UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: 26 September 2012

SUBJECT: Sulfoxaflor – New Active Ingredient Human Health Risk Assessment of Uses on Numerous Crops

PC Code: 005210
Decision No.: 439080
Petition No.: 0F7777
Risk Assessment Type:
Single Chemical, Aggregate
TXR No.: NA
MRID No.: NA

DP Barcode: D382604,
D382605, D382607
Registration No.: 62719-AGR,
62719-AEL, 62719-AEG
Regulatory Action: Section 3
Case No.: NA

CAS No.: 946578-00-3
40 CFR: 40 CFR 180.xxx

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1.0 Executive Summary

Dow AgroSciences has petitioned the Agency to grant registrations and establish tolerances for the new insecticide sulfoxaflor {CAS name: cyanamide, N-[methyloxido[1-[6-(trifluoromethyl)-3-pyridinyl]ethyl] λ^4-sulfanylidene]}. Sulfoxaflor is the first member of a new class of insecticides, the sulfoximines, which act through a unique interaction with the nicotinic acetylcholine receptor (nAChR) in insects. Sulfoxaflor is a highly efficacious agonist of that receptor. The registration request is for one technical-grade product (File Symbol 62719-AGR) and two end-use products, Transform™ WG (62719-AEL) and GF-2032 SC (62719-AEG). The requested use sites are identical for the two end-use products and include numerous agricultural crops (39 crops/crop groups in all), turfgrass (sod farms only), and ornamental plants. At this time, no uses have been requested that would result in residential exposure.

The toxicology database for sulfoxaflor is complete for risk assessment purposes, including the assessment of potential increased susceptibility of infants and children. Toxicity and mechanistic studies in rats, rabbits, dogs and mice indicate that the liver and the nervous systems are target organ systems. Liver effects in subchronic and chronic studies include organ weight and enzyme changes, hypertrophy, proliferation, and tumors; long-term studies resulted in hepatic effects at doses lower than in short-term studies. Based on the weight of evidence, including mode-of-action data, the Cancer Assessment Review Committee (CARC) determined that there is a "Suggestive Evidence of Carcinogenic Potential" for sulfoxaflor and that quantification of risk using a non-linear approach [i.e., reference dose (RfD)] will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to sulfoxaflor. The nervous system effects were observed as frank neurotoxicity as well as secondary developmental effects due to prolonged contracture of fetal muscle resulting in limb flexure and bent clavicle in fetuses, and decreased survival of neonatal rats. As these developmental effects were observed only in rats, the petitioner conducted numerous non-guideline studies to investigate the cause of these effects and to determine their human relevance. Those studies are adequate to delineate the mode of action of the developmental effects and provide information regarding interspecies uncertainty. The database indicates that the developmental effects are unlikely to be relevant to humans; however, an additional study is required in order to make a final conclusion. Therefore, pending that study, the developmental effects are considered to be relevant to humans. Data are sufficient to support reducing the interspecies uncertainty factor to 3X for the developmental effects. The developmental, neuromuscular, or liver effects are the basis for this risk assessment, depending on the duration of exposure and the population group of interest.

HED has recommended that the required 10X FQPA Safety Factor be reduced to 1X based on the completeness of the toxicological database, the adequacy of the submitted neurotoxicity and developmental neurotoxicity studies, the selection of endpoints and doses selected for risk assessment that are protective of observed developmental effects, special studies to address HED's concern regarding human relevance for the developmental effects, and exposure assessments that are unlikely to underestimate exposure.

No direct residential uses or commercial/professional uses at residential sites are being requested for sulfoxaflor at this time. Although there is a proposed use on ornamental plants, the proposed

use is only for nurseries and commercial greenhouses. Furthermore, exposure from homeowners buying turfgrass (sod) and planting it as a residential turf would be minimal. At this time, there are no uses of sulfoxaflor that will result in residential exposures. Therefore, if the requested registrations are granted, exposure to sulfoxaflor may occur via residues in food and/or drinking water. Data depicting the fate of sulfoxaflor in plants, animals, and the environment indicates that in systems that include an aerobic soil component (e.g., soil-directed applications, rotational crops, groundwater systems), breakdown of sulfoxaflor is quite extensive and rapid, with very little parent compound remaining. In those scenarios, the metabolite designated X11719474 is the major component. Contrary to that, the parent compound undergoes very little transformation in animals or via hydrolytic or photolytic processes. Some metabolism of the parent compound occurs in plants following foliar application, resulting in two major metabolites (X11719474 and X11721061). Plant metabolism studies also demonstrate that residues are distributed throughout the plant and are not restricted to treated surfaces. In residue field studies using foliar treatment (which is the requested use pattern), residues of sulfoxaflor were generally well above the limit of quantitation (LOQ) of 0.01 ppm in most commodities. The exception to that is in root crops and rotational crops where residues were approximately at or below the LOQ; presumably due to the involvement of the soil matrix in those situations. Typical food processing activities, such as baking, boiling and canning, are unlikely to significantly affect the nature and magnitude of sulfoxaflor residues. In processing studies, sulfoxaflor generally concentrated during production of dried foods and tended to be reduced in juices, though exceptions to those trends are evident in the database. In the case of drinking water, degradates are expected to be significantly greater in groundwater than in surface water. In addition, the residue profile for these two drinking water sources are expected to be quite different, with residue in surface water being comprised of sulfoxaflor almost exclusively and residues in groundwater being made up primarily of the metabolites X11719474 and, to a lesser extent, X11519540; parent sulfoxaflor is expected to be absent from groundwater. The different residue profiles, combined with toxicological information and risk assessment endpoints for sulfoxaflor versus these two metabolites, results in different strategies for risk assessment. In short, this assessment focuses on the following three combinations for evaluating aggregate exposure:

- Neuromuscular effects (acute dietary exposure, general population): Assess sulfoxaflor in food and the metabolites designated X11719474 and X11519540 in groundwater;
- Developmental effects (acute dietary exposure, women of child-bearing age; short- and intermediate-term dermal and inhalation exposure): Assess sulfoxaflor in food and surface water (note that at this time in the absence of residential uses, the dermal and inhalation exposure routes are not relevant to an aggregate assessment);
- Liver effects (chronic dietary exposure, all populations): Assess sulfoxaflor in food and the metabolites designated X11719474 and X11519540 in groundwater, factoring in information on the relative potency of the metabolites to elicit the liver effects. Relative to sulfoxaflor, these factors are 0.3X for X11719474 and 10X for X11519540.

HED has used moderately refined, health-protective estimates of residue levels in foods and upper-bound residue estimates for drinking water, taking into account the toxicological endpoint/residue profile considerations described above, in combination with an extensive database of food and water consumption for the U.S. population, to estimate dietary exposure and risk that may result from the use of sulfoxaflor. Across all representative population subgroups, including those of infants and children, the maximum dietary risk estimates are 16%

and 18% of the acute and chronic population-adjusted dose (aPAD, cPAD), respectively. These estimates are well below 100% and are, therefore, below HED's level of concern. In the absence of residential use sites, aggregate risk estimates are equivalent to dietary risk estimates and are, by extension, below HED's level of concern. There are no aggregate risk issues that would preclude granting the requested registrations and establishing tolerances for residues of sulfoxaflor at the levels recommended in Table 2.2.3.

HED has also estimated risks for persons exposed to sulfoxaflor during occupational activities. These activities include application-related tasks (mixing, loading, applying, etc.) as well as post-application tasks (scouting, weeding, irrigating, etc.). The current risk metric for assessing short- and intermediate-term occupational exposure to sulfoxaflor is a margin of exposure (MOE) that is less than 30. At a baseline level of personal protective equipment (PPE), estimated occupational MOEs range from 80 to 4,700,000, and the vast majority of the estimates are greater than 300. All estimated occupational MOEs indicate that risks are below HED's level of concern.

Although the proposed labels specify a 24-hour Restricted Entry Interval (REI), the acute toxicity categories for technical-grade sulfoxaflor indicate that a 12-hour REI is appropriate to protect agricultural workers from post-application exposures.

This risk assessment relies, in part, on data from studies in which adult human subjects were intentionally exposed to a pesticide or other chemical. These data, which include the Pesticide Handlers Exposure Database Version 1.1 (PHED 1.1); the Agricultural Handler Exposure Task Force (AHETF) database; the Outdoor Residential Exposure Task Force (ORETF) database; the Agricultural Re-entry Task Force (ARTF) database; other registrant-submitted exposure studies (MRID Nos. 47832415, 47832416); are subject to ethics review pursuant to 40 CFR 26, have received that review, and are compliant with applicable ethics requirements. For certain studies that review may have included review by the Human Studies Review Board. Descriptions of data sources as well as guidance on their use can be found at: http://www.epa.gov/pesticides/science/handler-exposure-data.html and http://www.epa.gov/pesticides/science/post-app-exposure-data.html.

2.0 HED Recommendations

HED is recommending for granting the registrations for use of sulfoxaflor on the requested crops and for the establishment of permanent tolerances for residues of sulfoxaflor as specified in Section 2.2.3. HED further recommends that the labels be revised as specified in Section 2.3.

2.1 Data Deficiencies/Conditions of Registration

None.

2.2 Tolerance Considerations

2.2.1 Enforcement Analytical Method

High performance liquid chromatographic (HPLC) methods with positive-ion electro spray (ESI) tandem mass spectrometry (LC/MS/MS) were developed for data collection and enforcement of sulfoxaflor residues and the two metabolites X11719474 and X11721061. Method 091116 was developed for plant commodities, and Method 091188 was developed for livestock commodities. Both methods use solvent extraction (acetonitrile+water, 80+20, v/v), solid-phase extraction clean up, and reverse-phase LC-MS/MS for separation and analysis. In addition, the plant method incorporates base- and enzymatic-hydrolysis steps to liberate conjugated residues. Successful validation of both methods was reported by the registrant and by an independent laboratory. Additionally, successful radiovalidation was reported for both methods. The lowest level of method validation (LLMV) was 0.01 mg/kg for each of sulfoxaflor, X11719474, and X11721061 in all matrices. The limit of detection (LOD) was reported as 0.003 mg/kg for each analyte in all matrices.

FDA multiresidue methods are not suitable for analysis of sulfoxaflor; however, data were provided which indicate that the DFG S-19 multiresidue method may provide satisfactory results.

2.2.2 International Harmonization

At this time, there are no MRLs for sulfoxaflor. As a result of the global joint review process, HED expects that sulfoxaflor MRLs covering the majority of the commodities addressed in this assessment will be established by Australia, Canada and Codex.

The U.S., Canada, and Australia have agreed on the residue definition for sulfoxaflor and the global partners are committed to harmonizing regulatory levels to the maximum extent allowed by each regulatory authority's policies. In addition, we note that sufloxaflor was presented at the Joint Meeting on Pesticide Residues (JMPR, 2011). As a pilot process to maximize harmonization, recommendations from that meeting were forwarded to the Codex Committee on Pesticide Residues (CCPR) prior to registration by any regulatory authority in an attempt to set benchmark MRLs. To date, final CCPR recommendations have not been made and negotiations are ongoing between the JMPR and CCPR. All tolerances recommended by HED are harmonized with the MRLs being recommended by Canada.

2.2.3 Recommended Tolerances

HED recommends that 40 CFR be amended by establishing tolerances for residues of sulfoxaflor in the plant and livestock commodities listed in the Table 2.2.3. As the residue definitions for plant and animal commodities are the same, HED recommends that the same entry be used for both types of commodities. The tolerances should be defined as follows:

> Tolerances are established for residues of the insecticide sulfoxaflor, including its metabolites and degradates, in or on the commodities in the table below. Compliance with the tolerance levels specified below is to be determined by measuring only sulfoxaflor (*N*-[methyloxido[1-[6-(trifluoromethyl)-3-pyridinyl]ethyl]-γ^4-sulfanylidene]cyanamide).

Table 2.2.3. Tolerance Summary for Sulfoxaflor.			
Commodity	Tolerance, ppm		Comments (correct commodity definition)
	Proposed	Recommended	
Almond, hulls	4.0	6.0	
Barley, grain	0.15	0.40	
Barley, hay	0.8	1.0	
Barley, straw	1.5	2.0	Tolerance is higher than the HAFT, but lower than the maximum residue. Since the CODEX Global MRL recommended by JMPR is 2.0 ppm, this value would be harmonized.
Bean, dry seed	0.25	0.20	
Bean, succulent	0.7	4.0	
Beet, sugar, dried pulp	--	0.07	
Beet, sugar, molasses	0.03	0.25	
Berry, low growing, subgroup 13-07G	0.06	0.70	
Cauliflower	0.08	0.08	Individual tolerance is required because it exceeds a 5x factor.
Citrus, dried pulp	0.9	3.60	
Cotton, hulls	0.4	0.35	
Cotton, gin byproducts	8.0	6.0	
Cottonseed subgroup 20C	0.2	0.20	
Fruit, citrus, group 10-10	0.6	0.70	
Fruit, pome, group 11-10	0.4	0.50	
Fruit, small, vine climbing, subgroup 13-07F, except fuzzy kiwi fruit	1.3	2.0	
Fruit, stone, group 12	0.6	3.0	
Grain, aspirated fractions	--	20.0	
Grape, raisin	5.0	6.0	The calculated tolerance was 5.4 ppm; however, HED recommends for a 6.0 ppm tolerance in order to harmonize with pending CODEX Global MRL.
Leafy greens, subgroup 4A	5.0	6.0	
Leafy petiole, subgroup 4B	1.0	2.0	
Nuts, tree, group 14	0.02	0.015	
Onion, bulb, subgroup 3-07A	0.01	0.01	
Onion, green, subgroup 3-07B	0.6	0.70	
Pistachio	0.02	0.015	Pistachio requires a separate tolerance. Data from tree nuts are translated to pistachio.
Plum, prune, dried	--	1.90	Processing factor for dried cherry used to calculate concentration of residues.
Rapeseed, meal	0.5	0.50	
Rapeseed subgroup 20A	0.3	0.30	
Soybean, seed	0.2	0.20	
Soybean, hay	1.8	3.0	
Soybean, forage	1.9	2.0	
Tomato, paste	1.6	2.60	
Tomato, puree	0.7	1.20	
Vegetable, *brassica*, leafy, group 5, except cauliflower	1.0 (5A) 1.6 (5B)	2.0	
Vegetable, cucurbit, group 9	0.3 (except	0.40	

Table 2.2.3. Tolerance Summary for Sulfoxaflor.			
Commodity	Tolerance, ppm		Comments (correct commodity definition)
	Proposed	Recommended	
	squash)		
Vegetable, fruiting, group 8-10	1.2	0.70	The calculated tolerance was 0.9 ppm; however, HED recommends for a 0.7 ppm tolerance in order to harmonize with CODEX.
Vegetable, leaves of root and tuber, group 2	4.0	3.0	Based on sugar beet and radish top data
Vegetable, legume, foliage, group 7	--	3.0	
Vegetable, root and tuber, group 1	0.01	0.05	
Watercress	--	6.0	Translated from subgroup 4A data, not a member of subgroup 4A.
Wheat, grain	0.07	0.08	
Wheat, forage	0.8	1.0	Tolerance is higher than the HAFT, but lower than the maximum residue.
Wheat hay	1.1	1.50	
Wheat, straw	2.0	2.0	
Livestock Commodities*			
Cattle, meat	0.1	0.15	
Cattle, fat	0.04	0.10	
Cattle, kidney	0.2	--	See Cattle, meat byproducts
Cattle, meat byproducts	0.25	0.40	
Milk	0.08	0.15	
Milk fat	NA	--	
Goat, meat	0.1	0.15	
Goat, fat	0.04	0.10	
Goat, kidney	0.2	--	See Goat, meat byproducts
Goat, meat byproducts (except kidney)	0.3	--	See Goat, meat byproducts
Goat, meat byproducts	--	0.40	
Hog, meat	0.01	0.01	
Hog, fat	0.01	0.01	
Hog, meat byproducts	0.04	0.01	
Horse, meat	0.1	0.15	
Horse, fat	0.04	0.10	
Horse, kidney	0.2	--	See Horse, meat byproducts
Horse, meat byproducts (except kidney)	0.3	--	See Horse, meat byproducts
Horse, meat byproducts	--	0.40	
Poultry, meat	0.01	0.01	
Poultry, fat	0.01	0.01	
Poultry, meat byproducts	0.03	0.01	
Poultry, eggs	0.01	0.01	
Sheep, meat	0.1	0.15	
Sheep, fat	0.04	0.10	
Sheep, kidney	0.2	--	See Sheep, meat byproducts
Sheep, meat byproducts (except kidney)	0.3	--	See Sheep, meat byproducts
Sheep, meat byproducts	--	0.40	

* Quantifiable residues of sulfoxaflor are not expected in poultry or swine commodities; for harmonization purposes, however, HED is recommending tolerances at the LOQ level (0.01 ppm) for sulfoxaflor in those commodities.

2.2.4 Revisions to Petitioned-For Tolerances

Many of the proposed tolerances are different from those recommended by HED. The reason for the differences is that the registrant determined the proposed tolerances using the NAFTA (North American Free-Trade Agreement) tolerance calculation procedures rather than by using the OECD calculation procedures. In addition, the registrant proposed tolerances for some crops as both an individual crop and as members of a crop group. The registrant should submit a revised Section F in which the proposed tolerances are the same as those recommended by HED.

2.3 Label Recommendations

2.3.1 Recommendations from Residue Reviews

The sulfoxaflor labels should be revised to specify that no more than two applications may be made to soybean forage, as supported by the available residue data.

2.3.2 Recommendations from Occupational Assessment

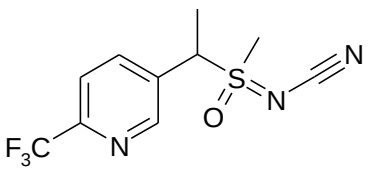
Sulfoxaflor is classified as Toxicity Category IV via the dermal route and for skin irritation potential. It is not a skin sensitizer. Short- and intermediate-term post-application risk estimates were not a concern on day 0 (12 hours following application) for all post-application activities. Under 40 CFR 156.208 (c) (2) (iii), ai's classified as Acute III or IV for acute dermal, eye irritation and primary skin irrigation are assigned a 12-hour REI. Therefore, the [156 subpart K] Worker Protection Statement interim REI of 12 hours is adequate to protect agricultural workers from post-application exposures to sulfoxaflor. It should be noted that the petitioner has proposed a REI of 24 hours for the proposed products based on their interpretation of the acute ocular toxicity study of the Transform™ WG formulated product resulting in a Toxicity Category II for eye irritation. HED recommends that RD ensure that the proper REI is listed on both proposed labels according to the acute toxicity profile of the active ingredient.

2.3.3 Recommendations from Residential Assessment

At this time, there are no residential uses for sulfoxaflor.

3.0 Introduction

3.1 Chemical Identity

Table 3.1. Sulfoxaflor Nomenclature.	
Chemical Structure	

Common name	Sulfoxaflor
Company experimental names	XDE-208
IUPAC name	1-(6-trifluoromethylpyridin-3-yl)ethyl](methyl)-oxido-λ^6-sulfanylidenecyanamide
CAS name	cyanamide, N-[methyloxido[1-[6-(trifluoromethyl)-3-pyridinyl]ethyl] λ^4-sulfanylidene]
Molecular formula	$C_{10}H_{10}F_3N_3OS$
CAS registry number	946578-00-3
End-use products (EP)	GF-2032 SC, Transform WG
Chemical Class	Sulfoximine
Known Impurities of Concern	None

3.2 Physical/Chemical Characteristics

The physical/chemical properties of sulfoxaflor (Appendix D) do not indicate that there are any special considerations that need to be made in conducting this risk assessment. The compound is not particularly volatile (vapor pressure $\leq 1.4 \times 10^{-6}$ Pa) and based on the K_{OW} (~6.35) it is not expected to partition into fatty tissue or bioaccumulate. In the soil, sulfoxaflor and its major degradate, X11719474, are expected to be highly mobile.

3.3 Pesticide Use Pattern

Sulfoxaflor products are intended to be used for insect control in a wide variety of crops as well as turf grass and ornamentals. Sulfoxaflor is generally applied 2-4 times with retreatment intervals ranging from 5 to 14 days, depending on crop, pest, and country/region. The end-use products may be applied through a variety of application methods including aerial, chemigation, ground-boom sprayer, and air-blast sprayer. The recommended application rates for agricultural crops range from 0.016-0.133 lb ai/A. A summary of the use directions is provided in Table 3.3.

Table 3.3. Summary of Proposed Directions for Use of Sulfoxaflor.

Applic. Timing, Type, and Equip.	Formulation	Applic. Rate, lb ai/A	Max. No. Applic. per Season	Max. Seasonal Applic. Rate, lb ai/A [g ai/ha]	PHI (days)	Use Directions and Limitations
Barley/Triticale/Wheat						
Ground, air blast, and aerial	SC/WDG	0.012 - 0.043	2	0.09 [101]	7 (forage/ fodder/hay) 14 (grain/ straw)	14-day retreatment interval.
Brassica (Cole) Leafy Vegetables						
Ground, air blast, and aerial	SC/WDG	0.023 – 0.090	4	0.266 [298]	3	7-day retreatment interval.
Bulb Vegetables (Crop Group 3-07)						
Ground, air blast, and aerial	SC/WDG	0.090	3	0.266 [298]	7	7-day retreatment interval.
Canola (Rapeseed) Subgroup 20A						
Ground, air blast,	SC/WDG	0.012 -	2	0.09 [101]	14	14-day retreatment

Table 3.3. Summary of Proposed Directions for Use of Sulfoxaflor.						
Applic. Timing, Type, and Equip.	Formulation	Applic. Rate, lb ai/A	Max. No. Applic. per Season	Max. Seasonal Applic. Rate, lb ai/A [g ai/ha]	PHI (days)	Use Directions and Limitations
and aerial		0.043				interval.
Citrus (Crop Group 10)						
Ground, air blast, and aerial	SC/WDG	0.023 - 0.133	4	0.266 [298]	1	7-day retreatment interval.
Cotton						
Ground, air blast, and aerial	SC/WDG	0.012 - 0.090	4	0.266 [298]	14	5-day retreatment interval.
Cucurbit Vegetables (Crop Group 9)						
Ground, air blast, and aerial	SC/WDG	0.023 - 0.090	4	0.266 [298]	1	7-day retreatment interval.
Fruiting Vegetables (Crop Group 8)						
Ground, air blast, and aerial	SC/WDG	0.022 - 0.090	4	0.266 [298]	1	7-day retreatment interval.
Leafy Vegetables (except Brassica) Crop Group 4						
Ground, air blast, and aerial	SC/WDG	0.023 - 0.090	4	0.266 [298]	3	7-day retreatment interval.
Leaves of Root and Tuber Vegetables (Crop Group 2)						
Ground, air blast, and aerial	SC/WDG	0.023 - 0.090	4	0.266 [298]	7	7-day retreatment interval.
Pome Fruits (Crop Group 11)						
Ground, air blast, and aerial	SC/WDG	0.023 - 0.090	4	0.266 (298)	7	7-day retreatment interval.
Root and Tuber Vegetables (Crop Group 1)						
Ground, air blast, aerial, and chemigation.	SC/WDG	0.023 - 0.090	4	0.266 [298]	7	7-day retreatment interval.
Small Fruit Vine Climbing, except Fuzzy Kiwifruit (Subgroup 13-07F)						
Ground, air blast, and aerial	SC/WDG	0.043 - 0.090	4	0.266 [298]	7	7-day retreatment interval.
Low Growing Berry (Subgroup 13-07G)						
Ground, air blast, and aerial	SC/WDG	0.043 - 0.090	4	0.266 [298]	1	7-day retreatment interval.
Soybean						
Ground, air blast, and aerial	SC/WDG	0.012 - 0.090	4*	0.266 [298]	7	7-day retreatment interval.
Stone Fruit (Crop Group 12)						
Ground, air blast, and aerial	SC/WDG	0.023 - 0.133	4	0.266 [298]	7	7-day retreatment interval.
Succulent, Edible Podded, and Dry Beans						
Ground, air blast, and aerial	SC/WDG	0.023 - 0.090	4	0.266 [298]	7	7-day retreatment interval.
Tree Nuts						
Ground, air blast, and aerial	SC/WDG	0.022 - 0.133	4	0.266 [298]	7	7-day retreatment interval.
Turf grass/ Ornamentals						
Ground and aerial (ornamentals only)	SC/WDG	0.043 - 0.133	Not specified	0.266 [298]	NA	7-day retreatment interval. Greenhouse

Table 3.3. Summary of Proposed Directions for Use of Sulfoxaflor.						
Applic. Timing, Type, and Equip.	Formulation	Applic. Rate, lb ai/A	Max. No. Applic. per Season	Max. Seasonal Applic. Rate, lb ai/A [g ai/ha]	PHI (days)	Use Directions and Limitations
						use.

*The residue data support two applications for soybean forage.

3.4 Anticipated Exposure Pathways

The requested uses may result in human exposure to sulfoxaflor and/or its metabolites in food and drinking water. At this time, there are no existing, pending, or requested registrations that would result in exposure from use in a residential setting.

Sulfoxaflor presents a somewhat unique situation regarding the food and drinking water exposure pathway. Environmental fate data indicate that use of sulfoxaflor is likely to result in different residue profiles in surface water and groundwater. In surface water, the principal residue is expected to be sulfoxaflor whereas in groundwater, the expected residues are the metabolites X11719474 and, to a lesser extent, X11519540.

3.5 Consideration of Environmental Justice

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," (http://www.eh.doe.gov/oepa/guidance/justice/eo12898.pdf). As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the USDA under the Continuing Survey of Food Intake by Individuals (CSFII) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrant. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas post-application are evaluated. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and farm workers as well as lifestyle and traditional dietary patterns among specific subgroups.

4.0 Hazard Characterization and Dose-Response Assessment

Sulfoxaflor is a member of the sulfoximine class of insecticides. It controls insects via a unique interaction with the insect nicotinic acetylcholine receptor. The proposed insecticidal mode-of-

action (MOA) is similar to neonicotinoid insecticides, as both are nicotinic acetylcholine receptor (nAChR) agonists. However, sulfoxaflor differs from the neonicotinoids because it is not susceptible to *in vitro* metabolism and is proposed to have a different adverse outcome pathway.

4.1 Toxicology Studies Available for Analysis

The hazard database for sulfoxaflor is extensive and provides sufficient information to complete a human health risk assessment. Sulfoxaflor was evaluated for acute, subchronic and chronic oral toxicity in rats, mice and dogs, dermal toxicity in rats, teratogenicity in rats and rabbits, reproductive and postnatal toxicity in rats, carcinogenicity in mice and rats, neurotoxicity in rats, and immunotoxicity in rats. Additional guideline studies included bacterial reverse-mutation test, micronucleus test, and an *in vitro* chromosome aberration test. The database does not contain a subchronic inhalation study. HED has determined that this study is not required for sulfoxaflor (TXR# 0056310).

During the conduct of the guideline studies, limb flexure and/or increased deaths in neonatal rats were observed in the developmental study, the reproduction and fertility study, and the developmental neurotoxicity study. In an attempt to further elucidate the biological processes underlying these effects and their potential relevance to humans, the registrant conducted mechanistic and pharmacokinetic studies beyond the guideline requirements. These studies included development and reproduction studies in the rat and rabbit, as well as *in vitro* studies in rat and human tissues. Mechanistic studies were also provided to support MOA proposals for Leydig cell tumors, hepatocellular tumors, and preputial gland tumors that were observed in the reproduction and chronic guideline studies. A detailed discussion of these studies can be found in the Cancer Assessment Review Committee (CARC) memorandum (TXR # 0056301). The guideline and mechanistic studies have been designed in such a manner as to be mutually supportive. For example: identical animal strains, vehicles, and routes of administration are used throughout the studies; plasma sulfoxaflor concentrations were measured in all studies for estimates of internal doses; and the same dose levels were used in multiple studies (e.g.,. the 2 generation reproduction study and the DNT were both conducted at 0, 25, 150, or 400 ppm). This allows for a more integrated evaluation of studies across the sulfoxaflor toxicity profile.

As described in the following section, sulfoxaflor is resistant to *in vivo* metabolism. However, X11719474 is a major plant metabolite and environmental degradate of sulfoxaflor with a potential for human exposure through drinking water. A battery of studies have been conducted including an oral acute study, absorption and metabolism studies in rats; 28-day and 90-day oral studies in rats, rabbits, and dogs; a reproduction study in rats; a complete battery of mutagenicity studies; and *in vitro* rat and human receptor binding studies. Limited studies are also available for several other metabolites.

4.2 Absorption, Distribution, Metabolism, & Elimination (ADME)

Several studies are available to evaluate the ADME of sulfoxaflor in the rat, including single-dose studies, repeated-dose studies, and an intravenous-dose study to determine systemic availability. In addition, sulfoxaflor plasma concentrations were determined in many of the

studies, including guideline and non-guideline studies in the rat, rabbit, and mouse. Offspring and fetal sulfoxaflor plasma concentrations were determined in the reproduction and development studies in the rat and rabbit. This information allows the comparison of toxicokinetics across species, route of administration, duration, and life-stages. Furthermore, data are available reflecting ADME of the plant/environmental metabolites X11719474 and X11519540. Note that these metabolites were not found in the rat metabolism study.

ADME of Sulfoxaflor in the Rat

Sulfoxaflor is extensively and quickly absorbed following an oral dose. In single- and repeated-dose studies using radiolabeled sulfoxaflor, absorption estimates ranged between 92 and 96%. Maximum plasma concentrations are reached within 2 hours. When compared to an intravenous dose, 92% of an oral dose is available systemically, confirming the high level of absorption. There were no significant differences between the low (5 mg/kg), high (100 mg/kg), or repeated (14 days @ 5 mg/kg) dose groups and plasma concentrations were dose-related indicating absorption was not saturated up to 100 mg/kg when given by oral gavage.

Following absorption, sulfoxaflor is widely distributed throughout the body. When tissue distribution was determined at C_{max} (i.e., maximum concentration of sulfoxaflor in the plasma), the tissues containing the largest proportions of radioactivity were the gastrointestinal tract, liver, kidney, and urinary bladder: all organs consistent with point-of-entry and excretion. By 168 hours, an average of <1.5% of the administered dose remained in the body and that was distributed throughout the body in minor quantities.

The primary route of elimination for sulfoxaflor is through urine. Within 24 hours of an oral dose, 89-94% of the administered dose is eliminated through urine, compared to 5-8% through feces. The removal of sulfoxaflor from plasma occurs in 2 distinct phases; a rapid phase with a half-life of 4-6 hours followed by a much slower phase with a half-life of 39-45 hours. In the 90-day oral study, the plasma half-lives were 8 and 9 hours in the males and females, longer than the 28-day study of 4-5 and 7-8 hours for the males and females, respectively. Despite the longer second phase and the observation that elimination of sulfoxaflor is marginally slower in longer-term studies, little sulfoxaflor remains *in vivo* after the initial 24 hours and therefore there is no concern for bioaccumulation.

In vivo metabolism of sulfoxaflor is minimal. As previously indicated, greater than 90% of an oral dose is eliminated within 24 hours; at least 93% of which is eliminated as the parent compound. A glucuronide conjugate of the urea metabolite was identified at 2-4%. Five additional metabolites were observed; however, they were not identified and each accounted for <1% of the administered dose. Similarly, no major metabolites were identified in either the rat or mouse 28-day and 90-day oral studies.

Fetal Exposure / Maternal Transfer in the Rat

To support the proposed MOA for developmental effects, the pharmacokinetics of sulfoxaflor were determined in a number of developmental and reproductions studies. Pharmacokinetics is a summation of the processes that control how sulfoxaflor is distributed *in vivo*, i.e., how it is

metabolized or eliminated, and, of critical importance to the developmental MOA, how it is presented to developing fetuses or newborn offspring. The distribution and clearance parameters of sulfoxaflor were described in the metabolism studies discussed above. The parameters relevant to fetal or offspring exposure were captured in the following reproduction and developmental studies.

In the 2-generation reproduction study in the rat, plasma concentrations were determined in offspring and dams 4 days after parturition. Offspring levels were ≈ 30% of maternal levels across all dose levels indicating that the offspring internal dose was ≈ 1/3 of the maternal internal dose. In a cross-fostering and a developmental toxicity study in rats, the maternal and fetal sulfoxaflor blood concentrations were similar on GD 21. Similar findings regarding the comparison of maternal vs. offspring sulfoxaflor blood concentrations were reported in the rabbit developmental study. This suggests that sulfoxaflor moves readily across the placenta.

Lactational transfer was also determined in the cross-fostering study in the rat. On Lactation Day (LD) 0, the measured concentrations of sulfoxaflor in milk from sulfoxaflor-exposed dams were approximately half the corresponding concentrations in plasma. Although milk concentrations were not determined in the 2-generation study, the offspring plasma concentrations of sulfoxaflor were approximately 30% of the maternal animals, supporting the observed reduction in lactational transfer. In a goat metabolism study, less than 2% of the dietary sulfoxaflor was found in the milk. Thus, lactational transfer ranges between 2 and 50% in goats and rats, respectively.

These studies clearly indicate that fetal plasma levels of sulfoxaflor are similar to the dams during gestation. However, after parturition when offspring exposure to sulfoxaflor is limited to lactational transfer through the milk, exposure to the rat pups is 2 to 3-fold lower than maternal doses.

Sulfoxaflor Metabolites X11719474 and X11519540

Despite the lack of *in vivo* metabolism, there is a potential for exposure to environmental degradates of sulfoxaflor. In particular, X11719474 is a major degradate found in environmental fate studies, and is expected to be the major degradate in drinking water from groundwater sources. The toxicokinetics of X11719474 are similar to the parent sulfoxaflor; following an oral dose, X11719474 is highly absorbed (>98%), reaches a maximum plasma concentration quickly (≈1 hour), and is quickly eliminated through the urine >90% within 12 hours. Limited ADME data are also available for a second metabolite, X11519540, which has been observed in limited amounts in field and animal feeding studies. The elimination half-life of X11519540 is approximately 27 hours, longer than sulfoxaflor and X11719474. Both metabolites are largely absorbed and excreted unmetabolized in *in vivo* studies.

4.2.1 Dermal Absorption

The Registrant submitted an *in vivo* study in rats and an *in vitro* study utilizing split-thickness rat and human skin in which sulfoxaflor was applied at doses of 2360 µg/cm^2, 4.8 µg/cm^2, and 0.25 µg/cm^2. These doses represent sulfoxaflor concentrate, the highest in-use concentration, and the

lowest in-use concentration, respectively. The *in vivo* data showed that skin-bound residues of sulfoxaflor were absorbed for up to 144 hours after skin washing. The dermal absorption factor (DAF) for sulfoxaflor after 10 hours of dermal exposure (that included absorbable skin-bound residues) was 11% for both the highest and lowest in-use concentrations. These values were larger in magnitude than the concentrate. The *in vitro* data for rat skin after 24 hours of dermal exposure showed the largest magnitude potentially absorbable dose for the lowest in-use concentration. The Agency considered comparison of data at the 24-hour time point in the *in vitro* study to the 10-hour time point in the *in vivo* study on the basis that the estimates at the 24-hour time point would be conservative surrogates for a 10-hour dermal exposure (because dermal absorption generally increases with time). The DAF for rat skin *in vitro* was 8%, a value that does not differ from the 11% DAF for rat skin from the *in vivo* study after standard errors in measurement are taken into account. This result indicated that the *in vitro* study design and execution was predictive of the *in vivo* results. The Agency's policy is that if an *in vitro* study is shown to be predictive of an *in vivo* result, the Agency will use human skin from the *in vitro* study to refine the DAF for human exposures. The DAF for human skin in the *in vitro* study at the lowest in-use concentration was 2.4% (Table 4.2.1). The Agency is using this human value of 2.4% as the DAF for determining dermal-equivalent doses in the sulfoxaflor risk assessment.

Table 4.2.1. Derivation of the Dermal Equivalent Dose for Human Health Risk Asssessment.

Parameters	*In Vivo* Rat			*In Vitro* Rat			*In Vitro* Human		
Study ID	MRID No. 47832413 TXR No. 0055507			MRID No.47832414 TXR No.0055507			MRID No.47832414 TXR No.0055507		
Test Material	SC Formulation (24%)			SC Formulation (24%)			SC Formulation (24%)		
Exposure	10 hours			24 hours			24 hours		
Doses tested ($\mu g/cm^2$)	Low	Mid	Hi	Low	Mid	High	Low	Mid	High
	0.25	4.8	2360	0.25	4.8	2360	0.25	4.8	2360
% Absorbed	10.77[a]	11.35	1.22	8.02[b]	8.72	1.67	2.38[b]	2.44	0.35
Point of Departure	Oral POD = Oral NOAEL = 1.8 mg/kg/day based on decreased neonatal survival at 7.1 mg/kg/day observed in the DNT								
Dermal Equivalent Dose (DED)	For short- and intermediate-term dermal exposure, the POD is 1.8 mg/kg/day. POD = oral NOAEL = 1.8 mg/kg/day DAF (*In Vivo* rat) ≈ DAF (*In Vitro* rat) ≈ 11% *In Vitro* Human dermal absorption = 2.4% Dermal Equivalent Dose = Oral POD/human DAF=1.8 mg/kg/day/ 0.024= 75 mg/kg/day								
Comments: Same formulation products were tested in all three studies. Doses are identical in the *in vitro* rat and human and the *in vivo* rat study. *In vitro* absorption predicts *in vivo* absorption in the rat. DED is calculated using the human dermal absorption factor.									

a – Absorbed dose after 144 hours
b- Potential absorbable dose after 24 hours

4.3 Toxicological Effects

Sulfoxaflor is a member of the sulfoximine class of insecticides. It controls insects via agonism of the insect nicotinic acetylcholine receptor (nAChR) leading to sustained muscle contracture, paralysis, and death. In mammals, the pattern of toxicity attributed to sulfoxaflor exposure includes developmental effects (manifested as neuromuscular abnormalities and neonatal death),

hepatotoxicity, and generalized toxicity (e.g., decreases in body weight, body weight gain, and/or food consumption).

4.3.1 Developmental/Reproductive Effects, Mode of Action, and Human Relevance

Developmental/offspring toxicity, manifested as skeletal abnormalities and neonatal deaths, was observed in rats only. The skeletal abnormalities, forelimb flexure, bent clavicles, and hindlimb rotation likely result from skeletal muscle contraction due to agonism of the muscle nAChR *in utero*. Similarly, contraction of the diaphragm muscle prevents normal breathing in neonates resulting in increased mortality. The skeletal abnormalities were observed at high doses in the developmental and reproduction studies and decreased neonatal survival was consistently observed in the reproduction and developmental neurotoxicity studies. These developmental effects were not observed in the rabbit.

4.3.1.1 Mode of Action Framework

Given the known insecticidal MOA for sulfoxaflor, it was hypothesized that these effects could be related to an interaction between sulfoxaflor and the nAChR. In an attempt to test this hypothesis and further elucidate the biological processes underlying these effects and their potential relevance to humans, the registrant conducted numerous mechanistic studies beyond the guideline requirements. These studies included but were not limited to development and reproduction studies in the rat and rabbit, as well as *in vitro* studies in rat and human tissues. These studies were designed to assess if sulfoxaflor binds and activates the nAChR in rats, rabbits, and humans. Ultimately, the objective was to determine if the differences observed in sulfoxaflor's developmental toxicity profile could be ascribed to a differential interaction with the nAChRs in these three species.

HED has used existing, peer reviewed frameworks in the analysis of the special data. In keeping with EPA's Framework for Determining a Mutagenic Mode of Action and the International Programme on Chemical Safety (IPCS) Framework for Analyzing the Relevance of a Non-Cancer Mode of Action for Humans, the Agency conducted a mode of action framework analysis considering the relevance of the mode of action (MOA) to humans. This is consistent with the approaches taken by the Joint FAO/WHO Meetings on Pesticide Residues (JMPR) and the Organization for Economic Cooperation and Development (OECD). The MOA analysis framework relies on a weight-of-evidence approach, which is based on modified Bradford Hill criteria for causality (Hill, 1965). In line with the 2007 NRC report on Toxicity testing in the 21st Century, some have considered the concept of adverse-outcome pathways (AOP) in addition to MOA. These concepts are fundamentally similar in that each considers key events (including an initiating event) leading from exposure to an adverse outcome. This document uses the MOA terminology instead of the AOP terminology. In brief, the MOA framework begins with a proposed set of key events and then describes the evidence for such events with particular focus on temporal and dose-response concordance. Consistent with the Bradford Hill criteria, the MOA analysis also takes into consideration the consistency, specificity, and biological plausibility of the findings. The analysis also involves explicit evaluation of alternative hypotheses, alternative MOAs, and remaining uncertainties.

Proposed Key Events

In light of observations in the guideline toxicity studies and the basic biological understanding of muscle nAChR ontogeny and function in mammalian systems, the registrant proposed a MOA by which the muscle effects (forelimb flexure, bent clavicles, and rotated hindlimbs) and deaths observed in rat neonates are caused by **sulfoxaflor's sustained agonism at the fetal-type muscle nAChR and subsequent sustained muscle contracture of the limb, shoulder girdle and diaphragm, respectively.** This proposed MOA can be expressed in terms of four key events depicted in the Figure 4.3.1.1:

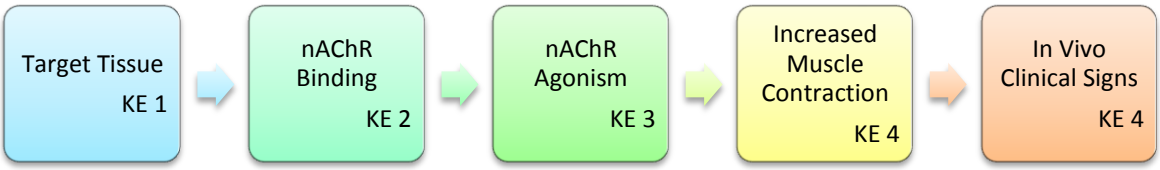


Figure 4.3.1.1. Key events of the sulfoxaflor MOA. Each box represents a key event, beginning with the availability of sulfoxaflor at the neuromuscular nAChR and concluding with the reproductive effects observed in the reproduction and development studies (KE=Key Event).

Summary of the Evidence for the Key Events:

- Key Event #1: Internal dose at the target tissue: As described in the ADME section of this document, sulfoxaflor is extensively and quickly absorbed following oral exposure and distributed systemically. It is, therefore, available to interact with the target tissue.

- Key Event #2: Muscle receptor binding: Two types of nAChRs are present in mammals, neuronal and muscle. The muscle nAChRs are found in the intramuscular junctions of skeletal muscles and are involved in muscle contraction; it is the muscle nAChRs which are the targets for sulfoxaflor. Disregulation of these receptors can result in improper muscle contraction, difficulties in breathing, and ultimately death (due to sustained diaphragm contracture). Two isoforms of the muscle nAChRs have been identified in mammals, a fetal isoform and an adult isoform. The transition from fetal to adult isoform occurs shortly after birth in rats and late during the 3rd trimester in humans. Furthermore, sulfoxaflor has been shown to bind the fetal isoforms of the nicotinic receptors of rats, rabbits, and humans in a series of mechanistic studies. These studies demonstrated a dose-dependent increase in sulfoxaflor binding to the fetal muscle nAChR in all three species.

- Key Event #3: Receptor agonism: Sulfoxaflor binds the nAChR in the rabbit, rat, and human. A study was undertaken to determine if sulfoxaflor causes activation of the receptors**.** Rat and human muscle nAChRs were expressed in *Xenopus* oocytes– a model system used to evaluate activation of receptors. Rabbit receptors were not evaluated because the encoding proteins are not currently available for this species. The ability of sulfoxaflor to activate both fetal and adult muscle nAChRs was examined. This study indicated that the rat fetal-type nAChR was activated by sulfoxaflor. However, there was no activation in the adult rat, fetal human, or the adult human receptors. The implications

for the lack of response in humans are discussed below in the human relevance section.

- Key Event #4: Muscle contraction and clinical signs: Sustained contraction of fetal rat diaphragm muscles in the presence of sulfoxaflor was demonstrated *in vitro*. Contraction of the diaphragm during late gestation leads to asphyxiation and the observed increase in neonatal deaths.

  The muscle nAChRs transition from the fetal to the adult isoforms and the timing of this shift provides insight into the potential role of the fetal nAChR in the developmental effects observed in rats after sulfoxaflor exposure. Both the skeletal effects and the offspring deaths in rats are limited to the very early postnatal period when the fetal isoform is the predominant isoform present. However, beyond post-natal day 4 there is no increase in pup death and the skeletal effects noted shortly after birth (forelimb flexure, bent clavicles, and rotated hindlimbs) begin to recover. Thus, it appears that there is a time-concordance between the appearance of the adult isoform and recovery from the developmental effects strongly suggesting that the fetal isoform of the muscle nAChR is critical to the manifestation of these types of effects. For a more detailed description of the agonism experiments refer to Appendix B.

- Alternative MOAs: As discussed in Appendix B, a variety of alternative hypotheses were considered (i.e., neuronal nicotinic receptor activation, muscarinic receptor activation, fetal nicotinic receptor inactivation, and ACE inhibition). However, none of these were consistent with sulfoxaflor's toxicological profile, particularly the adverse outcomes observed.

- Weight of the Evidence: In conclusion, there is compelling evidence that the proposed MOA is operative in the fetal rat and leads to the neonatal death and the skeletal effects seen in the guideline toxicity studies involving that species. As such, HED believes the developmental and reproduction MOA based on agonism of the fetal nAChR has been established.

4.3.1.2. Human Relevance

As summarized above and discussed in detail in Appendix B, the MOA for the neonatal death and limb flexures based on agonism of muscular nicotinic receptor has been established. Given this finding, HED has used the Human Relevance Framework (HRF) to consider the degree to which the developmental and reproduction MOA is relevant to humans and thus relevant for risk assessment. The HRF entails both a qualitative and quantitative evaluation and comparison of the key events in animals and humans. Like the MOA analysis, the HRF analysis includes an evaluation of uncertainties and consideration of alternatives. An HRF analysis may be undertaken for a variety of reasons including:

- Situations where the effects in animals would have potentially serious consequences if they occurred in humans, and
- Situations where there are interspecies differences in either the type of effect or the dose levels at which an effect occurs.

In addition to the guideline toxicity studies conducted with sulfoxaflor, an extensive mechanistic data set has been generated to further evaluate the interspecies sensitivity to sulfoxaflor's developmental effects. These studies include both binding and agonism *in vitro/ex vivo* studies to ascertain if: (a) sulfoxaflor binds to the muscle nAChR receptor in rats, rabbits, and humans and (b) if the bound sulfoxaflor can activate the receptor. Data from these experiments indicate that although sulfoxaflor does bind to the fetal nAChR in rats, rabbits, and humans, it does not activate the human receptor.

Table 4.3.1.1. Key Events of the Developmental and Reproduction Sulfoxaflor MOA based on agonism of the rat fetal nAChR and the supporting events observed in rats and human tissues.

Key Event	Evidence in Rodents	Evidence in Humans
#1 Binding to the fetal-type muscle nAChR	Yes	Yes
#2 Agonism at the fetal-type muscle nAChR	Yes	No; Plausible, but no agonism occurs with sulfoxaflor either at the fetal- or adult-type muscle nAChR.
#3 Sustained fetal-type muscle nAChR agonism/sustained muscle contraction	Yes	No data in humans. Presumably not possible via this MoA as key event #2 does not occur.
Apical Endpoints Forelimb Flexure, Hindlimb Rotation, Bent Clavicle, Neonatal Death	Yes	No data in humans. Presumably not possible via this MoA as key event #2 does not occur.

Close examination of the key events indicates significant differences in the key events between the rat and human.

- Key Event #1: Qualitatively, sulfoxaflor has demonstrated the ability to bind both rat and human nAChRs. As previously described, sulfoxaflor has been shown to bind the fetal isoforms of the nicotinic receptors of rats, rabbits, and humans.

- Key Event #2: Despite binding, sulfoxaflor does not activate the human fetal muscle nAChR. An *in vitro* study using fetal isoform for rat and human of the muscle nAChR expressed in *Xenopus* oocytes, examined the ability of sulfoxaflor to perturb the functionality of rat and human muscle nAChR isoforms. Treatment with sulfoxaflor resulted in increased response in the rat fetal isoform. In contrast, there was no indication of agonism by sulfoxaflor in the human isoform. Figure 4.3.1.2 is a graphical representation of the agonism of the rat and human fetal NAChRs by sulfoxaflor. Fetal rat and human nAChRs were expressed in *Xenopus* oocytes followed by exposure to sulfoxaflor (blue line). The endogenous agonist acetylcholine (ACh; red line) was also tested to ensure the integrity of the expressed receptors and for a quantitative comparison to sulfoxaflor-induced activity. ACh induced a clear and potent response at 10^{-6} M in the rat and human receptors. However, considerably higher concentrations of sulfoxaflor were required to initiate activity in the rat receptor (10^{-4} M), and the response was relatively low. It is especially important to note that the human receptor was refractory to sulfoxaflor-induced activity, even at concentrations exceeding 10^{-3} M, or approximately 500-fold greater than the concentrations that resulted in activation in the rat receptor.

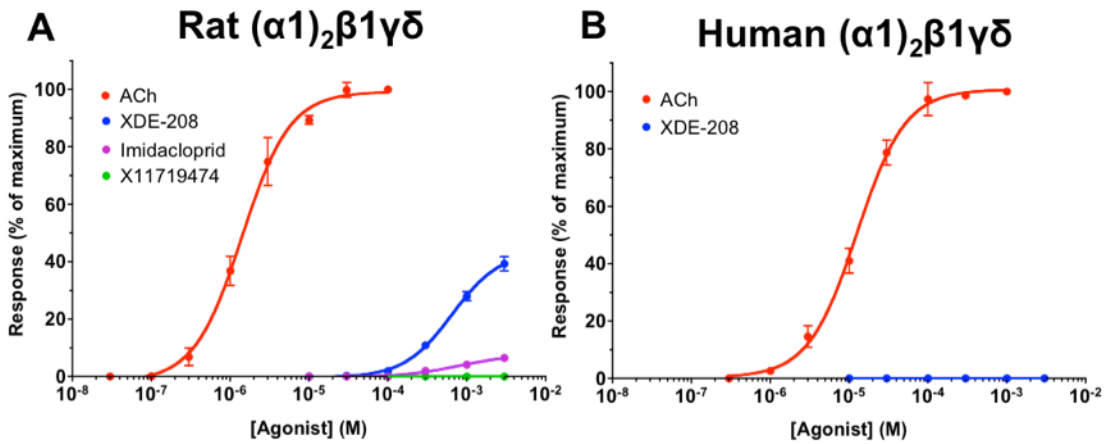


Figure 4.3.1.2. Agonist activation of muscle nAChRs expressed in *Xenopus* oocytes. Data are shown for the (A) rat fetal, (B) human fetal muscle nAChR. AChRs were expressed by microinjection of cDNA or cRNA in *Xenopus* oocytes. ACh = acetylcholinesterase; IMI = imidacloprid; Sulfoxaflor metabolite X11719474.

Although the technology for cloning the rabbit muscle nAChR is not yet available, the lack of developmental effects in the rabbit is an indirect indicator of the unique sensitivity of the rat. In the rabbit developmental study, (MRID 47832066) and non-guideline rabbit survival study (MRID 47832138) there is no indication of the fetal anomalies observed in the rat developmental toxicity study, in particular the forelimb flexure, bent clavicle, and hindlimb rotation seen in the rat developmental toxicity. Furthermore, the sulfoxaflor plasma concentrations on gestation day (GD) 27 were ≈ 4-fold higher than the plasma concentration associated with neonatal death and neuromuscular/skeletal effects in the rat pups, indicating that the absence of developmental effects in the rabbit studies is due to a pharmacodynamic rather than a pharmacokinetic difference between rabbits and rats.

- Key Events #3 and #4: Muscle contraction and *in vivo* clinical signs are not available for evaluation in humans. However, in the absence of Key Event #2, these final key events are not expected to occur in humans.

- Weight of Evidence: Based on the lack of receptor activity, there is compelling evidence that the developmental and reproduction MOA based on agonism of the fetal nAChR is not likely to be relevant to humans. Furthermore, a comparison of the amino acid sequence of the rat and human γ subunit (specific to the fetus) revealed that, although the two subunits are similar (approximately 90% identical), they contain 53 amino acid differences (Millar, 2010). There are several precedents for species-selective agonist activity of nAChR ligands. Given the evidence that as few as one or two amino acid differences can confer species-selective agonist activity upon nicotinic ligands, it seems entirely plausible that the differences in agonist activity of sulfoxaflor can be explained by differences in the amino acid sequence of the rat and human muscle nAChR γ subunits. The γ and ε subunits (fetal and adult isoforms, respectively) show even greater sequence differences than the human and rat γ subunit (even from the same species), where these subunits share only about 50% identity in amino acid sequence in the rat.

- Uncertainties: The use of *Xenopus* oocytes in the agonism study represents a residual uncertainty, albeit extremely low, that precludes the Agency from discounting the possibility of human relevance on a qualitative basis. The heterologous expression of mammalian receptors in *Xenopus* oocytes is a common technique that has been used for determining the structure and function of nicotinic receptors (Millar 2009). Expression in *Xenopus* oocytes offers several advantages over native mammalian cells including the following: limited expression of endogenous ion channels and receptors so that exogenous proteins can be studied with minimal contamination; some channels can only be expressed in oocytes and not in mammalian cells; and oocytes are well suited for electrophysiological recording. Past studies have indicated that the activity of muscle nAChRs expressed in either oocytes and HEK were similar for muscle (Stern et. al. 1994) and neuronal (Luetje and Patrick 1991) type receptors. However, the unique matrixes provided by the oocytes and mammalian cells can lead to differential expression of receptors or channels. For example, the expression of the human neuronal nAChR α3β4 results in different ratios of the α and β subunits for oocytes and HEK cells (Krashia e.t al. 2010; Boorman et. al. 2000). Altered subunit ratios have been shown to result in different sensitivities to the acetylcholine and other agonists (Krashia et al. 2010; Moroni et. al. 2006; Moroni et. al. 2008). Due to the severity of the endpoint (*i.e*, neonatal deaths), HED has a requested a confirmatory study; that is a study confirming the refractory response of a fetal human nicotinic receptor expressed in a mammalian cell. **Pending the results of the confirmatory study in human receptors expressed in mammalian cells, HED considers the MOA relevant to humans.**

4.3.1.3. Interspecies Uncertainty Factor

The available information comparing rat and human muscle nAChR response to sulfoxaflor has bearing on the interspecies uncertainty factor (UF_A) when assessing nAChR-mediated risks associated with sulfoxaflor exposure. HED recommends that the 10X interspecies factor be reduced to 3X for acute dietary risk assessment for women of reproductive age and for other scenarios assessed using the neonatal death endpoint (*i.e.*, dermal and inhalation risk assessments). This recommendation is based on the evidence gathered from the mechanistic data which indicate that rats may be uniquely sensitive to the developmental effects of sulfoxaflor. HED has used the 2005 IPCS document on Chemical Specific Adjustment Factors and the Agency's 2011 external peer review draft of the Guidance on Data Derived Extrapolation Factors. In short, both approaches endorse the use of mechanistic and pharmacokinetic data to inform data derived extrapolation factors for inter- and intra-species extrapolation. In both approaches, the typical 10X factor is split between a PD and PK component. USEPA typically uses an even split of 3X and 3X. As discussed in 4.1.1, the developmental and reproduction MOA based on agonism of the fetal nAChR has been established for skeletal anomalies (e.g., forelimb flexure, bent clavicle, and rotated hindlimbs) and neonatal deaths in the rat and HED cannot currently rule out this MOA as being not relevant to humans. As such, the neonatal mortality is relevant for POD selection (see above). Although, HED believes that additional data are necessary to fully demonstrate the lack of human relevance, the existing mechanistic are high quality and provide a robust evaluation of the response to rat and human tissues for the agonism of the nAChR. Moreover, the nature and quality of these data are consistent with the kinds of data recommended by the International Programme on Chemical Safety (IPCS) Chemical-

Specific Adjustment Factor (CSAF) and the EPA Data Derived Extrapolation Factors guidance documents.

Quantitatively, the sulfoxaflor concentration required to initiate receptor activation in human fetal nAChR is at least 500-fold greater than that required to activate rat fetal nAChR. The absence of nAChR activation precludes the manifestation of skeletal abnormalities and neonatal deaths. Therefore, the data indicate that rats are uniquely sensitive to the developmental and reproduction MOA leading to neonatal death and neuromuscular effects. The Agency is proposing that the PD portion of the interspecies UF be reduced to 1X. Pharmacodynamics can be defined as what the chemical does to the body. In the case of sulfoxaflor, PD is expressed as the ability of the sulfoxaflor to interact with the nAChR. Since agonism of the nAChR is the critical key event, interspecies extrapolation can be assessed using the agonism data (*i.e.*, data demonstrating sulfoxaflor-induced activity in the oocyte expressed rat and human nAChRs). As described in 4.3.1.2, sulfoxaflor does not elicit a response in the human nAChR at concentrations 500-fold higher than the concentrations eliciting a response in the uniquely susceptible fetal rat receptor. In other words, based on this assay, human tissue is at minimum 500-fold less sensitive than is the rat tissue. Since humans are clearly not more sensitive pharmacodynamically to sulfoxaflor compared to the rat, reduction of the UF for PD to 1X, which assumes an equal response between humans and rats, is conservative.

The PK portion of the inter-species factor is retained at 3X since there are PK studies in human tissues or physiologically-based pharmacokinetic models which allow for refinement of the PK component of the extrapolation factor.

4.3.2. Other Adverse Outcomes Observed for Sulfoxaflor

In terms of adults, sulfoxaflor systemic toxicity is manifested primarily in the form of hepatotoxicity in rat, mouse, and dog studies. Moreover, effects were observed following exposures as short as 8 days as well as in studies continuing for up to 2 years. Increased liver weight, hepatic hypertrophy, fatty liver, single cell necrosis, and changes in clinical chemistry (i.e., cholesterol, AST, or ALT) were consistently observed throughout the toxicology database. The longer term carcinogenicity studies resulted in hepatic effects at lower doses compared to the short-term studies. In addition, males appeared to be more sensitive to the hepatic toxicity. As indicated in section 4.2, male rats had a slower clearance rate compared to females. The slower clearance rate in males in essence increases exposure and might account for the increased male sensitivity to hepatotoxicity in the longer-term studies.

Aside from the developmental and hepatic effects, other effects seen in the sulfoxaflor database include decreases in food consumption and body weight as well as changes in the male reproductive system. Decreased body weight, body weight changes, and food consumption were observed during the first few days of several oral studies at the mid- and high-dose levels. As a result of decreased feeding early in the studies, body weights were typically lower in the mid- and high-dose groups compared to the controls, although the differences were not generally statistically significant. Decreased palatability is a likely contributor to this effect as body weight decreases were often observed at study initiation but were comparable to control animals within several weeks.

Effects in the male reproductive organs were observed in the chronic/carcinogenicity study in rats that included increased testicular and epididymal weights, atrophy of seminiferous tubules, and decreased secretory material in the coagulating glands, prostate, and seminal vesicles. Additionally, there was an increased incidence of interstitial cell (Leydig cell) tumors. The Leydig cell tumors observed after exposure to sulfoxaflor are not considered treatment related due to the lack of dose response, the lack of statistical significance for the combined tumors (unilateral and bilateral), and the high background rates for this tumor type in F344 rats. The primary effects on male reproductive organs are considered secondary to the loss of normal testicular function due to the size of the interstitial cell (Leydig Cell) adenomas. Consequently, the secondary effects to the male reproductive organs are also not considered treatment related.

Clinical indications of neurotoxicity were only observed at high doses in the acute neurotoxicity study in rats. At the highest dose tested, clinical signs included increased muscle tremors and twitches, convulsions, hindlimb splaying, increased lacrimation and salivation, decreased pupil size and response to touch, gait abnormalities and decreased rectal temperature. Decreased motor activity was also observed in the mid- and high-dose groups. While the muscle effects are consistent with perturbation of the neuromuscular nAChR, salivation, lacrimation, and pupil response are typically associated with muscarinic receptor activity. Since the neurotoxicity was observed only at a very high dose and is not entirely consistent with nicotinic receptor toxicity, it is unlikely that these effects are due to agonism of the nAChR.

Finally, tumors were observed in chronic rats and mouse studies. In the rat, a significant increase in the incidence of hepatocellular adenomas and combined adenomas and/or carcinomas in the high-dose males was observed when compared to controls. As previously indicated, Leydig cell tumors were also observed in the high-dose group, but it was determined that these were not related to treatment with sulfoxaflor. There was also a significant increase in the incidence of preputial gland tumors in males in the high-dose group. Marginal increases were also observed in the low- and mid-dose groups; however, the incident values for these groups were within the range of historical control values. In male mice, treatment-related changes in the high-dose groups consisted of increased incidences of hepatocellular adenomas and/or carcinomas.

4.3.3. Metabolites

As indicated previously, the petitioner has provided several additional studies on sulfoxaflor metabolites and degradates. Sulfoxaflor has six known primary metabolites; all are formed by metabolism of the parent's ethyl-sulfanylidene-cyanamide side chain and/or the side chain of its metabolites.

X11719474 is a major metabolite in terms of magnitude of residue and/or presence across matrices - soil, water and edible crops. The estimated half-life of X11719474 exceeds 1000 days in US soils and the compound has the potential to leach into ground water. Of the metabolites, X11719474 has the most complete toxicology database, which includes oral acute, subchronic, developmental, reproduction, and metabolism studies. Unlike sulfoxaflor, X11719474 does not cause agonism of the fetal rat muscle nAChR and prenatal exposure does not result in increased

neonatal deaths or skeletal abnormalities. The major target organ of X11719474 is liver and the LOAELs are based on hepatotoxicity.

X11519540 is a minor soil and animal metabolite. It is also found as a low-level impurity of the manufacturing process. Repeat-dose studies in the rat show that the primary target organ is the liver. In addition, X11519540 resulted in a dose-dependent increase in the adrenal gland weight. When the LOAELs for the 28-day oral studies for sulfoxaflor and X11519540 are compared, the LOAEL for X11519540 is 10-fold lower than the LOAEL for sulfoxaflor. The oral LD_{50} for this metabolite is approximately half of the LD_{50} for sulfoxaflor. Therefore, it is apparent that X11519540 is more potent than sulfoxaflor. The 28-day rat study also indicates that X11519540 has a longer half-life; 24-35 hours compared to 4-8 hours for sulfoxaflor, which could contribute to its increased potency.

Other metabolites include X11721061, X11596066, X11579457, and X11718922. None of these metabolites are expected to contribute significantly to the potential toxicity of sulfoxaflor.

As indicated above, the target organ for X11719474 and X11519540 is the liver. To determine if X11719474-induced hepatotoxicity is also mediated by nuclear receptor-mediated MOA, rats were given either 0 or 8000 ppm (583 mg/kg/day) X11719474 for 7 days in a non-guideline study. After 7 days, the key events identified in the sulfoxaflor MOA were determined to also occur after X11719474 exposure. Based on these observations, CARC determined that the metabolite X11719474 induced hepatocellular gene expression, enzyme activity, and proliferation in a manner very similar to sulfoxaflor. A similar study providing mechanistic data on the pathway leading to hepatotoxicity is not available for X11519540. However, due to structural similarity between X11519540 and X11719474, and the similar MOAs for X11719474 and sulfoxaflor, HED assumes that the events leading to hepatotoxicity in X11519540 are similar to those for sulfoxaflor and X11719474.

As previously indicated, there is a complete database of toxicological studies for sulfoxaflor and there are numerous studies describing the toxicity of X11719474. However, the database for X11519540 is limited to two studies, a 28-day oral toxicity study in the rat and an acute LD_{50} study, also in the rat. Since the rat 28-day oral toxicity studies are common to all three chemical databases and the studies' LOAELs are all based on the common toxic effect of hepatotoxicity, HED has estimated the relative potency of X11719474 and X11519540 based on results from the 28-day oral toxicity study in the rat. The study LOAELs are 79.4, 244, and 7.7 mg/kg/day for sulfoxaflor, X11719474, and X11519540, respectively. Therefore the relative potency factors with respect to liver toxicity are 0.3 for X11719474 and 10 for X11519540.

4.4 Safety factor for Infants and Children (FQPA Safety Factor)

HED has recommended that the required 10X FQPA Safety Factor be reduced to 1X based on the following considerations: 1) the toxicology database for sulfoxaflor is complete with regard to FQPA consideration, including the required developmental and reproductive toxicity studies; 2) the required neurotoxicity studies, including the developmental neurotoxicity study (DNT) have been submitted, are considered adequate; 3) although there is evidence of quantitative susceptibility in the DNT study, based on decreased survival of offspring up to postnatal day 4,

the endpoints and doses selected for risk assessment are protective for these effects; further, HED's degree of concern for human susceptibility is reduced based on the special studies submitted in support of the mode of action; and 4) although some refinements are used in the exposure assessment, the dietary and drinking water assessments still result in upper-bound estimates of exposure.

4.4.1 Completeness of the Toxicology Database

The toxicology database for sulfoxaflor is adequate for FQPA consideration including a developmental toxicity study in rats and rabbits, a 2-generation reproduction study in rats, and acute, subchronic, and developmental neurotoxicity studies in rats.

An acceptable guideline inhalation study is required for all food-use registrations; however in the case of sulfoxaflor, the use of an oral study is considered protective for inhalation exposure for the following reasons:

1) The acute inhalation toxicity (Category IV) is lower than the acute oral LD_{50} (Category III);
2) The vapor pressure is low ($\leq 1.4\times10^{-6}$ Pa = 1.05×10^{-8} mm Hg at 20°C);
3) There is adequate characterization of toxicity;
4) Oral absorption is >90% – substantially higher absorption *via* the inhalation route is not expected;
5) Oral absorption is rapid;
6) Sulfoxaflor is not subject to enterohepatic circulation, making the absorbed oral dose available systemically; and
7) Sulfoxaflor is not subject to *in vivo* metabolism and therefore potential first-pass effects are not relevant.

Therefore, the subchronic inhalation study is waived for sulfoxaflor. The lack of a subchronic inhalation study is not considered a data gap and a database uncertainty factor is not required (TXR# 0056310).

4.4.2 Evidence of Neurotoxicity

The level of concern for neurotoxicity is low because the effects are well characterized, the dose-response curve for these effects is well characterized, and clear NOAELs have been identified.

4.4.3 Evidence of Sensitivity/Susceptibility in the Developing or Young Animal

Although there was quantitative susceptibility observed in the DNT, there is no residual uncertainty because the effects are well characterized, a clear NOAEL was identified, and the endpoints chosen for risk assessment are protective of potential *in utero* and developmental effects. Quantitative susceptibility in the DNT was based on an increased rate of neonatal deaths at a dose where no maternal toxicity was observed. However, the apparent enhanced sensitivity may be due to the limited number of evaluations conducted in dams in the study rather than a true sensitivity of the young. Qualitative susceptibility was observed in the 2-generation reproduction study since neonatal deaths were observed at the same dose that resulted in

hepatotoxicity in parental animals. However, these effects occurred at a higher dose compared to the offspring effects observed in the DNT. Finally, there was no evidence of quantitative or qualitative susceptibility in the developmental studies in the rat or rabbit.

4.4.4 Residual Uncertainty in the Exposure Database

There are no residual uncertainties with regard to dietary and occupational exposure. The dietary exposure assessments are based on high-end health protective residue levels (that account for parent and metabolites of concern), processing factors, and percent crop treated assumptions (100%). Actual exposure to sulfoxaflor will likely be lower than the estimated exposure. Furthermore, conservative, upper-bound assumptions were used to determine exposure through drinking water sources, such that these exposures have not been underestimated.

4.5 Toxicity Endpoint and Point of Departure Selections

4.5.1 Dose-Response Assessment

Based on the use pattern and the toxicological profile of sulfoxaflor, HED selected endpoints and doses for acute and chronic dietary risk assessment. Endpoints and doses were not selected for non-dietary non-occupational exposures (i.e., incidental oral, dermal, or inhalation), since there are no proposed uses which would result in these exposure pathways. For occupational handlers and workers re-entering treated areas, HED selected endpoints and doses for dermal and inhalation exposure and risk assessment.

Endpoint Selection

Acute Dietary Endpoint for the General Population: The endpoint used for establishing the aPAD for the general population was selected from the acute neurotoxicity study in rats. An aPAD of 0.25 mg/kg/day was derived from a NOAEL of 25 mg/kg/day and a 100-fold factor that included 10x for inter-species extrapolations, 10x for intra-species variations, and a 1x FQPA SF. The LOAEL of 75 mg/kg was based on decreased motor activity on Day 1. More severe effects were observed at the high dose (750 mg/kg), including increased muscle tremors and twitches, convulsions, hindlimb splaying, increased lacrimation and salivation, decreased pupil size and response to touch, gait abnormalities and decreased rectal temperature. None of these effects were observed either 8 or 15 days after dosing. This endpoint is appropriate for the acute dietary exposure assessment because it is the result of a single dose, is an appropriate route of exposure (oral) and is protective of the general population.

Acute Dietary Endpoint for Females 13-49 years of age: The endpoint used for establishing the aPAD for females 13-49 years of age was selected from the DNT study in rats. An aPAD of 0.06 mg/kg/day was derived from a developmental NOAEL of 1.8 mg/kg/day and a 30-fold factor that included 3x for inter-species extrapolation, 10x for intra-species variation, and 1x FQPA SF. The endpoint of concern is decreased neonatal survival in PND 0-4 offspring at the LOAEL of 7.1 mg/kg/day. Through a series of non-guideline developmental studies in the rat, the critical window of susceptibility for skeletal effects and neonatal deaths was determined to be gestation days (GD) 19-20. Therefore, this endpoint is appropriate because a single dose during

GD 19-20 can be adverse. Furthermore, it is of a relevant route of exposure (oral), and is protective for effects seen in the most sensitive subgroup (fetuses).

Chronic Dietary: The endpoint used for establishing the cPAD was selected from the chronic/carcinogenicity combined study in the rat. A cPAD of 0.05 mg/kg/day was derived from a NOAEL of 5.13 mg/kg/day and a 100-fold factor that included 10x for inter-species extrapolation, 10x for intra-species variability, and a 1x FQPA SF. The LOAEL of 21.3 mg/kg was based on liver effects including increased blood cholesterol, liver weight, hypertrophy, fatty change, single-cell necrosis and macrophages in males and females.

Dermal Absorption Factor: HED is using 2.4% as the factor for determining dermal-equivalent doses in the sulfoxaflor risk assessment based on a comparison of a rat *in vivo* and rat and human *in vitro* studies (see Section 4.2).

Inhalation Absorption Factor: HED is assuming an inhalation absorption factor of 100%.

Dermal and Inhalation (short- and intermediate-term): The endpoint used for short- and intermediate-term dermal exposure was selected from the DNT study in rats. The endpoint of concern is decreased neonatal survival in PND 0-4 offspring with a NOAEL of 1.8 mg/kg/day and a LOAEL of 7.1 mg/kg/day. A 30-fold uncertainty factor that includes a 3X factor for inter-species extrapolation and a 10x factor for intra-species variability will be retained for risk assessment. Although a route-specific dermal study was available for endpoint selection, the developmental endpoint was chosen from the DNT because it is protective of reproductive and developmental parameters not measured in the dermal study. As previously noted, in the case of sulfoxaflor, the use of an oral study is considered protective for assessing inhalation exposures.

4.5.2 Recommendation for Combining Routes of Exposures for Risk Assessment

The developmental neurotoxicity study was selected for evaluating oral exposure as well as short- and intermediate-term dermal and inhalation exposures. Therefore, exposure estimates from these routes should be combined when assessing short- and intermediate-term risks based on the decreased neonatal survival endpoint.

4.5.3 Cancer Classification and Risk Assessment Recommendation

The CARC in accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), determined that there is a “Suggestive Evidence of Carcinogenic Potential” for sulfoxaflor, based on the preputial gland tumor response seen in rats. When there is suggestive evidence, the Agency does not attempt a dose-response assessment as the nature of the data generally would not support one. Therefore, the Agency has determined that quantification of risk using a non-linear approach (i.e., reference dose (RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to sulfoxaflor. The CARC considered the following factors in their weight-of-evidence deliberation on assessing the carcinogenic potential of sulfoxaflor.

(i) The liver tumors observed in male rats and male and female mice were considered to be treatment-related;

(ii) The hypothesized mode of action (CAR mediated, mitogenic) for liver tumors was adequately supported by studies that clearly identified the sequence of key events, dose-response concordance and temporal relationship to the tumor type. There is convincing evidence that the hepatocarcinogenic effects are not likely to occur below a defined dose range. The mode of action data met the criteria established by the Agency;

(iii) The Leydig cell tumors were not considered to be treatment-related in male rats because there is a high background rate for this tumor type in the particular strain of rats (F344) tested;

(iv) There was evidence of a response for carcinomas of the preputial glands in male rats. Preputial gland tumors are not commonly diagnosed in bioassay studies. The available data were inadequate to draw confident conclusions regarding this response due to the small sample size and lack of histopathology data on all animals. Thus, it was not possible to determine whether the preputial gland tumors are due to treatment. If the response is positive, this would be an unusual finding. Because the tumors could not be discounted, they were used in the suggestive classification; and

(v) There is no mutagenicity concern from the in vivo or in vitro genetic toxicity assays.

4.5.4 Summary of Points of Departure and Toxicity Endpoints Used in Human Risk Assessment

Table 4.5.4.1 Summary of Toxicological Doses and Endpoints for Sulfoxaflor for Use in Dietary and Non-Occupational Human Health Risk Assessments.

Exposure/ Scenario	Point of Departure	Uncertainty/ FQPA Safety Factors	RfD & PAD for Risk Assessment	Study and Toxicological Effects
Acute Dietary (General Population, including Infants and Children)	NOAEL= 25 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF= 1x	RfD = 0.25 mg/kg/day aPAD =0.25 mg/kg/day	Acute Neurotoxicity Study LOAEL = 75 mg/kg/day based on decreased motor activity.
Acute Dietary (Females 13-49 years of age)	NOAEL = 1.8 mg/kg/day	UF_A= 3x UF_H=10x FQPA SF= 1x	RfD = 0.06 mg/kg/day aPAD =0.06 mg/kg/day	Developmental Neurotoxicity Study LOAEL = 7.1 mg/kg/day based on decreased neonatal survival (PND 0-4)
Chronic Dietary (All Populations)	NOAEL= 5.13 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF= 1x	RfD = 0.05 mg/kg/day cPAD = 0.05 mg/kg/day	Chronic/Carcinogenicity Study - Rat LOAEL = 21.3 mg/kg/day based on liver effects including increased blood cholesterol, liver weight, hypertrophy, fatty change, single cell necrosis and macrophages.
Cancer (oral, dermal, inhalation)	Sulfoxaflor is classified as "Suggestive Evidence of Carcinogenic Potential." Quantification of risk using a non-linear approach (i.e., reference dose (RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to sulfoxaflor.			

Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). FQPA SF = FQPA Safety Factor. PAD = population adjusted dose (a = acute, c = chronic). RfD = reference dose. MOE = margin of exposure. LOC = level of concern. N/A = not applicable.

Table 4.5.4.2 Summary of Toxicological Doses and Endpoints for Sulfoxaflor for Use in Occupational Human Health Risk Assessments.

Exposure/ Scenario	Point of Departure	Uncertainty Factors	Level of Concern for Risk Assessment	Study and Toxicological Effects
Dermal Short-and Intermediate Term	NOAEL= 1.8 mg/kg/day DAF: 2.4%	UF_A=3x UF_H=10x	Occupational LOC for MOE = 30	Developmental Neurotoxicity Study LOAEL = 7.1 mg/kg/day based on decreased neonatal survival (PND 0-4).
Inhalation Short- and Intermediate Term	NOAEL= 1.8 mg/kg/day*	UF_A=3x UF_H=10x	Occupational LOC for MOE = 30	Developmental Neurotoxicity Study LOAEL = 7.1 mg/kg/day based on decreased neonatal survival (PND 0-4).
Cancer (oral, dermal, inhalation)	Sulfoxaflor is classified as "Suggestive Evidence of Carcinogenic Potential." Quantification of risk using a non-linear approach (i.e., reference dose (RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to sulfoxaflor.			

* Inhalation absorption assumed equivalent to oral absorption.

Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). MOE = margin of exposure. LOC = level of concern. N/A = not applicable.

5.0 Dietary Exposure and Risk Assessment

5.1 Metabolite/Degradate Residue Profile

5.1.1 Summary of Plant and Animal Metabolism Studies

HED has reviewed studies depicting the metabolism of sulfoxaflor in plants, livestock, and rats. In plants, metabolism was investigated with lettuce, pea, rice, and tomato (with foliar and soil application for each). In addition, metabolism was studied in radish, lettuce, and wheat grown as rotational crops. Livestock metabolism studies were conducted with lactating goat and laying hen with both sulfoxaflor and the plant metabolite X11719474. All of the submitted studies meet guideline requirements and are considered to be acceptable.

In plants, metabolism of sulfoxaflor was similar in all four target crops. Oxidation of the cyano-carbon bond results in the formation of X11719474, followed by loss of the sulfur side chain to form X11721061. X117211061 is then conjugated with glucose, which may then be conjugated with malonic acid. Metabolism continues through incorporation of the radiolabeled carbon into natural plant constituents, such as lignin and starch. A very minor pathway (<1%) included degradation of the X11719474 urea side chain to form X1159540 and X11479457, which are further reduced to give X11721061. The primary difference noted between the foliar and soil metabolism studies was that parent sulfoxaflor was the predominant residue found from foliar applications, while X11719474 was the major residue found after application of sulfoxaflor to

the soil. Similarly, X11719474 was the predominant residue observed in the rotational crop studies.

Metabolism studies with goats, hens, and rats consistently showed that sulfoxaflor is rapidly absorbed and eliminated (chiefly via urine in mammals), and undergoes very little *in vivo* transformation. The results of studies in which X11719474 was administered to goats and hens mirrored the studies conducted with sulfoxaflor in that the administered dose was rapidly absorbed and eliminated, primarily as unmetabolized X11719474.

5.1.2 Summary of Environmental Degradation

The fate of sulfoxaflor in the environmental is highly dependent on whether or not it is in a soil system, groundwater system, or surface water system. Environmental fate studies show sulfoxaflor to be stable to photolysis (soil and aqueous) as well as hydrolysis across a broad pH range (estimated $t_{½}$ = 141 days in aerobic water, 637 days in anaerobic water). In soil systems, however, sulfoxaflor is rapidly degraded to X11719474 (estimated $t_{½}$ = 0.4 days). Figure 5.1.2 depicts the fate of sulfoxaflor in the environment. In terms of drinking water, the fate pathways and their kinetic properties result in sulfoxaflor being the expected predominant residue in surface water. Contrariwise, and despite its high mobility, sulfoxaflor is not expected in groundwater due to its rapid degradation to X11719474 and, to a much lesser extent, X11519540.

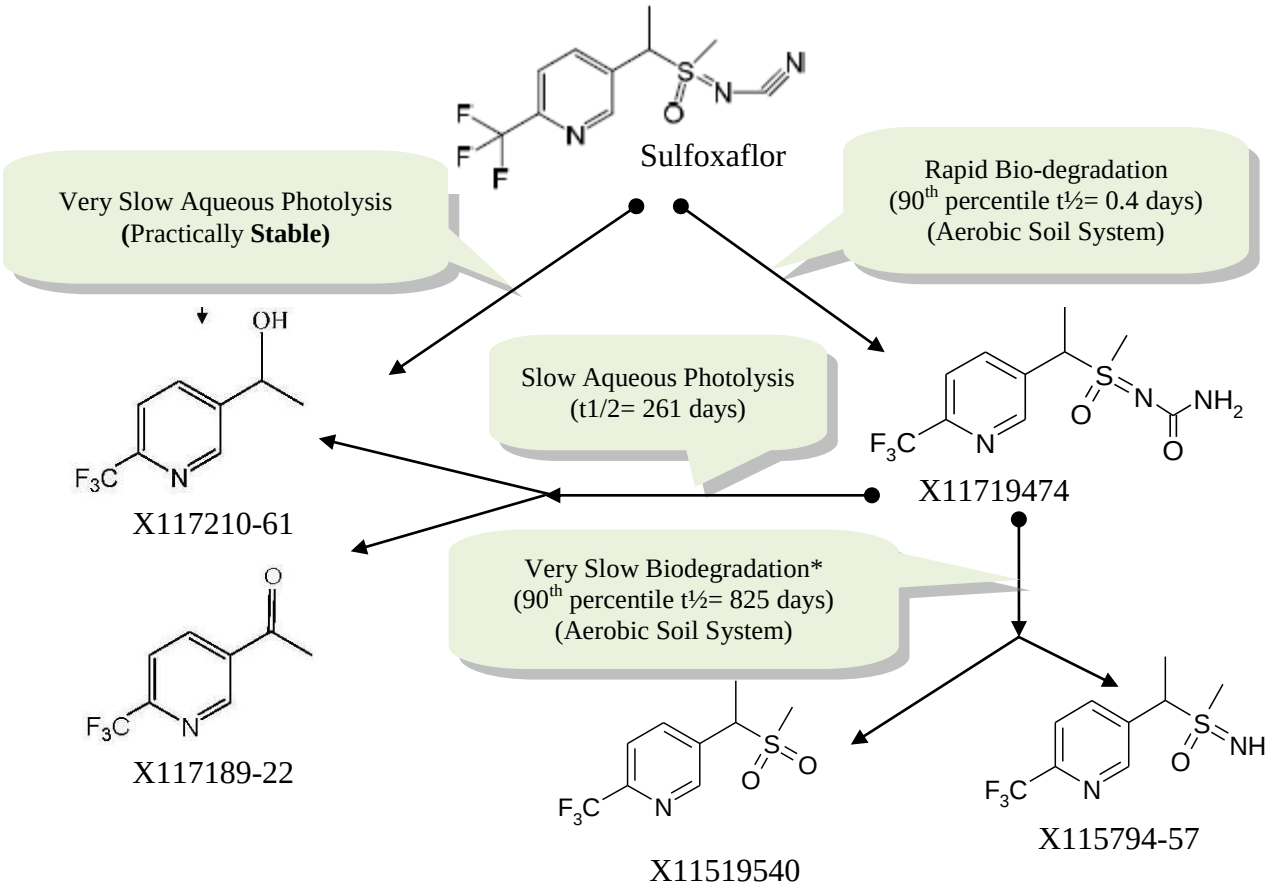


Figure 5.1.2. Expected environmental degradation pathways and transformation profiles for sulfoxaflor. Diagram taken from D382617, M. Ruhman, 1 August 2012.

5.1.3 Comparison of Metabolic Pathways

Although some metabolism was noted in studies with target crops (foliar application), livestock, and rats, sulfoxaflor does not generally show significant metabolism in those systems. Crops treated foliarly with sulfoxaflor tend to show more metabolism than the other two systems, but even then, there is little breakdown beyond oxidative loss of the cyano group and formation of X11719474. Environmental fate and rotational crop studies indicate that once sulfoxaflor is in contact with the soil, the parent compound is short lived and X11719474 predominates. X11719474 was not detected in livestock or rats.

5.1.4 Residues of Concern Summary and Rationale

E. Holman, 6 April 2012, D398294

HED's Residues of Concern Knowledgebase Subcommittee (ROCKS) has recommended the residues of concern summarized in Table 5.1.4 for sulfoxaflor. Although the global review partners concur with the recommended residue definition in terms of regulatory enforcement, risk assessment strategies differ in terms of metabolites of concern.

Table 5.1.4. Summary of Sulfoxaflor Residue Definitions for Risk Assessment and Tolerance Enforcement.			
Matrix		Risk Assessment	Tolerance Expression
Plants	Cotton	Sulfoxaflor	Sulfoxaflor
	Rotational Crop	Sulfoxaflor [1]	Sulfoxaflor
Livestock	Ruminant	Sulfoxaflor	Sulfoxaflor
	Poultry	Sulfoxaflor	Sulfoxaflor
Drinking Water		Sulfoxaflor + X11719474 + X11519540	NA

[1] Based on the proposed later season foliar application use pattern; decision may need to be revisited for future early season soil application uses.

Hazard Considerations

HED has determined that X11719474 does not share the reproductive MOA that was presented for sulfoxaflor (CARC Memo, TXR 0056301). However, exposure to X11719474 does results in hepatotoxicity comparable to that observed for sulfoxaflor. Based on several toxicology studies, the hepatotoxicity of X11719474 is 3- to 7-times less potent than sulfoxaflor. X11579457, a minor soil metabolite, exhibits no genotoxicity, and no acute oral toxicity. Structurally, it is quite similar to X11719474, and is therefore considered to be substantially less toxic than parent sulfoxaflor. The other primary plant metabolite, X11721061, is also considered to be significantly less toxic than the parent based on subchronic toxicity studies and is not mutagenic. Little toxicity data are available for X11596066, however, it has no structural alerts for genotoxicity, is negative in the Ames test, is structurally similar to X11721061, and therefore, is of low concern for human health. Contrary to the other metabolites, X11519540 appears to have a relative hepatotoxic potency that is greater than sulfoxaflor, as demonstrated in LD50 and 28-day studies in the rat.

Exposure Considerations

Water. Due to rapid degradation of sulfoxaflor to X11719474, for the purposes of drinking water modeling, initial X11719474 levels are expected to be essentially equivalent to the amount of parent that reaches the soil directly following application (not intercepted by foliage) and amounts washed-off foliage later. In the long term, there is potential for X11719474 to degrade to metabolites X11519540 and X11579457. Although not detected in U.S. aerobic soil metabolism studies, these metabolites were observed at higher levels in European studies. X11579457, a minor soil metabolite, is structurally quite similar to X11719474, which exhibits significantly less toxicity than parent sulfoxaflor. Therefore, X11579457 is excluded from the residue definition due to its decreased toxicity and low overall exposure levels. As previously noted, X11519540 appears to be more toxic than sulfoxaflor in terms of liver toxicity; therefore, despite the generally very low overall exposure to X11519540, this metabolite should be included, along with parent sulfoxaflor and X11719474, for the purposes of the drinking water risk assessment and modeling.

Plants (Primary Crops). The parent compound together with the two main plant metabolites, X11719474 and X11721061, are present in most of the primary crops tested in crop field trials. It is noted that all field trials were conducted at higher rates than those proposed for actual use, making the anticipated exposure levels to these metabolites even lower than recorded in the field studies. Due to the lower toxicity of X11719474 and X11721061, as well as generally lower overall exposure compared to parent sulfoxaflor, these metabolites are excluded from the residue

definition. The metabolite X11519540 was only observed at low concentrations (<1% of the TRR) in the lettuce metabolism study and was not observed at all in the other plant metabolism studies. Consequently, X11519540 is excluded from the residue definition due to low potential for exposure through plant matrices. Therefore, the inclusion of only parent sulfoxaflor should be adequate for dietary risk assessment. For tolerance enforcement, parent sulfoxaflor is also considered an adequate indicator of misuse. Although the parent may not be a good marker for misuse in crops like potatoes, the metabolites would not be much better indicators based on the field trial results showing that residues in root crops are likely to be very low (<0.04 ppm).

Rotational Crops. Although X11719474 is the predominant residue observed in rotational crops, assessing dietary risk based on exposure to parent only is appropriate. Field rotational crop studies demonstrate that the maximum amount of X11719474 taken up by succeeding crops is similar to the maximum amount present in primary crops after foliar treatments. As these summed levels of X11719474 are still low relative to the amount of parent sulfoxaflor in most primary crops, risk assessments based on parent sulfoxaflor will be protective for any X11719474 residues taken up by rotational crops. An exception to the preceding generalization regarding relative metabolite levels was noted for the root and tuber vegetables. However, for these crops, the maximum residue levels are low (<0.04 ppm). Thus, a separate residue of concern definition for rotational root and tuber vegetable crops was not considered necessary. Also, because there are few potential rotational crops that are not proposed for primary crop tolerances, concerns regarding rotational crop issues are low. Based on the above considerations combined with its substantially lower toxicity relative to sulfoxaflor, X11719474 is excluded from the residue definition. For tolerance enforcement, parent sulfoxaflor is considered an adequate indicator of misuse for the proposed application methods. Should there ever be soil directed applications of sulfoxaflor to primary crops, there is a potential for significant residues of X11719474 in rotated crops, requiring this decision to be revisited.

Livestock. Based on the available information, the inclusion of parent sulfoxaflor only should be sufficient for dietary risk assessment. With the exception of liver, the majority of the residue in milk, eggs, and edible tissues consisted of the parent compound, generally >80% of the residue. X11596066 was a major metabolite in ruminant and hen liver (14-18% TRR), but has been determined to have low overall toxicity; therefore, it is excluded from the residue definition.

X11519540 was detected at trace levels in most of the edible samples (<2% of the TRR in the ruminant and ≤ 8% of the TRR in the hens). The actual ppm levels of X11519540 were <0.01 ppm in all matrices. However, due to the potential for increased toxicity associated with X11519540, the sulfoxaflor team consulted with the HED Toxicology Science Advisory Council (ToxSAC) in order to adequately account for the relative potency of this metabolite. Following consultation with ToxSAC, the sulfoxaflor team has determined that it is appropriate to exclude residues of X11519540 in livestock commodities in the dietary risk assessment. It is noted that the draft 2011 JMPR decision excludes this metabolite from their residue definition due to the low overall observed concentrations. For tolerance enforcement, parent sulfoxaflor is considered an adequate indicator of misuse.

5.2 Food Residue Profile

D. McNeilly, 12 September 2012, D398756

The petitioner is requesting establishment of tolerances for residues of sulfoxaflor in/on 39 crops/crop groups. In support of that request, the petitioner has provided data from 583 field trials as well as supporting data related to metabolism, storage stability, analytical methods, processed commodities, residue transfer to livestock, and uptake in rotational crops. All of the studies are considered to be acceptable and adequate for evaluating regulatory levels and assessing risk.

Field trials were generally conducted in accordance with the proposed use directions; although application rates in the trials were somewhat exaggerated (1.4X) relative to the proposed labels. Generally, residues of sulfoxaflor were well above the limit of quantitation (LOQ) of 0.01 ppm in most commodities. The exception to that is in root crops where maximum residues were only slightly greater than the LOQ and rotational crops where residues were generally non-quantifiable. Studies indicate that residues are distributed throughout the crops and are not restricted to treated surfaces. Sulfoxaflor was stable in studies designed to mimic boiling, cooking, and pasteurization. Typical food processing activities, such as baking, boiling and canning, are unlikely to significantly affect the nature and magnitude of sulfoxaflor residues. In commodity-specific processing studies, sulfoxaflor generally concentrated during production of dried foods and tended to be reduced in juices, though exceptions to those trends are evident in the database. A summary of the field trial and processing study results are provided in Appendix D.

The request for registration of sulfoxaflor includes uses on a large number of commodities that are considered to be significant feed items. To account for transfer of sulfoxaflor residues from livestock feeds into livestock commodities, HED has derived maximum, reasonably balanced dietary burdens for beef cattle, dairy cattle, poultry, and swine and combined those estimates with data from dairy cattle and laying hen feeding studies in order to calculate anticipated residues in livestock commodities. For poultry and swine, quantifiable residues are not expected in any commodity. Nevertheless, HED is recommending for tolerances at the LOQ (0.01 ppm) for harmonization purposes. Quantifiable residues are more likely in commodities, including milk, derived from cattle, goats, horses, and sheep, and HED is recommending for tolerances ranging from 0.10 to 0.40 ppm in those commodities.

5.3 Water Residue Profile

M. Ruhman, 1 August 2012, D382617

Based on abiotic laboratory studies, sulfoxaflor is stable to photolysis on soil and to hydrolysis in acidic, neutral and alkaline systems. Aqueous photolysis is expected to be slow and may not be considered an important transformation route in the natural environment ($t_{½}$ >1,000 days in de-ionized water and 637 days in natural water). In the aerobic soil system, sulfoxaflor degrades rather quickly to its major degradate X11719474 (90^{th} percentile $t_{½}$= 0.4 days). Other residues, forming as a result of aerobic soil biotransformation, include limited amounts of X-1157957 and X11519540. Anaerobic soil data suggest that anaerobic conditions produces the main degradate, X11719474. In contrast to the rapid degradation of sulfoxaflor in aerobic soil systems, the

chemical degrades rather slowly to X11719474 in both aerobic and anaerobic aquatic conditions (90^{th} percentile t½= 141 days).

Sulfoxaflor is considered to be non-persistent in soil and exhibits low affinity to soil/sediment particles. The chemical is not expected to partition significantly into the air from wet or dry soils. Sources of surface water contamination include drift for parent and run-off for X11719474 and X11519540 following the rapid degradation of parent in the soil. Parent compound that drifts into surface water is expected to degrade rather slowly to X11719474. In contrast, the source of ground water contamination is expected to be leaching of parent and/or its degradation products X11719474 and X11519540, all of which are considered to be highly mobile.

Monitoring data depicting residues of sulfoxaflor in drinking water are not available. The Environmental Fate and Effects Division has used modeling to estimate upper-bound concentrations of sulfoxaflor in surface and ground water. Two scenarios were modeled: (1) Use of sulfoxaflor on non-aquatic row and orchard crops and (2) use of sulfoxaflor on watercress. For the non-aquatic crops, screening-level estimated drinking water concentrations (EDWCs) were calculated using Tier II PRZM (Pesticide Root Zone Model) and EXAMS (Exposure Analysis Modeling System) for surface water sources and the Tier I SCI-GROW (Screening Concentration in Ground Water) model was used for calculating the EDWCs in ground water sources. Of the requested non-aquatic-crop uses, those on citrus resulted in the highest EDWCs and are presented in Table 5.3.1a.

Table 5.3.1a. Estimated Drinking Water Concentrations (EDWCs) for Total Toxic Residues of Sulfoxaflor in Surface and Ground Waters from Use on Citrus.

Water Source*	Total Concentration, ppb [Sulfoxaflor + X11719474 + X11519540]		
	Acute Peak [1]	Non-cancer/Chronic [2]	Cancer [3]
Surface (PRZM/EXAMS)	26.4 [9.4 + 15.0 + 2.0]	13.5 [1.9 + 10.2 + 1.4]	9.3 [1.5 + 6.9 + 0.9]
Ground (SCI-GROW)	69.2 [0.0 + 60.9 + 8.3]		

* Surface water EDWCs include sulfoxaflor + X11719474 + X11519540. Due to rapid degradation of sulfoxaflor in aerobic soil systems (half-life = 0.4 days), the EDWC for groundwater includes X11719474 + X11519540.

[1] Peak value, from Florida citrus; [2] 1-in-10-year annual mean values, California citrus; [3] 1-in-10-year overall annual mean values, California citrus.

The Tier I rice model was used to obtain surface water EDWC from the use of sulfoxaflor on watercress. The results of those analyses are presented in Table 5.3.1b and include the accumulation of residues that is expected from multiple applications.

Table 5.3.1b. Estimated Drinking Water Concentrations (EDWCs) for Total Toxic Residues of Sulfoxaflor in Surface and Ground Waters from Use on Watercress.

Water Source*	Total Concentration, ppb [Sulfoxaflor + X11719474]		
	One Application	Two Applications	Three Applications
Surface (Tier 1 Rice Model)	91.3 [26.5 + 64.8]	182.5 [52.9 + 129.6]	273.8 [79.4 + 194.4]

* Aquatic-use EDWCs include sulfoxaflor + X11719474.

The values in Tables 5.3.1a and 5.3.1b represent upper-bound estimates of residue concentrations that might be found in drinking water due to the use of sulfoxaflor on non-aquatic crops and watercress, respectively.

Risk Assessment Considerations

Although the EDWCs associated with the use of sulfoxaflor on watercress are higher than those for the non-aquatic crops, HED's risk assessment is focusing on the non-aquatic-crop EDWCs. The 2007 census of agriculture estimates that approximately 680 acres of the U.S. are used for watercress production; thus, this use represents a very small fraction of the potential crop acreage that may be treated with sulfoxaflor. Moreover, the inputs to the Tier 1 rice model are quite conservative, especially with regard to application efficiency (the model assumes that there is no interception of the applied material by the watercress plants) and the 10-cm water column at the time of application (information from watercress growers indicates that watercress fields are drained prior to pesticide applications). Finally, the rice model predicts pesticide concentrations in water in the field and not drinking water *per se* where concentrations are expected to be lower due to dissipation processes such as degredation, stream flow, and dilution. While the use on watercress may impact drinking water for a few individuals, HED does not believe that the EDWCs and residue profiles associated with the watercress use give a representative depiction of the potential exposure profile for the vast majority of the U.S. population. HED has quantitatively estimated risks based on the watercress EDCWs and found the risk estimates to be below our level of concern (D. McNeilly, D401670, 12 September 2012). We note that if a future use for sulfoxaflor is requested on rice or cranberry, the EDWCs of primary interest would likely be those coming from the rice model.

Based on the modeled estimates associated with non-aquatic crops, the potential exposure through drinking water is much higher from groundwater than from surface water. Due to the different relative toxicities of the X11719474 and X11519540 metabolites, HED requested that EFED provide estimates for each compound separately. Environmental fate data indicate that the residue in groundwater will be comprised, predominantly, of the X11719474 metabolite. Quantitatively, the 69.2 ppb EDWC for groundwater can be divided into 60.9 ppb from X11719474 and 8.3 ppb from X11519450. HED has used these estimates and the relative toxicities of the metabolites to adjust the drinking water component of the dietary risk assessment, as outlined below. As noted earlier, sulfoxaflor presents a somewhat unique situation regarding the food and drinking water exposure pathway. Environmental fate data indicate that use of sulfoxaflor is likely to result in different residue profiles in surface water and groundwater, namely that sulfoxaflor is likely to be the predominant residue in surface water and X11719747/X11519540 will predominate in groundwater. When the residue profiles are coupled with the toxicological database, it becomes apparent that the EDWCs for assessing acute dietary exposure for the general population, acute dietary exposure for women of child-bearing age, and chronic dietary exposure for all populations need to be addressed differently. An explanation of the three scenarios and the rationale for the approaches taken by HED is provided below. The EDWCs used in the various dietary assessments are summarized in Tables 5.3.2a and 5.3.2b.

Acute Exposure

Separate acute endpoints were selected for the general population and females 13-49 years of age. For the general population, the point of departure is based on decreased motor activity observed in the acute neurotoxicity study. As there are no data available to examine the potency of X11719474 and X11519540 with respect to this endpoint, HED has assumed that the two metabolites possess similar toxicity relative to sulfoxaflor in order to assess acute dietary risk for the general population. The EDWC for groundwater is significantly greater than the acute estimate for surface water and, per HED policy, is being used in the acute dietary assessment for the general population. As it is a groundwater EDWC, it represents residues of the metabolites.

For females 13-49 years of age, the developmental endpoint of increased neonatal deaths was chosen because a single exposure during late gestation can adversely affect the developing fetus and the age group represents women of child-bearing age. As discussed in Section 4.3.4, the metabolites X11719474 and X1159540 are not likely to result in agonism of the muscle nicotinic receptor and, therefore, should be excluded from assessment scenarios using the developmental endpoint. Since the groundwater EDWC represents residues of these metabolites only, the acute surface water EDWC becomes the appropriate estimate for assessing dietary exposure for women of child-bearing age.

Chronic Exposure

The endpoint for assessing chronic dietary exposure is hepatotoxicity. As discussed in Section 4.3.4, HED has determined that it is appropriate to combine residues of sulfoxaflor, X11719474, and X11519540 when assessing chronic exposure and, furthermore, there is sufficient evidence to adjust the assessment to account for the different potencies of the metabolites. Based on NOAELs in the 28-day oral toxicity studies in rats, the potencies of the metabolites, relative to sulfoxaflor, are 0.3X for X11719474 and 10X for X11519540. To account for the relative toxicity, the EDWCs for each metabolite are multiplied by their respective potency factor.

Table 5.3.2a. Adjusted Estimated Drinking Water Concentrations (EDWCs) from Use on Citrus for Estimating Dietary Risk.					
Drinking Water Scenario	Water Source	Toxic Residue, ppm	Adjustment Factor	Adjusted Residue, ppm	Rationale
Acute – General Population	Groundwater	Sulfoxaflor ≈ 0	1	≈ 0	EDWC: Groundwater > Surface Water Endpoint is decreased motor activity. No information is available to rule out applicability or estimate relative toxicity.
		X11719474 = 0.0609	1	0.0609	
		X11519540 = 0.0083	1	0.0083	
			Total	0.0692	
Acute – Females 13-49 years old	Surface water	Sulfoxaflor = 0.0094	1	0.0094	Residues in groundwater are not appropriate for assessing the endpoint.
Chronic – All Populations	Groundwater	Sulfoxaflor ≈ 0	1	≈ 0	EDWC: Groundwater > Surface Water Endpoint is liver effects. 28-day studies are available for sulfoxaflor, X11719474, and X11519540 to derive relative toxicities.
		X11719474 = 0.0609	0.3	0.0183	
		X11519540 = 0.0083	10	0.083	
			Total	0.1013	

Table 5.3.2b. Adjusted Estimated Drinking Water Concentrations (EDWCs) from Use on Watercress for Estimating Dietary Risk.					
Drinking Water Scenario	Water Source	Toxic Residue, ppm	Adjustment Factor	Adjusted Residue, ppm	Rationale
Acute – General Population	Groundwater	Sulfoxaflor = 0.0794	1	0.0794	EDWC: Groundwater > Surface Water Endpoint is decreased motor activity. No information is available to rule out applicability or estimate relative toxicity.
		X11719474 = 0.1944	1	0.1944	
		X11519540 = 0.0	1	0.0	
			Total	0.2738	
Acute – Females 13-49 years old	Surface water	Sulfoxaflor = 0.0794	1	0.0794	Residues in groundwater are not appropriate for assessing the endpoint.
Chronic – All Populations	Groundwater	Sulfoxaflor = 0.0794	1	0.0794	EDWC: Groundwater > Surface Water Endpoint is liver effects. 28-day studies are available for sulfoxaflor, X11719474, and X11519540 to derive relative toxicities.
		X11719474 = 0.1944	0.3	0.0583	
		X11519540 = 0.0	10	0.0	
			Total	0.1377	

5.4 Dietary Risk Assessment

D. McNeilly, 12 September 2012, D401670

Acute and chronic aggregate dietary (food and drinking water) exposure and risk assessments were conducted using the Dietary Exposure Evaluation Model DEEM-FCID™ (v. 2.03). This model uses food consumption data from the U.S. Department of Agriculture's (USDA's) Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998. Based on analysis of the 1994-96, 98 CSFII consumption data, which took into account dietary patterns and survey respondents, HED concluded that it is most appropriate to report risk for the following population subgroups: the general U.S. population, all infants (<1 year old), children 1-2, children 3-5, children 6-12, youth 13-19, adults 20-49, females 13-49, and adults 50+ years old.

5.4.1 Description of Residue Estimates Used in Dietary Assessment

The data used in the acute assessment reflect several refinements relative to a screening-level assessment. Maximum residue values from field trials were used rather than tolerance-level residue estimates. For crop groups, the residue values were translated from representative crops to the other crops in the group using HED SOP 2000.1. For processed commodities, empirical processing factors were used for all commodities unless an empirical factor was not available, in which case the DEEM default estimate was used. Residue estimates for livestock were derived using maximum observed residues in the cattle and hen feeding studies.

For the chronic assessment, the same refinements were made as those described for the acute assessment, with two exceptions: (1) average residue levels from crop field trials were used rather than maximum values and (2) average residues from feeding studies, rather than maximum values, were used to derive residue estimates for livestock commodities.

Estimates of residues in drinking water were incorporated directly into the assessments. As previously discussed (Section 5.3), HED is focusing on the dietary risk estimates that incorporate the non-aquatic-crop EDWCs rather than the watercress EDWCs. Nevertheless, HED has estimated dietary risks using the watercress EDWCs and all estimates are below HED's level of concern.

5.4.2 Percent Crop Treated Used in Dietary Assessment

HED has assumed 100% of crops covered by the registration request are treated with sulfoxaflor.

5.4.3 Acute Dietary Risk Assessment

Acute dietary exposure was estimated for all of the standard, representative population groups described above. As previously noted, the assessment for women of child-bearing age (females 13-49 years old) is based on a different endpoint/point of departure and includes a different set of assumptions regarding residues in drinking water than were used for the other groups. Given the assumptions used in conducting the acute assessment, exposure and risk estimates (Table 5.4.6) should be considered moderately refined. The use of the highest residue values from field trials give some refinement in comparison to use of tolerance-level residues. However, crop field

trials are designed to result in worst-case residue levels and the assumption that all crops with proposed uses of sulfoxaflor are treated (100% crop treated) and that they bear residues at maximum levels is likely to overestimate actual acute exposure. Significant refinement to the dietary exposure estimates could likely be obtained by using the full distribution of field trial data in a probabilistic assessment.

Acute dietary risk estimates range from 4% to 16% of the acute population-adjusted dose (aPAD), with the highest risk estimates being for children 1-2 years old and females 13-49 years old. Generally, HED is concerned when exposure estimates exceed 100% of the population-adjusted dose (PAD). Even with the conservatisms in the assessment, acute dietary risk estimates are below HED's level of concern.

5.4.4 Chronic Dietary Risk Assessment

Chronic dietary exposure was estimated for all of the standard, representative population groups described in Section 5.4 above. The chronic dietary exposure estimates reflect significant refinement with respect to residue levels, although overall, the use of field trial data and the assumption of 100% crop treated results in estimates that are moderately refined. Monitoring data, if available, and incorporation of use statistics (i.e., % crop treated) have the potential to provide significant refinements to the assessment.

Chronic dietary risk estimates range from 5% to 18% of the chronic population-adjusted dose (cPAD). The highest risk estimate is for infants and all of the risk estimates are below HED's level of concern.

5.4.5 Cancer Dietary Risk Assessment

Sulfoxaflor is classified as having "suggestive evidence of carcinogenicity" and HED has concluded that assessments using a non-linear approach (e.g., a chronic RfD-based assessment) will adequately account for all chronic toxicity, including carcinogenicity that could result from exposure to sulfoxaflor. Chronic dietary risk estimates are below HED's level of concern; therefore, cancer risk is also below HED's level of concern.

5.4.6 Summary Table

Table 5.4.6. Summary of Acute and Chronic Dietary Exposure and Risk Estimates for Sulfoxaflor.

	Acute (95th Percentile)		Chronic		Cancer	
Population Subgroup	Estimated Exposure (mg/kg/day)	Estimated Risk, % aPAD	Estimated Exposure (mg/kg/day)	Estimated Risk, % cPAD	Exposure (mg/kg/day)	Estimated Risk
U.S. Population	0.015832	6	0.003343	7	See Chronic	
All infants	0.031158	12	**0.009110**	**18**		
Children 1-2 yrs	**0.044887**	**16**	0.007867	16		
Children 3-5 yrs	0.032523	13	0.006210	12		
Children 6-12 yrs	0.018338	7	0.003892	8		
Youth 13-19 yrs	0.010829	4	0.002456	5		
Adults 20-49 yrs	0.011137	4	0.002812	6		
Adults 50+ yrs	0.011446	5	0.002992	6		

Table 5.4.6. Summary of Acute and Chronic Dietary Exposure and Risk Estimates for Sulfoxaflor.						
	Acute (95th Percentile)		Chronic		Cancer	
Population Subgroup	Estimated Exposure (mg/kg/day)	Estimated Risk, % aPAD	Estimated Exposure (mg/kg/day)	Estimated Risk, % cPAD	Exposure (mg/kg/day)	Estimated Risk
Females 13-49 yrs	**0.009495**	**16**	0.002809	6		

Entries with the highest acute and chronic risk estimates are **bolded**.

6.0 Residential (Non-Occupational) Exposure/Risk Characterization

No residential uses, and no commercial/professional uses at residential sites are being requested for sulfoxaflor at this time; therefore, no residential risk assessment has been conducted. There are proposed uses on ornamental plants and turfgrass, but those uses are for nurseries/commercial greenhouses and for sod farms, respectively. For sulfoxaflor, HED believes that the post-application exposure for homeowners buying and planting ornamental plants or turfgrass (sod) would be minimal based on the exposure data available, the time between application and purchase, and the watering-in of turf. At this time, there are no uses of sulfoxaflor that will result in residential exposures.

6.1 Residential Bystander Post-Application Inhalation Exposure

Based on the Agency's current practices, a quantitative post-application inhalation exposure assessment was not performed for sulfoxaflor at this time primarily because of the acute inhalation toxicity (Toxicity Category III and IV), low vapor pressure ($\leq 1.4\times10^{-6}$ Pa = 1.05×10^{-8} mm Hg at 20°C), and the maximum seasonal proposed use rate (0.266 lb ai/A/season). However, volatilization of pesticides may be a source of post-application inhalation exposure to individuals nearby pesticide applications. The Agency sought expert advice and input on issues related to volatilization of pesticides from its Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) in December 2009, and received the SAP's final report on March 2, 2010 (http://www.epa.gov/scipoly/sap/meetings/2009/120109meeting.html). The Agency is in the process of evaluating the SAP report and may, as appropriate, develop policies and procedures to identify the need for and, subsequently, the way to incorporate post-application inhalation exposure into the Agency's risk assessments. If new policies or procedures are developed, the Agency may revisit the need for a quantitative post-application inhalation exposure assessment for sulfoxaflor.

6.2 Spray Drift

Spray drift is always a potential source of exposure to residents nearby to spraying operations. This is particularly the case with aerial application, but, to a lesser extent, could also be a potential source of exposure from the ground application method employed for sulfoxaflor. The Agency has been working with the Spray Drift Task Force, EPA Regional Offices and State Lead Agencies for pesticide regulation and other parties to develop the best spray drift management practices (see the Agency's Spray Drift website for more information at http://www.epa.gov/opp00001/factsheets/spraydrift.htm). On a chemical by chemical basis, the Agency is now requiring interim mitigation measures for aerial applications that must be placed on product labels/labeling. The Agency has completed its evaluation of the new database

submitted by the Spray Drift Task Force, a membership of U.S. pesticide registrants, and is developing a policy on how to appropriately apply the data and the AgDRIFT computer model to its risk assessments for pesticides applied by air, orchard airblast and ground hydraulic methods. After the policy is in place, the Agency may impose further refinements in spray drift management practices to reduce off-target drift with specific products with significant risks associated with drift.

Although a quantitative residential post-application inhalation exposure assessment was not performed as a result of pesticide drift from neighboring treated agricultural fields, an inhalation exposure assessment was performed for flaggers. This exposure scenario is representative of a worse case inhalation (drift) exposure and may be considered protective of most outdoor agricultural and commercial post-application inhalation exposure scenarios. The risk estimate for flaggers (MOE ≥ 4,200, Table 9.1) is not of concern.

7.0 Aggregate Exposure/Risk Characterization

As there are no registered or requested residential uses of sulfoxaflor, aggregate exposure is equivalent to the dietary exposure discussed in Section 5. Therefore aggregate risk estimates are below HED's level of concern.

8.0 Cumulative Exposure/Risk Characterization

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to sulfoxaflor and any other substances and sulfoxaflor does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that sulfoxaflor has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at: http://www.epa.gov/pesticides/cumulative/.

9.0 Occupational Exposure/Risk Characterization

Z. Figueroa, 18 September 2012, D382606

Two agricultural products are proposed for use on the various crops, turf grass and ornamentals: GF-2032 SC and Transform ™ WG.

GF-2032 SC is formulated as a suspension concentrate (SC) which contains 21.8% of the active ingredient, sulfoxaflor. The Personal Protective Equipment (PPE) statement on the proposed label requires applicators and other handlers to wear: long-sleeved shirt and long pants, and shoes plus socks.

Transform ™ WG is formulated as a water dispersible granule (WG) which contains 50% of the active ingredient, sulfoxaflor. The Personal Protective Equipment (PPE) statement on the proposed label adds protective eyewear to the PPE found on the GF-2032 SC label (i.e., long-

sleeved shirt and long pants, shoes plus socks, and protective eyewear) due to the Category II finding in the acute ocular toxicity testing with Transform ™ WG.

These end use products may be applied through a variety of application methods including: aerial, chemigation (for potatoes only), groundboom, airblast, backpack, and hand held equipment. There is a potential for occupational exposure associated with handler activities (i.e., mixing, loading, and applying) as well as with post-application activities (i.e., re-entering treated areas). No data on the number of exposure days per year was provided. Based on the proposed labels' use pattern and directions (e.g., 2-4 applications per season with a minimum 5- to 14- day spray interval), only short- (1 to 30 days) and intermediate-term (1 to 6 months) exposures are expected for handlers.

In the past HED has considered ornamentals and greenhouse uses to have the potential for long-term dermal exposure. However, the pests that are targets for sulfoxaflor have biological and life-stage properties that provide for short windows of opportunity for control. To avoid development of sulfoxaflor-resistant pests, the sulfoxaflor labels require switching periodically to another insecticide. Therefore, based on the proposed use pattern and resistance management strategies (i.e., avoid consecutive applications of insecticides with the same mode of action), long-term exposure is not expected to occur.

9.1 Short- and Intermediate-Term Handler Risk Estimates

HED uses the term handlers to describe those individuals who are involved in the pesticide application process. HED believes that there are distinct job functions or tasks related to applications, and that exposures can vary depending on the specifics of each task. Job requirements (e.g., amount of chemical used in each application), types of equipment used, the area treated or amount handled, and the level of protection used by a handler can cause exposure levels to differ in a manner specific to each application event. Detailed descriptions of the handler assessment are available in D382606 (Z. Figueroa, 18 September 2012) and a summary table of the handler exposure and risk estimates is provided in Appendix F. The occupational hander exposure and risk estimates rely on generic data from the Pesticide Handlers Exposure Database Version 1.1 (PHED 1.1), the Outdoor Residential Exposure Task Force (ORETF) database and the Agricultural Handler Exposure Task Force (AHETF) database.

All combined (dermal + inhalation) occupational handler scenarios for the proposed uses resulted in estimated MOEs that are greater than 30 (i.e., not of concern) at the baseline level of PPE (i.e., baseline clothing, no gloves, and no respirator) and engineering controls (enclosed cockpit) for aerial applications (Table 9.1). The highest risk estimate (MOE = 80) is associated with handlers conducting mixer/loader activities with dry flowable formulation (Transform™ WG) for aerial applications.

HED has no data to assess exposures to pilots using open cockpits. The only available data are for exposure to pilots in enclosed cockpits. Therefore, risks to pilots are assessed using the engineering control (enclosed cockpits) and baseline attire (long-sleeve shirt, long pants, shoes, and socks); pilots are not required to wear protective gloves. With this level of protection, there are no risks of concern for applicators.

Table 9.1. Summary of Occupational Handler Exposure and Risk Estimates for Sulfoxaflor.*								
Activity	Scenario	Use Site	Formulation	Dermal		Inhalation		
				Dose (mg/kg/day)	MOE (LOC=30)	Dose (mg/kg/day)	MOE (LOC=30)	Combined MOE (LOC=30)
Mixer/Loader	Groundboom Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	Transform ™ WG	0.00057	3,200	0.00089	2,000	1,200
			GF-2032 SC	0.00057	3,100	0.000023	79,000	3,000
		Ornamentals, Turf grass (Sod Farm)	Transform ™ WG	0.00088	2,100	0.00140	1,300	800
			GF-2032 SC	0.00085	2,100	0.000034	53,000	2,000
		Barley, Tricale, Wheat, Canola (Sub CG 20A)	Transform ™ WG	0.00077	2,300	0.00120	1,500	900
			GF-2032 SC	0.00069	2,600	0.000027	66,000	2,500
		Cotton, Soybean	Transform ™ WG	0.0014	1,300	0.00220	810	490
			GF-2032 SC	0.00140	1,300	0.000057	32,000	1,200
	Aerial Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	Transform ™ WG	0.00250	730	0.00390	460	280
			GF-2032 SC	0.00250	720	0.00010	18,000	690
		Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	Transform ™ WG	0.00380	470	0.00600	300	180
			GF-2032 SC	0.00370	490	0.00015	12,000	470
		Barley, Tricale, Wheat, Canola (Sub CG 20A)	Transform ™ WG	0.00460	390	0.00730	250	150
			GF-2032 SC	0.00410	440	0.00016	11,000	420
		Cotton, Soybean	Transform ™ WG	0.00850	210	0.01340	130	80
			GF-2032 SC	0.00860	210	0.00034	5,300	200
	Chemigation (AHETF)	Potatoes	Transform ™ WG	0.00250	730	0.00390	460	280
			GF-2032 SC	0.00250	720	0.00100	18,000	690

Table 9.1. Summary of Occupational Handler Exposure and Risk Estimates for Sulfoxaflor.*								
Activity	Scenario	Use Site	Formulation	Dermal		Inhalation		
				Dose (mg/kg/day)	MOE (LOC=30)	Dose (mg/kg/day)	MOE (LOC=30)	Combined MOE (LOC=30)
	Airblast Application (AHETF)	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	Transform ™ WG	0.00044	4,100	0.00069	2,600	1,600
			GF-2032 SC	0.00042	4,200	0.000017	110,000	4,100
Applicator	Groundboom Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Dry Podded, Beans	Transform ™ WG	0.00020	9,200	0.000034	53,000	7,800
			GF-2032 SC	0.00021	8,800	0.000035	51,000	7,500
		Ornamentals, Turf grass (Sod Farm)	Transform ™ WG	0.00030	5,900	0.000052	34,000	5,100
			GF-2032 SC	0.00030	5,900	0.000052	34,000	5,100
		Barley, Tricale, Wheat, Canola (Sub CG 20A)	Transform ™ WG	0.00027	6,700	0.000046	39,000	5,700
			GF-2032 SC	0.00024	7,300	0.000042	42,000	6,300
		Cotton, Soybean	Transform ™ WG	0.00049	3,700	0.000085	21,000	3,100
			GF-2032 SC	0.00051	3,500	0.000089	20,000	3,000
	Aerial Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Dry Podded, Beans	Transform ™ WG	0.000055	33,000	0.000030	61,000	21,000
			GF-2032 SC	0.000057	32,000	0.000031	58,000	25,000
		Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	Transform ™ WG	0.000084	21,000	0.000046	39,000	14,000
			GF-2032 SC	0.000084	21,000	0.000046	39,000	19,000
		Barley, Tricale, Wheat, Canola (Sub CG 20A)	Transform ™ WG	0.00010	18,000	0.000056	32,000	11,000
			GF-2032 SC	0.000093	19,000	0.000051	35,000	12,000
		Cotton, Soybean	Transform ™ WG	0.00019	9,600	0.0001	18,000	6,200
			GF-2032 SC	0.00020	9,200	0.00011	17,000	6,000

Table 9.1. Summary of Occupational Handler Exposure and Risk Estimates for Sulfoxaflor.*								
Activity	Scenario	Use Site	Formulation	Dermal		Inhalation		
				Dose (mg/kg/day)	MOE (LOC=30)	Dose (mg/kg/day)	MOE (LOC=30)	Combined MOE (LOC=30)
	Airblast Application (AHETF)	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	Transform ™ WG	0.00340	530	0.00036	5,000	480
			GF-2032 SC	0.00340	530	0.00036	5,000	480
Flagger	Flagging for Aerial Application (PHED)	All Low Acreage Crops	Transform ™ WG	0.00019	9,700	0.00024	7,600	4,200
			GF-2032 SC	0.00019	9,700	0.00024	7,600	4,200
		All High Acreage Crops	Transform ™ WG	0.00012	15,000	0.00015	12,000	6,700
			GF-2032 SC	0.00013	14,000	0.00016	11,000	6,300
Mixer/ Loader/ Applicator	Mechanically-pressurized Handgun Sprayer	Ornamentals, Greenhouses	Transform ™ WG	0.00169	1,100	0.00231	780	450
			GF-2032 SC	0.00169	1,100	0.00231	780	450
		Turfgrass (Sod farms)	Transform ™ WG	0.00047	3,800	0.00040	4,400	2,100
			GF-2032 SC	0.00047	3,800	0.00040	4,400	2,100
	Backpack Sprayer	Ornamentals	Transform ™ WG	0.00025	7,200	0.00011	16,000	5,000
			GF-2032 SC	0.00025	7,200	0.00011	16,000	5,000
		Turfgrass (Sod farms)	Transform ™ WG	0.00016	11,000	0.000002	900,000	11,000
			GF-2032 SC	0.00016	11,000	0.000002	900,000	11,000
	Manually-pressurized Handwand (PHED)	Ornamentals, Turfgrass (Sod farms)	Transform ™ WG	0.00193	930	0.000023	78,000	920
			GF-2032 SC	0.00193	930	0.000023	78,000	920

* From Z. Figueroa, 18 September 2012, D382606

9.2 Short- and Intermediate- Term Post-Application Risk Estimates

9.2.1 Dermal Post-Application Risk Estimates

Occupational workers who enter treated fields to perform post-application activities such as hand weeding and scouting may be exposed to sulfoxaflor residues. Based on the use pattern, workers may be exposed to short- and intermediate-term exposure durations.

Dislodgeable Foliar Residue Data: It is HED's policy to use the best available data to assess post-application exposure. The registrant submitted dislodgeable foliar residue (DFR) studies for wheat and broccoli in support of the proposed agricultural uses. Both studies demonstrated that dislodgeable foliar residue values declined over time for all sites and both formulations. The available DFR data were matched to the proposed agricultural crops (except cotton; see below) as surrogate data based on available information about foliage type, region of use, use pattern, etc. Dermal post-application exposure resulting from application of sulfoxaflor was estimated using the best available surrogate DFR-predicted Day-0 residue value; Day-0 DFR values were adjusted by application rate. Maximum single application rates for each crop/crop groups were used in this assessment.

Dislodgeable residue data were not submitted for cotton. Calculation of dermal post-application exposure resulting from application of sulfoxaflor to cotton during harvesting activities (e.g., module builder, picker operator, raker, tramper) requires an estimate of the "dislodgeable" residue on the cotton bolls. Since that information was unavailable, exposure was estimated using cotton field trial residues (see Appendix D). Additionally, since the proposed PHI for cotton is 14 days, the Day-14 residue value from the cotton field trials is considered adequate to estimate dermal post-application exposures and risks during harvesting activities. Adjusted to the proposed maximum seasonal application rate, the Day-14 residue is 0.000129 mg ai/g cotton. Note also that the cotton field trial residue data reflect solvent-extractable residues, which are likely to be significantly greater than dislodgeable cotton boll residue during harvesting activities. Therefore, use of the residue data from the cotton field trials, even after being reduced to account for the difference in application rate, results in a conservative estimate for the daily dose.

Turf transferable residue (TTR) data were not submitted for turfgrass (sod farms); therefore, default TTR values were used to assess dermal post-application exposure and risk.

Transfer Coefficients: Sources of generic post-application exposure data, used as surrogate data in the absence of chemical-specific data, are derived from Agricultural Reentry Task Force (ARTF) exposure monitoring studies, and, as proprietary data, are subject to the data protection provisions of FIFRA. The standard values recommended for use in predicting post-application exposure that are used in this assessment, known as "transfer coefficients, Tc" are presented in the "Science Advisory Council for Exposure (ExpoSAC) Policy 3" (http://www.epa.gov/pesticides/science/exposac_policy3.pdf), which, along with additional information about the ARTF data, can be found at: http://www.epa.gov/pesticides/science/post-app-exposure-data.html.

Dermal exposures during post-application activities were estimated using standard default and surrogate contact activity values (see Appendix G), chemical-specific DFR for all agricultural crops except cotton, default TTR for sod farms, and field trial data for cotton.

A summary of the post-application exposure and risk estimates for all proposed crops except cotton is provided in Table 9.2.1. Only risk estimates for the highest contact activity are shown below. Post-application dermal exposure and risk estimates resulted in MOEs greater than 30 and are not of concern on Day-0 (i.e., 12 hours after application).

A summary of the post-application exposure and risk estimates from use of sulfoxaflor on cotton during harvesting activities is provided in Table 9.2.2. Post-application dermal exposure and risk estimates resulted in MOEs greater than 30 and are not of concern.

Table 9.2.1. Post-Application Exposure and Risk Estimates for use of Sulfoxaflor.*					
Crop Group	Proposed Uses/Groups	Proposed Maximum Application Rate (lb ai/A)	Activity	Daily Dose (mg/kg/d)	MOE
DFR Data on Treated Wheat (MRID No. 47832415)					
Field / Row Crop, Low/Medium	Barley, Wheat Canola	0.047	Scouting	0.0000007	2,600,000
	Cotton	0.090	Scouting	0.0000003	7,000,000
	Soybean	0.090	Scouting	0.0000014	1,300,000
	Succulent Edible Podded and Dry Beans	0.090	Irrigation (Hand Set)	0.0000023	770,000
DFR Data on Treated Broccoli (MRID No. 47832416)					
Vegetable, head and stem Brassica	Brassica (Cole) Leafy Vegetables	0.090	Hand Weeding	0.00001	130,000
Vegetable, "Root"	Bulb Vegetables (Crop Group 3-07)	0.090	Hand Weeding	0.00001	130,000
	Leaves of Root and Tuber Vegetables (Crop Group 2); Root and Tuber Vegetables (Crop Group 1)	0.090	Irrigation (Hand Set)	0.000006	280,000
Tree Fruit, Evergreen	Citrus (Crop Group 10)	0.133	Thinning Fruit	0.00002	100,000
Vegetable, Cucurbit	Cucurbits Vegetables (Crop Group 9)	0.090	Irrigation (Hand Set)	0.00001	280,000
Vegetable, Fruting	Fruiting Vegetables (Crop Group 8)	0.090	Irrigation (Hand Set)	0.00001	280,000
Vegetable, Leafy	Leafy Vegetables (except Brassica – Crop Group 4 & Watercress)	0.090	Irrigation (Hand Set)	0.000006	280,000
Nursery/ Greenhouse (Unassigned)	Ornamentals	0.133	Irrigation (Hand Set)	0.00001	190,000
Tree, "Fruit", Deciduous	Pome Fruits (Crop Group 11); Stone Fruits (Crop Group 12)	0.133	Thinning Fruit	0.00002	100,000
Vine/Trellis	Small Fruit Vine Climbing (except fuzzy kiwifruit – Subgroup 13-07F)	0.090	Turning, Girdling	0.0001	28,000
Berry, Low	Low Growing Berry (Subgroup 13-07G)	0.090	Irrigation (Hand Set)	0.000006	280,000
Tree, "Nut"	Tree Nuts (Crop Group 14 & Pistachio)	0.133	Hand Harvesting	0.000007	260,000
Default TTR Approach					
Turf, Sod	Turfgrass, Sod farms	0.133	Maintenance, Harvesting, Slab Transplanting/ Planting	0.0000004	4,700,000

* Taken from Z. Figueroa, 18 September 2012, D382606

Table 9.2.2. Post-Application Exposure and Risk Estimates for use of Sulfoxaflor on Cotton during Harvesting Activities.*

Crop	Proposed Maximum Seasonal Application Rate (lb ai/A)	Activity	Days After Treatment	Residue from Cotton Field Trial [2] (mg ai/g cotton)	Daily Dose (mg/kg/d)[3]	MOE[4]
Cotton	0.266	Harvesting Mechanical (Module Builder)	14	0.000129	0.00034	5,300
		Harvesting Mechanical (Picker Operator; Raker)			0.00089	2,000
		Harvesting Mechanical (Tramper)			0.00189	950

* Taken from Z. Figueroa, 18 September 2012, D382606.

1. Transfer Coefficients from HED Policy No. 3.0., Non-Foliar Transfer Coefficient Table, March 2012 (p 122).
2. Residue Value from Cotton Field Trial (Table 9) adjusted to Max. Seasonal Application Rate (mg ai/g cotton) = (0.182 mg ai/kg cotton) (0.001 mg/g) ((0.266 lb ai/A)/(0.375 lb ai/A)) = 0.000129 mg ai/g cotton.
3. Dermal Dose (mg/kg/day) = [Cotton Field Trial Residue Value $_{adjusted}$ (mg ai/g cotton) x TC (g cotton/hr) x hr/day (8 hrs/day) x DAF (2.5%)]/ BW (69 kg).
4. Dermal MOE = NOAEL (1.8 mg/kg/day)/Dermal Dose (mg/kg/day); LOC = 30.

Restricted Entry Interval (REI)

Although acute toxicity testing of technical-grade sulfoxaflor and the GF-2032 SC end-use product support an REI of 12 hours, the petitioner has proposed REIs of 24 hours for both end-use products based on their interpretation of the Transform™ WG acute ocular toxicity study resulting in a Toxicity Category II. The petitioner believes that the higher toxicity of Transform ™ WG is due to an inert ingredient in that product. It is the petitioner's understanding that the REI for all products containing a particular active ingredient must be the maximum dictated by testing of the individual products.

HED recommends that RD ensure that the proper REI is listed on both labels according to the acute toxicity profile and the formulation for each product.

9.2.2 Inhalation Post-Application Risk Estimates

Based on the Agency's current practices, a quantitative post-application inhalation exposure assessment was not performed for sulfoxaflor at this time primarily because it has a low vapor pressure (1.05×10^{-8} mm Hg at 25°C) and it is applied at low application rates (maximum single application rate of 0.133 lb ai/A). However, volatilization of pesticides may be a potential source of post-application inhalation exposure to individuals nearby to pesticide applications. The Agency sought expert advice and input on issues related to volatilization of pesticides from its Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) in December 2009. The Agency received the SAP's final report on March 2, 2010 (http://www.epa.gov/scipoly/SAP/meetings/2009/120109meeting.html).

Although a quantitative occupational post-application inhalation exposure assessment was not performed, an inhalation exposure assessment was performed for occupational handlers. This assessment resulted in risk estimates that did not exceed HED's level of concern at baseline inhalation PPE. Handler exposure resulting from application of pesticides outdoors is likely to result in higher exposure than post-application exposure. Therefore, it is expected that these handler inhalation exposure estimates would be protective of most occupational post-application inhalation exposure scenarios.

Furthermore, the Worker Protection Standard for Agricultural Pesticides contains requirements for protecting workers from inhalation exposures during and after greenhouse applications through the use of ventilation requirements [40 CFR 170.110, (3) (Restrictions associated with pesticide applications)].

10.0 References

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Appendix A. Toxicology Profile and Executive Summaries

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for food use for Sulfoxaflor are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Study	Technical	
	Required	Satisfied
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (rodent)	yes	yes
870.3150 Oral Subchronic (nonrodent)	yes	yes
870.3200 21-Day Dermal	yes	yes
870.3250 90-Day Dermal	no	-
870.3465 90-Day Inhalation	yes	waived
870.3700a Developmental Toxicity (rodent)	yes	yes
870.3700b Developmental Toxicity (nonrodent)	yes	yes
870.3800 Reproduction	yes	yes
870.4100a Chronic Toxicity (rodent)	yes	yes
870.4100b Chronic Toxicity (nonrodent)	yes	yes
870.4200a Oncogenicity (rat)	yes	yes
870.4200b Oncogenicity (mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes
870.5100 Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300 Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5xxx Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.5xxx Mutagenicity—Other Genotoxic Effects		
870.6100a Acute Delayed Neurotoxicity (hen)	no	-
870.6100b 90-Day Neurotoxicity (hen)	no	-
870.6200a Acute Neurotoxicity Screening Battery (rat)	yes	yes
870.6200b 90-Day Neurotoxicity Screening Battery (rat)	yes	yes
870.6300 Develop. Neurotoxicity	yes	yes
870.7485 General Metabolism	yes	yes
870.7600 Dermal Penetration	yes	yes
870.7800 Immunotoxicity	yes	yes

Study	Technical	
	Required	Satisfied
Special Studies for Ocular Effects		
Acute Oral (rat)	no	-
Subchronic Oral (rat)	no	-
Six-month Oral (dog)	no	-

A.2 Toxicity Profiles

Sulfoxaflor Acute Toxicity Profile

Guideline No.	MRID (Annex II Point)	Route/ method	Species/ strain (sex)	Result	Classification
Sulfoxaflor					
870.1100	47832144 (5.2.1)	Oral/ gavage	Rat/ Fischer 344 (both)	LD_{50} =1000 mg/kg	Category III
	47832040 (5.2.1)	Oral/ gavage	Mouse/ CD1 (male)	LD_{50} =750 mg/kg	Category III
870.1200	47832051 (5.2.2)	Dermal/ topical	Rat/ Fischer 344 (both)	LD_{50} >5000 mg/kg	Category IV
870.1300	47832052 (5.2.3)	Inhalation/ nose only	Rat/ Fischer 344 (both)	LC_{50}>2.09 mg/L (4hr)	Category IV
870.2400	47832055 (5.2.5)	Eye/ instillation	Rabbit/ NZW (female)	Slight Irritation	Category III
870.2500	47832053 (5.2.4)	Dermal/ topical	Rabbit/ NZW (female)	Minimal Irritation	Category IV
870.2600	47832041 (5.2.6)	Dermal/ LLNA	Mouse/ CBA/J (female)	No Sensitization	None
Metabolites					
Metabolite X11719474					
Non-guideline	47832308 (5.8.1.2/1)	Oral/gavage 300 mg/kg/day	Rat/F344 (female)	No Observed Effects	Not Applicable
870.1100	47832131 (5.8.1.2/2)	Oral/gavage 2000, 5000 mg/kg/day	Rat/F344 (female)	No death LD50 > 5000. Signs for 48 hours	Category IV
Non-guideline	47832309 (5.8.1.2/3)	Dermal 1000 mg/kg/day	Rat/F344 (female)	No death. Irritation for 48 hours	Not Applicable
Non-guideline	47832309 (5.8.1.2/3)	Eye 0.1 ml (0.069 g)	Rabbit/NZW (female)	Conjunctivitis for 24 hours	Not Applicable

870.2600	47832130 (5.8.1.2/4)	Dermal 50% /rLLNA	CBA/J Mice (female)	Negative	Category IV
Metabolite X11721061					
870.1100	47832086 (5.8.2.2/1)	Oral/Gavage 1000, 2000, 5000 mg/kg/day	Rat/F344 (female)	LD50 > 2000	Category III
Metabolite X11596066					
870.1100	47832067 (5.8.3.2/1)	Oral/gavage 2000 mg/kg/day	Rat/F344 (female)	No deaths.; LD50 > 2000 Piloerection for 24 hours	Category III
Metabolite X11579457					
870.1100	47832145 (5.8.4.2/1)	Oral/gavage 2000 mg/kg/day	Rat/F344 (female)	No death; LD50 > 2000 Piloerection for 24 hours	Category III
Metabolite X11519540					
870.1100	47832069 (5.8.5.2/1)	Oral/gavage 320,1000,2000 mg/kg/day	Rat/F344 (female)	LD50 565; No death at 320. Death at 1000 and 2000	Category III

Sulfoxaflor Toxicity Profile Table

Guideline No.	Study	Dosages	Effects at lowest observed effect level
870.3050	Oral 28-day F344 Rat MRID 47832045 Annex 5.3.1/2 **Acceptable/Guideline**	0, 300, 1000, 2000, 3000 ppm M/F: 0/0, 24.8/26.5, 79.4/88.3, 155/170, 205/192 mg/kg/day	LOAEL – 88.3 mg/kg/day based on liver effects including ↑ serum cholesterol, liver weight and hepatocellular hypertrophy with altered tinctorial properties. NOAEL – 26.5 mg/kg/day
870.3050	Oral 28-day CD1Mouse MRID 47832042 Annex 5.3.1/4 **Acceptable/Guideline**	M: 0, 300/350, 1500, 3500 ppm F: 350, 1500, 3500 ppm 0/0, 43.9/53, 230/273, 524638 mg/kg/day	LOAEL – 230/273 M/F based on liver effects including ↑ ALT, AST, liver weight, hepatocellular hypertrophy in males and females. Fatty change, multifocal single cell necrosis and mitotic figures in males only NOAEL- 43.9/53 mg/kg/day M/F
Non-Guideline	Oral 28-day in Beagle Dogs MRID 47832043 Annex 5.3.1/6 **Acceptable**	Group 1 (dietary)- F: 500 ppm Group 2 (gelatin capsule)- F: 15 mg/kg/day Group 3 (oral gavage- analytical grade XDE 208 in Methocel)- F: 15 mg/kg/day Group 4 (dietary)- F: 100 ppm	The only tolerable dose and route was 15 mg/kg/day technical grade XDE-208 via oral gavage in Methocel vehicle. Exposure at 100 ppm via diet was well tolerated as determined by adequate food consumption, but test material intake was insufficient to justify its use as a high dose level for subsequent studies.

Guideline No.	Study	Dosages	Effects at lowest observed effect level
		Group 5 (oral gavage-technical grade XDE 208 in Methocel)- F: 15 mg/kg/day	
870.3100; 870.6200; 870.7800	90 day oral toxicity Neurotoxicity Screening Immunotoxicity Oral 90-day F344 Rat with 28-day Recovery MRID 47832046 Annex 5.3.2/1 **Acceptable/Guideline**	0, 100, 750, 1500 ppm M/F: 0/0, 6.36/6.96, 47.6/51.6, 94.9/101 mg/kg/day	LOAEL – 47.6/51.6 mg/kg/day (M/F) based on 11-13% ↓ body weight gain. Liver - ↑ serum cholesterol, liver weight, hepatocellular hypertrophy with altered tinctorial properties, and multifocal single cell necrosis;Fatty change in males. NOAEL = 6.36/6.96 mg/kg/day (M/F) **There were no indications of neurotoxicity (NOAEL ≥ 94.9 mg/kg/day)** **There were no indications of immunotoxicity (NOAEL ≥ 94.9 mg/kg/day)**
870.3100	Oral 90-day CD1Mouse MRID 47832057 Annex 5.3.2/2 **Acceptable/Guideline**	M: 0, 100, 750, 1250 ppm (0, 12.8, 98, 166 mg/kg/day) F: 0, 100, 1500, 3000 ppm (0, 16.2, 247, 489 mg/kg/day)	LOAEL – 98/247 mg/kg/day (M/F) based on liver effects including ↑ liver weight, hepatocellular hypertrophy with altered tinctorial properties, multifocal single cell necrosis. Adrenal - very slight hypertrophy/vacuolization of zona fasciculata. Females also had ↑ ALT, AST and triglycerides NOAEL – 12.8/16.2 mg/kg/day (M/F)
870.3150	Oral 90-day Dog/Beagle (gavage) MRID 47832058 Annex 5.3.3/1 **Acceptable/Guideline**	0, 1, 3, 6 mg/kg/day (10 mg/kg/day days 1-5)	LOAEL – 10 mg/kg/day based on ↓ food consumption NOAEL = 6 mg/kg/day
870.3200	28-day Dermal F344 Rat MRID 47832059 Annex 5.3.7/1 **Acceptable/Guideline**	0, 100, 500, 1000 mg/kg/day	LOAEL - > 1000 mg/kg/day ; Females: No effects Males: 17% ↑ serum cholesterol; 6.5% ↑ absolute and 4.4% ↑ relative liver weights; very slight centrilobular to midzonal hepatocellular hypertrophy with altered tinctorial properties. Although treatment-related, these effects were not considered adverse. NOAEL = 1000 mg/kg/day

Guideline No.	Study	Dosages	Effects at lowest observed effect level
870.3700	Developmental Toxicity in CD Rats (Definitive) MRID 47832140 Annex 5.6.10/2 **Acceptable/Guideline**	0, 25, 150, 1000 ppm (0, 2, 11.5, 70.2 mg/kg/day)	**Dam**: **LOAEL** – 70.2 mg/kg/day based on reduced feed consumption and body weight gain; increased liver weight. **NOAEL** = 11.5 mg/kg/day **Litter**: **LOAEL** – 70.2 mg/kg/day based on decreased fetal body weight; fetal abnormalities (forelimb flexure, bent clavicle, hindlimb rotation, convoluted/hydro-ureter). **NOAEL** = 11.5 mg/kg/day
870.3700	Developmental Toxicity Dietary in NZW Rabbits (Definitive) MRID 47832066 Annex 5.6.11/3 **Acceptable**	0, 30, 150, 750 ppm (0, 1.3, 6.6, 31.9 mg/kg/day)	**Dam**: **LOAEL** – 31.9 mg/kg/day based on decreased feed consumption (8-21%), body weight gain, (12%) and fecal output. **NOAEL** = 6.6 mg/kg/day **Litter**: **LOAEL** > 31.9 mg/kg/day **NOAEL** = 31.9 mg/kg/day **Toxicokinetics:** AUC α Dose, dose adjusted AUC similar to rat
870.3800; OECD 421	Reproduction/ Developmental Probe CD Rat MRID 47832143 Annex 5.6.1/1 **Acceptable/Guideline**	0, 100, 500, 1000 ppm	**Reproduction**: **LOAEL** = 39.5 mg/kg/day based on decreased neonatal survival. Postnatal day (PND) 4 survival 81.2% (95.4% in controls). **NOAEL** = 8.1 mg/kg/day (PND 4 pup survival 7.3%.) **Parental toxicity**: **NOAEL > 78.2 mg/kg/day** – Decreased body weight gain was observed at 500 and 1000 ppm in males and females. Increased liver weight in males at 500 and 1000 ppm. Hepatocellular hypertrophy in males at 500 and 1000 ppm and females at 1000 ppm. These effects were considered minimal and not adverse. **Offspring**: **LOAEL** – 39.5 mg/kg/day based on decreased neonatal survival (see above). **NOAEL** = 8.1 mg/kg/day

Guideline No.	Study	Dosages	Effects at lowest observed effect level
870.3800	2-Generation Reproduction in CD Rats MRID 47832142 Annex 5.6.1/2 **Acceptable/Guideline**	0, 25, 100, 400 ppm (1.52/1.91, 6.07/7.82, 24.6/30.5 mg/kg/day [m/f])	**Reproduction**: **LOAEL** – 24.6 mg/kg/day based on decreased neonatal survival (ca. 2-5%) **NOAEL** = 6.07 mg/kg/day **Parental toxicity**: **LOAEL** – 24.6 mg/kg/day based on increased liver weight in with correlating histopathologic changes **NOAEL** = 6.07 mg/kg/day **Offspring**: **LOAEL** – 24.6 mg/kg/day based on decreased neonatal survival and a slight delay in preputial separation (puberty onset) in F1 males **NOAEL** = 6.07 mg/kg/day
870.4100	Oral 1-year Beagle Dog (gavage) MRID 48288906 Annex 5.3.4/1 **Acceptable/Non-guideline**	0, 1, 3, 6 mg/kg/day	LOAEL > 6 mg/kg/day NOAEL = 6 mg/kg/day
870.4200	18-month carcinogenicity CD1 Mouse MRID 47832054 Annex 5.5.3 **Acceptable/Guideline**	♂: 0, 25, 100, 750 ppm 0, 2.54, 10.4, 79.6 mg/kg/day	**LOAEL** – 79.6 mg/kg/day based on liver effects including adenomas and carcinomas; Increased weight, hypertrophy with eosinophilia, fatty change, single cell necrosis, eosinophilic/ vacuolated foci, mitosis. **NOAEL** = 10.4 mg/kg/day
		♀: 0, 25, 250, 1250 ppm 0, 3.43, 33.9, 176 mg/kg/day	**LOAEL** – 176 mg/kg/day based on liver effects including carcinomas; increased weight, hypertrophy with eosinophilia, fatty change, single cell necrosis and eosinophilic/ vacuolated foci. **NOAEL** = 33.9 mg/kg/day

Guideline No.	Study	Dosages	Effects at lowest observed effect level
870.4300	2-year combined toxicity and carcinogenicity dietary F344 Rat MRID 47832060 Annex 5.5.1 & 5.5.2 **Acceptable/Guideline**	0, 25, 100, 500 ppm M/F: 0/0, 1.04/1.28, 4.24/5.13, 21.3/39 mg/kg/day	**Males:** *1-year:* **LOAEL** – 21.3 mg/kg/day based on liver effects including increased blood cholesterol, liver weight, hypertrophy, fatty change, single cell necrosis and macrophages. *2-year:* **LOAEL** – 21.3 mg/kg/day based on increased serum cholesterol concentrations, and histopathological liver effects in males; increased incidence and size of Leydig cell adenomas with secondary effects including preputial gland tumours; liver adenomas. **NOAEL** = 4.24 mg/kg/day **Females:** *1- and 2-year* **LOAEL** – 39 mg/kg/day based on liver effects including increased blood cholesterol, liver weight, hypertrophy, fatty change, single cell necrosis and macrophages. **NOAEL** = 5.13 mg/kg/day
870.5100	Bacterial Reverse Mutation Test MRID 47832047 Annex 5.4.1 **Acceptable/Guideline**	*S.typhimurium* TA 98, TA 100, TA 1535 & TA 1537 *E. coli*, WP2*uvr*A 100, 333, 1000, 2500, and 5000 μg per plate +/- S9	Negative
870. 5300	*In vitro* Mammalian Cell Gene Mutation Test MRID 47832049 Annex 5.4.3/1 **Acceptable/Guideline**	Chinese hamster ovary cells CHO/HGPRT 0, 173.3, 346.6, 693.3, 1386.5, and 2773 μg/ml +/- S9	Negative
870. 5375	*In Vitro* Mammalian Chromosome Aberration Test MRID 47832050 Annex 5.4.2/1 **Acceptable/Guideline**	Rat lymphocytes 4 hr treatment 0, 693.3, 1386.5, and 2773 μg/ml +/- S9; 24 hr treatment 0, 173.3, 346.6, and 693.3 μg/ml	Negative

Guideline No.	Study	Dosages	Effects at lowest observed effect level
870.5395	Mammalian Erythrocyte Micronucleus Test MRID 47832071 Annex 5.4.4	Mouse bone marrow polychromatic erythrocytes Male and female CD-1 mice, 6/sex/dose, single oral gavage on two consecutive days at 0, 100, 200, and 400 mg/kg/day	Negative
870.6200	Acute neurotoxicity in F344 Rats MRID 47832134 Annex 5.7.1/1 **Acceptable/Guideline**	0, 7.5, 75, 750 mg/kg; Additional motor activity work at 0, 2.5, 7.5, 25 mg/kg	**LOAEL** – 75 mg/kg/day. On Test Day 1, decreased motor activity in 750 and 75 mg/kg groups. Effect was considered equivocal in 7.5 mg/kg group. Clinical findings on test days 2,3, or 4 – decreased feces, red perioral soiling, perineal urine soiling (females) 750 mg/kg – muscle tremors and twitches, convulsions, splayed hindlimbs and perineal urine soiling, increased lacrimation and salivation, decreased pupil size and response to touch, decreased level of open-filed activity and gait abnormalities, decreased rectal temperature. One female death on day 1 – cause unknown No treatment-related histopathological findings. **NOAEL** = 25 mg/kg
870.6300	Developmental neurotoxicity in CD Rats MRID 47832133 Annex 5.7.5/1 **Acceptable/Guideline**	0, 25, 100, 400 ppm; 0, 1.8/1.9, 7.1/7.6, 27.7/29.8 mg/kg/day (gestation/lactation)	**Maternal:** LOAEL –> 27.7 mg/kg/day. No treatment-related systemic or reproduction related effects observed in the dams NOAEL = 27.7 mg/kg/day **Offspring:** LOAEL – 7.1mkd based on reduction in postnatal survival (PND 0-4) NOAEL = 1.8 mg/kg/day
Non-Guideline	Toxicokinetic – Rat and Mouse Probe looking at ADME: single oral dose Rat/F344 & Mouse/CD1 MRID 47832038 Annex 5.1.1/1 **Acceptable**	Oral gavage/ 100 mg/kg bw	Rapid and almost complete absorption and elimination without detectable metabolism. Most (rat: 87-98%; mouse: 80-85%) of the elimination was via urine. Plasma t½ was 9h in male rats and 7h in female rats. *report also includes integrated plasma and urine TK data from 28- and 90-day rat, and 28-day mouse dietary toxicity studies*

Guideline No.	Study	Dosages	Effects at lowest observed effect level
870.7485	Toxicokinetic – F344 Rat ADME: single oral dose with examinations at C_{max} and ½C_{max} MRID 47832039 Annex 5.1.2/1 **Acceptable/Guideline**	Oral gavage/ 5, 100 mg/kg bw	Rapid absorption, wide tissue distribution. Radioactivity detected mainly in portal of entry (GI tract, liver) and excretion (kidney, urinary bladder) tissues. No metabolite in tissues or plasma. Kinetics in tissues were parallel to plasma showing no potential for bioaccumulation.
870.7485	Toxicokinetic – F344 Rat ADME: oral – single and repeat dose; IV – single dose with examinations at 168 h (7 days) MRID 47832034 Annex 5.1.3/1 **Acceptable/Guideline**	Oral gavage/ 5 mg/kg bw for 1 and 15 days 100 mg/kg bw for 1 day Intravenous 5 mg/kg bw for 1 day	Rapid and almost complete (at least 92-96%) absorption of the oral dose. Low metabolism (<7%) and almost complete (89-94%) and rapid elimination of the oral dose in urine in the 1st 24 hours. No tissue accumulation, ≤1.2% remained in tissues 7 days after dosing.
Non-Guideline	*Ex vivo* gene expression and cell proliferation analyses CD1Mouse (♀) & F344 Rat MRID 47832033 Annex5.5.4/1 **Acceptable**	Mice: 0, 3000, 4500. Rats: 0, 2000	XDE-208-induced gene expression profile in mice and liver (hepatocellular) proliferation in both mice and rats characteristic of phenobarbital-like CAR agonism.
Non-Guideline	Targeted gene expression, cell proliferation and cytochrome P450 enzymatic activity in F344 MRID 47832061 Annex 5.5.4/2 **Acceptable**	0, 100, 750, 1500 for 3 or 7 days	XDE-208-induced liver effects were PB-like. Males were affected more than females. Neither AhR nor PPARα were involved.
Non-Guideline	Liver Weight Effects in crl:cd1(icr) CD1 Mice MRID 47832062 Annex 5.5.4/3 **Acceptable**	Males: 0, 500, 750. Females: 0, 1000, 1500 for 7 days	XDE-208-induced liver effects were consistent with CAR activation resulting in a PB-like MoA; males were more sensitive than females. Neither AhR nor PPARα were involved.

Guideline No.	Study	Dosages	Effects at lowest observed effect level
Non-Guideline	A Study to Characterize the Induction Profile of XDE-208 in the Livers Of C57BL/6J Mice MRID 47832276 Annex 5.5.4/4 **Acceptable**	0, 750, 1500 ppm	XDE-208-induced liver effects in C57Bl/6J mice were similar to previously observed effects in CD1 mice
Non-Guideline	Liver Effects Observed In Regulatory Toxicology Studies By Use Of Dual Car-PXR Knockout And Humanised Mice (Mouse/C57Bl/6J WT, Humanised and KO PXR/CAR) MRID 47832255 Annex 5.5.4/5 **Acceptable**	0, 750 ppm for 7 days	In WT C57BL/6J XDE-208 caused the same liver effects seen in CD1 mice. In PXR/CAR KO mice, XDE-208 did not induce any liver changes, demonstrating that activation of one or both of these receptors is required to elicit the liver effects seen in WT mice. In PXR/CAR humanised mice slight liver hypertrophic effects occurred but not hepatocellular proliferation. This study demonstrated that XDE-208, like PB, acts via a CAR-mediated MoA and that mice carrying the human PXR and CAR receptors did not develop hepatocellular proliferation responsible for liver tumor induction.
Non-Guideline	A Dietary Reproductive Toxicity Cross-Fostering Study in Crl:CD(SD) Rats MRID 47832063 Annex 5.6.12/1 **Acceptable**	Group 1: 0/0 ppm Group 2: 0/1000 ppm Group 3: 1000/0 ppm Group 4: 1000/1000 ppm	Groups 1 and 2 (negative control and lactation exposure only) had no effects Groups 3 and 4 (gestation exposure only and positive control) had decreased neonatal survival **Dam**: Reduced feed consumption and body weight gain when dams given 1000 ppm **Litter**: All offspring exposed prior to birth died by PND 4. No effect on neonatal survival with lactation-only exposure. Therefore, pup survival effect due to *in utero* exposure.
Non-Guideline	Neonatal Survival in New Zealand White Rabbits MRID 47832138 Annex 5.6.12/2 **Acceptable**	0, 750 ppm (0, 29 mg/kg/day)	**Dam**: Decreased feed consumption and body weight gain. **Litter**: No Effects

Guideline No.	Study	Dosages	Effects at lowest observed effect level
Non-Guideline	Characterization of the agonist effects of XDE-208 on mammalian muscle nicotinic acetylcholine receptors MRID 47832035 Annex 5.6.12/3 **Acceptable**	0-3mM	XDE-208 was an agonist to the rat fetal muscle nAChR XDE-208 was not an agonist to human fetal, human adult, or rat adult muscle nAChR
Non-Guideline	Phase 1; Critical window of exposure for fetal abnormalities and neonatal survival effects in Crl:CD(SD) rats MRID 47832137 Annex 5.6.12/4 **Acceptable**	Group 1: 0 ppm Group 2: 1000 ppm GD 6-16 Group 3: 1000 ppm GD 16-birth	**Litter**: 1000 ppm from GD 16-birth caused neonatal death and limb abnormalities in offspring. Also, all fetal abnormalities observed in developmental toxicity study reversed by PND 4. 1000 ppm from GD 6-16 produced no effects.
Non-Guideline	Phase 2; Critical window of exposure for fetal abnormalities and neonatal survival effects in Crl:CD(SD) rats MRID 47832136 Annex 5.6.12/5 **Acceptable**	Group 1: 0 ppm Group 2: 1000 ppm GD 16-18 Group 3: 1000 ppm GD 18-20 Group 4: 1000 ppm GD 20-22/LD0	**Litter**: 1000 ppm exposure for 48 hours starting on GD 20 caused neonatal death and limb abnormalities in offspring. All fetal abnormalities observed in developmental toxicity study reversed by PND 4. 1000 ppm from GD 16-18 or 18-20 produced no effects.
Non-Guideline	Observations on the effects of XDE-208 on the phrenic nerve-hemidiaphragm preparation from new-born rat. MRID 47832064 Annex 5.6.12/6 **Acceptable**	0, 0.1, 1.0mM	XDE-208 induced nAChR-dependent sustained contracture of the neonatal rat diaphragm.
Non-Guideline	Histopathological Evaluation Of Fetal Lung Samples From The Developmental Toxicity Study In Crl:Cd(Sd) Rats MRID 47832135 Annex 5.6.12/7 **Acceptable**	0, 1000 ppm	Fetal lungs from the rat developmental toxicity study were normal.

Guideline No.	Study	Dosages	Effects at lowest observed effect level
Non-Guideline (GF-3032 Formulation)	Comparative Dermal Absorption, *in vitro* rat and human skin MRID 47832414 Annex IIIA 7.6.2 **Acceptable**	0.024, 0.48, 2.4 g/L for 24 hours	% AD absorbed for 0.024, 0.48, 2.4 g/L, respectively; Human; 1.15, 1.54, 0.26% Rat: 4.34, 8.72, 1.3%
Non-Guideline (GF-3032 Formulation)	Dermal Absorption in the rat, *in vivo* MRID 47832413 Annex IIIA 7.6.3 **Acceptable**	0.024, 0.48, 2.4 g/L for 10hours then skin washed with mild soap. Tissue collected for up to 192 hours.	The amount of applied dose removed by washing was 96%, 71%, and 67% of the applied dose. After 10 hours, 2%, 11%, and 13% of the AD was absorbed across the dose groups, respectively.

Study type	Route/method Dose levels*	Effects at Lowest Observed Adverse Effects Level
1. Metabolite X11719474		
ADME probe in F344 Rats MRID 47832132 Annex 5.8.1.1/1 **Acceptable/Non-guideline**	Oral/gavage 100 mg/kg/day	n/a
870.3050 28-day Oral in F344 Rats MRID 47832099 Annex 5.8.1.3/2 **Acceptable/Guideline**	Oral/diet 0, 1000, 2000, 3000, 8000 ppm 0/0, 83.4/90.1, 167/184, 244/278, 662/734 mg/kg/day (M/F)	LOAEL= 3000 ppm (241mg/kg/day) based on decreased triglycerides in the males NOAEL= 2000 ppm (167 mg/kg/day).
870.3100 ; 870.3100 90-day Oral in F344 Rats MRID 47832147 Annex 5.8.1.3/3 **Acceptable/Guideline**	Oral/diet 0, 500, 1000, 5000 ppm	At 5000 ppm (71.8 mg/kg/day) ↑Cholesterol, ↑liver wt. with hypertrophy, eosinophilia, fatty change & SCN
870.3150 90-day Oral in Beagle Dogs (males) MRID 47832037 Annex 5.8.1.3/5 **Acceptable/Guideline**	Oral/gavage 0, 10, 25, 50 mg/kg/day	No Observed Effects
870.5100 Ames *S. typhimurium E. coli* MRID 47832097 Annex 5.8.1.4/1 **Acceptable/Guideline**	In vitro±S9 0 - 5000 μg per plate	Negative
870. 5375 Lymphocyte chromosome aberration in CD Rats (males) MRID 47832096 Annex 5.8.1.4/2 **Acceptable/Guideline**	In vitro± S9 0 - 2953 μg/ml	Negative
870. 5300 HGPRT Chinese hamster ovary MRID 47832095 Annex 5.8.1.4/3 **Acceptable/Guideline**	In vitro± S9 0 - 2953 μg/ml	Negative
Targeted Gene Expression, Cell Proliferation, and Cytochrome p 450 Enzymatic Activities in Rats. MRID 47832151 Annex 5.8.1.5/1 **Acceptable/Non-Guideline**	0 or 8000 ppm	Increased expression of several CAR-related transcripts, along with the pregnane X receptor (PXR)-related transcript Cyp3a, and PROD enzyme activity, suggests that X11719474 may be agonist ligand for CAR,
870.3550; OECD 421 Dietary reproduction & Developmental Toxicity Screening Test in CRL:CD(SD) Rats MRID 47832094 Annex 5.8.1.6/1 **Acceptable/Guideline**	0, 1000, 2000, 5000 ppm (0, 80.8/81.7, 162/167, 396/451 mg/kg/day (M/F)	No Observed Effect

Study type	**Route/method** **Dose levels***	**Effects at Lowest Observed Adverse Effects Level**
870.3700 Dietary Developmental Toxicity Study in CRL:CD(SD) Rats MRID 47832087 Annex 5.8.1.6/2 **Acceptable/Guideline**	0, 1000, 2000, 5000 ppm	No Observed Effect
2. Metabolite X11721061		
870.3050 28-day Dietary in F344 Rats MRID 47832036 Annex 5.8.2.3/2 **Acceptable/Guideline**	Oral/diet 0, 1000, 3000, 8000 ppm	LOAEL= 8000 ppm (662/734 mg/kg/day (M/F)) based on increased liver weights, hepatic hypertrophy, and increased cholesterol levels NOAEL = 3000 ppm (244/278 mg/kg /day (M/F)).
870.5100 Ames *S. typhimurium E. coli* MRID 47832085 Annex 5.8.2.4/1 **Acceptable/Guideline**	In vitro ± S9 0 - 5000 μg per plate	Negative
870. 5375 Lymphocyte chromosome aberration in CD Rats (males) MRID 47832078 Annex 5.8.2.4/2 **Acceptable/Guideline**	In vitro ± S9 0 - 1920 μg/ml	Negative
870. 5300 HGPRT Chinese hamster ovary MRID 47832077 Annex 5.8.2.4/3 **Acceptable/Guideline**	In vitro± S9 0 - 1920 μg/ml	Negative
3. Metabolite X11596066		
870.5100 Ames *S. typhimurium E. coli* MRID 47832076 Annex 5.8.3.4/1 **Acceptable/Guideline**	In vitro± S9 0 - 5000 μg per plate	Negative
4. Metabolite X11579457		
870.5100 Ames *S. typhimurium E. coli* MRID 47832070 Annex 5.8.4.4/1 **Acceptable/Guideline**	In vitro ± S9 0 - 5000 μg per plate	Negative
870. 5375 Lymphocyte chromosome aberration in CD Rats (males) MRID 48288932 Annex 5.4.2/2 **Acceptable/Guideline**	*In vitro± S9* 0 – 2525 μg/ml	Negative

Study type	Route/method Dose levels*	Effects at Lowest Observed Adverse Effects Level
870. 5300 HGPRT CHO MRID 48288933 Annex 5.8.4.4/3 **Acceptable/Guideline**	*In vitro± S9* 0 – 2525 μg/ml	Negative
5. Metabolite X11519540		
870.3050 28-day dietary in F344 Rats Annex 5.8.5.3/2 MRID 48288941 **Acceptable/Non-guideline**	0, 100, 300, 1000, 2000 ppm (0/0, 7.7/8.5, 23.1/24.9, 74/77, 140/152 mg/kg/day (M/F))	LOAEL = 300 ppm (23.1 mg/kg/day) based on changes increased liver weight and histopathology along with degeneration of kidney tubules (in males). NOAEL= 100 ppm (7.7 mg/kg/day).
870.5100 Ames *S. typhimurium E. coli* MRID 47832068 Annex 5.8.5.4/1 **Acceptable/Guideline**	In vitro± S9 0 - 5000 μg per plate	Negative
870. 5375 Lymphocyte chromosome aberration in CD Rats (males) MRID 48288935 Annex 5.8.5.4/2 **Acceptable/Guideline**	*In vitro± S9* 0 – 2540 μg/ml	Negative
870. 5300 HGPRT CHO Annex 5.8.5.4/3 MRID 48288934 **Acceptable/Guideline**	*In vitro± S9* 0 – 2540 μg/ml	Negative

A.3 Hazard Identification and Endpoint Selection

A.3.1 Acute Reference Dose (aRfD) - Females age 13-49

Study Selected: Developmental Neurotoxicity Study in the Rat
MRID No.: 47832133
Executive Summary: See Appendix A, Guideline § 870.6300
Dose and Endpoint for Risk Assessment: NOAEL = 1.8 mg/kg/day based on decreased neonatal survival PND 0-4 seen at LOAEL = 7.1 mg/kg/day.
Uncertainty Factors: 30 (3 X for interspecies (UF_A) and 10 X for intraspecies (UF_H) variability).
Comments about Study/Endpoint/Uncertainty Factors: The DNT was selected because the effect is a single dose effect, and can be provided the exposure occurs between gestation days 20 to birth. This endpoint is protective of decreased neonatal survival observed in the 2-generation reproduction study with a NOAEL of 6.63 mg/kg/day and offspring effects observed in the rat developmental study with a NOAEL of 11.5 mg/kg/day. This endpoint is appropriate for the females age 13-49 since it is protective of potential *in utero* and reproductive effects.

A.3.2 Acute Reference Dose (aRfD) - General Population, excluding females 13+

Study Selected: Acute Neurotoxicity Study in the Rat
MRID No.: 47832134
Executive Summary: See Appendix A, Guideline § 870.6200
Dose and Endpoint for Risk Assessment: NOAEL = 25 mg/kg/day based on decreased motor activity on day 1 seen at LOAEL = 75 mg/kg/day.
Uncertainty Factors: 100 (10 X for interspecies (UF_A) and 10 X for intraspecies (UF_H) variability).
Comments about Study/Endpoint/Uncertainty Factors: The ACN was selected because of the following: the effect was observed on the day of exposure, the effect, neurotoxicity, is consistent with the proposed MOA for sulfoxaflor; and the oral route of exposure is appropriate for risk assessment. This study is appropriate for the general population because the effect is not specific to a single sex or lifestage. Decreased food consumption was also observed within the first 4 days of exposure in several studies. However, decreased food consumption was attributed to the palatability of sulfoxaflor and was transient.

A.3.3 Chronic Reference Dose (cRfD) `

Study Selected: Combine Chronic/Carcinogenicity Study in the Rat
MRID No.: 47832060
Executive Summary: See Appendix A, Guideline § 870.4300

Dose and Endpoint for Risk Assessment: NOAEL = 5.13 mg/kg/day (females) based on liver effects including increased serum cholesterol, hypertrophy, necrosis of centrilobular hepatocytes, vacuolization, and increased severity of aggregates of macrophages/histiocytes observed in males (21.3 mg/kg/day) and females (39 mg/kg/day). Although the NOAEL for males was slightly lower at 4.24 mg/kg/day, HED is using the female NOAEL of 5.13 mg/kg/day as a PoD for risk assessment. HED considers 4.24 mg/kg/day to be artificially low as a result of dose-spread and the NOAEL of 5.13 is supported by similar rat NOAELs in the 2-generation reproduction study (6.07 mg/kg/day), the 90-day oral study (6.36 mg/kg/day), and the development study (11.5 mg/kg/day).

Uncertainty Factors: 100 (10 X for interspecies (UF_A) and 10 X for intraspecies (UF_H) variability).
Comments about Study/Endpoint/Uncertainty Factors: The combined chronic/carcinogenicity study was selected because the effects were observed of 2 years of exposure and the route of exposure of is appropriate. Hepatotoxicity was also observed in several other oral studies including the 28-day, 90-day, and chronic studies in the rat and mouse. However, the selected endpoint occurs at a lower dose and is therefore protective of the observed hepatotoxicity.

A.3.4 Dermal Exposure (Short- and Intermediate-Term)

Study Selected: Developmental Neurotoxicity Study in the Rat
MRID No.: 47832133
Executive Summary: See Appendix A, Guideline § 870.6300
Dose and Endpoint for Risk Assessment: NOAEL = 1.8 mg/kg/day based on decreased neonatal survival PND 0-4 seen at LOAEL = 7.1 mg/kg/day.
Uncertainty Factors: 30 (3 X for interspecies (UF_A) and 10 X for intraspecies (UF_H) variability).
Comments about Study/Endpoint/Uncertainty Factors: The endpoint used for short- and intermediate-term dermal exposure was selected DNT study in rats. The endpoint of concern is decreased neonatal survival in PND 0-4 offspring with a NOAEL of 1.8 mg/kg/day and a LOAEL of 7.1 mg/kg/day. This study is appropriate for the durations of exposure as well as it is protective for the populations of concern (fetuses *in utero* who may be exposed when mothers are exposed in occupational settings).

The dermal equivalent dose (DED) using a dermal absorption value of 2.5% is 25 mg/kg/day (1000 mg/kg/day x 0.025 = 25 mg/kg/day). The NOAEL for the route-specific 28-day dermal study is 1000 mg/kg/day (no effects in females up to the limit dose). The DED using the 28-dermal study is greater than the dose where offspring effects were observed in the DNT and is therefore not protective of the developmental effects observed in the DNT. As such, use of the 28-day dermal study is not appropriate for dermal exposure assessment.

A.3.5 Inhalation Exposure (Short- and Intermediate-Term)

Study Selected: Developmental Neurotoxicity Study in the Rat
MRID No.: 47832133

Executive Summary: See Appendix A, Guideline § 870.6300
Dose and Endpoint for Risk Assessment: NOAEL = 1.8 mg/kg/day based on decreased neonatal survival PND 0-4 seen at LOAEL = 7.1 mg/kg/day.
Uncertainty Factors: 30 (3 X for interspecies (UF_A) and 10 X for intraspecies (UF_H) variability).
Comments about Study/Endpoint/Uncertainty Factors: The endpoint used for short- and intermediate-term inhalation exposure was selected DNT study in rats. The endpoint of concern is decreased neonatal survival in PND 0-4 offspring with a NOAEL of 1.8 mg/kg/day and a LOAEL of 7.1 mg/kg/day. This study is an appropriate duration of exposure and it is protective for the populations of concern (fetuses in utero who may be exposed when mothers are exposed in occupational settings). A default inhalation absorption factor of 100% is assumed in the absence of an inhalation study.

A.4 Executive Summaries

A.4.1 Subchronic Toxicity

870.350 28-Day Oral Toxicity

IIA 5.3.1/2 28-Day Oral Toxicity *(feeding)* Fischer 344 Rats;
In a 28-day dietary toxicity study (MRID 47832045), groups of five male and five female Fischer 344 rats were given test diets formulated to supply 0, 300, 1000, 2000, or 3000 ppm sulfoxaflor for 28 days. These dose levels corresponded to 0, 24.8, 79.4, 155, or 205 mg/kg/day for males and 0, 26.5, 88.3, 170 or 192 mg/kg/day for females, respectively. Toxicokinetic analysis of blood plasma was completed.

Administration of sulfoxaflor to male and female rats at 3000 ppm resulted in excessive reductions in feed consumption (31% and 36%) and body weight loss (21% and 19%) compared to controls after 9 days of administration, respectively. The 3000 ppm dose groups were euthanized (day 9). The lower feed consumption was attributed to decreased palatability of rodent feed containing sulfoxaflor and was responsible for the decreased body weights. Animals in all other dose groups exhibited a dose-related decrease in feed consumption at the start of exposure, which was due to decreased palatability of treated diets containing sulfoxaflor. Males given 300, 1000 or 2000 ppm consumed 5%, 29% or 54% less feed than control animals after one day. Females given 300, 1000, or 2000 ppm consumed 7%, 26% or 48% less than control animals. Feed consumption increased in animals given 1000 or 2000 ppm for the remainder of the study. Feed consumption for males given 300 or 1000 ppm was comparable to controls by the end of the study and was 6% decreased at 2000 ppm. Females given 300, 1000 or 2000 ppm consumed 8%, 6% or 11% less than controls at the end of the study.
Animals given 1000 or 2000 ppm had decreased body weight after one day of exposure, and was considered a secondary effect of the reduced feed consumption. By day 28, males and females given 2000 ppm weighed 8.5% and 10% less, respectively, than controls. Males and females given 300 or 1000 ppm had body weights comparable to controls on day 28.
Males and females (1000 or 2000 ppm) had dose related increases in serum total cholesterol levels, and both sexes given 1000 or 2000 ppm, also had total serum protein levels higher than controls. Albumin and globulin levels were higher than concurrent and historical controls and were considered to be treatment related in males given 1000 or 2000 ppm and females given 2000 ppm.

Males and females given 2000 ppm had treatment-related decreases in final body weights. Males and females given 1000 or 2000 ppm had increased absolute and relative liver weights that were dose and treatment related.

There were a number of organ weights of males and females given 2000 ppm that were altered and included: relative brain (males and females), relative kidney (males), relative testes (males), relative thyroid (males and females), absolute heart (males), and absolute spleen (males and females). These differences in organ weights were considered to be secondary to the lower body weights of this dose group. This conclusion is supported by the absence of histopathological changes in these organs.

Treatment-related histological effects were observed in the livers of males and females given 1000 or 2000 ppm and consisted of a dose related increase in the severity (very slight to moderate) of hepatocellular hypertrophy, with altered tinctorial properties, involving the centrilobular to midzonal regions of the hepatic lobule. Effects were more prominent in males, compared to females, increasing to moderate severity in 2000 ppm males. Vacuolization, consistent with fatty change, involving hepatocytes primarily in the right lateral lobe was also occasionally seen in a multifocal distribution in males given 1000 or 2000 ppm and in one female given 2000 ppm. The restriction of this alteration to only one liver lobe, the minor nature of the effect (very slight or slight) and the lack of a clear dose response relationship in regards to severity suggests that this may not be a significant effect.

Toxicokinetic analysis of the plasma showed that levels of sulfoxaflor (AUC24h) were effectively proportional to dose; 3.3 to 3.6 fold increase between 300 and 1000 ppm groups and a 2.0-fold increase between 1000 and 2000 ppm dose groups. Females were more efficient in eliminating the test material than males. The 24 hour systemic dose as measured by the AUC24h was 21, 15 and 14% lower in females than males at 300, 1000 and 2000 ppm dose groups, respectively (corresponding to 210, 693, 1371 µg h/ml versus 167, 591 and 1183 µg h/ml at the low, middle and high doses, respectively). Plasma elimination half-life of sulfoxaflor in male rats was between 7 and 8 hours; whereas it was 32-43% lower in females (between 4-5 hours).

The no-observed-adverse-effect-level (NOAEL) for both sexes is 300 ppm (26.5 mg/kg/day) and the lowest-observed- adverse-effect-level (LOAEL) is 1000 ppm (88.3 mg/kg/day), based on enlarged livers (size and weight) with hepatocellular hypertrophy, slight vacuolization (males), and increased cholesterol levels. This study is Fully reliable (acceptable/non-Guideline): GLP compliant and acceptable for use as a range-finding study

IIA 5.3.1/4 28-Day Oral Toxicity *[feeding]* CD1(ICR) mice

In a 28 day toxicity study in mice (MRID 47832042, PMRA 1941272), groups of five male and five female CD-1 mice were fed diets supplying 0, 300, 1500, or 3500 ppm sulfoxaflor (purity, 98.1%; Lot # C2120-16, TSN105885), equivalent to 0, 44, 230, 524 mg/kg/day in males and 0, 53, 273, and 638 mg/kg/day in females, for at least 28 days. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, hematology, clinical chemistry, selected organ weights, and gross and histopathologic examinations.

Feed consumption values for males and females given 1500 or 3500 ppm were lower than the controls during days 1-2, whereas feed consumption values were comparable to the controls after day 4. Slight treatment-related decreases in body weights and body weight gains were observed in males and females given 1500 or 3500 ppm during the first week of the study, however, they were comparable to controls through the rest of the study.

Toxicokinetic analysis of the plasma showed that dose-proportional intake of sulfoxaflor was translated into a dose-proportional increase in plasma concentrations of sulfoxaflor. The systemic exposure of sulfoxaflor was ~40% higher in males than in females. The 24-hour urine

urinary elimination of sulfoxaflor in males was between 33 and 44% and in females was between 23 and 65% of what they consumed during a 24-hour period, indicating that the majority of the dietary administered sulfoxaflor was excreted essentially unchanged in the urine.

At termination on day 30, males and females given 1500 or 3500 ppm had treatment-related elevations in mean serum ALP, ALT, AST activities and triglycerides. There were treatment-related increases in the mean liver weights and liver histopathology of males and females given 1500 or 3500 ppm compared to controls. Males given 300 ppm had marginal treatment-related increases in liver weights over the respective control values, but were not associated with detectable hepatocyte hypertrophy or clinical chemistry changes and therefore, considered a non-adverse effect. Males given 3500 ppm had treatment-related elevations in absolute and relative adrenal gland weights that corresponded to hypertrophy of the zona fasciculata of the adrenal cortex. Males given ≥ 1500 ppm had treatment-related lower absolute and relative kidney weights. In summary, due to higher systemic exposure in male mice, higher toxicity of sulfoxaflor was observed.

The no-observed-adverse-effect-level (NOAEL) in male and female mice is 300 ppm (44 and 53 and mg/kg/day, respectively). The lowest-observed-adverse-effect-level (LOAEL) in male and female mice is 1500 ppm (230 and 273 mg/kg/day, respectively), based on increased liver weights, increased liver enzymes (ALT, AST), and hepatocellular hypertrophy in both sexes. Liver histopathology (necrosis) in male mice was also seen at this dose. At 3500 ppm (524 and 638 mg/kg/day, respectively), the same effects were seen along with increased triglycerides and ALP in both sexes, liver histopathology in males (mitotic cells, vacuolization/fatty change) and females (necrosis), and adrenal effects (increased weights and hypertrophy) in males.

This study is fully reliable (acceptable/guideline).

IIA 5.3.1/6 28-day Palatability/Tolerability Probe Study in Beagle dogs;

In a 28-day oral palatability probe study (MRID 47832043), sulfoxaflor (XDE-208 technical grade, 95.6% a.i.; lot no. E2162-34 or analytical grade, 99.7% a.i., lot no. 20062-464-9) was administered to Beagle dogs via dietary, capsule or oral gavage administration for up to 28 days. Using the same animals (with 1 or 2-week breaks of basal diet only between dosing regimens), routes of test material administration and dosing regimens were altered for a total of five dosing groups, during the course of the study as follows. Three female dogs received technical grade test material via the diet, *ad libitum*, for 6 consecutive days at a dose level of 500 ppm (Group 1). The dosing route for these animals was changed to analytical grade test material via oral gavage, once daily for 28 consecutive days, at a dose level of 15 mg/kg/day (Group 3). Another group of three female dogs received the technical test material via capsule, twice daily for 6 consecutive days, at a dose level of 15 mg/kg/day (Group 2). The dosing route for these animals was changed to dietary, *ad libitum* (technical grade) for 5 consecutive days, at a dose level of 100 ppm (Group 4). The dosing route for these animals was once again changed to oral gavage (technical grade), once daily for 28 consecutive days, at a dose level of 15 mg/kg/day (Group 5). For both oral gavage groups, the vehicle was 0.5% methylcellulose in deionized water and the dose volume was 10 mL/kg. A previous, preliminary palatability probe study (MRID 47832056) did not identify adequate oral acceptance by diet and capsule administration.

Observations for morbidity, mortality, injury and the availability of food and water were conducted twice daily for all animals. Clinical observations were conducted daily. Body weights and food consumption were measured and recorded daily. Blood samples for clinical pathology evaluations were collected from all animals pretest and on Days 6 and 29 (prior to necropsy), and urine samples were collected at necropsy from all animals on Day 29. Blood samples for determination of the plasma concentrations of the test article were collected from animals in Group 4 at designated time points on Day 5 (the last day of dietary dosing), and from animals in Groups 3 and 5 at designated time points on each respective Day 28 (prior to necropsy). At study termination, necropsy examinations were performed and organ weights were recorded. A complete set of tissues from Group 5 animals was sent to the Sponsor for microscopic evaluation. Findings were compared with historical range data due to the lack of concurrent controls.

All animals survived until the scheduled termination intervals. However, there were clinical findings and body weight findings that were associated with reduced food consumption (50% or more compared to pretreatment). In some instances, the reduced food consumption was accompanied by a lack of sufficient fluid intake which resulted in a loss of skin elasticity. In addition, intermittent emesis and fecal alterations (discolored, mucoidal, and soft/watery feces) was likely test material-related.

Oral gavage administration at 15 mg/kg/day was a tolerable route and concentration for the technical grade test material exposure as determined by adequate food consumption and tolerable in-life clinical signs. Exposure at 100 ppm via dietary route was well tolerated as determined by adequate food consumption, but the amount of test material consumed was not sufficient to justify it as a potential highest dose for subsequent studies. Exposure at 500 ppm via dietary route or 15 mg/kg/day via capsule route was not well tolerated as determined by insufficient food consumption. Therefore, the dietary route could not be considered a viable method of test material exposure over a sustained duration. Based on this information, oral gavage exposure was determined to be the most appropriate route for a sustained duration of test material exposure in Beagle dogs.

This non-guideline study is reliable with restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data. It was conducted to determine palatability and the appropriate method of oral administration for subchronic and chronic oral toxicity studies and not to satisfy guideline requirements.

IIA 5.8.1.3/2 X11719474: 28-Day Dietary Toxicity Study in F344/DuCrl Rats;
In a 28-day dietary toxicity study (MRID 47832099), five male and five female Fischer 344 rats per group were given test diets formulated to supply 0, 1000, 2000, 3000 or 8000 ppm X11719474 (Purity 99.5%; Lot # E-2695-1, TSN030626-0003) for 29 days. These concentrations corresponded to time-weighted average doses of 0, 83.4, 167, 244 or 662 mg/kg bw/day for males and 0, 90.1, 184, 278 or 734 mg/kg bw/day for females, respectively. Standard parameters were evaluated as well as toxicokinetic analyses of plasma and urine.

There were no treatment-related effects on clinical signs, ophthalmic observations, hematologic or coagulation parameters.

In the 8000 ppm dose group, males and females exhibited reduced body weight gain compared to controls during the first five days of treatment, and this effect was considered secondary to reduced feed consumption. After five days of treatment, body weight gains for males and females were 27% or 20% lower than controls, respectively. By Day 29, body weights and body weight gains were similar to control values. Decreased in feed consumption during the first five days of the study was considered to reflect decreased palatability of the treated diet. Males and females consumed ~11% or ~7% less feed than controls, respectively, during this period. However, feed consumption was comparable to controls by the end of the study. Feed consumption values for males and females given 0, 1000, 2000 or 3000 ppm were either slightly higher or comparable to controls throughout the study.

Also in the 8000 ppm dose group, blood cholesterol levels (25% in males and 22% in females), and liver weights (33% in males and 14% in females) were higher compared to controls. Triglyceride levels decreased 2-fold in males given 3000 and 8000 ppm X11719474. Males and females given 8000 ppm and males given 3000 ppm had slight treatment-related hypertrophy of hepatocytes. All other histopathologic observations were considered to be spontaneous alterations unassociated with exposure to X11719474.

Absorption of the X11719474 from the diet was dose-proportional for both genders. The ingested X11719474 was rapidly eliminated from plasma upon the removal of the fortified diet (fasting) and dropped below the limit of quantification at the time of sacrifice in most of the rats. The elimination half-life of X11719474 from plasma was between 2.7 and 3.6 hours. Male rats eliminated an average of 54 ± 21% of what they consumed during a 24-hour period (range: 40 to 85%), while elimination by females averaged 81 ± 10% (range: 66 to 89%) of the test material consumed in 24 hours. Overall, these data show that X11719474 was significantly absorbed following dietary administration and quickly eliminated, primarily via urine as parent compound. The ratio of diastereomers was not substantially different in the plasma and urine samples when compared with the matrix samples added with the test material.

The lowest-adverse-effect-level (LOAEL) was 3000 ppm (241mg/kg bw/day) based on decreased triglycerides in the males. The no-observed-effect-level (NOAEL) was 2000 ppm (167 mg/kg bw/day).

This subchronic toxicity study in the rats is Fully Reliable (acceptable/guideline) and satisfies the guideline requirement (OECD 407) for a repeat-dose oral study in rats.

IIA 5.8.2.3/2 X11721061: A 28-Day Oral Dietary Toxicity Study in Fischer 344/DuCrl Rats;

This study (MRID 47832036) was conducted to evaluate the potential toxicity of X11721061, a metabolite of the experimental insecticide sulfoxaflor, in rats when administered orally via the diet for 28 days. Three treatment groups of five male and five female Fischer 344 CDF® [F-344/DuCRL] rats were administered the test article at dose levels of 1000, 2000, 3000, and

8000 ppm. One additional group of five animals/sex served as the control and received untreated diet.

Standard parameters Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Cageside observations were conducted daily. Detailed clinical observations were conducted weekly. Body weights were measured and recorded prior to randomization (Day –2 and -3) and on Days 1, 5, 8, 15, 22, and 29. Food consumption was measured and recorded pretest (Days -4 to -1) twice during the first week, and once weekly for the remainder of the study. Compound consumption was calculated during each interval food consumption was measured, beginning on Day 1. Ophthalmoscopic examinations were conducted pretest and prior to the terminal necropsy. Blood and urine samples for clinical pathology evaluations were collected from all animals prior to the terminal necropsy. Blood samples for plasma analysis were collected from all animals at designated time points on Days 28 and 29. At study termination, necropsy examinations were performed and organ weights were recorded. Tissues were microscopically examined for animals at 0 (control) and 8000 ppm.

No treatment-related deaths occurred. There were no treatment-related effects on clinical signs, ophthalmic observations, hematologic or coagulation parameters. Slight reductions in food consumption noted in males and females at the highest dose (8000 ppm) during the first 5 days of treatment only were considered to reflect transient reduced palatability of the diet. No treatment-related clinical signs of toxicity or affects on body weight were observed.

Blood cholesterol levels for males and females given 8000 ppm were higher than the controls, statistically significant, and treatment related. Males and females given 8000 ppm had statistically-identified higher absolute (33% and 14%, respectively) and relative (37% and 18%, respectively) liver weights than the controls. These differences were interpreted to be treatment related. There were no other treatment-related alterations or statistical differences in the organ weights for males or females.

Males and females given 8000 ppm and males given 3000 ppm had a slight treatment-related hypertrophy of hepatocytes in the centrilobular/midzonal region of the hepatic lobules. This effect was not considered adverse at 3000 ppm since liver weight and clinical chemistry were not significantly impacted at this dose. All other histopathologic observations were considered to be spontaneous alterations unassociated with exposure to X11721061.

Under the conditions of this study, the lowest-observed-adverse-effect-level (LOAEL) for males and females was 8000 ppm (662/734 mg/kg/day (M/F)) based on increased liver weights, hepatic hypertrophy, and increased cholesterol levels. The no-observed-adverse-effect level (NOAEL) for males and females was 3000 ppm (244/278 mg/kg /day (M/F)).

IIA 5.8.5.3/2 X11519540: 28-day Dietary Toxicity Study in F344/DuCrl Rats;
In a 28-day repeated oral dose study (MRID 48288941), groups of five male and five female F344/DuCrl rats were given test diets formulated to supply 0, 100, 300, 1000 or 2000 ppm X11519540 (targeted doses of 0, 10, 30, 100, or 200 mg/kg/day, respectively) for at least 28 days. These dose levels corresponded to time-weighted average doses of 0, 7.7, 23.1, 74.0, and 140 mg/kg/day for males and 0, 8.5, 24.9, 77.2 and 152 mg/kg/day for females, respectively.

Standard parameters were evaluated, as well as toxicokinetic analysis of plasma and urine, and gene expression analysis of liver tissue.

Rats given 1000 or 2000 ppm had treatment-related lower body weights and body weight gains throughout the study, relative to controls. At study termination, the body weight gains of males given 1000 or 2000 ppm were 17.4 and 29.6% lower than controls, and the body weight gains of females given 1000 or 2000 ppm were 17.0 and 26.4% lower than controls, respectively. Males and females given 1000 or 2000 ppm also had treatment-related decrements in feed consumption. The lower feed consumption was interpreted to be caused by reduced palatability of the diets, resulting in lower body weight and body weight gains. There were no treatment-related effects on body weights, body weight gains, or feed consumption in male or female rats given 100 or 300 ppm.

Males and females given 1000 or 2000 ppm had treatment-related lower red blood cell counts, hemoglobin concentrations and hematocrit levels. The decrements in erythrocytic parameters were dose-related, and were interpreted to be secondary to lower feed consumption at these dose levels.

Males given 1000 or 2000 ppm had treatment-related higher serum alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, total protein, albumin, and cholesterol concentrations, relative to controls. In addition, males given 2000 ppm had a treatment-related higher gamma-glutamyl transpeptidase (GGT) activity. Males given 300 ppm had treatment-related higher total protein, albumin and cholesterol concentrations. Females given 1000 or 2000 ppm had treatment-related higher GGT activity, total protein, albumin, globulin and cholesterol concentrations. Females given 300 ppm had treatment-related higher total protein, albumin and cholesterol concentrations.

The urine pH of males given 300, 1000 or 2000 ppm, and of females given 1000 or 2000 ppm, was slightly lower than controls. Males given 2000 ppm had slightly higher protein concentration in the urine, relative to controls. The alterations in urine pH and protein were interpreted to be treatment related.

Treatment-related decreases in final body weights occurred in both males and females given 1000 (6.5 and 6.6%, respectively) and 2000 ppm (11.1% and 6.8%, respectively) when compared to controls. Treatment-related increases in the mean absolute and relative liver weights were noted in males and females given 100 (33.1 and 30.6%, and 19.9 and 25.7%, respectively), 300 (69.8 and 67.4%, and 53.3 and 59.8%, respectively), 1000 (101 and 116%, and 117.5 and 132.7%, respectively) or 2000 ppm (106.8 and 133.5%, and 143.4 and 161.4%, respectively), relative to controls. The higher liver weights corresponded with hypertrophy of centrilobular and midzonal hepatocytes at all dose levels.

Males and females from all dose levels had treatment-related higher absolute and relative adrenal gland weights. The higher adrenal weights corresponded with histopathologic findings of very slight or slight hypertrophy of the zona fasciculata in males and females given 300, 1000 or 2000 ppm, and very slight or slight increased vacuolization of cortical cells in males from all dose groups and females given 300, 1000 or 2000 ppm.

One female given 2000 ppm died, and one male given 1000 ppm and one female given 2000 ppm were euthanized in a moribund condition, following blood collection for toxicokinetic plasma samples on test day 28. All three animals had large axillary hematomas secondary to jugular vein bleeding. Excessive hemorrhage was interpreted to be the cause of moribundity or death in these animals, and may have been reflective of a treatment-related impairment of coagulation. However, the prothrombin times and activated partial thromboplastin times were unaffected by treatment in males and females at all dose levels.

The liver was the primary target organ. Male and female rats from all dose levels had treatment-related hepatocellular hypertrophy with altered tinctorial properties (increased cytoplasmic eosinophila) of centrilobular and midzonal hepatocytes. The severity of this alteration (very slight to moderate) increased in a dose-related manner. Other treatment-related liver effects consisted of: very slight increased numbers of mitotic figures (males at all dose levels, females at 300, 1000 or 2000 ppm), very slight multifocal necrosis of individual hepatocytes (males and females at 300, 1000 and 2000 ppm), and very slight multifocal hepatocellular necrosis with accompanying inflammation (males at 1000 and 2000 ppm and females at 1000 ppm). The other primary treatment-related effect was an increase in the incidence of very slight multifocal degeneration of tubules in the kidneys, which occurred in males given 300, 1000 or 2000 ppm, and in females given 2000 ppm.

Males from all dose levels had treatment-related very slight or slight diffuse follicular cell hypertrophy of the thyroid gland, and females given 1000 or 2000 ppm had very slight diffuse hypertrophy of the thyroid gland. The thyroid hypertrophy may have been caused by a treatment-related induction of liver microsomal enzymes responsible for biliary excretion of thyroid hormones, with resultant chronic stimulation of the thyroid by thyroid stimulating hormone through disruption of the hypothalamic – pituitary – thyroid axis.

Males and females given 300, 1000 or 2000 ppm had treatment-related very slight or slight hypertrophy of the zona fasciculata of the adrenal glands. In addition, males from all dose levels, and females given 300, 1000 or 2000 ppm had treatment-related very slight or slight increased vacuolization of the adrenal cortex (zona glomerulosa, zona fasciculata and zona reticularis). Males given 1000 or 2000 ppm and females given 2000 ppm had very slight diffuse acinar cell hypertrophy of the submandibular salivary gland. The adrenal and salivary effects were interpreted to be secondary, stress-related effects.

Males and females given 1000 or 2000 ppm had treatment-related very slight or slight erythroid cell hyperplasia of the bone marrow. Males given 2000 ppm had a treatment-related increase in the incidence of very slight extramedullary hematopoiesis (erythroid cell) of the spleen. The bone marrow and spleen effects were interpreted to be reflective of a regenerative response to the lower red blood cell counts, hemoglobin concentrations and hematocrits in males and females given 1000 or 2000 ppm.

Males and females given 1000 or 2000 ppm had treatment-related very slight or slight atrophy of the mesenteric adipose tissue. Females given 1000 or 2000 ppm had treatment related very slight or slight decreased size of the uterus. Females given 2000 ppm also had treatment-related

slight decreased size of the cervix and vagina. The atrophy of mesenteric adipose tissue and decreased size of the female reproductive tract were interpreted to be secondary to lower feed consumption and lower body weight gains of animals given 1000 or 2000 ppm.

Toxicokinetic analysis of plasma after 28 days of dietary administration to X11519540 showed that the systemic exposure of X11519540 (as plasma AUC_{24h}) deviated from linearity (in both sexes) between the low (100 ppm) and mid-low (300 ppm) dose groups. The mean plasma elimination half-lives of X11519540 were calculated to be 27 and 28 hours in male rats of the 1000 and 2000 ppm dose groups, respectively; plasma elimination half-lives in female rats, of the 1000 and 2000 ppm dose groups, were 35 and 24 hours, respectively. Plasma elimination half-lives could not be reliably calculated for animals from the 100 and 300 ppm dose groups as the X11519540 concentrations in plasma at terminal sacrifice were in most cases roughly equivalent to plasma levels measured at the time at which the test material formulated feed was withdrawn from the animals.

The amount of X11519540 eliminated in urine, over a 24 hour collection interval, deviated from linearity (in both sexes) between the low (100 ppm) and mid-low (300 ppm) dose groups. In male rats 47%, 31%, 18%, and 13% of the calculated daily TMI was eliminated over 24 hours in urine of low, mid-low, mid-high, and high dose groups. A similar pattern was observed in female rats with 51%, 28%, 15%, and 11% of the calculated daily TMI eliminated in urine of low, mid-low, mid-high, and high dose groups.

Targeted gene expression analysis of *Cyp2b1*, *Cyp2b2*, and *Cyp3a3* in the liver tissue showed significantly elevated transcripts at 100, 300 and 1000 ppm (2000 ppm was not tested for these endpoints). These data suggest that X11519540 may stimulate gene expression consistent with constitutive androstane nuclear receptor (CAR) activation, and were similar to those seen in previous studies with sulfoxaflor (the parent compound of X11519540) and X11719474 (another metabolite of sulfoxaflor).

The Lowest-observed-adverse-effect-level (LOAEL) is 300 ppm (23.1 mg/kg/day) based on changes increased liver weight and histopathology along with degeneration of kidney tubules (in males). The no-observed-adverse-effect-level (NOAEL) is 100 ppm (7.7 mg/kg/day).

This subchronic toxicity study in the rats is acceptable and satisfies the guideline requirement (OECD 407) for a repeat-dose oral study in rats.

II 5.8.1.3/2 X11719474: 28-Day Dietary Toxicity Study in F344/DuCrl Rats;
In a 28-day dietary toxicity study (MRID 47832099), five male and five female Fischer 344 rats per group were given test diets formulated to supply 0, 1000, 2000, 3000 or 8000 ppm X11719474 (Purity 99.5%; Lot # E-2695-1, TSN030626-0003) for 29 days. These concentrations corresponded to time-weighted average doses of 0, 83.4, 167, 244 or 662 mg/kg/day for males and 0, 90.1, 184, 278 or 734 mg/kg/day for females, respectively. Standard parameters were evaluated as well as toxicokinetic analyses of plasma and urine.

There were no treatment-related effects on clinical signs, ophthalmic observations, hematologic or coagulation parameters.

In the 8000 ppm dose group, males and females exhibited reduced body weight gain compared to controls during the first five days of treatment, and this effect was considered secondary to reduced feed consumption. After five days of treatment, body weight gains for males and females were 27% or 20% lower than controls, respectively. By Day 29, body weights and body weight gains were similar to control values. Decreased in feed consumption during the first five days of the study was considered to reflect decreased palatability of the treated diet. Males and females consumed ~11% or ~7% less feed than controls, respectively, during this period. However, feed consumption was comparable to controls by the end of the study. Feed consumption values for males and females given 0, 1000, 2000 or 3000 ppm were either slightly higher or comparable to controls throughout the study.

Also in the 8000 ppm dose group, blood cholesterol levels (25% in males and 22% in females), and liver weights (33% in males and 14% in females) were higher compared to controls. Triglyceride levels decreased 2-fold in males given 3000 and 8000 ppm X11719474. Males and females given 8000 ppm and males given 3000 ppm had slight treatment-related hypertrophy of hepatocytes. All other histopathologic observations were considered to be spontaneous alterations unassociated with exposure to X11719474.

Absorption of the X11719474 from the diet was dose-proportional for both genders. The ingested X11719474 was rapidly eliminated from plasma upon the removal of the fortified diet (fasting) and dropped below the limit of quantification at the time of sacrifice in most of the rats. The elimination half-life of X11719474 from plasma was between 2.7 and 3.6 hours. Male rats eliminated an average of 54 ± 21% of what they consumed during a 24-hour period (range: 40 to 85%), while elimination by females averaged 81 ± 10% (range: 66 to 89%) of the test material consumed in 24 hours. Overall, these data show that X11719474 was significantly absorbed following dietary administration and quickly eliminated, primarily via urine as parent compound. The ratio of diastereomers was not substantially different in the plasma and urine samples when compared with the matrix samples added with the test material.

The no-observed-adverse effect level (NOAEL) for males and females was 2000 ppm (167/184 mg/kg/day (M/F)) based on increased triglyceride at 3000 ppm. Increased cholesterol levels along liver changes (increased weight and histopathology) were observed at 8000 ppm. .
This subchronic toxicity study in the rats is Fully Reliable (acceptable/guideline) and satisfies the guideline requirement (OECD 407) for a repeat-dose oral study in rats.

870.3100 90-Day Oral Toxicity – Rat

IIA 5.3.2/1 90-Day Subchronic Oral Toxicity in rats;
In a 90-day oral toxicity study (MRID 47832046), ten male and ten female Fischer 344 rats per group were given test diets formulated to supply 0, 100, 750 or 1500 ppm sulfoxaflor (purity 96.6%; Lot # E2198-17, TSN106108) corresponding to time-weighted average concentrations of 0, 6.36, 47.6, or 94.9 mg/kg/day for males and 0, 6.96, 51.6, or 101 mg/kg/day for females, respectively. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, functional observational battery (FOB, pre-exposure and prior to necropsy, comprising cage-side, hand-held, and open field observations, rectal

temperature, fore- and hindlimb grip performance, landing foot splay, and motor activity), body weights, feed consumption, prothrombin time, hematology, urinalysis, clinical chemistry, selected organ weights and gross and histopathologic examinations, which included a specifically detailed review of the nervous system. The study also included integrated toxicokinetics and an assessment of immunotoxicity.

Males (all treated doses) and females (750 ppm and 1500 ppm) exhibited dose-related lower feed consumption, which was due to decreased palatability of diets containing sulfoxaflor. During the first four days males given 100, 750 or 1500 ppm consumed 5%, 12% or 24% less feed than controls, and females given 750 or 1500 ppm consumed 8% or 21% less feed than controls. However, feed consumption for males at all dose levels was comparable to controls by the end of the study. Female feed consumption in the 750 and 1500 ppm groups was 5% or 8% lower than controls at 90 days, respectively, and statistically identified.

Male and females given 750 or 1500 ppm gained less weight than controls during the first four days of treatment and were dose related. After four days of treatment, body weight gains of males given 750 or 1500 ppm were 24% or 45% lower than controls, respectively, and body weight gains of females given 750 or 1500 ppm were 13% or 60% lower than controls, respectively. These animals gained weight for the remainder of the study. By day 90, males and females given 750 or 1500 ppm weighed 8% or 9% and 3% or 8% less than controls, respectively. By day 90, the body weight gain of males and females given 750 or 1500 ppm was 11% or 13% and 9% or 20% less than controls, respectively. All body weight effects were considered secondary to the lower feed consumption due to decreased palatability of the test material in the feed. Males and females given 100 ppm had body weights comparable to the controls at the end of the 90-day study.
Serum cholesterol levels in males (127%) and females (83%) given 1500 ppm were significantly greater than controls and considered adverse. All other clinical pathology values were comparable to control values.

Rats given 750 or 1500 ppm had statistically identified higher absolute and relative liver weights that were dose related, and interpreted to be treatment related. The absolute liver weights of males and females given 750 or 1500 ppm was 6% or 5% and 25% or 17% higher than controls, respectively and the relative liver weights of males and females given 750 or 1500 ppm was 14% or 8% and 41% or 27% higher than controls, respectively. There were a number of additional differences in organ weights of males and females given 750 or 1500 ppm that were statistically identified. These differences in organ weights were secondary to the lower body weights of these dose groups and did not reflect a primary target organ effect of sulfoxaflor. This conclusion was supported by the absence of histopathological changes in these organs.

Treatment-related histological liver effects occurred in male and females given 750 or 1500 ppm and consisted of a dose-related increase in the severity (slight to moderate) of hepatocellular hypertrophy (with altered tinctorial properties) involving the centrilobular to midzonal regions of the hepatic lobule. Individual hepatocyte necrosis was also observed in the centrilobular region with a multifocal distribution to a very slight or slight degree. All effects were seen in both sexes but were more prominent in males compared to females. Vacuolization of hepatocytes, consistent with fatty change, was also observed in all males in the 750 and 1500 ppm groups at very slight, slight or moderate degrees. In addition, in the rats with the greatest degree of

hepatocellular hypertrophy, necrosis and vacuolization, there was an increase in the incidence of rats with multifocal aggregates of macrophages- histocytes. The microscopic changes were present in all three lobes of the liver examined in male and female rats; however, they were more readily apparent in the right lateral lobe. The microscopic changes in the liver were consistent with the increased liver weights and cholesterol levels noted for these rats.

Assessment of immunotoxicity as measured by immune responsiveness in the sheep red blood cell antibody-forming cell assay indicated there was no effect on immune responsiveness in female rats up to and including the high dose level of 1500 ppm. There was no effect on immune responsiveness for male rats in the 100 and 750 ppm groups, while the 1500 ppm group displayed a lower, non-statistically significant, response (26% lower) when compared to the vehicle control group. The lower AFC response in the high dose male group coincided with considerable general toxicity, including decreased body weights and increases in liver weight (absolute and relative), hepatocellular hypertrophy, necrosis, vacuolization consistent with fatty change, multi-focal aggregates of macrophages, and elevated serum cholesterol, for which the overall NOEL was 100 ppm. Therefore, the lower AFC response in the high dose males was considered secondary to systemic toxicity and thus does not reflect primary immunotoxic potential for sulfoxaflor.

The potential to recover from the effects induced by sulfoxaflor was demonstrated in male and female rats given 0 or 1500 ppm for 90 days, followed by control feed for 28 days. Nearly complete recovery was seen in body weights (only ~5% lower for both sexes). Feed consumption during the 28-day recovery period was comparable to controls in both sexes. Also, the serum cholesterol levels that were elevated during the 90-day study in both males and females were normal following the 28-day recovery period and a complete recovery was seen in the absolute and relative liver weights of males and females given 1500 ppm.
There was partial recovery of the microscopic hepatic effects. Two male rats in the 1500 ppm group still had recognizable hepatocellular hypertrophy of a very slight degree in the centrilobular and midzonal regions. One of these two rats also had multifocal, very slight individual hepatocellular necrosis. Multifocal, very slight or slight, hepatocellular vacuolization consistent with fatty change was present in most of the recovery males; however, the degree of involvement was substantially less severe in the recovery group. There were no microscopic treatment-related changes present in the liver in females given 1500 ppm.

Toxicokinetic analysis of the plasma showed that the systemic exposure of sulfoxaflor was dose proportional. An ~8-fold increase in AUC_{24h} was found between 100 and 750 ppm groups and a ~2-fold increase between 750 and 1500 ppm doses. Females were more efficient at eliminating the test material from their system than males. The 24-hour systemic dose as measured by the plasma AUC_{24h} was 15, 16 and 14 percent lower in females than males at 100, 750 and 1500 ppm nominal dose groups, respectively. Plasma elimination half-life of sulfoxaflor in male rats was ~9 hours; whereas, it was ~8 hours in females (12% lower). The chromatograms of the plasma samples taken from sulfoxaflor dosed rats (via diet) contained up to 5 minor peaks in addition to the parent compound. These peaks may represent metabolites of the test material or metabolites of test material impurities. Absolute quantitation of the minor metabolites could not be made, due to lack of reference standards. Elimination of sulfoxaflor in urine over 24 hours ranged between 51 and 61% of the ingested dose, with the exception of high dose in males which was 37% of the ingested

dose, 26 days after the initiation of the study. Elimination of test material in 0-24 hr urine on days 84 and 85 ranged between 52-69% for the lower two dose levels, but was somewhat lower at the high dose for both sexes (32-36% of ingested dose). In addition to parent sulfoxaflor, four urinary metabolites were detected. One peak was a known impurity in this lot of the test material. No definitive quantitation of the other three metabolites was obtained.

In conclusion, during the first few days of the study decreased feed consumption and body weight gain was observed at all dose levels. Subsequently feed consumption and body weight gain returned to normal in the 100 ppm group. There were no other observations noted in the parameters evaluated at this dose level. At 750 ppm and 1500 ppm the primary toxicologically significant findings were increased serum cholesterol levels, increased liver weight and histopathological findings in the liver. All of the effects due to ingestion of the test material had recovered during the 28-day recovery phase, except minor histopathologic liver effects noted only in males given 1500 ppm.

The lowest-observed-adverse-effect level (LOAEL) for systemic toxicity is based on the observation of decreased body weight, elevated cholesterol levels, hepatoxicity (increased weight, hypertrophy, necrosis, and vacuolization) at 750 ppm (47.6 mg/kg/day). The no-observed-adverse-effect-level (NOAEL) is 100 ppm (6.36 mg/kg/day).

There were no indications of neurotoxicity at any dose level, therefore, a LOAEL was not determined. The NOAEL is ≥ 1500 ppm (94.9 mg/kg/day) for neurotoxic effects.
There were no indication of immunotoxicity at any dose level, therefore, the LOAEL was not determined. The NOAEL is ≥ 1500 ppm (94.9 mg/kg/day) for immunotoxic effects.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408), subchronic neurotoxicity (OPPTS 870.6200; OECD 424), and immunotoxicity (OPPTS 870.7800) study in rats.

870.3100 90-Day Oral Toxicity - Mouse

IIA 5.3.2/2 90-Day Dietary Toxicity Study in Crl:CD1(ICR) – Mice;
In a 90 day oral toxicity study (MRID 47832057, PMRA 1941277), groups of ten male and ten female CD-1 mice were fed diets supplying 0, 100, 750 or 1250 ppm and 0, 100, 1500, 3000 ppm sulfoxaflor (purity 96.6%; Lot # E2198-17, TSN106108), respectively for at least 90 days. These concentrations supplied average doses of 0, 12.8, 98.0 or 166 mg/kg/day for males and 0, 16.2, 247 or 489 mg/kg/day for females. Parameters evaluated were: daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, hematology, clinical chemistry, selected organ weights, and gross and histopathologic examinations. In addition, toxicokinetic analyses were conducted on urine (day 80) and terminal blood plasma.

There were no treatment-related effects on clinical signs, ophthalmic parameters, body weights or feed consumption.

Toxicokinetic analysis of the plasma showed that dose-proportional intake of sulfoxaflor translated into a dose-proportional increase in plasma concentrations of sulfoxaflor only up to the mid dose for both male (750 ppm, 92 mg/kg/day) and female (1500 ppm, 227 mg/kg/day) mice. In males, the systemic exposure, as measured by the plasma concentration of sulfoxaflor, became supra-linear between the mid (92 mg/kg/day) and high (152 mg/kg/*day*) doses (3.9-fold increase instead of 1.6-fold expected from the difference in the test material intake between the mid and high doses). Plasma concentrations of sulfoxaflor in females reached a plateau, remaining almost unchanged between the mid (227 mg/kg/day) and the high (467 mg/kg/day) doses. Total elimination of sulfoxaflor in 24-hour urine remained dose-proportional only up to the mid dose and showed less than dose-proportional increase at the highest dose, both in male and female mice. These data are consistent with a saturation of elimination of sulfoxaflor in male mice at the highest dose and a saturation of absorption of sulfoxaflor from the gastrointestinal tract in female mice at the highest dose. On the basis of these results, the kinetically-derived maximum dose (KMD) *i.e.*, the dose above which kinetics become non-linear was considered to be 92 (750 ppm) and 227 (1500 ppm) mg/kg/day for male and female mice, respectively.

Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had treatment-related increases in absolute (20% and 74%; 36% and 54%, respectively) and relative liver weights (26% and 85%; 40% and 50%, respectively) compared to controls. Treatment-related organ weight changes consisted of increased absolute and relative adrenal weights in males given 1250 ppm, and lower absolute kidney weights in males given 750 or 1250 ppm. The lower kidney weights were however, considered non-adverse. Males given 1250 ppm had a treatment-related 200% increase in serum alanine aminotransferase (ALT), 43% increase in aspartate aminotransferase (AST) and 142% increase in alkaline phosphatase (ALP) activities. Serum total cholesterol and total bilirubin concentrations were decreased in males given 750 and 1250 ppm, and were attributed to treatment. Females given 1500 or 3000 ppm had treatment-related elevations in serum ALT (125% and 171% increase, respectively) and AST activities (44% and 31% increase, respectively), and decreased ALP activity (3000 ppm only) compared to controls. Serum triglycerides were elevated in females given 1500 or 3000 ppm and serum cholesterol concentration was elevated in females given 3000 ppm, and attributed to treatment. There was a minor, treatment-related reduction in hematocrit and hemoglobin concentration in females given 1500 or 3000 ppm.

Males given 750 or 1250 ppm had slight or moderate, and females given 1500 or 3000 ppm had very slight, slight or moderate, treatment-related, centrilobular to midzonal hepatocyte hypertrophy with altered tinctorial properties. Other treatment-related histologic liver effects consisted of an overall, very slight or slight increase in the numbers of mitotic figures (hepatocytes in mitosis) in the liver of males given 1250 ppm, and very slight or slight fatty change in hepatocytes of males given 750 or 1250 ppm. Males given 750 or 1250 ppm had treatment-related, very slight or slight necrosis of scattered, individual hepatocytes, whereas this change in females given 1500 or 3000 ppm was infrequent or minimal. Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had treatment-related, very slight hypertrophy of the zona fasciculata of the adrenal cortex. A very slight, treatment-related fatty change was also present in the zona fasciculata of the adrenal cortex in some females given 1500 or 3000 ppm.

Four out of ten females given 3000 ppm had very slight, treatment-related increase in extramedullary erythrocytic hematopoiesis in the spleen.

The No-Observed-Adverse-Effect Level (NOAEL) for males and females is 100 ppm (13 and 16 mg/kg/day, respectively). The Lowest-Observed-Adverse-Effect-Level (LOAEL) for males is 750 ppm (98 mg/kg/day), based on increased liver weights and hypertrophy, liver histopathology (necrosis/fatty change), increased cholesterol and bilirubin, and adrenal hypertrophy. At 1250 mg/kg/day (169 mg/kg/day), the same effects were observed, along with increased liver enzymes (ALT, AST, ALP), mitotic figures (hepatocytes), and increased adrenal weights.

The LOAEL in females is 1500 ppm (247 mg/kg/day), based on increased liver weights, liver hypertrophy, increased liver enzymes (ALT, AST), increased triglycerides, decreased hemoglobin and hematocrit, and adrenal changes (hypertrophy and fatty change/vacuolization). At 3000 mg/kg/day (489 mg/kg/day), the same effects were observed, along with decreased ALP, increased cholesterol, and hematopoiesis in the spleen. The toxicokinetics data indicates that there is a saturation of absorption from the intestinal tract at 1500 ppm in females and a maximal excretory level at 750 ppm in males.

This study is fully reliable (US EPA Acceptable/Guideline). The subchronic toxicity study in the mice satisfies the guideline requirement for a subchronic oral study (OPPTS 870.3100; OECD 408) in mice.

870.3150 90-Day Oral Toxicity – Dog

IIA 5.3.3/1 90-Day Oral Gavage Toxicity Study in Beagle Dogs;
In a 90 day oral dog study (MRID 47832058), sulfoxaflor (XDE-208 technical, 95.6% a.i., lot no. E2162-34) was administered by gavage to 4 beagle dogs/sex/dose at dose levels of 0 (vehicle only), 1, 3 or 10 mg/kg/day. The 10 mg/kg/day dose level was reduced to 6 mg/kg/day on Day 5 of the study due to intolerance (lack of food consumption). Controls received the vehicle, 0.5% methylcellulose (Methocel A4C) in water. The test material or vehicle was given once a day for 90 consecutive days, at a dose volume of 10 mL/kg.

Observations for morbidity, mortality, injury, availability of food and water were conducted twice daily. Clinical observations were recorded weekly. Food consumption was measured daily from Days 1 to 7 and 9 to 35, twice weekly from Week 6 to 12, and once during Week 13. Blood and urine samples were collected from all animals pretest and during Weeks 6 and 13. Blood and urine samples were collected from all animals at intervals during Week 13 for plasma and urine concentrations of the test material. At termination, necropsies were performed, organ weights recorded, and selected tissues were microscopically examined.

All animals survived the study and there were no treatment-related clinical signs. Treatment-related decreased mean body weights were observed at the high dose level of 10 mg/kg/day in both sexes, primarily during the first week of exposure. However, mean body weights at this exposure level did not have any significant decreases beyond Week 2, after the dose was reduced to 6 mg/kg/day on Day 5. By approximately Week 9, the mean body weights at this exposure level had returned to pre-exposure values. The transient nature of the decreased mean body

weights was a clear indication that the effect was related to exposure at 10 mg/kg/day and not related to exposure at 6 mg/kg/day.

Sulfoxaflor at 10 mg/kg/day was not tolerated, as determined by significant and unacceptable decreases in food consumption. However, reduction to 6 mg/kg/day after 5 days allowed for food consumption in the affected group comparable to controls for the remainder of the study. Treatment-related, decreased food consumption values at the high dose were observed in males and females during the first 2 weeks of the study. By Week 3, the mean food consumption values had stabilized and were similar to control values with males appearing to recover slightly sooner than females. Based on the data, the effect on food consumption was considered to be related to exposure at 10 mg/kg/day, with residual effects prolonging the instability in several animals into Week 3 of the study. There were no other notable food consumption findings related to intake of the test material for the remainder of the study. There were no other parameters under evaluation that showed treatment-related effects.

In Week 13, the steady-state systemic dose ($AUC_{24\,h}$) of sulfoxaflor to dogs after doses of 1, 3, 6 mg/kg/day was 32 ± 6, 84 ± 23 and 147 ± 13 µg*h/ml in males and 22 ± 3, 71 ± 26 and 119 ± 19 µg*h/ml in females, respectively. The increase in systemic dose was dose-proportional in female dogs and was approximately dose proportional in male dogs across all three dose levels. The dose-corrected AUC_{24} after oral gavage to dogs was 3- to 4-fold higher than that observed in dogs after 28 days of oral gavage and 3 to 5-fold higher than that observed in rats after 90 days of dietary exposure. Urinary elimination of sulfoxaflor was 70 ± 6, 76 ± 12, and 59 ± 33 percent of administered dose in male and 69 ± 4, 80 ± 6, and 74 ± 13 percent in female dogs at 1, 3, and 6 mg/kg/day doses, respectively. The mean plasma elimination half-life of XDE-208 in Week 13 of dosing was between 17 and 28 hours.

The LOAEL is 10 mg/kg/day, based on excessively reduced food consumption and body weight loss prior to reduction of the dose on Day 5. The NOAEL is 6 mg/kg/day.
This study is classified Fully Reliable (Acceptable/Guideline) and satisfies the guideline requirement for a subchronic oral toxicity (oral gavage) - Beagle dogs (OPPTS 870.3150 (nonrodent); OECD 409; EEC Part B.27; JMAFF Subchronic Oral Toxicity Study).

IIA 5.8.1.3/5 X11719474: A 90-Day Oral Gavage Toxicity Study In Male Beagle Dogs;
This study (MRID 47832037) was conducted for The Dow Chemical Company on behalf of Dow ArgoSciences, LLC., to act as a bridging study from the parent compound, sulfoxaflor, for which a full guideline compliant 90-day oral toxicity study in dogs was conducted. This study was required to enable a valid comparison of toxicity to be made between the test material, X11719474, and the parent compound using the minimum number of animals. In this respect, the study was limited to a single sex, males. Three treatment groups of four male beagle dogs were administered the test material at respective dose levels of 10, 25, and 50 mg/kg/day. One additional group of four animals served as the control and received the vehicle, 0.5% Methylcellulose (Methocel A4C) in reverse osmosis water. The vehicle and test material were administered to all groups via oral gavage, once a day for 90 consecutive days, at a dose volume of 10 mL/kg/dose.

Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Clinical observations were conducted and body weights

were measured and recorded weekly. Food consumption was measured and recorded weekly beginning with Week -1. Physical examinations were conducted by a veterinarian pretest to confirm good-health status of each animal placed on study. Blood and urine samples for clinical pathology evaluations were collected from all animals pretest and during Weeks 6 and 13. Blood samples for determination of the plasma concentrations of the test article were collected from all animals at 0.5, 2, 4, 24 hours postdose and once during Week 13. At study termination, necropsy examinations were performed, organ weights were recorded, and tissues were collected for microscopic examination.

No treatment-related effects were noted during clinical examinations and no early deaths occurred. Body weight, food consumption, and clinical pathology parameters were unaffected by treatment with X11719474. There were no treatment-related histopathologic effects at any dose concentration. Toxicokinetic analysis of plasma after 13 weeks of oral gavage administration of sulfoxaflor metabolite X11719474 to male dogs showed that the systemic exposure of sulfoxaflor metabolite X11719474 (AUC_{24hr}) was proportional across all dose levels. The mean plasma elimination half-lives of sulfoxaflor metabolite X11719474 were 8.4 ± 2.4 hours, 7.8 ± 1.0 hours, and 7.7 ± 1.1 hours from dogs of the 10, 25, and 50 mg/kg/day dose groups, respectively.

In summary, X11719474 administered orally at doses up to 50 mg/kg/day for 90 days was well tolerated by male beagle dogs. There were no treatment-related histopathologic effects at any dose concentration. The no-observed-adverse-effect level (NOAEL) is 50 mg/kg/day. A lowest-observed-adverse-effect-level (LOAEL) was not identified.

This study was conducted using the following guidance, but it does not meet the specific criteria of using both sexes of Beagle dogs (OPPTS 870.3150 (nonrodent); OECD 409; EEC Part B.27; JMAFF Subchronic Oral Toxicity Study; Acceptable/Non-Guideline).

870.3200 21/28-Day Dermal Toxicity – Rat

IIA 5.3.7/1 28-Day Dermal Toxicity Study in Rats;
In a repeated-dose dermal toxicity study (MRID 47832059), sulfoxaflor (XDE-208 technical grade, 95.6% a.i., lot no. E2162-34, TSN003725-0001) in 0.5% methylcellulose was applied dermally to 10 Fischer DuCrl 344 rats/sex/dose, exposed at a semi-occluded, shaved skin test site to 0, 100, 500 or 1000 mg/kg body weight/day for six hours per day for 28 consecutive days. Parameters evaluated were daily cage-side and weekly detailed clinical observations, dermal observations, ophthalmic examinations, body weight, feed consumption, hematology, clinical chemistry, urinalysis, toxicokinetics of blood plasma, selected organ weights, and gross and histopathologic examinations.

Systemic toxicity: At 1000 mg/kg/day, males showed marginal increases in absolute and relative liver weights (6.5% and 4.4%, respectively above controls, $p<0.05$). A treatment-related histopathologic change was observed in the livers of 6 of 10 males, consisting of very slight hepatocyte hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia), involving the centrilobular/midzonal regions of the hepatic lobule. These were minor changes, not associated with increases in liver enzymes in the blood and hence, considered adaptive and non-adverse. Recent mechanistic studies have demonstrated a phenobarbital-like mode of action for sulfoxaflor-induced liver effects. Mean serum cholesterol was 17% higher compared to

controls. This effect was considered treatment related but non-adverse. Toxicokinetic analysis showed that the average plasma concentration of test material at high dose (1000 mg/kg/day) was greater than dose proportional. Similar plasma concentrations were found prior to and 16 hr after test material removal, indicating some retention of test material at the application site. There were no treatment-related systemic effects observed in females at any dose. The NOAEL for systemic toxicity is 1000 mg/kg/day (mild liver effects observed in males indicated some dermal absorption, but were not considered adverse). A LOAEL for systemic toxicity was not determined (>1000 mg/kg/day).

Local dermal toxicity: There were no treatment-related gross or microscopic dermal effects at the application site. The NOAEL for local dermal toxicity is 1000 mg/kg/day. A LOAEL for local dermal toxicity was not determined (>1000 mg/kg/day).

This dermal toxicity study in the rat is classified as Fully Reliable (acceptable/guideline) and satisfies the guideline requirement for a repeat-dose dermal toxicity study (OPPTS 870.3200; OECD 410) in rats.

870.3465 90-Day Inhalation – Rat

A.4.2 Prenatal Developmental Toxicity

870.3700a Prenatal Developmental Toxicity Study – Rat

IIA 5.6.10/2 Prenatal Developmental Study in Rats;
In a prenatal developmental study (MRID 47832140), groups of 26 time-mated female Crl:CD(SD) rats were administered diets containing 0, 25, 150, or 1000 ppm sulfoxaflor (Purity 95.6%; Lot # E2162-34) on gestation day (GD) 6 through 21 corresponding to time-weighted average doses of 0, 1.95, 11.5, or 70.2 mg/kg/day, respectively, in order to evaluate the potential maternal and developmental toxicity of this compound. In-life maternal study parameters included clinical observations, body weight, body weight gain, and feed consumption. On GD 21, all rats were euthanized and each dam and fetus was examined for gross pathologic alterations. In addition, blood was collected from dams and fetuses to determine blood levels of the test material. Liver, kidneys and gravid uterine weights were recorded, along with the number of corpora lutea, uterine implantations, resorptions and live/dead fetuses. All fetuses were weighed, sexed and examined for external alterations. Approximately one half of the fetuses were examined for visceral and craniofacial alterations, while skeletal examinations were conducted on the remaining fetuses.

Administration of 1000 ppm sulfoxaflor in rodent feed resulted in maternal and developmental toxicity. Maternal toxicity was evidenced by decreases in body weight and body weight gains, relative to controls, with concomitant decreased feed consumption, throughout the treatment period, and increased relative liver weights. Developmental toxicity was evidenced by decreases in fetal body weight and gravid uterine weight. In addition, clear increases in several fetal abnormalities (forelimb flexure, bent clavicle, hindlimb rotation, convoluted ureter, and hydroureter) occurred, which have subsequently been shown to reverse by postnatal day four. The terminal plasma concentrations of sulfoxaflor in both dam and fetal blood were dose-

proportional throughout the entire range of dietary exposure concentrations with similar levels between the maternal and fetal blood compartments. Administration of 150 or 25 ppm sulfoxaflor in rodent feed produced no treatment-related maternal toxicity and no indications of embryo/fetal toxicity or teratogenicity.

The LOAEL is 1000 ppm (70.2 mg/kg bw/day) for developmental toxicity based on increased resorption, increased postimplantation loss, decreased number of viable fetuses/litter, decreased fetal weights, convoluted ureter and hydroureter, forelimb flexure and hindlimb rotation, and skeletal alterations including bent clavicles. The NOAEL is 150 ppm (11.5 mg/kg bw/day).

The developmental toxicity study in the rat is classified as Fully Reliable (acceptable/guideline) and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

IIA 5.8.1.6/2 X11719474: Dietary Developmental Toxicity Study in CRL:CD(SD) Rats;
In a dietary developmental toxicity study (MRID 47832087), groups of 26 time-mated female CD rats were given the sulfoxaflor metabolite X11719474 (purity 95.5% a.i. wt/wt, Lot # E2695-1, TSN030626-0003) in feed at concentrations of 0, 1000, 2000, or 5000 ppm on gestation days (GD) 6 through 21, which corresponded to time-weighted average doses of 0, 74, 152, or 368 mg/kg/day, in order to evaluate the maternal and developmental toxicity potential of this compound.

Treatment-related effects were limited to a transient decrease in body weight gain in the 5000 ppm group at the initiation of treatment, with a concomitant decrease in feed consumption. This finding was deemed of no toxicological significance as it was minor in nature, isolated to the first three days of treatment, and likely due to decreased palatability of the diet. There was no treatment-related maternal toxicity at 1000 or 2000 ppm. There was no treatment-related organ weight or gross pathologic findings at any tested dose level. Toxicokinetic analyses of dam and fetal plasma X11719474 concentrations revealed dose proportionality across all groups and concentrations that were similar in dams and fetuses from the same groups. There was no evidence of developmental toxicity at any dose level tested in this study.

There was no indication of maternal or developmental toxicity at the highest dose tested and therefore a no-observed-adverse-effect-level (LOAEL) was not identified. The no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 5000 ppm (368 mg/kg/day).

The developmental toxicity study in the rat is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement for a Prenatal Developmental Toxicity Study (OPPTS 870.3700; OECD 414) in rats.

870.3700b Prenatal Developmental Toxicity Study – Rabbit

IIA 5.6.11/3 Prenatal Developmental Study – Rabbit;
In a dietary developmental toxicity study (MRID 47832066), groups of 26 time-mated female rabbits were administered sulfoxaflor (purity 95.6% a.i. wt/wt, Lot # E2162-34, TSN003725-

001) at dietary concentrations of 0, 30, 150, or 750 ppm, which corresponded to time-weighted average doses of 0, 1.3, 6.6, or 31.9 mg/kg/day, on gestation days (GD) 7-28. In-life parameters evaluated for all rabbits included: clinical observations, body weight, body weight gain, and feed consumption. Maternal blood was collected for sulfoxaflor analysis from four rabbits/group over a 24-hour period starting on the morning of GD 27, and also at termination on GD 28. Fetal umbilical cord blood was also taken at termination. All rabbits surviving to GD 28 were euthanized and examined for gross pathologic alterations and changes in liver, kidney, and gravid uterine weight. The number of corpora lutea, uterine implantations, resorptions, and live/dead fetuses were determined. All fetuses were weighed, sexed, and examined for external and visceral alterations. Also, the heads were examined for craniofacial alterations by serial sectioning in approximately one half of the fetuses in each litter, while skeletal examinations were performed on all fetuses.

Animals in the 750 ppm dose group exhibited treatment-related maternal toxicity in the form of decreased feces in 7 of 26 animals, decreased mean body weight gain (55%) from GD 7-13, decreased mean body weight gain (12%) throughout treatment (GD 7-28), and decreased mean feed consumption (8-21%) from GD 7-17. There was no treatment-related maternal toxicity for animals in the 30 or 150 ppm dose groups. There was no treatment-related developmental toxicity in any dose group.

The daily systemic dose of sulfoxaflor on GD 27-28 was dose-proportional as indicated by the near identical mean dose-corrected $AUC_{24\,h}$ values of 18, 19, and 19 µg sulfoxaflor /h/kg^{-1} for animals given 30, 150, and 750 ppm, respectively. Levels of sulfoxaflor in maternal and fetal blood were similar. The daily systemic dose in this dietary study was similar to that measured in prior gavage studies with sulfoxaflor.

The maternal lowest-adverse-effect-level observed in this study was 750 ppm (31.9 mg/kg/day) based on decreased body weight and body weight gain. The no-observed-adverse-effect-level was 150 ppm (6.6 mg/kg/day). There was no treatment- related developmental toxicity; therefore the developmental NOAEL was > 750 ppm (31.9 mg/kg/day).

This study is fully reliable (Acceptable/Guideline) and fulfills the guideline requirements for a prenatal developmental study in rabbits (OPPTS 870.3700, OECD 414 Test Guideline).

IIA 5.6.12/1 A Dietary Reproductive Toxicity Cross-Fostering Study in Crl:CD(SD) Rats; In a non-guideline cross-fostering dietary developmental toxicity study (MRID 47832063), groups of female Crl:CD(SD) rats (n=16) were administered sulfoxaflor (purity 95.6% wt/wt, Lot # E2162-34, TSN003725-001) at dietary concentrations of 0 or 1000 ppm. The purpose of this study was to determine whether the previously observed decreased survival of pups born to sulfoxaflor-treated dams resulted from *in utero* and/or lactational exposure. As part of this study, effects on general toxicity, toxicokinetic analysis of blood and milk, reproductive function and prenatal/early neonatal growth and survival were assessed.

Groups of female Crl:CD(SD) rats were fed diets supplying 0 (control) or 1000 ppm sulfoxaflor for two weeks prior to mating through weaning on lactation day (LD) 21. As the control and treated females mated, they were subdivided into Foster dams and Donor dams. Cesarean-

section was performed on gestation day (GD) 21 Donor dams, at which time, one or more batches of two of their offspring/sex were immediately cross-fostered to a Foster dam(s) that had their own litter removed that day (i.e., on LD 0). After cross-fostering was complete, each control and sulfoxaflor-treated Foster dam had mixed litters comprised of two pups/sex that originated from control Donor dams (five litters) and two pups/sex that originated from sulfoxaflor-treated Donor dams (eight litters). This design controlled for litter of origin effects, and enabled comparison of the survival of pups exposed to sulfoxaflor during gestation alone or during lactation alone with unexposed control pups and pups exposed during both gestation and lactation.

Dams given 1000 ppm sulfoxaflor had treatment-related effects on body weight, body weight gain, and feed consumption consistent with effects seen at this dose level in the previous reproduction/developmental toxicity screening study (MRID 47832143). Time weighted average doses for treated animals were 81.2, 74.5, and 59.5 mg/kg/day in the pre-mating, gestation, and lactation periods, respectively. These corresponded to maternal sulfoxaflor blood concentrations of 23.0-29.3 µg/g plasma on GD 21 and 19.6-25.0 µg/g plasma on LD 0. The average measured plasma concentration of sulfoxaflor of male/female pups on GD 21 and LD 0 from these dams was 24.8/24.8 and 25.3/25.9 µg sulfoxaflor /g plasma, respectively. Thus, fetal and pup plasma levels of sulfoxaflor were very similar to one another, and very similar to dam plasma levels. The measured milk concentrations from the same dams on LD 0 were approximately half the corresponding plasma levels and ranged from 12.3-14.0 µg sulfoxaflor /g milk (mean = 13.3 µg/g).

All offspring from dams exposed to 1000 ppm sulfoxaflor prior to birth died by postnatal day (PND) 4, irrespective of whether they were cross-fostered to control- or treated-foster dams (see results Table 1 below). Consistent with reduced viability, some offspring were cold to the touch, had bluish skin, autolyzed and cannibalized, and stomach void of milk. Conversely, there was no effect on neonatal survival for pups exposed to sulfoxaflor only after birth. Furthermore, PND 1 pup body weights were significantly decreased in prenatally exposed offspring.

In conclusion, these data demonstrate that the effect of sulfoxaflor on pup survival was due to *in utero*, not lactational, exposure.

This cross-fostering study in the rat is classified as Fully Reliable (acceptable/non-guideline).

IIA 5.6.12/2 A Study of the Effect of Sulfoxaflor on Neonatal Survival in New Zealand White Rabbits;

In a non-guideline study (MRID 47832138), sulfoxaflor (purity 95.6%; Lot # E2162-34) , was offered on a continuous basis in the diet (with 0.5% apple flavoring) to a group of 12 litter-experienced, time-mated female New Zealand White [Hra:(NZW)SPF] rabbits from gestation day (GD) 7 through the initiation of parturition (25-26 consecutive days). Actual test material intake in the 750 ppm group was 29 mg/kg/day during GD 7-28. A concurrent control group of 12 time-mated females received the apple-flavored control diet on a comparable regimen.

With the exception of one F_0 female in the control and 750 ppm groups euthanized on LD 3 due to total litter loss, all females survived to the scheduled necropsies. No test substance-related maternal macroscopic findings were noted.

Lower mean body weight gains (24.2%) and food consumption (7.3%) were noted in the 750 ppm group during the gestation exposure period compared to the control group. Corresponding incidences of decreased defecation were noted for three females in this group. Although mean body weights remained within 2.9% of control group values throughout gestation, the reductions in mean body weight gains and food consumption were attributed to test substance exposure. Mean body weights, body weight gains, and food consumption in the 750 ppm group were similar to the control group during LD 1-4.

No test substance-related effects were observed on the mean number of offspring born, offspring survival, or the general physical condition of the offspring.

When sulfoxaflor was continuously in the diet from GD 7 through the initiation of parturition to pregnant New Zealand White rabbits, there was no indication of maternal or developmental treatment-related toxicity; therefore the lowest-adverse-effect-level (LOAEL) was not determined. The no-adverse-effect-level (NOAEL) is 750 ppm (29 mg/kg/day).

This study is Fully Reliable (acceptable/non-guideline)

A.4.3 Reproductive Toxicity

870.3800 Reproduction and Fertility Effects – Rat

IIA 5.6.1/1 Dietary Reproduction/Developmental Toxicity Screening Test in Crl:CD(SD) Rats;

In a reproduction/developmental toxicity screening study (MRID 47832143) sulfoxaflor (95.6% a.i. wt/wt, Lot # E2162-34, TSN003725-0001) was administered to Crl:CD (SD) rats (12/sex/dose group) at concentrations of 0, 100, 500 or 1000 ppm in the diet. These dose levels corresponded to time-weighted average doses for males of 0, 8.26, 40.7 or 79.1 mg/kg/day. The time weighted average doses for females during the various study phases ranged from 0, 8.12-8.30, 39.5-44.1, and 78.2-81.6 mg/kg/day, respectively. Males were fed the test diets for two weeks prior to breeding and continuing throughout breeding until termination. The females were fed the test diets for two weeks prior to breeding, continuing through breeding (up to two weeks), gestation, lactation and weaning; pups were weaned on postnatal day 21. Effects on gonadal function, mating behavior, conception, development of the conceptus, parturition and postnatal growth and survival were evaluated. In addition, a gross necropsy and histopathological examination of the adults were conducted with an emphasis on organs of the reproductive system. In the offspring, litter size, pup survival, sex, body weight and the presence of gross external morphological alterations were assessed.

Males in the 1000 and 500 ppm dose groups had a treatment-related decrease in food consumption and body weight (1000 ppm only) during the first week of treatment. Females in the 1000 and 500 ppm dose groups had treatment-related decreases in body weight gain during

the first week of gestation, and females in the 1000 ppm dose group had slightly lower food consumption during the pre-breeding and gestation phases. Body weight changes were minimal and not considered adverse.

Males of the 1000 and 500 ppm dose groups had increased absolute and relative liver weights that were dose and treatment related (liver weights for females at 1000 ppm were not recorded due to an effect on litter survival; there was no effect at 500 ppm). Treatment-related histological effects were observed in the livers of males given 1000 and 500 ppm and females given 1000 ppm and consisted of a dose-related increase in the severity of hepatocellular hypertrophy, with altered tinctorial properties, involving the centrilobular to midzonal regions of the hepatic lobule. Histological effects were very slight or slight severity in males and very slight in females. In addition, there was treatment-related multifocal hepatocyte vacuolization (slight severity) in 1000 ppm females. These hepatic effects were minimal and not considered adverse.

There were no reproductive or developmental toxicity effects observed in any group up to PND 0 (birth). Shortly after birth, there was a significant decrease in pup survival in the 1000 ppm dose group such that PND 1 survival was 46.3%, compared to 98.3% in controls. In addition, PND 1 pup body weights were significantly decreased (22-25%) relative to controls. By PND 4, eleven of twelve dams had total litter loss resulting in 7.3% pup survival, compared to 95.4% in controls. Because only one of twelve litters remained, this dose group was terminated on PND 6. Pup survival was also affected in the 500 ppm group with 4 of 12 dams losing approximately half of their litters by PND 4, which resulted in a pup survival rate of 81.2% compared to 95.4% in controls. There were no effects on pup body weight in the 500 ppm group. There were no treatment-related effects on any other reproductive parameters at 1000 or 500 ppm, and no reproductive effects whatsoever at 100 ppm.

The concentration of XDE-208 in pup and dam plasma was a dose proportionate from the 100, 500, and 1000 ppm groups. Sulfoxaflor plasma levels in PND 4 pups were ≈35% of adult plasma concentrations. Individual (n = 3) concentrations of sulfoxaflor were equivalent in dam plasma and milk from dams of the 1000 ppm dose group.

The systemic LOAEL is 1000 (78.2 mg/kg/day) based on decreased pup body weight. The NOAEL is 500 ppm (39.5 mg/kg/day).

The developmental LOAEL is 500 ppm (39.5 mg/kg/day) based on decreased pup survival. The NOAEL is 100 ppm (12.0 mg/kg/day).

This study is fully reliable (acceptable/guideline) and fully satisfies the guideline requirements for a reproduction/development screening study (OPPTS 870.3550; OECD 421) in rats.

IIA 5.8.1.6/1 X11719474: Dietary Reproduction/Developmental Toxicity Screening Test in CRL:CD(SD) Rats;

Groups of 12 male and 12 female Crl:CD(SD) rats were administered the sulfoxaflor metabolite X11719474 via the diet at concentrations supplying 0, 1000, 2000, or 5000 ppm, which corresponded to time-weighted average doses of 0, 80.8, 162, or 396 mg/kg/day for males, and ranged from 0, 81.7-114, 167-212, and 451-507 mg/kg/day during the female pre-breeding, gestation

and lactation phases (MRID 47832094). Males were fed the test diets for two weeks prior to breeding and continuing through breeding (up to two weeks) up until necropsy (test day 39). Females were fed the test diets for two weeks prior to breeding, through breeding (up to two weeks), gestation and lactation up until necropsy on post-partum day 22-24. Effects on gonadal function, mating behavior, conception, development of the conceptus, parturition, and postnatal growth and survival were evaluated. In addition, a gross necropsy and histopathologic examination of the adults were conducted with an emphasis on organs of the reproductive system. Offspring were evaluated through postnatal day (PND) 21 for litter size, survival, sex, body weight and the presence of gross external morphological alterations. Plasma concentrations of X11719474 were measured in PND 4 culled pups to demonstrate systemic exposure.

There were no treatment-related clinical findings or adverse effects on feed consumption at any dose level. Body weight and/or body weight gain in the 2000 and 5000 ppm groups were slightly lower than controls during isolated gestation and lactation intervals. However, these findings were considered of no toxicological significance based on the lack of a dose-response relationship, the small magnitude of change, and lack of consistency across intervals.

There was a treatment-related increase in the liver weights of males and females (15 and 7% increase in relative weight, respectively) in the 5000 ppm group. These findings corresponded with an increased incidence of very slight centrilobular and midzonal hepatocellular hypertrophy in all males and the majority of females (10/12) in the 5000 ppm group. There were no treatment-related organ weight or pathologic effects in the 1000 and 2000 ppm groups of either sex. Toxicokinetic data demonstrated dose-proportional systemic exposure in PND 4 pups.

There were no treatment-related effects on reproductive endpoints or development of the offspring at any dose level.

There was no indication of maternal toxicity at the highest dose tested of 5000 ppm (396 mg/kg/day) and therefore a lowest-observed-adverse-effect-level (LOAEL) was not identified. The no-observed-adverse-effect-level (NOAEL) for maternal toxicity was 5000 ppm (396 mg/kg/day). There was no indication of reproductive or developmental toxicity at the highest dose tested of 5000 ppm (396 mg/kg/day) and therefore a LOAEL was not identified. The reproductive and developmental NOAEL was 5000 ppm (396 mg/kg/day).

This study is fully reliable (acceptable/guideline) and satisfies the guideline requirements for a dietary screening reproduction/developmental study in rats (OPPTS 870.3550; OECD 421).

IIA 5.6.1/2 Reproduction and Fertility Effects – Rats;

In a two-generation dietary reproduction toxicity study (MRID 47832142) sulfoxaflor (95.6% a.i. wt/wt, Lot # E2162-34, TSN003725-0001) was administered to Crl:CD (SD) rats (27/sex/dose group) at concentrations of 0, 25, 100 or 400 ppm in the diet for approximately ten weeks prior to breeding, and continuing through breeding, gestation and lactation for two generations. In-life parameters included clinical observations, feed consumption, body weights, estrous cyclicity, reproductive performance, pup survival, pup body weights, puberty onset and anogenital distance. In addition, post-mortem evaluations included gross pathology and organ weights in weanlings, toxicokinetic analyses, gross pathology, organ weights, oocyte quantitation and sperm count, motility and morphology, and histopathology, in adults.

Parental toxicity at 400 ppm consisted of increased absolute and relative liver weights in the P1 (12.8 and 10.9%, respectively) and P2 (6.5 and 7.8%, respectively) males. This effect on liver weight correlated with histopathological findings of very slight to slight centrilobular hepatocyte hypertrophy, often with a very slight increase in individual cell necrosis of centrilobular hepatocytes. No other systemic effects were noted at 400 ppm, and there were no treatment-related effects on P1 or P2 parameters in male or female rats at 25 or 100 ppm.

Offspring effects were limited to 400 ppm and comprised slightly decreased neonatal survival in both generations; this in turn led to a lower percentage of live pups up to culling on PND 4. In addition, there was an apparent treatment-related delay in preputial separation (PPS) for 400 ppm F1 males. This external marker of male puberty onset is androgen dependent, but the underlying reason for how sulfoxaflor induced this finding is not known; however, there were no other indications of androgenic or anti-androgenic effects. This included no treatment-related effects on anogenital distance, no effects on testis or accessory sex gland (i.e., prostate, seminal vesicle, and epididymis) weight or histopathology, no evidence of malformations like hypospadias or ectopic testes, no effects on mating, fertility, time to mating, or gestation length, and no treatment-related effects on preputial separation at the same dose level in a developmental neurotoxicity study with sulfoxaflor. Taken together, the weight of evidence across androgen-sensitive endpoints led to the conclusion that the data do not support any other sulfoxaflor - mediated anti-androgenic effects. There were no effects on puberty onset or any other parameter of reproductive performance or offspring growth and survival at 25 or 100 ppm.

Toxicokinetic data from LD 4 dams and culled PND 4 pups in the second generation show dose-proportional systemic exposure to sulfoxaflor in dams and their offspring. Plasma concentrations of sulfoxaflor in rat pups were, on average, 32% of the levels measured in the dams.

The lowest-observed-adverse-effect-level (LOAEL) for systemic toxicity was 400 ppm (24.6 mg/kg/day) based on hepatic toxicity (increased weight, hypertrophy, and necrosis) in the P1 and P2 males. The no-observed-adverse-effect-level (NOAEL) was 100 ppm (6.07 mg/kg/day).

The reproductive LOAEL is established at 400 ppm (24.6 mg/kg/day) based on decreased neonatal survival (ca. 2-5%).

The offspring toxicity LOAEL is 400 ppm (24.6 mg/kg/day) based on decreased neonatal survival and delay in preputial separation in F1 females. The NOAEL is established at 100 ppm (6.07 mg/kg/day).

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 8700.3800; OECD 416) for a reproduction and fertility study.

A.4.4 Chronic Toxicity

870.4100a (870.4300) Chronic Toxicity – Rat

IIA 5.5.1/1 Combined Chronic Toxicity/Oncogenicity Study in F344/DuCrl Rats test by the oral route in the rat;

In a combined chronic carcinogenicity study in rats (MRID 47832060), groups of 60 male and 60 female F344/DuCrl rats were treated orally by dietary administration with sulfoxaflor (96.5%, Lot # E2162-34, TSN003725-0001) at dose levels of 0, 25, 100, 500 (males only) or 750 (females only) ppm for up to two years. The time-weighted average dosages ingested, based upon mean feed consumption and body weight data for 24-months were 0, 1.04, 4.24 or 21.3 mg/kg/day for males and 0, 1.28, 5.13 or 39.0 mg/kg/day for females. Ten rats/sex/dose level were necropsied after one year (chronic toxicity group) and the remaining 50 rats/sex/dose level were fed the respective diets for up to two years.

Animals were evaluated by daily cage-side examinations, bi-weekly clinical observations, periodic detailed clinical observations, body weights, feed consumption and toxicokinetics (plasma and urine). Clinical pathology examinations (hematology, clinical chemistry, and urinalysis) were conducted at regular intervals throughout the study. Ophthalmic examinations were conducted on all rats prestudy, and all surviving rats prior to the scheduled necropsies. All rats had a complete necropsy, with weights of multiple organs collected from all rats at the scheduled necropsies. Histopathological examination of an extensive set of organs was performed on all control and high-dose level rats and all rats that died spontaneously or were euthanized due to their moribund condition. Histopathological examination of rats from the low- and intermediate-dose levels of the 12-month chronic toxicity group was limited to the liver, adrenal glands, testes and relevant gross lesions. Histopathological examination of survivors from the low- and intermediate-dose levels of the 24-month oncogenicity group was limited to the liver, testes, epididymides, coagulating glands, prostate, seminal vesicles and relevant gross lesions.

No treatment related mortality or clinical signs were observed after sulfoxaflor exposure. Males given 500 ppm had decreases in body weights at most measurement intervals starting on Day 512 and continuing through Day 729. On Day 729, the mean body weight and body weight gain for males given 500 ppm were 5.0% and 5.7% lower than controls, respectively. The decreases were statistically significant and lower than historical control for most time points; therefore, they were considered treatment related. Females given 750 ppm had treatment-related, statistically significant decreases in body weights, first noted on Day 57, and continuing through Day 729. On Day 729, the mean body weight and body weight gain for females given 750 ppm were 6.3% and 9.1% lower than controls, respectively. The decreases were outside of the historical control range. The body weights of males and females given 100 or 25 ppm were unaffected by treatment with sulfoxaflor.

High-dose level males and females had treatment-related, statistically significant increases in cholesterol concentrations at 3, 6 and 12 months, and 3, 6, 12 and 18 months, respectively, with increases ranging from 17.5 to 32.9%.

The liver was the primary target organ for histopathologic effects for males given 500 ppm and females given 750 ppm at 12 and 24 months. The absolute and relative liver weights for high-dose males (500 ppm) and females (750 ppm) were only increased at 12 months, in the range of 3.2 to 17%. Non-neoplastic liver effects at 12 and 24 months consisted of hypertrophy of

centrilobular and midzonal hepatocytes, necrosis of individual centrilobular hepatocytes, vacuolization (females only) consistent with fatty change of hepatocytes, and an increase in the severity of aggregates of macrophages/histiocytes in both sexes at 500 and 750 ppm. An additional treatment-related liver effect in females given 750 ppm at 24 months consisted of a lower number of basophilic foci of altered hepatocytes. A treatment-related neoplastic liver effect at 24 months consisted of a statistically significant increase in the incidence of benign hepatocellular adenomas in males given 500 ppm. Females given 750 ppm did not have a treatment-related increase in the incidence of liver tumors. There were no treatment-related liver effects in males or females given 25 or 100 ppm. A phenobarbital (PB) like mode of action (MOA) has been postulated for sulfoxaflor induced rodent liver tumors and studies were conducted (MRID 47832062) with sulfoxaflor investigating the proposed MOA, including a study with dual CAR/PXR knockout mice. A formal framework analysis investigating the proposed MOA is available (MRID 48288939) and the data adequately supports a threshold based, mitogenic MOA for rodent liver tumors.

At 24 months, males given 100 or 500 ppm had statistically significant increases in absolute and relative testes weights, and statistically significant decreases in absolute and relative epididymal weights. Absolute testes weights of males given 100 or 500 ppm were approximately 46% and 62% higher than controls, respectively. The higher testes weights were reflective of larger interstitial (Leydig) cell adenomas in the testes of males at these dose levels.

The incidence of interstitial cell adenomas in at least one testis of males from all dose groups was comparable to controls at 24 months. However, males given 500 ppm had a statistically significant increase in the incidence of bilateral interstitial cell adenomas of the testes, and a corresponding decrease in the incidence of unilateral interstitial cell tumors, relative to controls. An additional testicular effect consisted of a statistically significant, increase in the incidence of severe bilateral atrophy of seminiferous tubules in males given 100 or 500 ppm. The seminiferous tubular atrophy was reflective of the generally larger interstitial cell adenomas in these dose groups.

At 24 months, males given 100 or 500 ppm had statistically significant decreases in absolute and relative epididymal weights. The lower epididymal weights were associated with a higher incidence of decreased spermatic elements (bilateral, severe) in the lumen of the epididymides of these males.

Males given 500 ppm had statistically significant increases in the incidence of decreased secretory material in the coagulating glands (severe), prostate (moderate), and seminal vesicles (severe) at 24 months. Males given 500 ppm also had an increased incidence of carcinoma of the preputial gland. The primary effect was interpreted to be the interstitial cell adenomas, and the effects on seminiferous tubules, epididymides, accessory sex glands were considered secondary to loss of normal testicular function due to the size of the interstitial cell adenomas, and possible alterations in the endocrine balance of these rats. Although the mode of action (MOA) for the effects on reproductive organ was not investigated as part of this study, MOA data for the interstitial cells were submitted separately (MRID 48288942). A similar (shared) MOA with interstitial cell adenomas was postulated for the increased incidence of preputial gland

carcinomas; however, the MOA remains unclear since the available data are not fully supportive of the proposed MOA.

Toxicokinetic analyses of plasma samples at 3 and 12 months indicated dose proportionality in systemic dose levels that were comparable between the two time points. There were no gender differences in plasma concentrations of sulfoxaflor across the dose levels and times analyzed. Urinary elimination of sulfoxaflor was also dose proportional for both male and female rats at 3, 6 and 12 months, representing 58-127% of the average test material consumed in a 24-hour period.

The no observed adverse effect level (NOAEL) is 100 ppm (4.24 mg/kg/day) and the lowest observed adverse effect level (LOAEL) is 500 ppm (21.3 mg/kg/day), based on increased serum cholesterol concentrations, and histopathological liver effects in males.

Note: Although male reproductive effects were seen at doses lower than the determined NOAEL, the effects were considered secondary to the increased incidence of interstitial cell (Leydig Cell) tumors. The CARC determined that the increased incidence of interstitial cell tumors were not treatment related; therefore, the male reproductive effects (seen in the presence of the tumors) are also considered to be not treatment-related. In contrast, PMRA determined that the LOAEL is 100 ppm, based on the male reproductive effects.

This study is fully reliable (US EPA Acceptable/Guideline): GLP compliant and fully compliant with the combined chronic/carcinogenicity study in rats (OPPTS 870.4300); OECD 453:

870.4100b Chronic Toxicity – Dog

IIA 5.3.4 Oral 1-year Chronic Toxicity (Dog);

In a chronic oral toxicity study (MRID 48288906), four Beagle dogs/sex/dose were administered sulfoxaflor (XDE-208 technical, 99.7% a.i., lot no. E2162-34) at doses of 0, 1, 3 or 6 mg/kg/day for 52 weeks. Test material was administered by a daily gavage dose in 0.5% aqueous methylcellulose vehicle (Methocel A4C; dosing volume 10 mL/kg). Dietary or capsule administration was not performed due to unsatisfactory food consumption using these methods. Observations for morbidity, mortality, injury and the availability of food and water were conducted twice daily for all animals. Toxicity was assessed by weekly detailed clinical observations, food consumption and body weight measurements, ophthalmoscopic examinations, and clinical pathology evaluations. Blood and urine samples for determination of plasma concentrations of the test material were collected from all animals at Weeks 13, 26 and 52. Toxicokinetic (TK) parameters were determined for the test article from concentration-time data. At study termination, necropsy examinations were performed, select organ weights were recorded, and tissues were preserved for subsequent microscopic examination.

No treatment-related deaths occurred. The increased frequency of soft and/or watery feces in two males at 6 mg/kg/day was considered treatment-related due to the high incidence in these animals relative to other groups, but was not considered adverse because these findings had no effect on food consumption or body weight/weight gain. A transient, treatment-related decrease in food consumption and body weight was observed in females at 6 mg/kg/day during the first two weeks

of dosing. The decreases in food consumption and body weight were considered non-adverse findings due to their transient nature. No definitive effects on hematological or clinical pathology parameters were noted. The systemic exposure of sulfoxaflor (AUC_{24h}) in plasma was proportional across all dose levels in both sexes. With the exception of females at 52 weeks of exposure (urine values of high dose females fell below the regression line fitted to the mean values), toxicokinetic analysis of parent in urine showed that the systemic exposure of sulfoxaflor (AUC_{24h}) was proportional across all dose levels and time points. There were no treatment-related organ weight changes or gross/histopathologic effects at any dose.

The NOAEL is 6 mg/kg/day (soft/watery feces in males and a transient decrease in food consumption and body weight in females during the initial two weeks were considered treatment-related, but not adverse). A LOAEL was not identified (> 6 mg/kg/day).

This study is classified fully reliable (US EPA acceptable/non-guideline) and satisfies the guideline requirement for a chronic oral toxicity study in the dog (OPPTS 870.4100; OECD 452).

A.4.5 Carcinogenicity

870.4200a Carcinogenicity Study – Rat (See 870.4100a (p. 106))

870.4200b Carcinogenicity (feeding) – Mouse

IIA 5.5.3/1 Carcinogenicity – Mice *[feeding]*;
In a carcinogenicity study in mice (MRID 47832054, PMRA 1941285), groups of 50 male Crl:CD1(ICR) mice were treated orally, by dietary administration, with sulfoxaflor (purity 95.6%, Lot # E2162-34, TSN003725-0001), at dose levels of 0, 25, 100, or 750 ppm for 18 months ; groups of 50 female Crl:CD1(ICR) mice were similarly treated at dose levels of 0, 25, 250, or 1250 ppm. These concentrations corresponded to time-weighted average doses of 0, 2.54, 10.4 or 79.6 mg/kg/day for males, and 0, 3.43, 33.9, or 176 mg/kg/day for females, respectively.

Animals were evaluated by daily cage side, bi-weekly observations, periodic handheld detailed clinical examinations, body weights, and feed consumption. Ophthalmic examinations were conducted pre-exposure and prior to necropsy. All mice had a complete necropsy examination with total white blood cell (WBC) and differential WBC counts, and organ weight recording at the scheduled necropsy. An extensive set of organs was examined histopathologically from all control and high-dose group mice, as well as those that died or were euthanized in moribund condition. The kidneys, liver, lungs, adrenal glands (females only) and all relevant gross lesions from the low- and intermediate-dose groups from the terminal necropsy were also examined histopathologically.

There were no treatment-related changes in clinical observations, body weights and body weight gains, feed consumption, ophthalmologic observations, or total and differential WBC counts in any of the sulfoxaflor treated groups.

Toxicokinetic analysis indicated that the daily systemic dose (steady-state plasma concentration) of sulfoxaflor in male and female mice remained essentially proportional to dose during the course of the study (3 and 12 months after study start) and concentrations of sulfoxaflor in urine also increased proportionally with dose for both male and female mice, and at both collection times (3 and 12 months).

The liver was the primary target of sulfoxaflor. The absolute and relative liver weights of males given 750 ppm were increased 87 and 79% respectively; in females given 1250 ppm they were increased 51 and 47%, respectively as compared to controls. At necropsy, there was a treatment-related increase in the incidence of mass nodules and multifocal pale foci in the liver of males given 750 ppm. Females given 1250 ppm had a treatment-related increased incidence of mass nodules in the liver, albeit at lower numbers compared to the high-dose males (750ppm). Histopathologic treatment-related changes consisted of hepatocellular adenomas and/or carcinomas in 60% of male mice given 750 ppm and in 10% of female mice given 1250 ppm. Although there were no statistically significant differences in the overall mortality rates between the controls and any of the sulfoxaflor treated groups, hepatocellular carcinomas or adenomas were attributed as the cause of death or moribundity in a small proportion of males (6 of 50) given 750 ppm. Treatment-related non-neoplastic liver effects consisted of increases in the incidences of eosinophilic and vacuolated foci of cellular alteration in males given 750 ppm; slight to moderate centrilobular/midzonal or panlobular hepatocellular hypertrophy with altered tinctorial properties (increased cytoplasmic eosinophilia) consistent with liver enzyme induction in males and females given 750 or 1250 ppm; very slight or slight multifocal individual cell necrosis of hepatocytes in males given 750 ppm and females given 1250 ppm (very slight); very slight fatty change in centrilobular/midzonal hepatocytes in males and females given 750 or 1250 ppm and increased incidence of hepatocytes in mitosis in males given 750 ppm.

The only treatment-related change in females given 250 ppm was an increased incidence of slight centrilobular/midzonal hepatocyte hypertrophy with altered tinctorial properties (increased cytoplasmic eosinophilia) consistent with liver enzyme induction. This was considered to be an adaptive response and non-adverse per se due to a complete lack of any associated changes including increase in liver weight or any other treatment-related histopathologic findings.

Males given 750 ppm had an exacerbation in the cumulative incidence of spontaneous dermatitis which is common in CD-1 mice. Histologically, these were characterized by subacute to chronic inflammation, variable epidermal ulceration and acanthosis. Associated with the ulcerative dermatitis was an increased incidence of reactive plasmacytosis of the local submandibular lymph nodes of males given 750 ppm. The exacerbation of spontaneous dermatitis in males given 750 ppm was interpreted to be secondary to the general unthriftiness of these mice due to the excessive stress induced by the malignant and/or benign hepatocellular neoplasms. In females, a non-statistically significant increase in lymphosarcoma was seen at the HDT of 1250 ppm.

A phenobarbital (PB) like mode of action (MoA) has been postulated for sulfoxaflor induced rodent liver tumors and several studies were conducted (MRID 47832062) with sulfoxaflor that investigate the proposed MoA, including a study with dual CAR/PXR knockout mice. Additionally, a formal framework analysis has been submitted (MRID 48288939). The

available toxicity data and the framework analysis will be used to evaluate the carcinogenic potential of sulfoxaflor.

The no-observed-adverse-effect levels (NOAELs) for males and females are 100 (10.4 mg/kg/day) and 250 ppm (33.9 mg/kg/day), respectively. The lowest-observed-adverse-effect level (LOAEL) for males is 750 ppm (79.6 mg/kg/day) and for females is 1250 ppm (176 mg/kg/day), based on increased liver weights, increased incidence of liver nodules, liver hypertrophy, and liver histopathology (necrosis, fatty change). In males, liver foci (eosinophilic and vacuolated) and gross (dermatitis) and histopathological changes (chronic inflammation, epidermal ulceration, and acanthosis) in the skin were also seen. This was accompanied by reactive plasmacytosis of the local submandibular lymph nodes.

This study is fully reliable (US EPA Acceptable/Guideline): GLP compliant and fully compliant with the carcinogenicity study in mice (OPPTS870.4200); OECD 451 (US EPA Acceptable/Guideline).

IIA 5.5.4/1 *Ex vivo* gene expression and cell proliferation analyses

The purpose of this study (MRID 47832033) was to obtain preliminary information on the potential mode of action responsible for the liver weight increases observed in mice and rats administered Sulfoxaflor [N-(Methyloxido(1-(6-(trifluoromethyl)-3 pyridinyl)ethyl)sulfanylidene)-cyanamide] via the diet. To accomplish this, specific gene expression was assessed by real-time PCR in liver samples from female CD1 mice exposed to 0 and 4500 ppm sulfoxaflor in diet for four days in a previously conducted palatability probe study (MRID 47832048). In total, eight genes were selected for this study. Five genes, primarily *Cyp2b10* but with four additional genes, were chosen to address whether sulfoxaflor induces a phenobarbital (PB)-like gene expression response. Two genes which are also induced by PB were selected specifically to investigate the effect on blood cholesterol seen in sulfoxaflor-treated rodents. One gene, *Cyp4a10*, was included as a marker of peroxisome proliferator-activated receptor alpha (PPAR alpha) to examine this potential mode of action. In addition, cell proliferation (seen as an early response to PB treatment) was assessed by Ki-67 immunohistochemical staining in liver tissue from male and female F344 rats from a 28-day rat study (MRID 47832045; 0, and 2000 ppm dose) and CD1 mice from the mouse palatability study (0, 3000, and 4500 ppm doses). Sulfoxaflor -treated mice showed gene expression similar to that reported in the literature following PB exposure. The primary PB-like marker gene, *Cyp2b10*, was induced 148.5-fold. In addition, three of the four remaining PB-like response genes (*Cyp3a11*, *Alas1*, and *NADPH-Cyp-reductase*) were induced. The remaining PB-like gene, *Slco1b2*, was not induced in sulfoxaflor-treated mice. Sulfoxaflor stimulated the cholesterol synthesis-related genes, *Dhcr7* and *Sqle1*, at levels similar to those reported in the literature following PB exposure. The lack of induction for both *Cyp4a10* and an internal homeostasis control gene, *Scd1*, suggests that sulfoxaflor is not acting as a peroxisome proliferator. Mice exposed to 3000 ppm sulfoxaflor for seven days showed increased proliferation in both the centrilobular and midzonal regions, whereas both sexes of rat exposed to 2000 ppm sulfoxaflor for 28 days showed increased proliferation only in the centrilobular region.

In conclusion, treatment with sulfoxaflor resulted in a gene expression profile similar to that reported for phenobarbital. Additionally, increased hepatocellular proliferation in both the centrilobulor and midzonal regions was seen in female mice following 3000 ppm treatment,

whereas both sexes of rats exposed to 2000 ppm sulfoxaflor showed increased proliferation only in the centrilobulor region. The carcinogenic potential of sulfoxaflor, along with the proposed MOA for liver tumors will be evaluated by the Cancer Assessment Review committee (CARC).

Although this study does not satisfy EPA guideline requirements, it provides supplementary information regarding the proposed mechanism of action for carcinogenicity of sulfoxaflor in rats and mice. This study fully reliable (acceptable/non-guideline).

IIA 5.5.4/2 Targeted gene expression, cell proliferation and cytochrome P450 enzymatic activity in rats;

In previous studies with sulfoxaflor, increased liver weight was observed. Targeted gene expression data in mice and hepatocellular proliferation data in both mice and rats suggest a possible phenobarbital (PB)-like mode of action (MoA) could be responsible for the observed liver weight increases, and subsequent liver tumors. The molecular response stimulated by PB is mediated by the constitutive androstane nuclear receptor (CAR), with peripheral involvement of the pregnane X receptor (PXR), and typically results in increased hepatocellular proliferation and hypertrophy, with associated increased liver weight, inhibition of apoptosis, hypertrophy, and eventual development of altered hepatic foci. In this study (MRID 47832061), five male and five female Fischer 344/DuCrl rats per group were given sulfoxaflor in the diet at 0, 100, 750, or 1500 ppm for 3 or 7 days. The primary endpoints examined were liver weight, targeted gene expression, liver enzyme analysis, and hepatocellular proliferation.

Decreased body weights and body weight gains were seen in males and females at 1500 ppm after 3 and 7 days. After 3 days, body weight was decreased 7% in males and 9% in females and body weight gain was decreased 60% and 100%, respectively. After 7 days, there was a 7% decrease in male body weight and 43% and 47% decrease in body weight gain in males and female, respectively. Food consumption in males at 750 and 1500 ppm for 3 days and 1500 ppm for 7 days was significantly lower (15 and 30% - day 3; 23% - day 7, respectively). A similar pattern of reduced food consumption was seen in females exposed to 750 and 1500 ppm for 3 days and 1500 ppm for 7 days (18 and 35%, day 3; 20% day 7, respectively).

Elevated cholesterol levels were seen in males on day 3 at 750 or 1500 ppm (19 and 29%, respectively) and day 7 at 750 or 1500 ppm (33% and 87%, respectively). In females, elevated cholesterol levels were seen after 7 days at 1500 ppm (41%). Relative liver weights were increased for males and females in the 1500 ppm group at 3 days (14 and 3%, respectively) and at 750 and 1500 ppm at 7 days (11 and 23% for males and 6 and 18% for females, respectively). Absolute liver weights were increased in male and females at 1500 ppm after 7 days (14 and 16%, respectively).

C*yp2b1* gene expression, the prototypical gene response following PB exposure, was induced over 800-fold in both male and female rats exposed to 1500 ppm sulfoxaflor for 3 and 7 days. To further examine similarities in PB-like response, *Cyp2b2* and *Cyp3a3* (CAR- and PXR-related genes, respectively) expression levels, together with PROD and BROD enzyme activity were evaluated at 3 and 7 days. These molecular markers were elevated in males and females exposed to 750 and 1500 ppm at both time periods in a PB-like manner. At 7 days, male rats exposed to 750 and male and female rats at 1500 ppm sulfoxaflor showed significant

hepatocellular proliferation; for males it occurred in the centrilobular and midzonal regions, whereas for females it was evident in the centrilobular region.

An additional aim of this study was to determine if other nuclear receptors might have played a role in sulfoxaflor-induced liver effects. Four nuclear receptors are primarily responsible for xenobiotic-induced liver weight increase; the aryl hydrocarbon receptor (AhR), CAR, PXR, and the peroxisome proliferator-activated receptor alpha (PPARα). *Cyp1a1* gene expression and EROD enzyme activity were slightly but significantly elevated at day 3; however, EROD enzyme activity returned to control levels by day 7. Furthermore, gene expression of *Cyp4a22* was not elevated in this study. From these data it is implied that sulfoxaflor is not likely an AhR or PPARα agonist.

In conclusion, the data suggests that sulfoxaflor may be an agonist ligand for CAR, and this activation results in the observed phenobarbital-like response in the liver. However, the carcinogenic potential of sulfoxaflor, along with the proposed MOA for liver tumors will be evaluated by the Cancer Assessment Review Committee (CARC).

This non-guideline study is reliable with restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data.

IIA 5.8.1.5/1 X11719474: Targeted Gene Expression, Cell Proliferation, and Cytochrome p 450 Enzymatic Activities in Rats;

The purpose of this study (MRID 47832151) was to generate information to aid in understanding the mode of action for liver effects in rats induced by X11719474 a metabolite of sulfoxaflor and to compare the results to those observed following exposure to sulfoxaflor. In this study, male F344/DuCrl rats were given test diets formulated to supply 0 or 8000 ppm X11719474 in the diet for 7 days which corresponded to time-weighted average doses of 0 or 583 mg/kg/day, respectively. The treatment level of 8000 ppm was the high dose level used in the rat 28-day toxicity study. The parameters evaluated were daily cageside observations, body weights, body weight gains, feed consumption, liver weights, histopathology of the liver, focused gene expression, liver cytochrome P450 enzyme activity, and hepatocellular proliferation using BrdU immunohistochemical methods. Results from this study were compared to an earlier work in which male F344/DuCrl rats were exposed to 1500 ppm sulfoxaflor for 7 days, resulting in a time-weighted average dose of 102 mg/kg/day.

At the end of the 7-day treatment period, males exposed to 8000 ppm X11719474 had body weight and body weight gains decrements of approximately 2% and 22% relative to controls, respectively. In addition, males given 8000 ppm X11719474 had statistically identified, treatment-related increases in liver weights (absolute: 16%; relative: 19%). X11719474 induced liver weight increases that correlated with treatment-related microscopic observations of very slight centrilobular/midzonal hypertrophy of hepatocytes that were present in all treated animals. At the end of the 7-day treatment period, males exposed to 8000 ppm X11719474 had the same types of treatment-related effects as parent sulfoxaflor, but were consistently less marked despite the almost 6-fold higher dose level.

The constitutive androstane receptor (CAR)-related transcripts, Cyp2b1 and Cyp2b2, along with the pregnane X receptor (PXR)-related transcript Cyp3a3, were increased 806, 32, and 4-fold, respectively. Sulfoxaflor -induced gene expression showed a similar profile of 848, 21, and 9-fold, respectively. The level of hepatocellular PROD enzyme activity, a CAR-associated biomarker, was increased 15-fold following X11719474 exposure. A similar level of induction (10-fold) was seen for sulfoxaflor -induced PROD enzyme activity. Hepatocellular proliferation, as measured by BrdU incorporation, was significantly elevated 4.5, 2.5, and 2.1-fold in the centrilobular, midzonal, and periportal regions, respectively. An earlier hepatocellular proliferation study with sulfoxaflor, as measured by Ki-67, showed increased proliferation in the centrilobular and midzonal regions of 3.4 and 2.9-fold, respectively.

In conclusion, the data suggests that sulfoxaflor and its metabolite X11719474 may be agonist ligand for CAR, and this activation results in the observed phenobarbital-like response in the liver. However, the carcinogenic potential of sulfoxaflor, along with the proposed MOA for liver tumors will be evaluated by the Cancer Assessment Review Committee (CARC).

This non-guideline study is reliable with restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data.

IIA 5.5.4/3 Mode of Action Study Investigating Liver Weight Effects in crl:cd1(icr) Mice;
In a non-guideline study, (MRID 47832062) 5 male and 5 female Crl:CD-1 (ICR) mice were exposed to different dose levels of sulfoxaflor in the diet for 7 days. In previous studies with sulfoxaflor, increased liver weight was observed. Targeted gene expression data in mice and hepatocellular proliferation data in both mice and rats suggested a phenobarbital (PB)-like mode of action (MoA) could be responsible for the observed liver weight increase. The molecular response stimulated by PB is mediated by the constitutive androstane nuclear receptor (CAR), with peripheral involvement of the pregnane X receptor (PXR), and typically results in increased hepatocellular proliferation and hypertrophy, with associated increased liver weight, inhibition of apoptosis, hypertrophy, and eventual development of altered hepatic foci. In this study, male CD-1® mice were exposed to 0, 500, or 750 ppm, while female mice were exposed to 0, 1000, or 1500 ppm sulfoxaflor in the diet for 7 days. Endpoints evaluated were daily cage-side observations, body and liver weights, feed consumption, serum clinical chemistries, focused gene expression, liver cytochrome P450 enzyme activity, and hepatocellular proliferation using BrdU and Ki-67 methods. Archived liver samples from previously conducted 28 and 90-day sulfoxaflor mouse studies were analyzed for targeted gene expression, liver enzyme activity, and hepatocellular proliferation (Ki-67).

In the 7-day study, males exposed to 750 ppm had a statistically identified increase (17%) in relative liver weights compared to controls. Females exposed to 1000 and 1500 ppm had a statistically identified increase in relative (38 and 43%, respectively) and absolute (43 and 47%, respectively) liver weights compared to controls. These liver weight increases correlated with treatment-related observations of centrilobular and midzonal hepatocyte hypertrophy with very slightly increased cytoplasmic eosinophilia. Analysis by Oil Red O stain demonstrated slightly

increased amounts of lipid in the hepatocytes in males exposed to 750 ppm sulfoxaflor, however no changes were observed in females.

Cyp2b10 expression, considered to be the prototypical gene response following PB exposure through activation of CAR, was induced 42.1 and 54.8-fold in males exposed to 500 and 750 ppm, respectively, and 20.0 and 30.8-fold, in females exposed to 1000 and 1500 ppm, respectively, relative to controls. *Cyp2b10* gene expression in males from the 28 and 90-day studies was increased 61.7 (300 ppm) and 56.5-fold (750 ppm), respectively. Females exposed to 1500 ppm showed elevated *Cyp2b10* expression at 28 and 90 days of 93.9- and 53.9-fold, respectively. *Cyp3a11*, a PB- and PXR-related gene, was significantly elevated in the 750 ppm males at 7 and 90 days, while in female mice it was significantly elevated at all doses and time periods (range of 3.4 to 6.6-fold). PROD and BROD liver enzyme activities, which give a measure of Cyp2b enzyme induction, were elevated in both male and female mice at all time-points (range of 2.6 to 9.5-fold).

Both BrdU and Ki-67 immunohistochemical techniques were used to measure hepatocellular proliferation in the 7-day study. For the BrdU analysis, males exposed to 500 ppm sulfoxaflor had elevated proliferation in the centrilobular region and at 750 ppm in both the centrilobular and midzonal regions. Females exposed to 1000 and 1500 ppm showed significant induction in all three regions. For Ki-67 analysis, males exposed to 500 and 750 ppm showed significant induction in the centrilobular region, however unlike the BrdU analysis, increased proliferation was not observed at 750 ppm in the midzonal region. In females, increased proliferation was not evident at any dose or zone by Ki-67 analysis. Ki-67 analysis of hepatocellular proliferation in the 28 and 90-day studies showed no induction at either time point in male or female mice.
An additional aim of this study was to determine if other nuclear receptors might have played a role in sulfoxaflor -induced liver effects. Four nuclear receptors are primarily responsible for xenobiotic-induced liver weight increase; the aryl hydrocarbon receptor (AhR; Cyp1a), CAR (Cyp2b), PXR (Cyp3a), and the peroxisome proliferator-activated receptor alpha (PPARα; Cyp4a). AhR-related EROD liver enzyme activity was slightly elevated in this study at all time-points in both male and female mice; however, the degree of induction was mild (none greater than 2.3-fold) and was most likely associated with the large induction of Cyp2b enzyme. *Cyp4a10*, a PPARα related gene, was not significantly altered in this study.

These data suggests that sulfoxaflor may be an agonist ligand for CAR, and this activation results in the observed PB-like response and increased liver weight. However, the carcinogenic potential of sulfoxaflor, along with the proposed MOA for liver tumors will be evaluated by the Cancer Assessment Review committee (CARC).

This non-guideline study is reliable with restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data.

IIA 5.5.4/4 Induction profile and gene expression in mice livers;

In an induction profile study (MRID 47832276), sulfoxaflor (95.6% pure; batch number E2162-34) was administered in the diet to 15 male mice at dose levels of 0, 750, or 1500 ppm (equivalent to 0, 160, and 310 mg/kg/day respectively) for 7 days.

Administration of dietary sulfoxaflor to mice for 7 days resulted in decreased body weight at the high dose of 1500 ppm compared to control. There was a treatment-related increase in absolute and relative liver weights and ALT following seven days of exposure to sulfoxaflor. However, no significant increases in plasma AST, cholesterol and triglycerides were seen. Administration of sulfoxaflor at 750 ppm and 1500 ppm elicited a 3- and 5- fold increase in total hepatic P450, respectively, a 33-fold increase in PROD activity at both concentrations, a 47- and 82- folding increase in BROD activity, respectively, and a 4-and 7-fold increase in BQ activity, respectively.

The study examined the expression of two genes, *Cyp2b10* and *Cyp3a11*. Gene expression indicated the presence of *Cyp2b10* mRNA in only the treatment groups as it is constitutively expressed. The change in expression of *Cyp2b10* from sulfoxaflor 750 ppm treatment to sulfoxaflor 1500 ppm treatment was 9.2-fold. *Cyp3a11* is constitutively expressed in the mouse, and results can be expressed as a relative fold change over control values. Administration of 750 and 1500 ppm sulfoxaflor resulted in a 2.4-and 5.6-fold increase in *Cyp3a11* relative to controls. Sulfoxaflor induced gene expression data was confirmed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

In conclusion, the data suggest that sulfoxaflor exerts its enzyme induction properties *via* CAR and possibly PXR. However, the carcinogenic potential of sulfoxaflor, along with the proposed MOA for liver tumors will be evaluated by the Cancer Assessment Review Committee (CARC).

This study is Reliable with Restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data.

IIA 5.5.4/5 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; The aim of this study (MRID 47832255) was to investigate: (1) if the constitutive androstane receptor (CAR) or pregnane X receptor (PXR) mediate sulfoxaflor-induced hypertrophy and hyperplasia in mice; and (2) if the human receptors (CAR and PXR) support these processes to a similar extent as the murine receptors. The mouse models used were wild type C57BL/6J (WT) mice, C57BL/6J mice null for PXR and CAR (PXRKO/CARKO) and C57BL/6J mice "humanised" for PXR and CAR (hPXR/hCAR).

In this study, groups of ten male mice of each strain were offered diets containing 0 or nominally 750 ppm sulfoxaflor for seven days. The measured dietary concentration of sulfoxaflor was 740 ppm (approximately 98.6% of target) with good homogeneity. Endpoints evaluated were daily clinical observations, body weights, body weight gain, feed consumption, plasma clinical chemistries, focused gene expression, protein quantification, liver cytochrome P450 enzyme activity, hepatocellular proliferation using nuclear incorporation of BrdU, and liver histopathology.

There were no treatment-related clinical observations or effects on body weight or body weight gain in any strain of mouse. There were treatment-related increases in absolute and relative liver weights in WT and hPXR/hCAR mice but not in the PXRKO/CARKO animals. Absolute liver weights for WT and hPXR/hCAR mice in the 750 ppm sulfoxaflor groups were 124 and 109% of controls respectively, and relative liver weights were 125 and 112% of controls respectively.

In WT mice, sulfoxaflor treatment increased hepatocellular proliferation (as measured by approximately 4-fold but no such changes in proliferation were seen in either the hPXR/hCAR or PXRKO/CARKO mice. Treatment-related hepatocyte hypertrophy was observed in WT and hPXR/hCAR mice while increased mitotic figures were observed only in WT mice. Neither hepatocyte hypertrophy nor increased mitotic figures were seen in PXRKO/CARKO mice.

Sulfoxaflor behaved as a phenobarbital-like inducer in WT mice. A marked induction of total cytochrome P450, markedly increased PROD and BROD (Cyp2b selective substrates), increased expression of *Cyp2b10* mRNA (demonstrated by RT-PCR), increases in Cyp2b10 protein (immunoblotting data) were observed after exposure to sulfoxaflor. However, in the hPXR/hCAR under the same conditions, induction of Cyp2b10 activity, protein and mRNA was markedly less than observed in the WT animals following treatment with sulfoxaflor. In PXRKO/CARKO animals, sulfoxaflor had no significant effect on Cyp2b10 expression or catalytic activity.

Sulfoxaflor-mediated Cyp3a11 induction, as determined by BQ activity (Cyp3a selective r**e**action), RT-PCR and immunoblotting was observed in the "humanised" and WT mice, to similar extents, but was not seen in the PXRKO/CARKO mice.

In conclusion, sulfoxaflor exhibited markedly more activity towards the mouse CAR than the human CAR and relatively weak activity towards the mouse and human PXR. This suggests that the difference in hepatic response between wild type and humanised mice in this study may be mediated via CAR. Additionally, the data show that the human CAR/PXR support sulfoxaflor-induced hypertrophy but not hyperplasia.

In conclusion, the data suggests that sulfoxaflor acts via a CAR-mediated mode of action in mice and it is possible that exposure would not result in human carcinogenicity. However, these findings, along with those in other mechanistic studies, will be used by the Cancer Assessment Review Committee (CARC) to evaluate the carcinogenic potential of sulfoxaflor and the proposed MOA (and human relevance) for liver tumors.

This non-guideline study is reliable with restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data.

A.4.6 Mutagenicity

Gene Mutation

IIA 5.4.1 *In vitro* bacterial (*Salmonella typhimurium* and *E. coli*)/mammalian activation gene mutation assay;
In independent trials of a reverse gene mutation assay (MRID 47832047), sulfoxaflor (Purity 96.6%; Lot/Batch No. E2198-17), prepared in dimethyl sulfoxide (DMSO), was tested in 4 strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and in *E. coli* WP2*uvrA* in a pre-incubation reverse mutation assay at concentrations ranging from 100 to 5000 µg/plate (both trials) with and without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

In both assays, the test material was not cytotoxic or mutagenic for any strain at concentrations up to the limit dose for this test system either in the absence or presence of S9 activation. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased.

Under the conditions of this study, sulfoxaflor did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to the limit dose for this test system.

The study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for in vitro mutagenicity (bacterial reverse gene mutation) data.

IIA 5.8.1.4/1 In vitro bacterial (*Salmonella typhimurium*)/mammalian activation gene mutation assay;
In independent trials of a reverse gene mutation assay (MRID 47832097), X11719474 (Purity 99.6%; Lot/Batch No. XS9-36891-82, TSN106429 and Lot/Batch No. XS9-37307-78, TSN030626-0001), prepared in dimethyl sulfoxide (DMSO), was tested in 4 strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and in *E. coli* WP2*uvrA* in a pre-incubation reverse mutation assay at concentrations ranging from 100 to 5000 µg/plate (both trials) with and without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

In both assays, the test material was not cytotoxic or mutagenic for any strain at concentrations up to the limit dose for this test system either in the absence or presence of S9 activation. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased.

Under the conditions of this study, X11719474 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to the limit dose for this test system.

The study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

IIA 5.8.2.4/1 *Salmonella –Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11721061;

In independent trials of a reverse gene mutation assay (MRID 47832085), [X11721061 Purity 99%; Lot/Batch No. GF908627 (TSN030701-0003)], prepared in dimethyl sulfoxide (DMSO) was tested in 4 strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and in *E. coli* WP2*uvr*A in a pre-incubation reverse mutation assay at concentrations ranging from 1.5 to 5000 µg/plate (Initial cytotoxicity-mutation assay) and 50 to 5000 µg/plate (Confirmatory assay) both with and without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

In the initial assay, there was some indication of a slight cytotoxic effect (reduced revertant colony counts) for several strains at the highest concentration; however, this was not reproduced in the confirmatory trial. Nevertheless, the data are in good agreement and indicate that the test material was not mutagenic in any strain at concentrations up to the limit dose for this test system either in the absence or presence of S9 activation. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased.

Under the conditions of this study, X11721061 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to the limit dose for this test system.

The study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

IIA 5.8.3.4/1 *Salmonella –Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11596066;

In independent trials of a reverse gene mutation assay (MRID 47832076), the sulfoxaflor metabolite X11596066 [Purity 98%; Lot/Batch No. SYN-6268-05-06 (TSN031296-0002)], prepared in dimethyl sulfoxide (DMSO), was tested in 4 strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and in *E. coli* WP2*uvr*A in a pre-incubation reverse mutation assay at concentrations ranging from 1.5 to 5000 µg/plate (Initial cytotoxicity-mutation assay) and 15 to 5000 µg/plate (Confirmatory assay) both with and without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

In both assays, concentrations ≥1500 µg/plate +/-S9 were cytotoxic for any strains. However, there were no increases in the number of revertant colonies at any noncytotoxic level, in any strains, both with and without S9. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased.

Under the conditions of this study, X11596066 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to those producing evidence of cytotoxicity.

The study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

IIA 5.8.4.4/1 *Salmonella –Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11579457;

In independent trials of a reverse gene mutation assay (MRID 47832070), the sulfoxaflor metabolite X11579457 (Purity 99%; Lot/Batch No. SYN-5866-65 (TSN030941-0002), was prepared in distilled H_2O, and tested in 4 strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and in *E. coli* WP2*uvrA* in a pre-incubation reverse mutation assay at concentrations ranging from 1.5 to 5000 µg/plate (Initial cytotoxicity-mutation assay) and 50 to 5000 µg/plate (Confirmatory assay) both with and without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

In both assays, the test material was not cytotoxic or mutagenic for any strain at concentrations up to the limit dose for this test system either in the absence or presence of S9 activation. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased.

Under the conditions of this study, X11579457 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to the limit dose for this test system.

The study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

IIA 5.8.5.4/1 *Salmonella –Escherichiacoli* /mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with X11519540;

In independent trials of a reverse gene mutation assay (MRID 47832068), the sulfoxaflor metabolite X11519540 (Purity 98%; Lot/Batch No. 37307-15 (TSN106498), prepared in dimethyl sulfoxide (DMSO), was tested in 4 strains of *S. typhimurium* (TA100, TA98, TA1535 and TA1537) and in *E. coli* WP2*uvr*A in a pre-incubation reverse mutation assay at concentrations ranging from 1.5 to 5000 µg/plate (Initial cytotoxicity-mutation assay) and 50 to 5000 µg/plate (Confirmatory assay) both with and without S9 activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

In both assays, the test material was not cytotoxic or mutagenic for any strain at concentrations up to the limit dose for this test system either in the absence or presence of S9 activation. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased.

Under the conditions of this study, X11519540 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to the limit dose for this test system.

The study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

Cytogenetics

IIA 5.4.3/1 In Vitro Gene Mutation assay in Chinese hamster ovary cells;

In independent trials of a mammalian cell forward gene mutation assay (MRID 47832049), Chinese hamster ovary (CHO/HGPRT) cells were exposed to sulfoxaflor (Purity 96.6% Lot # E2198-17, TSN106108), prepared in dimethyl sulfoxide (DMSO), at concentrations ranging from 173.3 to 2773 µg/ml in the absence and presence of S9. The S9 liver homogenate was derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. The highest concentration was the 10 mM limit for the assay system. Positive control chemicals used in this assay were ethyl methanesulfonate (EMS) in the absence of S9 and 20-methylcholanthrene (20-MCA) in the presence of S9.

Sulfoxaflor was tested up to the limit dose for this test system and failed to induce either a cytotoxic or mutagenic effect in either the absence or the presence of S9 activation. The expected responses were obtained with the negative and positive controls either with or without S9 activation. It was, therefore, concluded that sulfoxaflor was not active in this test system.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for in vitro mutagenicity (mammalian forward gene mutation) data.

IIA 5.8.1.4/3 Evaluation of X11719474 in the Chinese hamster ovary cell/ hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay;

In independent trials of a mammalian cell forward gene mutation assay (MRID 47832095), Chinese hamster ovary (CHO/HGPRT) cells were exposed to X11719474 (Purity 100%; Lot # XS9-36891-82, TSN106429), prepared in dimethyl sulfoxide (DMSO) at concentrations ranging from 184.6 to 2953 µg/ml in the absence and the presence of S9. The highest concentration was based on the 10 mM limit of the assay system. The S9 liver homogenate was derived from the livers of Sprague-Dawley rats Aroclor 1254-induced. The highest concentration was the 10 mM limit for the assay system. Positive control chemicals used in this assay were ethyl methanesulfonate (EMS) in the absence of S9 and 20-methylcholanthrene (20-MCA) in the presence of S9.

X11719474 was tested up to the limit dose for this test system and failed to induce either a cytotoxic or mutagenic effect in either the absence or the presence of S9 activation. The expected responses were obtained with the negative and positive controls either with or without S9 activation. It was, therefore, concluded that X11719474 was not active in this test system.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

IIA 5.8.2.4/3 Evaluation of X11721061 in the Chinese hamster ovary cell/ hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay;

In independent trials of a mammalian cell forward gene mutation assay (MRID 47832077), Chinese hamster ovary (CHO/HGPRT) cells were exposed to the sulfoxaflor metabolite X11721061 (Purity 99%; Lot # GF908627, TSN030701-0003), prepared in distilled H_2O, at concentrations ranging from 120 to 1920 µg/ml (both trials) in the absence and the presence of S9. The S9 liver homogenate was derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. The highest concentration was the 10 mM limit for the assay system. Positive

control chemicals used in this assay were ethyl methanesulfonate (EMS) in the absence of S9 and 20-methylcholanthrene (20-MCA) in the presence of S9.

X11721061 was tested up to the limit dose for this test system and failed to induce either a cytotoxic or mutagenic effect in either the absence or the presence of S9 activation. The expected responses were obtained with the negative and positive controls either with or without S9 activation. It was, therefore, concluded that X11721061 was not active in this test system.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OCSPP 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

IIA 5.8.4.4/3 Evaluation of X11579457 in the Chinese hamster ovary cell/ hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay;
In independent trials of a mammalian cell forward gene mutation assay (MRID No. 48288933), Chinese hamster ovary (CHO/HGPRT) cells were exposed to X11579457 (Purity 99%; Lot# SYN-5866-65, TSN030941-0002), prepared in distilled H_2O, at concentrations ranging from 157.8 to 2525 µg/mL (both trials) in the absence and the presence of S9. The S9 liver homogenate was derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. The highest concentration was the 10 mM limit for the assay system. Positive control chemicals used in this assay were ethyl methanesulfonate (EMS) in the absence of S9 and 20-methylcholanthrene (20-MCA) in the presence of S9.

X11579457 was tested up to the limit dose for this test system and failed to induce either a cytotoxic or mutagenic effect in either the absence or the presence of S9 activation. The expected responses were obtained with the negative and positive controls either with or without S9 activation. It was, therefore, concluded that X11579457 was not active in this test system.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

IIA 5.8.5.4/3 Evaluation of X11519540 in the Chinese hamster ovary cell/ hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay;
In independent trials of a mammalian cell forward gene mutation assay (MRID No. 48288934), Chinese hamster ovary (CHO/HGPRT) cells were exposed to the sulfoxaflor metabolite X11519540 (Purity 98%; Lot # 37307-15, TSN106498), prepared in dimethyl sulfoxide (DMSO) concentrations ranging 158.8 to 2540 µg/ml in the absence and the presence of S9. The highest concentration was based on the 10 mM limit of the assay system. The S9 liver homogenate was derived from the livers of Sprague-Dawley rats Aroclor 1254-induced. The highest concentration was the 10 mM limit for the assay system. Positive control chemicals used in this assay were ethyl methanesulfonate (EMS) in the absence of S9 and 20-ethylcholanthrene (20-MCA) in the presence of S9.

X1151940 was tested up to the limit dose for this test system and failed to induce either a cytotoxic or mutagenic effect in either the absence or the presence of S9 activation. The expected

responses were obtained with the negative and positive controls either with or without S9 activation. It was, therefore, concluded that X11519540 was not active in this test system.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data

Other Genotoxicity

IIA 5.4.2/1 Evaluation of sulfoxaflor in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes;

In an *in vitro* chromosome aberration test (MRID 47832050), primary rat lymphocytes, derived from 10-11 week old Sprague-Dawley male rats, were exposed to sulfoxaflor (Purity 96.6% Batch/Lot No. E2198-17, TSN106108) prepared in dimethyl sulfoxide (DMSO) at 0, 86.7, 173.3, 346.6, 693.3, 1389.5, and 2773 μg/mL without and with S9 activation for 4 hours and continuously for 24 hours at 0, 21.7, 43.3, 86.7, 173.3, 346.6, 693.3, 1386.5 and 2773 μg/mL without S9. The highest concentration tested approximates the limit dose of 10 mM and the S9 liver homogenate was prepared from Aroclor 1254-induced male Sprague-Dawley rats. Base on the analysis of mitotic indices (MIs), cultures treated for 4 hours with 0, 693.3, 1386.5, and 2773 μg/mL +/-S9 and cultures treated continuously for 24 hours with 173.3, 346.6 and 693.3 μg/mL –S9 were scored for structural and numerical chromosome aberrations.

Relative MIs (RMIs) were ≥ 50% of control at 2773 μg/mL –S9 (4-hour treatment); 2200 μg/mL +S9 (4-hour treatment); and 346.6μg/mL –S9 (24-hour treatment). There were no significant increases in the frequencies of cells with aberrations in either the presence or absence of S9 activation. Cultures treated with the positive control chemicals (mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in all assays.

Based upon these results, sulfoxaflor was not considered to be clastogenic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

This study is classified as totally reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mammalian cytogenetics (chromosome aberrations) data.

IIA 5.8.1.4/2 Evaluation of X11719474 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes;

In an *in vitro* chromosome aberration test (MRID 47832096), lymphocytes, derived from male Sprague-Dawley rats were exposed to X11719474 (Purity 100%; Batch/Lot No. XS9-36891-82, TSN106429), prepared in dimethyl sulfoxide (DMSO) at concentrations ranging from 184.6 to 2953 μg/mL for 4 hours in the presence and absence of S9 activation and for 24 hours without S9. The highest concentration was based on the limit dose of 10 mM in this assay system. The S9 was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. Metaphases, harvested from cultures treated with 738.25, 1476.5 and 2953μg/mL -S9 (4- and 24-hour treatments) and 369.1, 738.25 and 2953 μg/mL +S9 (4-hour treatment), were examined for the incidences of cells with structural aberrations and numerical aberrations.

X11719474 was not severely cytotoxic nor clastogenic at any concentration up to the limit dose for this test system following a 4-hour exposure. For the prolonged, 24-exposure, the MI was reduced to 32.3% of control at 2953 μg/mL –S9; however, the test material was not clastogenic at any non-cytotoxic concentration. The expected responses were induced by the non-activated and S9-activated positive controls, mitomycin C and cyclophosphamide, respectively. Based upon these results, X11719474 was considered not to be clastogenic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mammalian cytogenetics (chromosome aberrations) data.

IIA 5.8.2.4/2 Evaluation of X11721061 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes;

In an *in vitro* chromosome aberration test (MRID 47832078), lymphocytes, derived from male Sprague-Dawley rats were exposed to X11721061 (Purity 99%; Batch/Lot No. GF908627,TSN030701-0003), prepared in distilled water, at concentrations ranging from 30 to 1920 μg/mL for 4 hours in the presence and absence of S9 activation and at concentrations ranging from 15 to 1920 μg/mL for 24 hours without S9. The highest concentration was based on the limit dose of 10 mM in this assay system. The S9 was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. Metaphases, harvested from cultures treated with 480, 960 and 1920 μg/mL +/-S9 (4-hour treatment) and 120, 240 and 480 μg/mL –S9 (24-hour treatment), were examined for the incidences of cells with structural aberrations and numerical aberrations.

X11721061 was not severely cytotoxic nor clastogenic at any concentration up to the limit dose for this test system following a 4-hour exposure. For the prolonged, 24-exposure, MIs were ≥50% of control at concentrations ≤480 μg/mL –S9; however, the test material was not clastogenic at any non-cytotoxic concentration. The expected responses were induced by the non-activated and S9-activated positive controls, mitomycin C and cyclophosphamide, respectively. Based upon these results, X11721061 was considered not to be clastogenic in this in vitro chromosomal aberration assay utilizing rat lymphocytes.

This study is classified as totally reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mammalian cytogenetics (chromosome aberrations) data.

IIA 5.8.5.4/2 Evaluation of X11519540 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes;

In an *in vitro* chromosome aberration test (MRID 48288935), lymphocytes, derived from male Sprague-Dawley rats were exposed to X11519540 (Purity 98%; Batch/Lot No. 37307-15,TSN106498), prepared in dimethyl sulfoxide (DMSO), at concentrations ranging from 39.7 to 2540 μg/mL for 4 hours in the presence and absence of S9 activation and at concentrations ranging from 19.8 to 2540 μg/mL for 24 hours without S9. The highest concentration was based on the limit dose of 10 mM in this assay system. The S9 was derived from the livers of male

Sprague-Dawley rats induced with Aroclor 1254. Metaphases, harvested from cultures treated with 635, 1270 and 2540 μg/mL +/-S9 (4-hour treatment) and 158.8, 317.5 and 635μg/mL –S9 (24-hour treatment), were examined for the incidences of cells with structural aberrations and numerical aberrations.

X11579457 was not severely cytotoxic nor clastogenic at any concentration up to the limit dose for this test system following a 4-hour exposure. For the prolonged, 24-hour exposure, MIs were ≥50% of control at concentrations ≤635 μg/mL –S9; however, the test material was not clastogenic at any non-cytotoxic concentration. The expected responses were induced by the non-activated and S9-activated positive controls, mitomycin C and cyclophosphamide, respectively. Based upon these results, X11519540 was not clastogenic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

This study is classified as totally reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mammalian cytogenetics (chromosome aberrations) data.

IIA 5.4.2/2 Evaluation of X11579457 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes;

In an *in vitro* chromosome aberration test (MRID 48288932), lymphocytes, derived from male Sprague-Dawley rats were exposed to X11579457 (Purity 99%; Batch/Lot No. SYN-5866-65,TSN030941-0002), prepared in distilled H_2O, at concentrations ranging from 39.5 to 2525 μg/mL for 4 hours in the presence and absence of S9 activation and at concentrations ranging from 19.7 to 2525 μg/mL for 24 hours without S9. The highest concentration was based on the limit dose of 10 mM in this assay system. The S9 was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. Metaphases, harvested from cultures treated with 631.3, 1262.5 and 2525 μg/mL +/-S9 (all treatment times), were examined for the incidences of cells with structural aberrations and numerical aberrations.

X11579457 was neither cytotoxic nor clastogenic at any concentration up to the limit dose for this test system. The expected responses were induced by the nonactivated and S9-activated positive controls, mitomycin C and cyclophosphamide, respectively. Based upon these results, X11579457 was considered not to be clastogenic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mammalian cytogenetics (chromosome aberrations) data.

IIA 5.4.4/1 *In vivo* mammalian cytogenetics - erythrocyte micronucleus assay in mice;

In a bone marrow micronucleus assay (MRID 47832071), groups of 6 male and 6 female CD-1 mice were treated orally, by gavage, with 0, 100, 200 or 400 mg/kg/day of sulfoxaflor (Purity 95.6%; Lot/Batch No. E2162-34, TSN003725-0001) prepared in METHOCEL™ on 2 consecutive days. The highest dose level of 400 mg/kg bw was selected for the main assay based on the results of a range-finding test in which doses ≥1000 mg/kg bw/day caused more than 50% mortality in both sexes and 500 mg/kg bw/day caused unacceptable body temperature decreases

in males only. Therefore, both sexes were evaluated in the main study. Groups of animals were sacrificed at 24 hours after the second treatment for the collection of femoral bone marrow and evaluation of polychromatic erythrocytes (PCE, 2000 PCE/animal) with micronuclei (MN-PCE) from the first five animals in each group. The proportion of PCE was determined based upon 200 erythrocytes per animal and the results expressed as a percentage. Mice treated with 120-mg/kg bw cyclophosphamide monohydrate by a single oral gavage dose and sacrificed at 24 hours served as the positive control.

All animals survived to the end of the observation period. Treatment related clinical signs (decreased activity) occurred in 3/6 male mice at 400 mg/kg /day and two of these mice also had body temperature decreases of up to 5.8°C five hours post-dosing. There were no statistically significant increases in the frequencies of MN-PCE in groups treated with the test material as compared to the negative controls. There were no statistically significant differences in the percent PCE in groups treated with the test material compared to negative controls. By contrast, a significant increase in the frequency of MN-PCE and a significant decrease in the relative proportion of PCE: NCE ($p<0.05$) was seen in the positive control group as compared to the negative control group.

Under the experimental conditions used, sulfoxaflor was not genotoxic in the mouse bone marrow micronucleus test.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirement (USEPA OPPTS 870.5395; OECD 474) for an i*n vivo* mammalian cytogenetics – micronucleus assay in mice.

A.4.7 Neurotoxicity

870.6100 Delayed Neurotoxicity Study - Hen

870.6200 Acute Neurotoxicity Screening Battery

IIA 5.7.1/1 Acute Neurotoxicity – Rats;

In an acute neurotoxicity study (MRID 47832134), ten male and ten female F344/DuCrl rats per group were given a single, oral gavage dose of 0, 7.5, 75, or 750 mg sulfoxaflor/kg body weight to evaluate the potential for acute neurotoxicity. Body weights were recorded and a functional observational battery (FOB) and test for motor activity were conducted pre-exposure (baseline), the day of dosing (day 1, time-of-peak effect), day 8, and day 15. The FOB included hand-held and open-field observations as well as measurements of grip performance, landing foot splay and rectal temperature. Clinical observations were conducted on days 2, 3, and 4. At the end of the study all rats from the control and high-dose group and five rats/sex/low- and mid-dose group were perfused for histopathologic evaluation of the central and peripheral nervous systems, which was conducted on all of the control and high-dose group rats. A second motor activity study at dose levels of 0, 2.5, 7.5 and 25 mg/kg was conducted to investigate whether an apparent decrease in motor activity at 7.5 mg/kg was reproducible or treatment-related, and to

establish a clear no-observed-adverse-effect level (NOAEL). Motor activity was the only endpoint examined in this study phase.

One female rat given 750 mg/kg died following dosing on day 1, but the cause of death could not be determined. Treatment-related categorical observations on day 1 in males and females given 750 mg/kg included increased incidences of muscle tremors and twitches, convulsions, splayed hindlimbs and perineal urine soiling. Treatment-related ranked FOB observations on day 1 in males and females given 750 mg/kg were as follows: increased lacrimation and salivation, decreased pupil size and response to touch, increased level of urination (females only), decreased level of open-field activity and gait abnormalities. There were no treatment-related ranked or categorical FOB observations present on day 8 or day 15 in males or females given 750 mg/kg. There were no treatment-related ranked or categorical observations in males or females given 7.5 or 75 mg/kg during any FOB.

There was a treatment-related decrease in body weight of the 750 mg/kg group when compared to controls on days 8 and 15, which was more prominent in males than in females. There was a treatment-related decrease in rectal temperature of the 750 mg/kg group when compared to controls on day 1, which was not present in the subsequent examinations on days 8 and 15. There were no treatment-related effects in grip performance or landing foot splay.
There was a treatment-related decrease in the day 1 total motor activity and an effect on the distribution of motor activity counts of males and females given 75 or 750 mg/kg. The effect on total motor activity of animals given 7.5 mg/kg was considered equivocal on day 1. There were no effects on motor activity on days 8 or 15 in rats of any dose group. In the follow-up motor activity study, there were no treatment-related effects on total motor activity or on the distribution of motor activity counts for males and females given 2.5, 7.5 or 25 mg/kg when compared to controls.

Treatment-related clinical findings on days 2, 3 or 4 were limited to males and females given 750 mg/kg, and included decreased or absent feces, red perioral soiling, and perineal urine soiling (females only). There were no treatment-related gross or histopathologic findings in the central or peripheral nervous system.

The lowest-observed-adverse-effect-level (LOAEL) for neurotoxicity was 75 mg/kg based on decreased motor activity observed on day 1. The no-observed-adverse-effect-level (NOAEL) was 25 mg/kg. No treatment-related effects were observed for neuropathology; therefore the NOAEL for neuropathology was ≥ 750 mg/kg, the highest dose level tested.

The study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements (OPPTS 870.6200; OECD 424) for an acute neurotoxicity study in rats.

870.6200 Subchronic Neurotoxicity Screening Battery (See 870.3100 (p. 85))

870.6300 Developmental Neurotoxicity Study

IIA 5.7.5/1 Developmental Neurotoxicity Study

In a developmental neurotoxicity study in rats (MRID 478321333), sulfoxaflor (purity 95.6%; Lot # E2162-34) was offered on a continuous basis in the diet to 3 groups of 25 bred female Crl:CD(SD) rats daily from gestation day 6 through lactation day 21. Target test substance concentrations were 25, 100, and 400 parts per million (ppm), which corresponded to predicted dosage levels of 2, 8, and 32 mg/kg/day, respectively. Actual overall mean test substance consumption in the 25, 100, and 400 ppm groups was 1.8, 7.1, and 27.7 mg/kg/day through gestation and 1.9, 7.6, and 29.8 mg/kg/day through lactation, respectively. A concurrent control group composed of 25 bred females received the basal diet on a comparable regimen. Dams were approximately 13 weeks of age at the beginning of test diet exposure.
There were no test substance-related mortalities in the dams during the study. There were no test substance-related clinical findings noted during the daily examinations. Detailed clinical observation parameters, as well as maternal body weights and food consumption during gestation and lactation were unaffected by test substance exposure.

There were no test substance-related differences noted between groups when comparing the mean length of gestation, the process of parturition, and internal macroscopic pathologic findings. The mean numbers of former implantation sites and unaccounted for sites, as well as maternal kidney and liver weights were similar across groups.

There were no test substance-related effects on F_0 maternal parameters in this study. Therefore, the no-observed-adverse-effect level (NOAEL) for F_0 systemic toxicity and F_0 maternal reproductive toxicity (process of parturition and duration of gestation) of sulfoxaflor when administered orally in the diet was considered to be 400 ppm (equivalent to 27.7 mg/kg/day).

There were no test substance-related effects on the mean number of pups born, live litter size, or the percentage of males at birth at any maternal exposure level. However, there was a reduction in postnatal survival from birth to PND 4 compared to the control group in the 100 and 400 ppm dose groups. Furthermore, mean pup body weights in the 400 ppm group were 11.8% and 6.5% lower than the control group at birth (PND 1) and on PND 4, respectively. The reduced pup body weights resulted in a statistically significant delay in surface righting response for pups in the 400 ppm group. Pup body weights in the 400 ppm group did not differ from the control group values on PND 7 or later time points. The decrease in postnatal survival at 100 and 400 ppm is consistent with results from a previous probe reproduction study, in which dietary exposures of 500 and 1000 ppm resulted in decreased pup survival. The high dose level of 400 ppm in this study was based on the treatment-related decrease in survival observed in the probe study. Postnatal survival and pup body weights and body weight gains in the 25 and 100 ppm groups were unaffected by maternal test substance exposure. The age of attainment of surface righting response in the 25 and 100 ppm groups and eye opening in the 25, 100, and 400 ppm groups were similar to the control group. The attainment of sexual developmental landmarks (balanopreputial separation and vaginal patency) were unaffected by maternal test substance exposure.

No remarkable clinical observations or macroscopic findings were noted for offspring at any exposure level. No test substance-related effects were observed with respect to detailed clinical observations, locomotor activity, auditory startle response, and learning and memory. Furthermore, there were no test substance-related effects on morphometric parameters or histopathology of the brain and/or central and peripheral nervous systems for offspring on PND

21 and 72. On PND 72, slight, statistically significant changes in brain weight (a decrease of 5% in males relative to controls; an increase of 4% in females relative to controls)) and brain length (a decrease of 4% in males relative to controls) were noted at 400 ppm.

The LOAEL for offspring is 100 ppm (7.1 mg/kg bw/day) based on the reduction in pup survival. The NOAEL is 25 ppm (1.8 mg/kg bw/day).

The study is classified as fully reliable (acceptable/guideline) and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300; OECD 426 (draft)) due to the pending review of the positive control data.

A.4.8 Metabolism

870.7485 Metabolism – Rat

IIA 5.1.1/1 Single and repeat dose oral study in rats (and mice);

In a metabolism probe study (MRID 47832038), the absorption, distribution, metabolism and elimination of ^{14}C-sulfoxaflor (>97% radiochemical purity, specific activity 42.0 mCi/mmol; lot no. INV 2058) in Fischer 344 DuCrl rats and Crl:CD1 (ICR) mice was evaluated following a single oral gavage dose in 0.5% aqueous methylcellulose at a dosing volume of 5 mL/kg. One rat/sex and two mice/sex were administered 100 mg/kg ^{14}C-sulfoxaflor. Time-course blood and excreta were collected from rats, whereas only excreta was collected from mice up to 72 hours post-dosing. The only tissues collected at the 72 hr sacrifice were plasma and RBC. Urine samples collected during the first 12 hours post-dosing were analyzed for parent compound and metabolite(s). Additionally, two rats (1/sex) and four mice (2/sex) were orally dosed with 100 mg/kg ^{14}C-sulfoxaflor and plasma collected at C_{max} (2 hour post-dosing for rats and an estimated 1½ hour post-dosing for mice) and analyzed for parent and metabolite(s). This report also included results of the analysis of plasma and urine samples collected from 28-day rat, 90-day rat, and 28-day mouse dietary subchronic toxicity studies.

Orally administered ^{14}C-sulfoxaflor was rapidly absorbed from the GI tract both by rats and mice. Total urinary elimination accounted for 87-98% of the dose in rats and 80-85% of the dose in mice, most of which occurred within the first 24 hours post-dosing. Fecal elimination accounted for only 5% in rats and 13% in mice, mostly apparently representing unabsorbed dose due to its recovery in feces within the GI transit time of 24 hours. Negligible or no radioactivity was eliminated via expiration. Elimination of radioactivity from blood was mono-exponential with $t_{½}$ of 9 and 11 hours from the plasma and RBC of male rats, respectively; the $t_{½}$ of elimination of ^{14}C-sulfoxaflor from the plasma and RBC of female rats was 7 and 8 hours, respectively. Female rats eliminated ^{14}C-sulfoxaflor from the body at a 1.2-fold faster rate than males, resulting in the same difference in the AUC. No metabolism of the test material was observed following a single oral dose of ^{14}C-sulfoxaflor to rats or mice. Sulfoxaflor was excreted mostly as unmetabolized parent, with only low levels of metabolites identified. Both parent sulfoxaflor and the X11602627 (XR-208) urea metabolite were observed in rat and mouse urine samples, but only parent sulfoxaflor was observed in rat plasma (mouse plasma not evaluated due to low radioactivity). The concentration of the urea metabolite was similar to that

detected in the test material itself as an impurity. In addition to the urea metabolite, 5 additional minor metabolites in the rat plasma and 3 possible minor metabolites rat urine were observed, whereas one metabolite was observed in mouse urine. Metabolite profiles were similar in urine and plasma samples from the 28- or 90-day dietary studies.

This study is classified as reliable with restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data. It was conducted as a metabolism probe study and not to satisfy guideline requirements for sulfoxaflor.

IIA 5.1.2/1 Toxicokinetic studies – Second single dose, oral route, in rats;
In a supplemental rat metabolism study (MRID 47832039), the tissue distribution of sulfoxaflor (^{14}C-sulfoxaflor, radiochemical purity 99.3% a.i.; lot no. INV 027474-0001) was evaluated following a single oral gavage administration of 5 or 100 mg/kg body weight to groups of four F344/DuCrl rats/sex/dose/sacrifice time. The ^{14}C-sulfoxaflor test material was administered in 0.5% aqueous methylcellulose vehicle (dosing volume 5 mL/kg body weight). Animals were sacrificed at predetermined times of either C_{max} and ½C_{max} for plasma radioactivity levels (i.e., ranges 0.5-2 and 6-8 hours post-dosing, respectively). Radioactivity was also determined in urine, feces and cage wash for the ½ C_{max} groups. Additionally, the profile of sulfoxaflor-derived metabolites in three representative tissues (liver, kidney and plasma) was characterized by HPLC/ESI/MS for groups dosed at 100 mg/kg. A probe study was performed on 3 rats/sex at 100 mg/kg single gavage dose (sacrificed at C_{max}) to develop extraction procedures for liver, kidney and plasma for identification of metabolites in the main study.

Orally administered ^{14}C- sulfoxaflor was rapidly absorbed without any apparent lag time based on C_{max} times of 0.5 - 1 hr (5 mg/kg) and 2 hr (100 mg/kg). Total recovery of radioactivity from all the animals averaged 98% in both groups.

Test material-derived radioactivity (as µg ^{14}C- sulfoxaflor eq/g) was highest in the GI tract, liver, kidney and urinary bladder, consistent with portal of entry and primary excretion tissues. Plasma levels were approximately 50-60% of those found in the kidney and liver in both groups. Comparison of the C_{max} and $C_{1/2\ max}$ tissue radioactivity levels, together with comparisons of tissue to blood ratios, did not suggest the potential for bioaccumulation.

Parent sulfoxaflor, identified as its two diastereomers, was the only component detected in the kidney, liver and plasma samples above the limits of detection of 0.286, 0.563 and 0.146 µg equivalent sulfoxaflor /g tissue, respectively, indicating no metabolism of sulfoxaflor.

In summary, administered sulfoxaflor was rapidly absorbed, widely distributed without metabolism, with the highest levels in portal of entry and excretory tissues. Test material-derived radioactivity in tissues (other than portal of entry and excretory) tracked that of blood and did not indicate potential for bioaccumulation.

This study is classified Fully Reliable (acceptable/non-guideline). The study was conducted to provide supplemental information on tissue distribution and metabolite identification of

sulfoxaflor at C_{max} and ½C_{max} and on its own does not by itself satisfy guideline requirements for a general metabolism study in the rodent.

IIA 5.1.3/1 Toxicokinetic studies – Repeated dose, oral route, in rats;

In a rodent general metabolism study, (MRID 47832034), ^{14}C-sulfoxaflor (radiochemical purity 99.7% a.i.; lot no. INV 2066) was administered to four groups of male and female F344/DuCrl rats (4/group/sex) at the following doses: low dose of 5 mg/kg via gavage as a single dose or as multiple doses (14 daily doses of unlabeled sulfoxaflor followed by a single radiolabeled dose); single gavage high dose of 100 mg/kg, or a single intravenous (IV) low dose of 5 mg/kg). All oral doses were administered in 0.5% aqueous methylcellulose vehicle (5 mL/kg body weight). Animals were monitored for 168 hours (7 days) post-dosing to determine absorption, distribution, metabolism and excretion of ^{14}C- sulfoxaflor.

Orally administered sulfoxaflor was rapidly absorbed without any apparent lag time. The percent absorption of the administered dose in all three oral groups (single 5 mg/kg, multiple 5 mg/kg or single 100 mg/kg) was at least 92-96%, based on recovery in urine, non-GI tissues and expired air. Total recovery of radioactivity from all the animals averaged 102 ± 4 and 108 ± 4% in the oral and IV dose groups, respectively.

The oral doses were rapidly excreted in urine (92-97% of the administered dose) without any gender difference. The majority of the urinary elimination (89-94%) occurred within the first 24 hours post-dosing. Only a small percent (5-8%) of the oral dose was eliminated in feces, also mostly within the first 24 hrs post-dosing.

The IV-administered test material was also rapidly excreted in urine (97-101% of administered dose). The majority of the urinary elimination (91-95%) occurred in the first 24 hours post-dosing. Only a small percent (6-9%) of the IV dose was eliminated in feces, mostly in the first 24 hours post-dosing. Systemic bioavailability, calculated from the dose-corrected plasma AUC data for the low oral and IV dose groups, was 94% for both male and female rats.

Only 0.2-1.2% of the administered ^{14}C- sulfoxaflor (oral administration: low single, high single or multiple dose) remained in the tissues after 168 hours (7 days) post-dosing. An average of 0.6-1.3% of the IV dosed ^{14}C- sulfoxaflor remained in the tissues of the animals sacrificed 168 hours post-dosing.

In the rats dosed orally with ^{14}C- sulfoxaflor, elimination of the radioactivity from plasma was rapid during the α elimination phase ($t_{½\alpha}$ = 4-6 hours) followed by relatively slow elimination during the terminal β phase ($t_{½\beta}$ = 39-45 hours). The area under curve (AUC) and C_{max} of radioactivity in plasma were essentially dose proportional between the low and high dose groups, suggesting unsaturated kinetic behavior of sulfoxaflor up to 100 mg/kg body weight. The fate of the test-material derived radioactivity in RBC was similar to plasma. The time-course of radioactivity in RBC was essentially parallel to the plasma time-course concentration profiles with similar concentrations. Kinetics (absorption, elimination $t_{½}$, AUC, clearance) of radioactivity in RBC were similar to plasma, except that detectable levels of radioactivity were found in RBC for an extended period of time compared to plasma.

A total of seven radiolabeled components were identified in urine and/or fecal samples. Parent sulfoxaflor was the primary component in both urine and fecal samples, accounting for >93% of the administered dose. A glucuronide conjugate of sulfoxaflor metabolite X11721061 was tentatively identified in urine samples only, comprising 2-4% of the administered dose. Three other unidentified minor components, all <1% of administered dose, were found only in pooled urine samples. Two different minor components, both <1% of the administered dose, were present only in extracts of fecal samples.

In summary, sulfoxaflor was rapidly and highly absorbed, poorly metabolized and readily eliminated primarily in the urine from the rat, with low tissue residues. ADME of sulfoxaflor did not show significant gender differences.

This study is classified as fully reliable (acceptable/guideline) and satisfies the guideline requirements for a general metabolism study in the rodent.

IIA 5.8.1.1/1 X11719474: Probe Study to Determine Absorption, Metabolism and Elimination in F344/DuCrl Rats;

In a non-guideline metabolism probe study (MRID 47832132), absorption, metabolism and excretion of the sulfoxaflor metabolite ^{14}C-X11719474 (radiochemical purity 98.8% a.i., lot # XS9-100040-6, INV030930-0002) following a single oral dose of 100 mg test material/kg body weight was evaluated in 1 F344/DuCrl rat/sex. Pharmacokinetics, mass balance and urine metabolite profiles were determined. The profile of circulating metabolites in plasma was determined in 1 additional rat/sex.

Orally administered ^{14}C-X11719474 was rapidly absorbed without any apparent lag time. T_{max} occurred at 1 hour. Total absorption was 95% of the administered dose in the male rat and 98% in the female. A total of 95-97% of the administered dose was excreted in the urine after 168 hours post-dosing, with the majority (91-95%) rapidly eliminated by 12 hours post-dosing. A much lower percentage (2-3%) of the dose was eliminated in feces by 168 hours post-dosing. Total recovery of radioactivity from both animals was 98 and 99%.

Elimination of radioactivity from plasma was biphasic, with most occurring during the rapid (α) elimination phase ($t_{½\alpha} \leq 2$ hours), with a slower (β) elimination phase ($t_{½\beta}$ = 36 - 41 hours). The AUC of radioactivity in plasma was 505 and 351 µg h g^{-1} for male and female, respectively. Test-material derived radioactivity was also present in RBC, with C_{max} concentrations ~27-31% lower levels than seen in plasma. Elimination of the radioactivity from RBC was monophasic with all of the elimination occurring during the rapid (α) elimination phase ($t_{½\alpha}$ <3 hours). The AUC of radioactivity in RBC was 377 and 240 µg h g^{-1}, in the male and female, respectively. The absorbed test material was essentially un-metabolized in the male and female rat. The primary component found in urine was parent compound, with two metabolites, or test material impurities, present at less than 1% of the administered dose. Only parent compound was found in the C_{max} plasma samples.

In summary, administered ^{14}C-X11719474 was rapidly absorbed, mostly un-metabolized and eliminated very quickly from the rat.

This study is classified Fully Reliable (acceptable/non-guideline). It was conducted as a probe metabolism study on a metabolite of sulfoxaflor and was not submitted to satisfy guideline requirements for a rodent general metabolism study.

870.7600 Dermal Absorption – Rat

IIIA 7.6.2 Comparative dermal absorption, *in vitro* using rat and human skin;
GF-2032 is a suspension concentrate (SC) formulation containing the active substance sulfoxaflor, an experimental insecticide. The nominal concentration of the active substance in GF-2032 is 240 g/L. The highest in-use spray dilution concentration is 0.48 g/L and the lowest in-use spray dilution concentration is 0.024 g/L. In an in vitro dermal penetration study (MRID 47832414), sulfoxaflor was applied as GF-2032 to the dermal surface of groups of 5 human skin samples/group, or 8 rat skin samples/group at concentrations of 0.024, 0.48, or 2.4 g/L for 24 hours at an application volume of 10 µL/cm^2.

The overall recovery of the radiolabeled sulfoxaflor was good (95-97%) for all the groups. Application of 0.024 and 0.48 g/L resulted in similar rates of absorbed dose, 1.15, and 1.54 % of the administered dose in the human and 4.34 and 8.72% in the rat skin, respectively. The absorbed dose for the 240 g/L group was lower, 0.26 and 1.3%, in the human and rat skin, respectively, indicating saturation of the skin at this level. In all dose groups, a greater percentage of sulfoxaflor was absorbed through the rat skin compared to the human skin. The rates were most similar in the 0.48 g/L group, with a rat absorbing 2.6-fold greater percentage of the applied dose compared to human skin. The differences in absorption rates were greater in the 0.024 g/L (3.8-fold) and 240 g/L (5-fold) groups.

Although this study does not satisfy EPA guideline requirements, this dermal penetration study was done according to OECD 428 guidelines. This study fully reliable (acceptable/non-guideline).

IIIA 7.6.3 Dermal Absorption, *In Vivo* in the Rat;
5 groups of 4 male rats were dosed with radiolabeled sulfoxaflor as GF-2032 in a dermal penetration study (MRID 48732413). GF-2032 is a suspension concentrate (SC) formulation containing the active substance sulfoxaflor. The nominal concentration of the active substance in GF-2032 is 240 g/L. The highest in-use spray dilution concentration is 0.48 g sulfoxaflor/L.

The lowest in-use spray dilution concentration is 0.024 g sulfoxaflor/L.
Rats were dosed with 0.024, 0.48, or 240 g/L sulfoxaflor as GF-2032. [^{14}C]-Sulfoxaflor was applied to 10 cm^2 patch of shaved skin for 10 hours, and then washed off with a mild soap solution. Groups of rats were sacrificed at 24, 48, 96, 144, and 192 hours after dermal application and the extent of dermal penetration was determined. The amount of applied dose removed by washing the skin was 96%, 71% and 67% of the applied dose across the dose groups, respectively.

Following a dermal exposure period of 10 h to sulfoxaflor formulation concentrate (240 g/L), 93-99% of the applied radioactivity was readily removed from the skin surface by a mild detergent wash. Approximately 1-2% of the applied dose was absorbed over the exposure interval and

subsequent post exposure collection periods up to 192 h after exposure. The highest absorption was observed within the 48 hour group with 1.7%.

After a 10 h dermal exposure to the 0.48g/L spray dilution, the dosed material was not as readily removed from the skin surface with 62-79% of the applied radioactivity removed by a mild detergent wash. Approximately 2-3% of the applied radioactivity was absorbed after 24 h and 48 h, increasing to 8% at 96 h. By 144 h and 192 h the absorption levels were similar at 11%, indicating that absorption of the dose was essentially complete.

After a 10 hour dermal exposure to the 0.024g/L spray, the amount of dislodgeable dose was similar to the 0.48g/L spray dilution with 3-71% of the applied radioactivity removed from the skin surface by the detergent wash. Approximately 1-3% of the applied radioactivity was absorbed after 24 h and 48 h increasing to 6% at 96 h. By 144 h and 192 h the absorption levels were similar at 13% and 11%, respectively, indicating that absorption of the dose was essentially complete.

This study is considered Fully Reliable (acceptable/guideline) and fulfills the requirements (OPPTS 870.7600; OECD 427) for a dermal penetration study in the rat.

A.4.9 Immunotoxicity

870.7800 Immunotoxicity (See 870.3100 (p.85))

A.4.9 Special/Other Studies

IIA 5.6.12/3 Characterization of the agonist effects of XDE-208 on mammalian muscle nicotinic acetylcholine receptors;

Sulfoxaflor is a compound with insecticidal activity that acts as an agonist of insect nicotinic acetylcholine receptors (nAChRs). The aim of the work described in this report was to examine the influence of sulfoxaflor on mammalian muscle nAChRs. Competition radioligand binding was used to examine the ability of sulfoxaflor to bind to nAChRs from three mammalian species (human, rabbit and rat) (MRID 47832035). In addition, two-electrode voltage-clamp recording was used (with human and rat nAChRs) to examine whether binding of sulfoxaflor resulted in functional activation of muscle nAChRs. Radioligand binding experiments demonstrated that sulfoxaflor binds to human, rabbit and rat fetal muscle nAChRs. Electrophysiological studies revealed that sulfoxaflor is a partial agonist of the rat fetal muscle nAChR. In contrast, sulfoxaflor has no detectable agonist activity on the human fetal muscle nAChR or on the adult muscle nAChR (from either human or rat). In contrast to the clear agonist activity of sulfoxaflor on the rat fetal muscle nAChR, no agonist activity was observed with X11719474, a soil metabolite of sulfoxaflor.

This study demonstrates that sulfoxaflor is an agonist of the rat fetal muscle nAChR. In contrast, sulfoxaflor has no agonist activity on the equivalent human fetal nAChR or on the rat or human adult muscle nAChR. From these findings, it seems reasonable to conclude that the selective agonist activity of sulfoxaflor is due to species and age-dependent differences in the amino acid

sequences of the nAChR subunits. Furthermore, the soil metabolite, X11719474, had no agonistic activity on either the rat or human nAChR subtypes.

This study is reliable with restrictions (acceptable/non-guideline).

IIA 5.6.12/4 Investigation of the critical window of exposure for fetal abnormalities and neonatal survival effects in Crl:CD(SD) rats;

In a non-guideline reproductive/developmental toxicity study (MRID 47832137) sulfoxaflor (95.6% a.i. wt/wt; Lot # E2162-34, TSN003725-0001) was administered to pregnant female Crl:CD(SD) rats (12/group) at concentrations of 0 (Group 1, control) or 1000 ppm (Groups 2 and 3) in the diet (corresponding to doses of 0, 38.6, and 76.5 mg/kg/day for Groups 1, 2, and 3, respectively). Group 2 was fed the test substance from gestation day (GD) 6-16 to cover all of embryogenesis up to, but not including, the start of early fetal movements; Group 3 was fed the test substance from GD 16-birth to cover development of the muscle nAChR and its role in development of synchronized fetal limb movements up to onset of parturition.

Treatment-related decreases in body weight were observed in dams in Group 1, resulting in decreased body weight gain from GD 6-16; similarly Group 2 dams had treatment-related decreases in mean body weight and body weight gain during the test substance treatment interval. The body weight effects were accompanied by lower feed consumption (23.3% and 22.6% lower than controls during the treatment interval for Groups 2 and 3, respectively). No gross anatomical abnormalities were observed in any dams in any treatment group.

There were no treatment-related differences in the number of pups born alive or dead in Groups 2 or 3 when compared to the control. There was a treatment-related 18.8-20.8% decrease in Group 3 mean pup body weight in both sexes (alpha=0.05)

Offspring from animals in Group 2 did not display previously described fetal abnormalities or reduced neonatal survival. Offspring in Group 3 had the same gross effects of limb contractures and reduced neonatal survival (alpha=0.05) seen in previous studies that had treatment with 1000 ppm sulfoxaflor throughout gestation.

Daily examination of Group 3 offspring born with limb abnormalities indicated that these were fully reversible shortly after withdrawal of maternal dietary exposure to sulfoxaflor. In some cases, full reversal of the limb abnormalities was evident the day after birth and occurred for all affected animals that survived to postnatal day (PND) 4; reversal also occurred in some animals that subsequently died before PND 4. The visceral and skeletal findings of abnormal ureter and bent clavicle, the latter of which had a high incidence (30.1% of fetuses) in a previous definitive developmental toxicity study, were not present in this study at necropsy on PND 4 despite similar maternal sulfoxaflor blood concentrations and pup limb abnormality indices between these two studies.

This study demonstrates that the critical period of developmental susceptibility to sulfoxaflor-induced fetal abnormalities (forelimb flexure and hindlimb rotation) and reduced neonatal survival is between GD 16-birth, and that all of the fetal abnormalities are rapidly reversible after birth.

This study is Fully Reliable (acceptable/non-guideline).

IIA 5.6.12/5 Investigation of the critical window of exposure for fetal abnormalities and neonatal survival effects in Crl:CD(SD) rats (Phase 2);

In a non-guideline reproductive/developmental toxicity study (MRID 47832136) sulfoxaflor (95.6% a.i. wt/wt; Lot # E2162-34, TSN003725-0001) was administered in the diet to pregnant female Crl:CD(SD) rats (10/group) at concentrations of 0 ppm (Group 1, control) or 1000 ppm on gestation days (GD) 16-18 (Group 2), GD 18-20 (Group 3), or GD 20-22/LD 0 (Group 4), corresponding to mean intakes of 0, 63.9, 42.5, and 35.7 mg/kg/day for Groups 1, 2, 3, and 4 respectively.

Dams in all sulfoxaflor treatment groups (Groups 2-4) had treatment-related decreases in body weight gain during their respective treatment intervals. These body weight effects were attributed to lower feed consumption, consistent with decreased palatability of test material fortified diet observed in previous studies at 1000 ppm. No treatment- related clinical signs were observed in any dams in any test substance treatment group.

There were no sulfoxaflor treatment-related effects on gestation survival or sex ratios of the pups. There were no apparent treatment-related differences in the number of pups born alive or dead, pup body weight, or litter size.

Offspring from animals given 1000 ppm sulfoxaflor for 48 hours starting on the morning of GD 16 or 18 (Groups 2 and 3) were similar to controls and did not display previously described fetal abnormalities or reduced neonatal survival. Offspring given 1000 ppm sulfoxaflor for 48 hours starting on the morning of GD 20 (Group 4) had fetal limb abnormalities (forelimb flexure and hindlimb rotation) as well as reduced neonatal survival (89.6% survival), demonstrating that exposure shortly before birth (GD 21 or 22) is sufficient to induce developmental toxicity.

In addition, daily examination of surviving Group 4 offspring born with limb abnormalities indicated that these were fully reversible in surviving offspring shortly after withdrawal of maternal dietary exposure to sulfoxaflor. In some cases, full reversal of the limb abnormalities was evident the day after birth and occurred for all affected animals that survived to postnatal day (PND) 4; reversal also occurred in some animals that subsequently died before PND 4. Likewise, the visceral and skeletal findings of abnormal ureter and bent clavicle, the latter of which had a high incidence (30.1% of fetuses), in the definitive developmental toxicity study were not present in this study at necropsy on PND 4.

This study demonstrated that sulfoxaflor exposure between GD20-GD22/LD0 is sufficient to cause fetal abnormalities (forelimb flexure and hindlimb rotation) and reduced neonatal survival. Furthermore, these fetal abnormalities are reversible by PND 4.

This study is Fully Reliable (acceptable/non-guideline).

IIA 5.6.12/6 Observations on the effects of XDE-208 on the phrenic nerve-hemidiaphragm preparation from new-born rat;

Sulfoxaflor, a compound targeted to the insect nicotinic acetylcholine receptor (nAChR) has been shown to cause foetal limb contractions and reduced neonatal survival in rats following dietary exposure during gestation. The aim of the work described in this report was to make a qualitative investigation of the action of sulfoxaflor on isolated phrenic nerve-hemidiaphragm preparations from new-born rats (MRID 47832064).

Sulfoxaflor consistently (n=5) produced a reversible, concentration-dependent contracture of the diaphragm that was blocked by the selective muscle-type nAChR antagonist, tubocurarine (10 µM), showing that the contracture induced by sulfoxaflor is mediated via nAChR activation, rather than via a post-receptor mechanism. Furthermore, prolonged application of sulfoxaflor caused a sustained muscle contracture. Muscle twitches in response to phrenic nerve stimulation were not affected at low sulfoxaflor concentration (100 µM) but were reduced at high concentration (1 mM), demonstrating that sulfoxaflor can cause inhibition of nerve-evoked contraction of the diaphragm during sustained contracture, consistent with the observed impairment of respiration in the neonatal rat.

The results of these experiments demonstrate that sulfoxaflor caused a contracture of the new-born rat diaphragm by acting on the nAChR. Prolonged application caused a sustained muscle contracture and a contracture-associated inhibition of the phrenic nerve-evoked muscle twitch, which is considered analogous to the situation *in vivo* which resulted in foetal limb contractions (sustained muscle contractions) and compromised respiration at birth (contracture-associated inhibition of the muscle twitch).

Therefore, the results described in this report are entirely consistent with, and add additional support to, the hypothesis that sulfoxaflor causes neonatal death (and foetal abnormalities) via activation of the foetal muscle-type nAChR.

This study is Reliable with Restrictions (acceptable/non-guideline).

IIA 5.6.12/7 Histopathological Evaluation Of Fetal Lung Samples From The Developmental Toxicity Study In Crl:Cd(Sd) Rats;

Dietary administration of 1000 ppm sulfoxaflor to Crl:CD(SD) rats during gestation has been previously shown to cause neonatal pup death. In order to determine if morphological alterations (e.g., increased collagen deposition) in any region of the lungs were responsible for pup death, one fetus/sex from five control and four 1000 ppm litters (18 samples total) from the definitive developmental toxicity study were collected and preserved in neutral, phosphate buffered 10% formalin (MRID 47832135). Sections from these preserved tissues were processed such that each slide contained sections of the trachea, bronchi, bronchioles, and alveoli. Slides were stained with hematoxylin and eosin and evaluated for histopathological changes. Tissues were archived with the developmental toxicity study.

There were no sulfoxaflor induced lesions in the trachea, bronchi, bronchioles and alveoli in any of the treated fetuses examined. There were no treatment-related increases in collagen deposition around the airways or alveolar walls or any other changes. All observations were considered within normal limits.

This non-guideline study is reliable with restrictions (acceptable/non-guideline). The study is not GLP compliant. However, all experiments were done according to GLP standards. Additionally, the study was well conducted and the Agency has confidence in the scientific integrity of the data.

Appendix B. Sulfoxaflor Mode-of Action for Reproductive and Developmental Effects

The following Mode-of-Action was presented to an HED committee for comment on its acceptability and plausibility on Dec. 7, 2011. The general consensus of the committee was that the data supported the overall MOA in rats but the MOA was applicable to humans.

Sulfoxaflor is a member of a new class of insecticides, the sufoximines, with proposed uses on cotton, soybeans, cereals, citrus, leafy and fruiting vegetables, cole crops, grapes, apples, and a variety of other crops. It provides control for a broad spectrum of piercing/sucking insects. Sulfoxaflor is a nicotinic receptor agonist, similar to neonicotinoid insecticides such as acetamiprid, imidacloprid, and thiacloprid. However, unlike neonicotinoids, sulfoxaflor is not susceptible to *in vivo* metabolism by monooxygenase enzymes, which causes virtually all known cases of resistance in the field to this class of insecticides.

Toxicity and mechanistic studies in rats, rabbits, dogs and mice highlight the liver and the nervous systems as target organ systems. Liver effects in subchronic and chronic studies include organ weight and enzyme changes, hypertrophy, proliferation, and tumors. Neurological effects are manifested as limb flexure, bent clavicle and convoluted/hydroureter in rat fetuses and decreased neonatal survival. These effects were not observed at similar maternal and fetal systemic exposure levels in NZW rabbits. Finally, long term studies produced treatment related tumors in the rat and mouse including liver, Leydig, and preputial gland tumors.

In rats, sulfoxaflor exposure during gestation caused effects on neonatal survival and fetal abnormalities, but neither effect occurred in rabbits. Similar maternal and fetal blood data showed that the interspecies difference between rats and rabbits was not due to toxicokinetics; therefore, the species difference is likely due to toxicodynamics.

Since the insecticidal MOA is based on agonism of the nicotinic acetylcholine receptor (nAChR), it is likely that the developmental effects were also related to nAChR binding. There are two types of mammalian nAChRs, neuronal and muscular. The neuronal nAChRs are principally located in the central and peripheral nervous system and disregulation is often manifested as the modification of biological setpoints such as tachycardia, blood pressure, inflammation, cognitive learning, arousal motor control and analgeisa. The muscle nAChRs are found in the intramuscular junctions of skeletal muscles. Disregulation of these receptors can result in muscle contraction, difficulties in breathing, and ultimately death. Furthermore, there are two subtypes of muscle nAChRs, a fetal form and an adult form. The switch from fetal to adult occurs shortly before birth in humans and shortly after birth in rats. Based upon the types of skeletal effects observed and their appearance during early development, the registrant has proposed a MoA involving the interaction of sulfoxaflor with the fetal muscle nAChRs.

Postulated MOA for sulfoxaflor-induced muscle contracture and death in neonatal offspring

Given that sulfoxaflor targets the insect nAChR, and that functional expression of the fetal-type neuromuscular junction nAChR occurs in late gestation and is involved with limb muscle function, **it was hypothesized that the neonatal offspring abnormalities and deaths occur via a single MoA: sulfoxaflor's sustained agonism at the fetal-type muscle nAChR and**

subsequent sustained muscle contracture of the limb, shoulder girdle and diaphragm, respectively (MRID 48288940).

A single MOA is considered to be responsible for the neonatal offspring findings since the sulfoxaflor-induced limb contracture, clavicle abnormalities, and neonatal deaths occurred at the same dose with similar incidences (Figure 1). In order for a single mode of action to be plausible, the findings should have a similar incidence across doses from all studies. Based upon similar dose-response curves for both effects (i.e., limb abnormalities and neonatal deaths), the data from multiple studies have a high degree of correlation (R^2 values of 0.93 and 0.91), supporting a single MOA with different apical end points rather than a different MOA for each of the two major effects in rats.

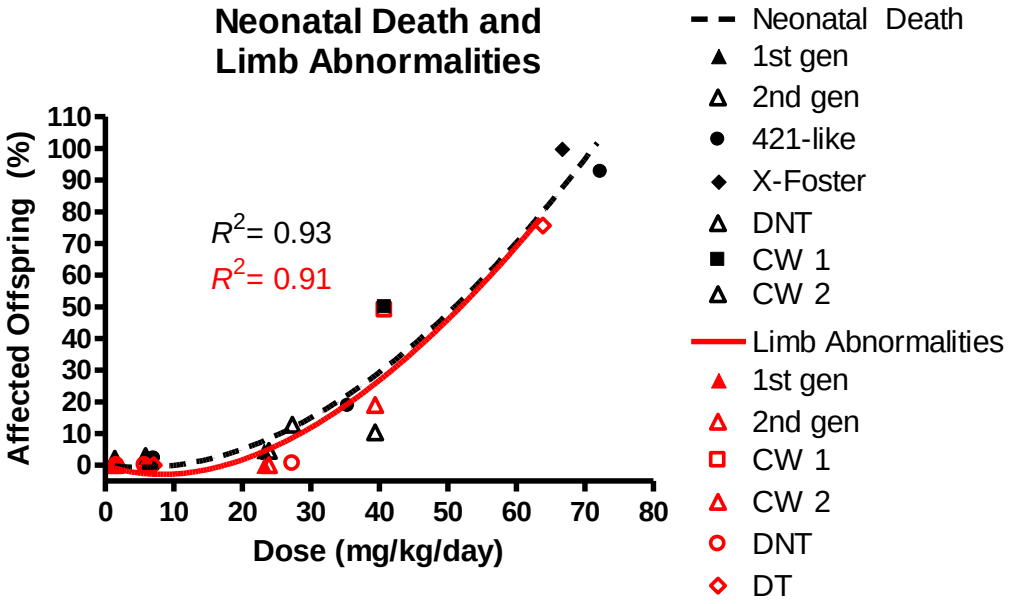


Figure 1. Dose-response curves with non-linear regression analyses for the percent of neonatal offspring affected by death and/or limb contracture in rats.
Study type abbreviations: 1st gen and 2nd gen, 1st and 2nd generations of the two-generation reproductive toxicity study; 421-like, reproductive toxicity screening study; X-foster, cross-foster study; CW 1, critical window study phase 1; CW 2, critical window study phase 2; DNT, developmental neurotoxicity study; DT, prenatal developmental toxicity study.

This MOA is proposed to progress through the following key events: (1) sulfoxaflor binding and (2) agonism at the fetal-type muscle nAChR, resulting in (3) sustained agonism/sustained muscle contracture in neonatal offspring (fetus and pup). This sustained muscle contracture results in limb contracture, bent clavicles, and abnormal neonatal respiration after birth with ensuing neonatal deaths.

The sequence of events in sulfoxaflor's proposed MOA for muscle contracture and neonatal pup death:

- Specific binding to the fetal-type muscle nAChR **(Key Event #1)**

- Agonism at the fetal-type muscle nAChR (**Key Event #2)**
- Sustained agonism/sustained muscle contracture **(Key Event #3)**

Key Event #1: Specific binding to the fetal-type muscle nAChR

Competition radioligand binding was used to examine the ability of sulfoxaflor to bind nAChRs from three mammalian species (human, rabbit and rat). Initial radioligand binding studies were performed with [^{3}H]-sulfoxaflor, the aim being to examine whether sulfoxaflor is able to bind to mammalian muscle nAChRs. However, technical difficulties were encountered in performing binding experiments with [^{3}H]-sulfoxaflor that were associated with high levels of non-specific binding. Therefore, a series of experiments was performed to examine whether unlabelled sulfoxaflor was able to displace binding of the high-affinity nAChR radioligand [^{3}H]-epibatidine from tissue homogenates prepared from rabbit fetal forelimb muscle, rat fetal forelimb muscle and human fetal recombinant nAChRs expressed in HEK (human embryonic kidney) cells (MRID 47832035).

Dose-dependent displacement of [^{3}H]-epibatidine binding was observed with increasing concentrations of sulfoxaflor for all three preparations examined (human, rabbit and rat). At higher concentrations of sulfoxaflor, almost complete displacement of bound [^{3}H]-epibatidine was observed (Fig. 2). Although data obtained with human nAChRs and rabbit muscle are well fitted by the single-site model (solid lines in Fig. 2), the binding data from rat muscle were fitted with a two-site model (dotted line in Fig. 2) that revealed two distinct binding sites of different affinities (0.01 mM and 8.9 mM). The ability of sulfoxaflor to bind either a single (human and rabbit) or multiple (rat) sites on the nAChR is ambiguous. However, demonstration of the capacity of sulfoxaflor to bind mammalian nAChRs and potentially affect the functionality of the receptors is of great significance.

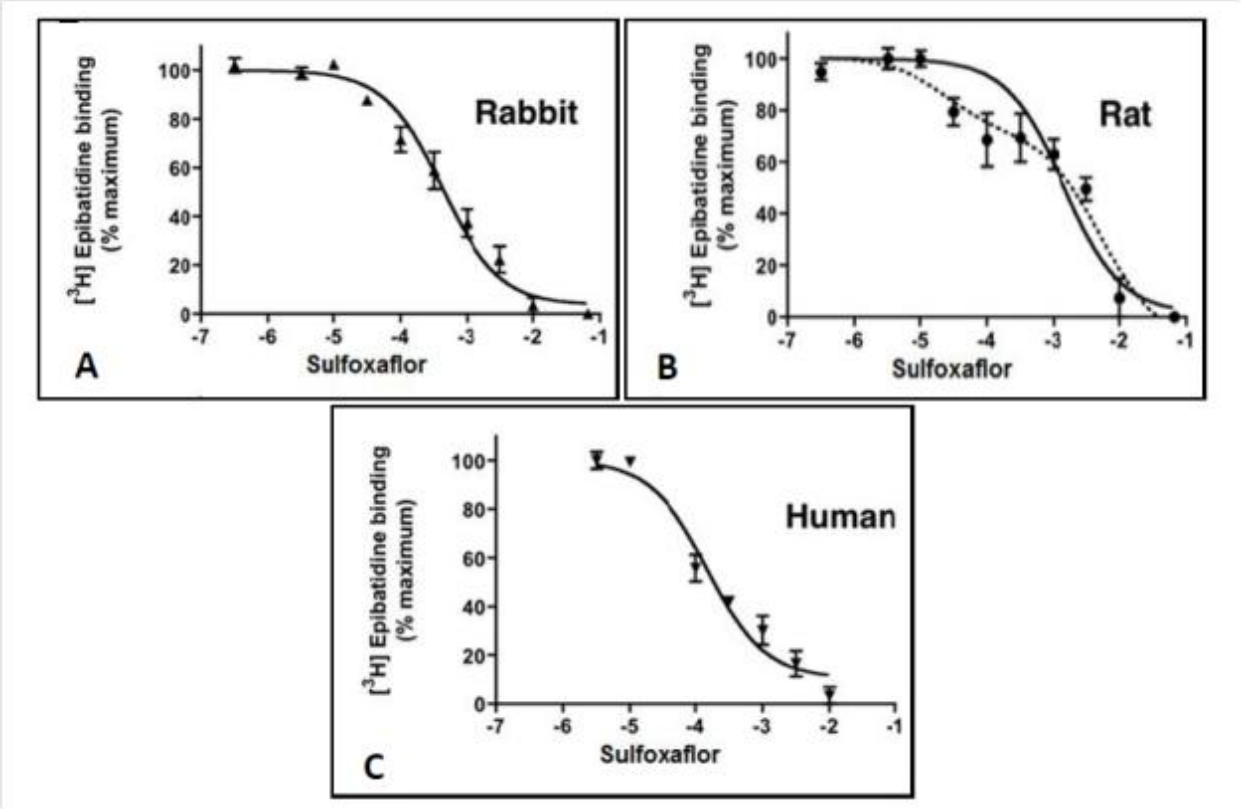


Figure 2. Sulfoxaflor displacement of [^{3}H]-epibatidine in (A) rabbit fetal muscle tissue, (B) rat fetal muscle tissue demonstrateing the 1-site and 2-site binding models, and (C) and in HEK-293 cells expressing recombinant human fetal nAChRs

Table 1. XDE-208: Dose Response for MoA Key Event #1: Fetal-type Muscle nAChR Binding

Dose ↓

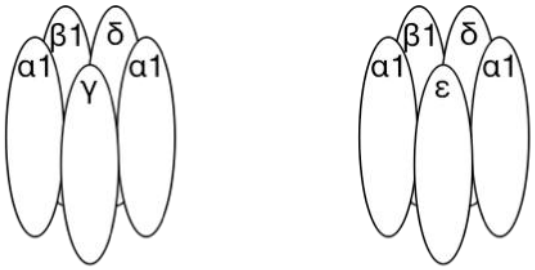
Media Conc. (µM)	Key Event 1 Fetal-type Muscle nAChR Binding[#]
0.3 µM	5[+]
3 µM	0
10 µM	0
30 µM	21
100 µM	31
300 µM	31
1000 µM	37
3000 µM	51

10000 µM	100

[#] Percent inhibition of maximum epibatidine binding
[+] Within background of system and not considered biologically

Key Event #2: Agonism at the fetal-type muscle nAChR

The ability of sulfoxaflor to act as an agonist of fetal and adult muscle nAChRs was examined by expression of rat and human nAChRs in *Xenopus* oocytes (MRID 47832035). In mammalian muscle cells, nAChRs are expressed at the neuromuscular junction (NMJ) and are composed of five nAChR subunits (α1, β1, γ, δ and ε). Transcription of the γ and ε subunits is differentially regulated during development, with the γ subunit expressed in "fetal" muscle and the ε subunit expressed in "adult" muscle (Mishina et al., 1986)(Figure 3).



Fetal-Type nAChR Adult-Type nAChR

Figure 3. Subunit composition of the fetal-type and adult-type muscle nAChR.

Expression of the fetal- and adult-type nAChRs was achieved by microinjection of cDNA or cRNA encoding the appropriate rat or human muscle subunits in *Xenopus* oocytes. To generate the fetal form of the muscle nAChRs, α1, β1, γ and δ cDNAs or cRNAs were injected, whereas α1, β1, δ and ε were injected to generate the adult form. In each case, functional responses (i.e, membrane currents) were detected in response to application of the endogenous agonist acetylcholine (ACh). There have been no reports of the molecular cloning of nAChR subunits from rabbit and so rabbit muscle nAChRs were not examined in this manner. Two-electrode voltage-clamp recording from *Xenopus* oocytes was performed 1-5 days after micro-injection with cDNA or cRNA.

Clear agonist-evoked responses were observed with sulfoxaflor (XDE-208) at the rat fetal-type nAChR (Fig. 4). Dose-dependent agonism at the fetal-type muscle nAChR was demonstrated; the lowest tested concentration having no agonism while incubation of the oocytes with higher concentrations of sulfoxaflor showed increasing agonism. There was no apparent agonism in either the adult rat or human (fetal and adult) type receptors (Table 2).

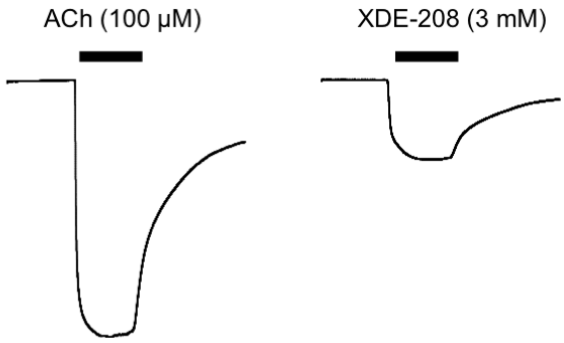


Figure 4. Representative whole-cell current responses in a *Xenopus* oocyte cell expressing fetal-type rat $(\alpha 1)_2\beta 1\gamma\delta$ nAChRs. Inward currents are show from the same oocyte in response to application of acetylcholine (100 µM) and XDE-208 (3 mM). The length of agonist application (5 secs.) is indicated by the horizontal bar. Agonist activation is associated with downward deflection in the trace. Note, there was recovery of response after ACh or sulfoxaflor were washed off (Millar, 2010).

Table 2. Agonist-activation of nAChRs expressed in *Xenopus* oocytes by sulfoxaflor (3mM).

	Maximum response (% ± SEM; normalized to max. ACh response)
Rat fetal muscle	39 ± 2.4%
Rat adult muscle	No response
Human fetal muscle	No response
Human adult muscle	No response

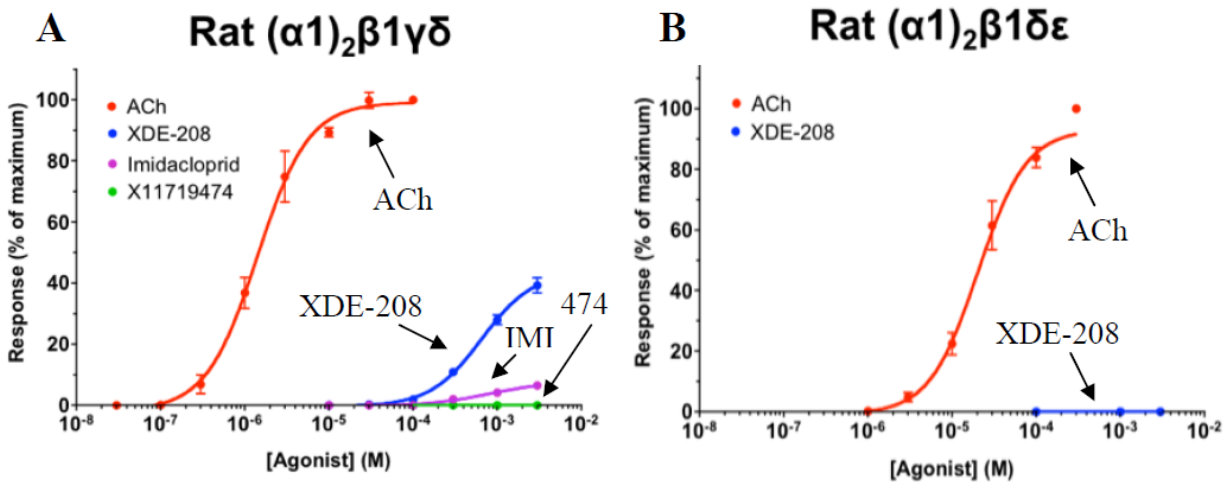


Figure 5. Agonist activation of nAChRs expressed in *Xenopus* oocytes. Data are shown for the rat fetal $(\alpha1)_2\beta1\gamma\delta$ nAChR (A) and rat adult $(\alpha1)_2\beta1\delta\varepsilon$ nAChR (B). AChRs were expressed by microinjection of cRNA in *Xenopus* oocytes. Dose-response curves are shown in which agonist-evoked responses are normalized to the maximal response detected with the endogenous agonist, acetylcholine (ACh). Note that no receptor desensitization occurs, which would have been seen as a diminished agonist response at higher doses. Data points are means of 3-7 responses. Adapted from Millar, 2010.

Table 3. Sulfoxaflor-induced agonism of rat Fetal-type muscle nAChRs expressed in *Xenopus* Oocytes

Media Conc. (µM sulfoxaflor)	% Response (normalized to maximum. ACh response)
10 µM	**0**
30 µM	**1.0**
100 µM	**5.1**
300 µM	**28**
1000 µM	**71**
3000 µM	**100**
10000 µM	**Not Determined**
Sulfoxaflor Agonism at the Adult-type Muscle nAChR	
100 to 3000 µM	**0**

Key Event #3: Sustained agonism at the fetal-type muscle nAChR and sustained muscle contracture

Sulfoxaflor readily perfuses into muscle from the blood (MRID 47832039) and therefore muscle sulfoxaflor concentrations would also be maintained at effective levels as long as dietary

treatment continued. At the neuromuscular junction, the neurotransmitter ACh binds to Ach receptors on muscle fibers causing contraction. ACh undergoes tightly regulated release followed by rapid hydrolysis by acetylcholinesterase (AChE), unlike sulfoxaflor which would remain at the nAChR synaptic cleft due to its lack of hydrolysis by AChE. Thus, upon fetal-type muscle nAChR activation, a sulfoxaflor-induced muscle contracture would be sustained for as long as sufficient sulfoxaflor molecules remain available for receptor binding.

To directly assess muscle contracture at the neonatal diaphragm, sulfoxaflor was tested for agonist action on the isolated phrenic nerve-hemidiaphragm preparation (Bulbring, 1946) from new-born rats (MRID 47832064). A number of issues were addressed in this study including **1) Concentration-dependence of the contracture produced by sulfoxaflor**, **2) Block of the response to sulfoxaflor by tubocurarine**, and **3) Response to prolonged sulfoxaflor application**. To accomplish this, diaphragm muscle preparations were mounted in a recording chamber and bathed in a physiological salt solution. A myograph wire attached to the muscle tendon was connected to a Harvard isometric strain gauge transducer to record muscle tension. The phrenic nerve was stimulated using a bipolar stimulating electrode placed at the nerve entry point to the muscle with supra-maximal rectangular voltage pulses of 0.2 ms duration at a frequency of 0.5 Hz. Strain gauge transducer and stimulating electrode were mounted on micromanipulators in order to allow accurate positioning relative to the muscle. Acetylcholine, tubocurarine, and sulfoxaflor were applied by manually switching taps controlling solutions flowing to the inflow manifold of the recording chamber. Recorded measures include 1) changes in muscle twitch, and 2) muscle contracture following test material application to the bath perfusion system.

1) Concentration-dependence of the contracture produced by sulfoxaflor

1mM sulfoxaflor consistently produced a contracture of the neonatal diaphragm muscle and a decrease in muscle twitch response. At 100 µM sulfoxaflor, there was little effect on twitch tension.

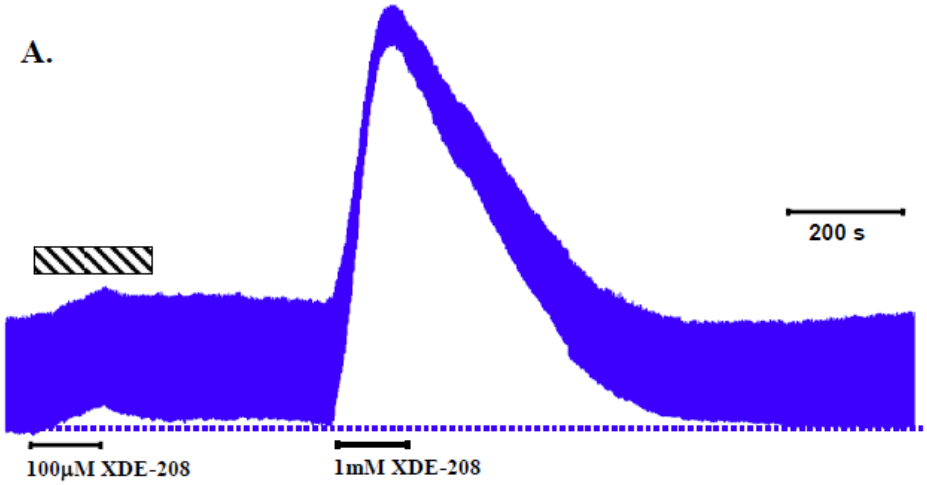


Figure 6. Rat fetal diaphragm tissue demonstrates contraction in the presence of sulfoxaflor.

Expanded time scale display of the sulfoxaflor response illustrates the muscle contracture and the accompanying decrease in twitch tension induced by sulfoxaflor. In five preparations, the muscle twitch response decreased to 34 ± 3.2% of control during responses to 1 mM sulfoxaflor.

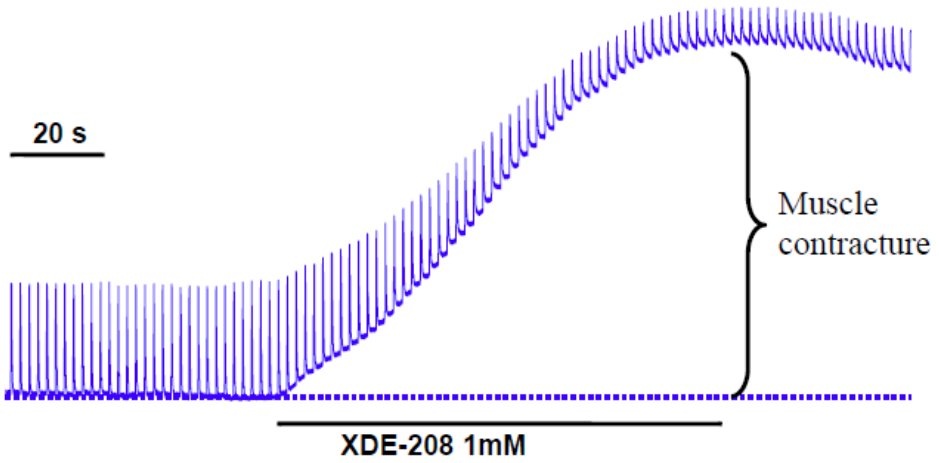


Figure 7. Expanded time scale demonstrating decreased twitch tension in the presence of sulfoxaflor.

2) Block of the response to sulfoxaflor by tubocurarine,

The response to sulfoxaflor was shown to be antagonized by the nicotinic antagonist, tubocurarine. Pre-incubation of the muscle tissue with tubocurarine prevents sulfoxaflor-induced muscle contractions, indicating that sulfoxaflor is acting via the nAChR and not via a post-receptor mechanism.

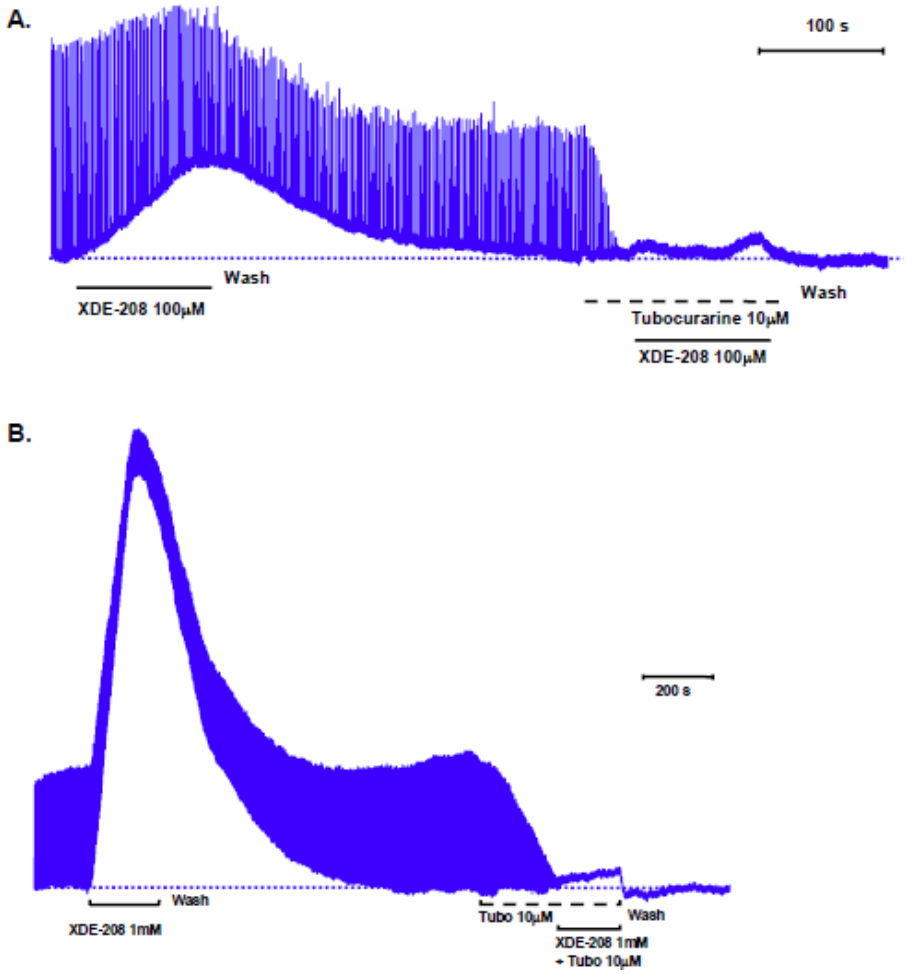


Figure 8. Pre-application of 10µM tubocurarine (Tubo) effectively blocks the muscle twitches and antagonises responses to 100µM or 1mM XDE-208 (Gibb, 2010).

3) Response to prolonged sulfoxaflor application

During application of a high concentration of sulfoxaflor (1mM), the muscle contracture is sustained, suggesting little desensitization of the muscle nAChRs in response to this agonist. Although the muscle contracture is sustained in the presence of sulfoxaflor, the muscle recovers normal function on removal of sulfoxaflor from the solution bathing the muscle.

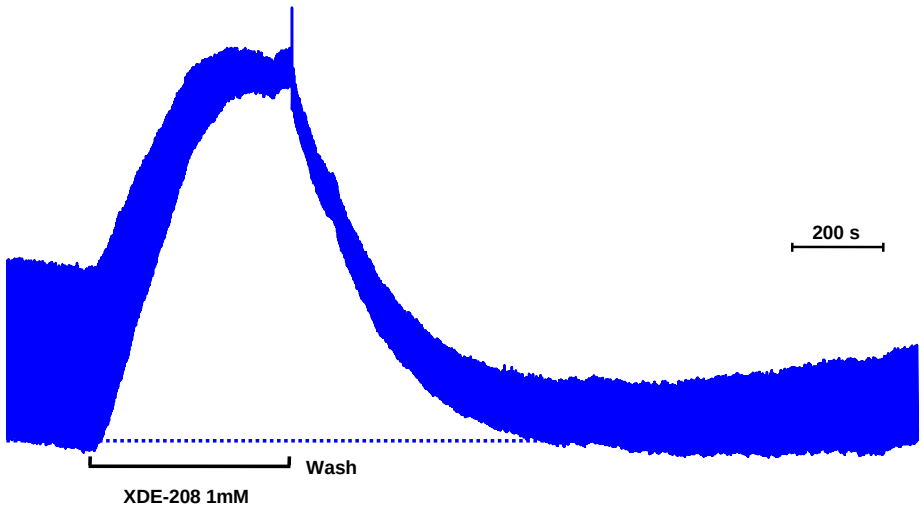


Figure 9. Prolonged application (7 minutes) of 1 mM XDE-208 shows a sustained contracture by the diaphragm muscle (Gibb, 2010).

Dose Response of sustained muscle contracture (i.e., forelimb flexure and hindlimb rotation)

Increased receptor response with increasing sulfoxaflor exposure has been demonstrated for several key events including receptor binding, receptor agonism, diaphragm muscle contraction, and fetal effects in vivo.

Table 4. Dose response observations from *in vitro* mechanistic studies

	Effect	Sulfoxaflor conc.(µM)
Key Event #1	Increased displacement of nAChR radiolignad [H3]-epibatidine from 0 to near 100%	0.3-1000
Key Event#2	Increased agonism in fetal-type nAChR from 0 to 100%	10-3000
Key Event #3	Greater relative contraction of fetal rat diaphragm muscle at 1mM compared to 100 µM sulfoxaflor	100 µM and 1 mM

Temporal concordance of sulfoxaflor levels, nAChR fetal-type muscle receptors, muscle flexures, and neonatal deaths

Ontogeny of fetal-type nAChRs

In rats, the muscle nAChR develops functional subunit expression at the neuromuscular junction between GD 15 and 17 (Kues *et al.,* 1995) resulting in synchronized fetal limb movements (Robinson and Smotherman, 1988) and diaphragmatic responsiveness between GD 16 and 17 (Bennett and Pettigrew, 1974), the latter being critical for the transition to extrauterine respiration. Replacement of the γ subunit by the ε subunit initiates late during the first postnatal week in rats and is largely complete by the end of the second postnatal week in limb and respiratory muscles (Missias *et al.,* 1996).

Cross fostering – Gestation

A cross-fostering study demonstrated that the effect of sulfoxaflor on pup survival was due to *in utero,* not lactational exposure (MRID 47832063). Groups of female Crl:CD(SD) rats were fed diets supplying 0 (control) or 1000 ppm sulfoxaflor for two weeks prior to mating through weaning on lactation day (LD) 21. As the control and treated females mated, they were subdivided into Foster dams and Donor dams. Cesarean-section was performed on gestation day (GD) 21 Donor dams, at which time, one or more batches of two of their offspring/sex were immediately cross-fostered to a Foster dam(s) that had their own litter removed that day (i.e., on LD 0). After cross-fostering was complete, each control and sulfoxaflor-treated Foster dam had mixed litters comprised of two pups/sex that originated from control Donor dams (five litters) and two pups/sex that originated from sulfoxaflor-treated Donor dams (eight litters).

All offspring from dams exposed to 1000 ppm sulfoxaflor prior to birth died by postnatal day (PND) 4, irrespective of whether they were cross-fostered to control- or treated-foster dams (see results Table 5 below). Consistent with reduced viability, some offspring were cold to the touch, had bluish skin, autolyzed and cannibalized, and stomach void of milk. Conversely, there was no effect on neonatal survival for pups exposed to sulfoxaflor only after birth. Furthermore, PND 1 pup body weights were significantly decreased in prenatally exposed offspring.

Table 5. Cross Foster or Treated Foster Dams Results

Foster Dams	Donor Pups	Hypotheses for Pup Survival	Outcome
Control	Control *in utero*	**No effect expected** (negative control)	No effect
	Treated *in utero*	If pups die, effect comes from treated **pups** (i.e. ***in utero* effect**)	All pups died By PND4
sulfoxaflor 1000 ppm	Control *in utero*	If pups die, effect comes from treated **dams** (i.e. **lactational effect**)	No effect
	Treated *in utero*	**Pups death expected** (positive control)	All pups died By PND4

Critical window studies – late gestation effect

The cross fostering study clearly indicated that sulfoxaflor–induced deaths were dependent upon *in utero* exposure. In order to appropriately frame the incidence data presented in this mode-of-action experiment, the findings presented here were compared to the guideline definitive developmental toxicity study in Crl:CD(SD) rats and the first critical window study. Treatment-related findings in the offspring were found at 1000 ppm in the developmental toxicity study (sulfoxaflor exposure GD 6-birth; MRID 47832140), Group 3 in critical window 1 (sulfoxaflor exposure GD 16-birth; MRID 47832137) and in Group 4 in the critical window 2 study (sulfoxaflor exposure GD 20-22/birth; MRID 47832136). See Table 6. When exposure was limited to between GD 6-20, regardless of dose, they were no treatment-related offspring effects. However, when exposure spanned the GD 20-birth, and were sufficiently high (i.e., 1000 ppm), offspring treatment related effects were observed.

Table 6. Comparison to Developmental Toxicity Study

	Developmental Toxicity Study(MRID 47832140)			**Critical Window 1 (MRID 47832137)**		**Critical Window 2 (MRID 47832136)**		
Group:	**Group 2**	**Group 3**	**Group 4**	**Group 2**	**Group 3**	**Group 2**	**Group 3**	**Group 4**
Dose (ppm):	**25**	**150**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
Treatment period:	**GD 6-21**	**GD 6-21**	**GD 6-21**	**GD 6-16**	**GD 16-Birth**	**GD 16-18**	**GD 18-20**	**GD 20-22/LD 0**
Avg.TMI (mg/kg/day)	1.95	11.5	70.2	76.5	38.6	62.9	42.5	35.7
TK plasma conc. (µg/g)	0.843 ± 0.09	4.938 ± 0.87	35.245 ± 5.43	35.4 – 40.9	32.1 - 43.2	16.4 - 33.3	23.0 - 30.2	5.41 - 16.1[a]
Offspring Effects	NO	NO	**YES**	NO	**YES**	NO	NO	**YES**

GD gestation day
[a]Three of the four sampled rats had undergone parturition prior to blood collection

Reversibility when sulfoxaflor removed from diet

The incidence of limb abnormalities decreased over the first two-to-four days of postnatal life. While a portion of the decreased incidence was due to pup deaths, reversibility of the limb abnormalities was directly demonstrated in the two critical window studies as evidenced by a 0% incidence of forelimb flexure and hindlimb rotation by PND 2 or 4 (Table 7). Reversal of the bent clavicles was indirectly demonstrated by an absence of bent clavicles in PND 4 pups (of 86 pups evaluated in the affected groups), which is significant as these findings were observed at a relatively high incidence (30.1%) in GD 21 fetuses from dams exposed to 1000 ppm sulfoxaflor in the developmental toxicity study. These reversibility data suggest that the limb abnormalities were transient, consistent with a pharmacologic MoA for sulfoxaflor.

Table 7. Reversibility of limb abnormalities in aging pups.

	Critical Window 1 (MRID 47832137)	Critical Window 2 (MRID 47832136)
Treatment period	GD 16-birth	GD 20-22/LD0
PND 0 to 1	11	5
PND 1 to 2	6	2
PND 2 to 3	3	0
PND 3 to 4	1	0
Total reversals	21	7

Furthermore, the timing of the neonatal deaths indicates a distinct change in the pharmacodynamic activity of sulfoxaflor after PND 4. In the 2-generation reproduction study (MRID 47832142), pup deaths were slightly, but significantly, greater in the high dose group (400 ppm) compared to controls; 95.4% v. 97.2% for the F1 and 93.0% v. 98.5% in the F2 generation prior to PND 4. However, survivability exceeded 99% for both generations after PND 4, despite the continued exposure of the pups to sulfoxaflor through LD 21.

Table 8. Concordance and dose response of offspring effects observed following treatment with sulfoxaflor

Dose ↓

Internal Dose (µM)	Applied Dose (ppm)	Exposure Days	KE #1 Fetal-type Muscle nAChR Binding[#]	KE #2 Fetal-type Muscle nAChR Agonism[$]	KE #3 Sustained Agonism & Sustained Muscle Contracture				Neonatal Apical Endpoints Forelimb Flexure, Hindlimb Rotation, Bent Clavicle, Death[&]
					Neonatal Diaphragm[$] (Fetal-type Muscle nAChR)	Reversible?	Neonatal Offspring Limb Contracture[^]	Reversible?	
2.3	25[1]	GD 6-21					-		-
4.0	25[2]	Premating-LD 21					-		-
10 µM *in vitro*[4]		-	-	-					
15	150[1]	GD 6-21					-		-
16	100[2]	Premating-LD 21					-		-
ND	100[3]	X					-		-
30 µM *in vitro*[4]			+	+					
57	400[2]	Premating-LD 21					-		+
ND	500[3]						ND[+]		+
60	1000[6]						+	Y	+
89	1000[7]						ND[+]		+++
100 µM *in vitro*[4]			++	+					
100 µM *ex vivo*[8]					+	Y			
101	1000[3]	X					ND[+]		+++
108	1000[1]	GD 6-21					+++		+++
136	1000[5]						++	Y	+++
300 µM *in vitro*[4]			++	++					
1000 µM *in vitro*[4]			+++	+++					
1000 µM *ex vivo*[8]					+++	Y			
3000 µM *in vitro*[4]			+++	+++					

[1]Rat Developmental Toxicity, [2]Two-Generation Reproduction, [3]One-Generation Reproduction, [4]Xenopus Oocytes-Recombinant Fetal-type Muscle nAChR, [5]Critical Phase 1 Mode of Action, [6]Critical Phase 2 Mode of Action, [7]Cross Foster, [8]Neonatal Diaphragm *Ex Vivo* Electrophysiology

[4$] Dose-response curve evaluated for muscle nAChR agonism (100-3000 µM).

[4*] Full dose-response data for fetal-type muscle nAChR binding and agonism showed in Table 3.
[9]Highest dose level from the One-Generation Reproduction and Rat Developmental Toxicity studies.
[#] Percent inhibition of epibatidine binding: +, 0-25%; ++, 25-50%; +++, 50-100%
[$]Percent of maximum XDE-208 response: +, 0-25%; ++, 25-50%; +++, 50-100%
[^] Incidence (fetal/pup basis): +, 0-25%; ++, 25-50%; +++, 50-100%
[&] Maximum incidence (fetal/pup basis): +, 0-25%; ++, 25-50%; +++, 50-100%
ND, No Data
ND[+], No data as early studies did not examine this parameter
Y, Yes

Biological plausibility and coherence

The postulated MoA for the neonatal deaths in rats is considered biologically plausible and coherent. The observed sulfoxaflor-induced limb and shoulder girdle contracture abnormalities and neonatal death are biologically consistent with the functional ontogeny of the fetal-type muscle nAChR in the rat.

Alternative Modes of Action

The registrant has proposed a number of alternative MoAs, including consideration of sulfoxaflor-induced limb abnormalities and neonatal offspring deaths due to: agonism at other AChR types (adult-type muscle nAChR, neuronal nAChR, muscarinic AChR), action downstream of the fetal-type muscle nAChR, antagonism at the fetal-type muscle nAChR, maternally-mediated fetal immobilization, inhibition of acetylcholinesterase and inhibition of angiotensin-converting enzyme. A more detailed examination of each of the alternative MoAs can be found in *Mode of Action Evaluation and Human Relevance Framework Analysis for XDE-208-Induced Fetal Abnormalities and Neonatal Death in Rats* (MRID 48288940).

a) Agonism at other Acetylcholine Receptor Types (Adult-type muscle nAChR, Neuronal nAChRs, Muscarinic AChR)

Adult-type nAChRs
In an *in vitro* study (MRID 47832035) in which recombinant adult-type nAChR were expressed in Xenopus oocytes, sulfoxaflor was found to bind to the receptor, however no functional agonism was demonstrated up to sulfoxaflor's limit of solubility. Furthermore, in *in vivo* studies in adult rats or rats older than PND 4 in the two generation reproduction study, there was no evidence of nACHr related toxicity such as muscle fasciculation or limb contractions. Rather, continued dietary exposure to sulfoxaflor in older animals resulted in hepatic toxicity along decreased body weight and body weight gains.

Neuronal nAChRs
Although the interaction of sulfoxaflor with neuronal nAChRs, as opposed to the proposed skeletal muscle nAChRs is plausible, the absence of historic neuronal nicotinic manifestations such as muscle weakness or flaccidity makes this MoA unlikely. In contrast, sulfoxaflor-induced limb contractures result in rigid, contracted limbs. Breathing movements are also known to be partially regulated by neuronal nAChRs in the brainstem. Although, prenatal nicotine exposure has been demonstrated to alter fetal lung development in rats (Maritz and van Wyk, 1997), it does not cause death in normoxic conditions (Geller, 1959; Sobrian et al., 1995). Increased deaths in neonatal rats following exposed to sulfoxaflor and normal histopathology of GD 21 fetal lung tissue (MRID 47832135) decrease the plausibility of this MoA.

Muscarinic AChRs
Agonism at muscarinic AChRs has also been explored as an alternative MoA. The most plausible target by which a compound could cause death in neonatal offspring would be via muscarinic activity would be the heart or lung. However, activity at these receptors would be accompanied by systemic clinical signs of mAChR activity such as salivation, lacrimation, and

convulsions, none of which were observed in the Guideline or MoA studies. Hearts were grossly normal on GD 21 visceral examination (including internal structures) in the prenatal developmental study in rats, and there were no adverse histological findings in the lungs.

b) Agonsim downstream of the fetal-type muscle nAChR

Downstream agonism of diaphragm muscle has been demonstrated with the herbicide cartap, causing respiratory failure and irreversible contracture of adult-type diaphragm preparations. Rather than acting at the muscle nAChR directly, the diaphragm contracture is via post-receptor induction of extracellular Ca2+ influx, release of internal Ca2+, and an inhibition of [3H]ryanodine binding to the Ca2+ release channel of sarcoplasmic reticulum (Liao *et al.*, 2000). In the phrenic-nerve hemidiaphragm experiments conducted with sulfoxaflor where sustained muscle contracture was demonstrated, a post-nAChR MoA would have been manifested by continued contraction in the presence of co-application with the nAChR antagonist tubocurarine. The sulfoxaflor-induced diaphragm contracture was completely eliminated with co-incubation with tubocurarine, thereby discounting a post-receptor MoA in the induction of the developmental effects of sulfoxaflor.

c) Fetal-type muscle nAChR inactivation

Nongenetic, pharmacologic antagonism of the fetal-type muscle nAChR also results in neonatal offspring death and limb abnormalities. A historical example of this includes the use of tubocurarine. Exposure to tubocurarine, a non-depolarizing neuromuscular blocking agent, during the last week of gestation in rats causes direct fetal paralysis capable of causing forelimb contracture, hindlimb rotation and death (Shoro, 1977). While this may appear similar to the effects seen with sulfoxaflor there are several important distinctions. First of all, neuromuscular blocking agents do not readily cross the placenta (Evans and Waud, 1973). Secondly, the limb contracture effects result in skeletal alterations in the limb cartilage, something that is not observed with sulfoxaflor. Finally, fetal paralysis additionally results in pulmonary hypoplasia, which has previously been mentioned to not occur following sulfoxaflor exposure.

d) Acetylcholinesterase Inhibition

Following sublethal exposures in rats, cholinergic signs of AChE toxicity are generally observed as clinical signs and in the absence of external, visceral, or skeletal abnormalities in fetuses. Therefore AchE inhibition does not fit the profile observed following sulfoxaflor exposure.

e) Angiotensin-converting enzyme (ACE) inhibition

Angiotensin-converting enzyme (ACE) inhibition presents another MoA that has been demonstrated to result in fetal limb contractures when exposure occurs during fetal development in humans (Buttar, 1997). The limb contactures which occur are secondary to renal failure associated oligohydramniosis. Other secondary fetal anomalies include potentially fatal hypotension, anuria, craniofacial deformities and hypoplastic lung development. Furthermore, rodents are relatively resistant to the teratogenic effects of ACE inhibitors and high doses of ACE inhibitors typically result only in fetal growth retardation and occasionally increased pup death.

Table 9. Summary Evaluation for Other Possible MOAs in the Rat

Alternative MoA	Example	MoA Characteristics	Plaubility/ Coherence	Evidence
Adult-type NMJ nAChR agonism	Acetyl-choline	Muscle fasciculations, tonic or clonic limb contractions; Fetal immobilization due to uterine contraction	- Plausibility/ Strong coherence across studies	XDE-208 causes no agonism at the adult-type NMJ *in vitro*; no clinical signs in dev. tox. and repro. tox. studies
Neuronal nAChR subtype agonism	Nicotine, Epibatidine	Clinical signs of cholinergic stimulation followed by desensitization (inhibition); Lung hypoplasia	- Plausibility/ Strong coherence across studies	No indication of representative clinical signs in adults or offspring; normal lungs (including histopathology) in GD 21 fetuses
Muscarinic nAChR agonism	Muscarine, Carbamyl-choline	Clinical signs of cholinergic stimulation and/or inhibition; death caused by action at heart or lungs	- Plausibility/ Strong coherence across studies	No indication of representative clinical signs in adults or offspring; normal hearts (including internal structures) and lungs (incl. histopathology) in GD 21 fetuses
Agonism downstream of the fetal-type NMJ nAChR	Cartap	Muscle contracture associated with intracellular calcium or ATP regulation; not blocked by NMJ AChR antagonists	- Plausibility/ Limited coherence, only evaluated in *ex vivo* study	Diaphragm contracture completely blocked by co-exposure to the NMJ nAChR antagonist, α-tubocurarine
Fetal-type NMJ nAChR inactivation	α-Tubocurarine	Decreased or eliminated fetal activity; Limb contracture characterized by hypotonia; Altered limb skeletal structures; Lung hypoplasia	- Plausibility/ Good coherence across studies; Agents have limited placental transfer, requiring direct fetal injection	XDE-208-induced decreased activity likely due to respiratory difficulties; Limb tone and skeletal structures normal; Lungs normal
AChE inhibition	Neostigmine	Clinical signs of cholinergic toxicity resulting in maternal	- Plausibility/ Strong coherence	No cholinergic clinical signs in adults or neonatal offspring in dev.

		death; Sublethal exposures generally without	across studies	tox. and repro. tox. studies; no maternal death
ACE inhibition	Captopril, Enalapril	In humans, limb contractures secondary to renal failure associated oligohydramniosis; In rats, fetal growth retardation and occasionally increased pup death without limb contractures	- Plausibility/ Strong coherence across studies	Lack of similarity in neonatal abnormalities and resistance of rodents to ACE inhibition-induced limb contractures

f) Conclusion of consideration of alternative MOAs

There is sufficient information to preclude many of the alternative **MOAs**. However, much of the evidence is indirect and does directly assess the mechanisms. For example, it is not likely that neuronal nicotinic receptors are responsible for the increased pup deaths; since the same isoforms of neuronal nicotinic receptors are present in both juveniles and adults, toxicity would be evident in adults as well as juveniles, which is not the case. Empirically determining the inability of sulfoxaflor to bind and adulterate the functionality of these neuronal receptors would provide a great deal more confidence in the decision to exclude this **MOA**.

Human Relevance

DAS has postulated that the proposed MoA is not relevant to humans based upon data demonstrating fundamental qualitative differences in the agonism of sulfoxaflor at the rat or human fetal-type muscle nAChR. Specifically, binding but no agonism was evident with sulfoxaflor at the human fetal-type or human adult-type muscle nAChR (MRID 47832035) (Figure 10.). Both muscle receptor types were examined as the transition from the fetal-type to adult-type human muscle nAChR occurs prenatally.

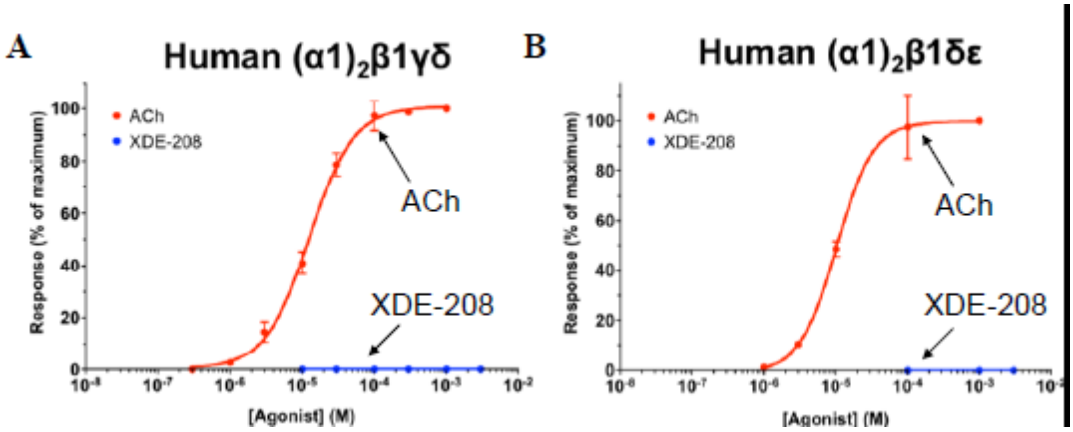


Figure 10. Agonist activation of nAChRs expressed in *Xenopus* oocytes. Data are shown for the human fetal (α1)2β1γδ nAChR (A) and human adult (α1)2β1δε nAChR (B). AChRs were expressed by microinjection of cDNA or cRNA in *Xenopus* oocytes. Dose-response curves are shown in which agonist-evoked responses are normalized to the maximal response detected with the endogenous agonist, acetylcholine (ACh). Data points are means of 3-7 responses. Adapted from Millar, 2010.

Table 10. Concordance of Key Events for Induction of Muscle Contracture and Associated Death in Neonatal Offspring in Rodents and Humans.

Key Event	Evidence in Rodents	Evidence in Humans
#1 Binding to the fetal-type muscle nAChR	Yes	Yes
#2 Agonism at the fetal-type muscle nAChR	Yes	No Plausible, but no agonism occurs with XDE-208 either at the fetal- or adult-type muscle nAChR.
#3 Sustained fetal-type muscle nAChR agonism/sustained muscle contraction	Yes	No data in humans. Presumably not possible via this MoA as key event #2 does not occur.
<u>Apical Endpoints</u> Forelimb Flexure, Hindlimb Rotation, Bent Clavicle, Neonatal Death	Yes	No data in humans. Presumably not possible via this MoA as key event #2 does not occur.

The species-specificity of the effects in the rat is supported by the finding that although sulfoxaflor binds to the fetal rabbit muscle nAChR, it does not induce any developmental effects in this species despite similar systemic exposure. Furthermore, the amino acid sequence of the

rat and human γ subunit indicate they are 90% identical, and therefore sufficiently unique that pharmacodynamic differences can be expected.

Conclusion
The Agency agrees that the proposed MoA is reasonable and likely. There is sufficient evidence with dose and time concordance to support the postulated MoA for skeletal abnormalities and increased neonatal pup deaths in rats. However, for the purposes of risk assessment, the Agency considers this MoA relevant to humans based on uncertainty regarding alternative MoAs.

Area of concern and potential study designs DAS should consider to reduce the uncertainty and increase confidence in the proposed MoA include the following:

- Demonstrate the inability of sulfoxaflor to cause sustained contracture in rabbit fetal diaphragm muscle. Alternatively, DAS can explore the functional response of rabbit nicotinic receptors (fetal and adult isoforms) expressed in HEK cells.
- Conclusively demonstrate that sulfoxaflor does not perturb the functionality of neuronal nicotinic receptors

III. *Sulfoxaflor Metabolite -474 Considerations*

X11719474 (-474) is the main soil metabolite of sulfoxaflor; it is found in treated crops and is predicted to have the potential to occur in ground and surface water ($t_{½}$ > 1000 days). Due to the high potential for exposure, numerous toxicity studies were conducted with -474 including acute studies, 28- and 90-day studies in the rat and dog, a mutagenicity battery, a reproduction and development study, liver toxicity mechanistic studies, and an nAChR binding study. -474 was consistently less toxic than sulfoxaflor, approximately 7-fold, and does not have acute or developmental toxicity. Unlike sulfoxaflor, -474 does not bind the rat fetal muscle nAChR (MRID 47832035) and therefore does not share the developmental MoA described for sulfoxaflor. Otherwise, -474 and sulfoxaflor have similar toxicity profiles; the most reliable indicators of the relative toxicity are effects to the liver (e.g., liver weight increase, hepatocellular hypertrophy, fatty change (vacuolation), single cell necrosis and aggregates of macrophages).

IV. *Global Partner Comments*

PMRA has reviewed the MoA characterizing the pup deaths as not relevant to humans. The overall evidence in support the proposed MOA for the fetal abnormalities and neonatal death was considered to be weak by the PMRA reviewer due to the inconsistencies noted in the findings as they relate to the proposed key events, and the fact that the possibility of alternate MoAs has not been fully discounted.

- Key Event 1: Specific binding to the fetal-type muscle nAChR **(Key Event #1)**

Sulfoxaflor binding to other AChR types was discussed in the MoA rationale; however, these alternate MoAs were not fully explored and could not be discounted. It was postulated in the Leydig cell MoA that there was binding of sulfoxaflor to neuronal nAChRs which increases dopamine release; this effect was demonstrated in the recently submitted microdialysis assay.

Furthermore, although there was a lack of muscarinic nAChR-mediated clinical signs, heart arrhythmia may be asymptomatic or the clinical signs could have been easily missed, especially when neonatal deaths occur very shortly after birth.

- Agonism at the fetal-type muscle nAChR (**Key Event #2)**

Expression of the recombinant rat and human (fetal and adult) muscle nAChRs in a mammalian cell culture would have provided a more native cellular environment compared to *Xenopus* ooctyes. Furthermore, the temperature at which the receptors are expressed would be significantly different (18^{o}C in ooctyes and 37^{o}C in mammalian cells) and may have a potential impact on the function of the receptor. Also, investigation of sulfoxaflor-induced agonist activity on fetal and adult rabbit muscle nAChRs could provide a useful comparison between species and additional weight of evidence to the proposed MoA.

- Sustained agonism/sustained muscle contracture **(Key Event #3)**

In the rat developmental toxicity and mechanistic studies, there was a lack of consistency in observations for fetal abnormalities;

  - Forelimb flexure and hindlimb rotation were not seen in the one-generation reproductive toxicity range-finding study
  - There were no treatment-related developmental findings reported in the reproductive toxicity range-finding study up to 1000 ppm
  - Necropsy of (PND 4) neonates in the critical window mechanistic studies did not reveal any observations of bent clavicle, convoluted ureter, or hydroureter. However, there are literature studies of chemicals demonstrating postnatal remodeling (based on delay ossification) which was found to be reversible by PND 6 (caffeine toxicity) and PND 63 (ethylene glycol).

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Appendix C. Metabolism Summary Table

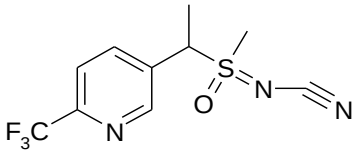
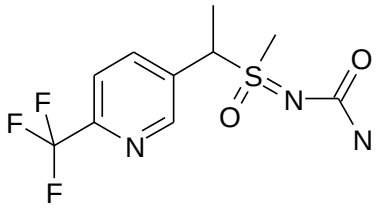
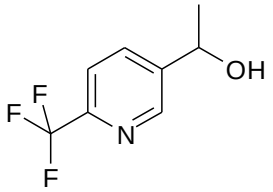
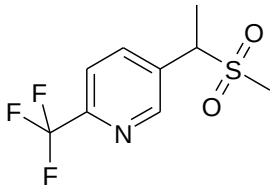
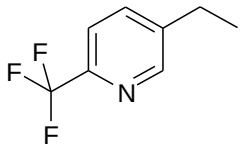
Table C.1. Tabular Summary of Metabolites and Degradates				
Chemical Name (other names in parenthesis)	Matrix	Approximate Percent TRR		Structure
		Matrices - Major Residue (>10%TRR)	Matrices - Minor Residue (<10%TRR)	
Parent	Tomato	33	--	
	Lettuce	17	--	
	Succulent Pea (pods)	60	--	
	Rice (grain)	35	--	
	Rotational Crops	--	< 3	
	Ruminant	> 80	--	
	Poultry	> 80	--	
	Rat (urine and feces)	> 93	--	
	Surface Water	~100	--	
	Groundwater	--	--	
X11719474	Tomato	20	--	
	Lettuce	31	--	
	Succulent Pea (pods)	13	--	
	Rice (grain)	--	9	
	Rotational Crops	~40-90	--	
	Ruminant	--	--	
	Poultry	--	--	
	Rat (urine and feces)	--	--	
	Surface Water	--	--	
	Groundwater	~88	--	
X11721061 (including glucose and glucose/malonyl conjugates)	Tomato	16	--	
	Lettuce	--	9	
	Succulent Pea (pods)	14	--	
	Rice (grain)	13	--	
	Rotational Crops	--	Generally < 9	
	Ruminant	--	< 3	
	Poultry	--	--	
	Rat (urine and feces)	--	< 4	
	Surface Water	--	--	
	Groundwater	--	--	
X11519540	Tomato	--	--	
	Lettuce	--	--	
	Succulent Pea (pods)	--	--	
	Rice (grain)	--	--	
	Rotational Crops	--	< 5	
	Ruminant	--	< 2	
	Poultry	--	< 8	
	Rat (urine and feces)	--	--	
	Surface Water	--	--	
	Groundwater	12	--	
X11596066	Tomato	--	--	
	Lettuce	--	--	
	Succulent Pea (pods)	--	--	
	Rice (grain)	--	--	
	Rotational Crops	--	--	
	Ruminant	< 18	--	

Table C.1. Tabular Summary of Metabolites and Degradates				
Chemical Name (other names in parenthesis)	Matrix	Approximate Percent TRR		Structure
		Matrices - Major Residue (>10%TRR)	Matrices - Minor Residue (<10%TRR)	
	Poultry	< 14	--	
	Rat (urine and feces)	--	--	
	Surface Water	--	--	
	Groundwater	--	--	

All crop metabolism studies were conducted at approximately a 2-fold exaggerated rate relative to the draft labels. Lettuce was harvested 7 days after the last treatment; tomatoes, peas, and rice were harvested 14 days after the last treatment. The proposed label lists PHIs ranging from 1 to 14 days, depending on the crop.

Livestock metabolism studies were conducted at levels of 12-14 mg/kg in the feed. The estimated dietary burdens are approximately 2 ppm for dairy cattle, 0.45 ppm for beef cattle, and 0.054 ppm for poultry.

Rats received a gavage dose at either 5 mg/kg (single and multiple doses) or 100 mg/kg, or a single intravenous dose at 5 mg/kg.

Appendix D. Physical/Chemical Properties and Summaries of Field Trial and Processing Study Results

Parameter	Value
Melting point/range (°C)	112.9
pH (24 ºC, 1.00% w/w aqueous solution)	5.82
Density (g/cm^3, Technical grade @ 19.7 ºC)	1.5378
Water solubility @ 20°C (g/L, 99.7% pure active ingredient)	
pH 5	1.380
pH 7	0.570
pH 9	0.550
Unbuffered	0.670
Solvent solubility @ 20°C (g/L, technical-grade material)	
Methanol	93.1
Acetone	217
p-Xylene	0.743
1,2-dichloroethane	39.6
ethyl acetate	95.2
n-heptane	0.000242
n-octanol	1.66
Vapor Pressure (Pa)	
(99.5% pure @ 25 ºC)	2.7×10^{-7}
(technical grade @ 20 ºC0)	1.1×10^{-7}
Dissociation constant (pK_a)	NA
Octanol/water partition coefficient Log(K_{OW})	
Unbuffered	NA
pH 5	0.806
pH 7	0.802
pH 9	0.799
UV/visible absorption spectrum	wavelength max absorption: 192, 211, and 260 nm

N/A = not available

Summary of Residues from the Crop Field Trials with Sulfoxaflor (SC formulation unless otherwise specified).									
Crop Matrix	Applic. Rate lb ai/A (g a.i./ha)	PHI (days)	n	Residues (ppm)					
				Min	Max	HAFT[1]	Median	Mean	Std. Dev
Crop Group 1 (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Carrot roots	0.357-0.376 (401-423)	7	24	<0.01	0.032	0.031	0.010	0.015	0.008
Potato roots	0.355-0.374 (399-420)	6-8	36	<0.01	<0.01	0.01	0.01	0.01	NA
Radish roots	0.360-0.363 (404-407)	7	12	<0.01	0.013	0.012	0.010	0.011	0.001
(WDG formulation)	0.360-0.364 (404-407)	7	6	<0.01	0.016	0.014	0.010	0.011	0.002
Sugar beet roots	0.351-0.374 (394-420)	7	26	<0.01	0.025	0.023	0.010	0.011	0.003
Crop Group 2 (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Carrot tops	0.360-0.366 (405-411)	7	8	0.311	2.283	2.069	0.515	0.857	0.764
Radish tops	0.360-0.364	7	12	0.183	0.506	0.478	0.258	0.297	0.108

Summary of Residues from the Crop Field Trials with Sulfoxaflor (SC formulation unless otherwise specified).									
Crop Matrix	Applic. Rate lb ai/A (g a.i./ha)	PHI (days)	n	Residues (ppm)					
				Min	Max	HAFT[1]	Median	Mean	Std. Dev
	(404-407)								
(WDG formulation)	0.360-0.364 (404-407)	7	6	0.242	0.465	0.441	0.398	0.370	0.099
Sugar beet tops	0.351-0.374 (394-420)	7	25	0.141	1.685	1.615	0.716	0.744	0.452
Crop Group 3 (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Dry Bulb Onion	0.357-0.365 (400-409)	7	12	<0.01	<0.01	0.01	0.01	0.01	NA
Green Onion	0.360-0.366 (404-410)	7-8	12	<0.01	0.440	0.387	0.105	0.132	0.130
Crop Group 4 (proposed use = 0.266 lb ai/A total application rate, 3-day PHI)									
Celery	0.360-0.361 (404-405)	2-3	24	0.058	0.804	0.771	0.143	0.229	0.235
Head Lettuce	0.328-0.388 (369-436)	2-3	27	<0.01	0.528	0.494	0.040	0.148	0.177
Leaf Lettuce	0.342-0.377 (384-424)	2-3	36	0.05	3.07	2.74	0.495	0.744	0.659
(WDG formulation)	0.360-0.363 (404-407)	3	6	0.379	0.885	0.806	0.577	0.595	0.189
Spinach	0.343-0.372 (385-418)	3	16	0.039	3.256	2.863	0.820	1.076	0.927
Crop Group 5 (proposed use = 0.266 lb ai/A total application rate, 3-day PHI)									
Broccoli	0.347-0.379 (390-426)	3	29	<0.01	1.600	1.584	0.070	0.201	0.395
Cauliflower	0.320-0.373 (360-419)	3-4	20	<0.01	0.07	0.055	0.014	0.020	0.014
Cabbage	0.341-0.383 (383-430)	2-3	28	<0.01	0.400	0.377	0.058	0.092	0.103
Mustard Greens	0.354-0.367 (397-411)	2-4	16	0.278	1.167	0.899	0.666	0.680	0.234
Crop Group 6 Beans (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Succulent edible podded bean	0.352-0.372 (393-411)	7	12	0.024	2.019	1.938	0.104	0.422	0.716
Dry shelled bean	0.350-0.365 (393-411)	7	12	0.020	0.112	0.104	0.078	0.068	0.029
Crop Group 7 (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Soybean, Forage	0.178-0.183 (200-205)	7-8	38	0.013	1.734	1.690	0.219	0.346	0.386
Soybean, Hay	0.358-0.368 (401-414)	6-8	38	0.057	1.378	1.238	0.725	0.670	0.383
Crop Group 8 (proposed use = 0.266 lb ai/A total application rate, 1-day PHI)									
Tomato	0.317-0.383 (356-430)	1	88	<0.01	0.762	0.602	0.051	0.091	0.123
Pepper, bell	0.321 - 0.364 (361-409)	1	24	<0.01	0.284	0.256	0.092	0.106	0.095
Pepper, non-bell	0.343 - 0.428 (385-481)	1	15	0.017	0.46	0.44	0.090	0.156	0.145
Crop Group 9 (proposed use = 0.266 lb ai/A total application rate, 1-day PHI)									
Cucumber (revised	0.355-0.374	1	36	<0.01	0.172	0.152	0.047	0.055	0.049

Summary of Residues from the Crop Field Trials with Sulfoxaflor (SC formulation unless otherwise specified).									
Crop Matrix	Applic. Rate lb ai/A (g a.i./ha)	PHI (days)	n	Residues (ppm)					
				Min	Max	HAFT[1]	Median	Mean	Std. Dev
from Tier II removing Zucchini samples)	(399-420)								
Squash, summer /Zucchini (revised from Tier II adding Zucchini samples)	0.357-0.367 (400-412)	1	10	<0.10	0.10	0.10	0.010	0.031	0.039
Squash, winter	0.357-0.364 (400-408)	1	6	<0.01	0.021	0.018	0.011	0.013	0.004
Melon	0.356-0.375 (400-421)	0-2	31	<0.01	0.304	0.266	0.031	0.054	0.067
Crop Group 10 (proposed use = 0.266 lb ai/A total application rate, 1-day PHI)									
Grapefruit	0.360 (404)	1	16	<0.01	0.186	0.130	0.014	0.041	0.056
Lemons	0.360 (404)	1	12	0.025	0.317	0.136	0.050	0.098	0.100
Oranges	0.263-0.368 (296-413)	1	61	0.037	0.46	0.44	0.114	0.167	0.120
Crop Group 11 Pome Fruit (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Apples	0.289-0.374 (325-420)	6-8	56	<0.01	0.297	0.266	0.070	0.086	0.057
Pears	0.279-0.379 (314-426)	7-8	32	0.044	0.267	0.261	0.142	0.146	0.063
Crop Group 12 (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Apricot	0.337-0.345 (379-388)	7	8	0.13	0.45	0.39	0.16	0.21	0.12
Cherry	0.337-0.365 (379-410)	7	32	0.26	1.60	1.49	0.820	0.824	0.379
Nectarine	0.341-0.351 (383-394)	7	18	0.074	0.247	0.232	0.150	0.154	0.045
Peach	0.338-0.373 (380-419)	6-8	48	<0.01	0.636	0.541	0.121	0.164	0.118
Plum	0.342-0.370 (384-415)	7	14	0.021	0.221	0.212	0.049	0.074	0.064
(WDG formulation)	0.358-0.366 (401-410)	7	12	0.014	0.362	0.358	0.076	0.115	0.117
Crop Group 15 (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Barley Grain	0.083-0.095 (94-108)	12-17	50	<0.010	0.37	0.32	0.048	0.060	0.060
Barley Hay	0.086-0.096 (97-108)	6-8	40	<0.010	0.643	0.642	0.213	0.249	0.174
Barley Straw	0.083-0.095 (94-108)	12-17	52	<0.010	2.2	1.46	0.189	0.322	0.413
Wheat Grain	0.084-0.098 (94-110)	12-17	67	<0.01	0.067	0.059	0.013	0.019	0.013
(WDG formulation)	0.089-0.093 (100-104)	14	6	<0.01	0.063	0.063	0.010	0.028	0.027
Wheat Forage	0.084-0.098 (94-110)	6-8	60	<0.01	1.400	1.400	0.108	0.187	0.225

Summary of Residues from the Crop Field Trials with Sulfoxaflor (SC formulation unless otherwise specified).									
Crop Matrix	Applic. Rate lb ai/A (g a.i./ha)	PHI (days)	n	Residues (ppm)					
				Min	Max	HAFT[1]	Median	Mean	Std. Dev
(WDG formulation)	0.084-0.098 (94-110)	7	6	0.041	0.221	0.197	0.095	0.111	0.073
Wheat Hay	0.084-0.096 (94-108)	6-8	60	0.024	1.024	0.848	0.246	0.292	0.202
(WDG formulation)	0.089-0.093 (100-104)	7	6	0.101	0.167	0.159	0.150	0.139	0.030
Wheat Straw	0.084-0.097 (94-109)	12-17	69	0.030	1.562	1.489	0.250	0.400	0.393
(WDG formulation)	0.088-0.093 (99-104)	14	6	0.060	1.641	1.600	0.105	0.588	0.784
Crop Groups 13-07F & 13-07G (proposed use = 0.266 lb ai/A total application rate, 1-day or 7-day PHI)									
13-07G Strawberries	0.339-0.369 (381-414)	1	26	0.02	0.50	0.49	0.184	0.182	0.109
13-07F Grape (all)	0.276-0.390 (310-438)	7-8	66	0.01	1.9	1.6	0.133	0.268	0.336
Crop Group 14 Tree Nuts (proposed use = 0.266 lb ai/A total application rate, 7-day PHI)									
Almond nutmeat	0.355-0.367	7	12	<0.01	0.013	0.123	<0.01	0.011	0.001
Almons hulls	0.355-0.367	7	12	1.04	3.12	3.05	1.68	1.86	0.681
Pecan nutmeat	0.360-0.363 (404-407)	7	12	<0.01	<0.01	<0.01	<0.01	<0.01	NA
Crop Group 20 (proposed use = 0.266 lb ai/A total application rate, 14-day PHI)									
Cotton gin byproducts (14-Day PHI)	0.346-0.375 (389-422)	14-15	42	0.037	4.20	4.03	0.476	0.869	1.12
Cotton, seed (14-Day PHI)	0.346-0.375 (389-422)	14-15	44	<0.01	0.182	0.176	0.019	0.037	0.038
Canola, seed (14-Day PHI)	0.078-0.099 (88.1-110.8)	13-15	36	<0.01	0.224	0.215	0.042	0.054	0.046
Canola, forage (14-Day PHI)	0.078-0.099 (88.1-110.8)	13-14	8	0.03	1.5	1.3	0.11	0.39	0.57
Soybean, Seed	0.357-0.371 (400-416)	6-8	36	<0.01	0.214	0.199	0.010	0.026	0.044

[1] HAFT = Highest Average Field Trial

Identification of Radioactivity in Hydrolyzed Buffered Water*.									
Analyte	14C-sulfoxaflor			14C-X11719474			14C-X11721061		
	pH 4	pH 5	pH 6	pH 4	pH 5	pH 6	pH 4	pH 5	pH 6
Sulfoxaflor	99.6	100.0	100.4	-	-	-	-	-	-
X11719474	-	-	-	99.0	96.9	89.1	-	-	-
X11721061	-	-	-	-	-	-	100.0	99.9	99.7
X11579457	-	-	-	0.4	3.8	11.6**	-	-	-
Accountability	99.6	100.0	100.4	99.4	100.7	100.7	100.0	99.9	99.7

*Only the pH is reported in this table. Durations and Temperatures were pH 4 = 20 minutes at 90°C, pH 5 = 60 minutes at 100°C, pH 6 = 20 minutes at 120°C. **Confirmed by LC/MS/MS.

Residue data from processing studies with Sulfoxaflor (XDE-208)					
RAC	Processed Commodity	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm) [b,c,d]	Processing Factor[e]
Apple	Apple Fruit RAC *	0.716 (802.2)	7	0.343	--
	Washed apples			0.246	0.7x
	Apple sauce			0.202	0.6x
	Juice			0.128	0.4x
	Wet pomace			0.365	1.1x
	Dry pomace			1.431	4.2x
	Canned apples			(0.0098)	0.03x
	Dried apples			0.118	0.3x
	* Apple fruit RAC samples analyzed after collection had similar residues (0.362 ppm, 0.028 ppm, and 0.009 ppm) to RAC samples analyzed prior to processing.				
Barley	Grain (RAC)*	0.277 (311.7)	16	0.174	--
	Pearl barley			0.130	0.7x
	Pot barley			0.152	0.9x
	Bran			0.167	1.0x
	Flour			0.132	0.8x
	Cleaned barley			0.165	0.9x
	Brewing malt			0.163	0.9x
	Malt sprouts			0.224	1.3x
	Beer			0.029	0.2x
	Spent grains			0.029	0.2x
	Brewer's yeast			0.026	0.1x
	* Barley RAC samples analyzed after collection had similar residues (0.16 ppm, 0.014 ppm, and 0.041 ppm) to RAC samples analyzed prior to processing.				
Cabbage	Heads (upon collection of field samples)	0.734 (824)	3	0.429	--
	Heads prior to processing (inc. wrapper leaves)			0.180	--
	Inner leaves			0.014	0.1x
	Outer (wrapper) leaves			0.322	1.8x
	Cooked head			(0.009)	0.1x
	Cooking liquid			(0.007)	0.1x
	Sauerkraut			0.017	0.1x
	Sauerkraut juice			0.013	0.1x
	These values are not used in calculation of the processing factor. The cabbage RAC was stored under "cool" conditions prior to processing, and was not kept in frozen storage.				
Oil seed, rape/ Taurus	Seeds prior to processing	0.264 (296.4)	14	0.033	--
	Cleaned seeds			0.037	1.1x
	Meal			0.063	1.9x
	Crude oil			ND	0.3x
	Refined oil			ND	0.3x
	Solvent-extracted meal			0.072	2.2x
	Canola RAC samples analyzed after collection had similar residues (0.038 ppm, ND, and <LOQ) to RAC samples analyzed prior to processing.				
Carrot	Carrot root (RAC)	1.092 (1,224)	7	0.0101	--
	Washed and peeled root			(0.0082), ND	<1.0x
	Cooked carrot			ND, ND	<1.0x
	Cooking liquid			0.0130, (0.0085) Avg. 0.012	1.1x
	Carrot juice			0.0234, 0.0246 Avg. 0.0240	2.4x

Residue data from processing studies with Sulfoxaflor (XDE-208)					
RAC	Processed Commodity	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm) [b,c,d]	Processing Factor[e]
	Canned carrot			ND, ND	<1.0x
Cherry	Whole cherries* (RAC)	0.715 (803)	7	0.765	--
	Washed cherries			0.648	0.8x
	Canned cherries			0.737	1.0x
	Juice			0.653	0.9x
	Jam			0.838	1.1x
	Dried cherries			3.950	5.2x
	*Cherry fruit RAC samples analyzed after collection had similar residues (0.616 ppm, 0.035 ppm, and 0.039 ppm) to RAC samples analyzed prior to processing.				
Cotton	Seed (RAC)	1.08 (1,213)	15	0.1001	--
	Aspirated seed fractions			2.3197	23x
	Delinted seed			0.0983	1.0x
	Hulls			0.1846	1.8x
	Meal			0.0822	0.8x
	Meal press cake			0.0781	0.8x
	Crude oil			(0.0059)	<0.1x
	Refined oil			ND	<0.1x
Grape/ Pinot Noir	RAC	0.739 (830)	7	0.243	--
	Raisins			0.856	3.5x
	Juice			0.164	0.7x
	Wine bottled			0.171	0.7x
	Pomace			0.237	1.0x
Head Lettuce	Heads with wrapper leaves (RAC)	0.748 (837.9)	3	0.592	--
	Wrapper leaves			0.593	1.0X
	Unwashed heads without wrapper leaves			0.354	0.6X
	Washed heads			0.146	0.2X
	Washings			0.045	0.1X
	Head lettuce RAC samples analyzed after collection had similar residues (0.507 ppm, 0.081 ppm, and 0.07 ppm) to RAC samples analyzed prior to processing.				
Leaf Lettuce	Unwashed lettuce (RAC)	0.728 (818)	3	0.654	--
	Washed lettuce			0.488	0.7x
	Washings			0.159	0.2x
Onion	Whole onion (RAC)	1.09 (1,225)	7	ND	--
	Peeled onion			ND	NC
	Dried onion			ND	NC
Orange (Valencia)	Pulp RAC*	1.79 (2.00)	1	(0.0039)	--
	Peel RAC*			0.3661	--
	Whole orange RAC* (peel +pulp)			0.0625	--
	Juice			(0.0042)	<0.2X
	Wet pulp			0.1567	2.5X
	Dried pulp			0.5159	8.3X
	Peel			0.5714	9.1X
	Oil			ND	<0.2X
	Marmalade			ND	<0.2X
	Canned slices			ND	<0.2X
	*Oranges were separated into peel and pulp prior to analysis. Processing factors were calculated using the whole orange RAC (peel + pulp) residues, which were calculated by the study reviewer as the sum of the peel and pulp residues, adjusted for the individual weights of the fractions.				

Residue data from processing studies with Sulfoxaflor (XDE-208)					
RAC	Processed Commodity	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm) [b,c,d]	Processing Factor[e]
Potato (Kuras)	Potato tubers* (RAC)	1.05 (1,176)	7	0.012	--
	Washed potatoes			0.014	1.2X
	Peeled potatoes			0.019	1.6X
	Peel			0.022	1.8X
	Potato flakes			0.030	2.5X
	Micro-waved potatoes			0.013	1.1X
	Boiled potatoes			0.012	1.0X
	Cooking water			(0.006)	<0.8X
	Potato chips			0.025	2.1X
	Dried potatoes			0.043	3.6X
	French fries			0.019	1.6X
	*Unpeeled and unwashed; sampled just prior to processing.				
Soybean	Seed (RAC)	1.10 (1,236)	7	0.0727	--
	AGF			6.8871*	95X
	Meal			0.0964	1.3X
	Hulls			0.1079	1.5X
	Pressed cake			0.0793	1.1X
	Expeller crude oil			0.0220	0.3X
	Solvent-extracted crude oil			0.0203	0.3X
	Refined oil			ND	<0.1X
	*Results are the mean of re-analysis.				
Strawberry	Unwashed strawberry	0.724 (812)	1	0.2003	--
	Washed strawberry			0.1670 0.1859 [0.1765]	0.9X
	Juice			0.0498 0.0564 [0.0531]	0.3X
	Canned strawberry			0.1215 0.1180 [0.1198]	0.6X
	Jam			0.0830 0.0702 [0.0766]	0.4X
	Samples were analyzed in duplicate; average residues are bolded and reported in brackets. Residues <LOQ were assigned a value of 0.01 ppm when averaged with a detectable residue.				
Sugar beet	Sugar beet root (RAC)	1.06 (1,191)	7	0.013	--
	Pulp			(0.005)	<0.8x
	Press water			(0.006)	<0.8x
	Raw juice			0.018	1.4x
	Thin juice			0.014	1.1x
	Lime sludge			(0.010)	<0.8x
	Thick juice			0.061	4.7x
	Raw sugar			0.023	1.8x
	White sugar			(0.006)	<0.8x
	Molasses			0.135	10x
	Dried pulp			0.039	3.0x
Tomato	Tomato RAC	0.719 (807.8)	1	0.119	--
	Fruit, washed and peeled			0.141	1.2X
	Juice			0.117	1.0X

Residue data from processing studies with Sulfoxaflor (XDE-208)					
RAC	Processed Commodity	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm) [b,c,d]	Processing Factor[e]
	Canned			0.093	0.8X
	Ketchup			0.250	2.1X
	Puree			0.235	2.0X
	Paste			0.518	4.4X
Wheat	Wheat Grain (RAC)	0.444 (499)	15	0.0650	--
	Aspirated Grain Fraction			1.3817	21X
	Total bran			0.0230	0.4X
	Germ			0.0307	0.5X
	Bran			0.0279	0.4X
	Middlings			0.0109	0.2X
	Shorts			0.0132	0.2X
	Whole meal flour			0.0136	0.2X
	Refined white flour			(0.0079)	<0.2X
	Whole grain bread			(0.0091)	<0.2X
	White bread			(0.0046)	<0.2X
	Gluten			ND	<0.2X
	Gluten feed meal			(0.0073)	<0.2X
	Starch			ND	<0.2X

[a] PHI = Preharvest Interval. ND = Non Detectable where residues were below the limit of detection (<LOD). LOD = 0.003 ppm.

[b] Residue values in parenthesis are <LOQ but >LOD. The LOQ was 0.010 ppm and the LOD was 0.003 ppm.

[c] Control specimens were collected and analyzed. No residues were detected for the unfortified control samples nor were any analytical interferences present in any untreated control sample.

[d] Processing factors were calculated using the equation: Processing Factor = [Measured residue for analyte in the processed fraction] / [Measured residue for analyte in the RAC prior to processing]. Residues <LOQ and ND were assigned a value of 0.01 ppm for calculation purposes.

[e] NC = Not calculated because residues were not detected above the LOQ in the RAC and processed fraction.

Appendix E. Review of Human Research

Aside from the Pesticide Handlers Exposure Database Version 1.1 (PHED 1.1), the Agricultural Handler Exposure Task Force (AHETF) database, the Outdoor Residential Exposure Task Force (ORETF) database, and the Agricultural Re-entry Task Force (ARTF) database, which were described at the end of the Executive Summary, no data from studies in which adult human subjects were intentionally exposed to a pesticide or other chemical were used in this risk assessment.

Appendix F. Occupational Exposure/Risk Summary Tables

Short- and Intermediate-term Occupational Handler Exposure and Risk Estimates for Proposed Uses of Sulfoxaflor (Transform ™ WG).										
Exposure Scenario	Crop or Target (Crop Group #)	Dermal Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Inhalation Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Max Single App Rate (lb ai/A or lb ai/gal)[2]	Area Treated or Amount Handled Daily [3]	Dermal		Inhalation		Combined MOE[8] (LOC=30)
						Dose[4] (mg/kg/day)	MOE[5]	Dose[6] (mg/kg/day)	MOE[7]	
Mixer/Loader										
Dry Flowable for Groundboom Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	0.227 (Baseline)	0.00896 (No respirator)	0.086	80 A	0.00057	3,200	0.00089	2,000	1,200
	Ornamentals, Turf grass (Sod Farm)	0.227 (Baseline)	0.00896 (No respirator)	0.133	80 A	0.00088	2,100	0.00140	1,300	800
	Barley, Tricale, Wheat, Canola (Sub CG 20A)	0.227 (Baseline)	0.00896 (No respirator)	0.047	200 A	0.00077	2,300	0.00120	1,500	900
	Cotton, Soybean	0.227 (Baseline)	0.00896 (No respirator)	0.086	200 A	0.0014	1,300	0.00220	810	490
Dry Flowable for Aerial Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	0.227 (Baseline)	0.00896 (No respirator)	0.086	350 A	0.00250	730	0.00390	460	280
	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	0.227 (Baseline)	0.00896 (No respirator)	0.133	350 A	0.00380	470	0.00600	300	180
	Barley, Tricale, Wheat, Canola (Sub CG 20A)	0.277 (Baseline)	0.00896 (No respirator)	0.047	1200 A	0.00460	390	0.00730	250	150
	Cotton, Soybean	0.277 (Baseline)	0.00896 (No respirator)	0.086	1200 A	0.00850	210	0.01340	130	80
Dry Flowable for Chemigation (AHETF)	Potatoes	0.227 (Baseline)	0.00896 (No respirator)	0.086	350 A	0.00250	730	0.00390	460	280
Dry Flowable for Airblast	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG	0.227 (Baseline)	0.00896 (No respirator)	0.133	40 A	0.00044	4,100	0.00069	2,600	1,600

Short- and Intermediate-term Occupational Handler Exposure and Risk Estimates for Proposed Uses of Sulfoxaflor (Transform ™ WG).										
Exposure Scenario	Crop or Target (Crop Group #)	Dermal Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Inhalation Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Max Single App Rate (lb ai/A or lb ai/gal)[2]	Area Treated or Amount Handled Daily [3]	Dermal		Inhalation		Combined MOE[8] (LOC=30)
						Dose[4] (mg/kg/day)	MOE[5]	Dose[6] (mg/kg/day)	MOE[7]	
Application (AHETF)	12), Tree Nuts (CG 14 & Pistachio)									
Applicator										
Sprays for Groundboom Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	0.0786 (Baseline)	0.00034 (No respirator)	0.086	80 A	0.00020	9,200	0.000034	53,000	7,800
	Ornamentals, Turf grass (Sod Farm)	0.0786 (Baseline)	0.00034 (No respirator)	0.133	80 A	0.00030	5,900	0.000052	34,000	5,100
	Barley, Tricale, Wheat, Canola (Sub CG 20A)	0.0786 (Baseline)	0.00034 (No respirator)	0.047	200 A	0.00027	6,700	0.000046	39,000	5,700
	Cotton, Soybean	0.0786 (Baseline)	0.00034 (No respirator)	0.086	200 A	0.00049	3,700	0.000085	21,000	3,100
Sprays for Aerial Application (PHED)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	0.005 (Engineering Controls - Enclosed cockpit)	0.000068 (Engineering Controls - Enclosed cockpit)	0.086	350 A	0.000055	33,000	0.000030	61,000	21,000
	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	0.005 (Engineering Controls - Enclosed cockpit)	0.000068 (Engineering Controls - Enclosed cockpit)	0.133	350 A	0.000084	21,000	0.000046	39,000	14,000
	Barley, Tricale, Wheat, Canola (Sub CG 20A)	0.005 (Engineering Controls - Enclosed cockpit)	0.000068 (Engineering Controls - Enclosed cockpit)	0.047	1200 A	0.00010	18,000	0.000056	32,000	11,000
	Cotton, Soybean	0.005 (Engineering Controls - Enclosed	0.000068 (Engineering Controls - Enclosed	0.086	1200 A	0.00019	9,600	0.0001	18,000	6,200

Short- and Intermediate-term Occupational Handler Exposure and Risk Estimates for Proposed Uses of Sulfoxaflor (Transform ™ WG).										
Exposure Scenario	Crop or Target (Crop Group #)	Dermal Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Inhalation Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Max Single App Rate (lb ai/A or lb ai/gal)[2]	Area Treated or Amount Handled Daily [3]	Dermal		Inhalation		Combined MOE[8] (LOC=30)
						Dose[4] (mg/kg/day)	MOE[5]	Dose[6] (mg/kg/day)	MOE[7]	
		cockpit)	cockpit)							
Sprays for Airblast Application (AHETF)	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	1.77 (Baseline)	0.00471 (No respirator)	0.133	40 A	0.00340	530	0.00036	5,000	480
Flagger										
Flagging for Aerial Application (PHED)	All Low Acreage Crops	0.011 (Baseline)	0.00035 (No respirator)	0.133	350 A	0.00019	9,700	0.00024	7,600	4,200
Flagging for Aerial Application (PHED)	All High Acreage Crops	0.011 (Baseline)	0.00035 (No respirator)	0.086	350 A	0.00012	15,000	0.00015	12,000	6,700
Mixer/Loader/Applicator										
Mechanically-pressurized Handgun Sprayer	Ornamentals, Greenhouses	3.5 (Baseline) (PHED)	0.120 (No respirator) (PHED)	0.00133	1,000 gal/day	0.00169	1,100	0.00231	780	450
	Turfgrass (Sod farms)	1.96 (Baseline) (ORETF)	0.042 (No respirator) (ORETF)	0.133	5 A	0.00047	3,800	0.00040	4,400	2,100
Backpack Sprayer	Ornamentals	13.2 (Baseline) (AHETF)	0.140 (No respirator) (AHETF)	0.00133	40 gal/day	0.00025	7,200	0.00011	16,000	5,000
	Turfgrass (Sod farms)	8.26 (Baseline) (MRID 44339801)	0.00258 (No respirator) (MRID 44339801)	0.00133	40 gal/day	0.00016	11,000	0.000002	900,000	11,000
Manually-pressurized Handwand (PHED)	Ornamentals, Turfgrass (Sod farms)	100 (Baseline)	0.030 (No respirator)	0.00133	40 gal/day	0.00193	930	0.000023	78,000	920

1. Occupational Pesticide Handler Unit Exposure Surrogate Reference Table (OPHED; March, 2012).
2. Application Rate is based on the proposed label.
3. Acres Treated per Day is based on Exposure SAC SOP #9: Standard Values for Daily Acres Treated.
4. Dermal Dose = [Dermal Unit Exposure (mg/lb ai) x Application Rate (lb ai/A) x Acres Treated (A/day) x % DAF (2.5%)]/%)/BW (69 kg).
5. Dermal MOE = Dermal NOAEL (1.8 mg/kg/day)/Dermal Dose (mg/kg/day); LOC = 30.
6. Inhalation Dose = [Inhalation Unit Exposure (mg/lb ai) x Application Rate (lb ai/A) x Acres Treated (A/day)]/)/BW (69 kg).
7. Inhalation MOE = NOAEL (1.8 mg/kg/day)/Inhalation Dose (mg/kg/day); LOC = 30.
8. Combined MOE = NOAEL (1.8 mg/kg/day)/Combined Dose (mg/kg/day); LOC = 30.

Short- and Intermediate-term Occupational Handler Exposure and Risk Estimates for Proposed Uses of Sulfoxaflor (GF-2032 SC).										
Exposure Scenario	Crop or Target (CG #)	Dermal Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Inhalation Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Max Single App Rate (lb ai/A or lb ai/gal)[2]	Area Treated or Amount Handled Daily [3]	Dermal		Inhalation		Combined MOE[8] (LOC=30)
						Dose[4] (mg/kg/day)	MOE[5]	Dose[6] (mg/kg/day)	MOE[7]	
Mixer/Loader										
Liquids for Groundboom Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	0.220	0.000219	0.090	80 A	0.00057	3,100	0.000023	79,000	3,000
	Ornamentals, Turf grass (Sod Farm)	0.220	0.000219	0.133	80 A	0.00085	2,100	0.000034	53,000	2,000
	Barley, Tricale, Wheat, Canola (Sub CG 20A)	0.220	0.000219	0.043	200 A	0.00069	2,600	0.000027	66,000	2,500
	Cotton, Soybean	0.220	0.000219	0.090	200 A	0.00140	1,300	0.000057	32,000	1,200
Liquids for Aerial Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	0.220	0.000219	0.090	350 A	0.00250	720	0.00010	18,000	690
	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	0.220	0.000219	0.133	350 A	0.00370	490	0.00015	12,000	470
	Barley, Tricale, Wheat,	0.220	0.000219	0.043	1200 A	0.00410	440	0.00016	11,000	420

Short- and Intermediate-term Occupational Handler Exposure and Risk Estimates for Proposed Uses of Sulfoxaflor (GF-2032 SC).										
Exposure Scenario	Crop or Target (CG #)	Dermal Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Inhalation Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Max Single App Rate (lb ai/A or lb ai/gal)[2]	Area Treated or Amount Handled Daily [3]	Dermal		Inhalation		Combined MOE[8] (LOC=30)
						Dose[4] (mg/kg/day)	MOE[5]	Dose[6] (mg/kg/day)	MOE[7]	
	Canola (Sub CG 20A)									
	Cotton, Soybean	0.220	0.000219	0.090	1200 A	0.00860	210	0.00034	5,300	200
Liquids for Chemigation (AHETF)	Potatoes	0.220	0.000219	0.090	350 A	0.00250	720	0.00100	18,000	690
Liquids for Airblast Application (AHETF)	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	0.220	0.000219	0.133	40 A	0.00042	4,200	0.000017	110,000	4,100
Applicator										
Sprays for Groundboom Application (AHETF)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F), Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans	0.0786 (Baseline)	0.00034 (No respirator)	0.090	80 A	0.00021	8,800	0.000035	51,000	7,500
	Ornamentals, Turf grass (Sod Farm)	0.0786 (Baseline)	0.00034 (No respirator)	0.133	80 A	0.00030	5,900	0.000052	34,000	5,100
	Barley, Tricale, Wheat, Canola (Sub CG 20A)	0.0786 (Baseline)	0.00034 (No respirator)	0.043	200 A	0.00024	7,300	0.000042	42,000	6,300
	Cotton, Soybean	0.0786 (Baseline)	0.00034 (No respirator)	0.090	200 A	0.00051	3,500	0.000089	20,000	3,000
Sprays for Aerial Application (PHED)	Brassica (Cole) Leafy Veg., Bulb Veg. (CG 3-07), Cucurbit Veg. (CG 9), Fruiting Veg. (CG 8), Leafy Veg. (Except Brassica CG 4 & Watercress), Leaves Root/Tuber Veg. (CG 2), Root/Tuber Veg. (CG 1), Small Fruit Vine (Sub CG 13-07F),	0.005 (Engineering Controls - Enclosed cockpit)	0.000068 (Engineering Controls - Enclosed cockpit)	0.090	350 A	0.000057	32,000	0.000031	58,000	25,000

Short- and Intermediate-term Occupational Handler Exposure and Risk Estimates for Proposed Uses of Sulfoxaflor (GF-2032 SC).										
Exposure Scenario	Crop or Target (CG #)	Dermal Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Inhalation Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Max Single App Rate (lb ai/A or lb ai/gal)[2]	Area Treated or Amount Handled Daily [3]	Dermal		Inhalation		Combined MOE[8] (LOC=30)
						Dose[4] (mg/kg/day)	MOE[5]	Dose[6] (mg/kg/day)	MOE[7]	
	Low Growing Berry (Sub 13-07G), Succulent, Edible Podded, Dry Beans									
	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	0.005 (Engineering Controls - Enclosed cockpit)	0.000068 (Engineering Controls - Enclosed cockpit)	0.133	350 A	0.000084	21,000	0.000046	39,000	19,000
	Barley, Tricale, Wheat, Canola (Sub CG 20A)	0.005 (Engineering Controls - Enclosed cockpit)	0.000068 (Engineering Controls - Enclosed cockpit)	0.043	1200 A	0.000093	19,000	0.000051	35,000	12,000
	Cotton, Soybean	0.005 (Engineering Controls - Enclosed cockpit)	0.000068 (Engineering Controls - Enclosed cockpit)	0.090	1200 A	0.00020	9,200	0.00011	17,000	6,000
Sprays for Airblast Application (AHETF)	Citrus (CG 10), Pome Fruits (CG 11), Ornamentals, Stone Fruits (CG 12), Tree Nuts (CG 14 & Pistachio)	1.77 (Baseline)	0.00471 (No respirator)	0.133	40 A	0.00340	530	0.00036	5,000	480
Flagger										
Flagging for Aerial Application (PHED)	All Low Acreage Crops	0.011 (Baseline)	0.00035 (No respirator)	0.133	350 A	0.00019	9,700	0.00024	7,600	4,200
Flagging for Aerial Application (PHED)	All High Acreage Crops	0.011 (Baseline)	0.00035 (No respirator)	0.090	350 A	0.00013	14,000	0.00016	11,000	6,300
Mixer/Loader/Applicator										
Mechanically-pressurized Handgun Sprayer	Ornamentals, Greenhouses	3.5 (Baseline) (PHED)	0.120 (No respirator) (PHED)	0.00133	1,000 gal/day	0.00169	1,100	0.00231	780	450
	Turfgrass (Sod farms)	1.14 (Baseline)	0.0019 (Baseline) (ORETF)	0.133	5 A	0.00047	3,800	0.00040	4,400	2,100

Short- and Intermediate-term Occupational Handler Exposure and Risk Estimates for Proposed Uses of Sulfoxaflor (GF-2032 SC).										
Exposure Scenario	Crop or Target (CG #)	Dermal Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Inhalation Unit Exposure[1] (mg/lb ai) (Mitigation Level)	Max Single App Rate (lb ai/A or lb ai/gal)[2]	Area Treated or Amount Handled Daily [3]	Dermal		Inhalation		Combined MOE[8] (LOC=30)
						Dose[4] (mg/kg/day)	MOE[5]	Dose[6] (mg/kg/day)	MOE[7]	
		(ORETF)								
Backpack Sprayer	Ornamentals	13.2 (Baseline) (AHETF)	0.140 (No respirator) (AHETF)	0.00133	40 gal/day	0.00025	7,200	0.00011	16,000	5,000
	Turfgrass (Sod farms)	8.26 (Baseline) (MRID 44339801)	0.00258 (No respirator) (MRID 44339801)	0.00133	40 gal/day	0.00016	11,000	0.000002	900,000	11,000
Manually-pressurized Handwand (PHED)	Ornamentals, Turfgrass (Sod farms)	100 (Baseline)	0.030 (No respirator)	0.00133	40 gal/day	0.00193	930	0.000023	78,000	920

1. Occupational Pesticide Handler Unit Exposure Surrogate Reference Table (OPHED; March, 2012).
2. Application Rate is based on the proposed label.
3. Acres Treated per Day is based on Exposure SAC SOP #9: Standard Values for Daily Acres Treated.
4. Dermal Dose = [Dermal Unit Exposure (mg/lb ai) x Application Rate (lb ai/A) x Acres Treated (A/day) x % DAF (2.5%)]/%)/BW (69 kg).
5. Dermal MOE = Dermal NOAEL (1.8 mg/kg/day)/Dermal Dose (mg/kg/day); LOC = 30.
6. Inhalation Dose = [Inhalation Unit Exposure (mg/lb ai) x Application Rate (lb ai/A) x Acres Treated (A/day)]/)/BW (69 kg).
7. Inhalation MOE = NOAEL (1.8 mg/kg/day)/Inhalation Dose (mg/kg/day); LOC = 30.
8. Combined MOE = NOAEL (1.8 mg/kg/day)/Combined Dose (mg/kg/day); LOC = 30.

Appendix G. Anticipated Post-Application Activities and Dermal Transfer Coefficients

Anticipated Post-Application Activities and Dermal Transfer Coefficients.†				
Proposed Crop	Crop Group	Transfer Coefficients, cm^2/hr	Activities	EPA MRID No.
Field / row crop, low/medium	Barley, Wheat	70	Hand weeding	42851302, 42689103, 42689104
		1,100	Scouting	45005904, 45005908
	Canola (Subgroup 20A)	1,100	Scouting	45005904, 45005908
	Cotton	70	Hand weeding	42851302, 42689103, 42689104
		210	Scouting	42851302
		900 g cotton/hr	Harvesting Mechanical (Module Builder)	42701601
		2400 g cotton/hr	Harvesting Mechanical (Picker Operator; Raker)	42701601
		5050 g cotton/hr	Harvesting Mechanical (Tramper)	42701601
	Soybean	70	Hand weeding	42851302, 42689103, 42689104
		1,100	Scouting	45005904, 45005908
	Succulent Edible Podded and Dry Beans	70	Hand weeding	42851302, 42689103, 42689104
		210	Scouting	42851302, 42974501
		1100	Scouting	45005904, 45005908
		1900	Irrigation (hand set)	45224801
Vegetable, head and stem Brassica	Brassica (Cole) Leafy Vegetables	230	Transplanting	45344501, 45469501, 45469502
		330	Scouting	42974501
		1400	Hand Weeding	45530102
		1900	Irrigation (hand set)	45224801
		4200	Weeding, Hand	45005906, 45005907, 45191701
Vegetable, "Root"	Bulb Vegetables (Crop Group 3-07)	330	Scouting	42974501
		1400	Hand Weeding	45530102
		1900	Irrigation (hand set)	45224801
		4200	Weeding, Hand	45005906, 45005907, 45191701
	Leaves of Root and Tuber Vegetables (Crop Group 2)	70	Weeding, Hand	42851302, 42689103, 42689104
		210	Scouting	42851302, 42974501
		230	Transplanting	45344501, 45469501, 45469502
		1100	Harvesting, Hand	43013003, 40966503, 40966502, 45530103
		1900	Irrigation (hand set)	45224801
	Root and Tuber Vegetables (Crop Group 1)	70	Weeding, Hand	42851302, 42689103, 42689104
		210	Scouting	42851302, 42974501
		1100	Harvesting, Hand	43013003, 40966503, 40966502, 45530103
		1900	Irrigation (hand set)	45224801
Tree Fruit, Evergreen	Citrus (Crop Group 10)	100	Hand weeding	43297602
		230	Transplanting	45344501, 45469501, 45469502
		580	Hand Pruning	45175102, 45480302

Anticipated Post-Application Activities and Dermal Transfer Coefficients.†				
Proposed Crop	Crop Group	Transfer Coefficients, cm^2/hr	Activities	EPA MRID No.
		1400	Hand Harvesting	42830002, 40975308, 40966501, 45138202, 45175101, 45432301,45432302
		3600	Thinning Fruit	42428101
Vegetable, Cucurbit	Cucurbits Vegetables (Crop Group 9)	90	Scouting	45005909
		230	Transplanting	45344501, 45469501, 45469502
		550	Turning	45480301, 45491902
		1900	Irrigation (hand set)	45224801
Vegetable, Fruting	Fruiting Vegetables (Crop Group 8)	70	Weeding, Hand	42851302, 42689103, 42689104
		90	Scouting	45005909
		210	Scouting	42851302, 42974501
		230	Transplanting	45344501, 45469501, 45469502
		550	Tying/Training, Hand Harvesting	45480301, 45491902
		1100	Harvesting, Hand	43013003, 40966503, 40966502, 45530103
		1900	Irrigation (hand set)	45224801
Vegetable, Leafy	Leafy Vegetables except Brassica – (Crop Group 4)& Watercress	70	Weeding, Hand	42851302, 42689103, 42689104
		210	Scouting	42851302, 42974501
		230	Transplanting	45344501, 45469501, 45469502
		1100	Harvesting, Hand	43013003, 40966503, 40966502, 45530103
		1900	Irrigation (hand set)	45224801
Nursery/Greenhouse (Unassigned)	Ornamentals	230	Harvesting, Hand	45344501, 45469501, 45469502
		1900	Irrigation (hand set)	45224801
Tree, "Fruit", Deciduous	Pome Fruits (Crop Group 11)	100	Orchard Maintenance, Propping, Hand Weeding	43297602
		230	Transplanting	45344501, 45469501, 45469502
		580	Scouting, Hand Pruning, Training	45175102, 45480302
		1400	Harvesting, Hand	42830002, 40975308, 40966501, 45138202, 45175101, 45432301, 45432302
		3600	Thinning Fruit	42428101
	Stone Fruits (Crop Group 12)	100	Orchard Maintenance, Propping, Hand Weeding, Thinning Fruit, Bird Control	43297602
		230	Transplanting	45344501, 45469501, 45469502
		580	Scouting, Hand Pruning, Training	45175102, 45480302
		1400	Harvesting, Hand	42830002, 40975308, 40966501, 45138202, 45175101, 45432301, 45432302
		3600	Thinning Fruit	42428101
Vine /Trellis	Small Fruit Vine Climbing (except fuzzy kiwifruit – Subgroup 13-07F)	230	Transplanting	45344501, 45469501, 45469502
		640	Scouting, Hand Pruning, Hand Weeding, Propagating, Bird Control, Trellis Repair, Tying/Training	45005910
		1900	Irrigation (hand set)	45224801

Anticipated Post-Application Activities and Dermal Transfer Coefficients.†				
Proposed Crop	Crop Group	Transfer Coefficients, cm^2/hr	Activities	EPA MRID No.
		5500	Leaf Pulling, Tying/Training, Hand Harvesting	43223907, 40985601
		10100	Leaf Pulling, Tying/Training, Hand Harvesting	45491901
		19300	Turning, Girdling	40975304
Berry, Low	Low Growing Berry (Subgroup 13-07G)	70	Hand Weeding, Hand Pruning (shears), Canopy Management	42851302, 42689103, 42689104
		210	Scouting	42851302, 42974501
		230	Transplanting	45344501, 45469501, 45469502
		1100	Hand Harvesting (ranking), Scouting	43013003, 40966503, 40966502 45530103, 45005904, 45005908
		1900	Irrigation (hand set)	45224801
Tree, "Nut"	Tree Nuts (Crop Group 14 & Pistachio)	100	Orchard Maintenance, Poling, Hand Weeding	43297602
		190	Harvesting, Mechanical (shaking)	41848604
		230	Transplanting	45344501, 45469501, 45469502
		580	Scouting	45175102, 45480302
		1400	Harvesting, Hand (net)	42830002, 40975308, 40966501 45138202, 45175101, 45432301 45432302
Turf/Sod	Turfgrass, Sodfarms	6700	Maintenance, Harvesting, Slab Transplanting/Planting	45432303

†The information in the table is based on proprietary and non-proprietary data.

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