CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
SULFOXAFLOR [synonyms: XDE-208, XR-208 or X11422208]

Chemical Code # 6109, Document Processing Number (DPN) # 53202
SB 950 # Not applicable
Feb. 12, 2014, revised Nov. 10, 2014

DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effects
Chronic toxicity, dog:	No data gap, no adverse effects
Oncogenicity, rat:	No data gap, no adverse effects
Oncogenicity, mouse:	No data gap, possible adverse effects
Reproduction, rat:	No data gap, no adverse effects
Developmental toxicity, rat:	No data gap, possible adverse effects
Developmental toxicity, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, no adverse effects
Chromosome effects:	No data gap, no adverse effects
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	No data gap, no adverse effects (acute rat neurotoxicity)

Toxicology one-liners are attached.

All record numbers for the above study types through 274764 (Document No. 53202-0462) were examined. This includes all relevant studies indexed by DPR as of Dec. 2, 2013.

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.

Lenny 11/10/14

Charles N Alborn
Nov. 10, 2014

File name: t20141110 sulfoxaflor
Original by Aldous.

NOTE: The following symbols may be used in the Table of Contents which follows:
** = data adequately address FIFRA requirement
† = study(ies) flagged as "possible adverse effect"
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

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** METABOLISM AND PHARMACOKINETICS

**53202-0371 269877 Hansen, S. C., A. J. Clark, E. L. McClymont, S. A. Saghir, and J. L. Staley, "XDE-208: pharmacokinetics and metabolism in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, 9/14/09. Laboratory Project Study ID 081065. Rats were administered sulfoxaflor, purity 95.6%, Lot No. E2162-34, containing suitable specific activity of C^{14} ring-labeled sulfoxaflor, purity 97.6%, with over 2% XR-208 ketone byproduct. Groups were (1) a single low (5 mg/kg) gavage dose, (2) a single high (100 mg/kg) gavage dose, (3) daily low doses of 5 mg/kg unlabeled sulfoxaflor, followed on day 15 with 5 mg/kg labeled sulfoxaflor, or (4) a single iv dose of 5 mg/kg. Sacrifice was at 168 hrs. Generally label was measured in blood, excreta, and tissues to assess kinetics. Metabolic residues were determined in urine and feces. Tissue residues were very low or below detection levels. About 65-70% of administered dose was excreted in urine within 12 hrs of dosing, with continuing rapid clearing. Feces comprised 4-5% of administered dose within 24 hrs. Carbon trap results were normally below detection. The pattern following 100 mg/kg sulfoxaflor was similar to that of the low dose. There was no apparent effect of 14-day pre-treatment with low doses. In all of these cases, about 92% of administered dose was excreted in urine and 5-7% in feces, with no apparent sex difference and no difference due to dose level. Intravenous dosing yielded 97-101% of estimated administered dose in urine, and 6-9% in feces. As with the gavage treatments, residues in blood or internal organs were minimal after 7 days. T_{max} estimates in plasma for single gavage

treatments were typically 1-2 hrs. Plasma elimination first phase $t_{1/2}$ regardless of dose or route ranged from 4-6 hrs, with second elimination phases of about 40 hrs duration. Patterns were comparable based on RBC's except that second phase $t_{1/2}$ ranged from about 50 to 75 hours. Two large adjacent peaks, identified as the two diastereomers of parent sulfoxaflor, comprised the bulk of radioactivity in excreta. The first of these peaks eluting in urine ("Peak F") comprised about 53% of administered dose, compared to about 37% for the second peak ("Peak G"). This ratio often exceeded 2:1 for the smaller amounts of radioactivity in feces. These results suggest that the labeled test article was not a racemic mixture, and further that metabolism of one isomer might be favored over the other. Other than one glucuronide (designated X11721061), no other metabolite exceeded 1% of administered dose. The glucuronide evidently involved cleavage between the sulfur and the methylene carbon of sulfoxaflor prior to conjugation at the cleavage site. None of the lesser peaks was characterized. This conjugate was only quantifiable in urine. Study is acceptable. Aldous, Feb. 12, 2014.

53202-0370 269876 Rick, D. L., S. C. Hansen, A. J. Clark, E. L. McClymont, L. L. McNalley, and J. L. Staley, "XDE-208: tissue distribution in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, March 5, 2010. Laboratory Project Study ID 081066. Four rats/sex/group were dosed once by gavage (5 ml/kg aq. suspension with 0.5% methylcellulose) with C^{14} ring-labeled sulfoxaflor at 5 or 100 mg/kg, with sacrifice at C_{max} (1 hr and 0.5 hrs after dosing for 5 mg/kg males and females, respectively: 7 hrs and 6 hrs after dosing for 100 mg/kg males and females, respectively) or at ½ C_{max} (2 hrs after dosing for 5 mg/kg males and females: 8 hrs and 7 hrs after dosing for 100 mg/kg males and females, respectively). Distribution studies found high concentrations in GI tract and urinary bladder, as expected, at C_{max} to ½ C_{max}. Liver, kidneys, and adrenals had specific activities consistently greater than plasma. Concentrations in most tissues were in the range of plasma concentrations. Lowest concentrations were commonly in bone, brain, and fat. This is a valid segment of the study regimen for metabolism and pharmacokinetics. Aldous, 9/24/13.

53202-0372 269878 Saghir, S. A., A. J. Clark, and E. L. McClymont, "11422208: probe study to determine absorption, distribution, metabolism, and elimination in in F344/DuCrl rats and CrlCD1(ICR) mice," The Dow Chemical Co., Midland, MI, Aug. 9, 2010. Laboratory Project Study ID 060493. This study tested 2 rats/sex and 4 mice/sex, each dosed orally once with 100 mg/kg of sulfoxaflor. Both species eliminated label quickly, primarily in urine. Two of those rats were fitted with jugular cannulae to determination peak plasma levels. Record provides supplementary data, needing no DPR worksheet. Aldous 2/12/14.

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

** Acute oral toxicity, rat (also mouse)

**53202-0321 269824 Brooks, K. J., C. M. Wiescinski, A. K. Andrus, and R. M. Golden, "XDE-208: acute oral toxicity study in F344/DUCRL rats (up and down procedure)," The Dow Chemical Co., Midland, MI, 8/19/08. Laboratory Project ID 081058. Typically one rat per sex at a time was dosed with Sulfoxaflor (purity 95.6%) by gavage, and a subsequent dose 48 hrs later was determined from that outcome. Starting dosages were 630 mg/kg for both sexes.

Detailed clinical observations (DCO's) were typically made daily, and hourly observations for neurological changes were done during the first 8 hrs after dosing. Estimated acute LD_{50} was 1405 mg/kg for males, and 1000 mg/kg for females. Common DCO signs were increased reactivity to stimuli, decreased response to touch, increased lacrimation, increased salivation, and inability to walk or poor coordination. Neurological phase observations included muscle tremors, twitches, convulsions, red peri-oral soiling, decreased feces, eyelids partially closed, piloerection, labored breathing, and moribund condition. Category III for both sexes. Acceptable. Aldous, Oct. 1, 2013.

**53202-0322 269825 Brooks, K. J., C. M. Wiescinski, and R. M. Golden, "XDE-208: acute oral toxicity study in CRL:CD1(ICR) mice (up and down procedure)," The Dow Chemical Co., Midland, MI, 8/19/08. Laboratory Project ID 081059. Only males were tested. One mouse at a time was dosed with Sulfoxaflor (purity 95.6%) by gavage, and the subsequent dose 48 hrs later was determined from that outcome. Evaluated doses were 560, 750, and 1000 mg/kg. Detailed clinical observations (DCO's) were made pre-test, twice on dosing day, and daily afterwards. Estimated acute LD_{50} was 750 mg/kg. Estimated acute LD_{50} was 750 mg/kg. Common DCO signs were increased reactivity to stimuli, decreased or increased response to touch, labored breathing, muscle twitches, tremors, and convulsions. All these signs were confined to day 1. Study is acceptable: Category III. Aldous, Dec. 11, 2013.

** Acute dermal toxicity

**53202-0329 269832 Durando, J., "[XDE-208]: Acute dermal toxicity study in rats – limit test," Eurofins, East Brunswick, NJ, May 8, 2008. Laboratory Project ID 080240. Five rats/sex were dosed at the limit test of 5000 mg/kg with Sulfoxaflor (purity 95.6%) in a 1:1 paste with distilled water, applied to shaved dorsal skin, and held under a dressing for 24 hours. Rats were monitored for 2 weeks. There was no evident toxicity. $LD_{50} > 5000$ mg/kg. Toxicity category is IV. Aldous, Dec. 11, 2013.

** Acute inhalation toxicity, rat

**53202-0331 269835 Krieger, S. M., and B. J. Radtke, "XDE-208: acute dust aerosol inhalation toxicity study in Fischer 344/DUCRL rats," The Dow Chemical Co., Midland, MI, 2/2/09. Laboratory Project ID 081101. Five rats/sex were exposed (nose only, 4 hours) at the highest practicable dose level of 2.09 mg/l of Sulfoxaflor (purity 95.6%), with MMAD of 3.60 µm, and an SD of 1.33. "Soiled coat" was observed in up to 2/5 females during latter stages of the exposure period. Detailed clinical signs observations found no abnormalities. In routine clinical observations, perineal urine staining was observed in up to 2 females, peaking on day 3. Net body weight losses between days 1 and 2 (mean body weight loss of 7.4 g for males) were plausibly treatment-related. Rats were grossly normal at termination. $LC_{50} > 2.09$ mg/L. Study is acceptable. Toxicity category: IV. Aldous, 12/11/13.

** Primary eye irritation, rabbit

**53202-0332 269836 Durando, J., "[XDE-208]: Primary eye irritation study in rabbits," Eurofins, East Brunswick, NJ, 5/15/08. Laboratory Project ID 080242. This study utilized 3 male rabbits. Sulfoxaflor (purity 95.6%) was used "as received:" 0.05 g was instilled into the right eye. The untreated left eye served as control. Ocular scoring was done at 1, 24 (fluorescein dye treatment and washing at that time), 48, and 72 hours after dosing. All rabbits experienced conjunctival redness, chemosis, and discharge (degree 1-2) by 1 hour, persisting in 2/3 rabbits through 48 hours, and clearing in all subjects by 72 hours. Iris scores were limited to "1:" positive iris response was evident in 2 rabbits at 1 hour and 1 rabbit at 24 hours, then clear thereafter. Toxicity category: III. Study is acceptable. Aldous, Oct. 2, 2013.

** Primary dermal irritation

**53202-0333 269837 Durando, J., "[XDE-208]: Primary skin irritation study in rabbits," Eurofins, East Brunswick, NJ, June 10, 2008. Laboratory Project ID 080241. Three female NZW rabbits were each dosed with 0.5 g of sulfoxaflor (purity 95.6%) in a 1:1 paste with water, applied to clipped dorsal skin. Material was contained under gauze and wrapped for 4 hours, then gently washed. Application sites were examined at 30-60 minutes after treatment, and then at 24, 48, and 72 hours. All subjects had erythema and edema scores of 1 during the first hour after treatment. All subjects were clear by 72 hours. Study is acceptable. Category IV. Aldous, Oct. 3, 2013.

** Dermal sensitization

**53202-0334 269838 Wiescinski, C. M., and L. K. Sosinski, "XDE-208 technical grade active ingredient: local lymph node assay in CBA/J mice," The Dow Chemical Co., Midland, MI, 7/22/08. Laboratory Project ID 071215. Investigators applied topically 25 µl of solution to the back of each ear at concentrations of 0 (DMSO vehicle), 5, 25, or 50% sulfoxaflor to 6 mice per group, once daily for 3 consecutive days. Positive control was α-hexylcinnamaldehyde as 30% solution in DMSO. Application sites were checked for possible irritation on days 2, 3, and 6. This test relies on relative uptake of label in adjacent lymph nodes, following iv injection of 20 µCi ^{3}H-thymidine on day 6. Five hours after injection, mice were euthanized, and auricular lymph nodes were collected. These were homogenized, pelleted, rinsed of supernatant, and assayed by LSC. Stimulation index was 1.0-1.1 in all sulfoxaflor groups, thus negative. Positive control was functional. Study is acceptable. Sulfoxaflor is not a sensitizer. Aldous, 12/11/13.

SUBCHRONIC STUDIES (including 4-week studies)

** Subchronic oral toxicity, rat:

**53202-0339 269843 Yano, B. L., T. L. Card, V. A. Marshall, J. F. Quast, A. K. Andrus, S. Saghir, M. R. Woolhiser, and D. R. Boverhof, "XR-208: 90-day dietary toxicity study in F344/DuCrl rats with a 28-day recovery in F344/DuCrl rats," The Dow Chemical Co., Midland,

MI, 8/27/09. Laboratory Project ID 071057. Groups of ten F344/DuCrl rats/sex/group were dosed in diet with Sulfoxaflor [(designated XR-208 in report), purity 96.6%, at 0, 100, 750, or 1500 ppm in a 90-day subchronic study. Estimated achieved dose levels were 6.4, 48, and 95 mg/kg/day in treated males, and 7.0, 52, and 101 mg/kg/day in females. An additional 10/sex/group were dosed with 0 or 1500 ppm for 3 months, then taken off treatment for 28 days for recovery. NOEL for subchronic study duration in males and females is 100 ppm, based on the following changes (in both sexes unless specified): reduced food consumption, reduced body weights (males), elevated cholesterol, elevated absolute and relative liver weights, and hepatocellular hypertrophy (centrilobular or midzonal, with altered tinctorial properties) and single cell necrosis (hepatocellular, centrilobular, multifocal). Vacuolization, consistent with fatty change (hepatocellular, multifocal) was elevated in 750-1500 ppm males only. Recovery rats approached normal body weights, and normal liver weights, and nearly fully restored liver microscopic appearance. An immunotoxicity antibody plaque-forming assay in rats administered sheep red blood cells found a slight reduction in plaques at the highest dose in males, likely to be a non-specific response. FOB and motor activity were evaluated, and were not altered. Plasma samples were evaluated to find an elimination $t_{1/2}$ of 8-9 hours. Acceptable, with no adverse effects. Aldous, 12/17/13.

53202-0336 269840 Yano, B. L., T. L. Card, E. L. McClymont, and S. A. Saghir, "X11422208: 28-day dietary toxicity study in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, Aug. 10, 2009. Laboratory Project Study ID 061170. Five rats/sex/group were initiated on study at 0, 300, 1000, 2000, and 3000 ppm. The highest dose was terminated after 9 days due to excessive toxicity. Mean achieved dose levels for the surviving groups were 25, 79, and 155 mg/kg/day for treated males, and 26, 88, and 170 mg/kg/day for females. NOEL < 300 ppm, based on statistically significantly increased cholesterol in males, and a non-significant increase in cholesterol in females (outside historical control range) at 300 ppm. Liver was the major target organ. Liver weights were markedly elevated in 1000-2000 ppm males and in 2000 ppm females. Hepatocellular centrilobular hypertrophy was observed in both sexes at 1000 to 2000 ppm. Hepatocellular vacuolization (indicating fatty change) was observed in 1000-2000 ppm males and in 2000 ppm females. In spleen, erythrocytic extramedullary hematopoiesis appeared to be treatment-related at 1000 to 2000 ppm in both sexes, but no splenic effects were observed in the 90-day study which followed at doses up to 1500 ppm (see Record No. 269843). Transitory body weight decrements were observed at 1000 ppm in both sexes, with more sustained food consumption decrements at 2000 to 3000 ppm. No adverse effects are indicated. Useful supplementary data. Aldous, July 8, 2013.

53202-0373 269879 Yano, B. L. *et al.*, "X11422208: Palatability Probe Study in Female F344/DuCrl Rats," Dow Chemical Co., Midland, MI, issued Oct. 2, 2007, and revised 9/23/09. Dow Study No. 060488. This probe study evaluated 5 female rats at targeted dose levels of 0, 250, 500, and 1000 mg/kg/day for 8 days. All doses led to marked reduction in food consumption. The 500 and 1000 mg/kg/day rats were humanely terminated due to marked body weight losses. Investigators determined that dose levels exceeding 250 mg/kg/day (equating to 2748 ppm) would not be sustainable. The above 28-day study (Record No. 269840) determined that the latter dose was excessive. There is no DPR worksheet on this probe study, since this study gives no information on NOEL nor on patterns of toxicity which would not be better addressed in other studies. Aldous, 10/11/13.

** Subchronic oral toxicity, mouse:

**53202-0340 269844 Thomas, J., A. K. Andrus, J. A. Murray, S. A. Saghir, and B. L. Yano, "XDE-208: 90-day dietary toxicity study in Crl:CD1(ICR) mice," The Dow Chemical Co., Midland, MI, 12/21/09. Laboratory Project Study ID: 071162. Groups of ten mice/sex/group were dosed in diet with Sulfoxaflor (purity 96.6%, Lot No. E2198-17) at 0, 100, 750, or 1250 ppm (M) or 0, 100, 1500, or 3000 ppm (F). Estimated achieved dose levels were 13, 98, and 166 mg/kg/day in treated males, and 16, 247, and 489 mg/kg/day in females. NOEL = 100 ppm. Liver was the chief target organ, with elevated liver weights, hepatocellular hypertrophy (centrilobular to midzonal), and hepatocellular single cell necrosis in both sexes at the highest two dose levels. Increased mitotic alteration was observed in 1250 ppm males. Vacuolization, indicating fatty change, was observed in 750-1250 ppm males. Liver-associated clinical chemistry showed males as most sensitive, based on increased ALT, AST, and ALP at 1250 ppm, and dose-response for decreasing cholesterol at 750 to 1250 ppm. Adrenal hyperplasia of zona fasciculata was slightly increased at 1250 ppm (M) and 1500-3000 ppm (F). Slight reduction of HCT and of Hb in 1500-3000 ppm females and very slight extramedullary hematopoiesis in spleens of 3000 ppm females were plausibly treatment-related. Limited urine and blood kinetics evaluations indicated possible saturation of absorption and/or excretion at respective highest dose levels. Systemic dose appeared highest in males. Acceptable subchronic study, supporting dose levels in the 2010 mouse oncogenicity study. Aldous, 12/17/13.

** Subchronic oral toxicity, non-rodent:

**53202-0342 269846 Stewart, C. W., "XDE-208: a 90-day oral gavage toxicity study in beagle dogs," MPI Research, Mattawan, MI, 7/27/10 (revised). Sponsor Study # 081054. Four dogs/sex/group were dosed with Sulfoxaflor (purity 95.6%, Lot No. E2162-34) by gavage (in 10 ml/kg 0.5% methylcellulose) in a subchronic study at initial dose levels of 0, 1, 3, and 10 mg/kg/day. Ten mg/kg/day was not sustainable in either sex, based on markedly reduced food consumption and body weight decrements. This dose was reduced to 6 mg/kg/day from day 6 onward. NOEL = 3 mg/kg/day, based on decreased food consumption and decreased body weight during the first 2 weeks. Study is acceptable, with no adverse effects. Aldous, 10/14/13.

53202-0376 269882 Stewart, C. W., "XDE-208: 28-day palatability/tolerability probe study in beagle dogs," MPI Research Mattawan, MI, 08/27/2009. Dow Study No. 081158. Two sets of 3 female beagles were treated sequentially with a series of dose levels and routes for sulfoxaflor in a pilot study to establish dose levels for the above subchronic study (DPR Record No. 269846). The two groups were subjected to a total of 5 treatment regimens, with off-treatment periods interspaced. There were no separate control groups, but temporal symptom changes allowed inferences. Diet and capsule treatments appeared to particularly suppress food consumption in this study, whereas gavage treatment appeared more sustainable. Gavage dose of 15 mg/kg/day was tolerated, but was associated with reduced body weight, reduced food consumption, and some clinical signs. Useful data, but only as a pilot study. Dose range in Record No. 269846 appeared justified, based on this probe study. No DPR worksheet. Aldous, Aug. 9, 2013.

53202-0375 269881 Stewart, C. W., "XDE-208: Palatability and tolerability probe in beagle dogs." MPI Research, Mattawan, MI, 7/28/10 (final revision). Dow Study No. 133-115. This study used 6 male and 3 female beagles to attempt to find dose and route most suitable for future subchronic studies. Most dosage periods varied from 3 to 10 days. Palatability proved limiting at all dose levels and treatment routes explores: powdered diet at 500 to 6000 ppm, bacon-flavored powdered diet, pelleted diet, gelatin capsules, powdered diet at low levels 50 to 500 ppm, and again in gelatin capsules at dose levels as low as 10 mg/kg/day. Food consumption was markedly reduced at each of these regimens. This probe study is not usable for dose-response evaluation, and warrants no DPR worksheet. Aldous, 10/11/13.

**Subchronic dermal toxicity, 21/28-day or 90-day:

**53202-0345 269850 Thomas, J., J. A. Murray, and S. A. Saghir, "XDE-208: 28-day dermal toxicity study in F344/DUCRL rats," The Dow Chemical Co., Midland, MI, 8/14/09, Laboratory Project Study ID: 081163. Ten rats/sex/group were dosed daily for 6 hrs/day with sulfoxaflor (95.6%) at 0, 100, 500, or 1000 mg/kg/day for 28 days. Application was in an aqueous suspension containing 0.5% methylcellulose secured under gauze during exposure periods. NOEL = 500 mg/kg/day (males), due to modestly elevated liver weights and "very slight" degree of hepatocellular hypertrophy. A modest increase in serum cholesterol in 1000 mg/kg/day males may have been related to this liver response. There were no treatment effects in females up to the highest dose of 1000 mg/kg/day. There were no treatment effects at application sites. There were no other notable responses. Rat plasma concentrations of test article were consistently non-linear between the two highest dose levels (several-fold increase in plasma concentration upon doubling dermal dose). Concentrations in 500-1000 mg/kg/day females just after the last daily dose were comparable to the same females 16 hours after exposure. Plasma concentration in 1000 mg/kg/day males 16 hrs after removal of last treatment was 4.37 µg/g (SD = 2.71 µg/g). Acceptable, with no adverse effects. Aldous, 9/12/13.

CHRONIC STUDIES

† ** Combined (Chronic and Oncogenicity), rat

† "Possible adverse effects" were significantly increased hepatocellular adenomas and bilateral testicular interstitial cell adenomas in 500 ppm (21 mg/kg/day) males only. There was a non-significant increase in preputial gland carcinomas at this dose, plausibly also treatment-related. Comments below attempt to draw together mechanistic study information which relates to the primary combined (chronic and oncogenicity study) below.

With reference to hepatocellular adenomas: males and females at respective highest dose levels of 500 ppm and 750 ppm had sharp increases or degrees of hepatocellular hypertrophy, hypertrophy individual cell necrosis, and increased degree of accumulation of aggregates of histiocytes/macrophages in the liver. Hepatocellular tumors in males and all of these increases in pre-disposing changes in both sexes were strictly limited to respective highest dose levels (21 and 39 mg/kg/day, respectively, in males and females). Thus NOEL's for these findings were

100 ppm (4.2 mg/kg/day and 5.1 mg/kg/day in males and females, respectively). The 2010 mechanistic study below by Geter, D. R. and T. L. Card (DPR Record No. 269884) showed that sulfoxaflor is a strong inducer of particularly Cyp2b gene expression, and greatly enhanced associated O-dealkylase activities. These changes are associated with up-regulation via nuclear receptor CAR. Thus investigators show correlation, but not proof, that sulfoxaflor effects on hepatocellular tumors arise from a non-genotoxic mechanism comparable to that of well-characterized phenobarbital up-regulation of CAR. Aldous, 10/24/13.

With reference to increased testicular interstitial cell adenomas and changes in male accessory tissues, several mechanistic studies summarized in this section address the possibility that sulfoxaflor effects male testes and accessory tissues by feedback mechanisms in which luteinizing hormone (LH) release is substantially increased, leading to over-stimulation of responsive tissues and in some cases, to enhancement of these benign tumors. What appears to be the strongest case for feedback loop effects by sulfoxaflor is the mechanistic study by Rowley and Heal (2011), DPR Record No. 269928. That study found that sulfoxaflor levels, presumed to be relevant to those of high dose rat studies, increased hypothalamic dopamine levels, which would be expected to reduce prolactin release. A series of steps outlined by Rasoulpour *et al.* (DPR Record No. 269926, below) and Stebbins *et al.* (DPR Record No. 269929, below) would be expected to lead to over-stimulation of LH-dependent tissues, with consequential functional alterations and tumors like those reported. Aldous, 10/24/13.

****53202-0354 269859** Stebbins, K. E., J. A. Murray, D. L. Rick, and S. A. Saghir, "XDE-208: Two-year chronic toxicity/oncogenicity study in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, 6/30/10. Laboratory Project ID: 071187. Groups of 50 rats/sex/group were dosed in diet for 732 to 743 days in an oncogenicity study at 0, 25, or 100 ppm Sulfoxaflor (designated XDE-208 in report), purity 95.6%, Lot No. E2162-34. High dose males and females (also 50/sex) received diets of 500 and 750 ppm, respectively. Estimated achieved dose levels for treated oncogenicity study males were 1.0, 4.2, or 21 mg/kg/day. Respective females received 1.3, 5.1, or 39 mg/kg/day. An additional 10 rats/sex/group were administered the same dose levels in a 1-yr chronic study. NOEL for males = 25 ppm, based on increased testicular weights and decreased epididymal weights, and bilateral atrophy of seminiferous tubules. NOEL for females = 100 ppm. High dose effects in both sexes included lower body weights, elevated serum cholesterol, increased liver weights, hepatocellular centrilobular necrosis, and hepatocellular hypertrophy. High dose males had increased incidences of hepatocellular adenoma and increased instances of bilateral of testicular interstitial cell adenomas. There were no interstitial cell carcinomas in any groups. A slight increase over background of the relatively common tumor of aged Fischer males, preputial gland carcinomas, was also plausibly treatment-related. There were no treatment-related increases in hepatocellular carcinomas. High dose males also displayed decreased secretory material in prostate, seminal vesicles, and coagulation gland; and decreased spermatic elements in epididymides. The above tumor responses indicate "possible adverse effects." Acceptable. C. Aldous, Jan 7, 2014.

53202-0457 271519 Carr, M. S. (2007). "XDE-208: Method validation and stability in rodent feed." Report of Toxicology & Environmental Research and Consulting, the Dow Chemical Company, Midland, Michigan. This report confirms stability of sulfoxaflor in rodent diet for at least 65 days. No DPR worksheet, but cited in the above combined study, 53202-0354 269859.

53202-0396 269926 Rasoulpour, R. J., C. Terry, M. J. LeBaron, R. G. Ellis-Hutchings, and B. B. Gollapudi, "XDE-208 (Sulfoxaflor): Mode of action and human relevance framework analysis for XDE-208-induced promotion of Fischer 344 Leydig cell tumors," The Dow Chemical Co., Midland, MI, 5/27/11. Laboratory Project ID 110101. Investigators cite a proposed mechanism for enhancement of testicular interstitial cell tumors following sulfoxaflor treatment, as follows: (1) increased dopamine release from the hypothalamus, causing (2) reduced prolactin secretion from the pituitary, in turn causing (3) down-regulation of the LH receptor in Leydig cells (which receptor function is dependent on prolactin), causing (4) transient decrease in serum testosterone, leading to (5) increased serum LH levels, causing (6) promotion of Leydig cell tumors. The present report did not provide clear methodology or group sizes, but did provide mean values of several parameters, usually at 2, 4, and 8 weeks of dietary administration at 0, 25, 100, or 500 ppm sulfoxaflor. Serum prolactin was unaffected at 2 and 8 weeks, but high dose prolactin levels at 4 weeks were lower than other groups at that interval (yet comparable to 2-week values). This single 4-week increase was considered by investigators to be treatment-related. LH receptor expression was stated to be meaningfully reduced at 4 weeks in high dose males, but there was no treatment effect at 8 weeks. Serum testosterone was not perceptibly affected at any measured interval (2, 4, and 8 weeks). Serum LH levels at 500 ppm were considered by investigators to be meaningfully elevated at 4 weeks, but not at 2 or 8 weeks. The elevated 4 week value was similar to 25 ppm group 2-wk value, and to 8 wk values of all group, hence not clearly biologically significant. This DPR reviewer does not consider the data to give strong support to the proposed mechanism of enhanced interstitial cell tumors. Aldous, 5/15/13.

53202-0396 269927, Toole, C., "XDE-208 technical: screening for estrogen receptor and androgen receptor binding and transactivation and aromatase inhibition," CeeTox, Inc., Kalamazoo, MI, 5/26/11. Report No. 9115-100297. Receptor binding for estrogen receptor a (ERa) and for androgen receptor (AR) were assessed by fluorescence polarization of fluormone from either receptor. Commercial kits provided androgen receptor ligand binding domain (i.e. not the entire protein). Sulfoxaflor caused 50% displacement of fluormone from the receptor at about 5×10^{-4} M, compared to about 6×10^{-9} M for dihydroxytestosterone. Sulfoxaflor was considered as a "potential binder" of AR under these conditions. There was no binding of sulfoxaflor to ERa under similar conditions. Cell lines to assess gene expression were used to evaluate estrogenic and androgenic agonism and antagonism: each utilizing a luciferase reporter gene. Sulfoxaflor had no perceptible influence on any of these functions. Sulfoxaflor had no aromatase inhibition effect, based on another assay utilizing a luciferase reporter. Collectively, these negative or marginal responses do not reveal a mechanism for interstitial cell tumors in the lifetime F344 rat study. Useful supplementary data. Aldous, 5/14/2013.

53202-0397 269928, Rowley, H. L. and D. J. Heal, "Effects of X11422208 infusion on hypothalamic dopamine, DOPAC and HVA efflux – a microdialysis experiment in freely-moving rats," RenaSci Consultancy Ltd, Nottingham, UK, 10/20/11. Report No. RS867. Investigators fitted male SD rats with microdialysis probes, stereotaxically placed into the hypothalamus, and charged probes with sulfoxaflor at 0 µM for 80 min (to set a baseline), then 400 µM for 120 min, and finally 2 mM for 120 min. Investigators estimated (based on previous experience) that extracellular concentrations of sulfoxaflor would be about 10% that of the probe, hence about 0, 40, and 200 µM. Finally a spike of 50 mM potassium solute was

introduced into perfusion fluid to depolarize dopaminergic neurons, leading to a release of dopamine if probe was properly placed. Extracellular fluids were collected at 20-min intervals, and were assayed by HPLC-electrochemical detection for dopamine and for major metabolites: DOPAC, and HVA. N = 7 rats were usable, based on post-treatment examination of tissue to confirm proper probe placement. Mean extracellular dopamine levels in the low and high sulfoxaflor treatments were elevated 15% and 26% above baseline, respectively. Potassium treatment elevated extracellular dopamine by 61%. Data suggest that if sulfoxaflor arrives in extracellular fluid of hypothalamus in concentrations achieved in this study, and if the short-term effects of this study are sustainable on a chronic basis, then sulfoxaflor enhancement of hypothalamic dopamine levels could be part of the mechanism of action leading to testicular tumors. Useful supplementary data. Aldous, 5/14/2013.

53202-0397 269929 Stebbins, K. E., R. J. Rasoulpour, and K Boekelheide, "XDE-208 (Sulfoxaflor): Mode of action and human relevance framework analysis of preputial gland carcinomas in the two-year F344/DuCrl rat carcinogenicity assay," The Dow Chemical Co., Midland, MI, Oct. 3, 2011. Laboratory Project ID 110175. This report effectively applies a mode of action sequence similar to that of Record No. 269926 to explain the non-significantly elevated incidence of preputial gland carcinomas in the rat combined study. The sequence proposed is: (1) increased dopamine release from the hypothalamus, causing (2) reduced prolactin secretion from the pituitary, in turn causing (3) reduced stimulation of prolactin receptors on Leydig cells, which reduces LH receptor density in Leydig cells, causing (4) down-regulation of LH receptor gene expression, causing (5) transient decrease in serum testosterone, leading to (6) increased serum LH levels, causing (7) increased serum testosterone via hypothalamic/pituitary/gonadal feedback loop, leading to (8) increased preputial gland carcinomas due to slightly increased higher circulating testosterone. Authors note that item (3) does not appear to relate to humans (which statement is consistent with published literature). DPR reviewer (Aldous) finds this mechanistic study not very convincing on items 6 and 7, since there does not appear to be evidence of sustained increases in serum testosterone. No new study was provided, and no DPR worksheet is provided at this time. Aldous, 5/15/2013.

53202-0378 269884 Geter, D. R. and T. L. Card, "XR-208: Targeted gene expression, cell proliferation, and cytochrome P450 enzymatic activity in rats," The Dow Chemical Co., Midland, MI, June 8, 2010 (revised). Laboratory Project ID: 070339. Five rats/sex/group were dosed in diet with sulfoxaflor (purity 96.6%) for 3 days or 7 days in a mechanistic study at 0, 100, 750, or 1500 ppm. Achieved dose levels for the 7-day study were 8.0, 59, and 102 mg/kg/day for males, and 7.8, 53, and 94 mg/kg/day for females. O-dealkylase activities, particularly to substrates 7-pentoxyresorufin (PROD), and 7-benzyloxyresorufin (BROD), were greatly enhanced. In gene expression tests, Cyp2b1 expression was greatly enhanced. There were much more modest responses by Cyp2b2 (which shares with Cyp2b1 an activation by the nuclear hormone receptor, CAR) and Cyp3a3 (which is controlled by the nuclear hormone receptor, PXR). Investigators justifiably considered that these responses are consistent with a phenobarbital-like up-regulation of CAR. Ki-67 staining to assess liver proliferation found the strongest response in 7-day males at 750 to 1500 ppm: staining increased over 2-fold at 750 ppm in centrilobular and midzonal regions, and increased 3- to 4-fold at 1500 ppm in the same regions. Useful supplementary data. Aldous, Feb 6, 2014.

53202-0377 269883 Geter, D. R. and H. L. Kan, "Gene expression and cell proliferation analyses in X11422208 exposed rats and mice," The Dow Chemical Co., Midland, MI, 2/15/08. Laboratory Project ID: 070158. Key results of this record are similar to those of study 53202-0378 269884, above. No DPR worksheet. Aldous, 2/12/14.

53202-0461 274756 LeBaron, M. J., D. R. Geter, R. J. Rasoulpour, B. B. Gollapudi, J. Thomas, M. Murray, H. L. Kan, A. J. Wood, C. Elcombe, A. Vardy, J. McEwan, C. Terry, and R. Billington, "An integrated approach for prospectively investigating a mode-of-action for rodent liver effects," *Toxicol. Appl. Pharmacol*. **270** (2013)164-173. This published article summarized results from several of the unpublished studies in support of sulfoxaflor relating to liver hypertrophy, hyperplasia, and hepatocellular neoplasias, which had been shown to be associated primarily with greatly enhanced Cyp2b gene expression (a CAR-mediated event), and associated enhancement of liver enzyme activities toward substrates (particularly PROD and BROD). Data in this article reflected several records investigating pre-neoplastic and neoplastic effects in mice and rats, namely DPR Record Nos. 269840, 269841, 269843, 269858, 269859, 269884, and 269885. Since the data summarize conclusions already advanced in the cited records, no DPR worksheet is relevant. Aldous, 11/25/13.

See records 269926 to 269929 for supplementary studies relating to male gonadal tissue histopathology.

** Chronic, dog

**53202-0459 274407 Heward, J., "XDE-208: A one-year oral gavage toxicity study in beagle dogs," MPI Research, Inc., Mattawan, Michigan, 1/25/11 (amended). Dow Study Number: 133-117. Four beagle dogs/sex/group were dosed by gavage at 0, 1, 3, or 6 mg/kg/day for 1 year with Sulfoxaflor, purity 96.6%, Lot No. E2-162-34. Periodic blood and urine collection was done to estimate tissue clearance and excretion patterns of parent sulfoxaflor. NOEL = 3 mg/kg/day, based on food consumption decrements in the first 2-3 weeks on study in both sexes, on increased soft or watery feces in males, and on a modest increase in tan vomitus in both sexes. Plasma concentration of parent sulfoxaflor over time peaked at about 2 hours, with about 40% of peak residues at 24 hours, with no obvious effect of sex on plasma levels. An estimated terminal plasma half-life of sulfoxaflor was about 20 hours for either sex. Typically about 60% to 80% of administered dose was recovered as parent sulfoxaflor in 24-hr urine collections. Ophthalmoscopy, gross and microscopic pathology, and other study parameters were not remarkable. Study is acceptable, with no adverse effects. Aldous, 10/23/13.

53202-0462 274764 Brzak, K. A., and L. M. Ito, "XDE-208: Method validation and stability in dog feed and 0.5% aqueous Methocel™," The Dow Chemical Company, Midland, Michigan, December 9, 2009. Laboratory Project Study ID: 081032. Dietary mixtures at room temperature were stable for at least 36 days at 0.001% to 10% concentration in diet, and for at least 65 days at 10% in diet. Suspensions in 0.5% aqueous Methocel™ were stable at room temperature for at least 25 days at concentrations of 0.1 to 250 mg/ml. These findings help validate the chronic dog and subchronic dog studies. Aldous, no DPR worksheet. 10/19/13.

† ** Oncogenicity, mouse (including related supplementary studies)

****53202-0353 269858** Thomas, J., V. A. Marshall, D. Rick, S. A. Saghir, and B. L. Yano, "XDE-208: oncogenicity study in Crl:CD1(ICR) mice," The Dow Chemical Co., Midland, MI, July 2, 2010. Laboratory Project Study ID: 081102. Mice received XDE-208 (sulfoxaflor), 95.6% purity, for 18 months at 0, 25, 100, or 750 ppm in males, and at 0, 25, 250, or 1250 ppm in females. Estimated achieved dose levels were 2.54, 10.4, and 80 mg/kg/day in treated males, and 3.43, 34, and 176 mg/kg/day in females. NOEL for males = 100 ppm, based primarily on liver responses at 750 ppm, such as increased liver weights, increased liver masses (associated with very significantly elevated incidences and/or multiplicity of hepatocellular adenomas and carcinomas), greatly increased single-cell necrosis, hypertrophy (with altered tinctorial properties; centrilobular/midzonal and panlobar), eosinophilic foci of cellular alteration, vacuolated foci of cellular alteration, and vacuolization with characteristics of fatty change. NOEL for females = 25 ppm, based on increased hepatocellular hypertrophy (with altered tinctorial properties; centrilobular/midzonal) at 250 ppm. Females at 1250 ppm had a small increase in hepatocellular carcinomas, and also had some of the common-associated non-neoplastic hepatocellular changes that were found in males, such as panlobar hypertrophy, single-cell necrosis, and a small increase in vacuolization with characteristics of fatty change. Acanthosis of the epidermis of the neck, with inflammation and ulceration, was also elevated in high dose males. In isolation, it is not clear that the findings in the neck area are of toxicological consequence. Study is acceptable, with hepatocellular tumors as a "possible adverse effect." Several commonly associated non-neoplastic effects, often noted for both sexes, are consistent with a non-genotoxic mechanism. Aldous, June 4, 2013.

53202-0337 269841 Thomas, J., M. D. Dryzga, S. A. Saghir, E. L. McClymont, J. F. Quast, "X11422208: 28-day dietary toxicity study in Crl:CD1(ICR) mice," The Dow Chemical Co., Midland, MI, 11/03/2008. Laboratory Project ID 071053. Groups of 5 mice/sex/group were dosed in diet with sulfoxaflor (purity 98.3%) in a range-finding study at 0, 300, 1500, or 3500 ppm. Estimated mean sulfoxaflor dose in treated males was 44, 230, and 524 mg/kg/day; compared to 53, 273, and 638 mg/kg/day in females. Apparent NOEL = 300 ppm, based on transitory decrements in food consumption and body weight during days 1-3, elevated liver weights, increased ALT and AST activities, and hepatocellular centrilobular hypertrophy (all these findings in both sexes at 1500 and 3500 ppm). Males also had multifocal increases in single cell necrosis and increased mitotic alteration, plus centrilobular or midzonal vacuolization (fatty change) at these dose levels. Plasma samples contained no residues of sulfoxaflor other than parent compound. An estimated 38-39% of administered dose in was found in urine. Urine samples contained mainly parent sulfoxaflor, plus one peak containing less than 2% of the amount of parent compound. This is a valid range-finding study. The dose levels selected for the subsequent mouse oncogenicity study were justifiable, based on the present study. Aldous, June 3, 2013.

53202-0374 269880 Thomas, J., and M. D. Dryzga "X11422208: Palatability and toxicokinetic probe study in CRL:CD1(ICR) mice," The Dow Chemical Co., Midland, MI. Original report was dated July 12, 2007, with revised report on June 7, 2010. Dow Study No. 060523. This was a probe study, which preceded the above 28-day study. The present study

determined that about 3000 ppm was a practical limit for definitive studies, based largely on palatability. No DPR worksheet. Aldous, 10/10/13.

53202-0379 269885 Geter, D. R., J. A. Murray, H. L. Kan, M. J. LeBaron, J. Thomas, "XDE-208: Mode of action study investigating liver weight effects in Crl:CD-1(ICR) mice," The Dow Chemical Co., Midland, MI, June 8, 2010 (revised). Laboratory Project ID: 080246. Five mice per group were dosed with Sulfoxaflor (purity 95.6%) in diet for 7 days and assessed for several parameters related to liver function. Doses in males were 0, 500, or 750 ppm (achieved dose in treated males being 89 and 128 mg/kg/day, respectively). Females received 0, 1000, or 1500 ppm (211 and 323 mg/kg/day, respectively). Liver weights were substantially elevated at 750 ppm in males and at 1000 to 1500 ppm in females. Nearly all mice at or above 750 ppm had "very slight" hepatocellular hypertrophy. Mitotic alteration and single-cell necrosis were observed to some degree in all treatment groups. Gene expression studies yielded the strongest increase in response over baseline for Cyp2b10 and a lesser but meaningful response to Cyp3a11. There were strong liver microsomal activity responses, particularly for O-dealkylase activities toward resorufin substrates BROD and PROD. BrdU as an indicator of hepatocellular proliferation showed noteworthy increases, mainly in centrilobular and midzonal regions. About 4-fold increases over controls were observed in both zones in both sexes at 750 ppm in males and at 1000 ppm in females. Ki-67 expression as an indicator of cell proliferation gave about 2-fold increases for centrilobular and midzonal regions. Investigators justifiably determined that these findings are consistent with a constitutive androstane receptor (CAR) response. NOEL's were not sought nor found in this study. These are useful supplementary data. Aldous, 9/17/13.

53202-0380 269886 Elcombe, B. M., "XDE-208: A study to characterize the induction profile of XDE-208 in the livers of C57BL/6J mice," CXR Biosciences Ltd., Dundee, UK, 7/19/10. Laboratory Project ID: CXR0821. This study was undertaken primarily to determine whether C57BL/6J mice would respond to sulfoxaflor similarly to CD-1 mice (see DPR Record No. 269885) with respect to induction of metabolic gene expression. Five male C57BL/6J mice per group were dosed at 0, 750, or 1500 ppm for 7 days, testing parameters similar to Record No. 269885. Testing confirmed that C57BL/6J mice had increased P450 content (3 to 5-fold), greatly increased PROD and BROD activities (for Cyp2b10), and lesser (7-fold) increase in benzyloxyquinoline activity (for Cyp3a11). mRNA analyses for Cyp2b10 found (as expected) no detectable Cyp2b10 in controls, and a 9.2-fold increase between 750 and 1500 ppm. Cyp3a11, which has constitutive activity, showed a 2.4-fold increase over controls at 750 ppm, and an additional 3.2-fold increase at 1500 ppm. Induction of both Cyp2b10 and Cyp3a11 was confirmed by Western blotting. Studies clearly showed qualitatively similar responses in the two mouse strains, and indicate that future specialized studies using C57BL/6J mice would be relevant to CD-1 mouse studies (including the oncogenicity study). Useful supplementary data. Aldous, 9/18/13.

53202-0381 269887 Ross, J., "XDE-208: a study to investigate the mode of action for liver effects observed in regulatory toxicology studies by use of dual CAR-PXR knockout and humanised mice," CXR Biosciences, Inc., Dundee, UK, 6/18/10. Laboratory Project ID 100125. All tests used male C57BL/6J mice: either wild type, humanized for key nuclear receptors CAR and PXR, (hPXR/hCAR), or null for those receptors (PXRKO/CARKO). There were 10 mice per treatment/model combination. Liver weights were elevated due to sulfoxaflor in wild-type

and humanized mice. BrdU labeling index was increased about 4x in wild-type sulfoxaflor mice compared to untreated wild-type mice. No other groups had a sulfoxaflor response on BrdU labeling. Hepatocellular hypertrophy was similarly elevated in wild-type and in humanized sulfoxaflor mice compared to controls. Hepatocellular hyperplasia was limited to wild-type sulfoxaflor mice. Null mice were unaffected by hypertrophy or hyperplasia. Hepatic P450 activities for (CAR-associated) substrates PROD and BROD were highly responsive to sulfoxaflor in wild-type mice (high induction over low control background). Responses for humanized mice were also significant, however control baseline activities were higher (indicating measurable constitutive activity in humans). Knockouts showed no influence of sulfoxaflor on activities toward these substrates. P450 activity for (PXR-associated) for benzoylquinoline (BQ) debenzylation showed about 2.5-fold increases for sulfoxaflor groups in wild-type and in humanized mice, with no response in knockouts. mRNA studies for (CAR-dependent) Cyp2b10 found a strong response to sulfoxaflor in wild-type mice, with no evidence of constitutive function. In humanized mice, there was a modest increase in Cyp2b10 mRNA due to sulfoxaflor over a low constitutive activity. In knockouts, there was no evident constitutive activity, and no detectable induction. In the case of Cyp3a11, there was an appreciable constitutive signal in all three models, with evidently modest induction in wild type and in humanized models. SDS-PAGE immunoblotting assays for Cyp2b10 found very strong bands following sulfoxaflor treatment of wild-type mice, an appreciably weaker response in humanized mouse microsomal preparations following sulfoxaflor, whereas other responses were much lower. Similarly, immunoblotting assays for Cyp3a11 reflected the mRNA data above. In summary, all parameters presented in this study are consistent with hepatocellular induction consistent with CAR-associated modulation. Useful mechanistic data. Aldous, 9/20/13.

See also a summary of the published LeBaron *et al*., "An integrated approach for prospectively investigating a mode-of-action for rodent liver effects," *Toxicol. Appl. Pharmacol*. **270** (2013)164-173. This was summarized with DPR worksheet by Aldous on 11/25/13.

GENOTOXICITY

** Bacterial reverse mutation

**53202-0355 269861 Mecchi, M. S., "*Salmonella-Escherichia coli* /mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with XR-208," Covance, Vienna, VA, Sept. 7, 2007. Laboratory Project Study ID: 071110. Study used *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 98, and *E. coli*: WP2 uvrA in a standard reverse mutation assay. Test material replicates, with or without S9, were plated at 100, 333, 1000, 3330, and 5000 µg/plate in all mutagenicity assays. There were 3 plates per strain/concentration combination, with and without S9, in each of the two tests which were readable (one test had contamination problems with some strains and was repeated in its entirety). All strains, with and without S9, in both trials found sulfoxaflor to be negative for mutagenicity. Positive controls were uniformly functional. Study is acceptable, with no adverse effects. Aldous, 9/30/13.

** *In vitro* mammalian cell assay

**53202-0361 269867 Schisler, M. R., D. R. Geter, and J. M. Trombley, "Evaluation of XR-208 in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay," The Dow Chemical Co., Midland, MI, 11/13/07. Laboratory Project Study ID: 071030. The CHO-K_1-BH_4 cell line was used with Sulfoxaflor (purity 96.6%) in a guideline CHO/HGPRT assay. Sulfoxaflor was tested at 2773 µg/ml (limit test equivalent to 10 mM), and at 2x dilutions down to 173.3 µg/ml (with and without S9 in all assays). There were duplicate cultures per dose in each test. Solvent controls and positive controls were also in duplicate. Positive controls were 621 µg/ml EMS (without S9), or 4 µg/ml and 8 µg/ml 20-methylcholanthrene (20-MCA) (with S9). For expression, each of the duplicate cultures was divided into 10 dishes, each containing 2 x 10^5 cells. There was no limiting toxicity for sulfoxaflor with or without S9. Sulfoxaflor did not elicit mutagenesis. Positive controls were effective. Study is acceptable, with no adverse effects. Aldous, 1/28/14.

**53202-0364 269870 Schisler, M. R., K. M. Kleinert, and D. R. Geter, "Evaluation of XR-208 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes," The Dow Chemical Co., Midland, MI, Jan. 10, 2011 (revised). Laboratory Project Study ID: 071029. Sulfoxaflor was tested to the limit test (10 mM, thus 2773 µg/ml for sulfoxaflor) with and without S9 in standard 4-hr exposures, and to limits to toxicity (maximum viable concentration of 693.3 µg/ml) without S9 in a 24-hr exposure. Sulfoxaflor was negative for chromosomal aberrations in all assays. Positive controls [cyclophosphamide (with S9) and mitomycin C (without S9)] were positive in all assays. Neither sulfoxaflor nor positive controls elicited polyploidy. Study is acceptable, with no adverse effects. Aldous, 1/29/14.

** *In vivo* cytogenetics

**53202-0367 269873 LeBaron, M. J. and M. R. Schisler, "Evaluation of XR-208 in the mouse bone marrow micronucleus test," The Dow Chemical Co., Midland, MI, 6/16/09. Laboratory Project Study ID: 071100. Six Crl:CD1(ICR) mice/sex/group were dosed by gavage twice at 24-hr intervals with Sulfoxaflor (purity 96.6%) at 0, 100, 200, or 400 mg/kg in a standard micronucleus test, or once with 120 mg/kg cyclophosphamide as positive control. Bone marrow samples were collected from femurs at sacrifice (24 hrs after final dosing). Aspirated marrow cells were collected and smears were made to assess micronucleated polychromatic erythrocytes (PCE's), and also to report ratios of PCE's with respect to normochromatic erythrocytes (NCE's). Five/sex/group were assayed for micronuclei. "Decreased activity" in 3 out of six 400 mg/kg males indicated adequacy of dose, as also marked body temperature reductions at 2 hrs post dosing in both sexes at 400 mg/kg. Micronucleated PCE's were greatly increased in positive controls, and not at all affected by sulfoxaflor treatment in either sex. Percent PCE's (PCE X 100)/(PCE + NCE) were normal in sulfoxaflor treated groups, but were greatly reduced in positive controls. Study is acceptable, with no adverse effects. Aldous, 9/25/13.

** † REPRODUCTIVE TOXICITY, RAT

† See also Rat Developmental Toxicity section. The high dose reproduction study (Record No. 269851) was flagged as showing a "possible adverse effect" due to very high neonatal mortality at 1000 ppm and modest but treatment-related neonatal mortality at 500 ppm, despite normal survival of fetuses to parturition. Nearly all of pup deaths in these groups occurred between live birth and PND 2. Mean pup weights were significantly reduced (about 24% reduction) at 1000 ppm. There was a sharp dose-response in neonatal survival, such that 400 ppm in the low-dose range study (Record No. 269857) had slightly reduced survival, with a NOEL of 100 ppm (about 7.8 mg/kg/day for dams). A cross-fostering study found that the critical window of exposure was during the gestation period (see also Rat Developmental Toxicity section), such that exposure to FOSTER dams, even at 1000 ppm, had no bearing on pup survival. The mechanistic study by Gibb (Record No. 269919) assessed possible effects of sulfoxaflor on pups' ability to breath via a phrenic nerve-hemidiaphragm preparation. That study found a diminished response to electrical stimulation at 1 mM sulfoxaflor, but not at 0.1 mM sulfoxaflor. Although there is no information on concentration of sulfoxaflor at the neuromuscular junction of PND 0 or PND 1 pups, investigators reported plasma concentration of 1000 ppm pups in Record No. 269851 (p. 71) to be about 15 µg/g at PND 4 (perhaps from exposure to milk, which appeared to have a slightly higher concentration of sulfoxaflor: about 30 µg/g, from p. 72). With a MW of 277 for sulfoxaflor, it would appear that the plasma concentration in PND 4 pups would be about 0.05 mM, i.e. below the threshold of expected dose-response based on the phrenic nerve-hemidiaphragm preparation. This reviewer (Aldous) suggests that data are insufficient to show that compromised respiration was the cause of early neonatal deaths, although this is a possibility. Aldous, 10/25/13.

**53202-0352 269857 Rasoulpour, R. J., C. L. Zablotny, J. W. Crissman, D. L. Rick, and J. Thomas, "XDE208: Two generation dietary reproductive toxicity study in Crl:CD(SD) rats," The Dow Chemical Co., Midland, MI, July 2, 2010. Laboratory Project Study ID 091023. Groups of 27 rats/sex/group were dosed in diet with Sulfoxaflor, 95.6% purity, at 0, 25, 100, or 400 ppm for 10 weeks pre-mating, and continuously through lactation of each generation. By exception to the above, dietary concentrations of sulfoxaflor were reduced to 50% and 33.3% of normal during weeks 2 and 3 of lactation to roughly normalize daily test article uptake. Thus achieved intakes of treated F0 parents during pre-mating (1.5, 6.0, and 25 mg/kg/day in males, and 1.9, 7.8, and 30 mg/kg/day in females) were similar to exposures in all phases of the study. Parental systemic toxicity NOEL = 100 ppm, based on hepatocellular hypertrophy and hepatocellular single-cell necrosis in virtually all high dose males. Parental reproductive effects NOEL = 400 ppm (no change at highest dose tested). Offspring viability and growth NOEL = 100 ppm, based on marginal decrease in early neonatal survival. There was a slight delay of preputial separation in high dose males, which was just outside historical control range. Study is acceptable, with no adverse effects. Aldous, 7/16/13.

****53202-0346 269851** Rasoulpour, R. J., A. K. Andrus, C. L. Zablotny, and B. L. Yano, "XDE208: Dietary reproduction/developmental toxicity screening test in Crl:CD(SD) rats," The Dow Chemical Co., Midland, MI, 1/28/10. Laboratory Project Study ID 081030. Twelve rats/sex/group were dosed with 0, 100, 500, or 1000 ppm sulfoxaflor in diet for 2 weeks prior to breeding, and until termination after the breeding period (males) or after weaning of one litter

(females). Concentrations in diet were reduced during lactation to approximate constant dosing on a mg/kg/day basis. Achieved dose levels in treated males were 8.3, 41, and 79 mg/kg/day, and in females during pre-mating: 8.3, 42, and 82 mg/kg/day. Achieved dose levels in dams during gestation and lactation were similar to those of pre-mating females. Parental systemic toxicity NOEL = 100 ppm [based on modest body weight gain decrements in dams during early gestation, modest transitory food consumption decrements in pre-mating males, increased liver weights in males (significant for relative weights), and dose-related increase in incidence and degree of hepatocellular hypertrophy (centrilobular or mid-zonal)]. Parental reproductive effects NOEL = 1000 ppm [no effects at highest dose tested]. Offspring viability and growth NOEL = 100 ppm [based on reduced early post-natal survival, a "possible adverse effect"]. Pup mortalities had a strong dose-response over the range of 500 to 1000 ppm, and appeared to occur in the absence of otherwise limiting maternal toxicity. The pattern of pup mortalities, only remarkable during the first four days of lactation, was not random, but rather was associated with individual litters (entire litter losses at 1000 ppm, or losses of 4 or more pups in 4 of 12 litters at 500 ppm). Useful supplementary information, demonstrating a strong dose-response for litter survival above the dose range of the primary reproduction study. Aldous, 10/24/13.

53202-0347 269852 Rasoulpour, R. J. and C. L. Zablotny, "XDE208: A dietary reproductive toxicity cross-fostering study in Crl:CD(SD) rats," The Dow Chemical Co., Midland, MI, July 1, 2010. Laboratory Project Study ID 081122. This cross-fostering study used four groups of 16 dams, namely donor controls, donor treated (Sulfoxaflor, purity 95.6%, 1000 ppm in diet), foster controls, and foster treated (1000 ppm) dams. Donor dams (control or treated) were anesthetized on gestation day 21. These pups were kept warm with a blanket pending availability of foster dams. Foster dams were allowed to deliver naturally. The natural-born pups of donor dams were removed and humanely killed. A given foster dam received two pairs of pups (one per sex per pair) from control donor dams, and two pairs of pups from a 1000 ppm treated donors. Thus each foster dam began lactation with eight pups. The primary objective was to assess viability of offspring, since other studies (particularly Record No. 269851) showed that the major effect at high dose levels was early post-natal deaths following near normal gestational survival. This study sought specifically to show whether exposure *in utero* or post-partum was the main determinant of high dose attrition. Five control foster dams and eight treated foster dams were usable. In every litter exposed to sulfoxaflor *in utero*, all pups were dead by day 4 (most were dead by lactation day 1), regardless of whether foster dams were treated during lactation. All pups which were not exposed to sulfoxaflor *in utero* survived, even if dams were treated during lactation. Day 0 pup body weights were reduced in groups with sulfoxaflor treatment to donor dams by about one gram, judged to be treatment-related. Toxicokinetic data showed consistent plasma content in donor dams, foster dams, and pups of about 25 µg/g plasma. Milk content on lactation day 0 was 13 µg/g. Useful supplementary data, showing that exposure of dam *in utero* was the principle or sole cause for early neonatal losses. Aldous, Sept. 4, 2013.

53202-0389 269919 Gibb, A. J., "XDE-208: Observations on the effects of XDE-208 on the phrenic nerve-hemidiaphragm preparation from new-born rat," University College London, UK, July 2, 2010. Laboratory Project Study ID: UCL Diaphragm. Investigators made isolated phrenic nerve-hemidiaphragm preparations from newborn rats. System included a muscle strain gauge transducer, a stimulating electrode fixed to the phrenic nerve, and tissue preparation anchored in a vessel which allowed changing of test solutions on demand. It was postulated that

sulfoxaflor would be an agonist toward the embryonic nicotinic acetylcholine receptor (nAChR). Initial tests confirmed muscle contraction and reduced twitch response to phrenic nerve stimulation at 100 µM ACh, with return to normal upon solution wash. Ten µM tubocurarine rapidly reduced muscle tension and twitch response. Sulfoxaflor caused muscle contraction similar to ACh: at 100 µM sulfoxaflor there was no evident change on nerve-stimulated muscle twitch, whereas at 1 mM sulfoxaflor the twitch response was reduced to about 34% of normal. Ten µM tubocurarine administered simultaneously with 1 mM sulfoxaflor blocked muscle contraction by about 50%. Pre-incubation with 10 µM tubocurarine essentially eliminated the muscle contraction by 1 mM sulfoxaflor. Prolonged exposure to 1 mM sulfoxaflor (7 min) led to sustained muscle contraction and reduction in twitch response, which was reversible on washing. Tests support the hypothesis that neonatal death could have arisen from diaphragm failure by impairing respiration. Useful supplementary information. Aldous, 1/17/14.

53202-0460 274755 Rasoulpour, R. J., R. G. Ellis-Hutchings, C. Terry, N. S. Millar, C. L. Zablotny, A. Gibb, V. Marshall, T. Collins, E. W. Carney, and R. Billington, "A novel mode-of-action mediated by the fetal muscle nicotinic acetylcholine receptor resulting in developmental toxicity in rats," *Toxicological Sciences* **127(2)**, 522-534 (2012). This published article summarizes several unpublished records reviewed for sulfoxaflor and included in this Summary of Toxicology Data. These are the guideline rat reproduction and rat developmental toxicity studies, plus several mechanistic studies, which collectively indicate that early neonatal deaths and developmental toxicity responses such as forearm flexure and bent clavicles appear to be consequences of hyper-excitation of some late gestational and neonatal skeletal muscle groups due to a unique and characteristic agonistic effect of sulfoxaflor on rat fetal nicotinic acetylcholine receptor, which is conformationally and functionally different from adult rat, fetal human, and adult human receptors. All key data in this report have been addressed in non-published reports, viewed by DPR under Record Nos. 269854, 269893, 269917, and 269918. Aldous: no worksheet for this record, 11/25/13.

DEVELOPMENTAL TOXICITY

† ** Rat Developmental Toxicity

† Rat developmental toxicity "possible adverse effect" is based on four malformations: forelimb flexure and hindlimb rotation (both grossly obvious in external observations), and also bent clavicle and convoluted ureter (see Record No. 269854, below). "Critical window of exposure" studies by Rasoulpour and Zablotny (Record Nos. 269917 and 269918, both using the top dose of the definitive study (1000 ppm), found that gestation days 20 to parturition constituted the window of vulnerability for forelimb flexure and hindlimb rotation in pups. Although many affected pups died during the pup examination period (delivery to PND 4), no survivors had forelimb flexure and hindlimb rotation at PND 4, and investigators determined mathematically that many survivors had shown reversal of these findings which had been evident at PND 0. A key mechanistic study showed that sulfoxaflor has an appreciable agonist effect on rat fetal muscle nicotinic acetylcholine receptors (nAChRs), but not on adult rat, fetal human, nor on adult human nAChRs. This could explain both the early neonatal deaths in rats if diaphragm is affected, as well as the reversible "malformations" which plausibly result from unnatural muscle

contraction of neonates (including forelimb flexure, hindlimb rotation, and bent clavicle). Thus there is evidence that these "malformations" may be irrelevant to humans. Aldous, 10/24/13.

****53202-0349 269854** Rasoulpour, R. J., V. A. Marshall, and S. A. Saghir, "XDE208: dietary developmental toxicity study in Crl:CD(SD) rats," The Dow Chemical Co., Midland, MI, 4/13/10. Laboratory Project Study ID 081024. Groups of 26 mated dams were dosed in diet with sulfoxaflor, 95.6% purity, Lot No. E2162-34, during gestation days 6-21, at 0, 25, 150, or 1000 ppm. Achieved dose levels were 0, 1.95, 11.5, and 70 mg/kg/day. Maternal NOEL = 150 ppm, based on marked reduction in food consumption and body weight, and elevated relative liver weights. Developmental NOEL = 150 ppm, based on 12% fetal weight decrement and several fetal alterations. Forelimb flexure, most commonly of "slight" degree, was seen in the majority of high dose fetuses. Bent clavicle was observed in 17/24 high dose litters. Hindlimb rotation, convoluted ureter, delayed ossification (parietal), and fused sternebrae were all observed in 4 to 7 out of 24 litters in high dose fetuses, being either absent or single incidences at lower dose levels. The presence of these many alterations in 1000 ppm offspring, many of which alterations are normally termed "malformations," warrants classifying this study as one showing "possible adverse effects." As discussed in this review, reproduction studies have shown that pups do not survive long after birth at this dose level, but the alterations described in this study are unlikely to be the reason(s) for the demise of the pups in the first days after birth. Aldous, 12/30/13.

53202-0382 269888 Rasoulpour, R. J., V. A. Marshall, and B. L. Yano, "XDE-208: dietary developmental toxicity probe study in CRL:CD(SD) rats," The Dow Chemical Co., Midland, MI, 10/22/08. Laboratory Project Study ID: 081023. Groups of 7 pregnant rats were dosed with 0, 500, 1000, 1500, or 2000 ppm sulfoxaflor (95.6% purity) over gestation days 6-21. The two highest dose levels caused such reductions in food consumption and body weight gain that these groups were terminated after day 12. There was dose-related reduction in food consumption at 500 to 1000 ppm, and increased liver relative weight at 1000 ppm. Reproductive endpoints appeared to be unaffected up to 1000 ppm. Useful pilot study, justifying the high dose used in the rat developmental toxicity study. Aldous, Sept. 10, 2013.

53202-0390 269920 Thomas, J. and V. A. Marshall, "XDE-208: histopathological evaluation of fetal lung samples from the developmental toxicity study in CRL:CD(SD) rats," The Dow Chemical Co., Midland, MI, 6/18/10. Laboratory Project Study ID: 100124. Lungs from selected pups from the rat developmental toxicity study [DPR Document No. 53202-0349, Record No. 269854: Dow Laboratory Project Study ID 081024] were examined microscopically to determine whether recognizable pathology could account for early neonatal losses in a supplementary rat reproduction study [DPR Document No. 53202-0346 and Record No. 269851: Laboratory Project Study ID 081030]. Lungs were examined in 10 control and eight 1000 ppm fetuses from Record No. 269854. Histopathology did not show any abnormal morphology. Study provides useful supplementary information. Aldous, 1/17/14.

53202-0387 269917 Rasoulpour, R. J. and C. L. Zablotny, "XDE-208: Investigation of the critical window of exposure for fetal abnormalities and neonatal survival effects in Crl:CD(SD) rats," The Dow Chemical Co., Midland, MI, 6/25/10. Laboratory Project Study ID: 090122. Twelve time mated female rats/group were dosed with 1000 ppm Sulfoxaflor in diet, purity

95.6%, either during gestation days 6-16 (Group 2) or days 16 to parturition (Group 3). There were 12 untreated controls. Investigators sought to determine how the timing of exposure influenced outcomes observed in rat developmental toxicity and reproduction studies: especially forelimb flexure and hindlimb rotation over the observation period (PND 0-4), early neonatal death, and evidences of convoluted ureters at PND 4. Food consumption was sharply decreased in Group 2 during the treatment period. Group 3 dams had greatly reduced food consumption on gestation days 19-21. Estimated mean daily exposure in Group 3 dams during gestation day 16 to delivery was 39 mg/kg/day. Body weight gains were reduced in treated dams, sometimes statistically significantly, during respective treatment periods. Clinical signs for dams were unremarkable. Previously observed findings of forelimb flexure and hindlimb rotation were common in Group 3 pups only. Signs such as "stomach void of milk" and other evidences of unhealthy pups were seen in Group 3 only. PND 4 survival was 47% for Group 3, with nearly all losses by PND 2. Group 2 was unaffected. Limb abnormalities (forelimb flexure and hindlimb rotation) were anticipated at this dose level, based on the main developmental toxicity study. Limb abnormalities were observed in 55/143 (38%) of Group 3 pups on PND 0. At least 21 pups which had limb abnormalities on PND 0 were clear of such changes by PND 4. Since convoluted ureter was a treatment effect in the main developmental toxicity study (about 13% of fetuses were affected in that study), PND 4 pups were examined for this finding. This involved a sampling of pups for Groups 1 and 2, and nearly all surviving pups (49 pups examined) for Group 3. There were no cases of convoluted ureter at PND 4, indicating that this change was reversible. Investigators proposed that observed findings may reflect interaction with the fetal nicotinic acetylcholine receptor. Useful supplementary data. Aldous, Sept. 6, 2013.

53202-0388 269918 Rasoulpour, R. J. and C. L. Zablotny, "XDE-208: Investigation of the critical window of exposure for fetal abnormalities and neonatal survival effects in Crl:CD(SD) rats (phase 2)," The Dow Chemical Co., Midland, MI, 6/24/10. Laboratory Project Study ID: 091049. Ten time mated female rats/group were dosed with 1000 ppm Sulfoxaflor in diet, purity 95.6%, either during gestation days 16-17 (Group 2), days 18-19 (Group 3), or days 20-21(or day 20 to parturition if litter was delivered prior to gestation day 22) (Group 4). There were 10 untreated controls in Group 1. Investigators assessed how the timing of exposure influenced outcomes observed in rat developmental toxicity and reproduction studies: especially forelimb flexure and hindlimb rotation over the observation period (PND 0-4), early neonatal death, and evidences of convoluted ureters at PND 4. Food consumption and body weight gains of dams was reduced significantly during treatment windows. Clinical signs were normal for dams. Litter clinical observations found forelimb flexure and hindlimb rotation in Group 4 only. These two changes vanished by PND 2. Survival of pups was slightly reduced in Group 4 only. Most losses were between lactation days 1-2. There were no observed convoluted ureters and bent clavicles in Group 4 fetuses as of PND 4 sacrifice. At least seven pups showed reversal from limb alterations. Useful supplementary data, showing the narrow late gestational window of vulnerability, and confirming reversibility of forelimb flexure and hindlimb rotation, of bent clavicles, and of convoluted ureters by PND 4 following an estimated achieved dose for Group 4 of 36 mg/kg/day. Aldous, Sept. 10, 2013.

53202-0386 269893 Millar, N. S., "XDE-208: Characterization of the agonist effects of XDE-208 on mammalian muscle nicotinic acetylcholine receptors," University College London, London, UK, June 7, 2010. Laboratory Project ID: UCL nAChR. Background: forelimb flexure,

hindlimb rotation, and misshapen clavicles, observed as sulfoxaflor effects in the primary rat developmental toxicity study [Rasoulpour *et al*., 2010, DPR Document No. 53202-0349 and Record No. 269854: Dow Laboratory Project Study ID 081024], were hypothesized to have arisen from agonist effects on fetal muscle via the nicotinic acetylcholine receptors (nAChRs) found in rat fetal skeletal muscle. nAChRs are composed of 5 oligomeric subunits, the specific conformations of which vary with age, species, and location of the receptors. Rat fetal muscle nAChRs are composed of a fixed stoichiometry and order of arrangement of subunits $(\alpha1)_2\beta1\gamma\delta$, whereas adult rat muscle nAChRs are composed of $(\alpha1)_2\beta1\delta\varepsilon$ (this transition is nearly complete by PND 14). The key experiment in the present study used Xenopus oocytes expressing oligomers for the above rat fetal nAChRs, rat adult nAChRs, for or oligomers needed to assembly of human fetal muscle or of human adult muscle. The oocytes were microinjected with cDNA or cRNA of the relevant subunits and were allowed 1-5 days for expression (i.e., to form functional nAChRs). Microelectrodes were used to assess changes in current caused by action potentials elicited by receptor binding. Response to the natural ligand (ACh, 100 µM) was compared to the highest practical concentration of sulfoxaflor (3 mM), of sulfoxaflor metabolite X11719474, or of another well-known nAChR agonist, imidacloprid (up to 3 mM). In oocytes expressing fetal rat muscle nAChRs, 3 mM sulfoxaflor gave a response 39% of that elicited by100 µM ACh. Imidacloprid gave a smaller response at 3 mM (6.5% of that of 100 µM ACh), whereas X11719474 gave no response at any dose tested in the oocytes expressing fetal rat muscle nAChR. Sulfoxaflor gave no agonist response to Xenopus oocytes expressing adult rat, human fetal, or human adult nAChRs. Results give qualitative support for the hypothesis that the mode of action of sulfoxaflor in rat fetuses is dependent on fetal muscle receptor type, and not relevant to human fetal or mature nAChR expression. Study provides useful supplementary data. Aldous, 10/24/13.

See also under “Reproductive toxicity, rat” (above) for a published article by Rasoulpour *et al*. in *Toxicological Sciences* **127(2)**, 522-534 (2012), which relates to rat reproduction and developmental toxicity study interpretation.

** Rabbit Developmental Toxicity (including supplementary studies)

**53202-0350 269855 Rasoulpour, R. J., K. J. Brooks, and S. A. Saghir, “XDE-208: Dietary developmental toxicity study in New Zealand White rabbits,” The Dow Chemical Co., Midland, MI, 8/17/09. Laboratory Project Study ID 081043. Groups of 26 does were dosed in diet with 0, 30, 150, or 750 ppm Sulfoxaflor (purity 95.6%, Lot No. E2162-34) on gestation days 7-28 in a guideline developmental toxicity study. Estimated achieved dose levels in treated does were1.3, 6.6, or 32 mg/kg/day, respectively. Maternal NOEL = 150 ppm, based on decreased food consumption, associated with decreased body weight and decreased feces. Developmental NOEL = 750 ppm (no response at the highest dose tested). Study is acceptable, with no adverse effects. Aldous, Jan. 8, 2014.

53202-0384 269890 Rasoulpour, R. J., K. J. Brooks, and S. A. Saghir, “XDE-208: Dietary developmental toxicity/palatability probe study in New Zealand White rabbits,” The Dow Chemical Co., Midland, MI, 4/14/09. Laboratory Project Study ID 081121. Groups of 5 does were dosed in diet with 0, 500, or 1000 ppm Sulfoxaflor (purity 95.6%) on gestation days 7-28 in

a pilot developmental toxicity study. One 1000 ppm doe consumed nearly nothing after day 8, and was sacrificed *in extremis*. This was attributed to treatment. Body weight gain and food consumption were reduced at 1000 ppm. The choice of dose levels in the primary study above (Record No. 269855) is supported by these results. Useful supplementary information. No DPR worksheet. Aldous, 8/13/13.

53202-0383 269889 Rasoulpour, R. J., K. J. Brooks, and S. A. Saghir, "XDE-208: Oral gavage developmental toxicity probe study in New Zealand White rabbits," The Dow Chemical Co., Midland, MI, 12/15/08. Laboratory Project Study ID 081042. Groups of 7 does were dosed by gavage with 0, 10, 15, 20, or 25 mg/kg/day Sulfoxaflor (purity 95.6%) on gestation days 7-27 in a pilot developmental toxicity study. The 20 and 25 mg/kg/day groups were terminated by gestation days 13 and 16, respectively, due to severe inanition. The 15 mg/kg/day dams had markedly reduced food consumption and reduced body weight gain. There were no apparent treatment effects at 10 mg/kg/day. The choice method of administration (diet vs. gavage) in the primary study above (Record No. 269855) is supported by these results, since a higher tolerated dose could be obtained by dietary administration. Useful supplementary information. No DPR worksheet. Aldous, 2/12/14.

53202-0385 269891 Kuhl, A. J., "A study of the effect of XDE-208 on neonatal survival in New Zealand White rabbits," WIL Research Laboratories, Ashland, OH, Aug. 4, 2009. Study No. 081197. Twelve "litter-experienced" does/group were dosed in diet with 0 or 750 ppm Sulfoxaflor (95.6% purity) from gestation days 1 through initiation of parturition. Estimated mean exposure of the treated group was 29 mg/kg/day. Study objective was to examine effects on survival of offspring through PND 4 of does treated during gestation. Other than "decreased defecation" and minor reductions in food consumption and body weight gain during the early treatment period, treated does thrived as well as controls. There was one litter per group with total litter loss before PND 4, but there were no treatment-related effects on litter viability. Useful non-guideline supplementary study. No adverse effects were indicated. No DPR worksheet. Aldous, 8/13/13.

NEUROTOXICITY

** Acute neurotoxicity, rat

**53202-0368 269874 Marty, M. S., A. K. Andrus, and K. E. Stebbins, "XDE-208: acute neurotoxicity study in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, May 4, 2010. Laboratory Project Study ID 081097. Ten rats/sex/group were dosed once by gavage with sulfoxaflor (purity 95.6%, Lot No. E2162-34) in an acute neurotoxicity study at 0, 7.5, 75 or 750 mg/kg. A follow-up study, assessing motor activity only, used 10/sex at 0, 2.5, 7.5, and 25 mg/kg. NOEL = 25 mg/kg, based on reduced motor activity at 75 mg/kg on day 1 (time of maximum response, about 2.5 hrs after dosing). Motor activity was greatly reduced on day 1 at 750 mg/kg in both sexes. The follow-up study found no treatment effects at or below 25 mg/kg. Eight-minute epoch data in the main study, Day 1, showed that 750 mg/kg caused a dramatic drop in counts during the first epoch, and a consistently low activity in the epochs which followed. At 75 mg/kg, first epoch counts were normal, but rats were subdued with very low counts by the end of the session. There were no treatment effects on days 8 or 15. Day 1 FOB

definitive effects were only in the 750 mg/kg group: increased lacrimation, constricted pupils, increased salivation, decreased level of activity, and decreased response to touch; each of these in both sexes. Tremors were noted in all 750 mg/kg males, and in 6/9 females. Convulsions, twitches, splayed hindlimbs, and perineal urine soiling were each seen in 1 to 5 high dose rats per sex, compared to none in other groups. Gait impairments ("unable to walk" or "slight incoordination") were noted in 4/10 high dose males and in 1/9 high dose females. Also on day 1, rectal temperatures were reduced by about 3 °C in both sexes. Clinical signs data from days 2 to 4 showed several rats/sex with decreased feces and with red perioral soiling. Also, perineal urine staining was increased in several 750 mg/kg females. Body weights were reduced in 750 mg/kg males and females by day 2, with normal gains thereafter. There were no necropsy or neurohistopathology effects. Acceptable, with no adverse effects. Aldous, 8/16/13.

90-day neurotoxicity, rat (see neurotoxicity components of rat subchronic study)

NOTE: There is no study specifically designed as a 90-day neurotoxicity study. The subchronic rat study addressed many facets of a standard subchronic neurotoxicity study. [See DPR Document No. 53202-0339, Record No. 269843: Yano, B. L., T. L. Card, V. A. Marshall, J. F. Quast, A. K. Andrus, S. Saghir, M. R. Woolhiser, and D. R. Boverhof, "XR-208: 90-day dietary toxicity study in F344/DuCrl rats with a 28-day recovery in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, 8/27/09. Laboratory Project ID 071057]. That study included evaluation of 9 cross-sections of brain, plus several peripheral and cranial nerves. Tissues were prepared by general-purpose techniques such as immersion fixation, paraffin embedding, and H&E staining (p. 35). Histopathology (pp. 188 ff.) found no unusual pattern of changes in nervous system tissues up to the highest dose of 1500 ppm. Motor activity and FOB were evaluated shortly before necropsy (week 12), and were uneventful. DPR reviewer (Aldous) recommends that these investigations be considered to suffice for requirements for a 90-day rat neurotoxicity study at this time. Aldous, 10/18/13.

** Developmental neurotoxicity, rat

**53202-0369 269875, Beck, M. J., "A dietary developmental neurotoxicity study of XDE-208 in rats," WIL Research Laboratories Inc., Ashland, OH, June 10, 2010. Sponsor Study #: 090339. Groups of 25 mated Crl:CD(SD) females were dosed in diet with baseline levels of 0, 25, 100, or 400 ppm Sulfoxaflor [purity 95.6%, Lot No. E2162-34], in a standard developmental neurotoxicity study. Dosing was from gestation day 6 through lactation day 21. Investigators sought fixed exposures of treated rats to 2, 8, or 32 mg/kg/day, so dietary concentrations were systematically reduced during lactation. Respective mean achieved dose levels in treated dams were 1.8, 7.1, and 28 mg/kg/day during gestation, and 1.9, 7.6, and 30 mg/kg/day during lactation. Dams were given detailed clinical observations (DCO's) on gestation days 10 and 15, and on lactation days 10 and 21. Selected litters meeting criteria were culled to 8 pups, with allocations to groups to evaluate neuropathology and morphometric changes in perfused rats at PND 21 and 72, achievement of maturational criteria, DCO's and motor activity at intervals, and learning and memory assessments. NOEL = 100 ppm. Early post-natal survival was reduced in 400 ppm pups, particular during the first four days post-partum. High dose pup body weights

were 0.6 g less than controls at PND 4, but substantially recovered by PND 7. Righting reflex criterion was reached one day later at 400 ppm than in controls, likely associated with this slight growth delay. Acceptable, with no adverse effects. Aldous, 6/11/13.

N/A: Delayed neurotoxicity, hen

This study type is not required at this time.

** IMMUNOTOXICITY

This requirement is addressed by the rat subchronic study (DPR Document No. 53202-0339, Record No. 269843), which included a valid SRBC plaque-forming assay.

N/A: ENDOCRINE DISRUPTOR STUDIES

This study type is not required at this time.

SUPPLEMENTAL STUDIES

53202-0456 270518 This is a printed reproduction of a slide presentation on general toxicity of sulfoxaflor, and is not a “reviewable” document. Aldous, 10/11/13.

53202-0456 270517 Journal article - species selectivity of a nicotinic acetylcholine receptor agonist is conferred by two adjacent extracellular B4 amino acids that are implicated in the coupling of binding to channel gating. This article reports specificity of nicotinic acetylcholine receptor binding, but the test articles are not sulfoxaflor, and a DPR worksheet is not needed at this time. Investigators make arguments that nicotinic acetylcholine agonist characteristics impacted rat reproduction and developmental toxicity study outcomes (see above supplementary studies above under those headings). Aldous, 10/11/13.

53202-0450 270004 XDE-208 MII Section 3 Toxicology (Pt.1-4) This is a 4-volume set of highlights of toxicology studies on sulfoxaflor. Since the individual studies have been reviewed as separate records, there is no DPR review of this summary. Aldous, 10/11/13.

Tests on Metabolites or Impurities

X11719474: Sulfanylidene urea soil metabolite

X11719474 Metabolism and pharmacokinetics

53202-0395 269925 Hansen, S. C., A. J. Clark, J. L. Staley, and K. A. Brzak, “X11719474: probe study to determine absorption, metabolism, and elimination in F344/DuCrl rats,” The Dow

Chemical Co., Midland, MI, May 3, 2010. Dow Study ID: 091048. A total of 2 rats/sex were dosed by gavage with X11719474 at 100 mg/kg to assess pharmacokinetics, urinary metabolite and tissue metabolite profiles. Peak plasma or RBC concentrations were observed at about 1 hour, with rapid subsequent clearance. Urine plus rinse accounted for over 90% of administered dose within the first 12 hours, with rapid disappearance of remaining label in the subsequent few hours. Feces comprised 2-3% of administered dose, mostly eliminated in the first 24 hours. Negligible residues were found in exhaled CO_2. Over 99% of urinary label in either sex was test article: X11719474. Useful supplementary data. This is an elective probe study, and there is no DPR worksheet. Aldous, 10/11/13.

X11719474 Acute Studies

53202-0324 269827 Durando, J., "[X11719474]: acute oral toxicity study in rats: acute toxic class method," Eurofins, East Brunswick, NJ, Jan. 12, 2010. Dow Study No. 090493. Female F344 rats were dosed by gavage with X11719474 (99.5% purity) at either 2000 mg/kg (1 rat) or 5000 mg/kg (3 rats). All rats survived the 14-day observation period. Transitory clinical signs were primarily limited to the first hours post-dosing in 5000 mg/kg rats (most commonly salivation and nasal discharge). Necropsy was uneventful. This is a useful supplementary study on this sulfoxaflor metabolite. Category IV. Acute oral LD_{50} > 5000 mg/kg. Aldous, 10/4/12.

53202-0323 269826 Golden, R. M., "X11719474: acute oral toxicity screening study in F344/DUCRL rats," The Dow Chemical Co., Midland, MI, Dec. 5, 2007. Laboratory Project Study ID 070419. Three female rats were dosed by gavage with 300 mg/kg of this metabolite in an aqueous suspension, with a 72-hr observation period. All survived, with no unusual clinical signs. Useful supplementary data on X11719474. Results suggest Category II for an oral LD_{50} > 300 mg/kg. Aldous, Dec. 11, 2013.

53202-0330 269834 Brooks, K. J., and R. M. Golden, "X11719474: acute dermal toxicity/skin irritation and eye irritation study screening studies," The Dow Chemical Co., Midland, MI, 3/31/08. Dow Study No. 080215. In this limited-scale, non-GLP study, 3 female F344 rats were dosed with 1000 mg/kg of sulfoxaflor metabolite X11719474 (99.5 to 100% purity), moistened with aq. 0.5% methylcellulose at unspecified ratio, for 24 hours before washing the site. Rats and exposure sites were examined for the balance of a 72-hr observation period. These rats had erythema or edema scores of 0-2 at 1 hour, and scores of 0 at 24 hours and beyond. Results correspond to Category 2 for dermal toxicity based on LD_{50} > 1000 mg/kg, and Category 4 for skin irritation. For eye irritation, one female NZW rabbit was dosed in the lower conjunctival sac of the right eye with 0.1ml (0.069 g) of neat X11719474. Eyes were examined at 1, 24, 48, and 72 hours. This treated eye had redness, chemosis, and ocular discharge at 1 hour, but showed no symptoms at later times. This corresponds to Category 4 for eye irritation. Results indicate low toxicity for parameters evaluated, although not admissible for or applicable to data requirements for sulfoxaflor. Aldous, Oct. 4, 2013.

53202-0335 269839 Wiescinski, C. M., and L. K. Sosinski, "X11719474: "Cut-down"/reduced local lymph node assay in CBA/J mice," The Dow Chemical Co., Midland, MI, 6/23/10 (revised), Laboratory Project ID 080113. After a screening study determined that mice could tolerate up to 50% concentration of X11719474 (a sulfoxaflor metabolite) on ear lobes without skin irritation, investigators undertook an abbreviated lymph node assay involving 6

controls and 6 treated mice (50% X11719474 in DMSO at 25 µl/ear). Procedures were similar to the local lymph node assay for sulfoxaflor by the same authors, however the present study did not have a concurrent positive control. Stimulation index was 1.3 in the treated group, far from the threshold of 3.0 or higher for a positive test. Findings indicate that this metabolite is not a sensitizer, although this is not a guideline study. Aldous, Oct. 4, 2013.

X11719474 Subchronic Studies

53202-0341 269845 Yano, B. L., J. A. Murray, K. E. Stebbins, D. R. Boverhof, A. K. Andrus, and D. L. Rick, "X11719474: 90-day dietary toxicity study in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, April 6, 2010. Laboratory Project ID: 081164. Groups of 10/sex/group were dosed in diet with the sulfoxaflor metabolite, X11719474, 99.5% purity, at 0, 500, 1000, or 5000 ppm. Estimated achieved dose levels were 32, 65, and 327 mg/kg/day in treated males, and 35, 72, and 352 mg/kg/day in females. NOEL = 1000 ppm. Findings at 5000 ppm included elevated liver weights in males, increased incidence or degree of liver hypertrophy at 5000 ppm in both sexes, single cell necrosis in 7/10 high dose males (not seen in other rats on study), and elevated cholesterol in both sexes. Other parameters, including a limited FOB, motor activity, and assessment of immune cell response in splenic cells, were negative. Valid supplementary study. Aldous, 5/30/13.

53202-0341 269845 Yano, B. L., K. J. Brooks, J. A. Murray, , and S. A. Saghir, "X11719474: palatability probe study in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, 3/23/2010. Laboratory Project ID: 080230. Three rats/sex were administered diets targeting 0, 125, 250, 500, and 1000 mg/kg/day of this metabolite for 7 days. Reduced body weight gains and reduced triglycerides were observed at 500 to 1000 mg/kg/day in both sexes. Liver hypertrophy was observed in both sexes at 1000 mg/kg/day and at 500 mg/kg/day in males only. This probe study warrants dose level selection for the 90-day study for this metabolite. Aldous, 10/11/13 (no DPR worksheet, as study was limited in scope, and not suitable to determination NOEL's).

53202-0343 269848 Heward, J., "X11719474: a 90-day oral gavage toxicity study in male beagle dogs," MPI Research, Inc., Mattawan, MI, 6/23/10 (revised). Sponsor Study # 091115. Four male beagles/group were dosed by gavage at 0, 10, 25, or 50 mg/kg/day in a standard subchronic study. There were no treatment effects at any dose tested. NOEL = 50 mg/kg/day. Viable supplementary data. Aldous, Aug. 9, 2013.

53202-0392 269922 Heward, J., "X11719474: A dose-range finding and tolerability study in male beagle dogs," MPI Research, Mattawan, MI, 06/16/2010. This was a range-finding study for the above 90-day gavage study with this metabolite of sulfoxaflor. Two males/group were administered either 50 or 100 mg/kg/day of X11719474 for 7 days. Dogs were examined for clinical signs, body weight, and food consumption: no other parameters were evaluated. Soft or mucoid feces and salivation, observed in one 100 mg/kg/day dog, were plausibly treatment-related. Choices of dose levels for the definitive study were justified. Useful supplementary data. No DPR worksheet. Aldous, Aug. 9, 2013.

53202-0338 269842 Yano, B. L., K. J. Brooks, J. A. Murray, and S. Saghir, "X11719474: 28-day dietary toxicity study in F344/DuCrl rats," The Dow Chemical Co., Midland, MI, 3/30/10. Laboratory Project ID 081075. Five rats/sex/group were dosed in diet with this sulfoxaflor soil

metabolite (sulfoxaflor cyanamide moiety is metabolized to a urea) at 0, 1000, 2000, 3000, or 8000 ppm in this 28-day toxicity study. Achieved dose levels were 0, 83, 167, 244, or 662 mg/kg/day for males, and 0, 90, 184, 278, or 734 mg/kg/day for females. Apparent NOEL = 2000 ppm (M) and 3000 ppm (F), based on liver hypertrophy. Liver weights were elevated in both sexes at 8000 ppm. Hepatocellular necrosis (very slight) was observed in two 8000 ppm males. Useful supplementary data. No adverse effects. Aldous, 8/13/13.

53202-0391 269921 This is the same as record as 53202-0341 269845, above, in this section.

X11719474 Developmental Toxicity Studies

53202-0351 269856 Rasoulpour, R. J. and V. A. Marshall, "X11719474: dietary developmental toxicity study in CRL:CD(SD) rats," The Dow Chemical Co., Midland, MI, 2/23/10. Laboratory Project Study ID 091029. Twenty-six time-mated dams/group were dosed in diet with X11719474 (99.5% purity) in a standard developmental toxicity study at 0, 1000, 2000, 5000 ppm on gestation days 6-21. Treated groups received about 74, 152, or 368 mg/kg/day X11719474. Maternal NOEL = 1000 ppm, based on minimal and transient decrement in body weight gain during the first few days of dosing. Since findings were not "adverse" at any dose level, a maternal NOAEL is 5000 ppm (highest dose tested). There were no developmental effects observed. Test article is a sulfoxaflor soil metabolite, hence study is supplementary by design. This study did not provide stability data for treated diets, and therefore is designated as "not acceptable." Aldous, Jan. 7, 2014.

53202-0348 269853 Rasoulpour, R. J., A. K. Andrus, R. G. Ellis-Hutchings, and B. L. Yano, "X11719474: dietary reproduction/developmental toxicity screening test in Crl:CD(SD) rats," The Dow Chemical Co., Midland, MI, 1/29/10. Laboratory Project Study ID 081153. Groups of 12 rats/sex/group were dosed in diet with X11719474 (metabolite of sulfoxaflor) from 2 weeks prior to mating through weaning in a supplementary reproduction study at 0, 1000, 2000, or 5000 ppm X11719474, equivalent to 0, 81, 162, or 396 mg/kg/day for males, and 114, 212, or 507 mg/kg/day for pre-mating females. Treated females received 82, 167, and 451 mg/kg/day during gestation, and 90, 171, and 446 mg/kg/day during lactation (step-wise dietary compound concentration reduction during lactation allowed near-constant exposure to females). Dietary concentrations for females were adjusted downward during lactation to maintain steady mg/kg/day exposure. There were no effects on reproductive indices or on pup growth. Relative liver weights were statistically significantly elevated in 5000 ppm males and females. Livers were grossly normal, but centrilobular/midzonal hepatocellular hypertrophy was observed in nearly all 5000 ppm rats. Thus parental systemic toxicity NOEL = 2000 ppm, and reproductive/neonatal viability NOEL = 5000 ppm (no effects at highest dose tested). Useful supplementary data, with no adverse effects. Aldous, 12/30/2013.

X11719474 Mechanistic Studies

53202-0393 269923 Geter, D. R., J. A. Murray, H. L. Kan, M. J. LeBaron, and K. E. Stebbins, "X11719474: Targeted gene expression, cell proliferation, and cytochrome P450 enzymatic activity in male F344/DuCrl rats to determine the mode of action for effects on the

liver," The Dow Chemical Co., Midland, MI, June 24, 2010. Laboratory Project ID: 090525. Six male rats/group were dosed with 0 or 8000 ppm of X11719474 in diet for 7 days prior to testing for parameters similarly evaluated for sulfoxaflor to assess mode of action. Estimated intake of X11719474 was 583 mg/kg/day. Absolute and relative liver weights were increased significantly. Hepatocellular hypertrophy (centrilobular/midzonal: very slight) was observed in all treated rats. Gene expression (compared to control) was increased 806-fold for Cyp2b1, 32-fold for Cyp2b2, and 4.4-fold for Cyp3a3. These responses were similar to those of 1500 ppm sulfoxaflor in another study. Hepatic PROD activity was increased 15-fold (compared to 10-fold for sulfoxaflor). BrdU hepatocellular proliferation studies found response of X11719474 similar to sulfoxaflor. Investigators justifiably concluded that this metabolite behaved similarly to sulfoxaflor in all evaluated respects, consistent with a CAR-mediated response, for which phenobarbital is a classic inducer. Useful supplementary data. Aldous, 1/23/14.

X11719474 Mutagenicity Studies

53202-0356 269862 Mecchi, M. S., "*Salmonella-Escherichia coli* /mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11719474," Covance, Vienna, VA, Sept. 2, 2008. Laboratory Project Study ID: 081017. This test on a sulfoxaflor metabolite was performed by the same team as the reverse mutation assay on sulfoxaflor itself. Study used *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 98, and *E. coli*: WP2 uvrA in a standard reverse mutation assay (20 minutes preincubation at 37°C prior to plate incorporation for 52 hours at 37°C). Test material replicates, with or without S9, were plated at 100, 333, 1000, 3330, and 5000 µg/plate in all mutagenicity assays. There were 3 plates per strain/concentration combination, with and without S9, in each of the two tests which were readable (one test had contamination problems with some strains and was repeated in its entirety). All strains, with and without S9, in both trials found X11719474 to be negative for mutagenicity. Positive controls were uniformly functional. Study is valid supplementary data on this metabolite, with no adverse effects. Aldous, 1/23/14.

53202-0365 269871 Schisler, M. R., K. M. Kleinert, and D. R. Geter, "Evaluation of X11719474 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes," The Dow Chemical Co., Midland, MI, 8/25/08. Laboratory Project Study ID 081022. X11719474 was tested to the limit test (10 mM, thus 2953 µg/ml for X11719474) with and without S9 in standard 4-hr exposures, and without S9 in a 24-hr exposure. X11719474 was negative for chromosomal aberrations in all assays. Positive controls [cyclophosphamide (with S9) and mitomycin C (without S9)] were positive in all assays. Neither test article nor positive controls elicited polyploidy. Study is acceptable, with no adverse effects. Aldous, 1/29/14.

53202-0362 269868 Schisler, M. R. and D. R. Geter, "Evaluation of X11719474 in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay," The Dow Chemical Co., Midland, MI, July 1, 2008. Laboratory Project Study ID: 081019. The CHO-K_1-BH_4 cell line was used with X11719474 in a guideline CHO/HGPRT assay. X11719474 was tested at 2953 µg/ml (limit test equivalent to 10 mM), and at several 2x dilutions (with and without S9 in all assays). There were duplicate cultures per dose in each test. Solvent controls and positive controls were also in duplicate. Positive controls were 621 µg/ml EMS (without S9), or 4 µg/ml and 8 µg/ml 20-methylcholanthrene (20-MCA)

(with S9). For expression, each of the duplicate cultures was divided into 10 dishes, each containing 2 x 10^5 cells. There was no limiting toxicity for X11719474 with or without S9. X11719474 did not elicit mutagenesis. Positive controls were effective. This is a valid supplementary study, with no adverse effects. Aldous, 9/27/13.

X11721061: 1-(6-(Trifluoromethyl)pyridine-3-yl)-ethanol

53202-0325 269828 Durando, J., "[X11721061]: Acute oral toxicity up and down procedure in rats, Eurofins | Product Safety Laboratories, East Brunswick, NJ (ESPL), Jan. 6, 2010. Dow Study No. 090520. Female F344 rats were dosed by gavage with this sulfoxaflor metabolite, X11721061 (98% purity, a liquid), (dosed as supplied) at 1000 mg/kg (N =2), 2000 mg/kg (N = 3), or 5000 mg/kg (N = 1). Survival by treatment group was: 2/2 at 1000 mg/kg, 1/3 at 2000 mg/kg, and 0/1 at 5000 mg/kg. Clinical signs were transient piloerection and hypoactivity at 1000 mg/kg. The 2000 mg/kg rats additionally displayed hunched posture. Survivors showed no clinical signs after day 1. Survivors were grossly normal at necropsy. Decedents had red color in intestines. Estimated LD_{50} = 2000 mg/kg, based on this dose with partial mortality. Useful supplementary data on this metabolite. Aldous, 10/10/13.

53202-0344 269849 Heward, J. "X11721061: a 28-day oral dietary toxicity study in Fischer 344/DuCrl rats," MPI Research, Inc., Mattawan, MI, 8/12/2010 (revised). Study No. 133-129. Groups of 5 rats/sex/group were dosed in diet with the sulfoxaflor metabolite, X11721061 (99% purity), at 0, 1000, 3000, or 8000 ppm. Respective achieved doses averaged 79, 236, and 622 mg/kg/day in treated males, and 82, 244, and 649 mg/kg/day in females. There were no treatment-related clinical signs. There were marginal decrements in food consumption and body weights at over treatment days 1-3, with substantial recovery thereafter. Liver absolute and relative weights were slightly **increased** in both sexes at 8000 ppm. There were no associated gross or microscopic changes. Useful information on this metabolite. Aldous, 12/20/13.

53202-0394 269924 Heward, J. K., "X11721061: A 1-week palatability study in male Fischer rats," MPI Research, Mattawan, MI, 06/29/2010. Dow Study No. 091108. This probe study found this metabolite to be well-tolerated at up to 10,000 ppm, and justifies dose levels used in the 28-day dietary study above (Record No. 269849). No DPR worksheet is needed. Aldous, 10/11/13.

53202-0366 269872 Schisler, M. R., and D. R. Geter, "Evaluation of X11721061 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes," The Dow Chemical Co., Midland, MI, 4/22/10. Laboratory Project Study ID 091111. X11721061was tested to the limit test (10 mM, thus 1920 µg/ml for X11721061) with and without S9 in standard 4-hr exposures, and up to 480 µg/ml (limited by cytotoxicity) without S9 in a 24-hr exposure. X11721061 was negative for chromosomal aberrations in all assays. Positive controls [cyclophosphamide (with S9) and mitomycin C (without S9)] were positive in all assays. Neither test article nor positive controls elicited polyploidy. Study is acceptable, with no adverse effects. Aldous, 1/29/14.

53202-0357 269863 Dakoulas, E. W. and VanDyke, M. R., "*Salmonella-Escherichia coli* / mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11721061," BioReliance, Rockville, MD, Dec. 2, 2009. Laboratory Project Study ID:

091106. Study used *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 98, and *E. coli*: WP2 uvrA in a standard reverse mutation assay on this sulfoxaflor metabolite. Test material replicates, with or without S9, were pre-incubated for 20 minutes at 37 °C and plated for 52 hours at 37 °C at approximately 3x steps up to 5000 µg/plate in all assays. There were 2 plates per strain/concentration combination, with and without S9, in the first test, and triplicate plates in the second. There were no precipitation issues nor were there clear limitations of cell viability at any concentration. All strains, with and without S9, in both trials found X11721061 negative for mutagenicity. Positive controls were functional. Study provides valid supplementary data on this metabolite, with no adverse effects. Aldous, 1/28/14.

53202-0363 269869 Schisler, M. R., "Evaluation of X11721061in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay," The Dow Chemical Co., Midland, MI, 4/26/10. Laboratory Project Study ID: 091110. The CHO-K_1-BH_4 cell line was used with sulfoxaflor metabolite X11721061 in a guideline CHO/HGPRT assay. X11721061was tested at 1920 µg/ml (limit test equivalent to 10 mM), and at several 2x dilutions lower (with and without S9 in all assays). There were duplicate cultures per dose in each test. Solvent controls and positive controls were also in duplicate. Positive controls were 621 µg/ml EMS (without S9), and two levels per test of 20-methylcholanthrene (20-MCA) (with S9). For expression, each of the duplicate cultures was divided into 10 dishes, each containing 2 x 10^5 cells. There was no limiting toxicity for X11721061 with or without S9. X11721061did not elicit mutagenesis. Positive controls were effective. This is a valid supplementary study, with no adverse effects. Aldous, 9/27/13.

X11596066

53202-0326 269829 Durando, J. "[X11596066]: Acute oral toxicity up and down procedure in rats," Eurofins | Product Safety Laboratories, East Brunswick, NJ (ESPL), 4/27/10. Dow Study No. 101004. Five female F344 rats were dosed by gavage with an aqueous (0.5% CMC) suspension of the sulfoxaflor metabolite, X11596066 (98% purity), at 2000 mg/kg. All rats survived, with transient piloerection in 2 rats as the only clinical sign, and normal appearance at necropsy. LD_{50} > 2000 mg/kg. Useful supplementary data on this metabolite. Aldous, 10/10/13.

53202-0358 269864 Dakoulas, E. W. and VanDyke, M. R., "*Salmonella-Escherichia coli* / mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11596066," BioReliance, Rockville, MD, 4/20/10. Laboratory Project Study ID: 091150. Study used *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 98, and *E. coli*: WP2 uvrA in a standard reverse mutation assay on this sulfoxaflor metabolite. Test material replicates, with or without S9, were pre-incubated for 20 minutes at 37 °C and plated for 52 hours at 37 °C at approximately 3x steps up to 5000 µg/plate in all assays. There were 2 plates per strain/concentration combination, with and without S9, in the first test, and triplicate plates in the second. Usually 5000 µg/plate of X11596066 was associated with absent or greatly reduced background lawn and no observed revertants. Often there was similar toxicity at 1500 µg/plate also. All strains, with and without S9, in both trials found X11596066 negative for mutagenicity at functional concentrations: normally maximum readable concentration was 500 or 1500

µg/plate). Positive controls were functional. Study provides valid supplementary data on this metabolite, with no adverse effects. Aldous, 1/28/14.

X11579457

53202-0359 269865 Dakoulas, E. W. and VanDyke, M. R., "*Salmonella-Escherichia coli* / mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11579457," BioReliance, Rockville, MD, 4/20/10. Laboratory Project Study ID: 101003. Study used *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 98, and *E. coli*: WP2 uvrA in a standard reverse mutation assay on this sulfoxaflor metabolite. Test material replicates, with or without S9, were pre-incubated for 20 minutes at 37 °C and plated for 52 hours at 37 °C at approximately 3x steps up to 5000 µg/plate in all assays. There were 2 plates per strain/concentration combination, with and without S9, in each of two tests. There were no precipitation issues (vehicle was water) nor were there limitations of cell viability at any concentration. All strains, with and without S9, in both trials found X11579457 negative for mutagenicity. Positive controls were functional. Study provides valid supplementary data on this metabolite, with no adverse effects. Aldous, 1/28/14.

53202-0327 269830 Durando, J., "[X11579457]: acute oral toxicity up and down procedure in rats," Eurofins, East Brunswick, NJ, 5/28/10. Dow Study No. 101005. Five female F344 rats were dosed once with 2000 mg/kg X11579457 in aqueous 0.5% methylcellulose suspension. All survived, and were without notable clinical signs an no necropsy changes. This is a valid supplementary study on a metabolite. LD_{50} > 2000 mg/kg. Technically, the acute oral toxicity data support a Toxicity Category III hazard designation. With recent changes in review practices at U.S. EPA (with which Medical Toxicology Branch concurs), label language for a Toxicity Category IV hazard is appropriate for this metabolite. Aldous, Oct. 3, 2013.

X11519540

53202-0360 269866 Dakoulas, E. W. and VanDyke, M. R., "*Salmonella-Escherichia coli* / mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11519540," BioReliance, Rockville, MD, 4/20/10. Laboratory Project Study ID: 101004. Study used *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 98, and *E. coli*: WP2 uvrA in a standard reverse mutation assay on this sulfoxaflor metabolite. Test material replicates, with or without S9, were pre-incubated for 20 minutes at 37 °C and plated for 52 hours at 37 °C at approximately 3x steps up to 5000 µg/plate in all assays. There were 2 plates per strain/concentration combination, with and without S9, in the first test, and triplicate plates in the second. There were no precipitation issues nor evident loss of cell viability at any concentration. All strains, with and without S9, in both trials found X11519540 negative for mutagenicity. Positive controls were functional. Study provides valid supplementary data on this metabolite, with no adverse effects. Aldous, 1/28/14.

53202-0328 269831 Durando, J., "[X11519540]: acute oral toxicity up and down procedure in rats," Eurofins, East Brunswick, NJ, 6/22/10. Dow Study No. 101006. Seven F344 female rats were dosed once by gavage with an aqueous suspension of this metabolite at 320, 1000, or 2000

mg/kg in 0.5% aqueous methylcellulose. All rats at ≥ 1000 mg/kg died, and all 320 mg/kg rats survived. Common clinical signs at 1000 mg/kg included piloerection, hyperactivity or hypoactivity, abnormal gait, somewhat impaired locomotion, excessive salivation, slow respiration, slight tremors, prone position, and hunched posture. Deaths usually occurred on day 1. Necropsies of decedents found "red" or "red/black" intestines in all cases. All 320 mg/kg rats showed minimal clinical signs and were grossly normal at day 14 necropsy. Estimated LD_{50} was 566 mg/kg. Useful supplementary data on this metabolite. Toxicity Category III. Aldous, December 11, 2013.

Historical Control Data

53202-269860 Record contains historical data relating to long-term mouse and rat studies for this compound, including tumor incidence, organ weight, clinical chemistry, and urinalysis. Aldous, 5/2/13 (no DPR worksheet).