose, and slight indication of toxicity with S9 at 2500 µg/plate in the pre-incubation study. Valid supplementary data for this metabolite, with no adverse effects. No DPR worksheet. Aldous, Jan. 2, 2007.

52976-0037 224158 Sokolowski, M., "*Salmonella typhimurium* reverse mutation assay with IR7863," RCC Cytotest Cell Research GmbH, Rossdorf, Germany, 9/19/03. Study No. 795502. IR7863 is evidently a metabolite of orthosulfamuron, but this test article was not further characterized. Material was tested with 5 strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537, and TA 102) by plate incorporation and by pre-incubation, each with 3 reps per dose level with and without S9, at 0, 33, 100, 333, 1000, 2500, and 5000 µg/plate. There were functional positive controls in all cases. There were no effects on mutation frequencies. There was no cytotoxicity observed. Valid supplementary data for this uncharacterized metabolite, with no adverse effects. No DPR worksheet. Aldous, Jan. 2, 2007.

52976-0034 224154 Poth, A., "*Salmonella typhimurium* reverse mutation assay with IR7825," RCC Cytotest Cell Research GmbH, Rossdorf, Germany, 7/14/03. Study No. 778802. IR7825 is evidently a metabolite of orthosulfamuron, but this test article was not further characterized. Material was tested with 5 strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537, and TA 102) by plate incorporation and by pre-incubation, each with 3 reps per dose level with and without S9, at 0, 33, 100, 333, 1000, 2500, and 5000 µg/plate. An additional low dose level was added (10 µg/plate) for the pre-incubation test, due to toxicity observed in the plate incorporation assay. The most sensitive strain to cytotoxicity was TA 102, with some toxicity as low as 1000 µg/plate. There were functional positive controls in all cases. There were no effects on mutation frequencies. Valid supplementary data for this uncharacterized metabolite, with no adverse effects. No DPR worksheet. Aldous, Jan. 2, 2007.

(Gene Mutation Studies with Metabolites or Contaminants of Orthosulfamuron): Mouse lymphoma thymidine kinase locus assay.

52976-0032 224152 Wollny, H. "Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with IR7825," RCC Cytotest Cell Research GmbH, Rossdorf, Germany, 6/24/03. Study No. 778803. IR7825 is evidently a metabolite of orthosulfamuron, but this test article was not further characterized. In the main test, 1 x 10^7 cells/flask were exposed to test article in RPMI medium (generally with 3% horse serum) for 4 hr (with and without S-9), at 0 (with and without DMSO, the solvent), 22, 44, 88, 175, and 350 µg/ml (limited in the latter

concentration by precipitation). Cells were washed and re-suspended, and incubated for expression for 72 hr, with assessments of cell viability and adjustments of cell density as required. Cells from each group were seeded at about 4 x 10^3 cells/well in duplicate microtiter plates in selective medium (with trifluorothymidine) for 10-15 days prior to evaluation for large and small colony counts. A second similar experiment involved a 24-hr incubation with test article at the same concentrations, only evaluated without S-9. Results were negative in this study for large and small colonies. There were functional positive controls in all cases. Valid supplementary data for this uncharacterized metabolite, with no adverse effects. No DPR worksheet. Aldous, 1/3/07.

52976-0038 224159 Poth, A. "Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with IR7863," RCC Cytotest Cell Research GmbH, Rossdorf, Germany, 10/27/03. Study No. 795503. IR7863 is evidently a metabolite of orthosulfamuron, but this test article was not further characterized. See above paragraph regarding Record No. 224152 (same lab, similar study date) for general techniques. Differences from that record included: (1) this study employed 2700 µg/ml as the highest dose level (essentially 10 mM, the limit dose), as there was no limiting toxicity or precipitation up to that dose level, and (2) test article was soluble in water, hence water was the solvent. Results were negative in this study for large and small colonies. There were functional positive controls in all cases. Valid supplementary data for this uncharacterized metabolite, with no adverse effects. No DPR worksheet. Aldous, 1/4/07.

52976-0043 224164 Krüger, I., "*In vitro* mammalian cell gene mutation assay (thymidine kinase locus/$TK^{+/-}$) in mouse lymphoma L5178Y cells with IR8181," BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany, 8/5/04. Study No. 040384. IR8181 (O-desmethyl metabolite of orthosulfamuron) was tested at 0, 40, 80, 156, 312, 625, 1250, 2500, or 5000 µg/ml for 4 hr (with and without S-9), then evaluated in a process similar to Record No. 224152 above (a different lab). A second similar experiment involved the above dose levels for a 4-hr exposure (as above) with S-9; or a 24-hr exposure period with test article at 0, 125, 250, 500, 1000, 2000, 3000, 4000, or 5000 µg/ml, without S-9. Limit dose of 5000 µg/ml showed no apparent cytotoxicity. Results were uniformly negative in this study for large and small colonies. There were functional positive controls in all cases. Valid supplementary data for this metabolite, with no adverse effects. No DPR worksheet. Aldous, 1/4/07.

CHROMOSOME EFFECTS

NOTE: There is a positive study with a key metabolite (Record No. 224163, below).

**52976-0011 211753 Golzio, L., "Study on the capacity of the test article IR5878 to induce chromosome aberrations in human lymphocytes cultured in vitro," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, 9/26/00. Laboratory Study #: R06410. Cultures of fresh lymphocytes from healthy male volunteers, 1 donor per trial, were maintained for about 48 hr prior to treatment with orthosulfamuron (IR5878), purity 98.54%, in three trials of a chromosomal aberrations study. There were duplicate cultures per concentration with 100 metaphases scored per culture. In the first trial, an

orthosulfamuron concentration of 2500 µg/ml caused precipitation, and there were reductions of mitotic index of 48% and 44% without and with S-9 at 1000 µg/ml. For this reason, concentrations of 0, 250, 500, and 1000 µg/ml were evaluated in this trial [3 hr exposure period, 24 hr from initiation of exposure until harvest, colcemid (0.1 µg/ml) added 2 hr before harvest to arrest cells in metaphase, 2 cultures evaluated per level with or without S-9, 100 metaphases read per culture]. A second trial conducted only without S-9 was performed at the same exposure levels, but with exposure duration increased to 24 hr. A third trial evaluated only 1000 µg/ml with or without S-9, employing a 3 hr exposure period and with harvest delayed until 48 hr after initiation of treatment, to account for possible cell cycle delays. All 3 trials were negative. Positive controls [mitomycin C (0.75 µg/ml) without S-9 and cyclophosphamide (25 µg/ml) with S-9] were functional. Study is acceptable with no adverse effects. Aldous, 11/3/04.

DNA DAMAGE

**52976-0011 211755 Honarvar, N., "IR5878: Micronucleus assay in bone marrow cells of the mouse," RCC - Cytotest Cell Research GmbH, Rossdorf, Germany, April 8, 2003. Laboratory Study No. 772101. Six fasted NMRI mice/sex/group were dosed once by gavage (in 10 ml/kg corn oil) with orthosulfamuron (purity 98.56%) and were sacrificed 24 hr after dosing. Five/sex were evaluated at 0, 500, 1000, or 2000 mg/kg for PCE's with micronuclei, assessed in cells flushed from marrows of the femurs. Two thousand PCE's were evaluated per animal. An additional 5/sex were evaluated 48 hr after dosing with 2000 mg/kg orthosulfamuron. Positive controls (5/sex) were dosed with cyclophosphamide, 40 mg/kg, 24 hr before sacrifice. All treatment groups (sexes combined) had PCE's with micronuclei in the range of 0.055% to 0.100%. Positive control had 1.205% PCE's with micronuclei. There were no apparent sex differences. Study is acceptable, with no adverse effects. Aldous, 11/3/04.

(Chromosomal Aberration or DNA Damage Studies with Metabolites or Contaminants of Orthosulfamuron):

****52976-0042 224163** Becker, T., "*In vitro* mammalian chromosomal aberration test in human lymphocytes with IR8181," BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany, 7/13/04. Laboratory Study # 040383. IR8181 (O-desmethyl metabolite of orthosulfamuron), purity 97% was evaluated in a standard protocol to assess chromosomal aberrations. The study used blood from healthy human female donors. Actively proliferating cultures were exposed for 4 hr to 0, 1250, 2500, or 5000 µg/mL IR8181, then cells were washed and maintained in fresh medium until harvest after 24 hr. Colcemid was added at least 2 hr before harvest. Two hundred metaphases were evaluated (100 for each of 2 cultures) per treatment combination. Positive controls (EMS without S-9, CPA with S-9) were functional. Mitotic index was reduced at 5000 µg/mL without S-9 (58% of control value) and with S-9 at 2500 and 5000 µg/mL (57% and 50% of control, respectively). **Treatment elicited chromosomal aberrations**: all treatment groups without S-9 activation were justifiably considered by investigators to be biologically relevant increases, each exceeding the concurrent control and the historical control range. Also, low and mid-dose groups with S-9 exceeded the concurrent control and the historical control range. Given the definitive positive result, no repeat test nor prolonged-exposure test was conducted. The present positive result for chromosomal

aberrations should apply to the profile of the parent compound, since this test article is the most abundant metabolite in rat excreta. Aldous, Jan. 8, 2007.

52976-0035 224156 Schulz, M., "Chromosome aberration test in human lymphocytes *in vitro* with IR7825," RCC Cytotest Cell Research GmbH, Rossdorf, Germany, 7/17/03. Study No. 778804. IR7825 is evidently a metabolite of orthosulfamuron, but this test article was not further characterized. Test article was evaluated up to limits of solubility of 350 µg/mL. Blood was obtained from a human male donor. The high dose was into a toxic range, eliciting a reduction of the mitotic index after a 46-hr exposure (32% of control index). Treatment groups were 114, 200, and 350 µg/mL. Exposure periods were 4, 22, or 46 hr without S-9. Exposures were 4 hr with S-9, followed by recovery periods of 18 or 42 hr. All tests were negative. Positive controls were functional. Valid supplementary data. No DPR worksheet. Aldous, Jan. 8, 2007.

52976-0039 224160 Schulz, M., "Chromosome aberration test in human lymphocytes *in vitro* with IR7863," RCC Cytotest Cell Research GmbH, Rossdorf, Germany, 11/10/03. Study No. 795504. IR7863 is evidently a metabolite of orthosulfamuron, but this test article was not further characterized. Test article was evaluated up to 2700 µg/mL (about 10 mM, a limit dose). Blood was obtained from a human male donor. Treatment groups for the main studies were 882, 1543, and 2700 µg/mL. Exposure periods were 4, 22, or 46 hr without S-9 (the latter group tested only at 2700 µg/mL). Exposures were 4 hr with S-9, followed by recovery periods of 18 or 42 hr. All tests were negative. Positive controls were functional. Valid supplementary data. No DPR worksheet. Aldous, Jan. 8, 2007.

52976-0044 224165 Krüger, I., "Mammalian micronucleus test of murine bone marrow cells with IR8181," BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany, 8/23/04. Laboratory Study # 041175. Male and female NMRI mice (5/sex/treatment/interval) were treated once 24 hr or 48 hr prior to sacrifice, with either IR8181 (O-desmethyl metabolite of Orthosulfamuron) or with cottonseed oil vehicle. Bone marrow cell suspensions were prepared for micronucleus evaluation. Cyclophosphamide was an effective positive control (24-hr treatment only). The 24-hr IR8181-treated female group had an elevated micronuclei count compared to concurrent controls ($p = 0.0159$), whereas 48-hr females and both IR8181-treated male groups were negative compared to corresponding controls. A similar comparison of 24-hr IR8181-treated females with 48-hr control females yields a non-significant p value of 0.15 (Mann-Whitney analysis by DPR reviewer). Taken together, there is insufficient reason to attribute the single significant result to treatment. This study should be considered as a valid and negative supplementary study. Aldous, Jan. 5, 2007.

NEUROTOXICITY

52976-0048 224169 This is a waiver request seeking that U.S. EPA not require neurotoxicity testing. The request notes relevant information from existing studies such as the rat subchronic study which includes FOB and motor activity data (Record No. 211727, below). Aldous, 1/9/07.

SUBCHRONIC STUDIES (including range-finding studies of subchronic duration)

**52976-0005 211727 Webley, L., "IR5878: toxicity study by dietary administration to Han Wistar rats for 13 weeks followed by a 4 week recovery period (Amended final report)," Huntingdon Life Sciences Ltd., Eye, Suffolk, England, 10/16/01. Laboratory Study # AGR 128/012174. Ten Han Wistar rats/sex/group were dosed with 0, 250, 1500, or 9000 ppm Orthosulfamuron (IR5878), purity 97.99% in a standard subchronic study, which included FOB and motor activity components. An additional 5/sex were dosed with 0 or 9000 ppm for 13 weeks, followed by a 4 week recovery period. Estimated achieved dose levels were 19, 113, and 706 mg/kg/day in low, medium, and high dose male groups, respectively, and 22, 131, and 773 mg/kg/day in corresponding females. NOEL in males = 250 ppm, based on centrilobular hepatocellular hypertrophy incidences of 0, 0, 1, and 7 in controls through 9000 ppm males, respectively (N = 10). Since that NOEL is based on a single event of an adaptive response, a suitable NOAEL for males is 1500 ppm. Relative liver weights were significantly elevated in 9000 ppm males, probably due to this hypertrophy. Slight but statistically significant increases in relative kidney weights were reported in 9000 ppm males, without associated histopathology. Very small but statistically significant reductions in RBC parameters were noted in 9000 ppm females (HCT, Hb, and RBC counts), thus the NOEL for females is 1500 ppm. None of these findings persisted through the recovery phase. The study is acceptable, with no adverse effects. Aldous, Dec. 7, 2004.

52976-0006 211729 Ammannati, E., "IR5878: 13-week oral toxicity study in dogs," Istituto di Richerche Biomediche, "Antoine Marxer" RBM, S.p.A., Colleretto Giacosa (Torino), Italy, Feb. 5, 2002. Laboratory Study #: R14570. Four beagle dogs/sex/group were dosed by gelatin capsule with 0, 150, 450, or 1000 mg/kg/day orthosulfamuron (IR5878), purity 98.01%, for 13 weeks in a study meeting most subchronic study guidelines. NOEL is 450 mg/kg/day, based on several indicators of liver responses. There were elevated liver weights and elevated alkaline phosphatase activity at 1000 mg/kg/day (each significant in both sexes). Hepatocellular centrilobular hypertrophy was observed in one male and in two females at 1000 mg/kg/day. Finally, cholesterol was consistently elevated in high dose males and females (although not statistically significant in either sex). A modest decrease in RBC parameters (RBC count, HCT, and Hb concentration) in 1000 mg/kg/day males is also a plausible treatment effect. This is a supplementary study (not required under FIFRA), with minor deviations from subchronic study guidelines, and useful for dose selection for the chronic study. No adverse effects are indicated. Aldous, Dec. 7, 2004.

52976-0004 211726 Webley, L., "IR5878: Preliminary toxicity study by dietary administration to CD-1 mice for 13 weeks," Huntingdon Life Sciences Ltd., Eye, Suffolk, England, 8/16/01. Laboratory Study #: AGR 129/012173. Ten CD-1 mice/sex/group were dosed in diet with

orthosulfamuron (IR5878), purity 97.99%, in a pilot study at 0, 250, 1250, or 6000 ppm. Estimated mean achieved dose levels were 36, 187, and 865 mg/kg/day in low to high dose males, respectively, and 47, 228, and 1096 mg/kg/day in corresponding females. A major difference between this study design and that of a FIFRA subchronic study was the limited histopathology performed: only brain, kidneys, spinal cord, and spleen were routinely examined microscopically. No definitive treatment effects were observed. Equivocal treatment effects included small but statistically significantly decreased heart weights in 6000 ppm females and a small (statistically non-significant) increase in liver weights in 6000 ppm males. Designation of NOEL's is inappropriate, since this study did not evaluate all key parameters included in a guideline subchronic study. No adverse effect is indicated, and no further information is required of this study. Aldous, 10/6/04.

METABOLISM

52976-0025 224145 McCombe, W. S. and L. Gedik, "The disposition of [^{14}C]-IR-5878 in the rat following single and repeated oral administration," Inveresk Research, Tranent, Scotland, July 9, 2002. Inveresk Project No. 201385. Groups of typically 4 Sprague-Dawley rats/sex/group were dosed by gavage with 5 mg/kg (low dose) or 1000 mg/kg (high dose) treatment with labeled orthosulfamuron as [^{14}C-U-phenyl]-IR-5878. Additional rats received 14-day multiple oral pre-treatment at 5 mg/kg followed by 5 mg/kg labeled orthosulfamuron. Disposition studies included analyses of urine and feces, whole blood, each over 72 hrs. Major tissues were sampled at times corresponding to maximum blood concentrations (1 hr or 2 hrs after dosing for low and high dose groups, respectively). Excretion profiles of biliary cannula treated rats were evaluated at 5 mg/kg only. Low dose pre-treatment had no influence on study parameters. At low dose levels, about 50 to 70% was excreted in feces, and the balance in urine. High dose administration increased the ratio of fecal/urinary excretion. Blood levels were near peak at about 1 hr for low dose rats, and at 2-4 hrs with high dose rats. Estimates of $t_{1/2}$ were 9 to 11 hr regardless of dose or sex. At times of peak blood concentrations, liver and kidney levels had several-fold higher concentrations than other tissues sampled (except, as expected, GI tract). In males, liver and kidney label concentrations were comparable. In females, liver concentrations were about 3-fold higher than in kidneys. Most fecal label represented products of biliary excretion: in cannulated rats, about 17% of administered dose was found in feces, whereas about equal amounts of label were found in bile (38 and 36 % for males and females) and urine (40 and 37% for males and females). This is a valid study for parameters studied. Aldous, 2/5/07.

52976-0027 224147 Kidd, G. G. and L. Gedik, "The disposition of [^{14}C-5-pyrimidinyl]-IR5878 in the rat following single and repeated oral administration," Inveresk Research, Tranent, Scotland, Nov. 12, 2002. Inveresk Project No. 21663. This is virtually a twin study to 52976-0025 224145, differences being that: (1) this study used ^{14}C-5-pyrimidinyl orthosulfamuron, whereas Record No. 224145 used ^{14}C label on the phenyl group, and (2) only Record No. 224145 included a biliary elimination study. The two studies were presented in the same format, and comparisons of the tables in the two studies show that radiolabel distribution patterns between the two studies were quite similar. There is no current need to perform a detailed worksheet on the present study. Aldous, 12/21/06.

52976-0026 224146 Rizzo, F., K. Mainolfi, and G. Pizzingrilli, "Profiling of radiolabelled metabolites of [^{14}C-U-phenyl] IR5878 in urine and faeces of rats following single and repeated oral administrations," Isagro Ricerca Srl, Novara, Italy, Oct. 4, 2002. Laboratory Study # MEF.01.13. Urine, feces, tissues, and bile samples for this study were shipped frozen from an Inveresk in-life study (DPR Record No. 224145). Metabolites were quantified primarily by TLC radiochromatograms. Peak identification was based on MS profiles and upon comparisons of TLC patterns with available standards. The most abundant fraction in urine, feces, and bile of low dose (5 mg/kg) groups was "C6", the O-desmethyl metabolite of the parent. C6 accounted for about 60% of administered dose in urine and feces combined following low dose administration. There were much smaller amounts of "C5", the N-desmethyl metabolite. A sulfoamino acid hydrolysis product (C3) probably was due to action of intestinal microflora: C3 was a major fecal metabolite, but only a minor urinary metabolite, and was not seen in bile. Several metabolites with very low mobility on the silica gel TLC plates comprised 10-15% of administered dose in bile duct-cannulated rats. These probably included several conjugates not characterized in this study (however see Record No. 224148 for description of two glucuronide conjugates, one of which would apply to the present study). Parent orthosulfamuron comprised over 40% of fecal label following high dose (1000 mg/kg) administration, indicating saturable absorption. This study adequately described the fate of [^{14}C-U-phenyl] IR5878. Aldous, 12/20/06.

52976-0028 224148 Rizzo, F., K. Mainolfi, and G. Pizzingrilli, "Profiling of radiolabelled metabolites of [^{14}C-5-pyrimidinyl] IR5878 in urine and faeces of rats following single and repeated oral administrations," Isagro Ricerca Srl, Novara, Italy, 2/13/03. Laboratory Study #: MEF.02.15. Other than the placement of the radiolabel and the absence of analysis of the biliary metabolites in this study, the present study and Record No. 224146 (which evaluated rats after dosing with phenyl-labeled orthosulfamuron) used equivalent designs and are complementary in results. The major unique contribution of the present study is a demonstration that at least 5-10% of administered label is found in conjugation with either glucuronide or sulfate on the pyrimidinyl ring. There were no characterized conjugation products with the phenyl ring. The majority of minimally mobile metabolites were not characterized, however no single component was abundant. These may have included a variety of conjugated products. Both this study and Record No. 224146 showed that some parent orthosulfamuron (up to 6%) was excreted unchanged, even at 5 mg/kg; especially in urine of females. This study adequately described the fate of ^{14}C-5-pyrimidinyl IR5878 in the rat. Aldous, 12/22/06.

52976-0023 224143 (Pilot study for excretion and distribution patterns of orthosulfamuron: no DPR worksheet is relevant, as the above primary studies are available). Aldous, 12/22/06.

52976-0024 224144 (Pilot study for metabolite characterization of orthosulfamuron: no DPR worksheet is relevant, as the above primary studies are available). Aldous, 12/22/06.

VARIOUS STUDIES OF ACUTE OR SUB-ACUTE DURATION

**52976-0029 224149 Chapman, M. J., "IR5878: Toxicity study by dermal administration to CD rats for 4 weeks," Huntingdon Life Sciences Ltd., 1/22/04. Laboratory Study # IGA 036/033374. Ten Crl:CD® (SD)IGS BR rats/sex/group were dosed with Orthosulfamuron (IR5878), Batch G009/02, purity 98.56%, by dermal application (in water, 2 ml/kg b.w.) to the clipped dorsal surface at 0 or 1000 mg/kg/day. Treatment was for 6 to 8 hr/day, 7 days/wk, for 4 weeks. Material was in contact by gauze dressing, which was removed with cleansing of the application site at the end of each exposure session. There was no definitive local effect. There were no effects on body weight, food consumption, or clinical signs. "Pale area(s)" of the liver were reported more commonly in treated males (6/10) than controls (1/10), but not in females (0/10 controls, 1/10 in treatment group). Liver histopathology was, however, not remarkable. Thus there were no adverse effects, and an apparent NOEL of 1000 mg/kg/day. Acceptable. Aldous, 12/11/06.

52976-0040 224161 Haist, I., "Acute oral toxicity: acute toxic class method with IR8181," BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany, 4/27/04. Study No. 040381. Three HsdBrlHan: WIST rats/sex were dosed by gavage orally once with 2000 mg/kg of IR8181 (the major rat metabolite of orthosulfamuron). Rats were observed for 14 days, then sacrificed and necropsied. There were no clinical signs, necropsy changes, nor body weight disturbances attributed to treatment. Data support Category III designation. Valid supplementary data. No DPR worksheet. Aldous, Jan. 9, 2007.

52976-0033 224153 Arcelin, G., "IR7825: acute oral toxicity study in rats," RCC Ltd., Füllinsdorf, Switzerland, July 2, 2003. Study No. 848210. Six female HanBrl: WIST (SPF) rats were dosed by gavage once with 2000 mg/kg IR7825 (evidently a metabolite of orthosulfamuron, but this test article was not further characterized). Rats were observed for 14 days, then sacrificed and necropsied. There were no clinical signs nor necropsy changes. Since there were no controls and since body weight gain appeared to be slight, no inferences can be made as to possible effects on body weight: initial weights were 180 to 196 g, and 2 weeks later weights ranged from 187 to 220 g. Data support Category III designation. Somewhat useful supplementary data. No DPR worksheet. Aldous, Jan. 9, 2007.

52976-0036 224157 Ott, M., "IR7863: acute oral toxicity study in rats," RCC Ltd., Füllinsdorf, Switzerland, 9/17/03. Study No. 850107. Three HanBrl: WIST (SPF) rats/sex were dosed by gavage once with 2000 mg/kg IR7863 (evidently a metabolite of orthosulfamuron, but this test article was not further characterized). Rats were observed for 14 days, then sacrificed and necropsied. There were no clinical signs nor necropsy changes. There did not appear to be any effects on body weight. Data support Category III designation. Useful supplementary data. No DPR worksheet. Aldous, Jan. 9, 2007.

**52976-0108 224244 Decker, U., C. Knuppe, and A. Ullrich, "IR5878 0.5 GR: 4-hour acute inhalation toxicity study in rats," RCC Ltd., Itingen and Füllinsdorf facilities, Switzerland, 5/14/04. RCC Study No. 849646. Five HanBrl:WIST(SPF) rats/sex were dosed once for 4 hours with an analytical concentration of 3.24 mg/L equivalents of the test article [IR5878 0.5 GR, Batch No. G 003/03, 0.49% orthosulfamuron on a w/w basis] by inhalation in a nose-only,

flow-past exposure design. Rats were examined for 14 days for possible clinical signs. Dose levels were as high as achievable and particle sizes were as low as practicable. The mass median aerodynamic diameter (MMAD) was 6.89 μm [GSD = 2.78]. There were neither unscheduled deaths nor clinical signs of toxicity. There was an equivocal treatment-related decrement in body weight gain during the first few days after treatment. Necropsy was negative. Acceptable. Category III for the inhalation LC_{50}. Aldous, 2/2/07.