EPA Reviewer: Edward Scollon, Ph.D.
RAB2, Health Effects Division (7509P)
EPA COR: Lori Brunsman
SIMB, Health Effects Division (7509P)

Signature: _____
Date: 11/5/13
Signature: _____
Date: 11/8/13

Revised by U. S. Environmental Protection Agency

IIA 5.7.5 Developmental Neurotoxicity Study

Report: Beck, M.J. A Dietary Developmental Neurotoxicity Study of XDE-208 in Rats (Study No. WIL-410013; 090339). WIL Research Laboratories, LLC, Ashland, OH (10 June 2010). MRID 47832133. Unpublished. PMRA 1941306.

Dates of work: 29-Jun-2009 to 5-Jan-2010

Guidelines: U.S. EPA OPPTS 870.6300
OECD 426

GLP: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Executive Summary: 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 28.8 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.

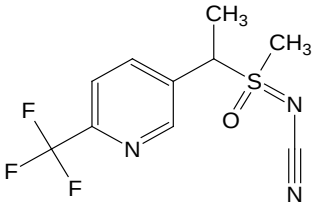
The LOAEL for offspring is 100 ppm (7.4 mg/kg bw/day) based on the reduction in pup survival. The NOAEL is 25 ppm (1.8 mg/kg bw/day).

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.

I. Materials and Methods

A. Materials:

1.	**Test material:**	Sulfoxaflor (XDE-208)
	Description:	White, fine powder
	Synonyms:	**Chemical Name:** N-(Methyloxido(1-(6-(trifluoromethyl)-3-pyridinyl)ethyl)sulfanylidene)-cyanamide **Synonyms:** [1-(6-Trifluoromethylpyridin-3-yl)ethyl](methyl)-oxido-λ4-sulfanylidenecyanamide, Sulfoximine, X11422208, XDE-208, XR-208
	Lot/Batch #:	E2162-34 (TSN003725-0001)
	Purity:	95.6% (wt/wt) as two diastereomers in 48.4/47.4% ratio
	CAS #:	946578-00-3

	Chemical Structure:	
2.	**Vehicle:**	PMI Nutrition International, LLC Certified Rodent LabDiet® 5002
3.	**Test animals:**	
	Species:	Rats
	Strain:	Crl:CD(SD)
	Age/weight at study initiation:	12 weeks at the time of mating/220 g to 303 g on gestation day 0
	Source:	Charles River Laboratories, Inc., Raleigh, NC
	Housing:	Upon arrival and until pairing, all animals were individually housed in clean, stainless steel wire-mesh cages suspended above cage-board. The animals were paired for mating in the home cage of the resident male. Following positive evidence of mating, the females were individually housed in plastic maternity cages with nesting material, ground corncob bedding. The females were housed in these cages through the scheduled day of necropsy. Following weaning on PND 21, pups were housed by litter in plastic maternity cages with nesting material until PND 28. On PND 28, surviving offspring were housed individually in suspended wire-mesh cages until euthanasia.
	Feed & Water:	The basal diet used in this study was PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 was provided *ad libitum*. Reverse osmosis-purified (on-site) drinking water was provided *ad libitum*.
	Environmental Conditions:	**Temperature:** 20.9°C to 21.9°C **Humidity:** 41.5% to 51.6% **Air changes:** minimum of 10 times/hour **Photoperiod:** 12-hour light
	Acclimation Period:	14 days prior to the start of the study.

B. Procedures and Study Design

1. In-life dates: 29-Jun-2009 to 5-Jan-2010

2. Study schedule: The control and test diets were offered *ad libitum* to the F_0 females daily from gestation day 6 to lactation day 21. The test substance was administered as a constant concentration (ppm) in the diet from gestation day 6 to lactation day 4. On lactation day 4 and 11, concentration was adjusted to 50% and 40%, respectively, of the dietary concentration offered from gestation day 6 to lactation day 4. The adjustments were to

account for the 2- to 3-fold increase in maternal food consumption that occurs during lactation days 7-21 in Crl:CD(SD) rats.

3. **Mating procedure:** At the discretion of the Study Director, each animal judged to be in good health and meeting acceptable body weight (minimum of 220 g) requirements at the time of cohabitation was placed in a suspended wire-mesh cage with a resident male from the same strain and source for mating. Resident males were untreated, sexually mature rats utilized exclusively for mating. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage and verified by a second biologist. Each mating pair was examined daily. The day on which evidence of mating was identified was termed gestation day 0, and the animals were separated.

4. **Animal assignment:** Following completion of the mating period, the bred females were assigned to groups using a WIL Toxicology Data Management System (WTDMS™) computer program which randomized the animals based on stratification of the gestation day 0 body weights in a block design.

Table 1. Animal Assignment

Test Group	Dose Level in Diet[a] (ppm)	Number of Females
Control	0	25
Low	25	25
Mid	100	25
High	400	25

[a]Diets were administered from gestation day 6 to lactation day 21
Source: p. 33 of the Study Report.

5. **Dose selection rationale:** Exposure levels were selected based on the results of a previous OECD 421 reproductive toxicity screening study in rats, in which neonatal survival was significantly decreased (MRID 47832143). In that study, 11 of 12 dams lost their entire litters by PND 4 at a dose of 1000 ppm, and 4 of 12 dams lost approximately half of their litters at a dose of 500 ppm. There were no effects on pup survival or other measured parameters at 100 ppm, indicating that this sulfoxaflor effect on neonatal survival has a relatively steep dose response curve. Based on test guideline requirements to avoid significant pup mortality, as seen at 500 ppm with sulfoxaflor, the highest exposure level selected for use in this study was 400 ppm. This exposure level was selected to ensure that adequate numbers of offspring were available to complete developmental neurotoxicity testing requirements. An exposure level of 400 ppm is also expected to produce increased relative liver weights in the dams. The mid- and low-exposure levels were expected to provide dose-response data for any treatment-related effects and to establish a no-observed-adverse-effect level (NOAEL). The exposure levels used in this study also corresponded to those used in the 2-generation reproduction toxicity study of sulfoxaflor (MRID 47832142).

6. **Dosage preparation and analysis:** For the control group, an appropriate amount of PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 was weighed twice (3 weeks apart) and placed in a labeled bag. For the test substance-exposed group, an appropriate amount of the test substance was weighed into tared weighing vessels for each group and transferred into a Hobart mixer with 5 kg of rodent feed (weight/weight). A portion of the

rodent feed was added to the weighing vessel; this was then scraped into the Hobart mixer to ensure complete transfer of the test substance. The formulation was then mixed for 5 minutes. The resultant formulation was termed pre-mix. The remainder of rodent feed to achieve the desired concentration was weighed and placed in a V-blender, and the pre-mix was then added to the blender. The diet was blended for 10 minutes (using an intensifier bar during the first and last 3 minutes) to achieve a total batch of homogeneous diet at the appropriate concentration per test group.

Results - Homogeneity Analysis: Analyses of dietary formulations indicated that the test substance was homogenously distributed in the feed based on relative standard deviations of 0.64% to 6.5%.

Stability Analysis: A previously conducted stability study has shown sulfoxaflor to be stable for a period of 65 days at dietary concentrations bracketing those used in the current study[1].

Concentration Analysis: Analyses of dietary formulations revealed mean concentrations ranging from 98.8% to 105% of targeted concentrations.

C. Observations

1. Maternal animals:

Daily In-Life Observations: All rats were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded daily for each dam from gestation day 0 until necropsy. The absence or presence of findings was recorded for individual animals. Females expected to deliver were also observed twice daily during the period of expected parturition and at parturition for dystocia (prolonged labor, delayed labor or other difficulties).

Detailed Clinical Observations: All dams in each group were observed outside of the home cage on gestation days 10 and 15 and on lactation days 10 and 21. The following parameters were evaluated:

Table 2. Detailed Clinical Observations in Dams

Ease of removal from cage	*Pupillary response*
Lacrimation/chromodacryorrhea	*General body posture*
Piloerection	*Salivation*
Palpebral closure	*Fur appearance*
Red/crusty deposits	*Respiratory rate/character*
Eye prominence	*Mucous membrane/eye/skin color*
Mobility	*Muscle tone*
Convulsions/tremors	*Gait*
Grooming	*Arousal*
Bizarre/stereotypic behavior	*Urination/defecation*
Ease of handling animal in hand	*Backing*

Source: p. 38 of the Study Report.

[1] Brzak, K.A.; Carr, M.S. X11422208: Method Validation and Stability in Rodent Feed. Report of Toxicology & Environmental Research and Consulting. The Dow Chemical Company, Midland, MI, 2007.

Body Weights/Body Weight Gains: Individual maternal body weights were measured on gestation days 0, 6, 9, 12, 15, and 20 and on lactation days 1, 4, 7, 11, 14, 17, and 21. Mean body weight changes during gestation were calculated for the pre-treatment period (gestation days 0-6), and thereafter by cumulatively normalizing daily body weight change from the first day of dose administration (gestation day 6). Mean body weight changes were also calculated for each corresponding interval of lactation, and for lactation days 1-21.

Feed Consumption: Individual maternal food consumption was recorded on gestation days 0, 6, 9, 12, 15, and 20 and on lactation days 1, 4, 7, 11, 14, 17, and 21. Mean test substance consumption (mg/kg/day) for each group was determined by multiplying the concentration of the test substance in the diet (mg/kg) by the g/kg/day food consumption value for each interval.

Parturition: All females were allowed to deliver naturally and rear their young to weaning (PND 21). During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. Beginning on the day parturition was initiated (PND 0), pups were sexed and examined for gross malformations, and the numbers of stillbirths and live pups were recorded. Individual gestation length was calculated using the date delivery was first observed.

2. **Litter observations:** Each litter was examined daily for survival and adverse changes in appearance or behavior. All deaths were recorded. A daily record of litter size was maintained. Each pup received a detailed physical examination on PND 1, 4 (prior to culling), 7, 11, 14, 17, and 21 and at weekly intervals thereafter until necropsy. Any abnormalities in nursing behavior were recorded. Pups were individually weighed on PND 1, 4, 7, 11, 14, 17, and 21; and at weekly intervals thereafter until necropsy and whenever they were removed from their cages for behavioral testing. Pups were individually sexed on PND 0 (when possible), 4, 11, and 21.

Table 3. Litter Observations

Observations	Time of Observation (lactation day)							
	Day 0	Day 1	Day 4	Day 7	Day 11	Day 14	Day 17	Day 21
Detailed physical examination		X	X	X	X	X	X	X
Sex of each pup (M/F)	X		X		X			X
Pup weight		X	X	X	X	X	X	X

Source: pp.41-42 of the Study Report.

Intact offspring dying from PND 0 to 4 were examined externally and sexed; the stomachs were examined for the presence of milk. A detailed gross necropsy was performed on any pup dying or euthanized *in extremis* after PND 4; tissues were saved in 10% neutral-buffered formalin for possible histopathological examination only as deemed necessary by the gross findings. The carcass of each pup was then discarded.

Culling: To reduce variability among the litters, 8 pups/litter, 4/sex when possible, were randomly selected on PND 4. Culled pups were weighed, euthanized by intraperitoneal

injection of sodium pentobarbital on PND 4 and discarded without macroscopic examination. If a litter consisted of less than 6 pups or did not meet the sex ratio criteria (at least 3/sex) for inclusion in the study, the litter was not used for neurobehavioral or neuropathological evaluation and the pups were necropsied on PND 4; the carcass of each pup was then discarded.

Weaning and Selection: Each dam and litter remained together until weaning on lactation day 21. Following standardization of litters (culling) on PND 4, 1 male and 1 female pup from each litter was randomly assigned to one of the following evaluation subsets (A-C). Subset A of 20 pups/sex/group was assigned to detailed clinical observations (PND 4, 11, 21, 35, 45, and 60), auditory startle response (PND 20 and 60), locomotor activity (PND 13, 17, 21, and 61), learning and memory (PND 62), and brain weight evaluations (PND 72). From this subset, 10 pups/sex from the control and 400 ppm groups were selected for neuropathological and brain morphometric evaluations on PND 72. A second subset (Subset B) of 20 pups/sex/group was selected for learning and memory (PND 22). A third subset (Subset C) of 20 pups/sex/group was selected for brain weight evaluations on PND 21; of these, 10 pups/sex from the control and 400 ppm groups were selected for neuropathological and brain morphometric evaluations on PND 21. All pups not selected for behavioral evaluations were euthanized and necropsied on PND 21. Offspring selected for learning and memory assessment beginning on PND 22 were necropsied following the completion of that assessment. The following table summarizes the allocation of offspring for behavioral tests, brain weights, and neuropathological evaluation.

Table 4. Allocation of Offspring for Behavioral Tests, Brain Weights, and Neuropathological Evaluation

No. Selected (Subset)	Age	Evaluation
20/sex/group (C)	PND 21	Brain weights, Neuropathological assessment, Morphometry***
20/sex/group (A)*	PND 4, 11, 21, 35, 45 and 60	Detailed clinical observations
20/sex/group (A)*	PND 20 and 60	Auditory startle response
20/sex/group (A)*	PND 13, 17, 21, and 61	Locomotor Activity
20/sex/group (B)	PND 22	Learning and Memory**
20/sex/group (A)*	PND 62	Learning and Memory**
20/sex/group (A)*	PND 72	Brain weights, Neuropathological assessment, Morphometry***

* - The same pup subset was used for detailed clinical observations, auditory startle response, locomotor activity, and learning and memory assessment at 62 days of age. Except as directed for learning and memory, the same pups were evaluated at multiple time points.
** - Different pups were evaluated on PND 22 and 62.
*** - Twenty pups/sex/group were examined for macroscopic neuropathology and had brain weights recorded. From these animals, microscopic neuropathology and brain morphometry evaluations were performed on 10 pups/sex/group in the control and high-dose groups.
Source: p. 43 of the Study Report.

3. Developmental landmarks, sensory function, and neurobehavioral observations:

Surface Righting Response: Each pup was evaluated for attainment of surface righting response beginning on PND 2. Each pup was placed in a supine position and allotted a

maximum of 2 seconds to return to an upright position. Failure to return to an upright position in the allotted time was considered a negative response. Pups were evaluated daily until demonstration of a positive surface righting reflex, or until culling for pups that were selected for culling and did not have a positive response by PND 4.

Eye Opening: Each pup was evaluated for eyelid separation beginning on PND 13 and continuing until both eyelids were fully opened or until the scheduled euthanasia. The characteristic was considered present when both eyelids were fully opened.

Balanopreputial Separation (Subset A): Each male pup was observed for balanopreputial separation beginning on PND 35. The day on which balanopreputial separation was first observed was recorded for each pup. Examination of the pups continued daily until balanopreputial separation was present. Individual body weights were recorded on the day of attainment of this landmark.

Vaginal Patency (Subset A): Each female pup was observed for vaginal perforation beginning on PND 25. The day on which the vaginal lumen was first observed to open was recorded for each pup. Examination of the females was continued daily until vaginal patency was present. Individual body weights were recorded on the day of attainment of this landmark.

Detailed Clinical Observations (Subset A): Twenty pups/sex/group were observed outside of the home cage on PND 4, 11, 21, 35, 45, and 60 by the same biologists whenever possible, without knowledge of the animal's group assignment. The same animals were examined at each interval, with the following exception. Any animal found dead between PND 4 and 21 was replaced with another pup of the same age and sex, if available (littermates were used preferentially when available). Parameters evaluated were identical to those evaluated for the F_0 dams (see Table 2), with the following exceptions. Piloerection, fur appearance, palpebral closure, eye color, eye prominence, pupillary response, mobility, gait, backing, and grooming were not recorded for pups on PND 4 and/or 11 due to the early stages of development (palpebral separation had not occurred, incomplete fur growth or inability to complete sufficient movement to determine gait, backing, or grooming).

Locomotor Activity (Subset A): Locomotor activity was assessed for 20 rats/sex/group (1 rat/sex/litter from 20 litters/group) on PND 13 (after assessment of eye opening), 17, 21, and 61. The same animals were tested at each interval. Locomotor activity was measured automatically using the Kinder Scientific MotorMonitor System (Kinder Scientific, LLC, Poway, CA). Each animal was tested separately. Data were collected in 5 minute epochs (print intervals) and the test session duration was 60 minutes. These data were compiled as six 10-minute subintervals for tabulation.

Data for total locomotor activity were tabulated. Total locomotor activity was defined as a combination of fine locomotor skills (i.e., interruption of a single photobeam) and ambulatory movement (e.g., interruption of 2 or more consecutive photobeams).

Auditory Startle Response (Subset A): An auditory startle response test was performed on 20 rats/sex/group (1 rat/sex/litter from 20 litters /group) on PND 20 and 60 using the Kinder Scientific Startle Response System (Kinder Scientific, LLC, Poway, CA). The same animals were tested at each interval.

Each test session consisted of a 5 minute acclimation period with a 65 ± 5 dB(A) broadband background white noise. The startle stimulus for each trial was a 115 ± 5 dB(A) mixed-frequency noise burst stimulus, approximately 20 milliseconds (ms) in duration. Responses were recorded during the first 100 ms following the onset of the startle stimulus for each trial. Each test session consisted of 50 trials, with an 8 second intertrial interval. Startle response data were analyzed in 5 blocks of 10 trials each. Startle response measurements obtained were maximum response amplitude (MAX) in Newtons (N) and latency to MAX (TMAX) in milliseconds (ms).

Biel Maze Swimming Trials (Subsets A and B): Beginning on PND 22 and PND 62, swimming ability and learning and memory were assessed for 20 rats/sex/group (assigned to Subsets B and A, respectively) using a water filled 8-unit T maze[2]. Animals were placed in the maze and were required to traverse the maze and escape by locating a submerged platform. For the learning and memory phases, the time required to traverse the maze and the numbers of errors for all trials were recorded. An error was defined as any instance when an animal deviated from the correct channel with all 4 feet. Testing for Subset B animals was initiated when each animal was 22 days of age; testing for Subset A animals was intiated when each animal was 62 days of age. Animals tested on PND 22 were not used for the second testing interval on PND 62.

Each testing interval consisted of 3 phases that were conducted over 7 consecutive days. Phase 1 was an evaluation of swimming ability and motivation to escape from the maze, and was performed on day 1 of the Biel maze procedure. For this evaluation, animals were placed in a straight channel opposite the escape platform, and the time required for each animal to escape was recorded. Each animal was allowed 4 trials to evaluate swimming ability and motivation. For each trial, animals were allowed 2 minutes to complete the trial. At the end of each trial, the animal was immediately placed at the starting position for another trial until all 4 trials were complete.

Phase 2 of the Biel maze procedure evaluated sequential learning. This evaluation was conducted on days 2-6 of the Biel maze procedure. Animals were allowed 2 trials per day for 2 days to solve the maze in path A. Animals were then allowed 2 trials per day for 3 consecutive days to solve the maze in path B (reverse of path A).

Phase 3 of the Biel maze procedure probed the animal for its memory to solve the maze when challenged in path A. This evaluation was conducted on day 7 of the Biel maze procedure. Each animal was allowed 2 trials to solve the maze in path A.

For each trial in Phases 2 and 3, animals were allowed 3 minutes to solve the maze. If an animal did not escape the maze within the allotted 3 minutes, the animal was placed on the escape platform for up to 20 seconds, then removed from the maze. The minimum intertrial interval for these phases was 1 hour.

Biel maze data were collected as the mean time to escape over all trials for each of the 3 phases (i.e., swimming ability and motivation, sequential learning and memory) of the Biel maze procedure. Also, the numbers of errors committed during Phases 2 and 3 were evaluated.

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[2] Biel, W.C. Early age differences in maze performance in the albino rat. *Journal of Genetic Psychology* 1940, *56*, 439-453.

4. Postmortem observations:

1) Maternal animals:

Macroscopic Examination: All dams with viable pups on lactation day 21, with total litter loss (within 24 hours of litter loss), that failed to meet the sex ratio criteria (lactation day 4) for inclusion in the study, and that did not deliver (post-mating day 25) were euthanized by carbon dioxide inhalation. A gross necropsy was performed for each of these females; this included examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities including viscera. For females that delivered or had macroscopic evidence of implantation, the numbers of former implantation sites (the attachment site of the placenta to the uterus) were recorded; numbers of corpora lutea were also recorded for females with total litter loss prior to lactation day 4. The number of unaccounted-for implantation sites was calculated by subtracting the number of pups born from the number of implantation sites observed.

Tissues were preserved in 10% neutral-buffered formalin for possible future histopathologic examination only as deemed necessary by the gross findings. The carcass of each female was then discarded.

Organ Weights: The liver and kidneys from all dams were weighed. Absolute weights and organ to final body weight ratios (for lactation day 21 females only) were reported.

2) Offspring: On PND 21, all offspring not selected for behavioral evaluations were euthanized by carbon dioxide inhalation and subjected to gross pathology examinations. The necropsy included examination of the external surface, all orifices and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Tissues were retained in 10% neutral-buffered formalin only as deemed necessary by the gross findings.

Offspring scheduled for euthanasia after completion of the learning and memory evaluations initiated on PND 22 (Subset B) were euthanized by carbon dioxide inhalation and subjected to a gross pathology examination. The necropsy included examination of the external surface, all orifices and the cranial, thoracic, abdominal, and pelvic cavities, including viscera. Organ and tissue samples were preserved in 10% neutral-buffered formalin as deemed necessary by the gross findings. The carcass of each animal was then discarded.

3) Neuropathology (Subsets A and C):

Macroscopic Examination: On PND 21, 1 male and 1 female pup from each litter (up to 20 rats/sex/group; Subset C), when possible, were macroscopically examined for neuropathology. At the termination of the study (PND 72), 1 male and/or 1 female from each litter (up to 20 rats/sex/group; Subset A) was also macroscopically examined for neuropathology. All animals were anesthetized by an intraperitoneal injection of sodium pentobarbital and perfused in situ with fixative (4% paraformaldehyde/1.4% glutaraldehyde) as described in WIL standard operating procedures. The whole brains were removed (including olfactory bulbs), weighed, and the dimensions (length [excluding olfactory bulbs] and width) were recorded for all Subset A and C animals. Any abnormal coloration or lesions of the external brain and spinal cord were recorded. On PND 21, brains from all animals were retained for microscopic examination; at study termination (PND 72), the central and peripheral nervous system and tissues were dissected and preserved for microscopic examination.

Microscopic Examination: Brains from all pups perfused on PND 21 and 72 were prepared for a qualitative histopathological examination by embedding in paraffin, sectioning, and staining with hematoxylin and eosin. Sections from all major brain regions (including olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain cerebellum, pons, and medulla oblongata) were examined from the first 10 rats/sex in the control and high-exposure groups that met the criteria for measurement. Specific measurements taken were determined by the pathologist, but included at least 2 measurements each from the neocortical, hippocampal, and cerebellar area.

Nervous system tissues from the offspring perfused in situ at study termination (PND 72, Subset A) were processed for neuropathological evaluation. The following tissues were microscopically examined for 10 rats/sex from 20 litters/group (10 rats/sex/group) in the control and high-exposure groups:

Table 5. Tissues Examined Microscopically from F_1 Pups on PND 72 (Subset A)

Spinal cord[a] - at cervical swellings C_3 - C_7 and at lumbar swellings T_{13} - L_4	Cervical spinal nerves
Trigeminal ganglion/nerves[b]	Sciatic nerves (mid-thigh region) [2][+]
Lumbar dorsal root ganglion at T_{13} - L_4*	Sciatic nerves (sciatic notch) [2][+]
Lumbar dorsal root fibers at T_{13} - L_4*	Sural nerves [2][+]
Lumbar ventral root fibers at T_{13} - L_4*	Tibial nerves [2][+]
Cervical dorsal root ganglion at C_3 - C_7*	Peroneal nerves [2][+]
Cervical dorsal root fibers at C_3 - C_7*	IIA 5.1.1Optic nerves[b]
IIA 5.1.2Cervical ventral root fibers at C_3 - C_7*	IIA 5.1.3Eyes with retina[b]
IIA 5.1.4Lumbar spinal nerves	IIA 5.1.5Skeletal muscle (gastrocnemius)

[a] = One cross and 1 longitudinal section
[b] = Both processed and evaluated microscopically
[+] = 1 processed for microscopic evaluation
[2] = Indicates that 2 sections (1 transverse and 1 longitudinal) of the tissue were evaluated from the right hind leg. The tissues from the left hind leg were collected and preserved for possible future evaluation.
* = 4-6 tissues were collected at necropsy; 2 tissues were evaluated microscopically.
Source: pp. 2330-2331 of the Study Report.

The central nervous system tissues were embedded in paraffin, and the peripheral nervous system tissues were embedded in plastic. Tissues were prepared for qualitative histopathological evaluation by sectioning and staining with hematoxylin and eosin.

Morphometric Analysis: Quantitative examinations of the brains from the selected PND 21 and PND 72 offspring were conducted using the Pax-It™ (MIS, Inc., Franklin Park, IL) image-capturing computer system and software (version 4.0). Specific levels analyzed were designated as Levels 1, 3, and 5. Measurements were made on homologous sections to ensure that the dimensions of the regions were comparable. In some cases, there was

deformation or irregularity of the desired region. As a result, morphometric analysis could not be made for some regions in a few animals.

D. Data Analysis

1. **Statistical analyses conducted by WIL Research Laboratories, LLC:** All statistical tests were performed using appropriate computing devices or programs and were in accordance with the published literature. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-exposed group to the control group. Each mean was presented with the standard deviation (S.D.), standard error (S.E.), and the number of animals (N) used to calculate the mean. Data obtained from the female whose pregnancy status was not determined were excluded from statistical analyses following the mating period. Where applicable, the litter was used as the experimental unit.

   Cumulative body weight gains (gestation days 6-9, 6-12, 6-15, and 6-20), gestation lengths, former implantation sites, unaccounted-for sites, day of attainment of balanopreputial separation or vaginal patency, body weight on the day of attainment, and continuous detailed clinical observation were analyzed by Dunnett's test to compare the test substance-exposed groups to the control group. The mean number of pups born and mean live litter size were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences between the control and test substance-exposed groups. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test substance-exposed groups to the control group. Mean litter proportions (percent per litter) of pup viability and males per litter were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test substance-exposed groups to the control group. Detailed clinical observation data for each test substance-exposed group which yielded scalar and descriptive data were compared to the control group by the Fisher's Exact Test.

2. **Statistical analyses conducted by BioStat Consultants, Inc.:** Statistical analyses conducted by BioSTAT Consultants, Inc., Portage, MI were performed using the SAS™ System version 9.1. All analyses were two-tailed, and unless otherwise specified, conducted at the 0.05 significance level. When used, Dunnett's test was conducted regardless of the outcome of the treatment (TRT) main effect.

   Maternal Body Weight, Food Consumption, and Organ Weight Data: Maternal body weight and food consumption data (gestation days 6 through 20 and lactation days 1 to 21) were analyzed separately with a repeated measures analysis of variance (RANOVA). Factors in the model included ANIMAL (random effect), fixed effects for treatment group (TRT), gestation or lactation day (TIME), and the TRT*TIME interaction term. The covariance structure was selected by comparing Akaike's Information Criterion (AIC). The Kenward-Rogers adjustment for denominator degrees of freedom was applied. If TRT*TIME was significant ($p<0.05$), pairwise comparisons for the interaction term were made for each individual test substance-exposed group with the control group through linear contrasts. If TRT*TIME was not significant, the TRT main effect was evaluated. If TRT was significant ($p<0.05$), pairwise comparisons for the main effect term were made for each individual test

substance-exposed group with the control group through linear contrasts. To adjust for multiple comparisons, pairwise comparisons with the control group were made at the 0.02 significance level.

Gestation body weight gain (gestation days 6-20) was analyzed with an analysis of covariance (ANCOVA). Factors in the model included a fixed effect for treatment group (TRT) with litter size as a covariate. Pairwise comparisons were made for each individual test substance-exposed group with the control group through Dunnett's test at the 0.05 significance level.

Organ weights were analyzed with an ANCOVA. Factors in the model included a fixed effect for treatment group (TRT) with final body weight as a covariate. Pairwise comparisons were made for each individual test substance-exposed group with the control group through Dunnett's test at the 0.05 significance level.

Offspring Body Weights: Pre-culling (PND 1 and 4) and post-culling (PND 4, 7, 11, 14, 17, and 21) pup body weights were analyzed separately with a RANOVA. Factors in the model included random effects for PUP and LITTER; fixed effects for TRT, SEX, and postnatal day (TIME); and the following interaction terms: TRT*SEX and TRT*TIME. Body weights for PND 28 through 72 were also analyzed separately with a RANOVA. Factors in the model included ANIMAL (random effect), fixed effects for TRT, SEX, and postnatal day (TIME) and the following interaction terms: TRT*SEX and TRT*TIME. For these parameters, the first examination was whether the TRT*SEX interaction was significant. If TRT*SEX was significant ($p<0.05$), the analysis was conducted by sex with a RANOVA; factors in the model included a random effect for PUP or ANIMAL, fixed effects for TRT and TIME, and the interaction terms TRT*TIME. The covariance structure for the RANOVA model(s) was selected by comparing AIC. The Kenward Rogers adjustment for denominator degrees of freedom was applied. In the final RANOVA model, TRT*TIME was evaluated and, if significant ($p<0.05$), pairwise comparisons for the interaction term were made for each individual test substance-exposed group with the control group through linear contrasts. If TRT*TIME was not significant, the TRT main effect was evaluated. If TRT was significant ($p<0.05$), pairwise comparisons for the main effect term were made for each individual test substance-exposed group with the control group through linear contrasts. To adjust for multiple comparisons, pairwise comparisons with the control group were made at the 0.02 significance level.

Pre-Weaning Developmental Landmarks: The mean day of attainment for surface righting response and eye opening were analyzed, individually, with a nested ANOVA. Factors in the model included LITTER (random effect), fixed effects for TRT and SEX, and the TRT*SEX interaction term. If TRT*SEX was significant ($p<0.05$), the analysis was conducted by sex, with a nested ANOVA including factors in the model for LITTER (random effect) and TRT (fixed effect). The Kenward-Rogers adjustment for denominator degrees of freedom was applied. In the final nested ANOVA model, pairwise comparisons were made for each individual test substance-exposed group with the control group through Dunnett's test.

Locomotor Activity across Sessions (PND 13, 17, and 21): Cumulative total counts were analyzed with a RANOVA. Factors in the model included random effects for PUP and LITTER; fixed effects for TRT, SEX, and SESSION; and the following interaction terms:

TRT*SEX and TRT*SESSION. The first examination was whether the TRT*SEX interaction was significant. If TRT*SEX was significant ($p<0.05$), the analysis was conducted by sex, with a RANOVA including factors in the model for PUP (random effect), fixed effects for TRT and SESSION, and the interaction term TRT*SESSION. The covariance structure for the RANOVA model(s) was selected by comparing AIC. The Kenward-Rogers adjustment for denominator degrees of freedom was applied. In the final RANOVA model, TRT*SESSION was evaluated and, if significant ($p<0.05$), pairwise comparisons for the interaction term were made for each individual test substance-exposed group with the control group through linear contrasts. If TRT*SESSION was not significant, the TRT main effect was evaluated. If TRT was significant ($p<0.05$), pairwise comparisons for the main effect term were made for each individual test substance-exposed group with the control group through linear contrasts. To adjust for multiple comparisons, pairwise comparisons with the control group were made at the 0.02 significance level.

Locomotor Activity within Sessions (PND 13, 17, and 21): Total counts from six 10-minute intervals were analyzed, by session, with a RANOVA. Factors in the model included random effects for PUP and LITTER; fixed effects for TRT, SEX, and time interval (TIME); and the following interactions terms: TRT*SEX and TRT*TIME. The first examination was whether the TRT*SEX interaction was significant. If TRT*SEX was significant ($p<0.05$), the analysis was conducted by sex, with a RANOVA including factors in the model for PUP (random effect), fixed effects for TRT and TIME, and the interaction term TRT*TIME. The covariance structure for the RANOVA model(s) was selected by comparing AIC. The Kenward-Rogers adjustment for denominator degrees of freedom was applied. In the final RANOVA model, TRT*TIME was evaluated and, if significant ($p<0.05$), pairwise comparisons for the interaction term were made for each individual test substance-exposed group with the control group through linear contrasts. If TRT*TIME was not significant, the TRT main effect was evaluated. If TRT was significant ($p<0.05$), pairwise comparisons for the main effect term were made for each individual test substance-exposed group with the control group through linear contrasts. To adjust for multiple comparisons, pairwise comparisons with the control group were made at the 0.02 significance level.

Locomotor Activity within Session (PND 61): Total counts from six 10-minute intervals were analyzed with a RANOVA. Factors in the model included PUP (random effect); fixed effects for TRT, SEX, and time interval (TIME); and the following interaction terms: TRT*SEX and TRT*TIME. The first examination was whether the TRT*SEX interaction was significant. If TRT*SEX was significant ($p<0.05$), the analysis was conducted by sex, with a RANOVA including factors in the model for PUP (random effect), fixed effects for TRT and TIME, and the interaction term TRT*TIME. The covariance structure for the RANOVA model(s) was selected by comparing AIC. The Kenward-Rogers adjustment for denominator degrees of freedom was applied. In the final RANOVA model, TRT*TIME was evaluated and, if significant ($p<0.05$), pairwise comparisons for the interaction term were made for each individual test substance-exposed group with the control group through linear contrasts. If TRT*TIME was not significant, the TRT main effect was evaluated. If TRT was significant ($p<0.05$), pairwise comparisons for the main effect term were made for each individual test substance-exposed group with the control group through linear contrasts. To adjust for multiple comparisons, pairwise comparisons with the control group were made at the 0.02 significance level.

Auditory Startle Response: Maximum force amplitude (MAX) was analyzed with a RANOVA. Factors in the model included random effects for PUP and LITTER (PND 20 only); fixed effects for TRT, SEX, and trial block (TRIAL); and the following interaction terms: TRT*SEX and TRT*TRIAL. The first examination was whether the TRT*SEX interaction was significant. If TRT*SEX was significant ($p<0.05$), the analysis was conducted by sex, with a RANOVA including factors in the model for PUP (random effect), fixed effects for TRT and TRIAL, and the interaction term TRT*TRIAL. The covariance structure for the RANOVA model(s) was selected by comparing AIC. The Kenward-Rogers adjustment for denominator degrees of freedom was applied. In the final RANOVA model, TRT*TRIAL was evaluated and, if significant ($p<0.05$), pairwise comparisons for the interaction term were made for each individual test substance-exposed group with the control group through linear contrasts. If TRT*TRIAL was not significant, the TRT main effect was evaluated. If TRT was significant ($p<0.05$), pairwise comparisons for the main effect term were made for each individual test substance-exposed group with the control group through linear contrasts. To adjust for multiple comparisons, pairwise comparisons with the control group were made at the 0.02 significance level.

Biel Maze: The number of errors for Learning Paths A and B and Memory Path A were statistically analyzed individually. Analyses were conducted with a RANOVA. Factors in the model included random effects for PUP and LITTER (PND 22 only); fixed effects for TRT, SEX, and TRIAL; and the following interaction terms: TRT*SEX and TRT*TRIAL. The first examination was whether the TRT*SEX interaction was significant. If TRT*SEX was significant ($p<0.05$), the analysis was conducted by sex, with a RANOVA including factors in the model for PUP (random effect), fixed effects for TRT and TRIAL, and the interaction term TRT*TRIAL. The covariance structure for the RANOVA model(s) was selected by comparing AIC. The Kenward-Rogers adjustment for denominator degrees of freedom was applied. In the final RANOVA model, TRT*TRIAL was evaluated and, if significant ($p<0.05$), pairwise comparisons for the interaction term were made for each individual test substance-exposed group with the control group through linear contrast. If TRT*TRIAL was not significant, the TRT main effect was evaluated. If TRT was significant ($p<0.05$), pairwise comparisons for the main effect term were made for each individual test substance-exposed group with the control group through linear contrasts. To adjust for multiple comparisons, pairwise comparisons with the control were made at the 0.02 significance level.

Brain Morphometry: The acceptable Type I error rate was set to 0.05, except as otherwise specified. The data were analyzed separately for PND 21 and PND 72. First, brain weight, brain length, and brain width were analyzed using a multivariate analysis of variance (MANOVA) with TRT, SEX, and TRT*SEX as fixed factors. The first examination was the TRT*SEX interaction. If that effect was not statistically significant, the overall TRT factor was tested for statistical significance for the pooled sexes. If TRT was statistically significant, linear contrasts, as part of the MANOVA across the dependant variables of brain weight, brain length, and brain width, were used to test for pairwise differences between each test substance-exposed group and the control group. The Type I error rate was corrected for multiple comparisons between the control group and each of the test substance-exposed groups by dividing the original Type I error rate of 0.05 by the square root of the number of comparisons. The Type I error rate for these comparisons was 0.02.

If the TRT*SEX interaction effect was statistically significant, the MANOVA analysis was conducted for each sex separately. The overall TRT factor was examined for statistical significance for each sex separately. If TRT was statistically significant, linear contrasts, as part of the MANOVA across the dependant variables of brain weight, brain length, and brain width, were used to test for pairwise differences between each test substance-exposed group and the control group. The Type I error rate was corrected for multiple comparisons between the control group and each of the test substance-exposed groups as described above. The Type I error rate for these comparisons was 0.02.

For microscopic measurements, only the control and high-dose groups were initially evaluated. If treatment-related effects were observed in the high-dose group, then the low- and mid-dose groups were examined. For the cases where TRT was statistically significant in the MANOVA, as described above, all the endpoints from the microscopic examination, which included at least 2 measurements each from the neocortical, hippocampal, and cerebellar areas, were analyzed using a MANOVA with TRT, SEX, and TRT*SEX as fixed factors. The first examination was the TRT*SEX interaction. If that effect was not statistically significant, the overall TRT factor was examined for statistical significance for the pooled sexes. If TRT was statistically significant, linear contrasts, as part of the MANOVA across the dependant variables from the microscopic examination, were used to test for pairwise differences between each test substance-exposed group and the control group. The Type I error rate was corrected for multiple comparisons between the control group and the test substance-exposed group(s) as described above. The Type I error rate for these comparisons was 0.02. If TRT was not statistically significant when analyzing microscopic parameters, no additional statistical analyses were conducted.

If the TRT*SEX interaction effect was statistically significant, the MANOVA analysis was conducted for each sex separately. The overall TRT factor was examined for statistical significance for each sex separately. If TRT was statistically significant, linear contrasts, as part of the MANOVA across the dependant variables from the microscopic examination, were used to test for pairwise differences between each test substance-exposed group and the control group. The Type I error rate was corrected for multiple comparisons between the control group and each of the test substance-exposed groups as described above. The Type I error rate for these comparisons was 0.02. If TRT was not statistically significant when analyzing microscopic parameters, no additional statistical analyses were conducted.

For the case where TRT was not statistically significant in the MANOVA analysis of perfused brain weight, brain length, and brain width, the endpoints from the microscopic examination were individually analyzed using ANOVA with TRT, SEX, and TRT*SEX as fixed factors. The first examination was the TRT*SEX interaction. If that effect was not statistically significant, Dunnett's test was conducted across the pooled sexes to test for pairwise differences between the test substance-exposed group(s) and the control group at each time point (PND 21 and 72). If the TRT*SEX interaction was statistically significant, the analysis was conducted for each sex and each time point separately. Dunnett's test was then conducted for each sex at each time point to test for pairwise differences between each test substance-exposed group and the control group.

3. Indices:

Offspring Viability Indices: The following viability indices were calculated from lactation records of litters in the study:

Mean Live Litter Size	=	Total no. of viable pups PND 0 / No. of litters with viable pups PND 0	
Postnatal Survival Between Birth and PND 0 or PND 4 (% Per Litter)	=	Sum of (Viable pups per litter on PND 0 or PND 4/No. of pups born per litter) / No. of litters/group	x 100
Postnatal Survival for All Other Intervals (% Per Litter)	=	Sum of (Viable pups per litter at end of interval N/Viable pups per litter at start of interval N) / No. of litters/group	x 100
Where N	=	PND 0-1, 1-4 (Pre-Selection), 4 (Post-Selection)-7, 7-14, 14-21 or 4 (Post-Selection)-21	

Source: p. 42 of the Study Report.

4. Historical control data: Historical control data were provided to allow comparison with concurrent controls. Historical control data was from previous reproductive studies and developmental neurotoxicity studies conducted in-house and was presented and analyzed in the same manner as the current study.

II. Results

A. Maternal Animals

1. Pregnancy status: The pregnancy rates in the control, 25, 100, and 400 ppm groups were 100%. However, 1 female (no. 49879) in the 100 ppm group failed to deliver and a pregnancy status was inadvertently not determined. Because the female that failed to deliver was most likely nongravid, it is assumed that the pregnancy status in the 100 ppm group was 96.0%.

2. Mortality and clinical signs: All dams survived to the scheduled necropsies. Female no. 49902 in the 400 ppm group had total litter loss on PND 2. Total litter loss was also noted for 2 females (nos. 49934 and 49916) in the control group on lactation days 0 and 9, respectively. No test substance-related clinical findings were noted during the daily examinations at any exposure level. Findings noted in the test substance-exposed groups, including hair loss, scabbing, and red material on various body surfaces, occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not exposure-related.

3. Detailed clinical observations: No test substance-related findings were observed in maternal animals at the detailed clinical observations. A significantly ($p<0.05$) lower number in the 100 ppm group were sitting or standing normally on gestation day 15 compared to the control group (8 vs. 16 animals); however, in the absence of a dose response, this decrease in normal body posture was not considered test substance-related. No other remarkable differences were apparent between the control and test substance-exposed groups when the detailed clinical observation data were evaluated on gestation days 10 and 15 and lactation days 10 and 21.

4. **Body weight:** Mean maternal body weights and body weight gains were unaffected by test substance exposure during gestation. Differences between the control, 25, 100, and 400 ppm groups were slight and not statistically significant.

   Mean maternal body weights and body weight gains were unaffected by test substance exposure during lactation. A significant (p=0.012) treatment-by-time interaction was noted in the analysis of mean body weights for the 100 ppm group; however, because there was no effect on mean body weights in the 400 ppm group, the difference was not considered test substance-related. Furthermore, the increase in mean body weights in the 100 ppm group were minimal (≤4.1% difference from the control group across the intervals measured) and not biologically meaningful.

5. **Food consumption:** Mean food consumption, evaluated as g/animal/day, was unaffected by test substance exposure during gestation. A significant (p=0.031) treatment-by-time interaction was noted for mean food consumption. When subsequent pairwise comparisons were conducted, the treatment-by-time interaction was significant (p=0.005) at 400 ppm. However, because the differences in mean food consumption between the control and 400 ppm groups were slight (1-3 g/animal/day) and in the absence of any effects on mean body weight gains during gestation at this exposure level, the statistically significant treatment-by-time interaction at 400 ppm was not considered test substance-related.

   Mean food consumption was unaffected by test substance exposure during lactation. Differences between the control, 25, 100, and 400 ppm groups were slight and not statistically significant.

6. **Test substance intake:** The average quantities of sulfoxaflor consumed during the maternal generation are presented in Table 6. Test substance was administered at a constant concentration from GD6 to LD 4. On LD 4 and 11 the concentration was adjusted to 50% and 40%, respectively, of the dietary concentration offered from GD 6 to LD 4. The adjustments were to account for the 2- to 3-fold increase in maternal food consumption that occurs during lactation days 7-21 in Crl:CD(SD) rats.

Table 6. Mean Test Substance Intake mg/kg/day[a]

Theoretical Dietary Level	Gestation	Lactation
25 ppm (2 mg/kg/day)	1.8	1.9
100 ppm (8 mg/kg/day)	7.1	7.6
400 ppm (32 mg/kg/day)	27.7	29.8

a = $\frac{\text{Summation of mean test substance consumption for the specified interval}}{\text{Number of days or intervals assessed}}$

Source: Table 3, pp.65, and Tables 14-15, pp. 162-164 of the Study Report.

7. **Gestation length and parturition:** No test substance-related effects were noted on mean gestation lengths or the process of parturition at any exposure level. Mean F_0 gestation lengths in the test substance-exposed groups were similar to the control group value. Differences were slight and not statistically significant. The mean gestation lengths in the 25, 100, and 400 ppm groups were 21.6, 21.9, and 21.6 days, respectively, compared to a

mean gestation length of 21.9 days in both the concurrent control group and WIL historical control data. No signs of dystocia were noted at any exposure level.

8. Maternal postmortem results:

a) Gross Pathology: No test substance-related internal findings were observed at any exposure level. The only macroscopic finding noted was dark red contents in the stomach for 1 female (no. 49902) in the 400 ppm group; this female had total litter loss on lactation day 2.

At the lactation day 21 necropsy, no test substance-related effects were observed on the number of former implantation sites and the number of unaccounted-for sites. The differences between the control and test substance-exposed groups were slight and not statistically significant.

b) Organ Weights: There were no test substance-related effects on maternal kidney or liver weights (absolute or relative to final body weight) at any exposure level. Differences in absolute weight between the control and test substance-exposed groups were not statistically significant.

B. F_1 Litter Data

1. PND 0 litter data and postnatal survival: Test substance-related effects on postnatal survival were noted at 100 and 400 ppm. Postnatal survival of the F_1 pups on PND 0 (relative to the number born) was unaffected by maternal test diet exposure. However, during PND 0-1 and 1-4 (pre selection), postnatal survival in the 100 ppm group (96.0% and 98.4% per litter, respectively) and the 400 ppm group (86.9% and 87.2% per litter, respectively) were lower than the concurrent control group (99.5% and 99.8% per litter, respectively); the difference was significant ($p<0.05$) during PND 0-1 for the 400 ppm group. As a result, postnatal survival in the 100 ppm and 400 ppm groups were 93.2 and 76.5% per litter, respectively from birth to PND 4 (pre-selection). Again, only the 400 ppm group was significantly ($p<0.01$) lower than the concurrent control group value (93.0% per litter) and the value was below the minimum mean value in the WIL historical control data (83.8% per litter).

However, when survival is considered on a pup and litter incidence basis, the increased number of dead or missing neonates from PND 1-4 (Tables 7a and 7b) is considered treatment-related and adverse in the 100 ppm and 400 ppm dose groups.

Table 7a. Incidences of Neonatal Deaths and Missing Neonates during PND 0-4

Postnatal day (PND)	Dose Levels (ppm)			
	0	25	100	400
Dead and missing neonates at PND 0 (litter incidence)	14 (6) 9 (5) if exclude TLL	3 (2)	5 (4)	6 (5)
Dead and missing neonates from PND 1-4 (litter incidence)	3 (3)	5 (5)	18 (9)	87 (17)* 71 (16) if exclude TLL
Dead and missing neonates from PND 0-4 (litter incidence)	17 (9) 12 (8) if exclude TLL	8 (7)	23 (11)	93 (19)* 77 (18) if exclude TLL

TLL – Total litter loss by PND 4
Source: Table A22, pp. 790-805 of the Study Report
*significantly different from control group at p=0.05 using Dunnet's test. Statistics generated by reviewer.

Table 7b. Incidences of Neonatal Deaths during PND0-4

Postnatal day (PND)	Dose Levels (ppm)			
	0	25	100	400
Neonatal deaths at PND 0 (litter incidence)	14 (6) 9 (5) if exclude TLL	3 (2)	5 (4)	6 (5)
Neonatal deaths from PND 1-4 (litter incidence)	1 (1)	3 (3)	12 (7)	54 (13)* 36 (11) if exclude TLL
Neonatal deaths from PND 0-4 (litter incidence)	15 (7) 10 (6) if exclude TLL	6 (5)	17 (11)	63 (15)* 41 (13) if exclude TLL

Source: Table A22, pp. 790-805of the Study Report
*significantly different from control group at the p=0.05 using Dunnet's test. Statistics generated by reviewer.

There was some variance in the survival of litters in the concurrent control group at 2 time points during the pre-weaning period. Three litters in the control group largely contributed to this increased variance in pup survival: on PND 0, litter nos. 49881 and 49934 had 70.6% and 0.0% survival, respectively, and during PND 7-14, litter no. 49916 had 0.0% survival (total litter loss on PND 9). Despite the reduced survival in the 3 aforementioned litters, mean postnatal survival values in the concurrent control group were still within the range of values in the WIL historical control data and greater than the values at 400 ppm, indicating a treatment-related effect on pup survival at 400 ppm.

The mean number of pups born, live litter size, and percentage of males per litter at birth in the 25, 100, and 400 ppm groups, and postnatal survival in the 25 and 100 ppm groups were unaffected by maternal exposure to the test diet. Differences from the control group were slight, were not statistically significant, and/or did not occur in an exposure-related manner.

Mean litter size and viability (survival) results from pups during lactation are summarized from the report in Table 8.

TABLE 8. Litter size and viability				
Observation	**Dose (mg/kg/day)**			
	Control (0)	**25**	**100**	**400**
F_1 Generation				
Mean implantation sites	16.2±1.44/24	15.7±2.27/25	16.4±2.30/24	15.9±1.62/24
Total number born (mean±SD)	14.8±3.14/25	15.2±2.08/25	15.3±2.14/24	15.2±1.75/25
Number born live (mean±SD)	14.2±3.84/25	15.0±2.09/25	15.1±2.12/24	14.9±1.87/25
Sex Ratio Day 0 (% males)	48.7±14.08/25	52.2±12.08/25	51.0±10.94/24	51.3±8.89/25
Mean litter size (%): (mean±SD/N) relative to number born				
Day 0	93.8±20.48/25	99.2±2.87/25	98.7±3.2/24	98.3±3.88/25
Day 1	99.5±1.76/24	98.9±2.51/25	96.0±7.82/24	86.9+±19.22/25
Day 4 [b]	99.8±1.20/24	99.8±1.11/25	98.4±3.77/24	87.2±24.82/25
Day 4-7[c]	99.5±2.61/23	100±0.0/25	99.5±2.61/23	100±0.0/21
Day 7-14	95.7±20.85/23	100±0.0/25	100±0.0/23	99.4±2.73/21
Day 14-21	100±0.00/22	100±.0.0/25	100±0.0/23	99.4±2.73/21
Live birth index (%)	96.0	98.8	98.7	98.0
Viability index (%)	93.0	97.9	93.2	76.5**
Lactation index (%)	95.1	100.0	99.5	98.8

SD = standard deviation N = Number of litters
[b] Before standardization (culling).
[c] After standardization (culling).
** Statistically different from control, p<0.01
Source: Tables 22, 24-25, pp. 171, 174-176 and Tables A23-A24, pp. 790-782 of the study report.

2. **General physical condition:** Pups (litters) that were found dead or euthanized *in extremis* numbered 24(8), 6(5), 17(11), and 59(15) in the control, 25, 100, and 400 ppm groups, respectively. Two (2), 2(2), 8(5), and 36(12) pups (litters) in the same respective groups were missing and presumed to have been cannibalized. In addition, malrotation of the left forelimb was noted for 2 pups in the same litter in the 400 ppm group during the week prior to weaning (on PND 14, 17, and/or 21); this observation was not apparent on PND 1, 4, 7, or 11 for either of these pups, both of which survived to the scheduled euthanasia on PND 21. The general physical condition of all F_1 pups in the 25 and 100 ppm groups was unaffected by maternal test substance exposure.

3. **Body weight:** Mean pup birth weights (PND 1) in the 400 ppm group were 11.8% lower than the control group. Mean pup weights in this group remained lower (6.5%) than the control group on PND 4 (pre- and post-culling), but were similar to the control group during the remainder of the pre-weaning period (PND 7-21). The recovery in pup body weights was likely due to the early deaths (prior to PND 4) of pups that generally had smaller body weights in the litters. The treatment-by-time interaction at 400 ppm was significant (p<0.001) during both the pre-culling and post-culling periods; however, with the exception of the post-culling weight on PND 4, pup body weights in the 400 ppm group were similar to the control group (i.e., within 5%) during the post culling period (PND 4-21). Mean body weight gains in the 400 ppm group were similar to the control group throughout the pre-weaning period.

   Mean pup body weights and body weight changes in the 25 and 100 ppm groups throughout the postnatal period were unaffected by maternal exposure to the test substance. The

significant (p<0.001) treatment-by-time interactions for mean pup body weights at 25 and 100 ppm during the post-culling period were attributed to sporadic, slightly higher (up to 4.1%) mean body weights that were not considered toxicologically important. Selected mean pup body weight data are presented in Tables 9a and 9b.

TABLE 9a. Mean (±SD) pre-weaning pup body weights (g)

Postnatal day	Dose (mg/kg/day)							
	0	25	100	400	0	25	100	400
	Males				Females			
1	7.00 (±0.578)	6.95 (±0.546)	6.93 (±0.570)	6.20 (±0.709)	6.59 (±0.527)	6.52 (±0.513)	6.43 (±0.510)	5.90 (±0.694)
4 [a]	9.53 (±1.27)	9.37 (±0.923)	9.49 (±1.12)	8.78 (±1.17)	8.95 (±1.16)	8.82 (±0.887)	8.84 (±0.951)	8.39 (±1.07)
7 [b]	12.16 (±3.71)	12.17 (±3.97)	12.31 (±3.98)	11.50 (±3.85)	11.39 (±3.45)	11.42 (±3.67)	11.58 (±3.87)	11.06 (±3.77)
11	22.35 (±2.19)	22.98 (±2.19)	22.92 (±1.73)	22.02 (±2.93)	20.87 (±2.35)	21.28 (±2.40)	21.94 (±1.82)	21.53 (±2.56)
17	35.74 (±3.26)	36.51 (±3.05)	35.74 (±3.35)	33.43 (±4.26)	34.00 (±3.09)	34.33 (±3.53)	34.56 (±3.16)	33.02 (±3.84)
21	44.81 (±4.16)	47.30 (±4.69)	46.15 (±5.23)	44.21 (±6.41)	43.03 (±4.72)	44.14 (±4.68)	44.23 (±4.47)	43.41 (±5.49)

[a] Before standardization (culling).
[b] After standardization (culling on day 4).
Source: Table 27-28, pp. 181-186 and Table A26-A27, pp. 1093-1166 in the study report.

TABLE 9b. Mean (±SD) post-weaning pup body weights (g)								
Postnatal day	**Dose (mg/kg/day)**							
	Control	**LDT**	**MDT**	**HDT**	**Control**	**LDT**	**MDT**	**HDT**
	Males				**Females**			
35	139.8 (±15.3)	143.3 (±15.7)	143.3 (±18.1)	127.05 (±23.2)	119.7 (±13.0)	120.3 (±12.7)	121.65 (±12.5)	121.35 (±12.0)
49	266.8 (±26.6)	271.7 (±23.3)	272.95 (±30.0)	249.95 (±36.7)	184.6 (±14.2)	182.3 (±19.7)	185.5 (±18.0)	185.85 (±17.1)
72	421.3 (±40.8)	426.8 (±36.9)	425.5 (±44.0)	394.6 (±44.0)	253.2 (±20.6)	251.8 (±29.5)	253.85 (±21.8)	249.7 (±25.8)

Source: Table 37-39, pp. 196-201 and Table 36, pp. 1353-1372 in the study report.

4. Offspring postmortem results:

a) Necropsies of Pups Found Dead or Euthanized *in Extremis*: The numbers of pups (litters) found dead or euthanized *in extremis* from PND 0 through the selection for evaluation subsets numbered 24(8), 6(5), 17(11), and 59(15) in the control, 25, 100, and 400 ppm groups, respectively. The increased number of pups found dead or euthanized *in extremis* at 100 ppm was interpreted to be test substance-related. No internal for gross pathological findings that could be attributed to parental exposure to the test substance were noted at the necropsies of pups that were found dead or euthanized *in extremis*.

b) Necropsies of Pups Not Selected for Neuropathological Evaluation (PND 21) and Pups Euthanized due to Sex Ratio Criteria not Met (PND 4): No internal findings that could be attributed to maternal exposure to the test substance were noted at the necropsy of pups euthanized on PND 4 due to sex ratio criteria not met or on PND 21. Aside from the presence of milk in the stomach, the only internal finding noted in the test substance-exposed groups was a dilated right renal pelvis for pup no. 49951-02 in the 25 ppm group. A dilated right renal pelvis, as well as a pale kidney, was also noted for pup no. 49937-02 in the control group. No other internal findings were noted.

C. Developmental Landmarks

1. Surface righting response: A statistically significant ($p<0.001$) delay in the mean age of attainment of surface righting response was noted in the 400 ppm pups (6.3 days) when compared to the concurrent control group value (5.3 days). The mean age of attainment at 400 ppm was also greater than the maximum mean age for males and females in the WIL historical control data (5.1 and 5.3 days, respectively). This test substance-related effect on surface righting response corresponded to reduced mean pup body weights that were noted on PND 1 and 4 in the 400 ppm group.

Surface righting response for the pups in the 25 and 100 ppm groups was not affected by F_0 maternal exposure to the test substance. The mean age of attainment was 5.2 days for pups in both the 25 and 100 ppm groups compared to 5.3 days in the control group; differences were not statistically significant.

2. Eye opening: Eye opening in the pups was not affected by maternal exposure to the test substance. The mean ages of attainment were 14.8, 15.1, 14.8, and 14.9 days for pups in the

control, 25, 100, and 400 ppm groups, respectively. The test substance-exposed group values were not statistically significantly different from the control group values.

3. **Balanopreputial separation:** Mean ages of attainment of balanopreputial separation and mean body weights at the age of attainment were unaffected by maternal exposure to the test substance. The mean ages of attainment of balanopreputial separation were 46.3, 46.1, and 47.6 days in the 25, 100, and 400 ppm groups, respectively, compared to 46.9 days in the concurrent control group; all values were within the WIL historical control data range (42.3 to 49.0 days of age). Mean body weights at the age of attainment were 247.1 g, 245.3 g, and 236.4 g in the same respective groups compared to 247.1 g in the concurrent control group and 228.8 g in the WIL historical control data. None of the differences from the control group were statistically significant.

4. **Vaginal patency:** Mean ages of attainment of vaginal patency and mean body weights at the age of attainment were unaffected by maternal exposure to the test substance. The mean ages of attainment of vaginal patency were 32.9, 32.7, and 32.6 days in the 25, 100, and 400 ppm groups, respectively, compared to 32.7 days in the control group. Mean body weights at the age of attainment were 106.0 g, 105.1 g, and 104.5 g in the same respective groups compared to 103.9 g in the control group. None of the differences from the control group were statistically significant.

D. Offspring

1. **Mortality and clinical signs:** Following weaning of the pups, male no. 49937-05 in the control group and female no. 49873-10 in the 400 ppm group were found dead on PND 22 and 28, respectively. No remarkable clinical observations or macroscopic findings were noted for either of these animals. Because of the mortality in the control group, the single death in the 400 ppm group was not considered test substance-related. All other offspring survived to the scheduled necropsies.

   No test substance-related clinical findings were noted during the weekly examinations of the pups. Findings noted in the test substance-exposed groups, including hair loss on the forelimbs and red material around the nose, mouth, and eyes, occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not exposure-related.

2. **Body weights:** Mean weekly post-weaning body weights and body weight gains in the offspring in the 25, 100, and 400 ppm groups were unaffected by maternal exposure to the test substance. Differences from the control group were slight and not statistically significant.

3. **Detailed clinical observations:** No consistent exposure-related trends were noted when detailed clinical observation data were evaluated for pups on PND 4, 11, 21, 35, 45, and 60. Findings in the test substance-exposed groups were noted infrequently, similar to the control group, and/or in a manner that was not exposure-related. On PND 60, red deposits around the nose were noted for 6 males in the control group compared to only a single male in the 400 ppm group; the difference was significant ($p<0.05$). However, a decrease in the number of males with red deposits around the nose is not considered toxicologically important. A significantly ($p<0.05$) higher number of females in the 25 ppm group were noted with alert body posture on PND 60 compared to the control group (9 vs. 2 females). In the absence of a

dose response, the increased number of alert females in the 25 ppm group was not considered test substance-related. Backing was observed for 1 male in the 25 ppm group on PND 45, and 1-2 males in the 100 ppm group on PND 11, 21, and 45. Because backing was also noted for 1 control group female on PND 21 and was not observed in the 400 ppm group, the sporadic occurrences of backing in the 25 and 100 ppm groups were not considered test substance-related.

TABLE 10. Functional observational battery results (incidence)

Observation	Dose (mg/kg/day)			
	0	25	100	400
Males				
Red deposits around nose				
-PND 4	--	--	--	--
-PND 11	--	--	--	--
-PND 21	--	--	--	--
-PND 35	--	--	--	--
-PND 45	--	--	--	--
-PND 60	6	--	--	1*[#]
Backing				
-PND 4	--	--	--	--
-PND 11	--	--	1[##]	--
-PND 21	--	--	1[##]	--
-PND 35	--	--	2[##]	--
-PND 45	--	1	--	--
-PND 60	--	--	--	--
Females				
Alert body posture				
-PND 4	--	--	--	--
-PND 11	--	--	--	--
-PND 21	--	--	--	--
-PND 35	--	--	--	--
-PND 45	--	--	--	--
-PND 60	2	9*[##]	--	--
Backing				
-PND 4	--	--	--	--
-PND 11	--	--	--	--
-PND 21	1	--	--	--
-PND 35	--	--	--	--
-PND 45	--	--	--	--
-PND 60	--	--	--	--

-- = Observation did not occur.
[#] Not considered toxicologically important.
[##] Not considered to be test related.
N = 10/sex/dose
Source: Table 49-50, pp. 296-299 in the study report.

4. **Locomotor activity:** Locomotor activity patterns (total activity counts) in pups were unaffected by maternal test diet exposure at all exposure levels when evaluated on PND 13, 17, 21, and 61. Values obtained from the 6 subintervals evaluated (0-10, 11-20, 21-30, 31-

40, 41-50, and 51-60 minutes) and the overall 60 minute test session values were generally comparable to the concurrent control values and within the WIL historical control data ranges. No remarkable shifts in the pattern of adaptation occurred in any of the test substance-exposed groups.

On PND 13, mean total counts in the 400 ppm group during the individual subintervals (0-10, 11-20, 21-30, 31-40, 41-50 and 51-60 minutes) were higher than the control group values. As a result, mean total counts in the 400 ppm group during the overall test session on PND 13 were 52.1% higher than the control group. However, these increases in motor activity were primarily attributed to 2 littermates (male no. 49861-07 and female no. 49861-10) in this group that had abnormally high total counts during the PND 13 test session. When these 2 outlier animals were excluded, mean total counts in the 400 ppm group for the overall test session on PND 13 were only 19.7% higher than the control group. Furthermore, in the absence of statistical significance across the treatment groups, the higher mean total counts noted in the 400 ppm group on PND 13 were not considered test substance-related.

On PND 17, there was a significant (p=0.029) treatment-by-time interaction for mean total counts. When subsequent pairwise comparisons were conducted, significance (p=0.007) was achieved at the low- and mid-exposure levels (25 and 100 ppm, respectively). However, statistical significance was not achieved at the high-exposure level (400 ppm), indicating the absence of a dose response. Furthermore, the significance achieved at 25 and 100 ppm was primarily the result of faster adaptation in these groups when compared to the control group, as mean total counts were slightly higher (15.9% to 21.6%) than the control group during the first 10-minute subinterval (0-10 minutes), but were approximately 18% to 27% lower than the control group during the second 10-minute subinterval (11-20 minutes) and approximately 25% lower than the control group during the last 10-minute subinterval (51-60 minutes).

A significant (p=0.044) treatment-by-sex interaction was noted when mean total counts were evaluated on PND 61; therefore, the repeated measures analysis of variance was conducted by sex. There were no statistically significant differences in mean total counts for males on PND 61. A significant (p=0.017) treatment-by-time interaction was noted for females when mean total counts were evaluated on PND 61. However, when subsequent pairwise comparisons were conducted, the treatment-by-time interaction was only significant (p=0.002) at 25 ppm. This non-dose-responsive decrease in mean total counts was not considered test substance-related. There was also a significant (p=0.002) treatment effect for females for mean total counts on PND 61; however, subsequent pairwise comparisons were not conducted because of the aforementioned treatment-by-time interaction in accordance with the protocol.

TABLE 11. Mean (±S.D.) motor activity data (total activity counts for session)				
Test Day	**Dose (ppm)**			
	0 (N=20)	**25 (N=20)**	**100 (N=20)**	**400 (N=20)**
Males+Females (pooled data)				
PND 13	1585±985.3	1499±111.7 (-5.4)	1688±897.7 (6.5)	2411±2732.8 (52.1)
PND 17	3130±2745.6	2687*±1630.4 (-14.2)	2855*±2561.5 (-8.8)	2339±1550.0 (-25.3)
PND 21	2498±806.5	2198±751.0 (-12.0)	2937±1624.9 (17.6)	2790±1350.6 (11.7)
PND [61]	5559±1475.1	4691±1386.1 (15.6)	5613±1923.6 (1.0)	4723±1403.9 (-15.0)
Males				
PND [61]	5043±1051.1	4306±1215.5 (-14.6)	4527±1376.5 (-10.2)	4499±1104.2 (-10.8)
Females				
PND [61]	6050±1668.8	5076*±1467.6 (-16.1)	6698±1794 (10.7)	4947±1649 (-18.2)

N = number of litters.
Number in brackets (#0=) is percent difference from control.
[*Include units for measurements, as needed.]*
* Statistically different from control, p<0.05
Source: Table 41, pp. 257-269 and Table A41, pp. 1571-1602, Statistics in Appendix J, pp. 2366-2378 in the study report.

5. **Auditory startle response:** The auditory startle response habituation paradigm was conducted as a longitudinal assessment with selected pups evaluated on PND 20 and again at sexual maturity (PND 60). Administration of 25, 100, and 400 ppm sulfoxaflor to the maternal animals had no significant effect on auditory startle responsiveness. At PND 20 and 60, the MAX and TMAX values for each of the 5 blocks of trials evaluated (trials 0-10, 11-20, 21-30, 31-40, and 41-50) were generally similar for the litters in the control and test substance-exposed groups. There was a significant (p=0.026) treatment-by-trial interaction for MAX on PND 60. When subsequent pairwise comparisons were conducted, significant treatment-by-trial interactions were noted at 100 (p=0.016) and 400 (p=0.019) ppm; however, the differences were the result of transient, higher MAX values at these dose levels during the second 10-trial block (trials 11-20) when compared to the control group. This transient increase in MAX values likely represented normal variability in auditory startle response measurements; during the subsequent 10-trial block (trials 21-30), the mean MAX values in the 100 and 400 ppm groups were slightly lower than the control group value. During this interval (trials 21-30) and all other trial blocks of the PND 60 test session (trials 0-10, 31-40, and 41-50), MAX values in the 100 and 400 ppm groups were similar to or slightly lower than the control group values. No other statistically significant differences from the control group were noted when MAX values were analyzed by a repeated measures analysis. No effects were noted in the pattern of the habituation response over the entire 50-trial test session in adult animals.

TABLE 12. Mean (±SD) overall (Blocks 1-5) acoustic startle peak amplitude (Newtons) and latency to peak (msec)[a]					
Dose (ppm)	**Parameter**	**Males**		**Females**	
		PND 20	**PND 60**	**PND 20**	**PND 60**
0	Peak Amp.	1.275±0.385	1.339±0.678	1.186±0.278	1.578±2.087
	Latency	61.02±2.911	45.040±10.390	58.910±4.246	49.180±7.431
25	Peak Amp.	1.385±0.270	1.054±0.566	1.300±0.327	1.126±0.519
	Latency	61.155±2.199	45.970±9.458	60.080±3.250	46.545±8.574
100	Peak Amp.	1.313±0.392	1.430±0.787	1.310±0.300	1.453±0.787
	Latency	61.120±3.780	49.385±12.811	60.720±2.721	50.115±8.930
400	Peak Amp.	1.423±0.390	1.780±1.447	1.422±0.370	1.319±0.662
	Latency	61.430±2.774	42.310±8.211	60.470±3.290	47.845±7.234

Mean and SD overall, calculated by reviewer; n=5.
Source: Table 42, pp. 270-273 and Table A42, pp. 1603-1618.

6. **Biel maze swimming trials:** Swimming ability on day 1 of the Biel maze assessment (PND 22 or PND 62) was similar between the control, 25, 100, and 400 ppm groups.

   There were no test substance-related effects on the mean numbers of errors committed in Path A (trials 1-4), Path B (trials 5-10), or the repeat of Path A (memory probe; trials 11-12) on PND 22 or 62. A significant (p=0.021) treatment-by-sex interaction was noted when the mean number of errors for Path B (trials 5-10) was evaluated on PND 62; therefore, the repeated measures analysis of variance was conducted by sex. When analyzed by sex, there were no statistically significant differences in the PND 62 mean number of errors committed between the control and test substance-exposed groups for Path B.

   There were no biologically meaningful trends for the times to criterion (mean time to locate the submerged platform) during the learning and memory trials between the F_1 males and females in the test substance-exposed groups and the control group beginning on PND 22 and 62.

TABLE 13. Biel swimming trials – Male+Female (mean ± S.D.)					
Test day/parameter		**Dose (ppm)**			
		0	**25**	**100**	**400**
PND [22] N=20					
Test day 1	Swimming ability (sec)	12.79±2.555	12.61±2.227	12.19±2.914	12.75±3.866
Test day 2 Path A	Time (sec) (first trial)	88.92±35.583	81.63±39.751	89.73±46.021	80.26±37.035
	Errors	16±6.8	15±7.7	17±8.3	15±8.5
	Time (sec) (second trial)	69.91±39.034	80.84±45.610	65.73±35.178	71.96±26.330
	Errors	12±7.5	16±9.3	14±7.9	14±4.9
Test day 3 Path A	Time (sec) (first trial)	71.31±39.335	61.22±29.189	61.25±33.513	63.97±33.002
	Errors	16±9.5	14±8.5	15±10.3	14±8.7
	Time (sec) (second trial)	63.10±40.897	57.83±33.304	55.67±29.876	51.44±37.843
	Errors	14±11.5	13±7.8	12±6.8	10±7.8
Test day 4 Path B	Time (sec) (first trial)	152.43±30.632	148.08±40.822	129.83±50.844	164.79±22.874
	Errors	32±8.9	32±10.9	28±12.1	33±8.1
	Time (sec) (second trial)	117.41±46.492	123.02±44.823	119.33±54.715	126.52±45.456
	Errors	25±11.1	25±8.6	26±13.5	26±10.3
Test day 5 Path B	Time (sec) (first trial)	112.70±48.097	103.65±46.663	110.99±43.428	107.17±50.284
	Errors	23±10.7	21±8.7	24±11.2	21±10.1
	Time (sec) (second trial)	83.06±45.041	95.73±55.043	89.31±41.685	84.38±48.819
	Errors	17±10.9	20±13.9	21±11.3	17±11.1
Test day 7 Recall	Time (sec) (first trial)	80.26±40.777	75.77±33.615	68.99±28.015	68.46±22.948
	Errors	21±10.6	21±10.7	19±7.6	20±6.7
	Time (sec) (second trial)	61.80±30.519	59.53±28.313	44.61±16.893	63.68±38.345
	Errors	15±8.5	15±8.2	11±6.0	16±9.7
PND [62] N=39 (control) and N=40 (25, 100, 400 ppm)					
Test day 1	Swimming ability (sec)	6.02±1.686	6.36±1.689	6.39±2.892	6.23±1.784
Test day 2 Path A	Time (sec) (first trial)	74.54±45.769	10.91±48.185	69.89±45.050	75.00±52.836
	Errors	17±11.4	15±10.9	16±11.6	16±11.7
	Time (sec) (second trial)	46.24±37.270	42.05±37.916	56.05±52.564	59.16±42.955
	Errors	11±9.5	8±7.9	13±11.5	14±10.2
Test day 3 Path A	Time (sec) (first trial)	38.62±31.186	40.47±36.716	50.13±48.495	38.33±37.901
	Errors	10±9.7	10±10.5	14±15.5	10±11.1
	Time (sec) (second trial)	23.06±11.527	26.96±29.753	29.78±33.291	26.90±21.442
	Errors	5±3.5	5±7.7	7±9.4	6±7.2
Test day 4 Path B	Time (sec) (first trial)	139.05±51.854	139.73±56.879	143.61±51.389	139.74±55.813
	Errors	31±12.3	31±13.8	33±13.3	31±12.9
	Time (sec) (second trial)	102.73±64.883	95.20±59.766	101.36±62.772	112.19±
	Errors	20±13.7	20±14.1	22±14.3	22±12.6
Test day 5 Path B	Time (sec) (first trial)	78.28±66.303	75.39±61.291	71.65±55.633	84.84±56.284
	Errors	17±15.9	17±14.6	15±11.7	19±12.0
	Time (sec) (second trial)	61.54±57.181	62.56±55.574	59.28±56.699	55.86±53.030
	Errors	13±13.4	13±12.5	11±10.4	12±13.9
Test day 7 Recall	Time (sec) (first trial)	70.73±48.544	67.42±49.533	85.53±55.901	60.29±41.260
	Errors	20±14.9	20±16.9	26±18.3	17±11.5
	Time (sec) (second trial)	43.90±44.282	46.86±36.058	47.77±40.279	51.81±44.617
	Errors	10±12.2	11±10.3	12±10.9	13±11.8

Path A = forward through maze; Path B = reverse through maze; Time = mean time to escape; Error = all four feet into an incorrect channel.
Source: Tables 43-48, pp.274-295 and Tables A43-A46, pp. 1619-1668 in the study report.

7. Offspring postmortem results:

a) Unscheduled Deaths: Male no. 49937-05 in the control group and female no. 49873-10 in the 400 ppm group were found dead on PND 22 and 28, respectively. No remarkable internal findings were noted for either of these animals at necropsy. All other animals survived to the scheduled necropsies.

b) Animals Euthanized Following PND 22 Learning and Memory (Subset B):

There were no internal findings related to maternal exposure to the test substance noted for Subset B animals euthanized following completion of the learning and memory assessments. Internal findings noted in the test substance-exposed groups consisted of a depressed area on the kidney, swollen spleen, a distended ureter, and clear contents in the uterus. These findings were observed in single animals, in a manner that was not exposure-related, and/or are common findings in laboratory animals. Dilated renal pelves were noted for 3 males in the 400 ppm group. Because this finding was also noted in a single control group female and is a common finding in this species, the slightly increased number of pups with dilated renal pelves in the 400 ppm group males was not considered test substance-related. No other internal findings were noted.

8. Neuropathology:

a) PND 21 (Subset C):

Macroscopic Examinations: No test substance-related gross findings were noted in the brain or spinal cord in offspring selected for brain weights on PND 21. In the 25 ppm group, 1 male had dark red material attached to the brain and 1 female had a depressed area on the brain; these findings were not observed at higher exposure levels.

Brain Weights/Brain Measurements: No test substance-related effects on mean brain weights or measurements were noted at any exposure level on PND 21. Mean brain width (combined-sex) in the 100 ppm group (14.4 mm) was significantly (p=0.002) higher than the control group value (14.2 mm); however, in the absence of a dose response, the difference was not considered test substance-related. Other differences between the control and test substance-exposed groups were slight and not statistically significant.

TABLE 14. Mean (±SD) brain weight and measurement data				
Parameter	**Dose (ppm)**			
	0	**25**	**100**	**400**
Males				
Day 21 [N=20]				
Terminal body weight (g)	44.81±4.16	47.30±4.69	46.15±5.23	44.21±6.40
Brain weight (g)	1.5337±0.08767	1.5862±0.07528	1.5458±0.07739	1.5415±0.07611
Brain-to-body weight ratio	3.42%	3.35%	3.35%	3.49%
Brain length (mm)	18.1 ± 0.40	18.4 ± 0.46	18.3 ± 0.54	18.0 ± 0.43
Brain width (mm)	14.4 ± 0.30	14.6 ± 0.20	14.6 ± 0.27	14.4 ± 0.27
Termination [N=19-20]				
Terminal body weight (g)	421.326±40.7786	426.75±36.8980	425.50±44.047	394.60±43.893
Brain weight (g)	2.1468±0.07311	2.1270±0.07540	2.0765±0.12149	2.0355*±0.14848
Brain-to-body weight ratio	0.510%	0.498%	0.488%	0.516%
Brain length (mm)	21.3 ± 0.65	21.1 ± 0.59	20.8 ± 0.70	20.5** ± 0.57
Brain width (mm)	15.4 ± 0.28	15.4 ± 0.31	15.3 ± 0.36	15.2 ± 0.33
Females				
Day 21 [N=20]				
Terminal body weight (g)	43.03±4.72	44.14±4.68	44.23±4.47	43.414±5.49
Brain weight (g)	1.4980±0.08739	1.5165±0.08105	1.5024±0.07790	1.4855±0.06444
Brain-to-body weight ratio	3.48%	3.44%	3.40%	3.42%
Brain length (mm)	17.5 ± 0.53	17.6 ± 0.48	17.6 ± 0.44	17.5 ± 0.49
Brain width (mm)	14.1 ± 0.33	14.2 ± 0.25	14.2 ± 0.30	14.2 ± 0.19
Termination [N=20]				
Terminal body weight (g)	253.15±20.5562	251.75±29.4563	253.85±21.777	249.70±25.787
Brain weight (g)	1.965±0.08630	2.0050±0.09417	1.9620±0.10144	1.9295±0.10531
Brain-to-body weight ratio	0.776%	0.796%	0.773%	0.773%
Brain length (mm)	20.0 ± 0.77	20.3 ± 0.65	20.4 ± 0.70	20.8 ± 0.47**
Brain width (mm)	14.7 ± 0.32	14.8 ± 0.39	14.7 ± 0.40	14.9 ± 0.36

* Statistically different from control, p<0.002
** Statistically different from control, p<0.001
Source: Tables 54 (pp.303-304) and 58 (pp. 319-320), , and TablesA52 (pp. 1990-1997) and A55 (pp. 2175-2182) in the study report.

Microscopic Examinations: There were no test substance-related histologic changes in the brain of males and females at any exposure level on PND 21. All histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There were no test substance-related alterations in the prevalence, severity, or histologic character of those incidental tissue alterations.

TABLE 15. Histopathology findings					
Parameter		**Dose (ppm)**			
		0 (N=10)	**25 (N=10)**	**100 (N=10)**	**400 (N=10)**
		Males			
		Day 21 [N=10]			
Basal Ganglia – ectopic tissue	minimal	1	NA	NA	0
		Day Termination [N=10]			
Lum spin nerve -degeneration, axonal	minimal	1	NA	NA	1
Lumbar dor. fib. -degeneration, axonal	minimal	0	NA	NA	2
Peroneal nerve -degeneration, axonal	minimal	1	NA	NA	3
Sciatic nerve unremarkable	minimal	3	NA	NA	4
	mild	1	NA	NA	0
Tibial nerve -degeneration, axonal	minimal	1	NA	NA	2
Trigeminal nerve -degeneration, axonal	minimal	0	NA	NA	1
		Females			
		Day 21 [N=10]			
None found					
		Termination [N=10]			
Lum spin nerve -degeneration, axonal	minimal	1	NA	NA	1
Lumbar dor. fib. -degeneration, axonal	minimal	1	NA	NA	1
Lumbar vent. fib- degeneration, axonal	minimal	1	NA	NA	0
Sciatic nerve degeneration, axonal	minimal	5	NA	NA	1

N = 10
NA = not applicable, none examined from this dose level.
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01
Source: Tables 55 and 59, pp. 305-308, 321-332 and Tables A53 and A56, pp. 1998-2015, 2183-2198 in the study report.

There were no differences between the control and 400 ppm rats in any of the mean brain morphometry measurements on PND 21.

b) PND 72 (Subset A):

Macroscopic Examinations: no test substance-related gross findings were noted in the brain or spinal cord in offspring selected for brain weights on PND 72. The only macroscopic finding noted in the test substance-exposed groups was dark red material attached to the brain for 2 males in the 25 ppm group; this finding was also noted for 1 male

in the control group, and was not observed at higher exposure levels. One male in the control group also had a dark red area on the brain and a small brain (olfactory bulb).

Brain Weights/Brain Measurements: A significant ($p<0.001$) treatment-by-sex interaction was noted when mean brain weights and measurements were evaluated on PND 72; therefore, the multivariate analysis of variance was conducted by sex. When analyzed for each sex separately, mean absolute brain weight ($p=0.002$) and brain length ($p<0.001$) for the 400 ppm group males were significantly lower (5% and 4%, respectively) than the control group (Table 14 above). However, in the 400 ppm group females, the mean absolute brain length was significantly ($p<0.001$) higher (4%) than the control group.

Microscopic Examinations: There were no test substance-related histologic changes in the brain of males and females at any exposure level on PND 72. There were instances of axonal degeneration in the peripheral nerves, particularly the sciatic nerve, and sometimes in the spinal nerve roots. This axonal degeneration was of minimal severity, typically with only a single 'digestion chamber', and consistent with incidental alterations. Minimal axonal degeneration in the peripheral nerves and spinal nerve roots is a common background lesion. In addition, the relative incidence of minimal axonal degeneration in the lumbar dorsal fibers and peroneal nerves for males in the 400 ppm group (20% and 30%, respectively) was similar to that noted in the WIL historical control data (17.8% and 36%, respectively).

There were no differences between the control and 400 ppm rats in any of the mean brain morphometry measurements on PND 21.

III. Evaluation, Summary and Conclusions by Regulatory Agency

A. Name of Authority: Health Effects Division/Office of Pesticides Program/US EPA

B. Reviewers Comments:

Reliability Rating: 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.

C. Conclusions:

The investigator proposed the alterations in brain lengths (4% decrease in males; 4% increase in females) in the 400 ppm group at 72 days were not treatment-related because there was no pattern of alteration in gross or microscopic brain structures and no differences in brain morphometric values. Additionally, there were no other indications of a sex-specific effects for any other parameter evaluated on study, thus, these minimal decreases and increases in mean absolute brain lengths in the 400 ppm males and females, respectively, were likely spurious. However, the Agency considers the change treatment-related and adverse, given that the brain parameters are normally conserved compared to other organ effects and there is no indication that these effects are not treatment-related. Additionally, gender differences are not uncommon toxicological findings.

There were no test substance-related effects on maternal parameters in this study. Therefore, the no-observed-adverse-effect-level (NOAEL) for systemic toxicity and maternal reproductive toxicity of sulfoxaflor when administered orally in the diet was 400 ppm (equivalent to 28.8 mg/kg/day).

The LOAEL for offspring is 100 ppm (7.4 mg/kg bw/day) based on the reduction in pup survival. The NOAEL is 25 ppm (1.8 mg/kg bw/day).

D. **Deficiencies:** As a minor deficiency in the study, pup birth weights (PND 0) were not measured, as recommended in guidelines. It would be advantageous to contact the company to inquire as to whether birth weights were measured.